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Editor: Lal S. Mian, Ph.D.
Department of Health Science & Human Ecology
California State University
San Bernardino, CA 92407-2397
Phone: (909) 537-7409
Fax: (909) 537-7037
E-mail: lmian@csusb.edu

Reviewers:

Bruce F. Eldridge, Ph.D., UC Davis; Minoo B. Madon, Greater L.A. Co. VCD; Steve Schutz, Ph.D., Contra Costa MVCD; William E. Walton, Ph.D., UC Riverside; James P. Webb, Ph.D., Orange County VCD; and Glenn M. Yoshimura, Sacramento-Yolo MVCD

Layout and Editorial Assistance: Emily Young, MVCAC

Mosquito and Vector Control Association of California
660 J Street, Suite 480
Sacramento, California 95814
Phone: (916) 440-0826 • Fax: (916) 442-4182
E-mail: mvcac@mvcac.org

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Dedication of the Seventy-Third Annual MVCAC Conference to Dr. William C. Reeves

December 2, 1916 - September 18, 2004

The Contribution of W.C. Reeves to MVCAC

Bruce F. Eldridge

University of California, Davis

In a few moments, Bill Reisen is going to review the brilliant scientific career of Dr. William C. Reeves, and I want to leave Bill as much time as I can to do that, so I'll try to be brief. I do, however, want to discuss the special relationship Bill Reeves had to MVCAC, and to illustrate some of his many important contributions to this association.

Dr. William C. Reeves was never an officer in MVCAC. He never worked for a mosquito abatement district. He was never a trustee of a mosquito abatement district. He was never an associate member. I know of only one committee he ever served on (Disaster Aid, 1985). Nevertheless, over the history of MVCAC and its predecessor organizations few individuals approach the impact he had on MVCAC and mosquito abatement in California and elsewhere.

Bill was a native Californian absolutely dedicated to the notion that protection of California citizens from viral encephalitides and other mosquitoborne diseases was tightly linked to mosquito abatement. It is hard to encapsulate the features of Bill's long career that resulted in his enormous impact on mosquito control, but I'll try. He had a rare ability to conceive and conduct original biological research, he firmly believed in the application of research results to important public health problems, and he aggressively pursued opportunities to work cooperatively with California mosquito abatement districts (MADs). But that tells only part of the story. I believe that to fully appreciate just what he accomplished during his 65-year career one must go back to the beginning of his entry into the world of mosquito biology and control in 1939.

Bill Reeves is first mentioned in the California Mosquito Control Association proceedings of its annual meeting in 1939. At the time he attended his first meeting he was Mr. Reeves, a research assistant working with Professor William B. Herms. In 1939, mosquito transmission of arboviruses was not an accepted concept, and the most of the experiments conducted on virus infection and transmission were with *Aedes aegypti*. At the 1940 meeting, Bill presented a paper on the biology of *Aedes varipalpus*. At this same meeting Malcolm Merrill presented a paper entitled "The mosquito as a possible vector of equine encephalitis." Bill made some remarks after Dr. Merrill's paper, but neither mentioned the mosquito *Culex tarsalis*. Also at this meeting, Thomas H.G. Aitken presented a paper summarizing the possible role of certain California mosquito species in transmission of equine and human encephalomyelitis, but the only *Culex* mosquito he mentioned was *Culex pipiens*.

In 1941, the course of mosquito abatement in California was changed forever, because Bill Reeves presented the results of his work in the Yakima Valley of Washington strongly suggesting that *Culex tarsalis* was the most likely vector of both western equine encephalomyelitis virus (WEE) and Saint Louis encephalitis virus (SLE). Coincidentally, in this paper he first presented his ideas about vector incrimination and overwintering of arboviruses. To appreciate the brilliance of this work, the clear and logical explanation of the data, and the persuasive interpretation of the results, I strongly urge you to find a copy of the 1940 Proceedings and read Bill's landmark paper, based on a paper he and his colleagues had published in the journal *Science* that same year. The significance of this paper is even more astonishing when you realize that Bill was only 23 years old at the time! I was interested to note that it was reported that Bill's paper was illustrated with lantern slides, and that Professor Herms gave him an "attaboy" with "That was a very excellent paper, Bill". That may be one of the greatest understatements in the history of MVCAC.

Articles Bill wrote for the Proceedings in the years just before and just after World War II exemplify Bill's philosophy of science and service; and set the stage for the contributions he made in the years following this period. The many scientific contributions he made will be reviewed by Bill Reisen, and I'll not dwell on them further. Bill's emphasis on applied research was presaged by an article which also appeared in 1941, titled "Some suggestions for the control of *Culex tarsalis* in California.

His ideas about cooperative studies between University of California faculty and mosquito abatement agencies are first found in a paper published in the 1946 Proceedings entitled "A preliminary report of the results of planned cooperative field studies." In this paper, Bill explained that scientists from the Hooper Foundation (where Bill worked at the time) and the Dr. Morris Mosquito Abatement District discussed misunderstandings stemming from one cooperating organization (a mosquito abatement agency) wanting as complete a control program as possible and another organization (UC) wanting mostly accumulation of scientific data on the biology and disease relationships of mosquitoes. This turned out to be the first of dozens of highly successful cooperative projects Bill engaged in with various mosquito abatement agencies.

Bill Reeves' direct contributions to MVCAC extend beyond research projects. He traveled all over the state to visit MADs and

examine practices such as placement of light traps and sentinel chicken flocks, and advised them on their most effective use. He participated in a number of evaluations of many other practices relating to mosquitoborne disease surveillance. He spoke to trustees. He presented lectures at continuing education sessions. Bill's role in the development and implementation of the California mosquito-borne disease surveillance program could be a subject all by itself.

From 1939 to 2004, I don't believe Bill Reeves missed a single annual conference, and he attended the vast majority of quarterly meetings. During this time he gave 67 presentations at annual conferences. They included talks on arbovirus surveillance, arbovirus ecology, disease prevention, and mosquito biology and control. Most of these talks summarized results of research, but in the later years of his career he presented many talks that expressed his views on the future of mosquito abatement in California.

On the basis of his dedication to mosquito abatement and prevention of mosquitoborne diseases he was the first recipient of the MVCAC Meritorious Service award (1981) and was elected an honorary member that same year.

I have really only skimmed the surface of Bill's contributions to MVCAC. I promised to be brief, but I do want to touch on Bill's lighter side. For some reason, Bill was considered somewhat gruff and unapproachable by some. Knowing Bill as I did, I find this difficult to understand. It may be that some people believed this because of a situation that eventually grew in the telling to become a MVCAC legend. This situation might be referred to as the legend of the orchids and Big Chicken and Little Chicken. Many of you know this story well, but there be many that do not. I don't think it would be appropriate for me to divulge the real names of Big Chicken and Little Chicken, but I will try to outline the main points of the story, and if you are interested in knowing all the details, I would refer you to our most recent past President, who knows the story well.

It seems that two mosquito abatement agency managers knew Bill had an impressive orchid greenhouse at his home, and asked him once if he had any spares that he would be willing to part with. Much to their surprise, the next time he saw them he had two beautiful potted orchid plants for them, and he provided them detailed information on how to care for them. The orchids were accepted with thanks and moved to the owners' respective district offices. Over the ensuing month or so, both plants were seriously neglected, and the two district managers were shocked to find that they had died. Each time Bill saw the two new orchid growers he asked them how the plants were doing and each time they both said "fine". This went on to such an extent that the managers' consciences began to bother them but neither could bring himself to confess to Bill that the orchid plants were gone. They even went to the extreme of having photographs of themselves taken posed in a nursery alongside a particularly health orchid plant, and sent copies of the photographs to Bill. He wasn't fooled. Eventually, they enlisted a third party to break the news to Bill. Because they wouldn't admit their malfeasance directly to Bill, he named them "Little Chicken" and "Big Chicken". Bill Reeves greatly enjoyed telling his version of the story, and he considered both "BC" and "LC" close friends.

Here is an anecdote of my own. Bill and I used to go on 3-4 day trips to various western states every year to collect larval mosquitoes. Bill owned a large stainless steel Thermos jug, and it was his practice to have it filled with fresh coffee at breakfast each morning, so we could take a mid-morning coffee break sitting on a log or a boulder in a forest or other scenic location somewhere. One morning, we were being served breakfast by a young blond woman in LeeVining, California. Bill had placed the Thermos on the table, but as the waitress brought us breakfast, and then more coffee or orange juice, she seemed to be ignoring it. Bill was kind of grumpy that morning because we had not been very successful the day before, and I could see he was getting impatient with her. When she approached with the check, Bill reached over to the Thermos and gave it a vigorous "tap, tap, tap". Without a word, the waitress reached over and gave it her own "tap, tap, tap". I could see the color rising in Bill's cheeks, and I was steeling myself for an eruption when she put her arm around Bill and said "Did you think I was going to forget your coffee, honey?" Have you ever watched a person melt?

It is easy to get hyperbolic when talking about Bill's importance to MVCAC and its programs, but it is also almost impossible to overstate his contributions. When Johnny Carson died, I was impressed by a number of things that were said about him. One in particular immediately made me think of Bill: If you knew Bill Reeves, consider yourself lucky, because another one like him will not come along this way in a very long time."

A Tribute to the Life and Times of William C. Reeves, 1916 - 2004

William K. Reisen[1]

Center for Vectorborne Diseases, Department of Pathology, Microbiology and Immunology
School of Veterinary Medicine, University of California, Davis

ABSTRACT: The 2005 meeting of the MVCAC is dedicated to Dr. William C. Reeves, Professor and Dean Emeritus, School of Public Health, University of California, Berkeley, who passed away on 18 Sep 2004. Bill was a leader in the field of arbovirology for over 6 decades at the state, national and international levels. He and his research teams discovered that western equine encephalomyelitis and St. Louis encephalitis viruses were a major cause of summer neurological illness and that these viruses were maintained in nature by *Culex* mosquitoes and wild birds. His field studies developed CO_2 as an attractant for trapping host-seeking mosquitoes, exploited the frequent infection of barnyard chickens as a sentinel system, were the first to use fluorescent dust to mark mosquitoes, and carefully resolved the transmission cycles of western equine encephalomyelitis, St Louis encephalitis and other viruses in California. These discoveries redirected mosquito control efforts to focus on *Culex tarsalis* Coquillett and led to the current integrated surveillance and control programs used by the MVCAC to this day. Bill's training program at Berkeley was the crucible which forged the careers of many of today's leaders in arbovirology, public health, and infectious disease epidemiology. The state of California honored his contributions and service to public health at his retirement in 1987 by California Legislature Resolution No. 127 of Commendation. He was very proud of the fact that he had attended every MVCAC annual conference and Department of Health Services Vector Control Advisory meeting ever held. The MVCAC has honored his contributions to mosquito control by bestowing the Meritorious Service Award in 1981, establishing the Reeves' Young Investigator Award in 1998, and issuing an Honorary Membership in 2003. His colleagues, the MVCAC and I especially will miss his continuous support, great ideas and endless enthusiasm.

INTRODUCTION

I was especially flattered and pleased when I was requested to share the podium with Bruce Eldridge to participate in today's tribute to Dr. William C. Reeves, Professor and Dean Emeritus, School of Public Health, University of California, Berkeley. Bill was my boss, mentor and friend for almost 25 years who, with Jim Hardy, taught me about arboviruses, how to think critically, and instilled in me the importance of applied research towards enhancing mosquito control in California.

My charge today was to provide an overview of Bill's illustrious 60 year career in public health and point out his important contributions that shaped our field. Detailed accounts of Bill's career and accomplishments have been summarized in the proceedings of a symposium published at his retirement as a supplement to the American Society of Tropical Medicine and Hygiene celebrating his career (Johnson 1987) and in his oral autobiographical history available through the Bancroft Library at UC Berkeley [Reeves, W.C. 1993. *William C. Reeves.* Regional Oral History Office, University of California, Berkeley. Available from the Online Archive of California; http://ark.cdlib.org/ark:/13030/kt3j49n66k].

BILL'S LIFE AND CAREER

William Carlisle Reeves was born on December 2, 1916 on a small orange ranch in Riverside, California where he spent his early life learning to love the outdoors and insects. An early love of entomology earned him the 'nick-name' of "Billy bugs" and directed his later education.

At Berkeley, Bill's summer work focused on USDA forestry surveys where he supervised field crews – undoubtedly an experience that shaped his management style that was always straight forward and direct. After completing his BS in Entomology at UC Berkeley, Bill's initial graduate studies focused on mosquitoes and factors that stimulated egg hatch in *Aedes sierrensis*

Synopsis of W.C. Reeves' Education

- 1934: HS Diploma - Riverside Polytechnical High School
- 1936: AA Riverside Junior College
- 1938: BS UC Berkeley – Entomology
- 1943: PhD UC Berkeley – Medical Entomology and Parasitology
- 1949: MPH UC Berkeley - Epidemiology

[1] Arbovirus Field Station, 4705 Allen Rd, Bakersfield, CA 93314

(Ludlow). Unfortunately for Bill, but fortunately for California and science in general, his results and unique ideas were 'scooped,' leaving him without a graduate project. About this time, Dr. William McD Hammon invited Bill to join an interdisciplinary team headed for the Yakima Valley of Washington to investigate recent outbreaks of human and equine encephalitis. These seminal studies [1941-42] unraveled the epidemiology of western equine encephalomyelitis [WEE] and St. Louis encephalitis [SLE] in the western USA and resulted in the first isolations of WEE and SLE from *Culex pipiens* L. *and Cx. tarsalis* Coquillett and the first incrimination of wild birds as reservoirs of these viruses (Hammon 1941; Hammon et al. 1941; Hammon et al. 1945; Hammon and Reeves 1947; Reeves et al. 1952; Reeves and Hammon 1944). These early studies formed a strong bond between Hammon and Reeves, expanded Bill's interests in mosquitoes to include the viruses they transmit, and launched his research and teaching career at UC Berkeley, that is summarized below.

WC Reeves' Career at University of California

- 1939, 1940: Agent, USDA, Forest Insect Investigations
- 1939-41: TA, Medical Entomology and Parasitology, Dept. of Entomology
- 1941-49: Research Entomologist, UC San Francisco, Hooper Foundation; also Lecturer at new UC Berkeley School of Public Health
- 1949-87: Professor of Epidemiology, UC Berkeley, School of Public Health
  - o 1967-71: Dean
  - o 1971-85: Head, Program in Epidemiology
- 1987-2004: Professor and Dean Emeritus, UC Berkeley, School of Public Health

Bill sorting mosquitoes at the Bakersfield Field Station ca. 1950

Upon completion of the Yakima studies, Bill and Hammon organized a similar interdisciplinary team to focus on summer encephalitis problems in Kern County, because of the unusually high incidence of summer neurological disease in humans and equines and the excellent cooperation by the Kern Mosquito Abatement District (MAD). Establishment of the Bakersfield Field Station and having in depth ecological and epidemiological studies underway positioned Bill and his team to carefully document conditions that led to the 1952 epidemic of WEE and SLE centered in the San Joaquin Valley.

These Kern County studies were summarized in Bill's first monograph that remains a classic to this day (Reeves and Hammon 1962). Recognition of the importance of quantitative sampling to track mosquito populations and virus activity resulted in the development of many of the surveillance techniques and virus isolation methods that we use today, including CO_2 to collect mosquitoes (Reeves 1951; Reeves 1953), sentinel chickens to monitor transmission (Hammon et al. 1948), precipitin testing to determine blood meal hosts (Reeves 1944; Reeves and Hammon 1944), fluorescent dusts to mark mosquitoes for dispersal studies (Reeves et al. 1948), and laboratory host competence studies to help incriminate vector mosquitoes (Hammon et al. 1943; Hammon and Reeves 1943a; Hammon and Reeves 1943b) and avian hosts (Hammon et al. 1951). Bill's ideas about combining climate variation and enzootic surveillance information into an early warning system to forecast years with high outbreak risk are still in place and form the basis of the current state-wide plan **[http://westnile.ca.gov/website/publications/2005_ca_mosq_response_plan.pdf]**

More recently Bill's team investigated a variety of encephalitis ecology and intervention approaches, including genetic control (Reisen et al. 1981; Reisen et al. 1982; Reisen et al. 1985a) and aerial low-volume adulticide applications (Reisen et al. 1984; Reisen et al. 1985b; Schaefer et al. 1985). These were followed by studies on the potential impact of global warming on vector-borne diseases (Reeves et al. 1994). Parallel investigations on mosquito biology combined with his previous and recent studies on the ecology and control of encephalitis viruses in California were summarized in Bill's second monograph (Reeves 1990). The following photo was taken at a party commemorating the completion of this second monograph.

Shown from left to right are: John Combs [manager of Delta MAD and initiator of the project], Bill Reeves, Bill Reisen, Marilyn Milby, Jim Hardy and Sister Monica Asman.

Even retirement did not seem to slow Bill down. After attaining Emeritus status, Bill, with Bruce Eldridge, Jim Hardy, Laura Kramer and a series of students launched in depth studies on the ecology, systematics and epidemiology of California-group viruses transmitted by snow pool mosquitoes (Campbell et al. 1991a; Campbell et al. 1991b; Eldridge et al. 1991; Eldridge and Reeves 1990; Kramer et al. 1992). Bill frequently contributed to this project by collecting mosquitoes at >10,000 ft elevations, quite a feat for a senior citizen with blood pressure problems!

In addition to his important research programs, administration of the School of Public Health and continued teaching responsibilities, Bill somehow found time to participate in state, national and international agency programs and planning. A sampling of these is listed below:

W.C. Reeves' Service to State, National and International Organizations (from a list of 33)

- 1945-78: Viral Commission, Armed Forces Epidemiological Board
- 1949-87: Consultant, Ecological Investigations Program, CDC
- 1966-73: Chair, Arbovirus Research Reagent Committee, NAIAD, NIH
- 1991-93: Committee on Microbial Threats to Health, Institute of Medicine, NAS
- 1995-96: Special Emphasis Panel on research on Emerging Viral Threats, Div. Int. Hlth., Institute of Medicine.
- 1960-2004: Expert Panel on Virus Diseases, WHO
- 1971-85: Advisory Scientific Board of Gorgas Memorial Inst. Trop. Med. Prev. Med.
- 1946-2004: Vector Control Advisory Committee, Calif. Dept Hlth Svcs
- 1977-2004: Advisory Committee on Mosquito Research to the President of the University of California

Bill was always focused on the future of public health, the need for continued vigilance against emerging problems, and the training of new scientists, as far back as his Presidential Address to the American Society of Tropical Medicine and Hygiene (Reeves 1972). This far forward thinking took place at a time when most of his colleagues were considering the eradication of infectious diseases, especially malaria, and the redirection of research and training resources to old age and related issues. History has proven him right and today our battle with emerging problems such as the invading West Nile virus, SARS and avian influenza, has intensified. Other examples of his participation in national level planning meetings are summarized below.

- *The U.S. Capacity to Address Tropical Infectious Disease Problems*. Board on Science and Technology for International Development, Office of International Affairs. National Research Council and the Institute of Medicine, National Academy of Sciences, 1987. National Academy Press, Wash., D.C., 1987. p. 1-172.
- W. C. Reeves. Concerns about the future of medical entomology in tropical medicine research. *Am. J. Trop. Med. Hyg.* 1989, 40:569-570.
- Lederberg, J., Shope, R. E. and Oaks, S. C., Emerging Infections Microbial Threats to Health in the United States. Institute of Medicine, Mate Academy Press, Washington, D.C., 1992, pp. 1-294.

Bill's internationally acclaimed research and training programs and accomplishments resulted in widespread acclaim and a large number of prestigious awards, some of which are listed below. Of particular interest was recognition of his contribution to both medical entomology and tropical medicine as indicated by his receiving both the Hoogstraal and Walter Reed Medals from the American Society of Tropical Medicine and Hygiene [ASTMH] in the same year.

W. C. Reeves Major Honors and Awards (selected from a list of 22)

- 1970-71: President, ASTMH
- 1973: RM Taylor Award for Achievement in Arbovirology, ACAV, ASTMH
- 1975: Certificate of Appreciation for Patriotic Civilian Service – US Army
- 1980-81: Distinguished Teaching Award, UC Berkeley
- 1981: Meritorious Service Award – CMVCA
- 1982: Medal of Honor for Distinguished Contributions to Mosquito Control, AMCA
- 1982: John Snow Award – APHA
- 1987: California Legislature Resolution No. 127 of Commendation
- 1987: Hoogstraal Award, ACME, ASTMH
- 1987: Walter Reed Medal, Meritorious Achievement in the field of Tropical Medicine, ASTMH
- 1988: Gold Headed Cane Award, AVES, AVMA
- 1991: Alumnus of the Year Award, School of Public Health, UC Berkeley

Bill continued to be active in our field practically to the day of his passing. In fact, his final publication focused on fine-tuning encephalitis virus surveillance in California by developing a test to see if sentinel chickens were being bitten by *Culex* mosquitoes (Trevejo et al. 2005). Bill's excellent ideas and practical and direct solutions to complex issues will be sorely missed and impossible to replace.

Thanks Bill, for the continuous support, the lasting friendship, the great ideas, and the endless enthusiasm . . .

Acknowledgement

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Introduction

William K. Reisen[1]

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

West Nile virus (Family: *Flaviviridae*, genus *Flavivurus*, WNV) invaded southern California during the summer of 2003 (Reisen et al. 2004), being found initially in Imperial Valley near El Centro during early July. WNV then amplified enzootically at wetlands along the margin of the Salton Sea in Riverside County and by late summer invaded eastern Los Angeles and San Bernardino in areas near large American crow roosts at Whittier Narrows along the San Gabriel River and at the Prado Basin along the Santa Ana River, respectively (Fig. 1). By November, WNV had amplified at and around these communal crow roosts and dispersed throughout southern California to the Mexican border. A single dead American crow found in Apple Valley north of the Santa Monica Mountains was the only detection of the virus indicating northward dispersal.

WNV successfully overwintered in southern California during 2003-04. The first detections of WNV activity were dead American crows found in February near the Whittier Narrows roost and positive *Culex tarsalis* Coquillett pools collected in April from the Coachella Valley near the Salton Sea. In early summer virus amplified to epidemic levels near crow roosts in east Los Angeles and San Bernardino and then at crow roosts in west Los Angeles along the Los Angeles River drainage system. In late June, the virus was detected north of the Tehachapi Mountains and by late summer, it was reported from every county in California.

The Arbovirus Unit within the Center for Vectorborne Diseases, University of California, Davis, in collaboration with the Coachella Valley, Greater Los Angeles County, Kern, and Sacramento-Yolo Mosquito and Vector Control Districts and the California Department of Health Services have been studying the invasion of California by WNV. At the 2004 annual meeting of the Mosquito and Vector Control Association of California, we summarized our findings during the first year of this invasion and described challenges in viral and serological diagnostics. The current symposium describes our continuing research during the second year of the invasion, focusing on virus dispersal and amplification and on factors enabling the success of this invading

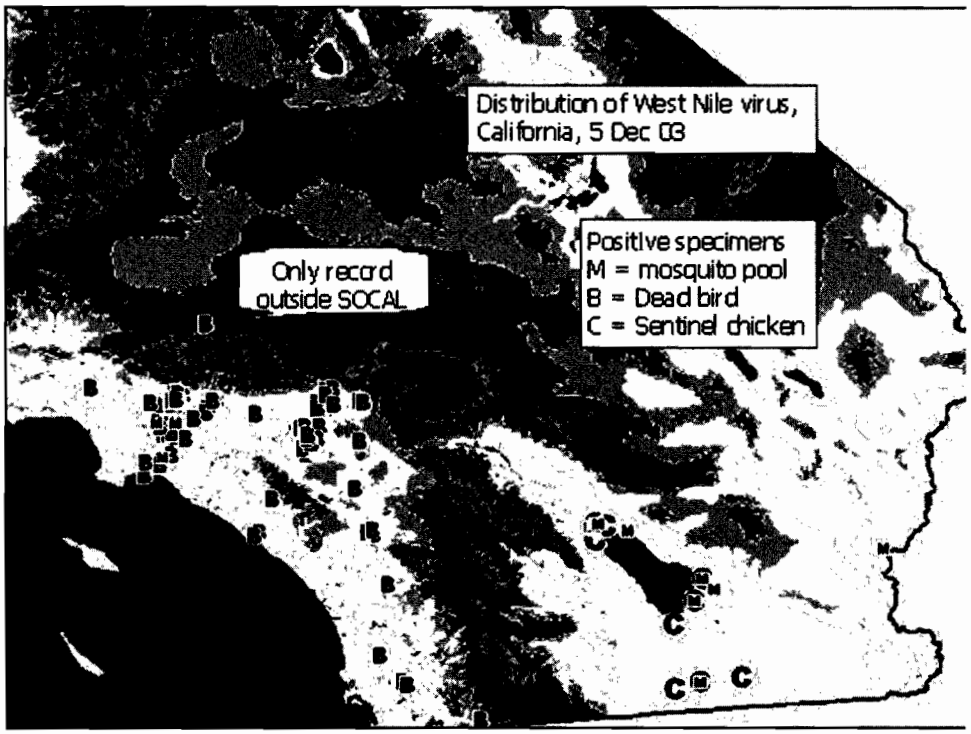

Figure 1. Distribution of WNV in California as of December 2003.

[1] Address correspondence to the Arbovirus Field Station, 4705 Allen Rd, Bakersfield, CA 93314

virus. The titles of the talks presented and the speakers are listed below. These talks summarize the combined efforts of a large number of University of California, Department of Health Services, and local agency personnel whose hard work produced the volume and quality of the data presented.

- West Nile Virus in Southeastern California, Hugh Lothrop
- Over Wintering and Amplification of WNV in South Los Angeles, Jennifer Wilson
- Dispersal and Amplification of WNV in North Los Angeles, Paul O'Connor
- Invasion of Kern County by West Nile Virus, Richard Takahashi
- Invasion of Sacramento and Yolo Counties, Veronica Armijos
- Vector and Host Competence: Importance of Virulence in Birds for WNV Transmission, William Reisen
- Impact of WNV on Wild Birds – Who Lives and Who Dies? Sarah Wheeler
- Conclusions, William Reisen

Acknowledgements

This research program described in our symposium was funded by grants from the National Institutes of Allergy and Infectious Diseases, NIH, Centers for Disease Control and Prevention, Office of Global Programs, NOAA, California Department of Health Services, and the University-wide Mosquito Research Program. Additional funds, research space and logistical support were generously provided by the Coachella Valley MVCD, Greater Los Angeles Co VCD, Kern MVCD and the Sacramento/Yolo MVCD. Dead birds were necropsied by the California Animal Health and Food Safety laboratories in San Bernardino and Davis. Dead bird tissues and mosquito pools were tested in the Arbovirus Laboratory at the Center for Vectorborne Diseases (CVEC) under the direction of Barbara Cahoon-Young. Avian sera were tested by Sandra Garcia and Ying Fang, CVEC. Sentinel chicken sera were tested by the Viral and Rickettsial Diseases Laboratory of the CDHS under the direction of Carol Glaser. Data on horse or human cases were provided by County Public Health Departments as well as CDHS. The map in Figure 1 was created by C.M. Barker, U.C. Davis.

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

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

[1]*Arbovirus Research Unit, Center for Vector-borne Disease Research, School of Veterinary Medicine, University of California, Davis, CA 95616*
[2]*Coachella Valley Mosquito and Vector Control District, 43420 Trader Place, Indio, CA 92201*

GEOGRAPHY

Arbovirus surveillance in the Coachella and Imperial Valleys is a collaborative effort by the California Department of Health Services (CDHS), University of California Davis, Center for Vector-borne Diseases (CVEC), the Coachella Valley Mosquito and Vector Control District (CVMVCD), and the Imperial County Health Department Vector Control (ICHDVC).

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

SURVEILLANCE METHODS

Surveillance in the Coachella Valley consisted of 10 flocks of 10 chickens each with 2 corresponding CO_2-baited CDC style traps (EVS traps), a grid of 40 EVS traps around the northern shore of the Salton Sea, 9 to 11 gravid traps located in urban areas including Mecca and upland communities, and the DHS dead bird surveillance program. Surveillance in the Imperial Valley was divided between 4 flocks located at Seeley, El Centro, Brawley, and Holtville, maintained by ICHDVC, and 3 flocks along the margin of the Salton Sea, maintained by CVEC and CVMVCD. Selection of these sites was based upon geographical and ecological parameters supported by historical arbovirus activity and juxtaposition to human populations. Flocks and EVS traps were sampled biweekly from March through November. Mosquitoes were identified, enumerated to species, pooled and sent to the CVEC for virus testing.

CHRONOLOGY

WNV overwintered successfully in southeastern California, with the first indication of the virus in the Coachella Valley found in 2 pools of *Cx. tarsalis* collected in EVS traps near North Shore on 14 April (Fig. 2). Positive pools continued to be collected in this area through early May. By the middle of May, 6 of 10 chickens seroconverted at North Shore and by the end of May, positive pools were found near the shoreline south of Mecca and on the west shore near Oasis. Two dead mallard ducks also were found in Cathedral City late in May. During early June, virus detection remained confined to the shoreline south of Mecca and on the West shore near Oasis. With the exception of a dead American Coot found at a managed wetlands, one positive chicken

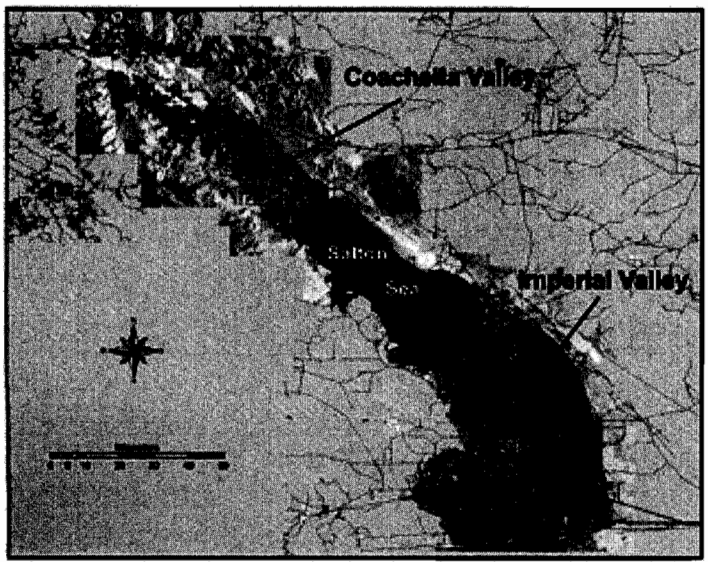

Figure 1. The Salton Sea basin showing the position of the Coachella and Imperial Valleys.

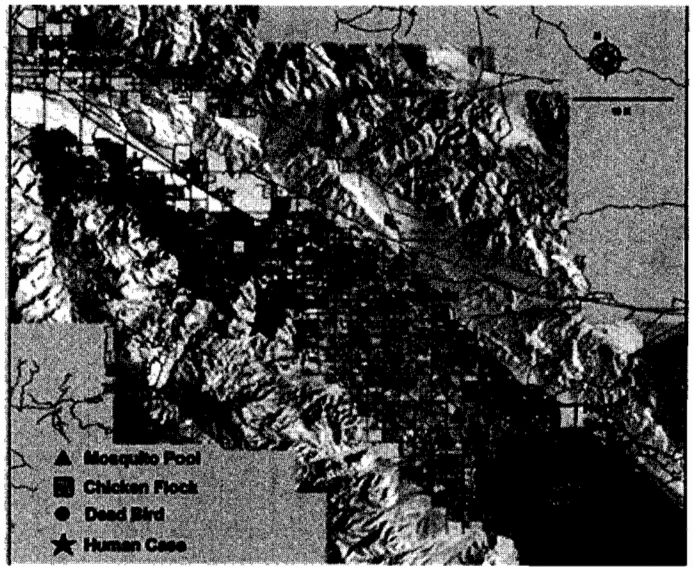

Figure 2. Coachella Valley surveillance showing all positive sites.

was detected at the Palm Desert flock. By the end of June, chickens were positive at Thermal, Mecca, Oasis, and along the central northern shoreline of the Salton Sea. During early July, evidence of virus activity was limited to the area from Mecca to Oasis in chickens and mosquito pools, but by the end of July the flock at the wetlands near Indio seroconverted coupled with the collection of positive pools at that site. At the same time, seropositive chickens and/or positive pools were found at Thermal, and south of Mecca near the Sea. The extent of virus activity remained the same in early August, but by the end of August it had disseminated into the residential areas of the valley and was detected in chickens at Cathedral City, mosquito pools of *Cx. quinquefasciatus* Say in Palm Desert and La Quinta, a dead owl in Palm Desert and a house sparrow in Indio. At the same time, chickens seroconverted at Indio, Mecca, and south of Mecca near the Sea. By early September, only chickens at Indio had seroconverted, but 7 human cases were reported from Palm Springs to south of Thermal. By the end of September, virus activity no longer was detected by the surveillance system, even though temperatures remained warm and *Cx. tarsalis* abundance increased in association with the flooding of wetlands south of Mecca.

In the Imperial Valley, WNV activity began in early June, as detected by positive mosquito pools and seroconversions in the flock at Wister Wildlife Refuge, near the Salton Sea shore north of Niland (Fig. 3) and in a mosquito pool north of Brawley, at the Finney-Ramer Refuge. By mid June, virus was detected in mosquito pools along the shore from Wister to west of Westmorland and at Finney-Ramer. During this time chickens seroconverted at Wister and Westmorland. By early July, virus was found throughout the Valley at Seeley and Holtville as well as Westmorland, southwest of Niland at Sonny Bono National Wildlife Refuge (NWR) and Finney-Ramer. At this time, the only human case for Imperial County was reported in a resident near Seeley. In late July, seroconversions continued in flocks at Westmorland, Sonny Bono, Brawley, Seeley, and Holtville. Virus activity in early August was detected at the Westmorland chicken flock and in a dead domestic goose south of El Centro. In early September, flocks at Westmorland, Seeley and El Centro had additional seroconversions while in late September only the Wister flock was positive in addition to a positive mosquito pool from Seeley. The last evidence of virus activity was a positive chicken at Sonny Bono NWR on November 1.

SUMMARY

In the Coachella Valley, arbovirus surveillance indicated that WN virus activity was focal and followed a similar pattern of amplification and dispersal as documented for St. Louis encephalitis (SLE) and western equine encephalomyelitis (WEE) in previous years. Consistent WNV activity was detected earlier than records for SLE or WEE. Early season activity was limited to mosquito pools collected around the northern margin of the Salton Sea with the exception of the ducks in Cathedral City and an American Coot in Indio and 1 surveillance chicken in Palm Desert. Midsummer, the virus dispersed up the valley and was found at the flock in Palm Springs by the beginning of September. Human cases were reported immediately following this dispersal and were predominantly limited to the upper valley where *Cx. tarsalis* is rare. Virus activity ended with the seroconversion of 1 chicken in

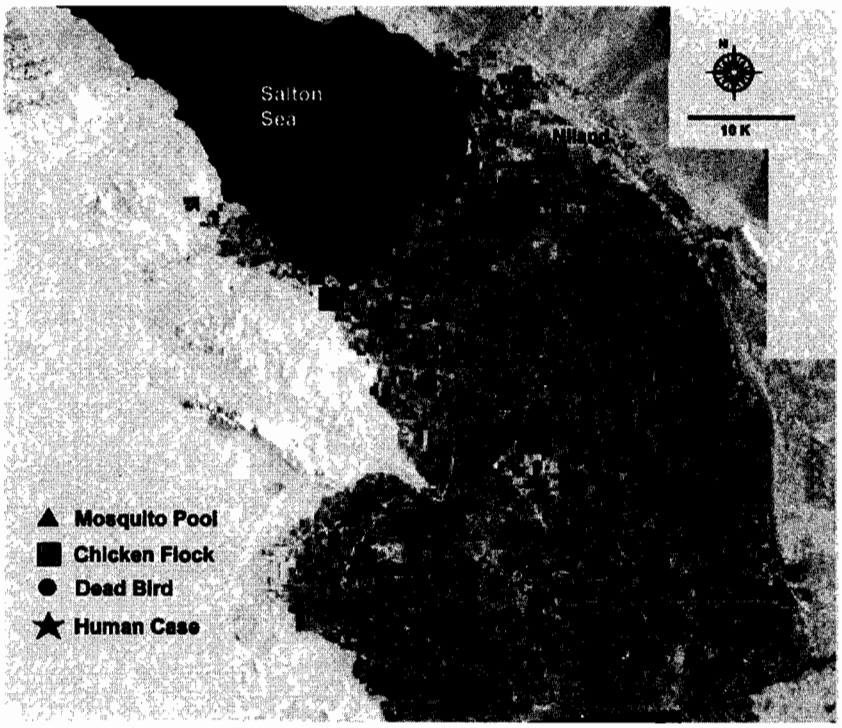

Figure 3. Imperial Valley surveillance positive sites.

Palm Springs on September 20.

In the Imperial Valley, WNV activity started later and was less focal. Although the first activity was detected along the shore of the Salton Sea in early June, other sites throughout the Valley became active within a month. Virus continued to be active throughout the valley until late September, as detected in surveillance flocks and mosquito pools. After September, there was little activity with only 1 mosquito pool at Seeley in October and 1 seropositive chicken at Sonny Bono NWR November 1.

There were no positive mosquito pools or chicken conversions for SLE or WEE in either valley or the state of California during 2004. Only 4 of 75 positive mosquito pools from the Coachella Valley and none of the 33 positive pools from the Imperial Valley were *Cx. quinquefasciatus*. The number of mosquitoes of each species tested for virus infection is shown in Table 1 and was largely proportional to species abundance, although greater emphasis was given to species in low abundance. This was the second year that WNV amplification failed to attain epidemic levels, although widespread enzootic transmission was detected in both valleys.

Table 1. Summary of mosquito numbers, virus positive mosquito pools and minimum infection rate per 1000 (MIR) for Coachella and Imperial Valleys.

Coachella	Total Number	Pools positive	MIR
Culex tarsalis	32402	71	2.19
Cx. quinquefasciatus	4939	4	0.81
Cx. erythrothorax	1086		
Culiseta inornata	600		
Aedes vexans	205		
Imperial			
Cx. tarsalis	14125	33	2.34
Cx. erythrothorax	3605		
Cx. quinquefasciatus	794		
Cx. erraticus	118		
Ae. vexans	1124		
Cs. inornata	252		
Ochlerotatus dorsalis	34		

Acknowledgements

This work was done in collaboration with the Coachella Valley Mosquito and Vector Control District, Don Gomsi Manager, who provided financial and logistical support. Additional funding came from the National Institutes of Health and the Centers for Disease Control and Prevention. Testing of chicken sera was done by the California Department of Health Services. Mosquito pool and dead bird testing was done by the UC Davis, Center for Vector-borne Diseases. Special thanks to Paul Johnson of the Imperial County Health Department Vector Control for sharing data.

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The Overwintering and Amplification of West Nile Virus in the Southern Portion of Greater Los Angeles

Jennifer Wilson[1], Minoo Madon[1], William K. Reisen[2]

[1] *Greater Los Angeles County Vector Control District, 12545 Florence Ave., Santa Fe Springs, CA 90670*
[2] *University of California, Davis, Arbovirus Field Station, 4705 Allen Road, Bakersfield, CA 93312*

ABSTRACT: West Nile virus (WNV) invaded the southern portion of the Greater Los Angeles County Vector Control District (GLACVCD) in September of 2003. The surveillance indicators of this invasion were peridomestic bird sera, followed by mosquito pools, and dead crows. Virus activity was centered in the Whittier Narrows crow roost as well as the Rio Hondo and San Gabriel River Corridors flowing out of the nature preserve (Fig.1). The 2004 season followed this progression of WNV amplification along the rivers and creeks in Los Angeles.

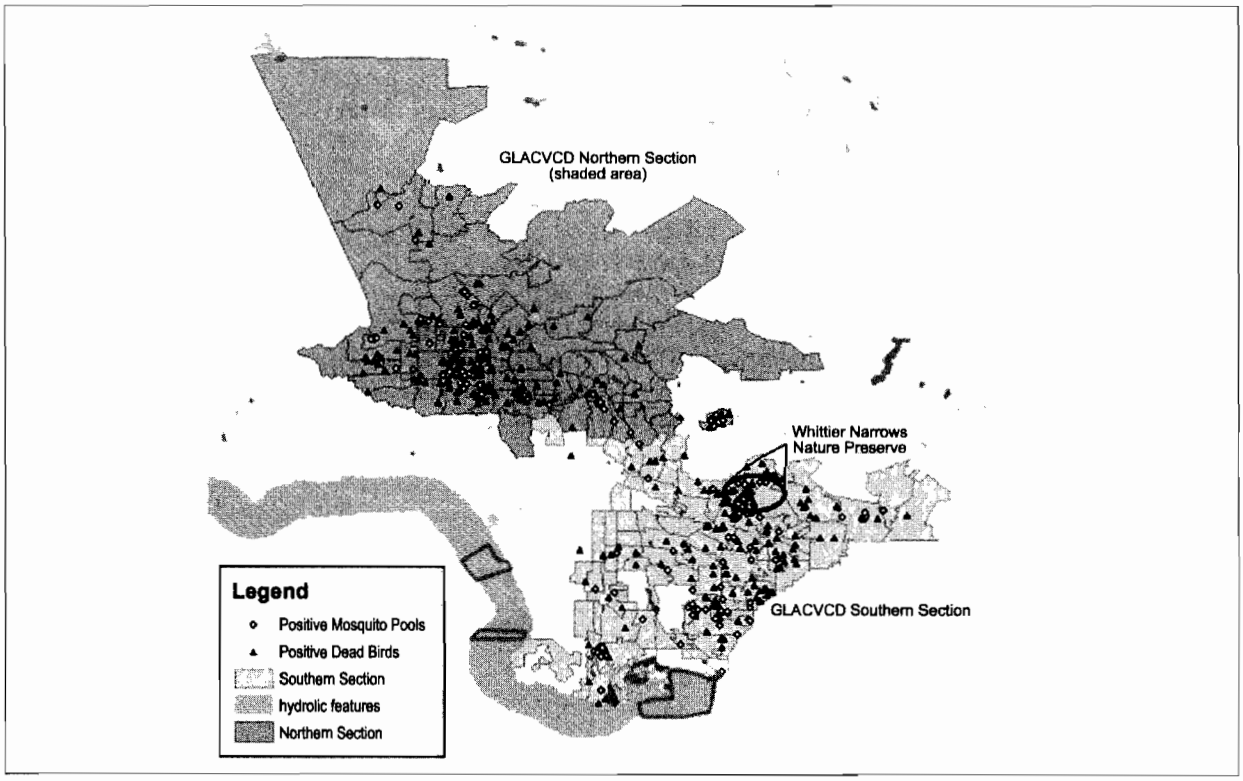

Figure 1. Map of GLACVCD boundary showing West Nile virus activity.

INTRODUCTION

West Nile virus (WNV) made its inroads to the southern area of the Greater Los Angeles County Vector Control District (GLACVCD) late in 2003. This area is highly urbanized with dense residential and commercial land use, with three river systems transecting in a north-south direction. A primary distinguishing feature of the southern GLACVCD landscape is the Whittier Narrows Nature Preserve, one of the largest open spaces reserved in this area, where the Rio Hondo and San Gabriel Rivers converge at the Whittier Dam. During the epizootic of 2003, it became apparent that the crow roosting behavior observed within this preserve was concentric to the marked crow die-off in the neighboring cities of Montebello, Pico Rivera, and Whittier. WNV activity was also detected along the two associated river corridors in 5 mosquito pools of *Culex quinquefasciatus* Say and at privately owned chicken ranches abutting the river systems.

MATERIALS AND METHODS

During the 2003 and 2004 surveillance seasons, WNV transmission activity was monitored using mosquito pools sampled with CO_2-baited encephalitis virus surveillance (EVS) traps and gravid traps (Reiter 1983; with rabbit chow/ brewer's yeast infusion as an attractant); sentinel chickens; participation in the California Department of Health Services' Dead Bird Surveillance Program; and sera samples from peridomestic birds captured in grain-baited modified Australian crow traps. Core sites were established in 3 representative areas of the district (Whittier Narrows, Machado Lake, and Rowland Heights), and all sampling methods were engaged and sampled twice monthly. In addition, random mosquito sampling was done along north-south and east-west transects of the district boundaries in residential and commercial properties.

Mosquitoes were identified to species, separated by sex and submitted to the Center for Vectorborne Diseases (CVEC) at the

University of California, Davis campus to test for western equine encephalomyelitis (WEE), St. Louis encephalitis (SLE) or WNV viral RNA by RT-PCR. Maximum likelihood estimations (MLE) were calculated bi-weekly using PooledInfRate 2.0 software (Biggerstaff, 2004). Peridomestic birds were captured, banded, sampled by jugular venipucture and withdrawal of 0.1cc blood, and released. The whole blood was diluted with 0.9 cc saline (0.9% sodium chloride), centrifuged, and the sera was frozen at -70° C prior to being shipped to CVEC for WNV, SLE, and WEE antibody screening by EIA. Sentinel chicken samples were taken by brachial venipuncture, samples were transferred onto filter paper and allowed to dry, then shipped to the California Department of Health Services (CDHS) Viral and Rickettsial Disease Laboratory (VRDL) for WNV, WEE, and SLE antibody screening by EIA and IFA. Dead birds were shipped for necropsy to the California Animal Health and Food Safety (CAHFS) laboratory, and subsequent tissue samples were sent to CVEC for WNV RNA screening by RT-PCR.

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

Winter activity was monitored through the use of CO_2-baited EVS traps placed in underground storm drain systems (USDS), continued bi-monthly trapping of core sites, and all avian sampling methods.

RESULTS AND DISCUSSION

The 2003 WNV epizootic spanned from 16 Sept to 4 Dec with detection in mosquito pools and 25 dead wild birds. The majority of the activity was centered along the northern portion of the Rio Hondo and San Gabriel River Corridors in the cities of Pico Rivera, Montebello, and Whittier (Wilson et al. 2004).

Though mosquito, sentinel chicken, peridomestic bird activity, and dead birds were monitored through the winter months, no further virus activity was detected until 8 Mar the following year, in two peridomestic bird serum samples collected from Whittier Narrows and Santa Fe Springs. During this period (from 4 Dec.'03 to 8 Mar.'04), 53 mosquito pools were submitted from core sites and USDS, 162 peridomestic bird serum samples, 13 dead birds and ~120 sentinel chicken samples were tested.

The 2004 WNV epizootic began near the San Gabriel River corridor in late April and progressed eastward along Coyote Creek and San Jose Creek, respectively. By the end of May, there were 12 WNV positive pools—11 urban *Cx. quinquefasciatus* and one *Cx. tarsalis* collected from Whittier Narrows, 78 WNV positive dead crows and one WNV positive black phoebe. The epidemic began in June when two WNV human cases occurred in the two cities of focal crow mortality and WNV positive *Cx. quinquefasciatus* pools within the southern area of GLACVCD.

The epizootic continued to spread southward along the San Gabriel River Corridor towards the coast of Long Beach and then westward with crows and *Cx. quinquefasiatus* mosquito pools preceeding human cases. Sentinel chickens did not provide an early indication of virus transmission, as the first seroconversions were detected in mid-July, corresponding only to the peak in WNV positive crows, and the beginning of the epidemic. On the contrary, WNV positive dead birds proved transmission 7 weeks before human cases occurred, but didn't provide the same locality of transmission information and therefore didn't focus control efforts.

In mid-August, MLEs for gravid trap collections of *Cx.quinquefasciatus* peaked at 39.8/1,000 (Fig. 2) while abundance was on the decline at 29 females/trap night. MLEs for EVS trap collections of *Cx. quinquefasciatus* peaked a month later, but also when abundance was low at 10 females/trap night (Fig. 2). This trend of infection rate being inversely related to abundance was true for all species in both trap types.

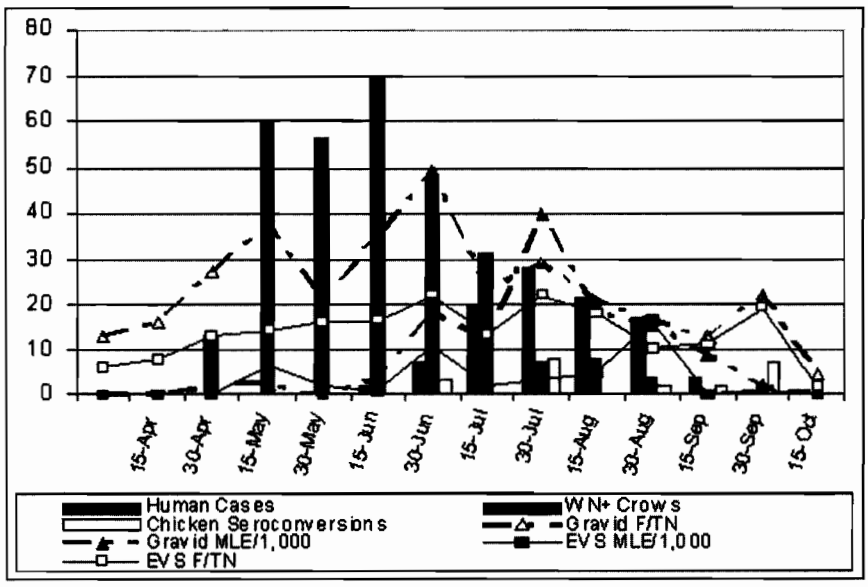

Figure 2. Bi-weekly West Nile virus activity in the southern area of GLACVCD. Mosquito data shown represent *Culex quinquefasciatus* only.

Culex quinquefasciatus infection rates dropped to 2.1/1,000 and lower in October for both trap types after a period of early rain, coinciding with the last WNV positive crow and human cases. No WNV activity was detected after 17 Oct in mosquito or avian sampling.

CONCLUSION

The abundance of WNV positive crows in urban areas with *Cx. quinquefasciatus* as the primary vector species fueled the amplification and spread of the WN epidemic in the southern part of GLACVCD. This amplification took place along the same river corridors where WNV first emerged in 2003, and the initial spread of the virus in 2004 continued along the river and creek systems of GLACVCD. Areas with a high incidence of WNV positive dead birds also had higher MLEs and incidence of human cases.

Acknowledgements

We thank the Scientific-Technical Services staff of GLACVCD, including Jacqueline Spoehel, Paul O'Connor, Susanne Kluh and Saeed Tabatabaeepour for their efforts in disease surveillance activities.

Support was provided by funds from NIAID, NIH, CDC, University of California Mosquito Research Program, and GLACVCD.

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Dispersal and Amplification of West Nile Virus in the Northern Section of the Greater Los Angeles County Vector Control District

Paul O'Connor[1], Jacquie Spoehel[1], Minoo Madon[2], William Reisen[3]

[1]*Greater Los Angeles County Vector Control District, 16320 Foothill Blvd, Sylmar, CA 91342*
[2]*Greater Los Angeles County Vector Control District, 12545 Florence Ave., Santa Fe Springs, CA 90670*
[3]*Center for Vectorborne Diseases, School of Veterinary Medicine, University of California, Old Davis Rd, Davis, CA 95616*

ABSTRACT: West Nile Virus (WNV) activity was first found in the Greater Los Angeles County Vector Control District in 2003, but initially remained confined to the southern portion of the District. The over-wintering and subsequent amplification of WNV in this part of the District are addressed in a separate paper in this symposium. This paper describes the dispersal and amplification of West Nile virus in the northern section of the Greater Los Angeles County Vector Control District GLACVCD).

The first indication of WNV activity occurred in the latter part of May 2004, with the recovery of a dead American crow from the San Fernando Valley community of North Hills, that tested positive for WNV. This event was followed, in order, by the collection of WNV positive mosquito pools, human infections, and lastly, chicken seroconversions. *Culex quinquefasciatus* Say was the most widespread and frequently infected species of mosquito collected in the area. No further WNV activity was detected after October by any of the surveillance methods employed.

INTRODUCTION

The northern region of GLACVCD contains the areas located north of the intersection of the Golden State Freeway (Interstate Highway 5) and the Pasadena Freeway (State Highway 110), and includes incorporated areas of the City of Los Angeles in the San Fernando Valley, and the cities of Glendale, Burbank, San Fernando, and Santa Clarita (Fig. 1). In order to present a more complete picture of arbovirus activity within the District, and to highlight differences that occurred between the northern and southern regions of our District, data are presented separately from the southern portion.

MATERIALS AND METHODS

Study Areas. The valley areas are circumscribed by sections of the Transverse Ranges, including the Santa Monica, San Gabriel, Santa Susanna and Verdugo Mountains, and the San Rafael Hills. Although technically part of the Los Angeles metropolitan area, the area covered is largely a suburban residential area, with some dense commercial districts and light industry. The mostly concrete-lined Los Angeles River flows through the region, and a network of concrete-lined flood control channels and underground storm drains carry street runoff to the river.

Sepulveda Basin and Hansen Dam are mixed-use wetlands that are associated with flood control basins for the Los Angeles River. Both areas contain large parks and golf courses. Two other two wetlands, Chatsworth and Van Norman, are closed to public use. Covering more than 4,100 acres, Griffith Park, one of the largest municipal parks in the Unites States, contains a major zoo, golf courses, equestrian trails, and natural areas among foothills

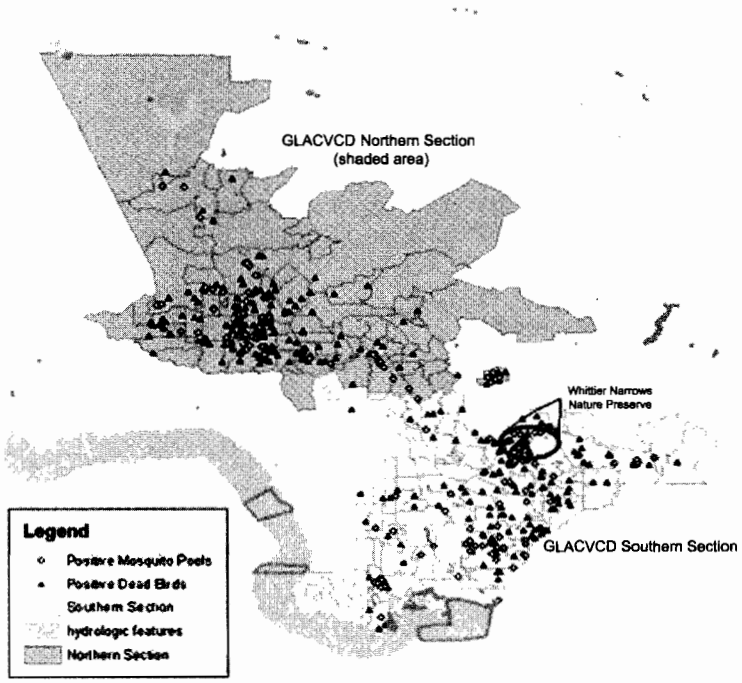

Figure 1. Northern section of the GLACVCD referred to in the text, with major core trapping sites identified.

[3]Address correspondence to the Arbovirus Field Station, 4705 Allen Rd, Bakersfield, CA 93314

and canyons at the eastern edge of the Santa Monica Mountains. The park borders the Los Angeles River west of the City of Glendale.

Four methods of surveillance were employed to detect arbovirus activity: sentinel chicken flocks, collection of adult mosquitoes, dead bird collection, and sera sampling of peridomestic birds.

Three sentinel chicken flocks were located in Griffith Park, Encino (Sepulveda Basin), and Vista Valencia Golf Course in Santa Clarita. Sentinel chicken blood samples were collected on filter paper, dried, and sent to the California Department of Health Services (CDHS), Viral and Rickettsial Disease Laboratory (VRDL) to test for antibodies to WNV, WEE, and SLE by EIA and IFA (CDHS 2005).

Mosquito surveillance utilized CO_2-baited CDC traps, and Reiter gravid female traps (Cummings 1992) using an aged ground rabbit chow and brewer's yeast infusion as an attractant. Mosquito pools were sent to the UC Davis Center for Vectorborne Diseases (CVEC) where they were screened for WEE, SLE, and Viral RNA by RT-PCR (CDHS, 2005). Additionally, 5 New Jersey Light traps were operated as part of the State's surveillance program. Monthly maximum likelihood estimations (MLE) of infection rates were calculated for mosquito monthly pool data using PooledInfRate, version 2.0 software (Biggerstaff, 2004). Three core sites, established at the Los Angeles Zoo in Griffith Park, the Sepulveda Basin in Encino, and Chatsworth Reservoir, were sampled on a biweekly basis for population estimates and submission of mosquito pools for testing. Transects, some of which were several miles long, were established for trapping adult mosquitoes, in order to get a better region-wide sample in residential and other urban habitats. These transects were trapped approximately twice a month, as were other historical trap sites in known mosquito-prone habitats.

The District also conducted dead bird surveillance in conjunction with the CDHS WNV Dead Bird Surveillance Program. Dead birds were sent to the California Animal Health and Food Safety (CAHFS) laboratories where necropsies were preformed. Tissue samples were then sent to CVEC where they were tested for WNV by RT-PCR (CDHS, 2005).

RESULTS

The first indication of WNV activity occurred in northern GLACVCD in the latter part of May 2004, with the recovery of a dead American Crow from North Hills that tested positive for WNV by RT-PCR. As much as 138 dead birds tested positive for WNV in July, the highest number for any one-month period (Fig. 2). However, bird mortality, especially in American Crow populations, continued to be high throughout the summer, and the apparent peak in July may have been artificial, since testing of dead birds from known positive areas declined due to the large numbers of birds being reported. From May to October, a total of 277 dead birds tested positive for WNV.

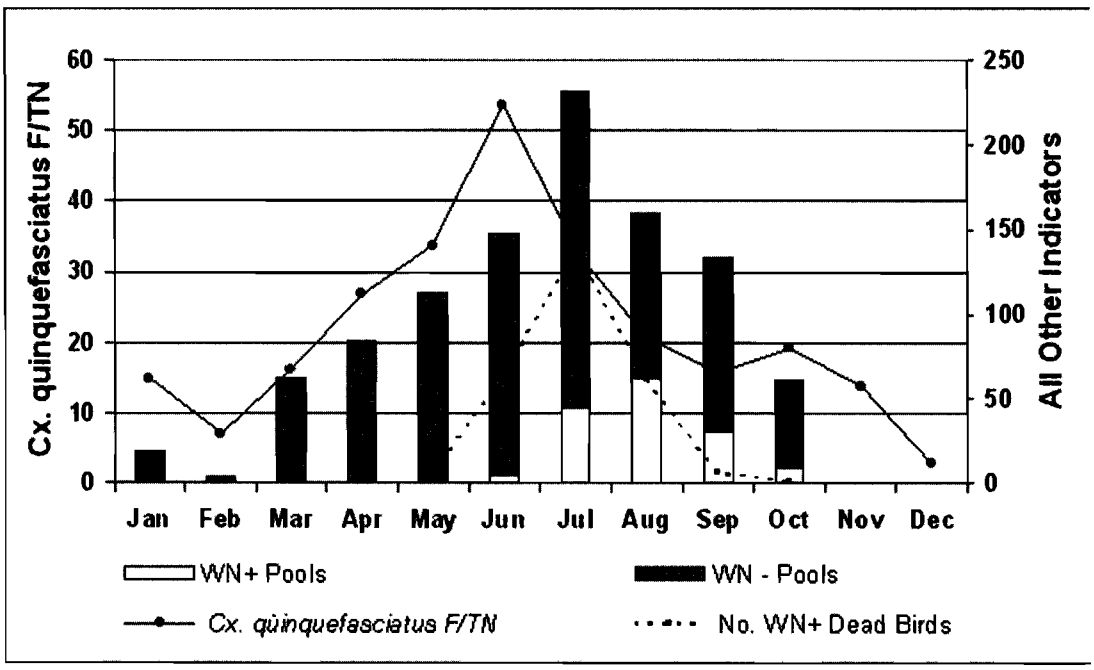

Figure 2. 2004 West Nile virus activity in the northern section of the GLACVCD. Pool numbers represent collections from all species submitted. The vast majority of positive pools were from *Cx. quinquefasciatus.*

A WNV positive pool of 21 gravid *Culex quinquefasciatus* Say was collected in early June, approximately 2 weeks after the first positive dead bird was collected. There was a total of 153 WNV positive pools collected from six mosquito species during the period from May to October (Table 1). The number of positive pools increased sharply from 5 in June to 45 in July, while the MLE for these months remained relatively low, 0.9/1000 and 6.02/1000, respectively. August had the highest number of positive pools (63) and the highest MLE (33.6/1000). The number of positive pools declined in September (31), but the MLE remained relatively high (14.8/1000) compared to June and July. In October, the number of positive pools further declined to 17 (Figure 2), and the MLE was 4.7/1000.

Culex quinquefasciatus numbers peaked in June with 53.5 females/trap night, and remained far more abundant throughout the year than the other two species shown (Fig. 3). *Culex stigmatosoma* numbers varied little throughout the period, with females/trap night ranging from 1.7 to 4.5. *Culex tarsalis* numbers

Table 1. WNN positive mosquito pools by species from the two northern GLACVCD core sites compared to northern valley residential and wetland habitats.

	Griffith Park	Sepulveda Basin	Valley (residential)	Valley (wetlands)	WN+Total	Total Submitted
Culex quinquefasciatus						
(CDC CO_2 Trap)	0	16	16	1	33	161
(Gravid Trap)	8	37	58	5	108	511
Cx. stigmatosoma	3		1	1	5	45
Cx. tarsalis		1		1	2	42
Cx. erythrothorax				2	2	138
Cx. thriambus				2	2	22
Cx. restuans						2
Anopheles hermsi				1	1	27
Culiseta incidens						39
Cu inornata						5
Cu particeps						7
WN+ Total:	11	54	75	13	153	
			Total northern section pools submitted:			999

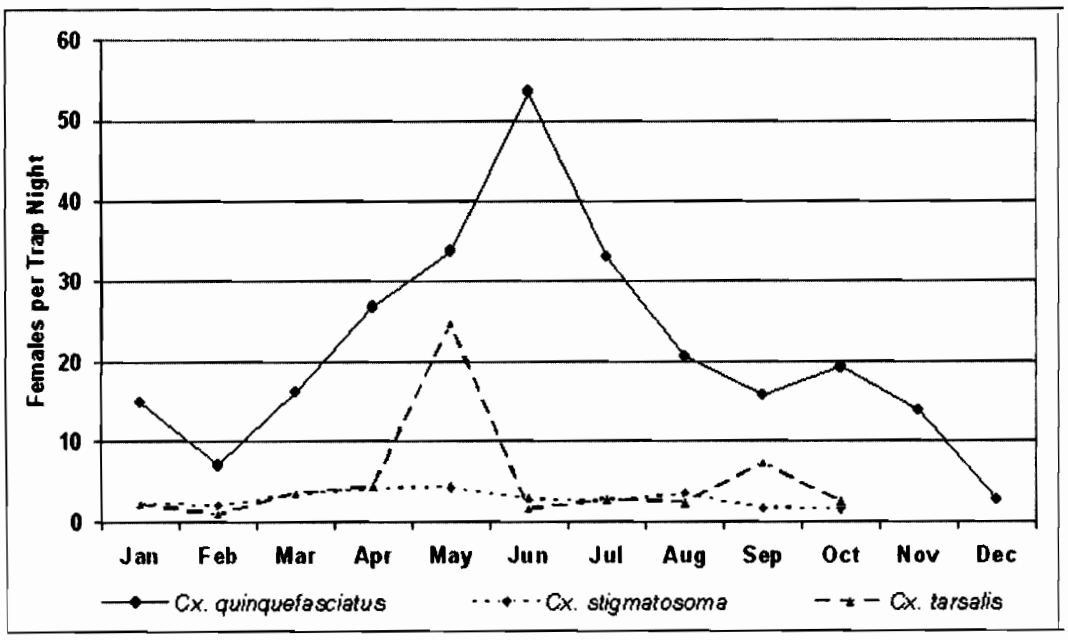

Figure 3. 2004 Monthly mosquito abundance for three species with the highest number of WN+ pools in northern GLACVCD.

were about the same as *Cx. stigmatosoma* for most of the year, except for peaks of 24.8 and 7.3 females/trap night that occurred in May and September. These peaks were caused by higher than normal populations of this species at Chatsworth Reservoir.

The first human cases in the area appeared in July, with a likely date of onset in the early part of the month. There were a total of 76 cases during a 4-month period from July to October, with a peak of 43 cases in August. Human infections closely followed the mosquito infection rates estimated by the MLEs (Figure 4).

The first sentinel chicken seroconverted in late July, approximately three weeks after the suspected onset of the first human case. Because of the biweekly bleeding schedule, it is possible that sentinel chickens were infected concurrently with the onset of human infection; however, these data did not provide an early warning of virus activity. A total of 15 chickens seroconverted between July and September at Griffith Park and in the Sepulveda Basin. No chickens at the Vista Valencia flock in Santa Clarita converted, but there were low numbers of WNV positive dead birds and crows from this area.

Early season WNV positive dead bird and mosquito pool samples suggested a clustering near crow roosts in the North Hills and Van Nuys area. As the season progressed, positive dead bird finds spread rapidly throughout the area. West Nile virus positive mosquito pools and human cases appeared to radiate out more slowly from this central cluster. The greatest number of positive mosquito pools and case onset dates occurred in August. September and October saw a gradual decline in all categories of surveillance indicators, and no WNV activity was detected in the area in November or December.

Dead birds and mosquito pool collections were much better surveillance systems than sentinel chickens. The role and efficacy of the peridomestic bird sampling program is still being evaluated and is not addressed here.

DISCUSSION

The invasion of West Nile virus into the northern region of the GLACVCD occurred somewhat earlier and with greater intensity than Saint Louis encephalitis epidemics that occurred in the past (Murray et al. 1985, Reisen et al. 1992). In this region, WNV presently has a decidedly suburban and urban disease cycle, with *Cx. quinquefasciatus* as the main vector. It will be interesting to see what future role, if any, that wetland species such as *Cx. erythrothorax* may play in the maintenance of WNV in the region.

Acknowledgements

We thank Dr. B. Cahoon-Young and the CVEC laboratory staff for testing of the mosquito pools and dead bird tissues, Y. Fang, CVEC, for confirming wild bird serology, E. Baylis and C. Cossen at VRDL for testing of sentinel chicken samples, and R. Carney and the DHS staff at the WNV Dead Bird Surveillance Program for their patience and support. We would also like to thank Jennifer Wilson, UC Davis for her data management and field support; Susanne Kluh and Saeed Tabatabaeepour at GLACVCD for their ideas and field support: Bruce Cameron of GLACVCD for assistance with mapping; and the CAHFS staff for their support and work on dead bird necropsies.

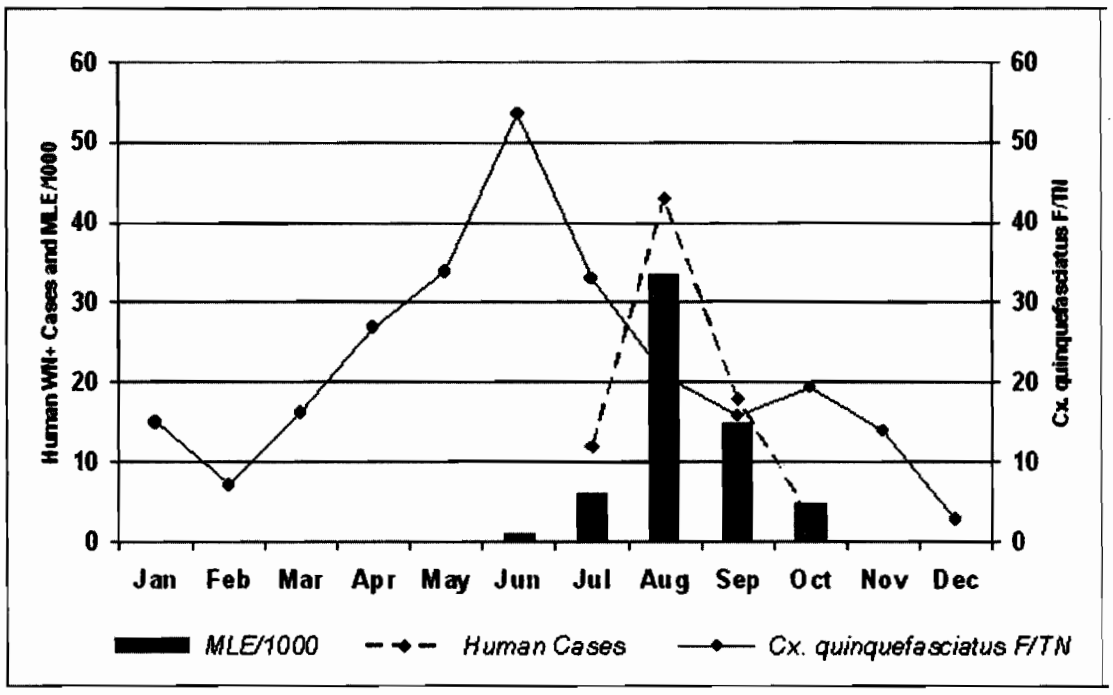

Figure 4. 2004 WN+ human cases, MLE/1000, and *Cx. quinquefasciatus* F/TN in the northern section of the GLACVCD. MLEs represent estimates from all species submitted. The vast majority of positive pools were from *Cx. quinquefasciatus*.

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Invasion of Kern County by West Nile Virus

Richard M. Takahashi[1], William K. Reisen[2], and Christopher M. Barker[3]

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

ABSTRACT: West Nile virus (WNV) invaded Kern County during late-June/early-July 2004 and was concurrently detected by all surveillance methods. Human cases were detected concurrently with enzootic transmission indicators. Human and horse cases were clustered within Bakersfield; however, highest incidence rates were found in the small rural farming communities of Arvin and Shafter. WNV was amplified simultaneously in rural and urban transmission cycles and minimum infection rates in *Culex tarsalis* and *Cx. quinquefasciatus* exceeded 5 infected females/ 1,000 during August and September.

INTRODUCTION

During 2003, West Nile virus (WNV) invaded southern California from Imperial to Los Angeles counties, including San Bernardino County bordering eastern Kern County (Kern). The virus was detected in mosquito pools (July 16, in Imperial County), sentinel chickens (August 4, in Imperial and Riverside counties), dead birds (September 3, in Los Angeles County), a single horse (onset October 17, San Diego County) and three locally acquired human cases in Imperial, Riverside and Los Angeles counties. During 2003, only a single dead bird from the Mojave Desert (north of the San Bernardino Mountains) tested positive. By late June 2004, WNV appeared in California north of the Tehachapi mountain range and in the Central Valley for the first time. This paper discusses the introduction of WNV Virus into Kern, its detection by various surveillance and reporting methods, and its progressive dispersal throughout the county during 2004.

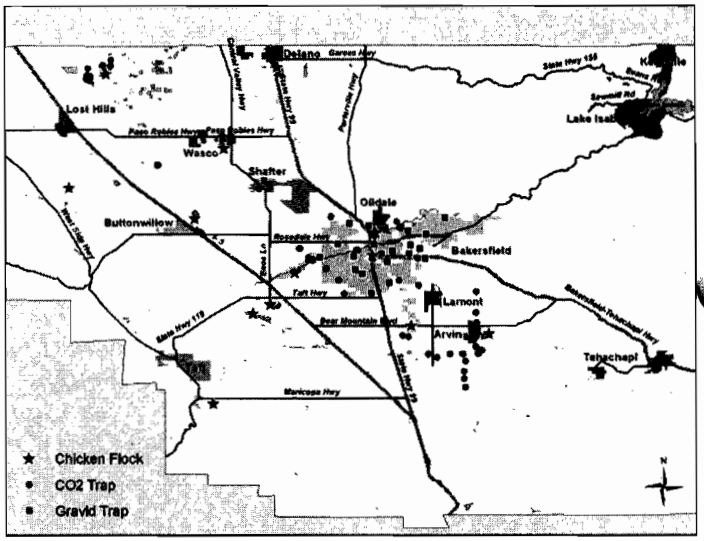

Figure 1. Map of western Kern County showing the locations of sampling sites during 2004.

MATERIALS AND METHODS

Background. Surveillance information in Kern was gathered by several entities including four separate mosquito & vector control agencies, the Environmental Health section of Kern County Department of Public Health, Edwards Air Force Base in eastern Kern County, and the Arbovirus Field Station (ABFS) of the University of California, Davis (UCD). Data and results in this report were collected largely within the boundaries of the Kern Mosquito and Vector Control District (KMVCD), the largest mosquito control district in Kern County covering 1,650 square miles. Other mosquito control agencies include Delano Mosquito Abatement District (MAD), South Fork MAD, and West Side MVCD. Sampling locations are shown in Fig. 1.

Dead Birds. KMVCD and other agencies in Kern participated in the California WNV Dead Bird Surveillance Program administered statewide by the California Department of Health Services, Vector-Borne Disease Section (CDHS-VBDS). Dead birds reported to the CDHS-VBDS dead bird hotline by the public were picked up by the vector control agency and submitted to the California Animal Health and Food Safety (CAHFS) Central laboratory at UCD for necropsy. Tissues were forwarded to the UCD Center for Vectorborne Diseases (CVEC) laboratory for testing by reverse transcriptase-polymerase chain reaction (RT-PCR) and/or virus isolation from Vero cell culture.

Mosquitoes. Mosquitoes were collected by dry ice-baited CDC traps (Sudia and Chamberlain 1962) and by Reiter/Cummings gravid traps (Cummings 1992). Personnel at KMVCD and the ABFS placed the traps throughout Kern County. Collections were identified to species and processed for virus in accordance with "Procedures for Processing Mosquitoes for Arbovirus Detection – 2004", an annual protocol published by CDHS. The pools were submitted to CVEC for testing by multiplex RT-PCR (Chiles et al. 2004).

Sentinel Chickens. Chickens from 15 flocks were bled biweekly per CDHS "Instructions for Sentinel Blood Samples" (January, 2004). Individual blood samples were collected from each chicken on filter paper strips and sent to CDHS Viral & Rickettsial Disease Laboratory (VRDL) for testing for IgG antibody by an indirect enzyme immunoassay (EIA). Positives were

confirmed by indirect fluorescent antibody assay (IFA) and end-point plaque reduction neutralization test (PRNT)

Humans and Equines. Human case data were provided by the Epidemiology section of the Kern County Department of Public Health and by the California Arbovirus Surveillance Bulletins published by CDHS-VBDS. Equine case data were provided from reports generated by the CDHS/Veterinary Public Health Section, Division of Communicable Disease Control (DCDC).

RESULTS

WNV was detected initially in the Central Valley in a western scrub jay collected in Kern on June 22, 2004. Overall, 87 dead birds tested positive for WNV in Kern during 2004. Most of the positive birds were western scrub jays (28) and American crows (25); others represented were Cooper's hawks (6), Brewer's blackbirds (4), common ravens (3), barn owls (3), house sparrows (3) Steller's jays (3), American robins (2), northern mockingbirds (2), hermit thrush (1), lesser nighthawk (1), northern waterthrush (1), red -tailed hawk (1), American kestrel (1), western bluebird (1) and woodpecker (1).

Sixty-five of the 87 positive birds found in Kern were collected in or near the City of Bakersfield at the southern end of the San Joaquin Valley (SJV) (Table 1). Nine were found in the SJV cities of Delano and McFarland, Wasco, Shafter and McKittrick located north and west of Bakersfield, whereas 7 positives were found in the desert cities of Edwards, North Edwards, Mohave and Ridgecrest, east and northeast of the Tehachapi mountain range. Three positives were found in the cities of Glennville and Kernville located in the Sierra Nevada mountain range northeast of Bakersfield. Two were found near the city of Tehachapi, east of Bakersfield, in the Tehachapi mountain range.

Table 1. Dead birds testing positive from different areas of Kern County.

City	# Birds
Bakersfield	65
Delano, McFarland, McKittrick, Shafter & Wasco	9
Edwards, North Edwards, Mohave & Ridgecrest	7
Glennville & Kernville	3
Tehachapi	2

Chronologically, the first 8 dead birds were found within the city of Bakersfield. The next bird was found in the city of Tehachapi, a city relatively close to other California counties with WNV activity the previous year. Subsequently, positive dead birds were found scattered around the county with no clear pattern. Because the dead bird surveillance program relies on the public to report dead birds, Bakersfield may have had the highest number of positive birds simply because it is the most populated county that is primarily rural.

From March through May, 2004, 957 *Culex quinquefasciatus* Say, 3,065 *Cx. tarsalis* Coquillett, and 4,467 *Ochlerotatus melanimon* Dyar submitted in 229 pools tested negative for WNV. However, on June 30, 2004, a pool of *Cx. tarsalis* collected near the city of Arvin tested positive for WNV. The day after discovery of this initial positive mosquito pool, a gravid trap set in a NW Bakersfield suburban yard collected WNV-infected *Cx. quinquefasciatus*. During 2004, 214 out of 1,333 Kern mosquito pools tested positive for WNV (Table 2). West Nile virus was detected in 4 of the 6 species submitted for testing: *Cx. tarsalis, Cx. quinquefasciatus, Oc. melanimon* and *Cx. thriambus Dyar*. Minimum infection rates per 1,000 (MIRs/1,000) for *Cx tarsalis* and *Cx. quinquefasciatus* approached or exceeded 5/1,000 during the July –September period of epidemic activity.

A total of 101 chickens from 15 flocks seroconverted to WNV in Kern; and 89% of the seroconversions were within the boundaries of KMVCD. The first chicken seroconversion in Kern occurred before July 19, 2004 east of the city of Arvin, southeast of Bakersfield. During the following two weeks the entire Arvin flock seroconverted to WNV. By the end of August 2004, 7 additional flocks from Arvin north to the city of Delano (Kern's northern border) had seroconverted in a northerly trend. By the end of September, flocks in and around the Kern National Wildlife Refuge on the northern fringe of Kern had seroconverted to WNV. Transmission continued at these sites throughout the summer, as evidenced by flocks that seroconverted early in the year and continued to convert through late October when the surveillance was halted.

The first reported human infection with WNV in Kern was an asymptomatic 63 year-old male initially detected by a Bakersfield blood bank and confirmed by CDHS on July 27, 2004. The earliest symptomatic WNV-attributed human case with a known date of onset was a 34 year-old Bakersfield female whose symptoms initially manifested on June 19. Subsequently, 60 human cases were reported in Kern by CDHS. Thirty-one of these cases were classified as West Nile Fever (WNF), 14 as West Nile neuroinvasive disease (WNND) and 15 were of unknown status (due to incomplete reporting). The mean age of the cases was 48 (range 7-83) and 68% were between the ages of 39 and 70. Twenty-five of the cases were females and 33 cases were males. Two of the cases were of unknown gender. Forty-four (73%) of Kern's human cases were from the city of Bakersfield (Table 3). Bakersfield's infection rate was 15.7/100,000, while Shafter, a nearby smaller city, had an infection rate of 43.8/100,000 (Table 3). The infection rate for Kern County as a whole was 8.3/100,000, compared to California's rate of 2.4/100,000.

The first confirmed equine case of WNV in Kern County occurred in a northwest Bakersfield suburb just outside the city limits. The onset of symptoms was July 15, 2004, and the horse survived. Subsequently, 46 Kern County equine cases were reported by DCDC, of which 20 resulted in death or euthanization of the horse. Thirty-five of the 46 Kern County cases were stabled within the city of Bakersfield (Table 4).

Table 2. West Nile Virus minimum infection rates in Kern County mosquito species, 2004

Month	Species	# Pools	Total Mosquitoes	WN + Pools	MIR/1000
March	*Culex tarsalis*	15	580	0	0
	Ochlerotatus melanimon	22	1038	0	0
April	*Culex quinquefasciatus*	23	496	0	0
	Culex tarsalis	35	1166	0	0
	Ochlerotatus melanimon	41	1871	0	0
May	*Culex quinquefasciatus*	21	461	0	0
	Culex tarsalis	40	1319	0	0
	Ochlerotatus melanimon	32	1558	0	0
June	*Culex quinquefasciatus*	59	2166	0	0
	Culex tarsalis	72	2790	1	0.4
	Ochlerotatus melanimon	10	354	0	0
July	*Culex quinquefasciatus*	104	4054	11	2.7
	Culex stigmatosoma	1	38	0	0
	Culex tarsalis	95	1078	20	4.9
	Ochlerotatus melanimon	22	925	0	0
August	*Culex erythrothorax*	2	67	0	0
	Culex quinquefasciatus	140	5486	42	7.7
	Culex tarsalis	101	4274	38	8.9
	Culex thriambus	2	47	1	21.3
	Ochlerotatus melanimon	12	441	1	2.3
September	*Culex quinquefasciatus*	190	7679	59	7.7
	Culex stigmatosoma	4	83	0	0
	Culex tarsalis	166	7165	32	4.5
	Ochlerotatus melanimon	23	1148	0	0
October	*Culiseta inornata*	4	91	0	0
	Culex quinquefasciatus	58	2402	6	2.5
	Culex tarsalis	26	1058	3	2.8
	Ochlerotatus melanimon	13	598	0	0

Table 3. West Nile Virus Human Cases per 100,000 in Kern County, California

Entity	Population [1]	# Human cases	Cases per 100,000
Bakersfield	279,672	44	15.7
Shafter	13,692	6	43.8
Arvin	14,499	4	27.6
Lamont	13,300	2	15.0
Wasco	22,858	2	8.8
Kern County	724,900	60[2]	8.3
California	34,144,000	818	2.4

[1] Population statistics from Kern Council of Governments via California Department of Finance Records

[2] Includes two cases of unknown location and with incomplete reporting.

Table 4. Distribution of equine cases within Kern County.

Entity	No. Equine Cases	Dead or Euthanized
Bakersfield	35	14
Arvin	3	0
McFarland	1	1
Rosamond	1	1
Shafter	2	0
Taft	1	1
Tehachapi	2	2
Wasco	1	1
Kern County	46	20
California	536	228

DISCUSSION

The invasion of Kern County was sudden with little time between the initial detection of enzootic activity and the onset of human cases. Virus was detected in dead birds and mosquitoes during the last week of June in both urban and rural locations, followed within 2 weeks by seroconversions in sentinel chickens and the first horse case. Considering the time required for a diagnostic IgG antibody rise in chickens and the 2-week bleeding schedule, these hens probably were infected close to the date of the initial detection of virus in mosquitoes. Human cases were reported immediately after enzootic transmission was detected and before emergency control efforts could be mounted to interrupt transmission. Sampling weekly may have allowed a slight earlier warning, but would have doubled surveillance costs.

Dead birds proved to be effective in early detection of WNV, providing the first indication of WNV in Kern and continued to provide evidence of viral amplification throughout the season. Dead birds were the only indicator of the virus in areas where other surveillance methods were not being deployed. With 87 WNV-positive birds collected, Kern ranked 10th among the 58 counties in the state. This was unexpected because American crow populations were low and widely dispersed in most of the county. Since the introduction of WNV into the United States in 1999, crows were shown to be susceptible and indicators of virus activity.

Mosquito collections were also a good indicator for early detection of WNV, with positive pools collected 8 days after the first positive bird was collected. Kern ranked second (with 214 positive mosquito pools) in terms of the number of WNV-positive pools among the 58 counties in California. Minimum infection rates per 1,000 peaked during August and September during which Kern was near or above an MIR of 5/1000 with *Cx. tarsalis* and *Cx quinquefasciatus*. Most positive *Cx. tarsalis* pools were collected in rural areas, whereas *Cx. quinquefasciatus* were infected most frequently in urban areas, suggesting the occurrence of parallel and concurrent rural and urban cycles. The rapid invasion of Bakersfield was unexpected and did not follow the gradual amplification and dispersal of virus from rural to urban environments as documented previously (Reisen 1984).

Kern had 101 WNV antibody-positive sentinel chickens. Generally the flocks seroconverted following a south to north trend. All chicken flocks of KMVCD and some of Delano MAD and West Side MAD had been infected by the end of November. Near the Arvin focus, the entire original sentinel flock and additional replacement birds became infected.

Although the city of Bakersfield had the most human cases of WNV, some of the smaller outlying cities in Kern had a much higher incidence per 100,000 population. One small city (Shafter) had an incidence of 43.8/100,000 and the county's only death of 2004. Overall, Kern had a human incidence of 8.2/100,000, which was higher than the state average of 2.4/100,000 and higher than the 3.8/100,000 reported in Los Angeles County. In terms of the number of WNV-positive cases per county, Kern ranked 5th in the state with 60 human cases. Interestingly, the 14 cases with confirmed WNND reported during the 2004 WNV outbreak were actually fewer than the 29 cases that occurred during the 1989 St. Louis encephalitis outbreak (Tueller 1990).

Kern had one of the highest number of equine cases in the state with 46. Twenty of the equines either died or were euthanized by their owners. The number of WNV-affected horses ranked Kern County 3rd among California counties.

The 2004 epidemic occurred during La Niña hot and dry conditions. Currently we are experiencing an El Niño-driven cool and wet winter and much of the southern SJV is currently flooded. It will be interesting to see if WNV will again amplify to epidemic levels or if cool weather and acquired immunity within the wild bird populations will result in decreased transmission levels during the summer of 2005.

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West Nile Virus in Sacramento and Yolo Counties, 2004

Veronica Armijos[1], Stan Wright[2], William Reisen[1], Kara Kelley[2], Sheryl Yamamoto[2], Dave Brown[2]

[1]*Center for Vector borne Diseases, School of Veterinary Medicine, University of California, Davis, CA 95616*
[2]*Sacramento-Yolo Mosquito and Vector Control District, Elk Grove, CA 95624*

ABSTRACT: West Nile virus (WNV) arrived in the Sacramento Valley region in June 2004. WNV infection was detected in 21 mosquito pools, 165 dead birds, 26 chickens and 23 wild birds from June through December in the counties of Sacramento and Yolo. During the first year of WNV invasion, it appeared that early in the season migrant birds arrived in Sacramento from areas where WNV was active. Detection of WNV in dead birds and mosquito pools demonstrated the presence of the virus in Sacramento and seroconversion in chickens was evidence that the virus was being transmitted locally.

INTRODUCTION

Although West Nile virus (WNV) invaded California during 2003, activity was restricted to areas south of the Tehachapi Mountains (Reisen et al. 2004). During the summer 2004 WNV amplified to epidemic levels in southern California and then rapidly progressed northward throughout California. Although western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses previously were endemic in the Sacramento Valley (Reeves 1990), their occurrence has been intermittent in recent years, with the most recent increase in WEEV enzootic transmission detected in 1993 (Reisen et al. 1995). The current paper describes the introduction of WNV into Sacramento and Yolo counties during the summer 2004 and identifies mosquito and avian host species initially involved in enzootic transmission.

MATERIALS AND METHODS

West Nile virus surveillance in mosquitoes, dead birds, and chickens was conducted at different sites throughout the two counties. Mosquito surveillance was conducted from May to October 2004 at 15 sites / week, with a total of 283 sites sampled throughout Sacramento and Yolo counties. Encephalitis virus surveillance (EVS) traps baited with CO_2 (Rohe and Fall 1979) and gravid female traps (Cummings 1992) were operated from dusk through dawn. Samples were transported to the SYMVCD laboratory for species identification, enumeration and pooling for virus testing. Pools consisting of ≤ 50 female of the same species were tested at the UC Davis Center for Vectorborne Disease Arbovirus Laboratory (CVEC) using a multiplex RT-PCR system that tested simultaneously for WNV, WEEV and SLEV.

Dead birds reported by the public to the California Department of Health Services (CDHS) Dead Bird Hot Line were picked up by SYMVCD personnel and transported to the California Animal Health and Food Safety (CAHFS) laboratory at Davis for necropsy. Tissues then were sent to CVEC for testing for WNV by a singleplex RT-PCR assay.

Ten chicken flocks (10 hens each) were sampled biweekly from May through October and then monthly from November to April. Sera were screened by enzyme immunoassay [EIA] at SYMVCD and then confirmed by DHS personnel using a PRNT.

Free-ranging bird seroprevelance was monitored at Stone Lakes National Wildlife Refuge (SLNWR), Center for Equine Health (CEH) at the University of California-Davis, and Laguna Creek. SLNWR is located in south Sacramento County. The CEH is an equine research facility located in the south UC Davis campus and housed a herd of 250 horses. Laguna Creek is located in the city of Elk Grove in the Sacramento County and is surrounded by ponds and trees on the Sacramento-Yolo MVCD property. Wild birds were captured year-round using 10 to 15 Japanese style mist nets (3x12 m), 2 modified Australian crow traps and 4 ground traps baited with grain (McClure 1984). Birds were banded with a unique USGS metal band and a 0.1 cc blood sample taken from jugular or brachial veins. Sera were screened for WEE or *Flavivirus* antibody by enzyme immunoassay (EIA) (Chiles and Reisen 1998) and then confirmed and identified by end point PRNT.

RESULTS

WNV invaded Sacramento and Yolo counties during the summer 2004. Vector abundance and seroprevalence of WNV in surveillance indicators across time are plotted in Figures 1 and 2. The first indication of WNV activity occurred on June 24 2004, when sera from 2 of 20 Cliff swallows and 1 of 10 Purple martins tested positive for the WNV antibody. Both bird species are summer residents, and therefore it was not possible to determine the site or timing of these infections. On July 8, a dead Western scrub jay collected from the town of Wilton tested positive establishing the presence of WNV. Mosquito traps were set around the Wilton area, and on July 23, three pools of *Culex tarsalis* Coquillett and two of *Cx. pipiens* L. tested positive, indicating an ongoing transmission in this area.

By August, pools of *Cx. tarsalis* (7) and *Cx. stigmatosoma* (1) tested positive for WNV. Of 163 dead birds tested, 52 were WNV-positive, with most being corvids (n= 46, 88.5%). Sentinel chickens remained negative, but three of 201 wild bird sera tested positive for WNV infection.

West Nile virus continued to amplify during September, with 7 of 251 mosquito pools and 78 of 274 wild birds testing positive. Nineteen chickens from 4 flocks seroconverted on September 8. These WNV-seropositive chickens were infected most likely during early to late August, concurrent with the increased number of dead wild birds.

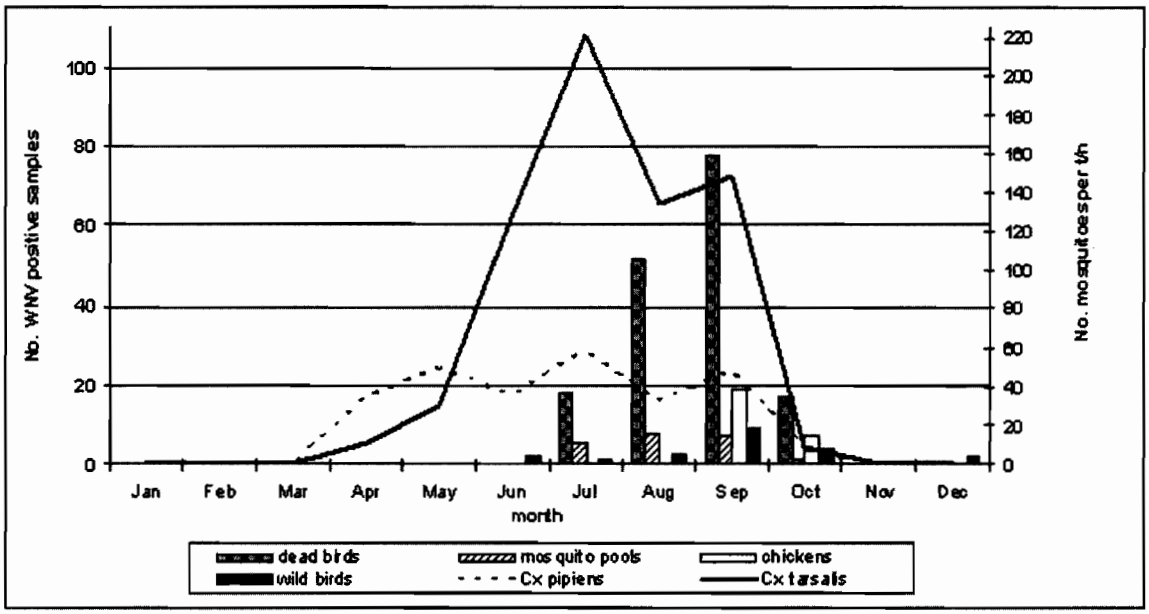

Figure 1. WNV seasonal activity in Sacramento and Yolo counties during 2004.

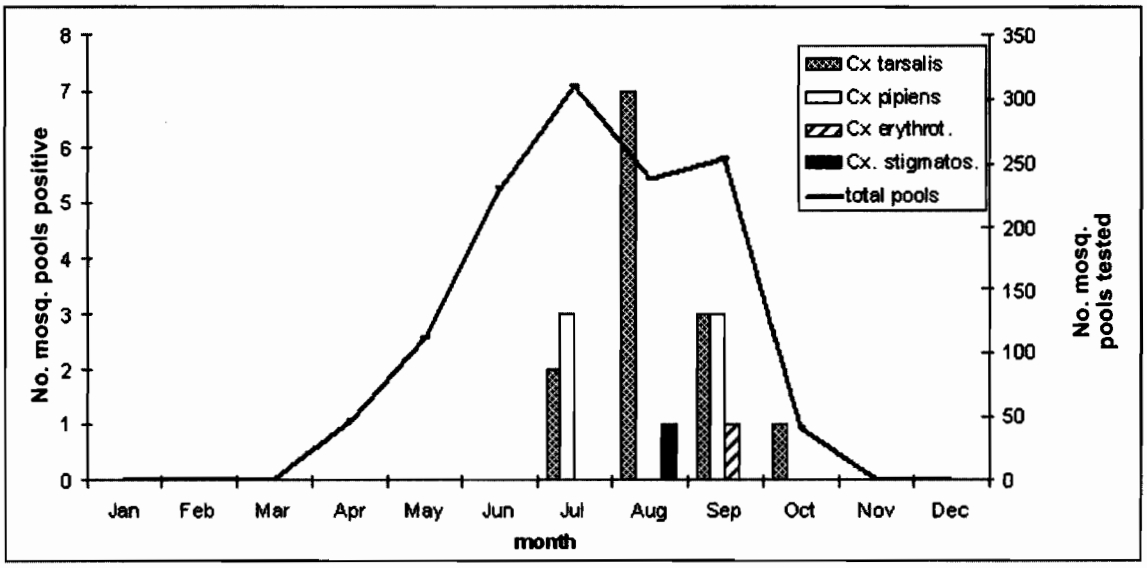

Figure 2. WNV positive mosquito pools for Sacramento and Yolo counties during 2004.

In October, WNV activity diminished as host-seeking and gravid female mosquito populations declined (Fig. 1) with the onset of diapause. Although the number of positive mosquito pools was low and the number of positive dead birds declined to 17, sentinel chickens continued seroconverting (7) and four additional wild bird samples tested positive for WNV. Although WNV surveillance continued into November, no positives were detected. In December, sera from a golden-crowned sparrow and a fox sparrow tested positive for WNV antibodies. Both birds are winter residents and presumably became infected after entering California.

During 2004, 21 (2%) of 1,231 mosquito pools tested positive for WNV. The highest numbers of positive pools were found in *Cx. tarsalis* (13) (60%) and *Cx. pipiens* (6) (30%); other positive species included *Cx. stigmatosoma* (1) (5%) and *Cx. erythrothorax* (1) (5%) (Fig. 2).

Dead birds were frequently reported by the public; 165 of 446 dead birds tested positive for WNV during the summer 2004. Overall, 92% (n = 165) of positive birds were corvids (Table 1), including Western scrub jay (47% of total tested), American crow (25%) and Yellow-billed magpie (52%).

Table 1. WNV positive dead bird species found in Sacramento and Yolo counties from January to December 2004.

Species	# tested	# positive	%
American crow	117	29	25
Western scrub jay	150	70	47
Yellow-billed magpie	102	53	52
Barn owl	5	1	20
Red-tailed hawk	6	6	100
Other species	66	6	9
GRAND TOTAL	446	165	37

Although chickens did not seroconvert until September, by the end of the year 26 chickens in 7 of 10 flocks tested positive for antibodies to WNV. The flocks that had positive seroconversions were Merrit (6 chickens), Winters (5), Natomas (4), Galt (4), Folsom (3), Elk Grove (3), and Hood (1).

Flavivirus antibody was found in 1% of 2,340 wild bird samples (11 positive species), with highest number of positives (10) found in rock pigeons (Table 2). Positive samples were collected from resident species (Rock pigeons, House finch, Black phoebe, Western scrub jay, Song sparrow, American goldfinch), winter residents (Golden-crowned sparrow, Fox sparrow) and summer residents (Cliff swallow, Purple martin, Ash-throated flycatcher) (Table 2).

Table 2. Positive WNV/SLE wild birds in Sacramento and Yolo counties from January to December 2004.

Species	# tested	# positive	%
Cliff swallow	41	2	4.8
Purple martin	10	1	10.0
Rock pigeon	141	10	7.0
Golden-crowned sparrow	219	1	0.5
Ash-throated flycatcher	14	2	14.3
House finch	320	2	0.6
Fox sparrow	90	1	1.1
Black phoebe	90	1	1.1
Western scrub Jay	51	1	1.9
Song sparrow	299	1	0.3
American goldfinch	13	1	7.6

DISCUSSION

The first indication of West Nile Virus in Sacramento County was the detection of antibodies in two migrant, colonial bird species, the cliff swallow and purple martin. These two closely related species, which winter in Central and South America, are summer residents in Sacramento County. The colonial nest sites of these two species are occupied every year by the same individuals and their offspring, as demonstrated by multiple recaptures of banded birds from these colonies. Blood samples from previous years gave no indication of WNV in the populations. So it appeared that members of the Sacramento population were likely first exposed to WNV either on their winter grounds in 2003, during their spring migration through southern California, or at their breeding grounds in Sacramento during the spring 2004. Because these birds were serologically positive, we conclude that they survived previous infection and most likely were no longer viremic. We do not know if they were viremic upon arrival in Sacramento County. However, the detection of antibody in spring migrants does provide proof of principal that some avian migrants from sites with active WNV transmission did arrive in Sacramento County prior to the detection of WNV in either resident birds or mosquitoes. Further studies are required to establish the role of migrants in virus introduction.

The sequence of WNV detection in the different surveillance systems illustrated the progression of WNV amplification in Sacramento County. Although antibody was detected in spring migrants, the arrival of WNV was established first by the detection of RNA in dead birds and then in mosquitoes. Serological indicators such as antibody-positive wild birds and sentinel chicken seroconversions occurred later, most likely because of the focal nature of sampling and the delay required after infection for a diagnostic rise in antibody titer. Some of the dead bird species included raptors such as the Red-tailed hawk which are known to prey on birds, especially those too ill to escape predation. Antibody rates were highest in Rock pigeons and adult chickens, neither of which develop significant viremias or die from WNV infection. Seroconversions among marked pigeons and chickens verified that virus was being locally transmitted. Although mosquito populations and virus activity declined by December, WNV antibody was

detected in bird species that winter in Sacramento County. This progression of events describes the mode of introduction and local amplification of WNV in Sacramento and Yolo counties. How WNV overwinters and becomes established locally will be the focus of investigations during 2005.

Acknowledgements

We especially thank Dr. B. Cahoon-Young and the CVEC laboratory staff for testing the mosquito pools and dead bird tissues; Y. Fang, CVEC for confirming wild bird serology; and C. Cossen and the VRDL staff for confirming sentinel chicken sera. We thank the Sacramento-Yolo Mosquito and Vector Control District for financial support and district personnel for their logistical support, especially Glenn Yoshimura, Gary Goodman and Ken Boyce. We also our appreciation to the volunteers of Stone Lakes National Wildlife Refuge and U.S. Fish and Wildlife Service for their frequent field work participation. This research was funded by grants from the National Institutes of Health.

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Vector and Host Competence: Importance of Virulence in Birds for West Nile Virus Transmission[1,2]

William K. Reisen[3], Ying Fang, and Vincent Martinez[4]

[4]Arbovirus Laboratory,Center for Vectorborne Diseases,School of Veterinary Medicine,University of California, Davis, Old Davis Rd,Davis, CA 95616

ABSTRACT: Experimental infections of *Culex* mosquitoes from southern California with West Nile virus (WNV) showed that *Cx. stigmatosoma* was the most competent laboratory vector followed by *Cx. tarsalis* and *Cx. quinquefasciatus*. Although estimates varied markedly among and within populations, mosquitoes generally were less susceptible to oral infection with WNV than closely related St. Louis encephalitis virus, requiring viremia titers >5 $\log_{10}$ plaque forming units (PFU) of WNV per ml for infection. Experimental infection of 13 species of California birds indicated that 7 species were competent hosts; 6 of these species were in the order Passeriformes including Western scrub jays. Species in the orders Galliformes and Columbiformes were not competent hosts. Mortality among bird species was significantly correlated with average peak viremia titer on days 2 – 4 after infection. Field infection rates in *Cx. quinquefasciatus* and the incidence of human cases were related to the distribution of corvids, being highest in Los Angeles and Kern counties and lowest in the Coachella Valley.

INTRODUCTION

West Nile virus (WNV; Family *Flaviviridae*, genus *Flavivirus*) invaded southern California during 2003, successfully overwintered and then amplified to unprecedented epidemic levels within southern California (including Kern County) during 2004. Among the ecological and epidemiological factors that have influenced the success of this invading virus, the competence of avian and mosquito hosts for WNV infection seemed especially important because *Culex* vector mosquitoes have required greater viral titers for infection (Goddard et al. 2002, Turell et al. 2002, Reisen et al. 2004a) than closely-related St. Louis encephalitis (SLE) (Hardy and Reeves 1990). Transmission of WNV has seemed effective because infected birds (especially corvids) produce extremely elevated viremias in response to infection (Komar et al. 2003, Brault et al. 2005). However, this elevated viremia response comes at the price of increased mortality (Komar et al. 2003; Reisen et al. 2005) and therefore possible depopulation (Caffrey et al. 2003). In the current paper we describe the importance of elevated avian WNV viremia in the infection of California *Culex* mosquitoes with WNV and relate these data to mosquito field infection rates and the occurrence of human disease.

MATERIALS AND METHODS

Virus. The NY99 strain of WNV was used in both mosquito and bird infection experiments. The quantity of virus within avian blood, mosquito tissue and expectorate samples was detected by plaque assay using Vero cell culture (Kramer et al. 2002).

Mosquitoes. Our study focused on the three *Culex* species that were infected most frequently in nature during 2003 (Reisen et al. 2004b): *Culex tarsalis* Coquillett, *Cx. pipiens* L., *Cx. quinquefasciatus* Say and *Cx. stigmatosoma* Dyar. Mosquitoes were collected as immatures or adults from Riverside, Los Angeles, Kern and Yolo counties and transported to the Arbovirus Field Station. Eggs were collected from field-collected adult females and reared under insectary conditions so that all mosquitoes tested were nulliparous and approximately 3 – 8 d old when infected. Mosquitoes were infected by feeding on either donor birds (House finches or House sparrows on 2 – 3 days post infection (dpi)] or cotton pledgets soaked with a solution of virus, heparinized chicken blood and 2.5% sucrose. Engorged mosquitoes were held for 2 weeks at 26°C, after which transmission was attempted using the capillary tube method (Aitken 1977). Median infectious dose was estimated by interpolation after plotting the percentage infected following feeding on a 10-fold dilution series of WNV as a function of viral dose.

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[1] Most of this research has been accepted for publication elsewhere (Reisen et al. 2005).

[2] The collection and infection of wild birds was done under Protocol 11184 approved by the Animal Use and Care Administrative Advisory Committee of the University of California, Davis, California Resident Scientific Collection Permit No. 801049-02 from the State of California Department of Fish and Game, and Federal Fish and Wildlife Permit No. MB082812-0 from the Department of the Interior. Animal Use and Care Administrative Advisory Committee Protocol No. 11187 approved procedures for using wild birds and chickens for mosquito infection experiments. 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.

[3] Address correspondence to: Arbovirus Field Station, 4705 Allen Rd, Bakersfield, CA 93314

Avian infection. Thirteen species of California birds were collected in Kern or Riverside Counties and transported to the Arbovirus Field Station for experimental infection. Birds were inoculated subcutaneously in the cervical region with ca. 1,000 plaque forming units (PFU) of WNV and then bled daily for 5 – 8 dpi to monitor the viremia response and mortality.

RESULTS AND DISCUSSION

Mosquito infection. *Culex stigmatosoma* was the most competent laboratory vector for WNV, followed in descending order by *Cx. tarsalis* and *Cx. quinquefasciatus*. When the percentage of females infected with WNV was plotted as a function of decreasing viral dose, more *Cx. stigmatosoma* were infected after feeding on less virus than were *Cx. tarsalis* and *Cx. quinquefasciatus* (Fig. 1). Data for *Cx. tarsalis* were averaged over estimates for Coachella Valley, Los Angeles and Kern County and data for *Cx. quinquefasciatus* averaged over Coachella Valley and Los Angeles, thereby providing an overview of these species susceptibilities to WNV infection. The Kern *Cx. quinquefasciatus* population seemed refractory to infection with WNV and was plotted separately as a possible outlier.

Data on infecion and transmission after feeding on single doses ranging from 6 to 7 $\log_{10}$ PFU/ml were summarized for multiple populations in Fig. 2. These data depicted considerable variability within and among different geographical areas and different species. After combining data over collection sites, infection rates varied significantly among species ($Chi^2 = 15.4$, df = 2, $P<0.001$), being highest for *Cx. stigmatosoma* (90%, n = 19) and lowest for *Cx. tarsalis* (43%, n = 122); *Cx. quinquefasciatus* was intermediate (57%, n = 99) (Reisen et al. 2005). Transmission rates by infected females did not vary significantly among species ($P>0.05$), being 30% (n = 53) for *Cx. tarsalis*, 14% (n = 57) for *Cx. quinquefasciatus* and 18% (n = 17) for *Cx. stigmatosoma*. These data generally agreed with our previous reports (Goddard et al. 2002, Reisen et al. 2004a).

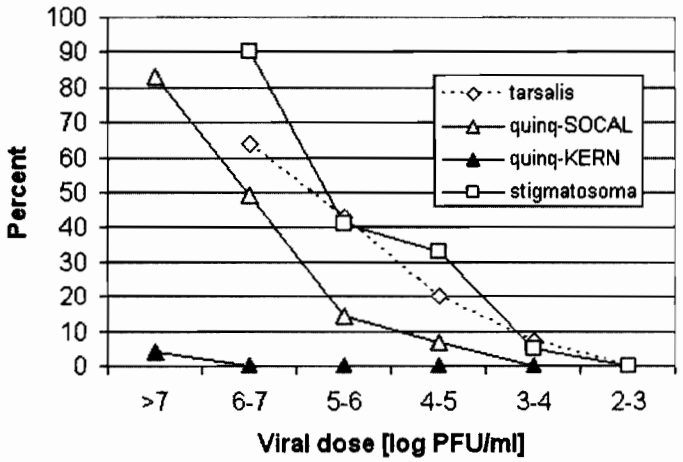


Figure 1. Percent of 3 species of *Culex* collected during 2003 from 3 localities in southern California infected with WNV after feeding on a 10-fold dilution series in $\log_{10}$ plaque forming units (PFU) per ml. n=17–60 females per dose per species, quinq = Cx. *quinquefasciatus*.

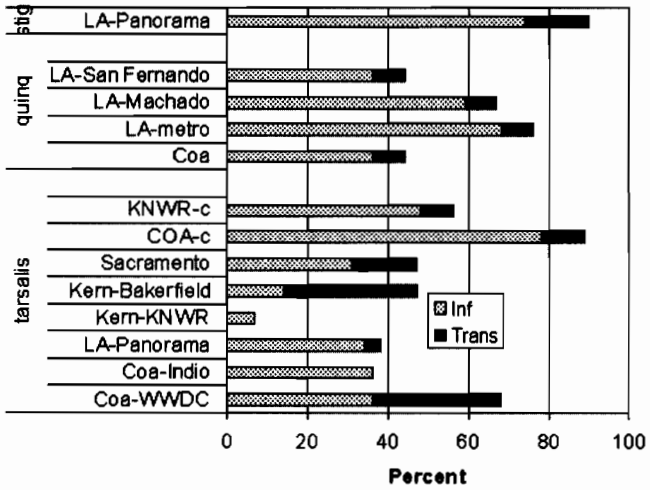


Figure 2. Percentage of *Culex stigmatos*oma (stig), *Cx. quinquefasciatus* (quinq) and *Cx. tarsalis* (tarsalis) from Coachella Valley (COA), Los Angeles (LA), Kern and Sacramento that became infected (Inf) and transmitted (Trans) WNV after feeding on a mixture of 6 – 7 $\log_{10}$ PFU/ml of WNV, blood and 2.5% sucrose and surviving 2 wks extrinsic incubation at 26°C. Figure drawn from previously published tabular data (Reisen et al. 2005).

Bird infection. Viremia responses of birds to infection with WNV varied markedly among the 13 bird species tested (Fig. 3). Corvids (American crows and Western scrub jays) had the highest viremia levels, whereas galliform birds (chickens and quail) had the lowest viremias. One of two adult chickens (24 wks) had a low level fleeting viremia that was well below the threshold required for mosquito infection, thereby ensuring that chickens were safe to use as sentinel birds near humans. Seven bird species had peak

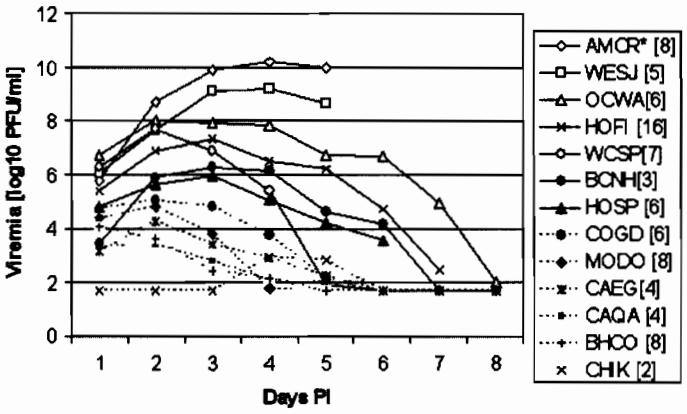


Figure 3. Viremia profiles for 13 species of California birds (redrawn and extended from (Reisen et al. 2005)). Data for American crow (AMCR), Western scrub jay (WESJ), Orange-crowned warbler (OCWA), House finch (HOFI), White-crowned sparrow (WCSP), Black-crowned night heron (BCNH), House sparrow (HOSP), Common ground dove (COGD), Mourning dove (MODO), California quail (CAQA), Brown-head cowbird (BHCO) and adult chicken (CHIK). Numbers in brackets were the number of birds tested per species. Species represented by a dashed line probably would not be competent hosts for most *Culex* mosquitoes. *Crow data are redrawn from Komar et al. (2003).

viremias during days 2-3 post inoculation of >5 $\log_{10}$ PFU/ml and were considered to be competent hosts, whereas 6 species (indicated by dashed lines) had lower viremias and were considered incompetent hosts (Fig. 3). Viremia levels for each species were correlated significantly ($r = 0.82$, $df = 10$, $P<0.05$) with mortality observed during each experiment (Fig. 4); i.e., bird species producing high viremias also exhibited the high mortality. Mortality among orange-crowned warblers was 100%, agreeing well with their elevated viremia profiles as well as our repeated inability to detect antibodies in field-collected birds during their northern or southern migration (Wheeler et al. 2003; Wheeler et al. 2004). These vernal migrants could be very important in virus dispersal, because during their northbound migration they frequently rest at wetlands in southern California when virus activity is beginning each year. If these birds became infected they could continue their northern migration while viremic. When they become too weak from the infection to continue the migration, they remain alive for a day or so and thereby could distribute virus to host-seeking *Culex* at the site where they became sick. Such a mechanism could explain the rapid wide scale dispersal of WNV into northern California during 2004.

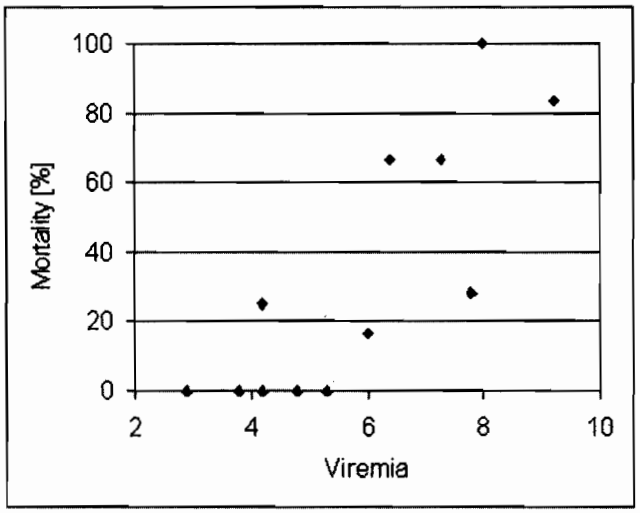
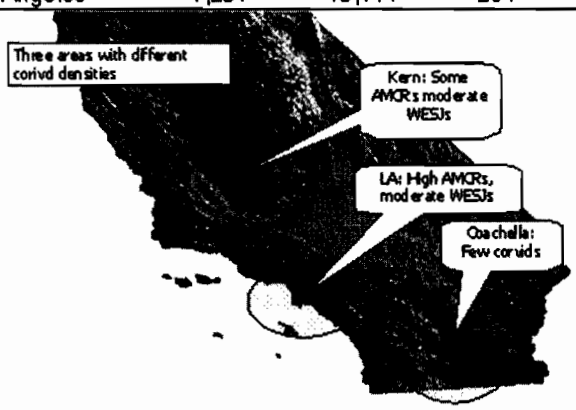


Figure 4. Avian mortality (% dead) plotted as a function of average maximum viremia post infection ($\log_{10}$ PFU WNV/ml).

Relationship to human disease. Because *Culex* required elevated avian viremias for effective infection and transmission, the geographical distribution of resident avian species having elevated viremias may impact *Culex* minimum infection rates and therefore transmission to humans. Three biomes of southern California where WNV was active during 2004 were characterized by different avian communities associated with different *Culex* infection and WNV case incidence rates during 2004 (Fig. 5). Based on maps provided by the bird breeding survey (http://www.mbr-pwrc.usgs.gov/), the SE California deserts had few corvids, Los Angeles elevated American crow populations, and Kern County moderate Western scrub jay and American crow populations. In accordance with these measures of corvid density, Coachella Valley had the lowest *Culex* infection rates and the lowest human WNV incidence rates within California. Virus amplification here seemed to rely on House finch and House sparrow populations and infections in *Cx. tarsalis*; few *Cx. quinquefasciatus* pools were positive (6% of total positive). In marked contrast, elevated corvid populations in Kern and Los Angeles Counties resulted in combined *Cx. tarsalis* and *Cx. quinquefasciatus* MIRs >5/1,000 for the summer and a markedly higher incidence of human infection. These higher case incidence rates were related to higher density human populations as well as the extensive involvement of urban *Cx. quinquefasciatus* populations in transmission. In Kern and Los Angeles counties, *Cx. quinquefasciatus* accounted for 50 and 92% of the positive *Culex* pools detected during the 2004 transmission season, respectively.

Locality	Pools tested	Total mosquitoes tested	WNV positives	Infection Rate per 1,000
Coachella Valley	556	18,269	67	3.67
Kern County	816	32,218	171	5.31
Los Angeles	1,201	43,444	294	6.77



Area	Population size per 100,000[a]	WNV cases	Incidence per 100,000
California	33,871	808	2.39
Coachella Valley	336	7	2.08
Los Angeles County	9,519	322	3.38
Kern County	662	59	8.91

a Based on 2,000 census figures

Figure 5. Areas of southern California having avian communities with varying levels of American crows (AMCRs) and Western scrub jays (WESJs). Tables show associated *Culex* mosquito infection rates during May – September 2004 and the incidence of laboratory confirmed human cases through October 2004.

Based on our laboratory and field findings in southern California, areas of the Central Valley supporting large populations of American crows, Western scrub jays and Yellow-billed magpies may be especially vulnerable to WNV amplification during 2005. Areas around in the Sacramento Valley and along the Sacramento and Stockton Rivers supporting large crow populations (Fig. 6) experienced WNV introduction during 2004 and may have virus amplification reach epidemic levels during 2005.

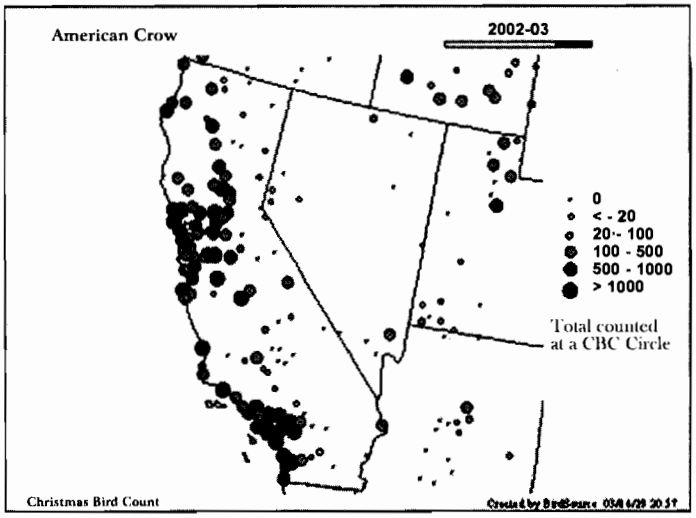


Figure 6. Abundance of American crows in California as estimated by the Audubon Society's Christmas bird count (http://www.audubon.org/bird/cbc/hr/index.html)

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We especially thank Brian Carroll and Sarah Wheeler for collection of the wild birds; Hugh Lothrop (Coachella Valley MVCD) and the staffs of the Greater Los Angeles County VCD, and Kern and Sacramento-Yolo MVCDs for collecting the mosquitoes for vector competence testing; Scott Hallam and Leah Kosaroff for mosquito and bird maintenance; and Sandra Garcia, Sira Ansari and Marzieh Shafii for laboratory testing.

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West Nile Virus in Wild Birds: Who Lives and Who Dies?

Sarah S. Wheeler[1], Ryan Carney[2], Brian Carroll[1], Stan Wright[4], Veronica Armijos[4, 1], Jennifer Wilson[1, 3], Sandra Garcia[1], Ying Fang[1], William K. Reisen[1, 5]

[1]*Center for Vectorborne Diseases, School of Veterinary Medicine, University of California, Davis, CA 95616*
[2]*California Department of Health Services, Vector-Borne Disease Section, 850 Marina Bay Parkway, Richmond, CA 94804*
[3] *Greater Los Angeles County Vector Control District, Santa Fe Springs, CA 90670*
[4] *Sacramento-Yolo Mosquito and Vector Control District, 8631 Bond Rd, Elk Grove, CA 95624*
[5]*Arbovirus Field Station, 4705 Allen Rd., Bakersfield, CA (address correspondence here)*

ABSTRACT: Data on the prevalence of West Nile virus (WNV) antibodies in wild birds was compared with concurrently collected data on dead birds testing positive for WNV as part of the California Dead Bird Surveillance Program. Field data were related to the results of experimental laboratory infections. A total of 12,198 birds were tested for *Flavivirus* antibody during 2004, of which 831 individuals were considered *Flavivirus* positive. House sparrows, House finches, 2 species of quail, and 3 species of doves were most frequently WNV antibody positive. American crows and three other corvid species predominated the dead bird species reported from California. Western scrub jays, House sparrows, House finches, as well as Mourning doves and California quail were collected alive and WNV antibody positive and also reported dead and WNV positive. Of these species Mourning doves and California quail were found to survive WNV test infections. Orange-crowned warblers infected with 10^3 plaque-forming units of the NY99 strain of WNV succumbed to infection within 4-7 days post infection. Interestingly, these birds produced an average peak viremia of 10^8 PFU/ml, sufficient to infect most mosquitoes and thereby amplify virus.

INTRODUCTION

The rapid dispersal of West Nile virus (WNV) throughout California during 2004 stimulated much concern as to the impact of this virus on populations of wild birds. To address this concern, we compared data on the prevalence of antibodies in wild birds that survived WNV infection with concurrently collected data on dead birds testing positive for WNV as part of the California Dead Bird Surveillance Program. Field data were related to the results of experimental laboratory infections.

MATERIALS AND METHODS

Wild bird sampling stations were located in the Coachella Valley, Greater Los Angeles, Kern County, and Sacramento-Yolo counties. Birds were collected by mist nets and/or grain baited traps and a blood sample taken by venipuncture and diluted 1:10 in saline. Sera were tested for antibodies against *Flavivirus* (either St .Louis encephalitis (SLE) or WNV) using an enzyme immunoassay (EIA), with positives confirmed and specifically identified using a plaque reduction neutralization test (Chiles et al. 1998). Serologically positive birds survived infection and developed a detectable immune response. Birds reported to the California Department of Health Services (CDHS) by the public were necropsied by the California Animal Health and Food Safety laboratories. Selected tissues or buccal swabs were submitted to the Center for Vectorborne Diseases (CVEC) laboratory for testing for WNV RNA using a singleplex assay with a TaqMan platform (Chiles et al. 2004). Birds that tested positive for WNV represented individuals that did not survive WNV infection, or died from other causes while infected with WNV.

RESULTS AND DISCUSSION

A total of 12,198 birds from 216 species (Table 1) were tested for *Flavivirus* antibody during 2004, of which 831 individuals exhibited a positive/negative ratio >2 in our EIA and were considered *Flavivirus* positive. House sparrows, house finches, 2 species of quail and 3 species of doves were most frequently *Flavivirus* positive, agreeing with observations during 2003 when WNV first entered southern California (Wheeler et al. 2004, Wilson et al. 2004). New species found positive for antibody included western scrub jays and white-crowned sparrows. The collection of WNV antibody positive western scrub jays was of particular interest because of the extreme susceptibility of many members of the family Corvidae to WNV infection. The repeated sampling of WNV antibody positive white-crowned sparrows was unexpected, because this winter resident species has never tested positive for antibodies against SLE or western equine encephalomyelitis virus (WEE) (Milby and Reeves 1990, Reisen et al. 2000). Species that were occasionally antibody positive indicated that some individuals survived WNV infection, but these data do not provide enough information to quantify survivorship.

American crows and three other corvid species were the predominant dead bird species reported from California (Table 2). Several factors account for the high numbers of dead American crows. Firstly, this species is highly susceptible to WNV and almost all individuals succumb to infection 5- 6 days post infection (Komar et al. 2003, Brault et al. 2005). Secondly, CDHS instructed the public to focus on reporting dead crows and initially only American crows, Raptors, House finches and House sparrows were being tested from southern California until November 1, 2004 when testing was opened to all avian species. All species reported from

Table 1: Combined serological findings from the Coachella Valley, Greater Los Angeles, Kern, and Sacramento-Yolo wild bird sampling stations.

		EIA RESULTS		PRNT RESULTS		
Species	**Family**	**Number Sampled**	***Flavirus* Positive**	**WNV Positive**	**PRNT Neg.**	**Pending**
Sharp-shinned hawk *Accipiter striatus*	Accipitridae	2	1	0	0	1
Cooper's hawk *Accipiter cooperii*	Accipitridae	9	2	0	0	2
Great blue heron *Butorides virescens*	Ardeidae	1	1	1	0	0
Least bittern *Ixobrychus exilis*	Ardeidae	25	13	8	5	0
Black-headed grosbeak *Pheucticus melanocephalus*	Cardinalidae	7	2	1	1	0
Ringed turtle-dove *Streptopelia risoria*	Columbidae	1	1	0	1	0
White-winged dove *Zenaida asiatica*	Columbidae	7	3	0	2	1
Common ground-dove *Columbina passerina*	Columbidae	96	25	5	4	16
Rock pigeon *Columba livia*	Columbidae	485	80	35	15	30
Mourning dove *Zenaida macroura*	Columbidae	1,032	81	24	25	32
Western scrub jay *Aphelocoma californica*	Corvidae	208	20	10	0	10
Abert's towhee *Pipilo aberti*	Emberizidae	42	1	1	0	0
California towhee *Pipilo crissalis*	Emberizidae	33	1	0	0	1
Golden-crowned sparrow *Zonotrichia atricapilla*	Emberizidae	123	1	0	0	1
Lincoln sparrow *Melospiza lincolnii*	Emberizidae	65	1	0	1	0
Savannah sparrow *Passerculus sandwichensis*	Emberizidae	69	1	0	0	1
Song sparrow *Melospiza melodia*	Emberizidae	534	2	0	2	0
White-crowned sparrow *Zonotrichia leucophrys*	Emberizidae	847	16	0	0	16
American goldfinch *Carduelis tristis*	Fringillidae	13	1	1	0	0
House finch *Carpodacus mexicanus*	Fringillidae	2,060	177	61	24	92
Cliff swallow *Petrochelidon pyrrhonota*	Hirundinidae	41	1	0	0	1
Purple martin *Progne subis*	Hirundinidae	10	1	1	0	0
Great-tailed grackle *Quiscalus mexicanus*	Icteridae	5	1	1	0	0
Red-winged blackbird *Agelaius phoeniceus*	Icteridae	13	1	1	0	0
Brown-headed cowbird *Molothrus ater*	Icteridae	193	4	1	3	0

Continued »

Species	Family	EIA RESULTS		PRNT RESULTS		
		Number Sampled	*Flavirus* Positive	WNV Positive	PRNT Neg.	Pending
Loggerhead shrike *Lanius ludovicianus*	Laniidae	24	1	0	0	1
Northern mockingbird *Mimus polyglottos*	Mimidae	56	4	0	0	4
California thrasher *Toxostoma redivivum*	Mimidae	68	4	0	0	4
Common yellowthroat *Geothlypis trichas*	Parulidae	92	1	0	0	1
House sparrow *Passer domesticus*	Passeridae	1,731	242	80	11	151
Domestic chicken	Phasianidae	7	4	1	0	3
California quail *Callipepla californica*	Phasianidae	450	40	0	0	40
Gambel's quail *Callipepla gambellii*	Phasianidae	668	96	47	18	31
Bewick's wern *Thryomanes bewickii*	Troglodytidae	39	1	0	0	1
Black pheobe *Sayornis nigricans*	Tyrannidae	90	1	0	0	1
Ash-throated flycatcher *Myiarchus cinerascens*	Tyrannidae	14	2	0	0	2
180 other species	various	3,038	0	0	0	0
Grand Total		**12,198**	**831**	**280**	**112**	**443**

Table 2: Dead birds found WNV positive by the CA dead bird surveillance program in 2004.

Common Name	Scientific Name	Family	Total
American crow	*Corvus brachyrhynchos*	Corvidae	1,669
Western scrub-Jay	*Aphelocoma californica*	Corvidae	624
Yellow-billed magpie	*Pica nuttalli*	Corvidae	303
Steller's jay	*Cyanocitta stelleri*	Corvidae	68
House finch	*Carpodacus mexicanus*	Fringillidae	38
Common raven	*Corvus corax*	Corvidae	35
House sparrow	*Passer domesticus*	Passeridae	35
Cooper's hawk	*Accipiter cooperii*	Accipitridae	31
Red-tailed hawk	*Buteo jamaicensis*	Accipitridae	27
Barn owl	*Tyto alba*	Tytonidae	27
Brewer's blackbird	*Euphagus cyanocephalus*	Icteridae	20
American robin	*Turdus migratorius*	Turdidae	19
Lesser goldfinch	*Carduelis psaltria*	Fringillidae	18
Sharp-shinned hawk	*Accipiter striatus*	Accipitridae	17
American kestrel	*Falco sparverius*	Falconidae	15
European starling	*Sturnus vulgaris*	Sturnidae	15
Golden-crowned sparrow	*Zonotrichia atricapilla*	Emberizidae	13
Northern mockingbird	*Mimus polyglottos*	Mimidae	13
Red-shouldered hawk	*Buteo lineatus*	Accipitridae	11
California towhee	*Pipilo crissalis*	Emberizidae	11
Fox sparrow	*Passerella iliaca*	Emberizidae	10
Western tanager	*Piranga ludoviciana*	Thraupidae	10
Black-headed grosbeak	*Pheucticus melanocephalus*	Cardinalidae	9
California quail	*Callipepla californica*	Phasianidae	9

Continued »

Common Name	Scientific Name	Family	Total
Great Horned owl	*Bubo virginianus*	Strigidae	9
Mourning dove	*Zenaida macroura*	Columbidae	8
Acorn woodpecker	*Melanerpes formicivorus*	Picidae	8
Western screech-owl	*Otus kennicottii*	Strigidae	7
Western bluebird	*Sialia mexicana*	Turdidae	7
Mallard	*Anas platyrhynchos*	Anatidae	6
Black-billed magpie	*Pica hudsonia*	Corvidae	6
Spotted towhee	*Pipilo maculatus*	Emberizidae	6
Pine siskin	*Carduelis pinus*	Fringillidae	6
Hermit thrush	*Catharus guttatus*	Turdidae	6
Swainson's thrush	*Catharus ustulatus*	Turdidae	6
Northern flicker	*Colaptes auratus*	Picidae	5
White-tailed kite	*Elanus leucurus*	Accipitridae	4
White-crowned sparrow	*Zonotrichia leucophrys*	Emberizidae	4
Anna's hummingbird	*Calypte anna*	Trochilidae	4
Black phoebe	*Sayornis nigricans*	Tyrannidae	4
Osprey	*Pandion haliaetus*	Accipitridae	3
domestic goose		Anatidae	3
Cedar waxwing	*Bombycilla cedrorum*	Bombycillidae	3
Dark-eyed junco	*Junco hyemalis*	Emberizidae	3
Song sparrow	*Melospiza melodia*	Emberizidae	3
American goldfinch	*Carduelis tristis*	Fringillidae	3
Yellow-rumped warbler	*Dendroica coronata*	Parulidae	3
Rufous hummingbird	*Selasphorus rufus*	Trochilidae	3
Pacific-slope flycatcher	*Empidonax difficilis*	Tyrannidae	3
Northern goshawk	*Accipiter gentilis*	Accipitridae	2
Turkey vulture	*Cathartes aura*	Cathartidae	2
Rock pigeon	*Columba livia*	Columbidae	2
Savannah sparrow	*Passerculus sandwichensis*	Emberizidae	2
Evening grosbeak	*Coccothraustes vespertinus*	Fringillidae	2
Purple finch	*Carpodacus purpureus*	Fringillidae	2
Hooded oriole	*Icterus cucullatus*	Icteridae	2
Red-winged blackbird	*Agelaius phoeniceus*	Icteridae	2
Loggerhead shrike	*Lanius ludovicianus*	Laniidae	2
Common yellowthroat	*Geothlypis trichas*	Parulidae	2
Orange-crowned warbler	*Vermivora celata*	Parulidae	2
Wilson's warbler	*Wilsonia pusilla*	Parulidae	2
domestic chicken		Phasianidae	2
Mountain quail	*Oreortyx pictus*	Phasianidae	2
Western sandpiper	*Calidris mauri*	Scolopacidae	2
Swainson's hawk	*Buteo swainsoni*	Accipitridae	1
domestic duck		Anatidae	1
Great Blue heron	*Ardea herodias*	Ardeidae	1
Green heron	*Butorides virescens*	Ardeidae	1
Snowy egret	*Egretta thula*	Ardeidae	1
Common nighthawk	*Chordeiles minor*	Caprimulgidae	1
Lesser nighthawk	*Chordeiles acutipennis*	Caprimulgidae	1
Killdeer	*Charadrius vociferus*	Charadriidae	1
Pinyon jay	*Gymnorhinus cyanocephalus*	Corvidae	1
Black-chinned sparrow	*Spizella atrogularis*	Emberizidae	1
Nutmeg mannikin	*Lonchura punctulata*	Estrildidae	1
Peregrine falcon	*Falco peregrinus*	Falconidae	1
Bank swallow	*Riparia riparia*	Hirundinidae	1
Barn swallow	*Hirundo rustica*	Hirundinidae	1

Continued »

Common Name	Scientific Name	Family	Total
Cliff swallow	*Petrochelidon pyrrhonota*	Hirundinidae	1
Western meadowlark	*Sturnella neglecta*	Icteridae	1
California gull	*Larus californicus*	Laridae	1
Ring-billed gull	*Larus delawarensis*	Laridae	1
Townsend's warbler	*Dendroica townsendi*	Parulidae	1
Yellow warbler	*Dendroica petechia*	Parulidae	1
Double-crested cormorant	*Phalacrocorax auritus*	Phalacrocoracidae	1
Bronze turkey		Phasianidae	1
Ruffed grouse	*Bonasa umbellus*	Phasianidae	1
Red-breasted sapsucker	*Sphyrapicus ruber*	Picidae	1
Pied-billed grebe	*Podilymbus podiceps*	Podicipedidae	1
American coot	*Fulica americana*	Ralidae	1
Common moorhen	*Gallinula chloropus*	Ralidae	1
Virginia rail	*Rallus limicola*	Ralidae	1
Pygmy nuthatch	*Sitta pygmaea*	Sittidae	1
Varied thrush	*Ixoreus naevius*	Turdidae	1
Grand Total			**3,230**

zip codes near our wild bird collection stations were reported and tested. Finally, crows are large conspicuous birds that generally live in urban areas, thus succumbing to infection where there is a greater chance that someone will report the carcass.

In contrast to urban Los Angeles and Bakersfield, where there were respectively 256 and 56 dead birds that tested positive for WNV, the dead bird program did not function well in the Coachella Valley. Here only 6 WNV positive dead birds were reported despite high levels of WNV enzootic activity detected by wild bird serology, sentinel chicken seroconversions, and positive mosquito pools (Lothrop 2005). The likely cause of this under-reporting by the dead bird program is that most virus activity was detected in rural southern Coachella Valley where there are very few American crows and low human population density.

Western scrub jays, House sparrows, House finches, Mourning doves and California quail were collected alive and seropositive and also were reported dead by the public and tested WNV positive. Because these species appeared on both lists, it may be inferred that not all individuals succumbed following infection. These conclusions were supported by our experimental infection studies (Table 3). Interestingly, all Mourning doves and California quail survived experimental WNV infections, producing only moderate viremias. Appearance of these species on the dead bird list suggest s that although WNV does not generally cause death in these species some may more susceptible, such as old or otherwise immune compromised birds, or birds weakened by co-infection with other parasites. Wild House finches were frequently found infected with several species of avian malaria (Reisen et al. 2001).

Neither the free ranging wild bird serology nor dead bird

Table 3: Test infection finding where each bird was infected with 1,000 plaque forming units (PFU) of NY99 WNV.

Species	Number Infected	Dead (%)	Peak Viremia (log 10 PFU/ml)
Western scrub jay	12	83	92.0
Orange-crowned warbler	6	100	8.0
White-crowned sparrow	7	29	7.8
House finch	36	67	7.3
Black-crowned night heron	3	67	6.4
House sparrow	6	17	6.0
Common ground-dove	6	0	5.3
Mourning dove	20	0	4.8
Brown-headed cowbird	9	0	4.2
Cattle egret	4	25	4.2
California quail	6	0	3.8
Chicken	5	0	2.3

programs provided much information on endangered species and neotropical migrants. We have no information regarding WNV seroprevalence in endangered species because the wild bird sampling stations do not handle endangered species and there were none found positive by the dead bird program. Among neotropical migrants, the survivorship of warblers (family Parulidae) is of great concern. In the Coachella Valley, 294 neotropical warblers from 9 species were tested for antibodies with negative results. Negative serological findings may indicate that a species is not frequently bitten by mosquitoes or that it succumbs rapidly to infection. Only 11 WNV-positive dead warblers were reported statewide: 3 Yellow-rumped warblers, 2 Orange-crowned warblers, 2 Wilson's warblers, 2 Common yellowthroats, 1 Yellow warbler, and 1 Townsend's warbler.

We recently found that Orange-crowned warblers infected with 10^3 plaque-forming units (Table 3) of the NY99 strain of WNV succumbed to infection within 4-7 days post infection. Interestingly, these birds produced an average peak viremia of 10^8 PFU/ml, sufficient to infect most mosquitoes and thereby amplify virus (Reisen et al. 2005). Therefore, it may be possible that we do not detect antibody positive warblers because they are not surviving WNV infection. It could also be possible that in the spring when most migratory warblers were sampled, these birds had not come in contact with virus, or had contracted the virus locally but did not have time to form detectable antibodies before moving out of the area. If infected in southern California, these birds could disperse virus to northern California before succumbing to infection. We intend to investigate this possibility during 2005 by testing migrants collected in the Coachella Valley for the presence of virus in addition to antibodies to determine if locally infected birds are being missed by our current methods. Migrants traversing the Salton Sea and the surrounding desert during northward migration frequently rest at the north shore area for several days before continuing their movement up the inland route of the Pacific flyway. Sacramento-Yolo and Kern stations will also attempt to track these birds and their infection status as they progress northward.

SUMMARY

In conclusion, our sampling stations provided information about seroprevalence rates of sampled species, but data were limited to those species that could be caught by mist nets or grain-baited traps and to those species that survive WNV infection. In contrast, the California dead bird program has provided new information about species succumbing to WNV infection, but is biased towards large conspicuous species.

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Summary

William K. Reisen[1]

Center for Vectorborne Diseases, School of Veterinary Medicine, University of California, Old Davis Rd, Davis, CA 95616
[1] Address correspondence to the Arbovirus Field Station, 4705 Allen Rd, Bakersfield, CA 93314

West Nile virus [WNV] over wintered successfully in southern California during 2003 - 2004, but the mechanism for persistence was not established. Three mechanisms seemed to be possible:

1] Continued transmission. Because *Culex quinquefasciatus* Say do not enter winter diapause, but rather undergo a cold-induced quiescence, low level transmission by infected females may continue in southern California. Mosquito gonotrophic activity and transmission would subside during cool weather and be reinitiated immediately following periods of warm temperature. In agreement RT-PCR positive dead American crows were collected in Los Angeles as early as February, however, positive mosquito pools were not recovered from this area until May 2004, despite the testing of 8,430 *Cx. quinquefasciatus* females in 231 pools.

2] Vertical transmission. Because *Culex* females that undergo reproductive diapause do not take blood meals, infection of these females must occur by vertical transmission from their infected female parent. Vertical transmission by intrathoracally inoculated females has been demonstrated in the laboratory (Dohm et al. 2002; Goddard et al. 2003) as well as in the field by the collection of infected males (Miller et al. 2000), and this was considered to be the mechanism responsible for the infection of over wintering *Cx. pipiens* females collected in NY (Nasci et al. 2001). Although vertical infection has been detected in the field in California during summer by the isolation of WNV from *Cx. quinquefasciatus* males collected as immatures (unpublished), attempts to detect virus in overwintering adults have been unsuccessful in Coachella Valley, Los Angeles, and Kern County.

3] Chronic infections in birds. Although persistent WNV infections in wild birds can be detected at a low rate for >6 weeks after experimental infection (unpublished), we have no data to indicate that these infections ever relapse and produce peripheral viremias suitable to infect blood feeding mosquitoes. In proof of principal re-infection experiments with St. Louis encephalitis virus, House finches produced a rapid antibody response following challenge with homologous virus resulted in sterilizing immunity (Reisen et al. 2003a). Attempts to experimentally compromise the avian immune system to trigger relapses also were not successful (Reisen et al. 2003b).

Susceptible *Cx. tarsalis* seemed capable of WNV maintenance and amplification throughout southern California and exhibited comparable minimum infection rates among geographical areas with different avian communities. In contrast, elevated viremias in infected American crows and Western scrub jays seemed critical in driving WNV into less susceptible *Cx. quinquefasciatus* populations and this may have been critical for the epidemic transmission of WNV in Los Angeles and Bakersfield. These data collectively indicated that there may be parallel or overlapping transmission cycles of WNV in California involving House finches and *Cx. tarsalis* in rural areas such as the Coachella Valley and within Kern County and American crows and *Cx. quinquefasciatus* in suburban and urban areas of Los Angeles and Bakersfield.

Surveillance methods to monitor virus within these cycles likewise seemed to differ in effectiveness. Testing *Cx. tarsalis* collected by dry ice-baited traps and sentinel chickens seemed to work best in rural areas; dead bird reports and testing were less effective because there were few residents to report dead birds as well as lower densities of corvids. In contrast, testing *Cx. pipiens* complex females collected by gravid female traps and dead birds were most effective in urban areas, yielding the earliest and most frequently positive findings. Results from both sentinel chicken and free-ranging wild bird serology were delayed, because of the 2 week period necessary for the birds to produce diagnostic serological titers following infection and because separating antibody due to WNV and SLE infections was problematic.

Amplification of WNV during the spring of 2004 was followed in early summer by rapid virus dispersal throughout the Central Valley and eventually all of California. Amplification was greatest in Kern County, especially in the Bakersfield area and surrounding small towns such as Arvin and Shafter. Rapid dispersal of virus from foci in southern California to the remainder of California may have been associated with infection in northbound migrating birds. One species of warbler was found to be highly susceptible to infection producing elevated viremias as well as mortality.

Depopulation of corvids and acquired immunity by surviving resident birds seemed critical in slowing transmission during late summer, especially in Los Angeles where 39% of 117 birds tested for antibody during December 2004 were *Flavivirus*-positive by an enzyme immunoassay. WNV transmission in Coachella Valley as detected by positive mosquito pools or new seroconversions in sentinel chickens declined by late August and did not increase in response to the resurgence of *Cx. tarsalis* populations associated with the flooding of wetlands managed for waterfowl.

Based on our observations in southern California, it may be possible to predict what may happen in California during 2005. Most likely the epidemic will subside south of the Tehachapi Mountains, because many birds have died or are now immune. In contrast, enzootic transmission most likely will intensify to epidemic levels in Central Valley, driven by American crows, Western scrub jays and Yellow-billed magpies and increased mosquito abundance associated with the wet northern California climate. Peak WNV transmission will occur later in summer in the Central Valley than in Southern California because relatively cooler spring temperatures will delay mosquito abundance increases and virus extrinsic incubation. Most human involvement will remain focal, periurban and associated with communal crow roosts that 'drive' virus into peridomestic *Cx. pipiens* populations.

Collectively, these data indicate that the ongoing WNV epidemic could worsen in California during 2005.

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Evaluation of RAMP West Nile Virus Test in Northern Tulare County

Yolanda Lourenco

Delta Vector Control District, P.O. Box 310, Visalia, CA 93279

ABSTRACT: This paper will reflect test results and data relating to first year utilization of the RAMP West Nile Virus test by the Delta Vector Control District. The RAMP system was used primarily for early detection of West Nile Virus in the wild bird population. Collection methods, preservation, processing and confirmation of results by the California Department of Health Services will be presented. The District provides surveillance and control to the northwest quadrate of Tulare County, encompassing 1851.2 km^2 (712 mi^2), which is dedicated primarily to agriculture. The District serves the cities of Visalia, Dinuba, Exeter, Farmersville, and Woodlake.

The Rapid Antigen-Capture Assay to Detect West Nile Virus in Santa Clara County, CA 2004

Noor Tietze, Daniel Strickman, Michael Stephenson, Victor Romano, William Shipway, Mike Phillips and Paul Robinson

Santa Clara County Vector Control District, 976 Lenzen Avenue, San Jose, CA 95126

ABSTRACT: During 2004 about 240 dead birds were sampled for West Nile virus (WNV) in Santa Clara County. Detection of WNV was attempted from 31 bird species using the VecTest antigen assay, PCR or sometimes both. The VecTest yielded 23 WNV positive birds: 18 American crows and 5 Western scrub jays. There were 184 negatives using the VecTest taken from 21 bird species, which included other corvids (Raven, Yellow billed magpie and Steller's Jay, raptors, House sparrows, etc...). PCR-based results (California Department of Health Services, Vector-Borne Disease Section) yielded 41 WNV positives from 9 bird species and 37 negative birds from 13 bird species. In American crows, the VecTest had an overall accuracy of 79.3%; sensitivity of 66.6% and specificity of 93.3% based on a comparison to PCR results. The Western scrub jay had a VecTest accuracy, sensitivity and specificity of 57, 50 and 100%, respectively. The Positive Predictive Value for the VecTest on crows was 88.9%, while that of Western scrub jays was 100%. The Negative Predictive Values for crows and scrub jays were 78 and 37.5% respectively. These data strongly suggest restricting future WNV VecTest sampling to the American crow and Western scrub jay.

Using the RAMP Test to Detect West Nile Virus in Dead Birds and Mosquitoes in the San Gabriel Valley, California

Angela Brisco

San Gabriel Valley MVCD, 1145 N. Azusa Canyon Road, West Covina, CA 91790

ABSTRACT: In 2004, southern California residents faced an unprecedented epidemic of infections with West Nile virus. Staff from the San Gabriel Valley Mosquito and Vector Control District used Response Biomedical Corporation's RAMP test to assess whether dead birds and samples of adult mosquitoes were infected with the virus. The rapid turnaround helped direct surveillance and control activities when a disease problem severely taxed resources throughout the State.

Preliminary Evaluation of Immunochromatographic Tests for West Nile Virus

Min-Lee Cheng, Ph.D. and Amanda J. Colombo

West Valley Mosquito & Vector Control District,13355 Elliot Avenue, Chino, CA 91710

Two types of commercially available lateral flow immunochromatographic tests have been used to screen mosquito pools and dead crows for the presence of West Nile virus. The results of preliminary evaluation of these two tests and their potential usefulness in disease surveillance will be the focus of discussion.

West Nile Virus Testing in San Mateo County

Angela Rory and Chindi A. Peavey

San Mateo County Mosquito Abatement District, 1351 Rollins Rd. Burlingame, CA 94546

INTRODUCTION

West Nile virus (WNV) is a mosquito-borne pathogen that can cause serious illness in humans and horses. This virus was first detected in North America in 1999 in New York City. It spread across the United States over the next 4 years, arriving in southern California in July, 2003. The San Mateo County Mosquito Abatement District (SMCMAD) began surveying for the virus in wild birds and mosquitoes in 2002. The District participates in a statewide surveillance program. Wild bird carcasses are submitted to the University of California, Davis, Center for Vectorborne Diseases (UCD, CVEC) for testing by PCR TaqMan assay and virus isolation. Results from these tests may take 2 weeks to a month to obtain. In 2003, SMCMAD began conducting in-house testing for WNV in dead birds and mosquito pools. Two rapid immunochromatographic assays that detect WNV antigen are available: VecTest® (Medical Analysis Systems, Inc. Camarillo, CA) and RAMP® (Rapid Analyte Measurement Platform, Response Biomedical Corp.; Burnaby, British Columbia, Canada). These tests give results within 15 minutes to 2 hours, enabling an immediate operational response. The VecTest was purchased in July of 2003. In 2004, SMCMAD purchased the RAMP test, reported to have greater sensitivity and specificity (Fong, et al. 0000).

West Nile virus was detected for the first time in San Mateo County in August of 2004. This paper discusses the district's experience with in-house testing for WNV. A description of the virus distribution in bird species, seasonality, and geography within the county is also reviewed.

MATERIAL AND METHODS

Submissions to CVEC

The SMCMAD received 135 calls from local residents reporting dead birds during 2003 and 2004. Sixty-four of these birds met submission criteria set forth by the California Health Services Department (CDHS) and were sent to California Animal Health and Food Safety (CAHFS). The first positive bird carcass in the county was collected on July 28, 2004. Virus was detected in the crow, by PCR. A total of 14 birds and 3 squirrels had virus detectable by PCR in 2004.

In 2004, 31 mosquito pools were submitted to CVEC for testing. Mosquito species tested included *Culex pipiens* L., *Cx. tarsalis* Coquillett, *Cx. erythrothorax* Dyar, and *Ochlerotatus dorsalis* Meigen. None of the mosquitoes submitted to date have tested positive for WNV.

In-House Testing

The SMCMAD began conducting in-house testing in July of 2003, using the VecTest. This test is relatively inexpensive, gives results within 15 minutes, and does not require any specialized equipment. It is used by agencies throughout the state and has been recommended by both the CDC and CDHS. This test was used on 24 wild birds during 2003 and 2004. Carcasses of 17 birds were submitted to CAHFS for confirmatory testing. Three of the 17 were positive by PCR (Table 1). The VecTest failed to detect virus in any of the birds tested (Table 2).

Table 1. Number of birds tested by PCR that were also tested by VecTest or RAMP.

	# of Birds Tested	# Also Tested by PCR	# Positive by PCR
VecTest	24	17	3
RAMP	29	22	9

Table 2. Results of Vectest and RAMP on birds testing positive by PCR

	# Birds Tested	# Positive
VecTest	24	0
RAMP	29	0

The VecTest was also used on 76 mosquito pools. Mosquito species tested included *Cx. pipiens, Cx. tarsalis*, and *Cx. erythrothorax*. All pools tested negative for the virus by this test.

In 2004, the district purchased the RAMP West Nile Virus Test. The RAMP test requires a $3000 investment in equipment. However, it is reported to be ten times more sensitive than the VecTest (Fong, et al. 0000). The test is read by a machine that measures the amount of fluorescence emitted by the sample, and is displayed in quantitative units. Results for the VecTest are determined visually, by the appearance of a line on a dipstick.

Twenty-nine birds were tested by the District using the RAMP system in 2004. Carcasses from 22 of these were submitted to CVEC for confirmatory testing, 9 tested positive for WNV (Table 1). The RAMP test failed to detect virus in any of the birds tested (Table 2).

The RAMP system was used on 71 mosquito pools belonging to 3 species: *Cx. pipiens, Cx. tarsalis,* and *Cx. erythrothorax*. All mosquitoes were negative for WNV by this test.

Distribution of WNV in Wild Birds

Species Infected: The species distribution of birds positive for WVN is shown in Figure 1. Scrub jays and Stellar's jays were the most commonly infected species, accounting for 50% of the positive birds. Virus was detected in 7 of the 15 (47%) jays tested (Fig. 1). Crows made up 55% of the birds tested by PCR. However, only 3 (9%) of the 35 tested had detectable virus. Nine squirrels were submitted for PCR testing, 3 (33%) tested positive. Two Doves, one Owl and one Thrush also tested positive for WNV by PCR.

Seasonality: In 2004, the district began receiving calls regarding dead birds in February (Fig. 2). The peak number of calls was received in August. This was probably influenced by press coverage following the first positive bird on July 28th. The number of birds testing positive for virus also peaked in August (Fig. 2). This correlates with the peak in density of *Cx. pipiens* adults collected in CO_2 - baited traps. This species is the most prevalent mosquito in San Mateo County in summer months and is expected to be the most significant vector of WNV in this area.

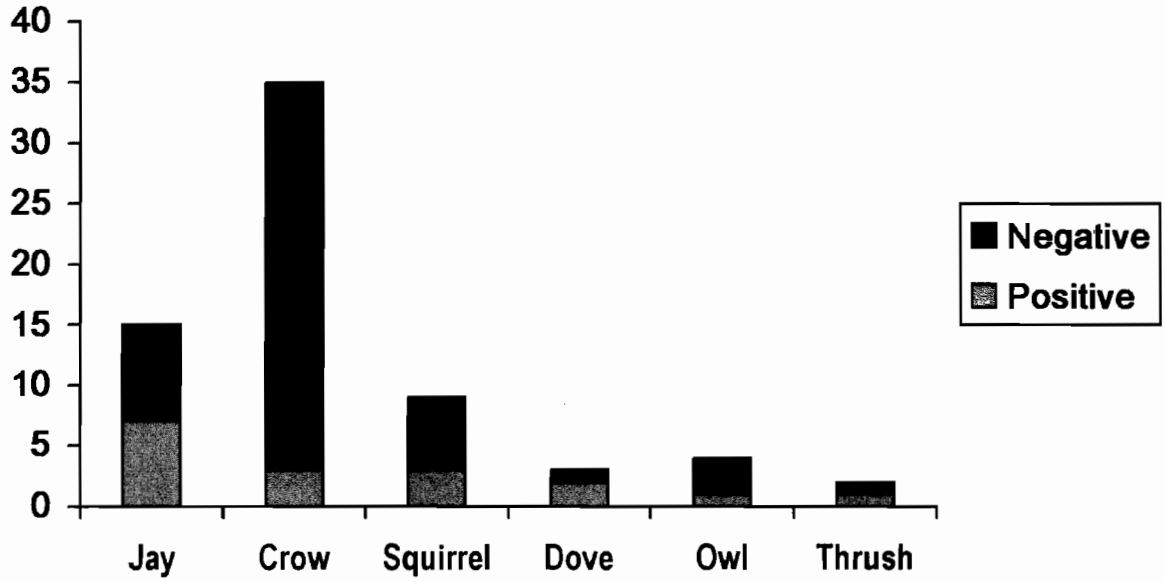

Figure 1. Species distribution of animals tested for West Nile virus by PCR.

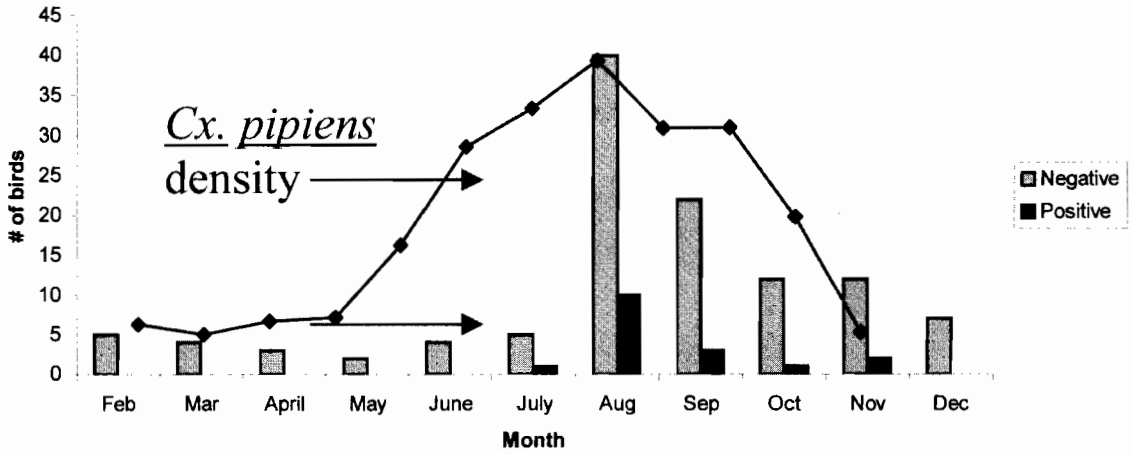

Figure 2. Temporal distribution of birds submitted for testing by residents of San Mateo County and host-seeking *Culex pipiens* females as measured in carbon dioxide traps in 2004.

Geographic distribution: The majority of positive birds were found in the most densely populated areas of the county (Figure 3). There were limited numbers of calls reporting dead birds in the less populated areas. However, 1 positive bird was found along the coast; and the virus is assumed to be present throughout the county.

CONCLUSIONS

Neither RAMP nor VecTest detected the presence of virus in wild birds or mosquito pools in San Mateo County. Both tests are known to be less sensitive than PCR for detection of WNV; however, the number of specimens was small. Furthermore, much of the testing was done early in the season before WNV had been detected in the county by PCR.

In San Mateo County, jays appear to be more important than crows or ravens as indicators of the presence of WNV. Squirrels may also be important sentinels, although the number tested to date is small.

Based on information received from other districts, the district has decided to continue to use the RAMP system for surveillance. This test gives rapid results and is less subjective than the VecTest system. RAMP testing will be limited to crows, ravens and mosquito pools.

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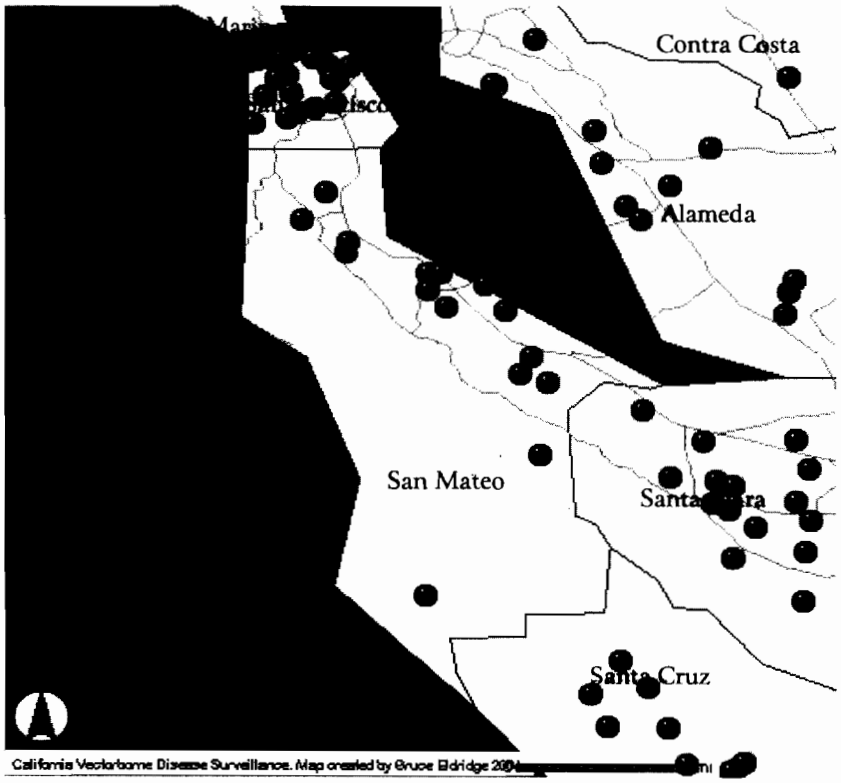

Figure 3. Geographic distribution of dead birds testing positive for West Nile virus in San Mateo County in 2004.

To VecTest™ or Not and Can We RAMP© It Up?

Jodi Holeman

Consolidated Mosquito Abatement District, 2425 Floral Ave.,P.O. Box 278, Selma, CA 93662

In 2004, the Consolidated Mosquito Abatement District (CMAD) intensified its surveillance program by incorporating the use of both the VecTest™ and the RAMP© system as tools to detect West Nile virus (WNV) within the district. With WNV rapidly spreading through southern California and expected to reach the Central Valley by mid season 2004, the surveillance program was expanded to include in-house testing of both mosquitoes and dead birds.

Dead birds were collected from within the district, as well as from outlying areas in Fresno County that do not have mosquito or vector control programs. The district encompasses 1058 sq miles in the Central Valley, primarily in Fresno County. Any birds called into the Department of Health Services (DHS) WNV dead bird hotline within this area were picked up by CMAD.

Following the 2004 season, the district evaluated the use of both the VecTest™ as well as the RAMP© system to determine whether either test will be used in the 2005 season.

VECTEST™

At the time of collection the bird's condition was evaluated for in-house testing. If the bird appeared to have died within the last 48 hours, not desiccated, covered in maggots or ants, and did not have blood in its oral cavity it was suitable for in-house testing. Birds were tested using the VecTest™ from mid July to the end of the season in mid October.

The VecTest™ is a dipstick immunochromatographic assay that uses monoclonal antibodies bound to colloidal gold (Burkhalter et al. 2003) to indicate the presence of WNV antigens. A homogonenized sample of up to 50 female mosquitoes or an oral swab from a dead bird is mixed with grinding solution. From this solution a 250ìl sample is taken to be used with the dipstick. The monoclonal antibodies with attached colloidal gold bind to any WNV antigen present in the sample. This new complex migrates through the test strip until it is blocked by WNV proteins. At this point if the sample contains WNV a reddish-purple line is visible (Burkhalter et al. 2003).

The VecTest™ is simple to use and the results are ready within 15 minutes. The VecTest™ can be performed in less than ten steps from beginning to end. There is a 30 minute waiting period while the swab sample is incubated in grinding solution and a 15 minute waiting period to read the results. The test is simple enough to be conducted by all personnel handling dead bird pickups. Instructions are followed and proper lab techniques are applied to minimize error and contamination.

Results are determined by the presence or absence of a purple line. Much like a pregnancy test, a single line indicates a sample is WNV negative and two lines indicate it is WNV positive. However, interpretation of results is subjective. The purple control line was always clearly visible; however, the second purple line in the test zone wasn't always as clearly defined. Test zones displaying a faint purple haze often lead to incorrect interpretation of the results.

The VecTest™ presented another potential problem because it is recommended by DHS only for testing crows and mosquitoes. In 2004 the district had 85 dead birds positive for WNV. Of those birds, 46 were Western scrub jays (*Aphelocoma californica*) and 28 were American crows (*Corvus brachyrhynchos*). Approximately 54% of all WNV positive birds in the district were Western scrub jays, while only 33% were American crows. Dead bird results (RT-PCR) provided to the district by the University of California, Center for Vectorborne Diseases (CVEC) were then compared with in-house VecTest™ results.

Of the 67 birds that were tested using the VecTest™, 51 were confirmed RT-PCR positives. Of those 51 positives the VecTest™ was able to detect 18. The primary bird species tested were American crows and western scrub jays (Fig 1).

Western Scrub Jay

		RT-PCR True	RT-PCR False
VecTest™	**True**	15	1
	False	13	3

Sensitivity = 53%
Specificity = 75%

American Crow

		RT-PCR True	RT-PCR False
VecTest™	**True**	2	0
	False	12	4

Sensitivity = 14%
Specificity = 100%

Figure 1. Sensitivity and specificity of VectTest vs. Rt-PCR results in western scrub jays and American crows

Other birds tested included a Black-headed grosbeak (*Pheucticus melanocephalus*), Steller's jay (*Cyanocitta stelleri*), Loggerhead shrike (*Lanius ludovicianus*), Northern mockingbird (*Mimus polyglottos*), Cockatiel, and Mallard duck (*Anas platyrhynchos).* The VecTest™ was able to detect the virus in the mallard duck.

The VecTest™ provided a quick and easy way to detect WNV in dead birds. This enabled CMAD staff to potentially test every bird that was called into DHS within the district boundaries, without consuming the entire day.

RAMP©

The RAMP© system offered similar advantages and disadvantages to the VecTest™. The RAMP© was also simple to use, required minimal training and results were available within 90 minutes. Although the incubation time for solution absorption and drying was longer, there was better correlation with the RT-PCR results from CVEC.

The RAMP© system eliminated the problem of subjectivity by utilizing the RAMP reader. The RAMP© reader measures fluorescence in RAMP© immunoassay applications (Burkhalter et al. 2003). The RAMP© reader is always consistent in determining ratio values between the test zone and the control zone. The results are given in units and preset values have been established to determine the range of WNV positives and negatives.

Although the RAMP© system is recommended by the manufacturer, Response Biomedical, for mosquitoes and crows, the results indicated an acceptable level of sensitivity for other corvids such as the western scrub jay. The RAMP© system yielded better correlation with RT-PCR results than did the VecTest™ (Fig 2), however significantly fewer tests were run.

During the 2004 season the district used the RAMP© system to test 16 mosquito pools. Two of the 16 pools were positive for WNV. A second sample from those pools was never submitted for RT-PCR confirmation. A separate pool was submitted to DHS from one of the areas that RAMP© tested positive, but returned negative for WNV.

SUMMARY

It is clear that both the VecTest™ and the RAMP© system are valuable tools in the fight against WNV. To draw any definite conclusions on the use of either test within the district would be premature given the limited number of tests conducted by CMAD. However, some initial value assessments can be made with results available. Both tests have shown the ability to rapidly detect WNV to some degree. This small window of opportunity can result in immediate mosquito abatement response to areas where the virus is present and help prevent transmission of the disease to humans. Furthermore, a statewide effort to collect and compile results on the use of both tests could prove even more valuable in the long-term efforts to combat West Nile Virus.

REFERENCES CITED

Burkhalter, K.L., R. Lindsay, R. Anderson, A. Dibernardo, H. White, M. Drebot, W. Fong, and R. Nasci. 2003. Evaluation of Commercial Assays for Detecting West Nile Virus Antigen. Response Biomedical Technical Papers. http://www.responsebio.com.

Western Scrub Jay

		RT-PCR	
		True	**False**
RAMP©	**True**	4	0
	False	3	1

Sensitivity = 57%
Specificity =100%

American Crow

		RT-PCR	
		True	**False**
RAMP ©	**True**	0	0
	False	1*	2

Sensitivity = 0%
Specificity = 100%
*14.0 units
**The RAMP system was also able to detect the virus in a loggerhead shrike.

Figure 2. Sensitivity and specificity of RAMP vs. Rt-PCR results in western scrub jays and American crows

RAMP and VecTest: A Comparative Study

Piper Kimball

Marin/Sonoma Mosquito and Vector Control District, 595 Helman Lane, Cotati, CA 94931

ABSTRACT: The West Nile virus (WNV) epidemic of 2004 became the main concern for all organized mosquito control districts in California and reliable surveillance tools were of the utmost importance. While many districts were provided with the VecTest (Medical Analysis Systems) by the California Department of Health Services, other districts decided to try out another commercially available antigen assay, RAMP (Response Biomedical). Both assays were developed to detect WNV in mosquito pools and corvids. This presentation focuses on comparing the sensitivity and specificity of both assays from data compiled by nine mosquito control districts throughout California.

Diagnostic Assays for Detecting West Nile Virus in Oral Swabs from Dead Birds: Evaluation of RT-PCR and Commercial Immunochromatic Assays

Kerry Padgett[1], Barbara Cahoon-Young[2], Ryan Carney[1], Leslie Woods[3], Deryck Read[4], Stan Husted[1], and Vicki Kramer[5]

[1] *California Department of Health Services, Vector-Borne Disease Section, 850 Marina Bay Parkway, Richmond, CA 94804*
[2] *Center for Vectorborne Diseases,, University of California, Old Davis Road, Davis, CA 95616*
[3] *CAHFS Central Lab, University of California, West Health Sciences Drive, Davis, CA 95616*
[4] *CAHFS San Bernardino Lab, 105 West Central Avenue, San Bernardino, CA 92408*
[5] *Department of Health Services, Vector-Borne Disease Section, 1616 Capitol Avenue, MS 7307, P.O. Box 997413, Sacramento, CA 95899*

ABSTRACT: Oral swabs were evaluated as a diagnostic sample for detecting West Nile virus (WNV) in dead birds in California. To assess whether oral swabs in viral transport media (VTM) could replace or supplement kidney tissue for WNV testing, oral swabs and kidney tissue from American Crows were tested in parallel by RT-PCR at the UC Davis, Center for Vectorborne Diseases. Oral Swab samples were submitted by either a state veterinary pathology laboratory (California Animal Health and Food Safety Laboratory – CAHFS) or by local vector control agencies. RT-PCR of oral swabs submitted by CAHFS and by local vector control agencies yielded similar results as kidney tissue. Local vector control agencies and health departments also tested avian oral swabs with two commercial antigen-based immunochromatic assays (VecTest and RAMP) and results were compared to RT-PCR of kidney tissue to assess sensitivity and specificity. VecTest and RAMP assays were most sensitive and specific when used for detecting WNV in oral swabs from American Crows. False negative results were common for other bird species. Both VecTest and RAMP assays were highly specific for WNV with few false positive results. Testing dead bird oral swabs by either commercial immunochromatic assays or by RT-PCR may increase efficiency, allow for faster reporting of PCR results, and can save valuable resources.

Highlights of 2004 West Nile Virus Surveillance Activities in Southern California

Renjie Hu

California Department of Health Services, Vector-Borne Disease Section, 2151 Convention Center Way, Suite 218B, Ontario, CA 91764

West Nile virus (WNV) is a mosquito-transmitted pathogen that may potentially cause severe human illness, and in some cases even death. In nature, the virus is maintained in mosquito-bird transmission cycle. Because certain bird-feeding mosquito species may also feed on human, horse, and most of other mammals, they can inadvertently transmit the virus to these hosts upon blood feeding, resulting in their illness. In 2004, statewide surveillance efforts indicated that WNV activities began and most intensive in southern California. The virus was first detected in February from a dead American crow collected in Los Angeles County. In the following months, WNV was also detected from dead birds collected in nearby counties. Both WNV positive mosquito pool and chicken serum were first detected in May from Riverside County. During the same month, the first 5 human cases were diagnosed from San Bernardino County. Of 830 human cases identified in the state during 2004, more than 85% were from the southern region.

Due to the early detection of WNV activities in southern California, local health departments and vector control agencies immediately began the execution of their plans in dealing with this significant public health threat. Both WNV surveillance and control efforts were enhanced by these agencies. Based on surveillance data, areas with greater exposure risks of WNV were identified. Prompted by potentially severe consequences of WNV infection, several vector control agencies decided to conduct mosquito adulticiding in some of the areas with increased exposure risks to WNV and frequent public use to lower the mosquito populations. Results from these applications are provided elsewhere in this publication by those agencies involved and should be very helpful to guide other agencies faced with similar decisions in the coming years. Some counties in southern California also conducted monthly county-wide multi-agency WNV Task Force meetings. These provided great opportunities for information sharing and for further planning in control and prevention of WNV transmission. Members attending these meetings included individuals from county department of health/environmental health, public health veterinarian, animal control, office of emergency services, legal council, law enforcement, agriculture commissioner's office, vector control program or districts and local universities. Staff from California Department of Health Services, Vector-Borne Disease Section and State Office of Emergency Services also participated in these meetings and provided coordination and assistance.

The following is the list of other speakers, their affiliations, and the titles of their presentations at the symposium entitled "West Nile Virus Outbreak and Mosquito Control Strategies in Southern California":

Jack Hazelrigg, Ph.D., Greater Los Angeles County Vector Control District.

Greater Los Angeles County Vector Control District Response Strategies to West Nile Virus Occurrence in 2004.

Kenn Fujioka, Ph.D., San Gabriel Valley Mosquito and Vector Control District

West Nile Virus in the San Gabriel Valley, Los Angeles County, CA in 2004.

Robert Saviskas, Los Angeles County West Vector Control District

West Nile Virus Experience at the Los Angeles County West Vector Control District in 2004.

Karen Mellor, Antelope Valley Mosquito and Vector Control District

West Nile Virus Activity in Antelope Valley, Los Angeles County, CA in 2004.

Nelson Kerr, City of Long Beach Vector Control Program

West Nile Virus in Southern California: A Local Health Department's response.

Lawrence Shaw, Orange County Vector Control District

Mosquito Control at Orange County Vector Control District during a WNV Crisis.

Joanna Wisniewska-Rosales, Ph.D., Northwest Mosquito and Vector Control District

West Nile Virus Surveillance and Mosquito Control at Northwest Mosquito and Vector Control District.

Branka Lothrop, Ph.D., Coachella Valley Mosquito and Vector Control District

Control Measures and West Nile Virus in the Coachella Valley, California in 2004.

J. Wakoli Wekesa, Ph.D., San Bernardino County Vector Control Program

West Nile Virus and Mosquito Control in San Bernardino, California in 2004.

West Nile Virus in the San Gabriel Valley, Los Angeles County, California 2004

Kenn K. Fujioka[1], Kelly M. Middleton, M. Angela Brisco, Warthuro Campugan, Brian Reisinger, Melvin C. Cook, and Steve A. West

San Gabriel Valley Mosquito and Vector Control District, 1145 N. Azusa Canyon Road, W. Covina, CA 91790

ABSTRACT: In 2004, a significant epidemic of infections with West Nile virus occurred in southern California. The jurisdiction of the San Gabriel Valley Mosquito and Vector Control District (the District) experienced the highest incidence rate (7.9 per 100,000 population overall) of the five vector control districts in Los Angeles County despite mosquito trap counts that did not exceed 30/ trap night. The District expended significant resources beyond its normal operating budget to prevent additional human cases in its jurisdiction and participated in a regional education campaign to preserve public health.

INTRODUCTION

In 2004, California took its turn as the epicenter of West Nile virus (WNV) activity in the USA; 831 human cases and 26 deaths were reported (California and County of Los Angeles Department of Health Services, 2005). The first infected bird and human case reported from Los Angeles County occurred in the jurisdiction of the San Gabriel Valley Mosquito and Vector Control District (the District).

The District is approximately 805 km^2 with a population of 1.36 million. It comprises 23 cities and unincorporated county areas and is located in east Los Angeles County along the foothills of the San Gabriel Mountains. In 2003, 34 birds infected with WNV were found within the jurisdiction of the District, but no infected mosquitoes were found and no human infections were reported. The numbers increased substantially in 2004. The incidence of infection in the District (7.9 per 100,000 pop.) was considerably higher than those for the county and state (3.1 and 2.3 per 100,000 pop., respectively). Here we describe the epidemic of infections with WNV in the District.

THE EPIDEMIC

Dead Birds: Dead birds were the first indication in 2004 that WNV was being transmitted in the District; they began dying several weeks before the rest of the county (Fig. 1). The first WNV-positive bird was collected on Feb 24 (week 9) but test results were not available until April 1 (week 14). The portion of the 500 cases that were reported to the District during week 22 overwhelmed our telephone system. Table 1 illustrates the magnitude of infection among wild birds during this time; testing samples was largely discontinued because of the high percentage of WNV-positives that was observed.

Sentinel Chickens: As part of the State's encephalitis surveillance program, the District maintains 11 flocks of six sentinel chickens placed throughout its jurisdiction. In 2004, all of the chickens developed antibodies to WNV. The first seroconversion was detected on June 8 (week 24) by an in-house EIA. Although it appears that infected mosquitoes, chickens, and humans all became

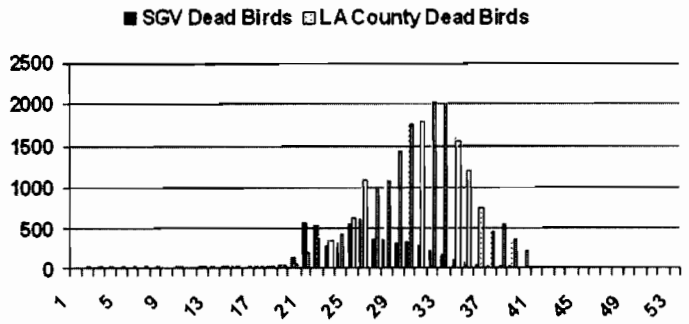

Figure. 1. Dead birds by week of reporting, San Gabriel Valley Mosquito and Vector Control District versus Los Angeles County, California, 1 Jan-30 Sep 2004.

Table 1. Summary of birds tested by the State of California Department of Health Services for infections with West Nile virus in the San Gabriel Valley Mosquito and Vector Control District, Los Angeles County California, 2-29 May 2004.

Week	Date	Birds Reported	Birds Tested	WNV+ Birds
19	2-8 May	47	14	9
20	9-15 May	140	33	29
21	16-22 May	549	28	27
22	23-29 May	517	16	15

detectable at once (Fig. 2), only the chickens were identified timely; the mosquitoes and human were not confirmed until the end of June (week 27).

Human Infections: The first case of human illness in Los Angeles County was reported from the District. The date of onset was 9 June, but the case was not confirmed until late in the month (Fig. 2). The overall incidence in the District was 7.9 per 100,000 pop. which was considerably higher than the rates for the County (3.1 per 100,000 pop.) and the State (2.3 per 100,000 pop.). Incidence rates among individual cities varied from 0 to 27.7 per 100,000 pop.; the case fatality rate for the District (2.80 percent) was lower than the rate for the rest of the County (4.98 per cent) and the rest of the State (3.32 percent). Cases did not appear clustered spatially or temporally.

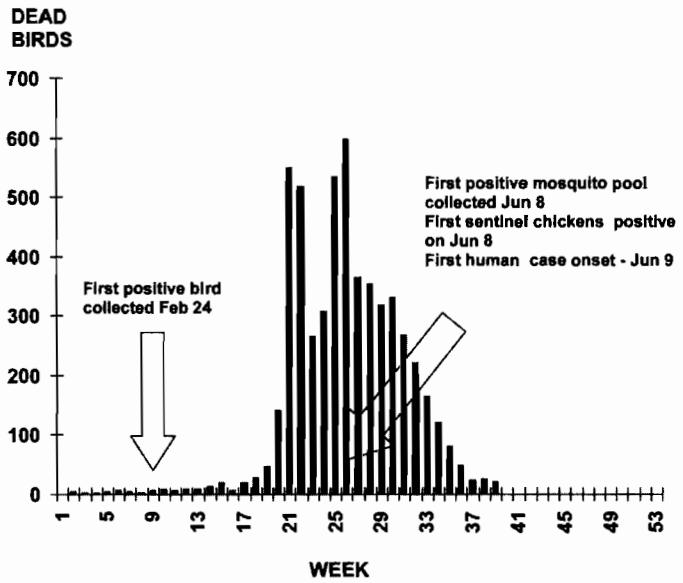

Figure 2. Dead birds (week of reporting) and events related to West Nile virus in the San Gabriel Valley Mosquito and Vector Control District, Los Angeles County, California, 1 Jan to 30 Sep 2004.

Because tests used to confirm human infections and the vagaries associated with reporting cases, a three week lag between disease onset and disease identification was typical. In many cases, by the time we were able conduct surveillance in the vicinity of a reported case who had given the health department permission to be contacted, no mosquitoes were present. As more cases occurred, it became impossible to visit each one.

Timely follow-up of human cases was also hampered by our having to learn about and accommodate the Health Insurance Portability and Accountability Act (HIPAA). The interpretation of the law designed to protect confidentiality affected the manner and type of data we received and which data we were able to distribute. In retrospect, we spent too much time attempting to reconcile human case data we received from various agencies; during this epidemic wild bird mortality gave us the earliest indication of the problem.

Mosquitoes: Prior to 2004, the District had never collected within its jurisdiction a pool of mosquitoes that was positive for any mosquito-borne virus. In June-July 2004, mosquitoes infected with WNV were collected from at least half of the 24 cities in our jurisdiction, but trap counts did not exceed 30 per trap. Minimum infection rates during these months were particularly high despite a low number of mosquitoes (*Culex quinquefasciatus*Say - 20.4, *Culex tarsalis* Coquillett - 41.7). Both carbon dioxide and gravid traps used were equally effective at collecting WNV-positive mosquitoes.

Monitoring Temperature: The last epidemic of St. Louis encephalitis (SLE) occurred in southern California in 1984. Because data that are collected from other sites vary in reliability, the records from the Los Angeles Civic Center are regularly used to make comparisons between 1984 and a given year. We have observed that the closer a temperature curve for a given year approximates the one for 1984, the more likely it is that we will have problems with SLE (Fig. 3). March through May 2004 closely approximated 1984, and may have helped create conditions favorable for the proliferation of WNV.

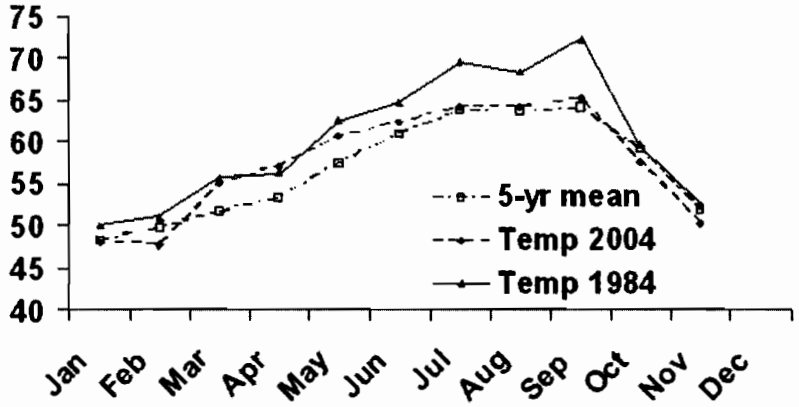

Figure 3. Mean night temperature by month, Los Angeles Civic Center 2004, 1984, and 5-year-mean.

EFFECTS ON DISTRICT OPERATIONS

Equipment and Staff: West Nile virus certainly impacted the District's operations. As yet another example, the District's web site received 45,489 hits in August 2004 followed by 29,719 in September and 21,817 in October from throughout the world. Two full-time staff and twice the normal number of summer help were hired, a new telephone system was installed, and radio time and newspaper space were purchased. The expense of these modifications added 10.5 percent to our operating budget and was funded by reserves.

Public Education: The impact of WNV on the public and on our program was significant. This District began a WNV outreach program in 2001 in anticipation that WNV would eventually impact Southern California. Our ongoing program revolves around constant interactions with city and community leaders, the creation and distribution of literature, attendance at local fairs and events, classroom presentations, speaking engagements, agency staff safety training, and work with local and regional media.

In 2004, we expanded our outreach activities and participated with several other Districts and Health Departments in the southern California area in a regional "WipeOut West Nile" public relations campaign designed to pool our financial resources and reach this broad media market with the same, comprehensive message. As the campaign targeted regional media, all phone calls were directed to the California Department of Health Services' toll-free number to alleviate the confusion that multiple phone numbers would cause. From there, residents were able to report birds, get answers, and find their local mosquito and vector control district.

Figure 4 depicts our in-house outreach efforts compared to more typical years. Note that in 1999 when Africanized honey bees were first detected in the San Gabriel Valley; an unprecedented workload was produced until 2004.

Our underlying goal was to saturate the public with information concerning WNV through all available media, i.e., the radio, print, in retail outlets, and throughout the community.

In 2004, we spoke directly to nearly 66,000 people and provided over 953,000 pieces of literature. We created a 4-page full color newspaper insert that was delivered to every household in the District either through the newspaper delivery or through direct mail, and printed well over 100,000 WNV brochures that were distributed in bulk across the district.

During the height of the season, we received multiple calls most days from both local and regional media for interviews. Coverage overall was fair and relatively accurate; however less experienced reporters sometimes caused confusion by making incorrect statements or generalizations.

A coordinated regional "WipeOut West Nile" outreach campaign was spearheaded by the Greater Los Angeles County Vector Control District. Several vector control agencies in southern

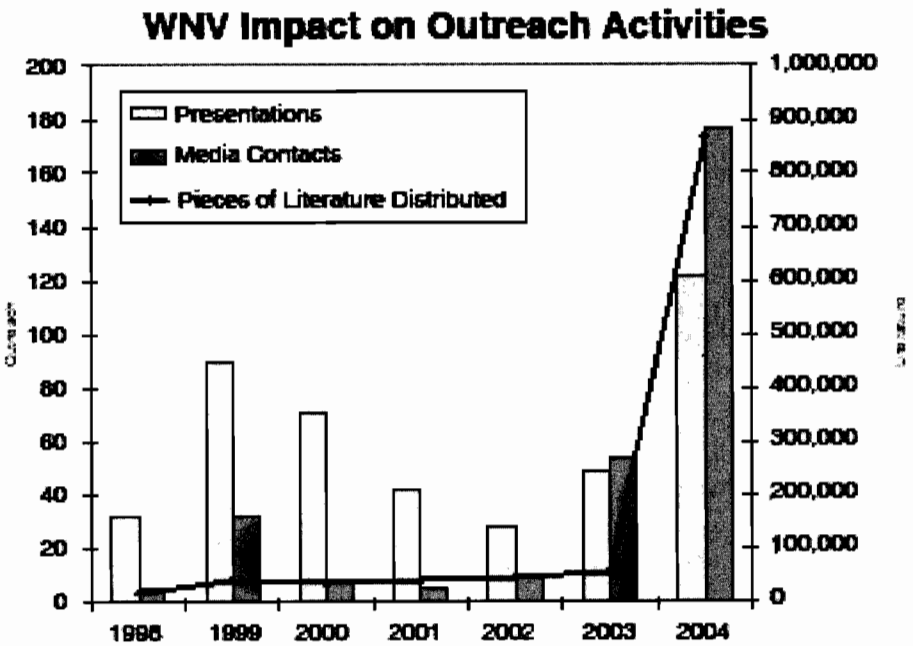

Figure 4. Impact of West Nile virus on outreach activities in the San Gabriel Valley Mosquito and Vector Control District, Los Angeles County, California 2004.

California pooled their resources and hired a firm to produce a campaign that no single agency had the time or resources to accomplish. Figure 5 summarizes the success of the "WipeOut West Nile Campaign." Much of the value of the program was in the time and resources donated by businesses and media outlets. Radio time was additionally purchased to ensure multilingual reach to the broadest of audiences.

Although the total cost of the campaign was $219,500, the firm estimated the total value including sponsorships, organizations, paid media, and public service announcements at $1,455,483. Total estimated reach (impressions) was 23,509,859 people.

In 2004, the Board of Trustees authorized a $55,000 expenditure beyond our current outreach budget (of $20,000) giving us the ability to reach all of our households directly (direct mail), and huge numbers of residents from throughout southern California indirectly (regional media campaign) with the same critical message.

Evaluating the effectiveness of any outreach program is a difficult and expensive exercise which we could not justify in lieu of using those funds for education. One option is to look at incidence rates of WNV infection in people to evaluate which areas were impacted the most. Not withstanding the effects of Mother Nature, two major factors contributed to decreased infection rates for a given environment – the effectiveness of surveillance and control measures, and the public's willingness to follow the recommended precautions, i.e., success of outreach.

Colorado was the state hardest hit in 2003 with an incidence rate of nearly 64.7 cases per 100,000 pop. California, and particularly, southern California led the nation in WNV cases thisyear although our incidence rate was significantly lower at 2.3 per 100,000 pop.

We saw our first indication of WNV transmission in Los Angeles County on Feb. 24 and would have expected to see higher incidence rates as a result of the longer season. The low incidence rate in Los Angeles County is most certainly a testament to the efforts and dedication of mosquito and vector control staff.

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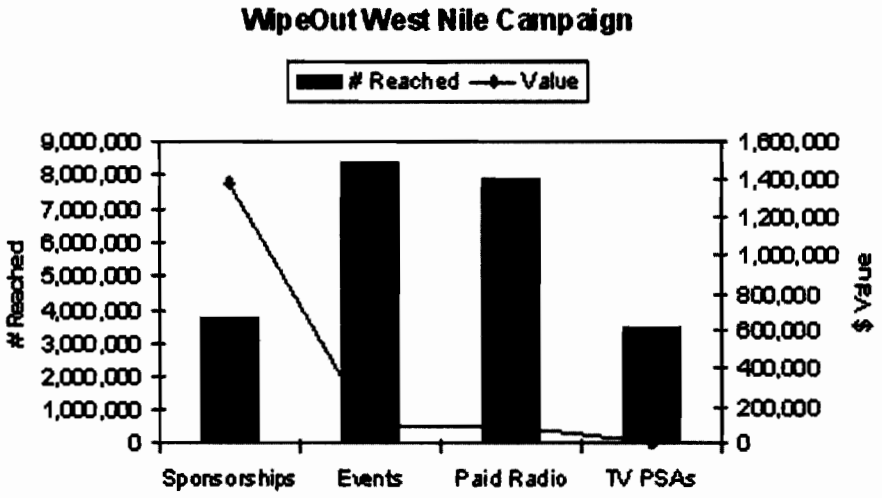

Figure 5. A summary of the "WipeOut West Nile" campaign in southern California, 2004

Mosquito Control at Orange County Vector Control District During a WNV Crisis

Lawrence H. Shaw

Orange County Vector Control District, 13001 Garden Grove Boulevard, Garden Grove, CA 92868

ABSTRACT: During the 2004 season, the Orange County Vector Control District (OCVCD) stepped up efforts in the control of mosquitoes due to the introduction of WNV into Orange County. Twenty years earlier the District had set aside funds for just such an emergency. This funding allowed the District to increase staffing. Seasonal staff started work in April and worked through October. Full time operational staff concentrated efforts on mosquito control, completing other vector service request as time permitted. Coordination between laboratory and operational divisions worked to identify mosquito-breeding sites. The drainage cycle had been reduced to an average of 7 days. Mosquito trap counts recorded a 72% reduction in adult mosquito populations throughout Orange County.

Mosquito and West Nile Virus Surveillance at Northwest Mosquito and Vector Control District in 2002-2004

Joanna Wisniewska-Rosales and Gregory A. Williams

Northwest Mosquito and Vector Control District, 1966 Compton Ave., Corona CA 92881-3318

ABSTRACT: The results of a 3-year long mosquito and West Nile virus (WNV) surveillance program at the Northwest Mosquito and Vector Control District in Corona, California are summarized. Mosquito abundance throughout the District area in 2002, 2003 and 2004 was measured with 12 New Jersey light traps (NJLTs) and 25 CO_2-baited encephalitis virus surveillance traps (EVSTs). Of the three years, mosquitoes were most abundant in 2003 and least numerous in 2004. While the numbers of all *Culex* species were reduced markedly in 2004, *Culex quinquefasciatus* Say dominated EVST catch in 2002 and 2003 and *Culex erythrothorax* Dyar were most commonly collected in EVSTs in 2004. The change in the mean numbers and species composition of mosquitoes in 2004 is attributed to the urbanization of rural areas in the District (dairies being replaced with housing tracks) and to the District's increased mosquito abatement efforts. The antibodies to WNV were first detected in the District area in a live House finch (*Carpodacus mexicanus*) in September, 2003. Then, in late October through early November, 2003, the virus was isolated from 4 dead birds including 3 American crows (*Corvus brachyrhynchos*) and one House finch. The first WNV-positive mosquito pool was collected on May 13, 2004 and the first sentinel chicken seroconversions occurred between May 20 and June 3, 2004. Overall, in 2004, 42 dead birds, 15 live wild birds (tested for the antibody to WNV only), 56 sentinel chickens and 22 mosquito pools tested positive for WNV. Most and the earliest of the positive WNV-surveillance results were obtained from mosquitoes and birds collected near and around the Santa Ana River.

INTRODUCTION

The Northwest Mosquito and Vector Control District (NWMVCD) has been providing mosquito surveillance and control services in the cities of Norco, Corona, Lake Elsinore, parts of the city of Riverside and several adjoining unincorporated communities for over 40 years. The NWMVCD service area encompasses approximately 605 km^2 with nearly 400,000 residents. West Nile virus (WNV) was first detected in California in July 2003, in a pool of *Culex tarsalis* Coquillett collected near El Centro, Imperial County (Reisen et al. 2004). Since then, it has spread through the Imperial and Coachella Valleys to the city of Riverside and within the NWMVCD boundary. Antibodies to WNV were first identified in NWMVCD area in a blood sample collected from a live House finch from one of the wild-bird traps operated by NWMVCD in the Canyon Crest area in the city of Riverside on September 22, 2003. West Nile virus was first isolated in the District from a dead crow on October 21, 2003.

In 2003 and in 2004, increased efforts were exerted by the District to better survey for mosquitoes and WNV as well as to control mosquitoes. The surveillance program included mosquito collections with NJLTs and carbon dioxide-baited encephalitis virus surveillance traps (EVSTs) as well as testing of mosquito pools, sentinels chickens, wild birds and bird carcasses collected throughout the District. Mosquito control efforts were mostly focused on increased mosquito breeding-source inspection and treatment. However some adulticiding was employed in select areas.

MATERIALS AND METHODS

New Jersey-Style Light Traps

The population dynamics of adult mosquitoes in 2002, 2003 and 2004 were monitored with NJLTs (Mulhern, 1942) at 12 fixed locations throughout the District (Fig. 1). The traps were set at 3 urban, 6 suburban and 3 rural areas as described by Mian and Reed (2002) and were checked weekly from April through October and biweekly from January through March and in November and December. The traps were equipped with 25-watt incandescent light bulbs (235 lumens) and placed approximately 2.4 m above ground level. The mosquitoes trapped were counted and sorted according to sex and species with a report submitted to the California Department of Health Services (CDHS) to be included in the state-wide adult mosquito occurrence report.

Encephalitis Virus Surveillance Traps

Host-seeking female mosquitoes were monitored in 2002, 2003 and 2004 using carbon dioxide-baited EVSTs without light or rain shields (Cummings and Meyer 1999). Each trap was operated at an approximate height of 1.25 m and CO_2 was presented in a 3.7-liter Styrofoam®-insulated bucket with 4 to 5 openings at the bottom (diameter = 4 mm). The openings were located 18 cm above trap entry.

A total of 25 standard fixed trap locations were selected to best monitor mosquito-infested areas within the District (Fig. 1). The traps were operated from dusk to dawn June through November in 2002, April through December in 2003 and all year in 2004. In

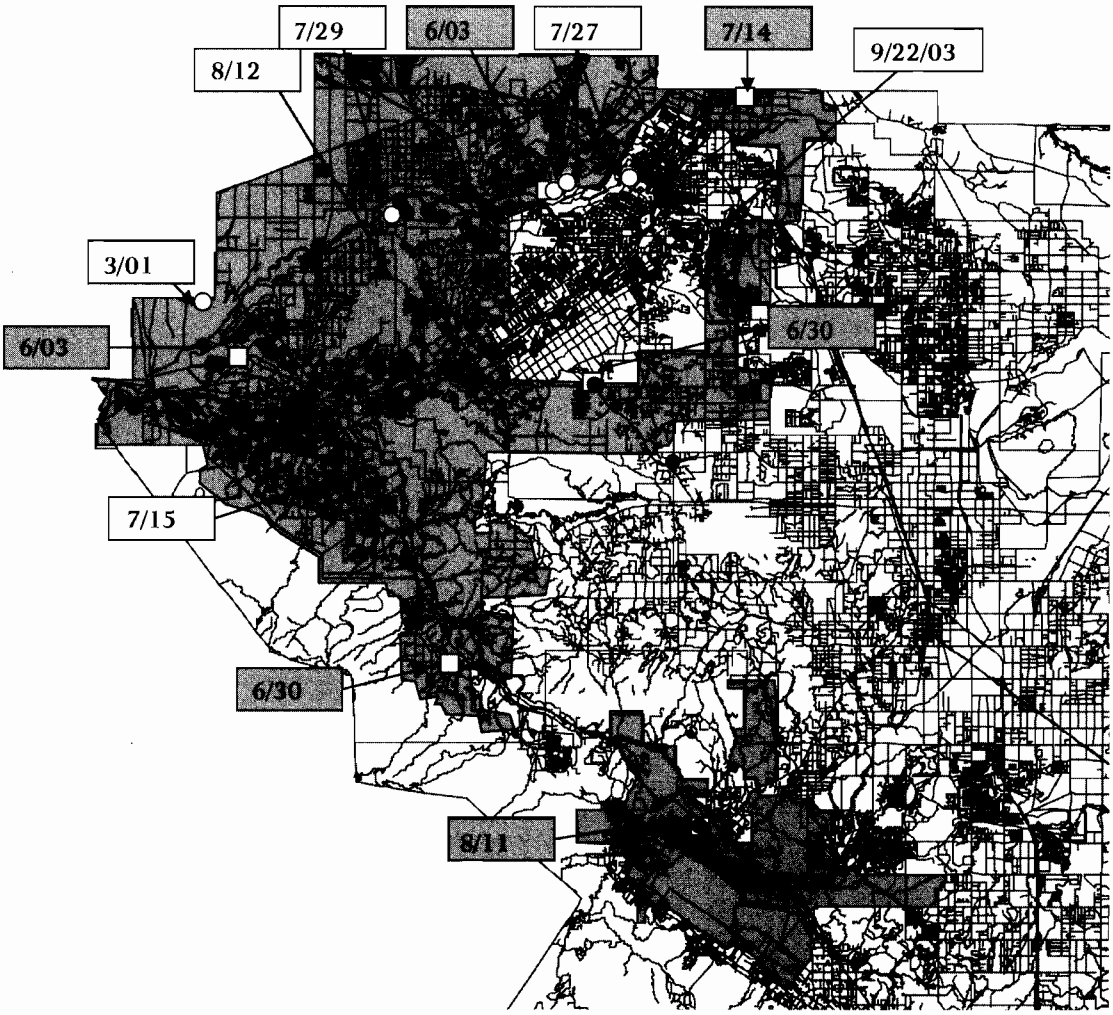

Figure 1. Distribution of sentinel chicken flocks (□) and wild bird traps operated by NWMVCD (▲) and SAWA-OCWD) (● and ❍) within the boundaries of NWMVCD (dark gray area) in 2003 and 2004. Open circles (❍) mark SAWA-OCWD-operated traps where birds positive for WNV antibodies were found. Arrows point to locations where birds positive for WNV antibodies were found. Tags attached to the arrows indicate dates of first seroconversions at those locations. Grey tags correspond to sentinel chicken seroconversions while white tags demarcate wild bird seroconversions. Tags where no year is indicated indicate seroconversions that occurred in 2004.

2002, the traps at different locations were set on alternating weeks so that each location was sampled once every two weeks. In 2003 and 2004, traps at all locations were deployed weekly May through October; due to much lower mosquito abundance, they were deployed biweekly in January, February, November and December. Beginning August 26, 2004, twenty additional locations along the Santa Ana River (Fig. 1) were monitored weekly with EVSTs.

All mosquitoes collected in EVSTs were anesthetized with triethylamine (TEA) and sorted by species and sex. Pools of 12 to 50 mosquitoes were shipped overnight on dry ice to the University of California Davis Center for Vectorborne Diseases (CVEC) for testing. Female *Culex erythrothorax* Dyar, *Culex quinquefasciatus* Say, *Culex stigmatosoma* Dyar and *Cx. tarsalis* Coquillett were included for virus isolation.

Sentinel Chicken Flocks

Six sentinel chicken flocks, comprised of ten white leghorn birds each, were maintained at different locations throughout the District (Fig. 2). Blood samples were collected biweekly from April through October in 2002, April through December in 2003 and January through December in 2004. The samples were placed on filter-paper strips, air dried and submitted to the DHS Viral and Rickettsial Disease Laboratory (VRDL) for testing.

Wild Birds

Beginning in April 2003, four modified Australian Crow traps (McClure, 1984) were built and set up in Corona, Norco, Canyon Crest and Lake Elsinore (Fig. 2). The traps were baited with wild bird seed (Golden State Commodities, P.O. Box 458, Oakdale, CA 95361) and water to attract House finches and House sparrows (*Passer domesticus*). They were checked twice a week. The birds were identified to species and sex, banded, bled and released at the site. We also collected and tested blood samples of Brown-headed cowbirds (*Molothrus ater*) obtained from modified Australian crow traps operated by the Least Bell's Vireo Conservation Project of the Santa Ana Watershed Authority (SAWA) and by the Orange County Water District (OCWD). Bird blood samples (0.1 - 0.2 ml from each bird) were collected from the jugular vein with a 1-ml insulin syringe fitted with a 28 gauge, ½ inch hypodermic needle. Each sample, dissolved in 0.9 ml of 0.75% bovine serum albumin/ PBS (phosphate-buffered saline) diluent, was submitted to the Orange County Vector Control District Laboratory for Saint Louis encephalitis (SLE) and western equine encephalomyelitis (WEE) antibody testing by serum hemagglutination inhibiton as described by Gruwell at al. (2000). The samples were also tested for antibodies specific to the WNV by a blocking ELISA developed by Jozan et al. (2003).

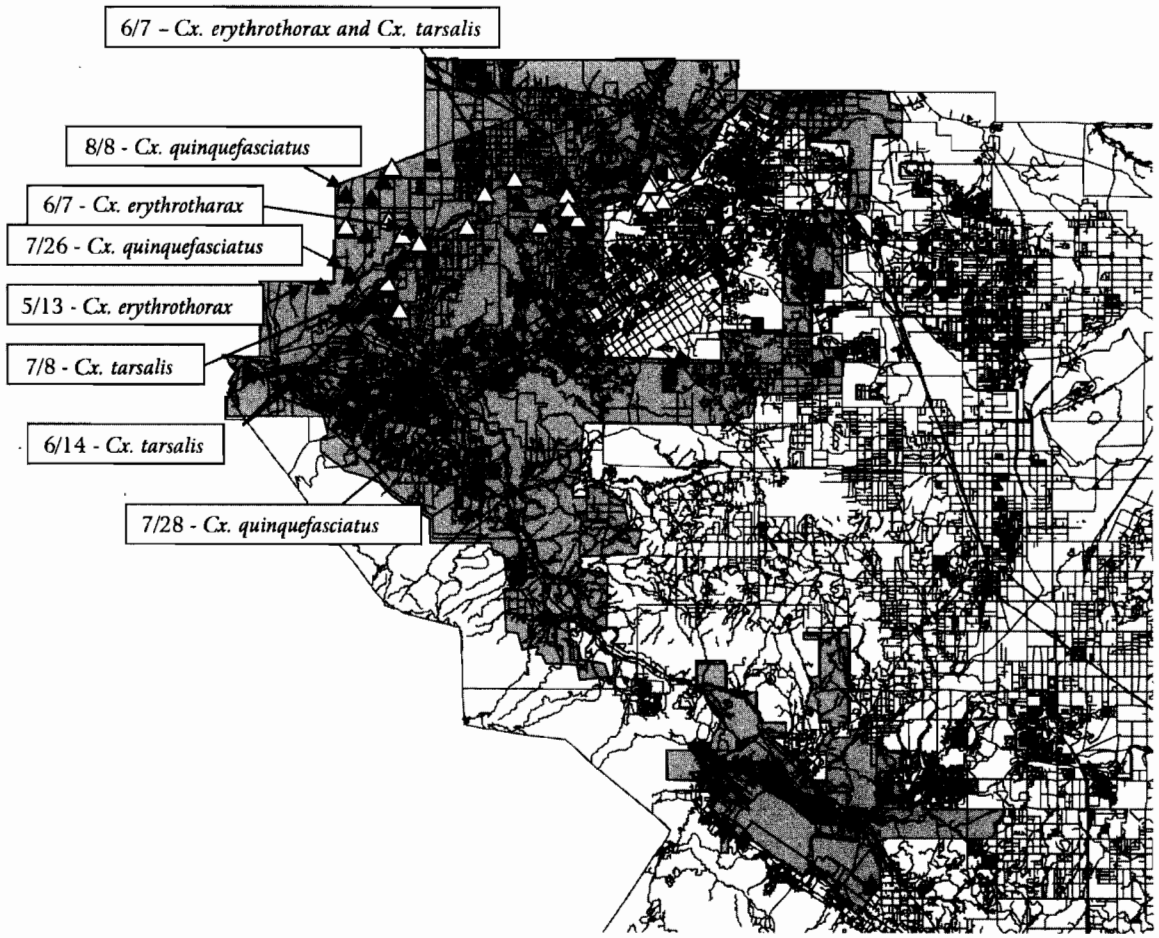

Figure 2. Distribution of New NJLTs (■) and EVSTs (▲) within the boundaries of the NWMVCD (dark gray area) in 2004. Open triangles mark trap locations along the Santa Ana River where trapping begun in late August. Arrows point to locations where mosquito pools positive for the WNV were found. Tags attached to the arrows indicate dates (in 2004) and species of first WNV-positive mosquito pools.

Dead Birds

Through the participation of NWMVCD with the CDHS Dead Bird Surveillance Program, dead birds reported to the District were picked up and submitted to the California Animal Health and Food Safety (CAHFS) Laboratory in San Bernardino for tissue processing and to CVEC for WNV testing.

Data Analysis

For year to year comparisons (Table 1; Figs. 3 and 4), mosquito abundance data were blocked by year and analyzed using repeated measures ANOVA with the collection year as the main effect. Abundance measurements were repeated within each trap location. Student-Newman-Keuls method was utilized for multiple comparisons of means. For monthly comparisons, the data were blocked by year and month and analyzed with 2-way repeated measures ANOVA. The Holm-Sidak test was employed for multiple comparisons of means.

Table 1. Female mosquitoes collected in the carbon dioxide-baited Encephalitis Virus Surveillance Traps (EVST) and New Jersey Light traps (NJLT) at standard locations in 2002, 2003 and 2004. Mosquito numbers callected in the same trap type that share the same letter are not significantly different ((á =0.05).

Mosquito Species	Mean Number of Females per Trap Night							
	EVST				NJLT			
	2002	2003	2004	**All**	2002	2003	2004	**All**
Anopheles franciscanus	0.00	0.00	0.00	**0.00**	0.00a	0.01a	0.00a	**0.04**
Anopheles hermsi	1.52a	1.18a	0.76a	**1.02**	0.02a	0.07a	0.03a	**0.00**
Culex erythrothorax	8.77a	24.48a	10.66a	**15.11**	0.02a	0.13b	0.03a	**0.06**
Culex quinquefasciatus	35.39a	45.28a	3.16b	**22.32**	0.03a	0.30b	0.07a	**0.13**
Culex stigmatosoma	9.39a	4.76ab	0.49b	**3.26**	0.09a	0.14a	0.05a	**0.10**
Culex tarsalis	19.61a	16.87a	8.84b	**13.18**	0.09a	0.24b	0.13ab	**0.15**
Culiseta incidens	0.05a	0.22a	0.04a	**0.10**	0.04a	0.09b	0.04a	**0.05**
Culiseta inornata	0.08a	0.31a	0.06a	**0.15**	0.01a	0.03b	0.00a	**0.01**
Culiseta particeps	0.43a	0.25a	007a	**0.19**	0.01a	0.04a	0.01a	**0.02**
Ochlerotatus washinoi	0.00a	0.02a	0.03a	**0.02**	0.00a	0.01a	0.00a	**0.00**
Total	**75.25a**	**93.37a**	**24.12b**	**55.35**	**0.31a**	**1.04b**	**0.37a**	**0.56**

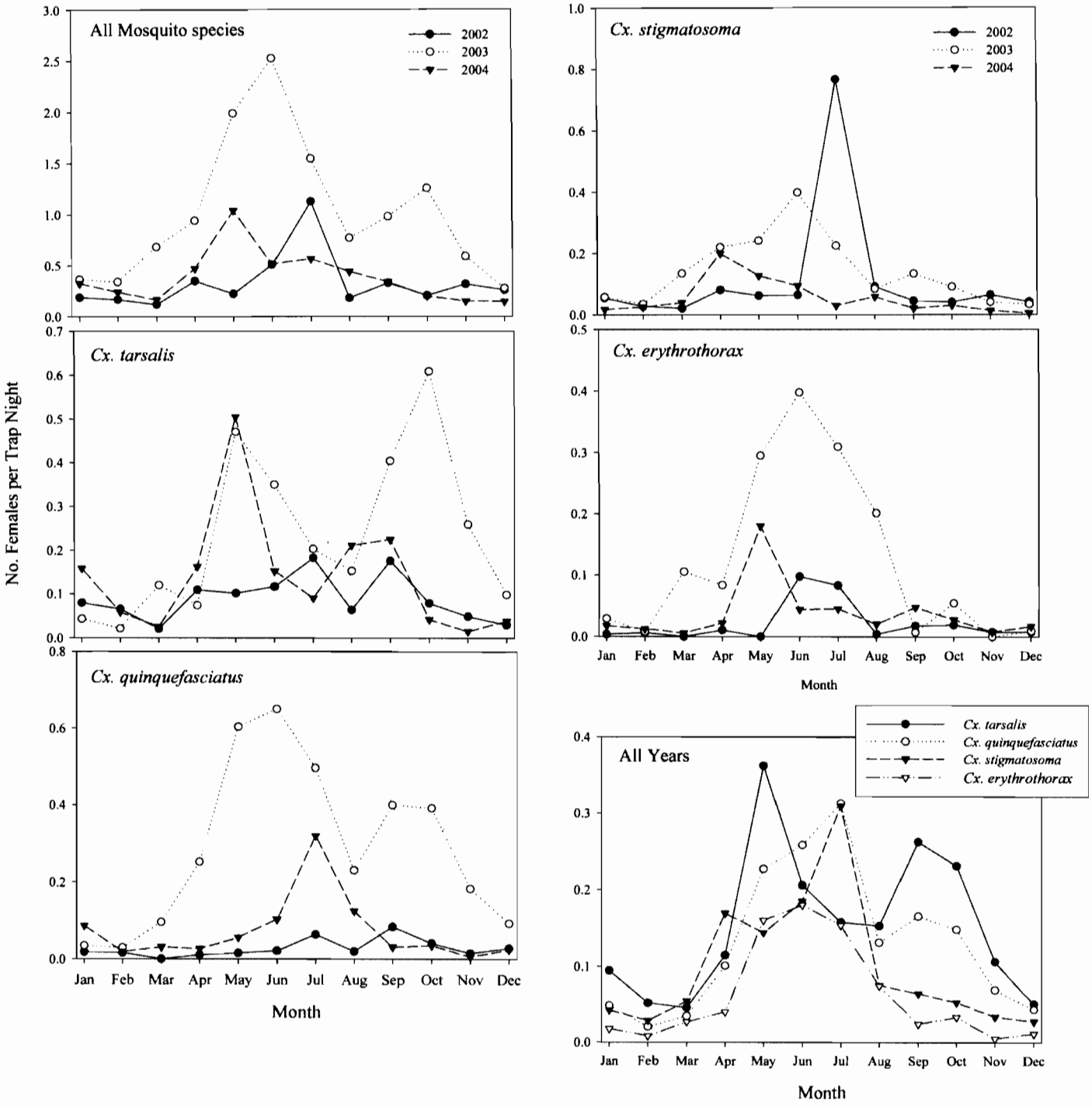

Figure 3. Mean numbers of female *Culex spp.*/trap night collected in 12 standard NJLTs in 2002, 2003 and 2004.

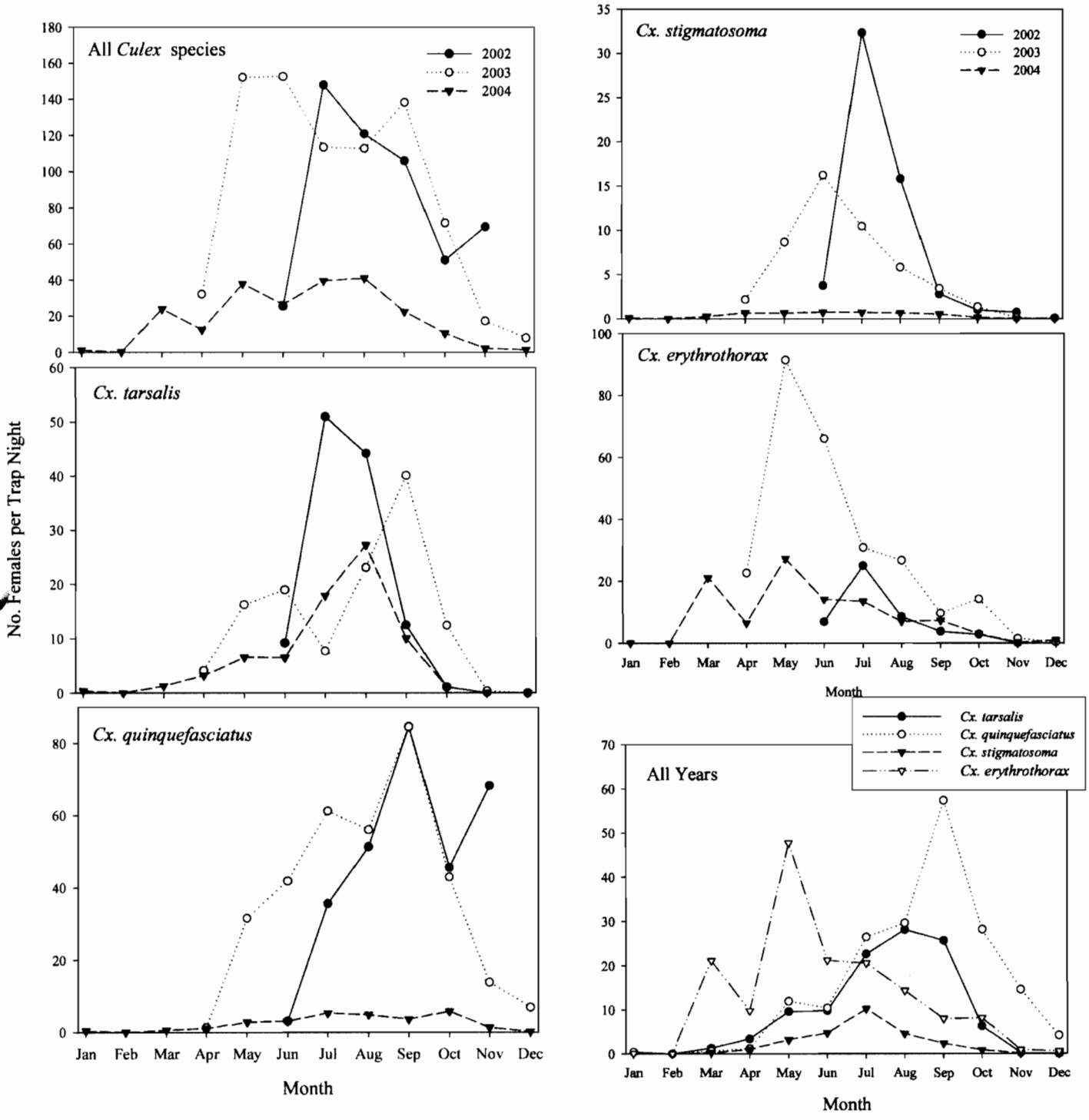

Figure 4. Mean numbers of female *Culex spp.*/trap night collected in 25 standard CO_2 –baited EVSTs in 2002, 2003 and 2004.

RESULTS

Mosquito Surveillance

Mosquitoes were most abundant in the District service area in 2003 with 1.04 and 93.37 females/ trap night captured in the NJLTs and EVSTs respectively (Table 1). Based on the EVST results, mosquitoes were least abundant in 2004 (alpha = 0.05) with the year mean of 24.12 females/trap night. There were 0.37 females/ trap night collected in NJLTs in 2004. The overall means for 2002 were 75.25 and 0.31 females/trap night in EVST and NJLT respectively. Mosquito species collected in NJLTs and EVSTs included *Culex quinquefasciatus*, *Cx. tarsalis, Cx. stigmatosoma, Cx. erythrothorax, Culiseta inornata* Williston, *Cs. particeps* (Adams), *Cs. incidens* (Thomson), *Anopheles hermsi* Barr & Guptavanji, *An. franciscanus* McCracken and *Ochlerotatus washinoi* Lanzaro & Eldridge. In all years, *Culex* species were most abundant comprising 79% of NJLT and 98% of EVST collections. Of the remaining species, *An. hermsi* contributed 1.8% to the EVST collections while 18% of the NJLT catch was comprised of *Culiseta spp.* Based on the EVST results, *Cx. quinquefasciatus* were the most abundant species in 2002 and 2003 while *Cx. erythorthorax* dominated trap catch in 2004 and there was a marked decrease in the abundance of all *Culex* species in 2004 (Table 1). In all years, *Cx. tarsalis* mosquitoes were most commonly collected in NJLTs followed by *Cx. quinquefasciatus*, *Cx. stigmatosoma* and *Cx. erythrothorax*. *Culiseta incidens* were also abundant in NJLT catch. Temporal patterns of abundance of the four *Culex* species varied among years with the lowest numbers of mosquitoes occurring in January through March and in November through December (Figs. 3 and 4; alpha = 0.05). Generally, based on the EVST data, the mean numbers of *Cx. erythrothorax* peaked earliest in the season (around May), followed by *Cx. tarsalis* July through September, *Cx. stigmatosoma* in July and *Cx. quinquefsciatus* in September (Fig. 4). Those temporal patterns did not exactly match those of the NJLTs: *Cx. tarsalis* had one peak in May and one in September/October and *Cx. quinquefasciiatus* numbers were highest in July (Fig. 3).

For the additional 20 EVST sites along the Santa Ana River, the mean number of females/trap night was 8.26 for *Cx. erythrothorax*, 4.88 for *Cx. tarsalis*, 1.53 for *Cx. quinquefasciatus* and 0.42 for *Cx. stigmatosoma*. All species were most abundant at the beginning of the sampling period (in August) and then their numbers decreased gradually (Fig. 5) in manner similar to that observed for the standard EVSTs (Fig. 4).

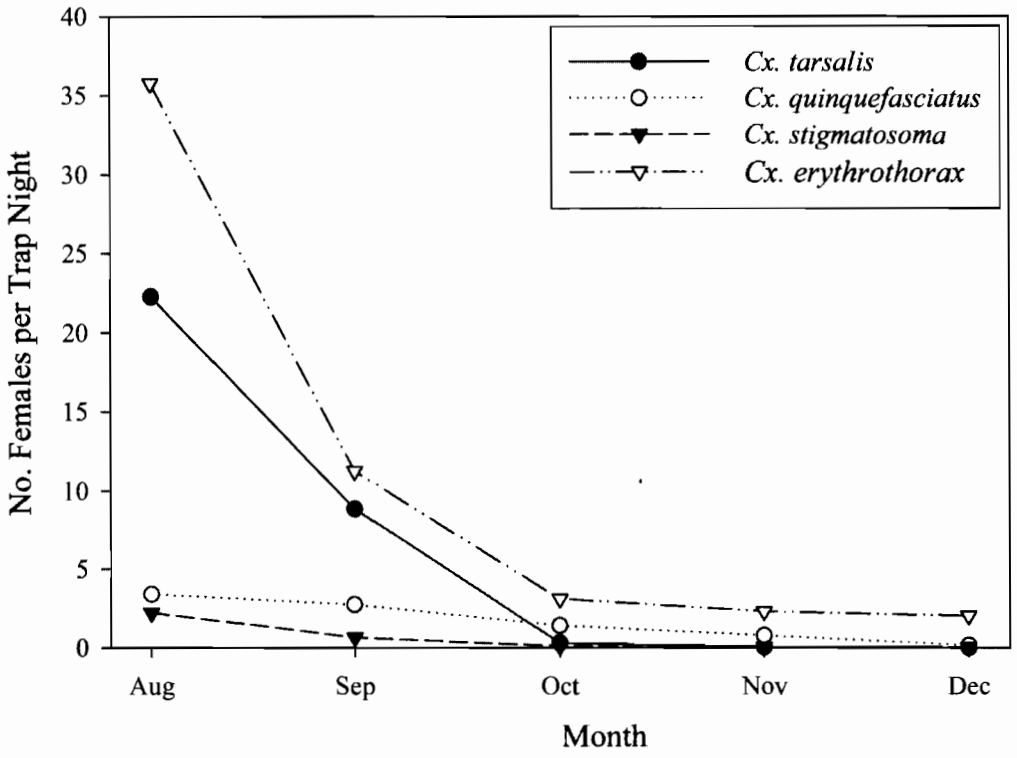

Figure 5. Mean numbers of female *Culex spp.*/trap night collected in 20 CO_2 –baited EVSTs along the Santa Ana River in 2004.

West Nile Virus Surveillance

A total of 1,118 mosquito pools comprised of 43,068 mosquitoes were submitted to CVEC for WNV testing in 2002, 2003 and 2004 (Table 2). Even though the greatest number of mosquitoes were submitted for testing in 2003, WNV was isolated only from the pools of mosquitoes collected in 2004. The first WNV-positive mosquito pool in the District service area was collected at Prado Basin on May 13 (Fig. 1). It was one of the 12 pools of *Cx. erythrothorax* submitted from that area on that date. Additional WNV-positive mosquito pools were then collected from June 7 through September 2, from other riparian and wetland habitats along the Santa Ana River (Fig. 1). Only one WNV-positive mosquito pool was found outside of that general area. It consisted of 15 *Cx. quinquefasciatus* collected in La Sierra on July 28. Even though *Cx. erythrothorax* was the most common mosquito species collected in 2004. *Culex tarsalis* had a much higher minimum infection rate per 1000 mosquitoes (MIR/1000) with *Cx. erythrothorax* MIR/1000 = 0.73 and *Cx. tarsalis* MIR/1000 = 4.98. Moreover, with only 5 pools of 190 *Cx. quinquefasciatus* mosquitoes submitted, the MIR/1000 for that species equaled 5.26.

None of the blood samples from our sentinel chickens submitted in 2002 and 2003 tested positive for WNV. In 2004, 56 out of 76 chickens maintained in 6 sentinel flocks throughout the District area (Fig. 1) tested positive for the virus (Table 3). The first infected chickens, with probable seroconversion date of June 3, were identified in two flocks closest to the Santa Ana River (Fig. 2). Two additional flocks became infected on June 30, further south of the River. A flock in Highgrove, north of the Santa Ana River seroconverted on July 17 and, finally, chickens in Lake Elsinore flock further south of the river became infected on August 11. Transmission of WNV continued through early December as shown by a seroconversion of a chicken in the Corona airport flock

Table 2. Mosquito pools submitted for West Nile Virus (WNV) testing in 2002, 2003 and 2004 and WNV-test results

Year	No. Mosquito Pools* Submitted					No. WNV-Positive Pools				
	Cxt	*Cxq*	*Cxs*	*Cxe*	***All Culex***	*Cxt*	*Cxq*	*Cxs*	*Cxe*	***All Culex***
2002	86(3,115)	124(5,229)	48(1,721)	N/A	**258(10,065)**	0	0	0	N/A	**0**
2003	135(4,923)	189(7,631)	65(2,089)	134(6,233)	**523(20,876)**	0	0	0	0	**0**
2004	94(2,612)	42(1,115)	5(190)	196(8,212)	**337(12,127)**	13	3	1	6	**23**
Total	**315(10,650)**	**355(13,975)**	**118(4,000)**	**330(14,445)**	**1,118(43,068)**	**13**	**3**	**1**	**6**	**23**

* *Cxe—erythrothorax, Cxq—Cx. quinquefasciatus, Cxs—Cx. stigmatosoma, Cxt—Cx. tarsalis.*

Table 3. Sentinel chicken cage locations and West Nile virus testing results for 2004.

Cage Location	*No. of Chickens in Cage*	*No. Positive for WNV*	*Probable Date of Infection*
Corona Airport	15	13	Jun-3 (1)*; Jun-15 (3); Jun-30 (3); Jul-14 (2); Jul-28 (1); Sep-22 (1); Oct-22 (1); Dec-8 (1)
Corona (Temescal Forest Fire Station)	10	10	Jun-30 (1); Jul-14 (4); Jul-28 (3); Aug-25 (2)
Lake Elsinore (Water Treatment Facility)	10	5	Aug-11 (1); Aug-25 (1); Sep-8 (2); Oct-6 (1)
Riverside (Highgrove)	19	9	Jul-14 (4); Jul-28 (4); Aug-11 (1)
Riverside (Rancho Jurupa Park)	14	11	Jun-3 (1); Jun-15 (3); Jun-30 (5); Jul-14 (1); Sep-22 (1)
Riverside (Woodcrest)	8	8	Jun-30 (2); Jul-14 (3); Aug-11 (2); Sep-22 (1)
Total	**76**	**56**	

* Numbers in parentheses refer to the number of chickens infected on the corresponding dates

on December 8. Two flocks (Temescal Forest Fire Station and Woodcrest), showed no new seroconversions after August 25 and September 22 respectively because there were no new chickens to infect after those dates (all chickens at those sites seroconverted and none were replaced).

In 2003, one adult male House finch tested positive for the WNV antibody (Wisniewska-Rosales et al. 2004). In 2004, additional 15 birds tested antibody positive (Table 4). These included 8 Brown-headed cowbirds, 1 California Towhee and 6 House finches. The first three Brown-headed cowbirds were bled at the Orange County Water District in the Prado Basin (Fig. 2). They were bled on March 1, March 8 and April 14. Additional birds included two juvenile House Finches trapped on July 15 on the Northwest MVCD premises, another juvenile House finch captured on July 16 at Canyon Crest, three Brown-headed cowbirds trapped at Rancho Jurupa Park on July 27, a Brown-headed cowbird caught at Hidden Valley Nature Reserve on July 29, two House finches trapped on NWMVCD premises on August 6, and a House finch and a California towhee caught at Norco Animal Shelter on August 12.

During 2003, 53 dead birds were submitted to the CAHFS laboratory. Of these, three American crows (one collected October 20 and two November 3) and a House finch (collected Oct. 22) tested positive for WNV. All four birds were found in the portion of the city of Riverside serviced by the District. In 2004, we collected and submitted a total of 105 dead birds including 90 American crows. Forty two of these birds were WNV-positive which comprised 50% of all dead birds tested. The last WNV-positive crow was found on July 7, 2003. After July 7, CDHS halted further testing of dead birds in our area. Forty one of the WNV-positive birds were American crows. One was an American raven. The majority (13) of the WNV-positive dead birds were collected in the areas of the city of Riverside served by the District. A great majority of the birds were found in close proximity to the Santa Ana River, especially earlier in the season.

DISCUSSION

Mosquito Surveillance

In comparison to the 2002 and 2003 collections, the mean numbers of mosquitoes collected in EVSTs in 2004 were much lower (Table 1). The pattern was not the same for the NLJTs where mosquito collections were lowest in 2002 and highest in 2003. The difference in NJLT catch between 2002 and 2004 was entirely due to the larger numbers of *Cx. tarsalis* and *Cx. quinquefasciatus* collected in those traps in 2004. This difference may be attributed to the relocation, in 2004, of two suburban NJLTs with the lowest trap catch to nearby locations further away from competing light sources. Competing light sources create background illumination that may greatly reduce NJLT count especially in the urban and suburban areas (Milby and Reeves 1989). When the mean numbers of mosquitoes/trap night were calculated for NJLTs with data from those two locations excluded, the mean number of females/trap night was 0.30 for both 2002 and 2004 with the mean for *Cx. tarsalis* dropping from 0.13 to 0.09.

The main reason for the marked decrease of mean numbers of females/trap night in EVSTs in 2004 may have been the decreasing

Table 4. Birds captured, bled and tested for the West Nile Virus (WNV) antibodies in 2003 and 2004.

	No. Birds Bled		No.(%) Recaptured*				No. WNV+	
			Multiple		Single			
Bird Species	2003	2004	2003	2004	2003	2004	2003	2004
Barn swallow	0	1	0	0	0	0	0	0
Brown-headed cowbird**	60	163	0	55(33.7)	0	95(58.3)	0	8
California towhee	5	16	0	4(25.0)	0	4(25.0)	0	1
European starling	0	1	0	0	0	0	0	0
Green-tailed towhee	0	1	0	0	0	0	0	0
House finch	160	151	28(17.5)	22(14.6)	56(35.0)	26(17.2)	1	6
House sparrow	72	70	7(9.7)	10(14.29)	8(11.1)	20(28.6)	0	0
Red-winged blackbird	2	2	0	0	0	0	0	0
Savannah sparrow	0	1	0	0	0	0	0	0
Song sparrow	0	9	0	0	0	0	0	0
Spotted towhee	1	0	0	0	0	0	0	0
White-crowned sparrow	0	1	0	0	0	0	0	0
Total	**300**	**416**	**35(11.7)**	**91(21.0)**	**64(21.3)**	**145(34.9)**	**1**	**15**

* In multiple recaptures, birds captured multiple times are counted once. In single recpatures, birds captured multiple times are counted each time.

** For Brown-headed cowbirds recaptures refer to multiple bleedings of sentinel birds in cages.

number of dairies in the area. The number of dairies in 2002 was 73 and down to 70 in 2003. It dropped to 43 in 2004 and, by the end of the year, it was close to 24. The dairies in the District service area contain barn-wash ponds that breed large numbers of *Cx. qinquefasciatus*. As the dairies move out, barn-wash ponds are drained and the areas are cleared and populated with housing tracks. Also, the decreased number of cows results in a decreased amount of organic pollutants being discharged into the Santa Ana River. This, in turn, results in fewer nutrients available in the river for mosquito breeding. The dramatic decrease in the *Cx. quinquefasciatus* collections in 2004 supports this hypothesis. In 2002 and 2003, *Cx. quinquefasciatus* dominated EVST catch while *Cx. erythrothorax* were most prevalent in 2004. *Culex erythrothorax* mosquitoes are commonly found in wetland areas containing cattails (*Typha spp.*) and bullrush (*Schoenoplectus californicus*) and not in street drains in residential areas or in barn-wash ponds.

The decreased number of mosquitoes/trap night in 2004 may also be attributed to the increased efforts of our vector control technicians in mosquito breeding source inspection and treatment. Close to 35,000 source inspections were conducted in 2004 as compared to 23,670 inspections in 2003 and 13,655 in 2002. Also, in 2004, increased efforts were exerted in inspecting catch basins that are becoming more common in the area. In 2004, there were 7,026 catch basin inspections as compared to 2,679 and 1,874 in 2003 and 2002 respectively. Finally, mosquito breeding sources in 2004 were treated with methoprene formulations of greater longevity (Altosid® 30-day briquets were replaced with Altosid®XR 90-day briquets, partially in 2003 and almost totally in 2004).

West Nile Virus Surveillance

Since the introduction of WNV in California in June, 2003, antibodies to WNV were first detected in the NWMVCD area in a live House Finch on September 22, 2003 in the city of Riverside (Fig. 2). Subsequently, between October 21 and November 3, 2003 the virus was isolated from 3 American crows and a House finch that were found within or in close proximity to the city of Riverside. In 2004, 42 additional WNV-positive dead crows were collected in the NWMVCD service area between May 13 and July 7. The first WNV-positive mosquito pool was collected on May 13, 2004 and the first sentinel chicken seroconversions occurred between May 20 and June 3 (Table 3). The first horse was diagnosed with a WNV infection on June 20, 2004 in the city of Norco. The first human WNV case occurred in Riverside County on June 13, 2004. It may be then concluded that testing of live birds for the antibodies to the WNV proved to be the most sensitive method of detection of the arrival of the virus, followed by the dead bird surveillance and testing of mosquito pools and sentinel chickens.

Most and the earliest detection of WNV-positive mosquito pools, dead birds, live birds and sentinel chicken seroconversions in NWMVCD area occurred in the riparian and wetland habitats surrounding the Santa Ana River. Human and horse WNV cases seemed to have followed a similar pattern. This could only be expected since these habitats breed a lot of mosquitoes and there was a crow roost located in the Hidden Valley Nature Reserve area along the river. The roost may have contributed to the disease amplification in that area due to the thousands of crows arriving there nightly from neighboring habitats and, most probably bringing the virus with them. Infected crows at the roost may have then transmitted the virus to healthy crows and other bird species through mosquitoes feeding on them, and also through direct exchange of body fluids with healthy crows or through the sharing of food and water (Komar *et al.*, 2003). Birds infected at or near the roost would then be able to take the virus out of the area to other locations along their flight routes and home habitats where they would be able to infect mosquitoes that would in turn transmit the virus to other birds or other animals such as horses and to humans. Such pattern of WNV amplification and infection would result in the virus being most prevalent along the river and appearing in other areas further away at a later time which is the pattern we observed. The existence of the pattern is further corroborated by the fact that although the riparian /wetland habitat along the Temescal wash and near Lake Elsinore Water District facility bred plenty of mosquitoes, Lake Elsinore was the last area where sentinel chicken seroconversions occurred. There were no WNV-positive mosquito pools or dead birds found in that area in 2003 and 2004 and none of the live bird blood samples collected there tested positive for the WNV.

Acknowledgements

We would like to thank Tanya Posy, Harold Morales and Chris Mullens for their assistance in our mosquito and vector surveillance program throughout the years. We thank Dr. Thomas Scott of University of California, Berkeley, for providing information about crow roosts and crow behavior and for assistance in creating maps of the District and trap locations. We also thank Loren R. Hays of the U.S. Fish and Wildlife Service, the biologists of SAWA Least Bell's Vireo Conservation Project and Pat Tennant of the OCWD for their cooperation in maintaining and supplying Brown-headed cowbirds to test for the WNV antibodies. We gratefully acknowledge the assistance of Robert F. Cummings and Dr. James P. Webb, Jr. for providing invaluable guidance and support in our wild bird surveillance program. We thank Dr. Martine Jozan and Carrie L. Fogarty of OCVCD for testing of avian blood sera for arboviruses.

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Control Measures and West Nile Virus in the Coachella Valley, California, 2004

Branka B. Lothrop, Ph.D, James Saulnier, Tianyun (Steven) Su, and Donald Gomsi

Coachella Valley Mosquito and Vector Control District, 43-420 Trader Place, Indio, CA 92201

ABSTRACT: Appropriate and timely response to surveillance data is the key to preventing human and animal diseases associated with West Nile virus (WNV) and other arboviruses. The response must be immediate and effective mosquito control, if increasing levels of virus activity are detected in the bird or mosquito surveillance systems. In 2003, southeastern California had a high probability to be the first region to detect WNV in California, and that prediction was proven. In spring 2004 WN virus activity continued and the Coachella valley Mosquito and Vector Control District (CVMVCD) organized a comprehensive and timely control program in the Coachella Valley that included, ground and aerial larviciding and adulticiding, and at the same time encouraged evaluation of mosquito fish use as a control measure in specific habitats such as duck ponds. All control measures were based on surveillance data for the Coachella Valley and risk assessment values calculated by using the CVMVCD Mosquito-Borne Virus Surveillance and Emergency Response Plan, as well as the CVMVCD WNV Action Plan.

West Nile Virus and Mosquito Control in San Bernardino County, California in 2004

J. Wakoli Wekesa, Chris Nwadike, Joe Krygier and Joan Mulcare

San Bernardino County vector Control Program, 2355 E. 5th Street, San Bernardino, CA 92410

ABSTRACT: The first activity of West Nile virus (WNV) in San Bernardino County was a positive crow collected in Rialto in October 2003, and by the year-end 9 additional birds had tested positive. In 2004, WNV-positive dead birds were collected in the same area of the county as the previous year. The spatial-temporal distribution pattern of dead birds overlapped with that of WNV-positive mosquito pools, sentinel chicken seroconversions, horse infections, and human cases. The outbreak caused significant impact on our communities and influenced our operation and surveillance activities. We will provide information on how effective our comprehensive WNV plan worked in responding to this epidemic and discuss strategies for the next season.

Surveillance for Mosquito-Borne Encephalitis Virus Activity and Human Disease, Including West Nile Virus in California, 2004

Albert Hom, Lauren Marcus, Vicki L. Kramer, Barbara Cahoon[1], Carol Glaser, Cindi Cossen, Elizabeth Baylis, Cynthia Jean, Evelyn H. Tu, Bruce Eldridge[1], Ryan Carney, Kerry Padgett, Ben Sun, William K. Reisen[1], Leslie Woods[2] and Stan Husted

Division of Communicable Disease Control, California Department of Health Services, 1616 Capital Ave. MS 7307, P.O.Box 997413, Sacramento, CA 95899-7413
[1]U.C. Davis Center for Vectorborne Diseases, University of California, Davis, CA 95616
Arbovirus Field Station, Center for Vectorborne Disease Research, University of California, Davis, Bakersfield, CA 93312
School of Public Health, University of California, Berkeley, CA 94720
[2] California Animal Health and Food Safety Laboratory (CAHFS), University of California at Davis, CA 95616

The California Arbovirus Surveillance program involves cooperative efforts by many groups and individuals from the local mosquito and vector control districts; the University of California—Davis, Center for Vectorborne Diseases (CVEC); the California Animal Health and Food Safety Laboratory (CAHFS); the Mosquito and Vector Control Association of California (MVCAC); county and local public health departments; physicians and veterinarians throughout California; the Department of Food and Agriculture (CDFA); and the Division of Communicable Disease Control, including the Vector-Borne Disease Section (VBDS), the Veterinary Public Health Section, and the Viral and Rickettsial Disease Laboratory (VRDL) of the California Department of Health Services (CDHS).

The invasion of West Nile virus (WNV) was in full force in 2004. The epicenters of activity were in southern California but the invasion rapidly spread northward and by the end of September, evidence of WNV activity was detected in all of the 58 counties in CA. The ability of California's extensive arbovirus surveillance program to indicate risk of transmission to humans and horses prior to the onset of cases was thoroughly tested. Collectively, the use of sentinel chickens, mosquito collections, wild bird serology, and dead wild bird reporting validated the effectiveness of the surveillance program. The season's first detection of WNV activity was a dead crow collected on 2/24 in the city of San Gabriel. The surveillance program provided early warning of virus activities in various regions of the state and helped focus mosquito control efforts in the most critical areas.

Surveillance program elements include: 1) mosquito population monitoring and testing for St. Louis encephalitis (SLE), West Nile virus (WNV) and western equine encephalomyelitis (WEE) virus infections; 2) serological monitoring of sentinel chickens for evidence of encephalitis virus activity; 3) surveillance, submission, and diagnostic testing of bird carcasses for WNV; 4) testing of domestic animals that exhibit clinical symptoms compatible with SLE, WNV, or WEE infection; 5) capture, release, and recapture of wild bird serology; and 6) serological testing of patients presenting symptoms of viral meningitis or encephalitis. Table 1 indicates the local agency participation in the statewide program and Table 2 lists the arbovirus diagnostic procedures used by the testing laboratories.

Table 1. Mosquito pools and sentinel chicken flocks tested for Arboviruses, 2004.

		Adult Mosquito Surveillance	WNV Surveillance						
			Mosquitoes			Sentinel Chickens			
County	Agency	No. of New Jersey Light Traps	No. pools tested	No. mosquitoes tested	WNV + pools	No. flocks	No. chickens	No. sera tested	WNV + sera
Alameda	Alameda Co. MAD	15	157	6,851		3	21	294	
Alpine									
Amador									
Butte	Butte Co. MVCD	26	19	765	1	7	77	912	50
Calaveras									
Colusa	Colusa MAD	4				1	11	140	
Contra Costa	Contra Costa MVCD	18	417	20,649		4	41	560	
Del Norte									
El Dorado									
Fresno	Consolidated MAD	23	122	4,524	13	6	64	883	24
Fresno	Fresno MVCD	8	39	1,601	1	2	21	320	1
Fresno	Fresno Westside MAD	10	56	2,674		2	20	340	
Glenn	Glenn Co. MVCD	5				2	26	306	19

Continued »

		Adult Mosquito Surveillance	WNV Surveillance						
			Mosquitoes			Sentinel Chickens			
County	Agency	No. of New Jersey Light Traps	No. pools tested	No. mosquitoes tested	WNV + pools	No. flocks	No. chickens	No. sera tested	WNV + sera
Humboldt									
Imperial	Coachella Valley MVCD					3	65	620	42
Imperial	Imperial Co. Environmental Health		245	9,753	33	4	53	280	14
Inyo	Owens Valley MAP		55	1,617		3	30	383	
Kern	Arbovirus Field Station		211						
Kern	Delano MAD	8				2	19	239	10
Kern	Kern MVCD	20	1,134	53,697	214	9	119	1,273	90
Kern	South Fork MAD					1	10	110	
Kern	Westside MVCD	17	3	150		3	30	421	1
Kings	Kings MAD	9	3	150		4	40	509	2
Lake	Lake Co. VCD		365	16,935	17	2	20	272	1
Lassen									
Los Angeles	Antelope Valley MVCD	13	6	34		8	62	587	14
Los Angeles	Greater Los Angeles Co. VCD	17	2,437	87,257	342	5	146	1,193	45
Los Angeles	Long Beach Environmental Health		432	13,848	30	4	50	479	23
Los Angeles	Los Angeles Co. West VCD		238	8,611	12	20	130	41	39
Los Angeles	San Gabriel Valley MVCD		71	1,966	24	11	70	99	46
Madera	Madera Co. MVCD	5	20	1,000		2	21	196	
Marin	Marin-Sonoma MVCD	12	48	2,145		5	55	431	
Mariposa									
Mendocino									
Merced	Merced Co. MAD	18	305	12,929	1	6	36	509	
Modoc									
Mono									
Monterey	North Salinas MAD	18	1	12		3	34	388	
Napa	Napa MAD	18				6	64	834	
Nevada									
Orange	Orange Co. VCD		1,886	59,534	164	1	10	138	3
Placer	Placer Co. VCD	15	165	3,216	4	6	60	828	25
Plumas									
Riverside	Coachella Valley MVCD	8	1,595	57,437	71	10	197	1,565	70
Riverside	Northwest MVCD	12	430	15,842	23	6	80	906	55
Riverside	Riverside Co. Environmental Health	13	303	13,156	9	6	94	974	33
Sacramento	Sacramento-Yolo MVCD	23	1,227	38,528	16	5	50	16	15
San Benito									
San Bernadino	San Bernardino Co. VCP	23	280	9,297	63	9	152	1,517	71
San Bernardino	West Valley MVCD		267	8,538	65	7	30	485	37
San Diego	San Diego Co. Dept of Health		92	4,410		3	30	500	
San Francisco									
San Joaquin	San Joaquin Co. MVCD	23	531	20,410	2	6	72	963	11
San Luis Obispo	San Luis Obispo Co.		207	9,633	1	3	31	370	
San Mateo	San Mateo Co. MAD	6	29	1,136		4	30	380	
Santa Barbara	Santa Barbara Coastal VCD		198	7,705		6	64	998	
Santa Clara	Santa Clara Co. VCD	36	16	718		4	41	526	4
Santa Cruz	Santa Cruz Co. MVCD	7	24	915		1	10	140	
Shasta	Burney Basin MAD	6				2	20	180	
Shasta	Shasta MVCD	20	146	6,605	11	5	55	708	5
Sierra									
Siskiyou									
Solano	Solano Co. MAD	25				2	24	311	
Solano	Sacramento-Yolo MVCD								
Sonoma	Marin-Sonoma MVCD	7				2	22	633	
Stanislaus	East Side MAD					2	16	225	3
Stanislaus	Turlock MAD	21	635	24,816	3	7	85	1,340	9
Sutter	Sutter-Yuba MVCD	20	307	14,382	8	5	50	974	12
Tehama	Tehama Co. MVCD	9	4	169		2	22	197	12
Trinity									
Tulare	Delta VCD	12	76	2,827	3	6	72	856	3
Tulare	Tulare MAD	10				2	20	284	8
Tuolumne									
Ventura	City of Moorpark	5				1	5	95	
Ventura	Ventura Co. Environmental Health	19	7	204		4	40	709	1
Yolo	Sacramento-Yolo MVCD	14			5	5	50	12	11
Yuba	Sutter-Yuba MVCD	19				2	20		
Total		**617**	**14,809**		**1,136**	**253**	**2,787**	**29,498**	**809**

Table 2. Arbovirus diagnostic procedures for California.

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

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

Assays: EIA, enzyme immunoassay
PRNT, plaque reduction neutralization test
IFA, immunofluorescent antibody

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

The 2004 surveillance season began in April with the deployment of sentinel chicken flocks and the beginning of mosquito collection data for the Adult Mosquito Occurrence Report (AMOR). Thirty-four weekly Arbovirus Surveillance bulletins and 44 adult mosquito occurrence reports with a five-year average summary were disseminated to all program participants to provide detailed surveillance summaries. Positive findings including serology, mosquitoes, and dead birds were communicated immediately to submitting agencies.

HUMAN DISEASE SURVEILLANCE

In 2002, the CDHS Viral and Rickettsial Disease Laboratory (VRDL) initiated a regional public health laboratory network to enhance human WNV testing in California. The regional laboratory network consists of VRDL as well as 33 county public health laboratories. Specimens for patients that met diagnostic testing guidelines were tested for WNV within the regional laboratory network. The local laboratories tested for WNV with an immunofluorescent assay and/or a capture IgM MAC-ELISA assay, then forwarded positive specimens to VRDL for repeat and confirmatory testing. Over 2,800 specimens for 2,389 patients were tested for WNV at VRDL in 2004.

VRDL also collaborated with three major commercial reference laboratories and the regional laboratories of Kaiser Permanente to ascertain additional suspect WNV cases. In addition, specimens were submitted through the California Encephalitis Project (CEP), which provides comprehensive testing for many agents known to cause encephalitis, including WNV. Asymptomatic blood donors infected with WNV were identified through blood banks, which tested all donations for WN and reported to the CDHS Infectious Diseases Branch (IDB).

The first human WNV case of 2004 was identified in early June, in a 40-year-old female from San Bernardino County who had an onset of West Nile fever (WNF) in mid-May. In total, 829 human WNV infections were identified from 23 counties in California in 2004, compared with 3 WNV infections from 3 counties in 2003. Sixty-six of the 829 WNV infections were detected in blood donors, fifteen of which later developed clinical symptoms consistent with WNF.

Of the 779 WNV-positive patients with symptoms, 391 were classified as WNF, 284 as West Nile neuroinvasive disease (WNND, i.e., encephalitis, meningitis, or acute flaccid paralysis), and 104 were of unknown clinical presentation (Table 3). The median age for all cases, where data were available, was 52 years (range: 2 – 94 years). The median age for WNF cases was 50 years (range: 2 –91 years), and for WNND cases 58 years (range: 4 – 91 years). Of the 779 cases, 483 (62%) were male. There were twenty-seven WNV-related fatalities reported. The median age of the 27 fatalities was 76 years (range: 26-91 years). Figure 1 shows the incidence per 100,000 populations.

Table 3. Human cases of infection with West Nile virus

	Clinical Classification			
County	WNF	WNND	Unknown	Total
Butte	2		5	7
Fresno	1	4	6	11
Glenn	2	1		3
Imperial	1			1
Kern	41	15	3	59
Lake	1			1
Lassen			1	1
Los Angeles	129	125	52	306
Merced	1			1
Orange	20	29	13	62
Placer			1	1
Riverside	63	40	6	109
Sacramento		2	1	3
San Bernardino	124	62	1	187
San Diego		2		2
San Joaquin	2			2
San Luis Obispo		1		1
Santa Clara	1			1
Shasta		1	4	5
Tehama	1		9	10
Tulare		1	2	3
Ventura	1	1		2
Yolo	1			1
Total	**391**	**284**	**104**	**779***

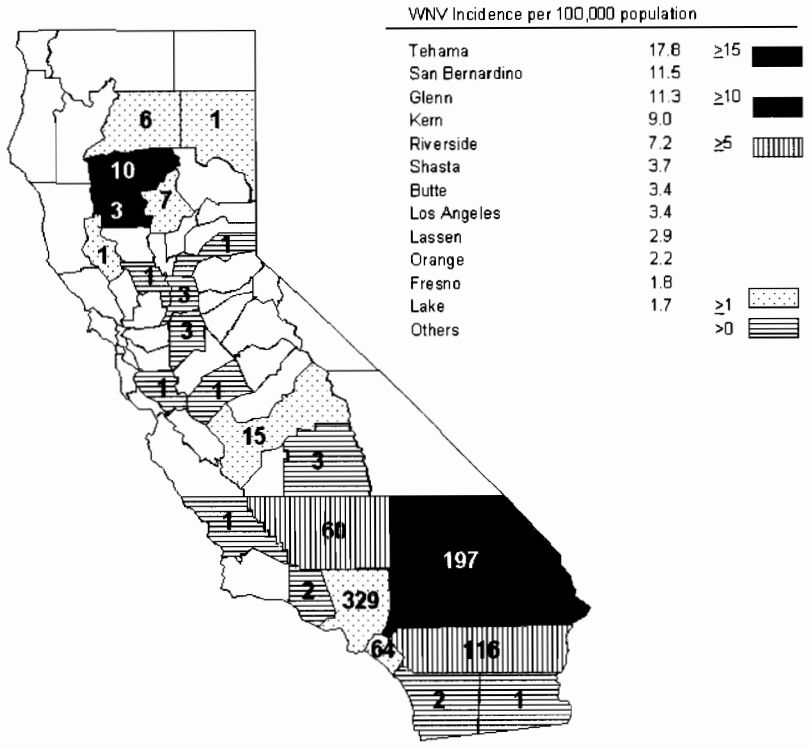

Figure 1. Incidence of Human WNV cases in 2004

EQUINE SURVEILLANCE

A total of 141 sera and brain tissue specimens from horses displaying neuroogical signs were submitted to CAHFS laboratories and CVEC for arboviral testing.

West Nile virus infection was detected in 540 horses from the following 32 counties: Alpine (3), Butte (18), Colusa (6), Fresno (21), Glenn (12), Inyo (3), Kern (47), Kings (1), Lake (4), Lassen (4), Los Angeles (16), Mendocino (3), Merced (3), Orange (2), Placer (26), Riverside (102), Sacramento (84), San Bernardino (36), San Diego (2), San Joaquin (19), Shasta (30), Siskiyou (5), Solano (1), Sonoma (1), Stanislaus (7), Sutter (11), Tehama (44), Trinity (1), Tulare (13), Ventura (3), Yolo (1), and Yuba (11) counties (Table 4 and Fig 2). Positive equines provided the first indication of WN in two counties: Alpine and Inyo. Of the 540 infected horses, 228 (43%) died or were euthanatized. Follow-up investigations revealed that among the 540 horses, 12 were properly vaccinated with the WNV vaccine; 115 were improperly vaccinated; 395 were unvaccinated; and 18 were of unknown vaccination history.

Table 4. Summary of WN virus Surveillance in California, 2004

County	Humans	Horses	Dead Birds	Mosquito Pools	Sentinel Chickens
Alameda			23		
Alpine		3	3		
Amador			9		
Butte	7	18	118	1	50
Calaveras			10		
Colusa		6	21		
Contra Costa			19		
Del Norte			3		
El Dorado			22		
Fresno	15	21	116	14	25
Glenn	3	12	75		19
Humboldt			16		
Imperial	1		1	32	56
Inyo		3	12		
Kern	60	47	87	214	101
Kings		1	11		2
Lake	1	4	30	17	1
Lassen	1	4	13		
Los Angeles	331	16	840	408	167
Madera			7		
Marin			18		
Mariposa			6		
Mendocino		3	13		
Merced	1	3	29	1	
Modoc			1		
Mono			6		
Monterey			12		
Napa			6		
Nevada			26		
Orange	64	2	225	164	3
Placer	1	26	47	4	25
Plumas			26		
Riverside	116	102	139	104	158
Sacramento	3	84	153	21	15
San Benito			5		
San Bernardino	197	36	289	128	108
San Diego	2	2	34		
San Francisco			14		
San Joaquin	3	19	57	2	11
San Luis Obispo	1		15	1	
San Mateo			15		
Santa Barbara			7		
Santa Clara	1		46		4
Santa Cruz			36		
Shasta	6	30	90	11	5
Sierra			3		
Siskiyou		5	34		
Solano		1	17		
Sonoma		1	49		
Stanislaus		7	82	3	12
Sutter		11	28	8	12
Tehama	10	44	115		12
Trinity		1	9		
Tulare	3	13	48	3	11
Tuolumne			34		
Ventura	2	3	23		1
Yolo	1	1	26		11
Yuba		11	13		

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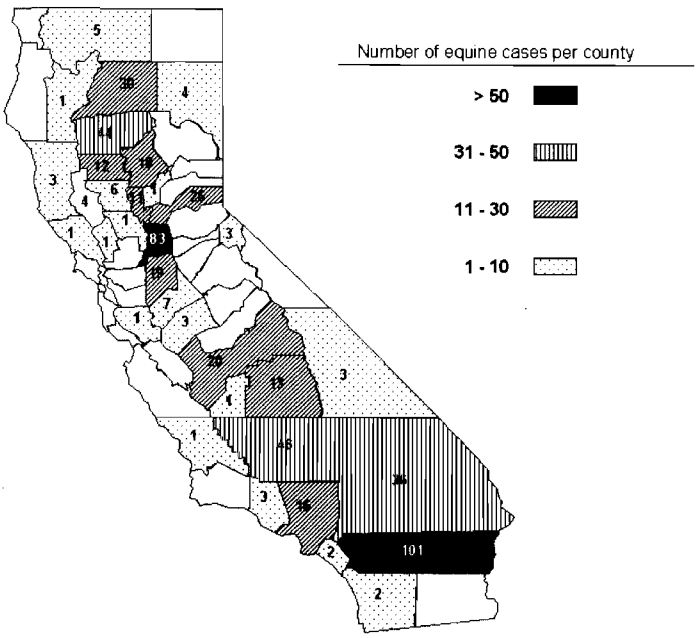

Figure 2. Equine WNV cases in 2004

ADULT MOSQUITO SURVEILLANCE

Forty-two local agencies from 33 counties initiated mosquito collections using New Jersey light traps in April 2004. Trap catch counts from these sources were forwarded to VBDS and collated weekly into the AMOR from April 7 to November 3.

MOSQUITO TESTING

Forty-one local mosquito control agencies submitted a total of 554,724 mosquitoes (14,809 mosquito pools) collected from dry ice-baited and Gravid traps, for virus isolations. This submission rate sets the record for the largest number of pools submitted from any previous years. Mosquito pools were tested for arboviruses at CVEC using a Taq Man multiplex RT-PCR (Tables 5,6,7,8, and 9). Of these, 1136 pools tested positive for WNV. No other arboviruses (e.g., western equine encephalomyelitis and St. Louis encephalitis) were detected in 2004.

The first WNV positive mosquito pool of the season was obtained from 2 pools of *Culex tarsalis* Coquillett collected on April 14th from North Shore (Riverside County) and the last from a pool of *Cx. quinquefasciatus* Say collected on November 4th from Westminster (Orange County). A total of 1136 mosquito pools representing 3 genera and 9 species collected from 20 counties tested positive for WN. Of the 1136 positives, 741 were *Cx. quinquefasciatus*; 315 *Cx. tarsalis*; 41 *Cx. stigmatosoma* Dyar; 19 *Cx. erythrothorax* Dyar; 13 *Cx. pipiens* L.; 3 *Cx. thriambus*; 2 *Ochlerotatus melanimon*; 1 *Oc. squamiger*; and 1 *Anopheles hermsi* Barr & Guptavanji. WN isolation from *An. hermsi*, *Cx. thriambus*,

Table 5. Mosquitoes (*Culex spp.*) tested for WN, WEE, and SLE viruses by submitting county and agency, 2004.

		Cx erythrothorax		*Cx pipiens*		*Cx quinquefasciatus*		*Cx stigmatosoma*		*Cx tarsalis*		Total	
County	Agency	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.
Alameda	ALCO	67	3,331	68	2,817					20	649	155	6,797
Butte	BUCO	1	13	1	7					7	245	9	265
Contra Costa	CNTR	91	4,540	29	1,420					278	13,823	398	19,783
Fresno	CNSL	7	296			75	2,506			39	1,701	121	4,503
Fresno	FRNO					11	385			28	1,216	39	1,601
Fresno	FRWS					9	450			43	2,034	52	2,484
Imperial	IMPR	50	2,358			18	769			155	5,805	223	8,932
Inyo	INYO	22	708							31	834	53	1,542
Kern	KERN	2	67			598	22,764	5	121	551	22,470	1,156	45,422
Kern	WEST					3	150					3	150
Kings	KNGS									3	150	3	150
Lake	LAKE	35	1,639					24	909	261	12,317	320	14,865
Los Angeles	ANTV	1	10			2	14			1	10	4	34
Los Angeles	GRLA	420	19,686			1,555	56,363	71	1,348	177	5,380	2,223	82,777
Los Angeles	LONG	63	2,621			256	7,874	3	41	110	3,312	432	13,848
Los Angeles	LACW					183	6,360			4	108	187	6,468
Los Angeles	PASA									1	17	1	17
Los Angeles	SGVA	1	33			50	1,537			9	181	60	1,751
Madera	MADR			18	900					2	100	20	1,000
Marin	MARN			43	1,967			1	23	2	58	46	2,048
Merced	MERC	3	97	124	4,800					141	6,225	268	11,122
Monterey	NSAL			1	12							1	12
Orange	ORCO	268	10,389			1,184	39,575	39	890	160	5,291	1,651	56,145
Placer	PLCR	6	270	30	903			10	47	56	1,625	102	2,845
Riverside	COAV	77	3,513			231	5,273			1,186	46,857	1,494	55,643
Riverside	NWST	258	11,115			52	1,405	3	56	113	3,233	426	15,809
Riverside	RIVR	179	8,688			17	344	16	539	82	3,170	294	12,741
Sacramento	SAYO	71	3,062	352	9,501			19	277	573	23,307	1,015	36,147
San Bernardino	SANB	15	533			81	2,527	15	294	138	5,489	249	8,843
San Bernardino	WVAL	9	299			216	7,161	24	572	18	506	267	8,538
San Diego	SAND	34	1,700			5	84			40	1,993	79	3,777
San Joaquin	SJCM	2	61	271	10,929	1	45			209	7,655	483	18,690
San Luis Obispo	SLOC	132	6,364	17	730			4	111	4	110	157	7,315
San Mateo	SANM	4	184	13	523					5	142	22	849
Santa Barbara	SBCO	25	1,103			12	478	3	46	75	3,451	115	5,078
Santa Clara	STCL			12	573					4	145	16	718
Santa Cruz	SCRZ	16	671	7	225					1	19	24	915
Shasta	SHAS	2	43	63	2,918			3	105	77	3,531	145	6,597
Solano	SAYO											0	0
Stanislaus	TRLK	121	5,762	273	10,594	8	327	9	151	194	7,171	605	24,005
Sutter	SUYA	1	50	5	137					232	11,579	238	11,766
Tehama	TEHA									4	169	4	169
Tulare	DLTA					59	2,433	2	33	15	361	76	2,827
Ventura	VENT			2	65					5	139	7	204
Yolo	SAYO											0	0
Yuba	SUYA	1		1						13		15	0
Total		**1984**	**89206**	**1330**	**49021**	**4626**	**158824**	**251**	**5563**	**5067**	**202578**	**13,258**	**505,192**

Table 6. Mosquitoes (*Culex spp.*) tested for WN, WEE, and SLE viruses by submitting county and agency, 2004.

		Cx erraticus		*Cx restuans*		*Cx thriambus*		Total	
County	Agency	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.
Kern	KERN					2	47	2	47
Los Angeles	GRLA			2	26	22	724	24	750
Placer	PLCR					2	6	2	6
Riverside	COAV	4	83					4	83
San Diego	SAND					1	50	1	50
Total		**4**	**83**	**2**	**26**	**27**	**827**	**33**	**936**

Table 7. Mosquitoes (*Aedes vexans*, *Coquillettidia perturbans*, *Culiseta* spp., *Orthopodomyia signifera*, and *Psorophora columbiae*) tested for WN, WEE, and SLE viruses by submitting county and agency, 2004.

		Ae vexans		*Cq perturbans*		*Cs incidens*		*Cs inornata*		*Cs particeps*		*Ps columbiae*		Total	
County	Agency	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.
Contra Costa	CNTR							1	18					1	18
Fresno	CNSL					1	21							1	21
Imperial	IMPR	18	705									2	82	20	787
Kern	KERN							8	126					8	126
Lake	LAKE	4	178			1	22							5	200
Los Angeles	GRLA					133	3,394	7	189	7	147			147	3730
Los Angeles	LACW					50	2,105	1	38					51	2143
Los Angeles	SGVA					10	215							10	215
Orange	ORCO					108	2,250	14	233	6	89			128	2572
Placer	PLCR	1	14			11	28	2	41					14	83
Riverside	COAV	29	731					67	974			1	6	97	1711
Riverside	NWST									1	22			1	22
Riverside	RIVR	9	415											9	415
Sacramento	SAYO	18	652			29	469			1	12			48	1133
San Bernardino	SANB	2	20			17	374	3	60					22	454
San Diego	SAND					4	183							4	183
San Joaquin	SJCM	22	859			1	28							23	887
Santa Barbara	SBCO					6	172			7	250			13	422
Shasta	SHAS			1	8									1	8
Solano	SAYO													0	0
Stanislaus	TRLK					1	5	1	7					2	12
Yolo	SAYO													0	0
Total		**103**	**3,574**	**1**	**8**	**372**	**9,266**	**104**	**1,686**	**22**	**520**	**3**	**88**	**605**	**15,142**

Table 8. Mosquitoes (*Ochlerotatus spp.*) tested for WN, WEE, and SLE viruses by submitting county and agency, 2004.

		Oc dorsalis		*Oc melanimon*		*Oc nigromaculis*		*Oc sierrensis*		*Oc squamiger*		*Oc taeniorhynchus*		*Oc tahoensis*		*Oc washinoi*		Total	
County	Agency	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.
Alameda	ALCO	2	54															2	54
Butte	BUCO			10	500													10	500
Contra Costa	CNTR	5	230	13	618													18	848
Fresno	FRWS			4	190													4	190
Imperial	IMPR	2	34															2	34
Inyo	INYO			2	75													2	75
Kern	KERN			179	8,102													179	8,102
Lake	LAKE			39	1,853			1	17									40	1,870
Marin	MARN	2	97															2	97
Merced	MERC			37	1,807													37	1,807
Orange	ORCO									7	235	5	155			15	427	27	817
Placer	PLCR			7	226			1	4					2	52			10	282
Riverside	NWST															1	11	1	11
Sacramento	SAYO			38	1,136	1	13	3	80							1	19	43	1,248
San Diego	SAND											8	400					8	400
San Joaquin	SJCM			25	833													25	833
San Luis Obispo	SLOC	17	835							3	123					29	1,360	49	2,318
San Mateo	SANM	7	287															7	287
Santa Barbara	SBCO									2	20	14	668			32	1,517	48	2,205
Stanislaus	TRLK			28	799													28	799
Solano	SAYO																	0	0
Sutter	SUYA			28	2,616													28	2,616
Yolo	SAYO																	0	0
Yuba	SUYA			27															
Total		**35**	**1,537**	**410**	**18,755**	**1**	**13**	**5**	**101**	**12**	**378**	**27**	**1,223**	**2**	**52**	**78**	**3,334**	**570**	**25,393**

Table 9. Mosquitoes (*Anopheles spp.*) tested for WN, WEE, and SLE viruses by submitting county and agency, 2004.

		An franciscanus		*An freeborni*		*An hermsi*		*An punctipennis*		Total	
County	Agency	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.
Los Angeles	ANTV	2									
Los Angeles	GRLA					43	1,340			43	1,340
Orange	ORCO					73	1,825				1,825
Placer	PLCR			35	841			2	12		853
Riverside	NWST					2	27				27
Sacramento	SAYO			119	4,301			2	81		4,382
San Bernardino	SANB	2	23	1	19	6	67				109
San Luis Obisp	SLOC	1	38								38
Santa Barbara	SBCO	1	19			21	975				994
Total		**6**	**80**	**155**	**5,161**	**145**	**4,234**	**4**	**93**	**43**	**9,568**

Oc. melanimon and *Oc. squamiger* represented the first time the virus has been isolated from these species in the nation (Table 10).

SENTINEL CHICKEN SURVEILLANCE

In 2004, fifty-five local mosquito and vector control agencies maintained 253 sentinel chicken flocks in 38 counties (Table 1). Chickens were bled bi-weekly and dry blood spots were sent to VRDL for testing. A total of 29,498 chicken sera were tested for antibodies to SLE, WNV, and WEE by VRDL. The Sacramento-Yolo Mosquito and Vector Control District (1600 samples), Los Angeles County West Vector Control District (2290) and the San Gabriel Valley Mosquito and Vector Control District (1498 samples) tested their own sentinel chicken flocks for antibodies to SLE, WEE, and WNV for a total of (5388) additional serum

Table 10. WN virus isolated from mosquito pools, 2004.

Mosquito species	County	Agency	WNV	
			pools	mosqs.
Anopheles hermsi	Los Angeles	GRLA	1	20
Culex erythrothorax	Lake	LAKE	1	48
	Los Angeles	GRLA	4	195
		LONG	1	30
	Orange	ORCO	1	50
	Riverside	NWST	5	154
		RIVR	3	150
	Sacramento	SAYO	1	50
	San Bernardino	WVAL	3	129
Culex pipiens	San Joaquin	SJCM	1	50
	Shasta	SHAS	4	186
	Sacramento	SAYO	6	188
	Stanislaus	TRLK	1	46
	Yolo	SAYO	1	50
Culex quinquefasciatus	Fresno	CNSL	13	476
		FRNO	1	26
	Kern	KERN	119	5263
	Los Angeles	GRLA	312	11,497
		SGVA	22	616
		LACW	12	430
		LONG	21	667
	Orange	ORCO	153	5,946
	Riverside	COAV	4	78
		NWST	3	93
	San Bernardino	SANB	25	895
		WVAL	55	2,073
	Tulare	DLTA	1	46
Culex stigmatosoma	Lake	LAKE	9	390
	Los Angeles	GRLA	6	78
Culex tarsalis	Imperial	IMPR	33	1,034
	Kern	KERN	94	4,363
	Lake	LAKE	7	311
	Los Angeles	GRLA	17	594
		LONG	8	197
		SGVA	2	74
	Merced	MERC	1	50
	Orange	ORCO	4	141
	Placer	PLCR	3	131
	Riverside	COAV	67	2,901
		NWST	14	364
		RIVR	5	62
	Sacramento	SAYO	8	438
	San Bernardino	SANB	30	1,063
	San Joaquin	SJCM	1	44
	Shasta	SHAS	6	211
	Stanislaus	TRLK	2	74
	Sutter	SUYA	7	311
	Tulare	DLTA	1	50
	Butte	BUCO	1	50
	Yolo	SAYO	4	160
Culex thriambus	Los Angeles	GRLA	2	94
	Placer	PLCR	1	2
Ochlerotatus melanimon	Kern	KERN	1	50
	Sutter	SUYA	1	50
Ochlerotatus squamiger	San Luis Obispo	SLCO	1	50
Totals			**1,136**	**44,081**

Continued »

samples, to bring the total number of chicken sera tested to 34,886.

The first WNV seroconversions occurred on May 17th. Dry blood spots and sera collected on 5/17 from 6 chickens in a flock located near North Shore (Riverside County) tested positive for flavivirus on initial ELISA screening and the sera confirmed this result. The first bleed date for these birds was April 19th. A total of 809 seroconversions to WNV were recorded among 133 flocks from 22 counties (Tables 4 and 11): Imperial (56), Los Angeles (167), Riverside (158), San Bernardino (108), Glenn (19), Kern (101), Butte (50), Sutter (12), Tehama (12), Orange (3), San Joaquin (11), Ventura (1), Placer (25), Sacramento (15), Shasta (5), Yolo (11), Tulare (11), Fresno (25), Santa Clara (4), Stanislaus (12), Kings (2), and Lake (1). Detection of antibodies to WNV in sentinel chickens was the first indication of WNV in Glenn County. Figure 3 shows seroconversions for SLE, WEE, and WNV by year since 1995.

DEAD BIRDS

The CDHS West Nile virus dead bird surveillance program (DBSP), a collaborative program between CDHS and over 130 local agencies and supported by a CDC grant, was established in 2000. In 2004, the DBSP was a critical component of the arbovirus surveillance program as it was the only surveillance element to detect early WNV activity in every county in California.

Public education strategies were utilized to educate local agencies and the public about WN throughout 2004. The "*Fight the Bite*" campaign materials, originally designed for the outbreak in Colorado, were modified for use in California. Additionally, presentations by CDHS biologists were given to local agencies and the general public to encourage participation in the dead bird surveillance program.

Table 11. Sentinel chicken seroconversions to WN, 2004.

County	Agency	Total flocks	Positive flocks	Seroconversions
Butte	BUCO	7	7	50
Fresno	CNSL	6	4	24
Fresno	FRNO	2	1	1
Glenn	GLEN	2	2	19
Imperial	COAV	3	3	42
Imperial	IMPR	4	4	14
Kern	DLNO	2	1	10
Kern	KERN	9	9	90
Kern	WEST	3	1	1
Kings	KNGS	4	1	2
Lake	LAKE	2	1	1
Los Angeles	ANTV	8	3	14
Los Angeles	GRLA	7	6	45
Los Angeles	LACW	20	11	39
Los Angeles	LONG	4	3	23
Los Angeles	SGVA	11	11	46
Orange	ORCO	1	1	3
Placer	PLCR	6	4	25
Riverside	COAV	10	10	70
Riverside	NWST	6	6	55
Riverside	RIVR	6	6	33
Sacramento	SAYO	5	5	15
San Bernardino	SANB	9	9	71
San Bernardino	WVAL	7	3	37
San Joaquin	SJCM	6	2	11
Santa Clara	STCL	4	1	4
Shasta	SHAS	5	3	5
Stanislaus	EAST	2	1	3
Stanislaus	TRLK	7	2	9
Sutter	SUYA	5	5	12
Tehama	TEHA	2	2	12
Tulare	DLTA	6	2	3
Tulare	TLRE	2	1	8
Ventura	VENT	4	1	1
Yolo	SAYO	5	2	11
Totals		**192**	**134**	**809**

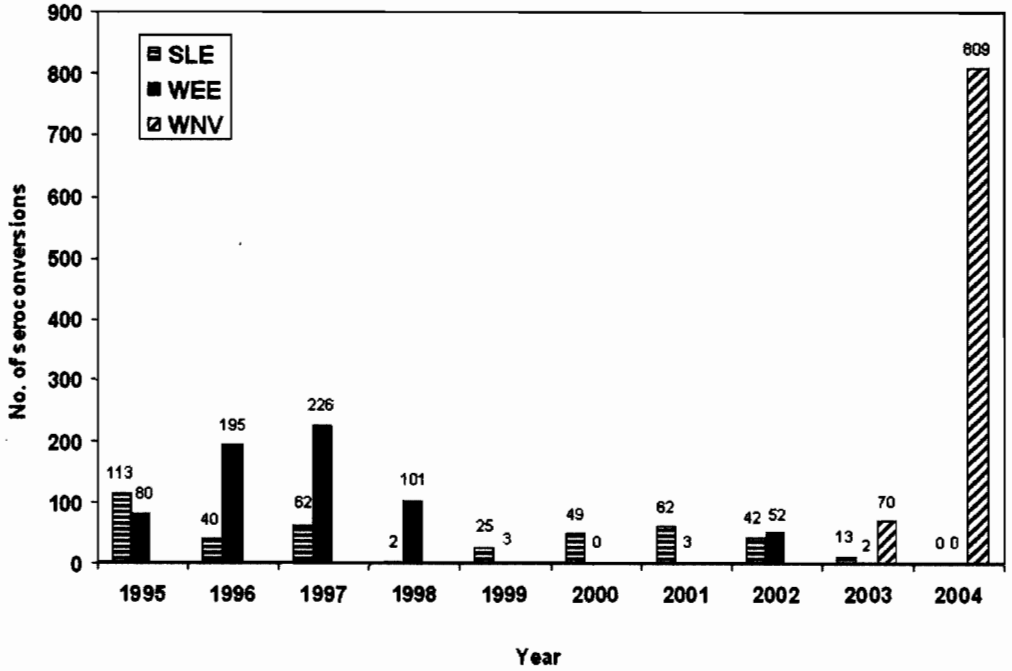

Figure 3. Sentinel flock seroconversions to arboviruses, 1995-2004.

The toll-free hotline **(1-877-WNV-BIRD)** received over 137,000 calls and generated 93,055 dead bird submission reports from 58 counties¾ an 11-fold increase as compared with the previous year 8,650 reports, from 45 counties. Figure 4 shows the number of calls to the hotline, dead birds reported, tested, and positives by month.

In 2004, the number of dead birds submitted for testing was 8,195; of these 56.4% were positive for WNV (3,232 positive/ 5,729 tested) (Table 12). These numbers far surpass the throughput of WNV testing in dead birds in 2003. In 2003, only 5.4% of birds tested (96 positive/1,765 tested) for WNV were positive, representing 5 out of 51 counties (Table 12). Dead bird reporting via the California West Nile Virus Website (www.westnile.ca.gov) was encouraged as an alternate to alleviate the high volume of calls to the hotline.

In 2004, local agencies began screening birds for WNV using two commercially available rapid assays RAMP® (Rapid Analyte Measurement Platform), Response Biomedical Corp., and VecTest, Medical Analysis Systems. To ensure these assays were reliable for WNV surveillance, CDHS and CVEC compared results of rapid assays and RT-PCR of tissues. In August, it was determined that VecTest was a valid test for WNV in American crows; the specificity and sensitivity were comparable to the RT-PCR gold standard. At this time, CDHS accepted VecTest results of American crows. Of the 3,232 dead birds that were positive for WNV, 85 were tested via VecTest and 3,147 were tested via RT-PCR.

In total, WNV activity was detected in every county via a dead bird (Table 12). WNV was found in 3,232 bird carcasses: Alameda (23), Alpine (3), Amador (9), Butte (118), Calaveras (10), Colusa (21), Contra Costa (19), Del Norte (3), El Dorado (22), Fresno (116), Glenn (75), Humboldt (16), Imperial (1), Inyo (12), Kern (87), Kings (11), Lake (30), Lassen (13), Los Angeles (840), Madera (7), Marin (18), Mariposa (6), Mendocino (13), Merced (29), Modoc (1), Mono (6), Monterey (12), Napa (6), Nevada (26), Orange (225), Placer (47), Plumas (26), Riverside (139), Sacramento (153), San Benito (5), San Bernardino (289), San Diego (34), San Francisco (14), San Joaquin (57), San Luis Obispo (15), San Mateo (15), Santa Barbara (7), Santa Clara (46), Santa Cruz (36), Shasta (90), Sierra (3), Siskiyou (34), Solano (17), Sonoma (49), Stanislaus (82), Sutter (28), Tehama (115), Trinity (9), Tulare (48), Tuolumne (34), Ventura (23), Yolo (26), and Yuba (13).

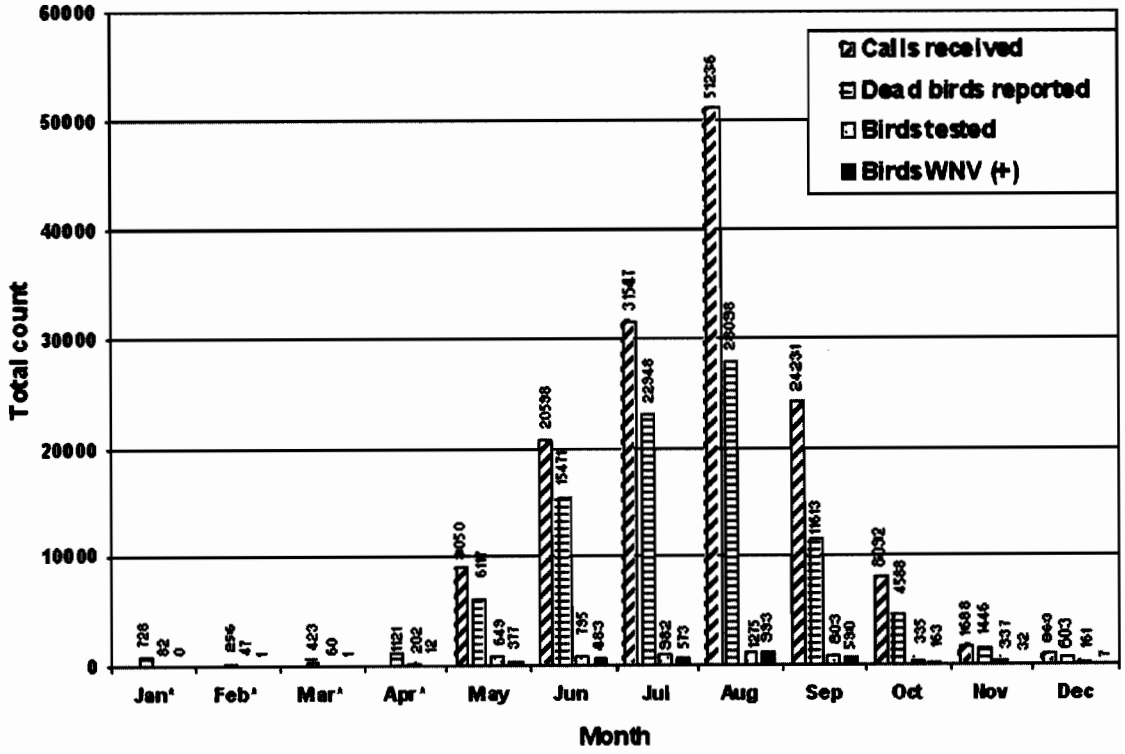

Figure 4. Summary information from the Dead Bird Surveillance Program, 2004.

*The number of calls was not tallied for January through April.

WEST NILE VIRUS ACTIVITY IN SOUTHERN CALIFORNIA

Southern California was the center of the West Nile virus epidemic. The virus was first detected in February from a dead American crow in Los Angeles County. The virus was also detected from dead birds collected from neighboring Imperial, Orange, Riverside, San Bernardino, San Diego, and Ventura counties. In May, mosquito pools and chicken sera from Riverside County tested positive for WN and 5 human cases were diagnosed in San Bernardino County. These events triggered rapid and intensified surveillance and mosquito control efforts by local agencies. The southern counties also had the largest share of the human WNV

Table 12. Dead birds reported and tested for West Nile virus, 2004

	Corvid[1]			Non Corvid			All birds		
County	Reported	Tested	Positive	Reported	Tested	Positive	Reported	Tested	Positive
Alameda	326	37	5	1172	46	18	1498	83	23
Alpine	8	1	1	5	2	2	13	3	3
Amador	28	6	3	70	8	6	98	14	9
Butte	1267	142	115	367	5	3	1634	147	118
Calaveras	30	2	2	177	10	8	207	12	10
Colusa	49	19	18	22	3	3	71	22	21
Contra Costa	527	56	9	1624	49	10	2151	105	19
Del Norte	8	1	0	14	6	3	22	7	3
El Dorado	222	30	20	385	11	2	607	41	22
Fresno	1181	133	99	1362	40	17	2543	173	116
Glenn	293	81	72	57	3	3	350	84	75
Humboldt	71	17	4	129	32	12	200	49	16
Imperial	11	0		32	1	1	43	1	1
Inyo	60	16	5	82	13	7	142	29	12
Kern	672	92	61	1081	67	26	1753	159	87
Kings	110	19	9	146	6	2	256	25	11
Lake	159	37	29	120	4	1	279	41	30
Lassen	31	9	7	55	10	6	86	19	13
Los Angeles	24107	1064	818	7130	89	22	31237	1153	840
Madera	60	10	4	106	6	3	166	16	7
Marin	351	39	7	436	21	11	787	60	18
Mariposa	6	2	1	41	7	5	47	9	6
Mendocino	132	29	13	95	4	0	227	33	13
Merced	210	36	18	235	36	11	445	72	29
Modoc	4	0		20	2	1	24	2	1
Mono	23	7	4	46	4	2	69	11	6
Monterey	151	14	3	467	19	9	618	33	12
Napa	50	19	3	71	17	3	121	36	6
Nevada	125	28	22	203	4	4	328	32	26
Orange	3555	287	179	1077	97	46	4632	384	225
Placer	653	59	42	738	11	5	1391	70	47
Plumas	31	13	6	64	28	20	95	41	26
Riverside	5044	200	131	1810	49	8	6854	249	139
Sacramento	3284	256	142	2140	49	11	5424	305	153
San Benito	28	7	0	93	12	5	121	19	5
San Bernardino	8505	359	288	2918	27	1	11423	386	289
San Diego	462	172	21	862	162	13	1324	334	34
San Francisco	39	8	1	278	27	13	317	35	14
San Joaquin	599	80	46	694	30	11	1293	110	57
San Luis Obispo	161	29	10	510	25	5	671	54	15
San Mateo	184	29	10	476	20	5	660	49	15
Santa Barbara	191	49	6	304	13	1	495	62	7

continued »

County	Corvid[1]			Non Corvid			All birds		
	Reported	Tested	Positive	Reported	Tested	Positive	Reported	Tested	Positive
Santa Clara	439	106	28	1118	65	18	1557	171	46
Santa Cruz	108	18	11	442	41	25	550	59	36
Shasta	667	94	87	366	7	3	1033	101	90
Sierra	2	1	1	7	3	2	9	4	3
Siskiyou	38	11	9	85	31	25	123	42	34
Solano	307	31	14	517	7	3	824	38	17
Sonoma	477	66	24	767	51	25	1244	117	49
Stanislaus	983	109	79	804	13	3	1787	122	82
Sutter	268	57	25	204	5	3	472	62	28
Tehama	539	117	113	166	8	2	705	125	115
Trinity	4	0		33	11	9	37	11	9
Tulare	503	66	45	515	9	3	1018	75	48
Tuolumne	19	8	7	91	31	27	110	39	34
Ventura	642	63	18	737	42	5	1379	105	23
Yolo	654	58	21	364	12	5	1018	70	26
Yuba	133	18	13	107	0		240	18	13
unknown	136	0		91	0		227	0	0
Total	58791	4317	2729	34037	1411	503	92828	5728	3232

cases. Among the 829 human cases reported in 2004 in California, 330, 197, 116, and 64 cases were from Los Angeles, San Bernardino, Riverside and Orange counties, respectively.

Because of the early detection of WNV in 2004 and the detection in 2003, local health departments and vector control districts started early in preparation and execution of their response plans. Several counties, including Los Angeles, San Bernardino, and Riverside, conducted monthly county level multi-agency WNV Task Force meetings. These meetings provided opportunities for information update and exchange as well as for further planning in control and prevention of WNV. Staff from CDHS/VBDS and the State Office of Emergency Services also participated in these meetings and provided coordination and assistance.

Several local agencies used adult mosquito control measures to lower the adult mosquito populations in some of the areas with increased risks to WN by the public. For example, San Bernardino County Vector Control Program staff applied adulticides (either Scourge® or Pyrenone 25-5) in Fontana, Colton, Loma Linda, Grand Terrance, Needles, Redlands, and San Bernardino. San Gabriel Valley Vector Control District personnel applied Scourge® at the Los Angeles County Arboretum and Santa Anita Racetrack in Arcadia, and Greater Los Angeles County Vector Control District staff applied Scourge® along the San Gabriel and Rio Hondo Rivers. These applications decreased mosquito populations, reducing the risk of WNV transmission to humans and domestic animals.

WEST NILE VIRUS IN THE UNITED STATES

The end of 2004 had identified WNV activity in 47 states, Puerto Rico and the District of Columbia. The 2004 WNV epidemic and epizootic transmission resulted in reports of 2,432 human cases of WNV disease (87 fatal). Significant human disease activity was recorded from these following states: California (829), Arizona (390), Colorado (276), and Texas (152).

In addition, 5,660 dead corvids and 1,414 other dead birds with WNV infection have been reported from 46 states and New York City. WNV infections have been reported in horses in 37 states; 1,429 sentinel chicken flocks in 14 states; and 8,263 mosquito pools in 38 states, DC, and New York City.

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The authors are grateful to Curtis Fritz, VRDL; the CDHS Infectious Disease Branch especially Kristeen De Fuentes, Jason Stull, and Bethanne Palermo; CVEC and Chris Barker; participating local mosquito and vector control agencies; CAHFS including Leslie Woods, DVM; local health departments including the Los Angeles County Health Department (Roshan Reporter, MD, and Karen Ehnert, DVM); the CDFA Animal Health Branch; and physicians and veterinarians who submitted specimens from clinical cases.

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Factors Affecting the Probability of Mosquito-borne Virus Activity in California Vector Control Districts, 1983-2003

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

[1]*Center for Vectorborne Diseases, School of Veterinary Medicine, University of California, Davis, CA 95616*
[2]*Department of Entomology, University of California, Davis, CA 95616*

ABSTRACT: California vector control agencies have an ongoing need for identification of risk factors contributing to the probability of western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) activity within a particular year. This study used logistic regression analysis to identify important predictors of WEE and SLE activity in five vector control agencies in California. The variables considered included seasonal trap counts for *Culex tarsalis* Coquillett, meteorological factors (temperature and precipitation), indicators of agency surveillance effort (budget and number of sentinel chicken flocks) and the Southern Oscillation Index. This study shows that early-season (winter and spring) predictors have significant effects on the probability of subsequent seroconversion to WEE and SLE. Winter precipitation and spring mosquito abundance had the greatest effect on the probability of WEE seroconversion, while winter temperature and summer mosquito abundance had the greatest effect on the probability of SLE seroconversion. The potential effect of El Niño events on the probability of WEE seroconversion is also discussed.

INTRODUCTION

Since the formal inception of a California state-level arbovirus surveillance program in 1969, the activity of two mosquito-borne encephalitis viruses of public and veterinary health interest, western equine encephalomyelitis (WEE, family *Togaviridae*) and St. Louis encephalitis (SLE, family *Flaviviridae*), has been monitored intensively by vector control agencies, public health personnel, and university researchers. Response plans and surveillance guidelines have been drafted periodically to provide guidelines for responses by appropriate agencies during periods of increased risk for virus activity (Walsh 1987, Reisen 1995a), but most of these documents have not provided specific, quantitative estimates of virus transmission risk. A notable exception is the semi-quantitative risk model introduced in the recent California Mosquito-borne Virus Surveillance and Response Plan (California Department of Health Services et al. 2004), but this plan gives equal weight to all risk factors without accounting for the varying degrees to which each component risk factor contributes to overall risk.

Many factors affect arbovirus amplification and transmission. Temperature affects the rates of mosquito development and of virus replication, and precipitation affects availability of mosquito breeding habitat directly as rainfall and indirectly through winter snowpack in the Sierra Nevada mountains that later appears as runoff that creates breeding habitats. Broader influences such as the El Niño-Southern Oscillation (ENSO) affect weather patterns throughout California to varying degrees. In many areas, humans also play an important role in determining the amount of available mosquito breeding habitat through irrigation strategies and control of water releases from reservoirs. The surveillance effort of each individual mosquito or vector control agency also is expected to affect the likelihood of virus detection. Development of a model that incorporates these factors to predict periods of virus activity would enable mosquito and vector control districts to prepare for periods when WEE or SLE activity is most likely and will allow agencies to conserve financial resources during periods when the probability of arbovirus activity is lower. Other studies have used multiple logistic regression to identify important predictors for vector-borne diseases such as Ross River Virus (Woodruff 2002) and visceral leishmaniasis (Elnaiem 2003).

In this study, logistic regression was used to quantify the effects of a number of potential predictors on the probability of detection of WEE and SLE in five areas of California with the following objectives: 1) to determine the effect of spring and summer mosquito abundance on the probability of detecting activity of two arboviruses, WEE and SLE, and 2) to identify other important predictors of WEE and SLE activity (e.g., vector control district effort, meteorological factors such as precipitation and temperature, or ENSO signals).

MATERIALS AND METHODS

Study Areas. This study was conducted using data from five vector control agencies in California (Fig. 1). These agencies were selected because they represent a variety of ecological zones and each agency has had a well-documented and extensive surveillance program for mosquitoborne viruses during the period from 1983-2003. Coachella Valley Mosquito and Vector Control District (CVMVCD) is located in California's warm southeastern desert, where annual precipitation is very low and temperatures are very high. Greater Los Angeles Vector Control District (GLACVCD) is in an urban coastal region with moderate annual rainfall and moderate temperatures. Kern MVCD is in the southern end of California's Great Central Valley, where annual rainfall is low and temperatures are high, particularly in summer. Sacramento-Yolo

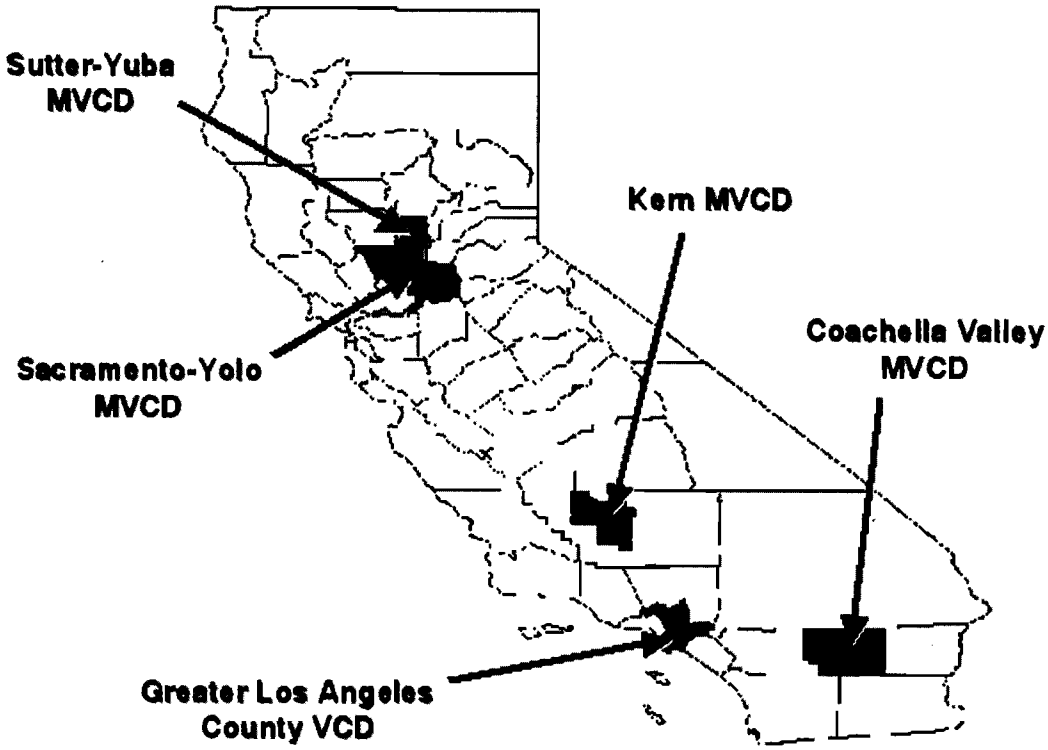

Figure 1. Vector control agencies included in this study.

and Sutter-Yuba MVCDs are in the north-central portion of the Great Central Valley, where annual rainfall is low-to-moderate and temperatures are cool during winter and hot during the summer. Sacramento-Yolo MVCD and Kern MVCD also include the Sacramento and Bakersfield urban areas, respectively, within their boundaries. In all of these agencies, there is very little rainfall from May-October.

Data Collection. Information on sentinel chicken seroconversions and numbers of flocks for each agency was primarily obtained from annual reports by the California Department of Health Services-Vector-borne Disease Section (CDHS-VBDS) published in the annual Proceedings and Papers of the Mosquito and Vector Control Association of California (Proc. & Papers, MVCAC) and from long-term summaries by Hui et al. (1999) and Steinlein et al. (2003). Supplemental information was found in the Annual Progress Reports of the Arthropod-borne Virus Research Unit (AVRU) of the University of California Berkeley's School of Public Health, weekly Arbovirus Surveillance Bulletins published by CDHS-VBDS and other electronic data sources previously transferred from tape backups from AVRU.

The mosquito collection data used in this study were obtained from historical archives maintained by the individual vector control agencies. Paper and/or electronic trap-by-trap mosquito collection records were obtained from each agency for the period from 1983-2003. For records that were in paper format, the collections were entered into a Microsoft Access 2000 database (Microsoft Corporation, Redmond, WA). Numbers of *Culex tarsalis* Coquillett, the principal vector of WEE and SLE (Reeves and Hammon 1962), were averaged over months and quarters of each year to get an average number of mosquitoes/trap-night. These monthly and quarterly averages of *Cx. tarsalis*/trap-night for each year were expressed as a percentage of the long-term average from 1983-2003. These percentages were the variables considered for inclusion in the logistic regression model.

Agency annual budgets and land areas covered were obtained from MVCAC yearbooks. Each budget was adjusted to 2003 U.S. dollars before dividing by the area to get a budget/ square mile. The conversion to 2003 U.S. dollars was done by multiplying the budget's proportion of the U.S. GDP per capita in the study year by the U.S. GDP per capita in 2003 (Williamson 2004).

Precipitation and temperature data were obtained from selected representative stations within each agency (UC IPM 2004; Table 1). Using these temperature data, degree-days also were calculated for each study area using the single sine method with a lower threshold of 7.3°C, the average of the lowest and highest estimates of the zero development threshold for *Cx. tarsalis* (4.6-10.0°C; Reisen 1995b). Degree-days were summed over quarters for potential inclusion in the logistic regression model, and precipitation was summed over months and from October-March, January-March, and March-May for potential inclusion in the models.

Table 1. Meteorological stations selected to represent the study areas. Small numbers of missing observations from the primary stations were replaced by neighboring backup stations (see table footnotes).

Study Area	Temperature & Precipitation[a]
Coachella Valley MVCD	Indio Fire Station[b]
Greater L.A. County VCD	Santa Ana Fire Department[c]
Kern MVCD	Bakersfield Airport[d]
Sacramento-Yolo MVCD	Sacramento Executive Airport[e]
Sutter-Yuba MVCD	Marysville Fire Department[f]

[a] data source: UC Statewide IPM, 2004 – see references.
[b] cities for backup stations: Indio and Thermal, CA.
[c] cities for backup stations: Santa Ana and Tustin, CA.
[d] cities for backup stations: Shafter, CA.
[e] cities for backup stations: Davis, CA.
[f] cities for backup stations: Yuba City and Nicolaus, CA

A summary of all potential predictors considered for inclusion in the models is presented in Table 2.

Data analysis. Separate logistic regression models were fit for WEE and SLE using R version 2.0.1 (R Development Core Team 2004). To account for the differences in baseline probability of seroconversion detection for each agency, due to factors such as coop design, fixed effects for the agencies were included in all models with Kern MVCD as the reference agency. Also, because virus activity in one year seemed to increase the potential for virus activity the following year, a single autoregressive term was included in all models as a binary indicator of seroconversion to the respective virus during the previous year.

For each virus, all other variables were added singly to the baseline model. Each of these models was compared to the baseline model using a likelihood ratio test, and factors producing significant reductions in deviance (alpha=0.05) were retained for further consideration in a forward stepwise selection procedure. Factors causing the largest change in deviance were added first, then combined with other significant predictors to determine if they resulted in a further significant reduction in deviance. Biological and practical importance was also considered in addition to statistical significance in selecting the final model for each virus. Model fit was assessed using the Hosmer-Lemeshow goodness of fit statistic (Hosmer and Lemeshow 2000).

RESULTS

WEE regression model. The model selection procedure for WEE resulted in a model with the baseline autoregressive and agency terms plus January-March cumulative precipitation and April-June average Cx. tarsalis females/trap-night as a percentage of the 21-year average. This model had a deviance of 91.18 on 95 d.f., compared to 110.43 on 97 d.f. for the baseline WEE model, meaning that the likelihood ratio test statistic comparing the two models was equal to 19.25 on 2 d.f. ($p<0.001$). The Hosmer-Lemeshow goodness-of-fit statistic was equal to 7.85 with $p = 0.45$, suggesting that the model fit the observed data well. Adding the

Table 2. Potential predictor variables for detection of WEE or SLE seroconversions in sentinel chickens.

Variable	Description
Adjusted budget	Agency's annual budget /sq. mile, adjusted to 2003 U.S. dollars
Agency variables	4 binary agency variables (1 if agency, 0 else)
Seasonal degree-days	Number of degree-days for winter, spring, or summer quarter[a]
Southern Oscillation Index	Mean of monthly December-February standardized SOIs
Number of chicken flocks	Number of sentinel chicken flocks maintained by the agency
Cx. tarsalis females, seasonal	*Cx. tarsalis* females/trap-night for spring or summer quarter[b]
Cx. tarsalis females, monthly	Monthly *Cx. tarsalis* females/trap-night for April-October[b]
Precipitation, fall-winter	Cumulative water-year precipitation (cm) from October-March
Precipitation, winter	Cumulative precipitation (cm) from January-March
Precipitation, winter-spring	Cumulative precipitation (cm) from March-May
Precipitation, monthly	Monthly cumulative precipitation (cm) for January-May
WEE previous year	WEE seroconversions in previous year (1 if yes, 0 if no)
SLE previous year	SLE seroconversions in previous year (1 if yes, 0 if no)

[a] method = single sine, lower threshold = 7.3°C
[b] expressed as a percentage of the 21-year average from 1983-2003.

December-February average Southern Oscillation Index to the model instead of January-March precipitation yielded a fit similar to that of the model chosen (deviance = 90.392 on 95 d.f.), but the model with precipitation was selected because precipitation is a more familiar and interpretable predictor.

SLE regression model. The model selection procedure for SLE resulted in a model with the baseline autoregressive and agency terms plus January-March cumulative degree-days and July-August average *Cx. tarsalis* females/ trap-night as a percentage of the 21-year average. This model had a deviance of 61.48 on 95 d.f., compared to 72.20 on 97 d.f. for the baseline WEE model, meaning that the likelihood ratio test statistic comparing the two models was equal to 10.72 on 2 d.f. ($p<0.01$). The Hosmer-Lemeshow goodness-of-fit statistic was equal to 5.13 with $p = 0.74$, suggesting that the model fit the observed data well. Adding the April-June degree-days to the model instead of July-August *Cx. tarsalis* counts yielded a fit similar to that of the model chosen (deviance = 61.852 on 95 d.f.), but the model with *Cx. tarsalis* was selected because of correlation between the two quarterly degree-day factors (r = 0.63).

WEE effect measures. Probabilities of WEE seroconversion are plotted against January-March cumulative precipitation in Fig. 2. The probability of WEE seroconversion increases with precipitation and is higher for a year in which the preceding year had at least one WEE seroconversion. Likewise, the probability of WEE seroconversion also increases with April-June *Cx. tarsalis* abundance, and the probability of WEE seroconversion is highest for the CVMVCD (Fig. 3). Odds ratios and confidence intervals are shown in Table 3.

SLE effect measures. Probabilities of SLE seroconversion are plotted against January-March cumulative degree-days in Fig. 4. The probability of SLE seroconversion increases with degree-days, approaching certainty for the higher numbers of degree-days in the CVMVCD. The point estimates indicate that the probability of SLE seroconversion is higher for a year in which the preceding year had at least one SLE seroconversion, but the confidence interval for the odds ratio includes 1, indicating that this effect is not significant for the sample size in this study (Table 4). The probability of SLE seroconversion decreases with increasing July-August *Cx. tarsalis* abundance, and based on point estimates, the probability of SLE seroconversion is highest for the CVMVCD (Fig. 5), although the wide confidence intervals for the odds ratios show that this effect was not significant in this study (Table 4).

DISCUSSION

The risk for both WEE and SLE seroconversions depends on *Cx. tarsalis* abundance as measured by New Jersey light trap counts, but the effect of the abundance is most significant at different times of the year for the two viruses. The probability of seroconversion for WEE is affected positively by *Cx. tarsalis* abundance in the spring (April-June), which is the period of enzootic amplification for the virus. During this period, the virus is not normally detected by surveillance tools, such as sentinel chickens, but this may be the most critical period for controlling the vector populations to limit the probability of subsequent WEE seroconversions. Conversely, the probability of SLE seroconversion is negatively associated with July-August *Cx. tarsalis* abundance, which was an unexpected result. The July-August period is the transmission period following the spring amplification period of the SLE annual cycle and is the period during which the virus is often detected by surveillance tools. In areas like the CVMVCD where summer temperatures are very high, mosquito survival often declines during the hottest summer months (July-September), reducing their abundance. This same temperature increase also increases the rate of virus replication and thus decreases the virus's extrinsic incubation period, so perhaps this is the reason for the negative association between late summer *Cx. tarsalis* abundance and the probability of SLE seroconversion. In any case, it seems illogical that having fewer mosquitoes alone results in greater risk for SLE transmission, so this variable must be a surrogate for another variable that we have not included. We will be examining this in additional studies.

We also found that both SLE and WEE seroconversion probabilities are affected by winter meteorological factors. This is encouraging in light of the need for early prediction of virus activity to allow vector control agencies to prepare appropriately for periods of elevated risk. With early warning of conditions favorable or unfavorable to activity of WEE or SLE, agencies would know whether to supplement or conserve financial resources and whether an increase in early season vector control efforts is needed.

Based on this study, risk for WEE seroconversion depends more heavily on winter precipitation than temperature, while the opposite is true for SLE. This agrees with previous research that indicates that SLE requires a higher temperature for replication and transmission than WEE (15 and 10°C, respectively; Reisen et al. 1993). Perhaps most years meet the temperature requirements for sufficient amplification of WEE, meaning that whether or not a particular year has WEE activity would be principally dictated by other factors, while the temperature requirements for SLE activity are met less frequently and thus are subject to a greater temperature influence than WEE. Winter precipitation (and resulting snowpack) only partially determines the extent of mosquito breeding habitat during the following virus transmission season. Water for irrigation and wildlife habitats is intensively managed throughout most of California, and decisions about water allocation and method of delivery undoubtedly modify the direct effects of precipitation on mosquito habitat availability. We will examine these factors more closely in future studies.

Another interesting effect that was not included in the selected model for WEE was the effect of the December-February Southern Oscillation Index (SOI) on the probability of subsequent WEE seroconversion. The SOI is the standardized difference between sea level pressure at Tahiti and Darwin, Australia and is an important indicator of El Niño/Southern Oscillation events that influence weather events in California and elsewhere. A negative SOI is associated with El Niño events that are generally associated with a precipitation increase in California. For the WEE model that included the December-February SOI instead of January-March precipitation, the association of the SOI with the odds of WEE seroconversion was negative and significant, indicating that WEE seroconversions are likely to be associated with El Niño conditions. The predictive value of the SOI for WEE was similar to the value of California Department of Health Services, Mosquito and Vector

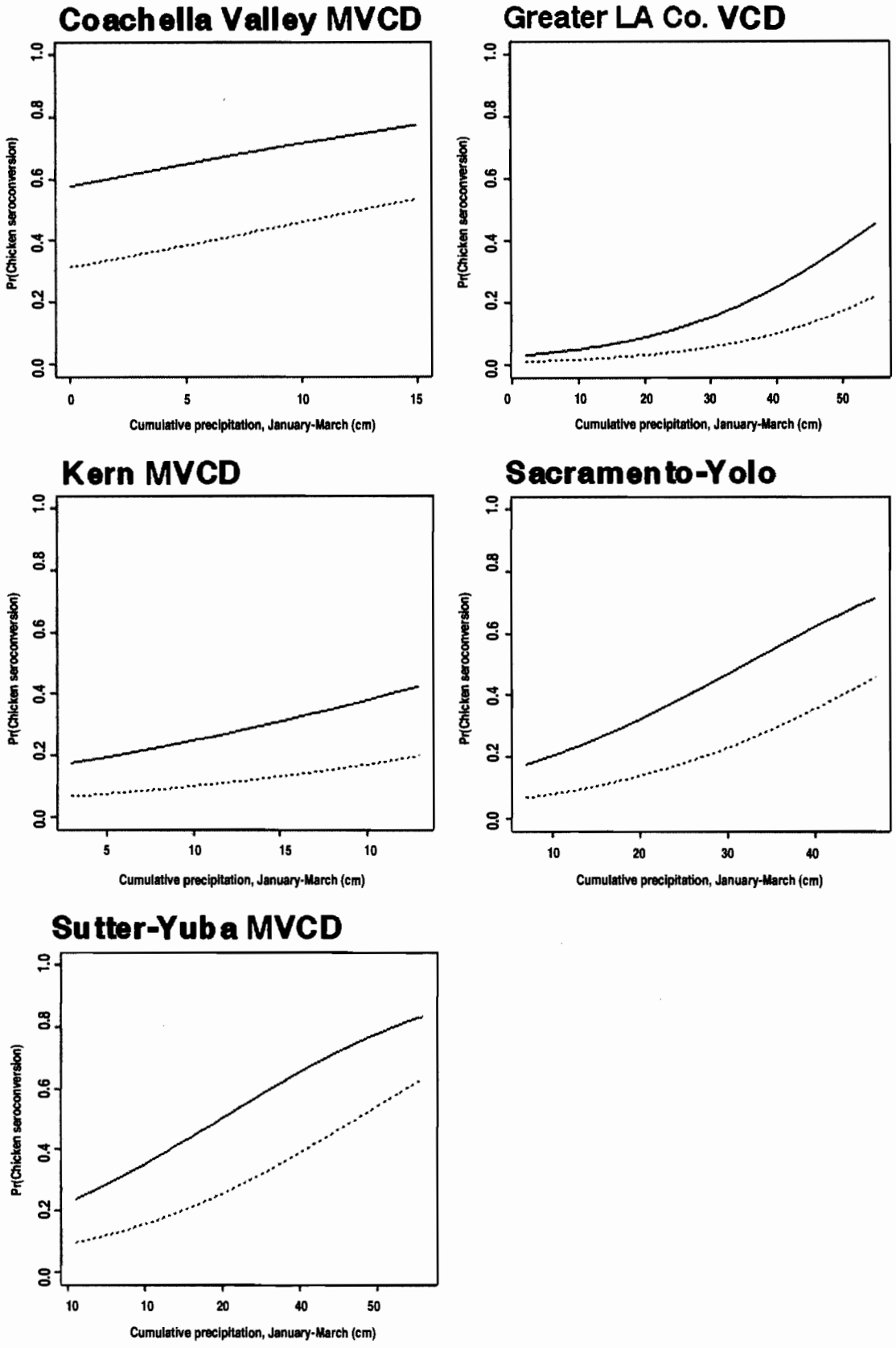

Figure 2. Probabilities of seroconversion for WEE by January-March cumulative precipitation for years in which the previous year had at least one WEE seroconversion (solid line) and in which the previous year had no WEE seroconversions (dashed line). Probability calculations are based on the median April-June *Cx. tarsalis* counts for each agency, and each chart is plotted over the respective agency's precipitation range between 1983 and 2003.

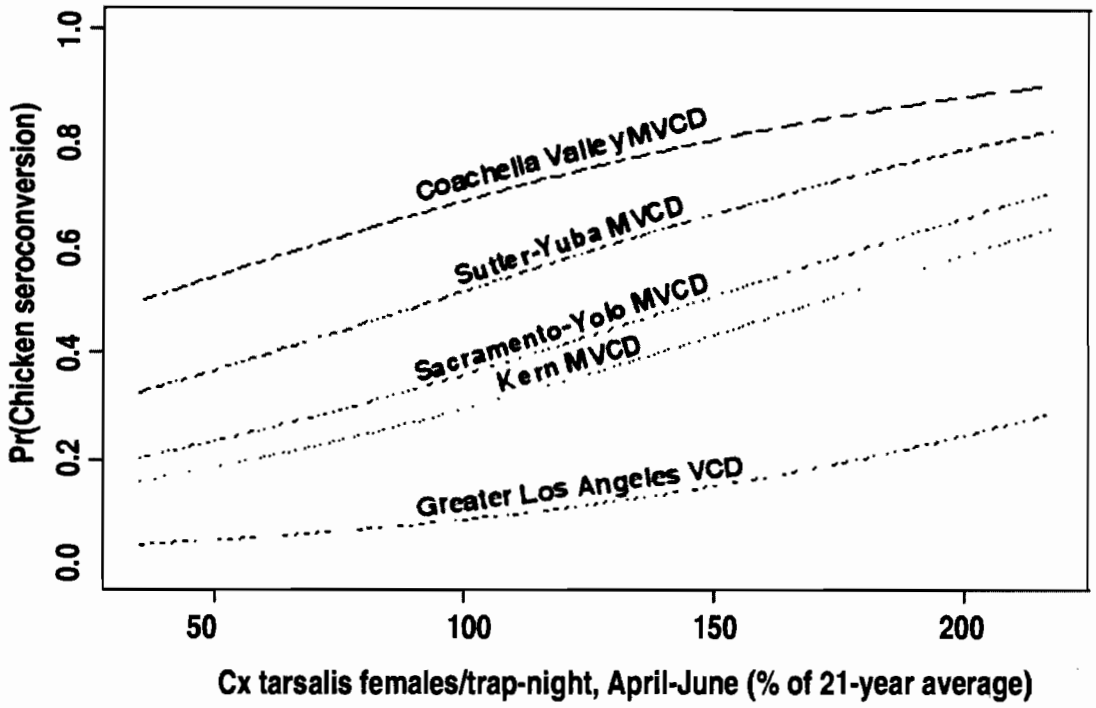

Figure 3. Probabilities of seroconversion for WEE by relative April-June *Cx. tarsalis* counts for a year in which the preceding year had a WEE seroconversion for each agency. Probability calculations are based on the median January-March cumulative precipitation for each agency, and the chart is plotted over a range of percentage values shared by all agencies.

Table 3. Odds ratios and confidence intervals for the selected WEE model.

Factor	Odds Ratio	95% CI	*p*-value*
Intercept			<0.001
WEE-previous year	2.94	(1.01, 8.51)	0.047
Coachella	7.92	(1.47, 42.85)	0.016
Los Angeles	0.14	(0.01, 1.67)	0.120
Sacramento-Yolo	0.61	(0.09, 4.10)	0.610
Sutter-Yuba	0.97	(0.13, 7.12)	0.975
Jan-Mar precipitation	1.36[a]	(1.05, 1.76)	0.018
Apr-Jun *Cx. tarsalis*	1.13[b]	(1.04, 1.22)	0.003

* based on the Wald statistic (bhat/Se(bhat))

[a] based on an increase of 5 cm

[b] based on an increase of 10% in *Culex tarsalis* relative to the 21-year average

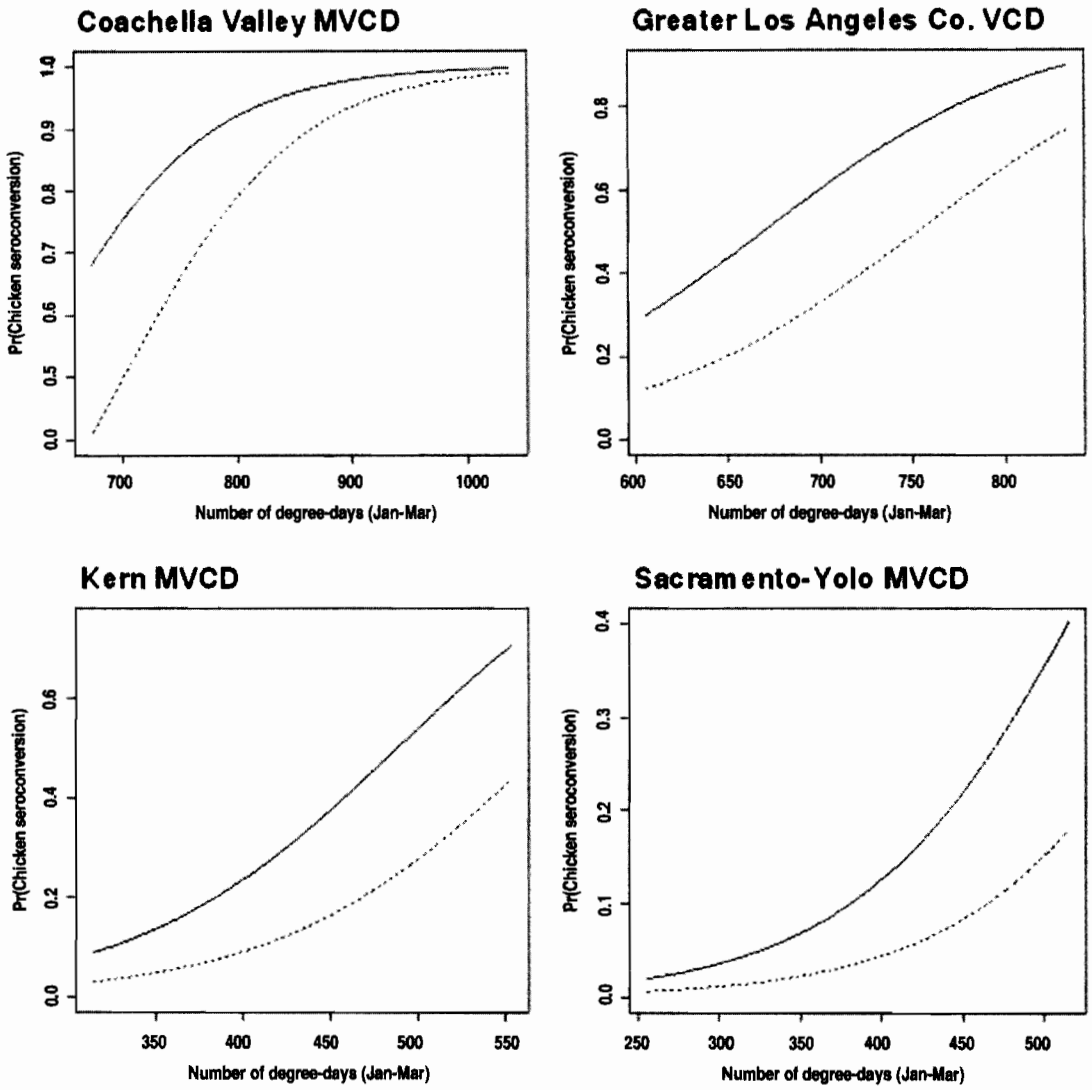

Figure 4. Probabilities of seroconversion for SLE by January-March degree-days for years in which the previous year had at least one SLE seroconversion (solid line) and in which the previous year had no SLE seroconversions (dashed line). Probability calculations are based on the median July-September *Cx. tarsalis* counts for each agency, and each chart is plotted over the respective agency's degree-day range between 1983 and 2003. Sutter-Yuba MVCD did not have any SLE seroconversions during the study period and is not included.

Table 4. Odds ratios and confidence intervals for the selected SLE model.

Factor	Odds Ratio	95% CI	*p*-value*
Intercept			0.018
SLE-previous year	3.06	(0.70, 13.34)	0.1364
Coachella	0.17	(0.00, 11.81)	0.413
Los Angeles	0.10	(0.00, 2.71)	0.174
Sacramento-Yolo	0.59	(0.05, 7.17)	0.677
Sutter-Yuba	N/A	N/A	N/A
Jan-Mar deg-days	1.95 [a]	(1.10, 3.46)	0.023
Jul-Sep *Cx. tarsalis*	0.88[b]	(0.78, 0.99)	0.040

*based on the Wald statistic (bhat/Se(bhat))

[a] based on an increase of 50 degree-days

[b] based on an increase of 10% in *Culex tarsalis* relative to the 21-year average

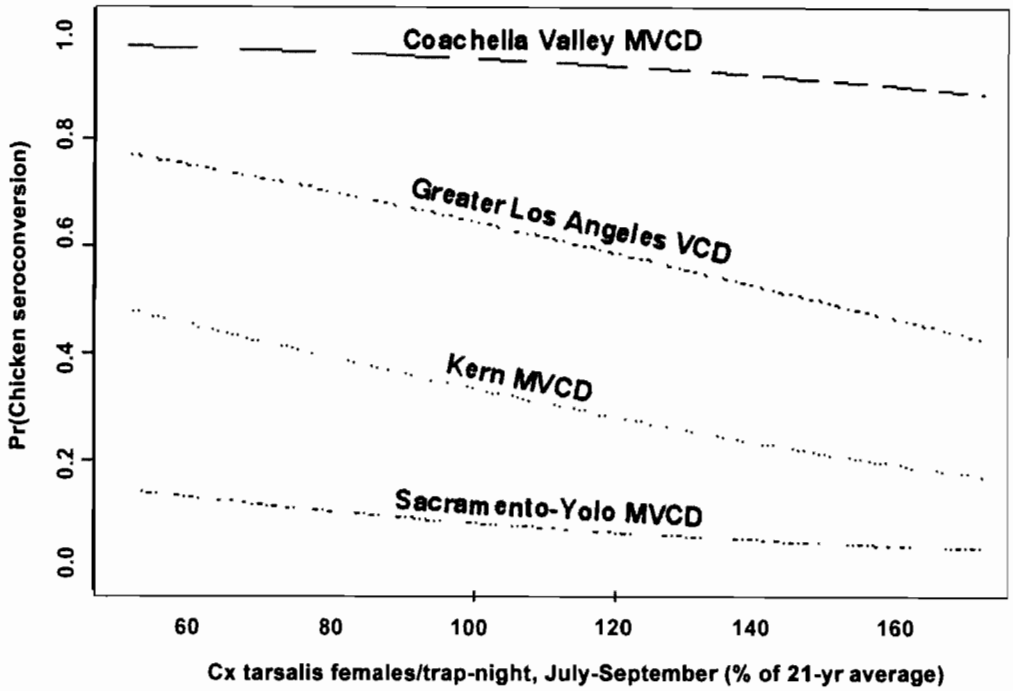

Figure 5. Probabilities of seroconversion for SLE by relative July-September *Cx. tarsalis* counts for a year in which the preceding year had an SLE seroconversion for each agency. Probability calculations are based on the median January-March cumulative degree-days for each agency, and the chart is plotted over a range of percentage values shared by all agencies. Sutter-Yuba MVCD did not have any SLE seroconversions during the study period and is not included.

January-March precipitation, but precipitation was included because of familiarity and ease of interpretation.

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Getting Connected: Progress for Surveillance Data Exchange

Bruce Eldridge

Department of Entomology and Center for Vectorborne Diseases, University of California, Davis. CA 95616

The current outbreak of human diseases caused by the West Nile virus in California has proven that the rapid exchange of surveillance data associated with viral activity is a vital component of the prevention of human cases through mosquito abatement. This is not only true of data exchanged among various state and local agencies involved in prevention and control, but also of data supplied to the public in a timely manner. The electronic California Vectorborne Disease Surveillance System, a collaborative effort among MVCAC, California Department of Health Services (CDHS), and the University of California Center for Vectorborne Diseases (CVEC) continued to evolve in 2004, and to improve its service to agencies needing both contemporary and historical surveillance data. One of the most important improvements for 2004 was the establishment of high speed data connections between the surveillance servers maintained by CVEC at UC Davis and selected testing laboratories and research collaborators. High speed connections should greatly improve the rapid and efficient use of this system. Figure 1 shows the current status of surveillance servers and databases and the agencies currently connected via direct connections and virtual private networks (VPN).

High speed connections to the surveillance servers. The surveillance servers at CVEC are a cluster of individual machines running software programs (called servers) that produce the general surveillance website, the West Nile virus website, maps that appear on the websites, and data that are stored in SQL databases. The maps and the websites depend on the databases for much of their information, and the databases can also be accessed directly. Most of the new connections use VPN operating over the Internet. With this type of connection, data are encrypted at the sending end and decrypted at the receiving end, so that interception of data by snooping parties is difficult, if not impossible. Connections of the CDHS Viral and Rickettsial Disease Laboratory Branch (VRDL) and the CDHS Vector-Borne Disease Section (VBDS) in Richmond to the surveillance servers were installed using special dedicated lines operating through firewalls at both ends of the connection. Agencies with VPN connections include the CVEC Arbovirus Research Laboratory (ARL), the Sacramento-Yolo Mosquito and Vector Control District, the Placer Mosquito Abatement District, the Arbovirus Field Station in Bakersfield, and the Scripps Institution of Oceanography in La Jolla.

Data input. Until this year, surveillance testing data were input to the surveillance servers using a variety of mechanisms, largely involving transfer via email of entire databases containing tables of test data. Although these connections were faster than using conventional mail or faxes, some time invariably elapsed before the email recipient downloaded the data, and it took additional time to manually append the data received into the central database. If the emails were sent over a weekend, the time lag was

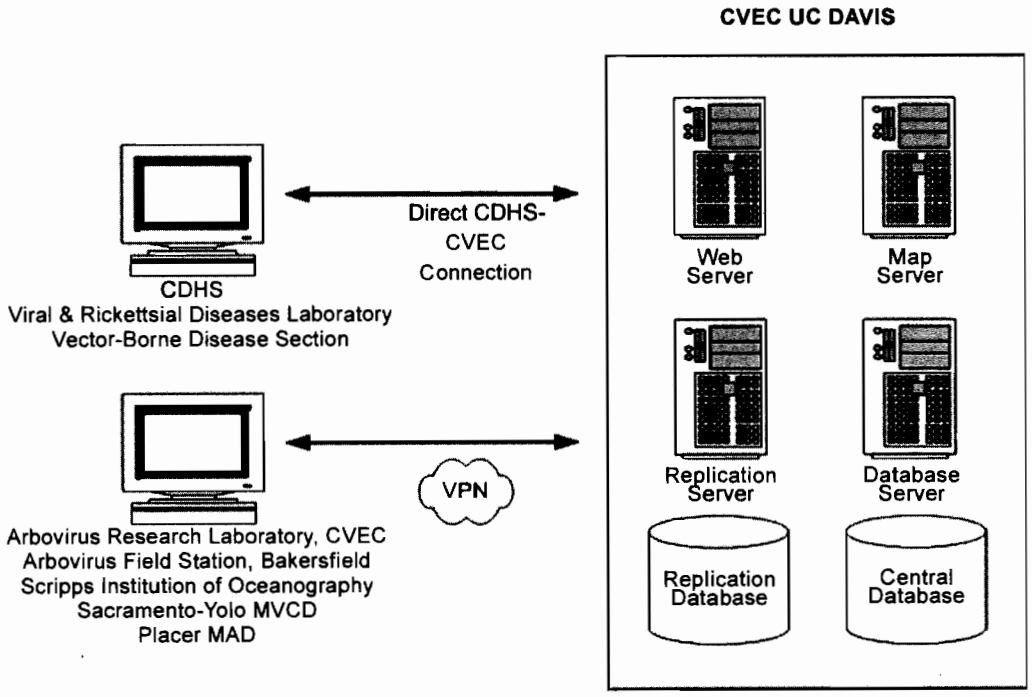

Figure 1. High speed data connections to and from the surveillance servers at UC Davis.

even longer. With the implementation of direct connections, new data can be entered into the central database directly by the testing laboratories. Direct data entry has several advantages other than speed. Because each data record must be entered only once, errors are reduced and resources needed to both enter and edit data are saved. The summer of 2004 marked the beginning of direct entry of mosquito pool test data into the central database, and arrangements for direct entry of sentinel chicken data from VRDL should be in place by February 2005.

Data output. In parallel with the establishment of direct connections for data input, several VPN connections have been installed permitting access to both current and historical surveillance data. To protect the integrity of the central database, data for output are continually replicated to a second database (the replication database), which is an exact copy of the data from the central database. The most straightforward approach for users to obtain data is by using Microsoft SQL client software. Because the surveillance data are hosted by Microsoft SQL Server software, data downloads are easy by sending simple SQL statements to the server over the VPN connection. Other choices are the use of dedicated front ends such as Vector Control Management System, and in-house front ends built with programming languages such as Visual Basic, Delphi, or Java. Data access is also easy using projects created in MS Access.

Websites. The general surveillance website (http://vector.ucdavis.edu) and the West Nile virus (http://westnile.ca.gov) websites are both hosted on the surveillance servers, but the content for the former is maintained by Chris Barker of CVEC; the latter by Lauren Marcus of DHS. Before the advent of the direct DHS connection, maintenance of the WN virus website required e-mailing of updated HTML documents from VBDS to CVEC. Now the content is updated directly, and new data are available virtually immediately.

The transition from static snapshot-style surveillance maps to dynamic interactive maps, created with Environmental Systems Research Institute's (ESRI's) ArcIMS, also was completed during early 2004. Now, visitors to the California vectorborne disease surveillance website can view maps in a window that will allow them to control the look of the map. Users can add or remove layers, zoom to the scale of their choice, or query the data on the map to find collection dates, species, or a variety of other information.

The Development of the California Department of Health Services West Nile Virus Website (www.westnile.ca.gov) as a Tool for Local Agencies, Information Distribution to the Public, Passive Surveillance, and Public Educators

Lauren Marcus[1], Bruce Eldridge[2], Stephen Lewis[3], Chris Barker[3], Teresa Nastoff[4], Carol Crawford[5], Lisa Richman[6], Rosanna Westmoreland[1], Stan Husted[1], and Vicki Kramer[7]

[1] *California Department of Health Services, Vector Borne Disease Section, 850 Marina Bay Parkway*
[2] *Department of Entomology, University of California at Davis, Old Davis Road, Davis, CA 95616*
[3] *Center for Vectorborne Diseases, University of California at Davis, Old Davis Road, Davis, CA 95616*
[4] *Division of Health Education and Promotion, Center for Disease Control*
[5] *Emergency Communication System (ECS), Office of Communication, Centers for Disease Control and Prevention*
[6] *Northrop Grumman – Center for Disease Control Information Technology Support Contract (CITS)*
[7] *California Department of Health Services, Vector Borne Disease Section, 1616 Capitol Avenue, MS 7307, P.O. Box 997413, Sacramento, CA 95889*

ABSTRACT: The California West Nile Virus Website, initially developed in 2002, has been expanded into a multifaceted tool for enhanced surveillance and information distribution. In 2004, the website was redesigned, with the aid of the Centers for Disease Control and Prevention (CDC), to increase usability. A new, local agency toolbar provides confirmation of submission data from local agencies to testing sites. Additional information on protection techniques and mosquito repellent use was added and us now available in a variety of languages. Public educators now have access to the above, current California West Nile virus data, pamphlets, and public service announcements to circulate within communities. These improvements have allowed the California Department of Health Services (CDHS) to streamline our surveillance program and reach a broader audience for prevention purposes.

Using Remote Sensing and Geographic Information Analysis Techniques for Surveillance and Abatement of Mosquitoes in Relation to West Nile Virus Activity in Monterey County, California

Krista Hanni[1], Darryl Tyler[2], Dennis Boronda[3], and Cindy Schmidt[4]

[1] *Monterey County Health Department, 1270 Natividad Road, Salinas, CA 93906*
[2] *Monterey County Information Technology, 1550 Moffet Road, Salinas, CA 93905*
[3] *Northern Salinas Valley MAD, 342 Airport Boulevard, Salinas, CA 93905*
[4] *NASA Ames Research Center, Mail Stop 242-4, Moffett Field, CA 94035*

ABSTRACT: West Nile Virus (WNV) surveillance, abatement, and response are primarily the responsibility of local government agencies, including public health departments and mosquito abatement agencies. It has been difficult for these agencies to plan localized abatement and surveillance activities for WNV due to the temporal and spatial variability of WNV hosts (reservoir and accidental) and the environment; and statewide predictive models often lack specificity for local agency decision-makers. A risk assessment was conducted in 2003 by local agencies in Monterey County, CA, and NASA that used geographic information systems and remote sensing technology to predict areas of potential highest WNV morbidity and mortality for affected human populations and for targeting limited resources for WNV abatement and surveillance activities. Landsat 7 images and geographic layers were used to map the extents of potential 1) WNV vector (mosquito) breeding source areas, 2) adult vector habitat areas, and 3) "higher-risk" mosquito vector source and habitat areas in Monterey County, California. The predictive capabilities of the model were assessed using 2004 WNV activity, as determined by human and animal surveillance, and vector activity, using mosquito reports to and activities of the county mosquito abatement and public health agencies. Areas of WNV activity in 2004 generally correlated with the model's predictions. The model thus provided an excellent assessment for enhancing ongoing surveillance activities prior to local arrival of WNV.

Developing and Using a Digital Inspection, Recording, and Management System for Monitoring West Nile Virus and Other Vector-borne Agents.

Kenn Fujioka, Ph.D.

San Gabriel Valley Mosquito and Vector Control District, 1145 N. Azusa Canyon Road, West Covina, CA 91790

ABSTRACT: This presentation covers the technical (hardware and software) aspects of automating the paper-based inspection and treatment recording method currently used by most mosquito and vector control districts. The benefits of using a Structured Query Language (SQL) database to effectively aid in reporting and managing outbreaks of West Nile virus (WNV) and other vector-borne diseases is discussed. The Spatial Data Server (SDS) displays information contained in the SQL Database as a map. When the system is adopted more broadly, then data can be aggregated to give a region, state, or nationwide view of the spread and effect of treatments on WNV.

Use of Gravid Traps for Collection of California West Nile Virus Vectors

Julie A. Christiansen[1], Charles Smith[2], Minoo B. Madon[3], John Albright[4], William Hazeleur[4], Jack Hazelrigg[3], Susanne Kluh[3], Rory D. McAbee[1], F.S. Mulligan III[2], Walter Leal[5] and Anthony J. Cornel[1*]

[1]*Mosquito Control Research Laboratory, Department of Entomology, University of California at Davis, 9240 S. Riverbend Avenue, Parlier, CA 93648, USA*
[2]*Consolidated Mosquito Abatement District, 2425 Floral Ave, Selma, CA 93662*
[3]*Greater Los Angeles County Vector Control District, 12545 Florence Ave, Santa Fe Springs, CA 90670*
[4]*Shasta Mosquito and Vector Control District, PO Box 990331, Redding, CA 96099*
[5] *Honorary Maede-Duffey Laboratory, Department of Entomology, University of California, Davis, CA 95616, USA*

ABSTRACT: Most California mosquito abatement districts use carbon dioxide baited traps to collect predominantly host seeking *Culex* females for arboviral surveillance and to assess overall abundance of disease vector and nuisance mosquitoes. The invasion of West Nile virus (WNV) has called for the need to isolate virus from and monitor population sizes of more diverse mosquito vector species and particularly *Culex pipiens sensu latu.* This past season we evaluated the efficiency of gravid traps using Bermuda grass and rabbit chow infusions in collection of *Cx. tarsalis* and *Cx. pipiens* complex and other WNV vectors in Shasta, Fresno and Los Angeles Counties. Bermuda grass and rabbit chow infused gravid traps were higher but not significantly more efficient in collection of *Cx. pipiens s.l.* and much less efficient in collection of *Cx. tarsalis* than CO_2 –baited traps in all three counties in both urban and rural locations. However, proportionately more WNV infected *Cx. pipiens s.l.* were collected in the gravid traps than in the CO_2-baited traps. Gravid traps using appropriate oviposition attractants are desperately needed to enhance collection efficiency of virus infected *Cx. tarsalis*

INTRODUCTION

For many years mosquito abatement districts in California have used carbon dioxide baited CDC-style light traps (Pfunter 1979) to collect mosquitoes in order to survey for abundance and presence of arboviruses in mosquito populations. This typically focused on collecting *Culex tarsalis* Coquillett, which is the most important vector of Western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) in California (Reeves 1990, Goddard et al. 2002). In 2004, however, West Nile virus (WNV) established itself as a threat to public health in California. Mosquito abatement districts responded to this threat with increased surveillance and control of many more mosquito species, focusing on what is believed to be the primary vector in other parts of the country, *Culex pipiens* (*sensu latu*). (Turell et al. 2000, Goddard et al. 2002). With *Cx. pipiens s.l.* and *Cx. tarsalis* the potential major vectors of this disease in California, a need has arisen for efficient methods to trap these mosquitoes.

The use of CDC traps is inherently flawed for virus surveillance as they primarily (>65%) collect unfed, nulliparous females (Barr et al. 1986, Reisen and Pfunter 1987, Reisen et al. 1995) which have not had contact with a viremic host. A more effective means of virus surveillance would be to sample with gravid traps (Reiter 1983), which should collect proportionately more gravid females that have ingested a blood meal and survived through the virus eclipse phase (Hardy et al. 1983). Previous studies conducted by others have shown that currently available gravid traps use attractants that do not readily attract *Cx. tarsalis*, even in areas where they are abundant as indicated by CO_2 traps. In addition, these attractants have offered very little improvement in collection of *Cx. pipiens s.l.* over CDC-style CO_2, New Jersey light traps (Mulhern 1942), or resting boxes (Myer 1985). The purpose of this study was to confirm the above findings and obtain base line data for later evaluation using novel attractants. We performed experiments to evaluate the efficacy of gravid traps using Bermuda grass and rabbit chow infusions, as well as CO_2-baited CDC light traps, to collect *Cx. pipiens s.l.* and *Cx. tarsalis* mosquitoes in urban and rural sites in Shasta, Fresno and Los Angeles Counties during the spring and summer of 2004. These three areas of California were selected as they represent all members of the *Cx. pipiens* complex as indicated by DV/D ratios (Sundararaman 1949) and diagnostic PCR assay (Smith and Fonseca 2004). In northern California, *Cx. pipiens* is the only member present, in southern California, *Cx. quinquefasciatus* predominates, while both forms as well as hybrids occur in the Central Valley (Tabachnik and Powell 1983, Urbanelli et al. 1997, Cornel et al. 2003).

MATERIALS AND METHODS

Trapping

Trapping was performed in northern (Shasta Co.), southern (Los Angeles Co.), and central (Fresno Co.) California by respective mosquito abatement district personnel. Districts used modified gravid traps based on Reiter (1983), and CO_2-baited CDC light traps (Pfunter 1979). Each district had four collection sites, two

each in rural and urban settings, at least 2 miles apart from one another. These collection sites were also selected based on a history of having abundant numbers of both *Cx. tarsalis* and *Cx. pipiens s.l.* At each collection site the three oviposition traps and the CO_2 trap were separated by roughly 100 m.

Starting in March, only two of the three oviposition traps at each site were used. One was infused with with rabbit chow + yeast, and the other with rabbit chow + yeast + lactalbumen hydrolysate. In June when Bermuda grass became available, all three traps at each site were used. The infusions tested from then on until the end of the season were rabbit chow + yeast, Bermuda grass + yeast, and Bermuda grass + yeast +lactalbumen hydrolysate. Oviposition traps were set out every two weeks and counts were taken of each mosquito collected, adults drowned on the attractant surface, and egg rafts laid. Oviposition attractants were rotated among the three traps located at each site every two week collecting period.

Attractant Preparation

The rabbit chow infusions were used based on rabbit chow efficacy in previous studies (Beehler and Mulla 1993, Lampman and Novak 1996). The rabbit chow + yeast infusion (RC) mix consisted of 12.1 g Purina Rabbit Chow (Purina Mills, St. Louis, MO) and 6.9 g of yeast (US Biochemical Corp., Clevland, OH) in 10 L of tap water. The rabbit chow + yeast + lactalbumen hydrolysate (RC+L) was prepared as above except for the addition of 6.0 g lactalbumen hydrolysate (US Biochemical Corp., Cleveland, OH).

The Bermuda grass + yeast infusion (BG) was prepared following the methods described by Isoe et al. (1995), which consisted of 22.5 g of dried, ground Bermuda grass (collected in Visalia, California, 2004) and 1.35 g of yeast in 10 L of tap water. The Bermuda grass + yeast + lactalbumen hydrolysate (BG+L) was prepared as above except for the addition of 1.35g of lactalbumen hydrolysate.

All four of the infusion mixes were allowed to mature for 7-12 days at its working concentration, at which time 2.5 L of each infusion was used in each of the four oviposition traps per trapping location.

Virus Isolation

Pools of mosquitoes (£50) collected within the jurisdiction of the Consolidated Mosquito Abatement District were sent to the University of California Davis, Center for Vectorborne Diseases (CVEC) for examining the presence of WN virus RNA by RT-PCR. For assessment of virus activity throughout the district, mosquitoes for virus surveillance were collected from gravid and CDC-style CO_2 traps from both inside and outside the study areas. Mosquitoes of each species that were collected in CDC-style CO_2 traps were kept separate from mosquitoes collected in gravid traps so that minimum infection rate comparisons could be made between the two trap types. Minimum infection rates were calculated on the basis of minimum numbers infected per thousand.

RESULTS AND DISCUSSION

Early season trapping (March through May) yielded no significant difference between rabbit chow (RC) infusion and rabbit chow with lactalbumem hydrolysate (RC+L) (data not shown). This prompted us to discontinue the use of RC+L once Bermuda grass infusion became available. When the data from all three California locations were pooled together, there was no significant difference (P=0.272) between the number of adult females collected with RC, BG, or BG+L (Fig. 1).

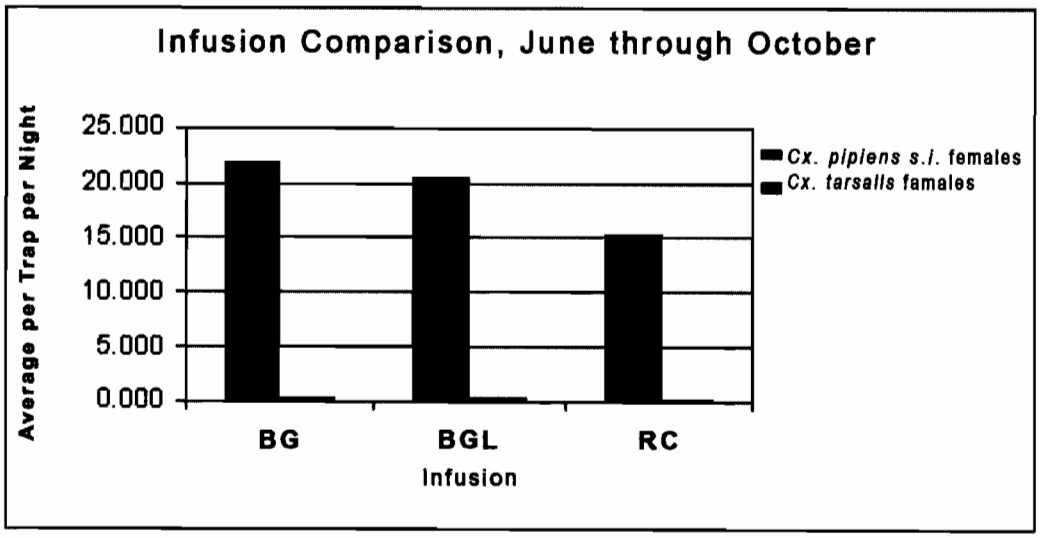

Figure 1. Comparison of adult females collected from June through October 2004, from oviposition traps containing Bermuda grass infusion (BG), Bermuda grass + lactalbumen hydrolysate (BGL), or rabbit chow.

All infusion mixtures collected low numbers of *Cx. tarsalis*. Carbon dioxide CDC-style traps collected similar numbers of *Cx. pipiens s.l.* as the RC oviposition trap did from March to October for all locations. CDC-style traps collected significantly ($P=2.27x10^{-14}$) more *Cx. tarsalis* than the RC baited oviposition trap for the same period (Fig. 2).

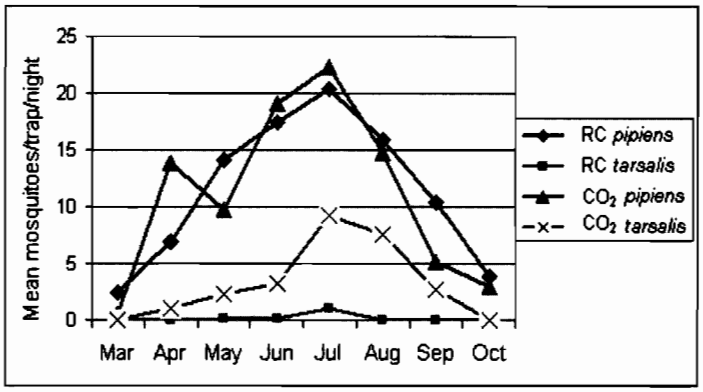

Figure 2. Comparison of adult mosquitoes collected from rabbit chow-baited oviposition traps (RC) versus CDC-style CO_2 baited light traps from March to October, 2004.

Both mosquito populations peaked in July, as indicated by each trap type. This indicates that oviposition traps with these attractants were not more efficient at collecting *Cx. pipiens s.l.* mosquitoes, and were much less efficient at collecting *Cx. tarsalis* females than CDC-style CO_2 traps. While the oviposition and CDC-style CO_2 traps collected similar numbers of *Cx. pipiens s.l.*, minimum infection rates from pools of *Cx. pipiens s.l.* mosquitoes collected in central California were consistently higher from oviposition traps than in CDC-style CO_2-baited light traps (Fig 3).

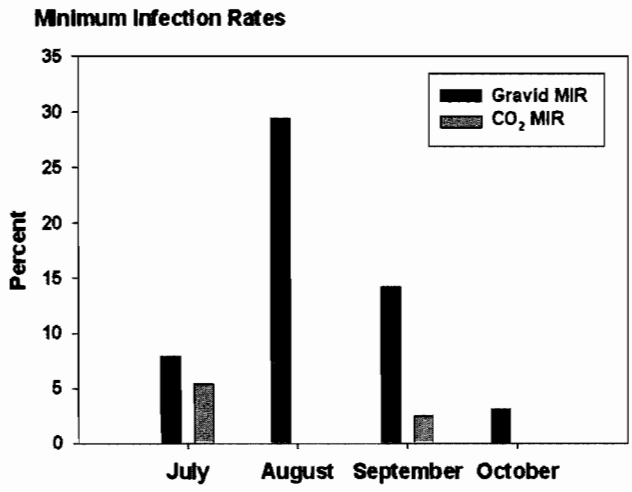

Figure 3. Minimum infection rates of *Culex pipiens s.l.* mosquito pools collected by Consolidated Mosquito Abatement District from CDC-style CO_2 baited light traps (CO_2 MIR) and oviposition traps (Gravid MIR).

The traps showed variability in collection of *Cx. pipiens s.l.* when each of the three locations were examined separately. Trapping numbers from Redding (Shasta Co.) indicate that there was no significant difference between BG and BG+L for urban or rural sites and that the bermuda grass infusions collected more than RC infusions in both urban and rural settings. The CO_2 – baited traps, however, outperformed all gravid trap infusions in rural sites, while performing poorly in urban areas (Fig. 4). In the Central Valley, there was also no difference between BG and BG+L for both urban and rural sites. However, the CO_2 traps performed poorly in both urban and rural sites (Fig. 5). In Los Angeles, there was similarly no significant difference between the BG and BG+L traps for rural or urban areas. In Los Angeles, the RC trap was the best at collecting urban mosquitoes, while the CO_2 trap was significantly the worst. In rural areas, the BG and BG+L did not collect significantly more mosquitoes than the CO_2 trap, while the RC collected the least (though not significantly less than the CO_2 trap)(Fig. 6).

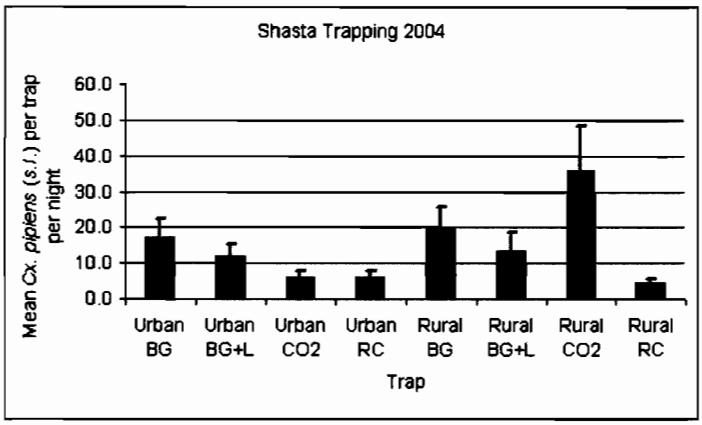

Figure 4. *Culex pipiens s.l.* collected from oviposition traps containing Bermuda grass infusion (BG), Bermuda grass + lactalbumen hydrolysate (BG+L), rabbit chow (RC), or CDC-style CO_2 baited light traps, in urban and rural locations in northern California.

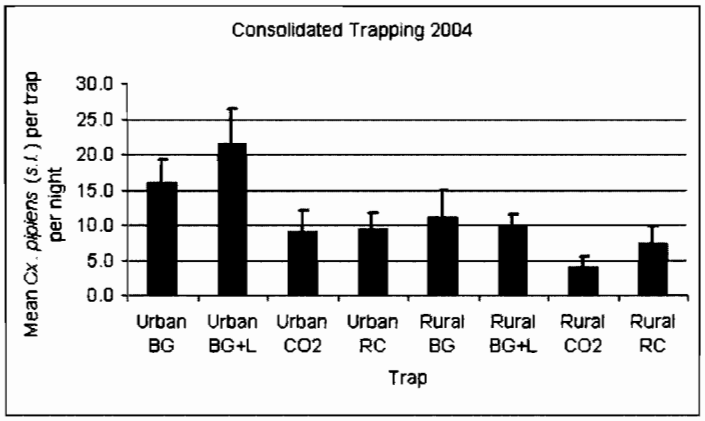

Figure 5. *Culex pipiens s.l.* collected from oviposition traps containing Bermuda grass infusion (BG), Bermuda grass + lactalbumen hydrolysate (BG+L), rabbit chow (RC), or CDC-style CO_2 baited light traps, in urban and rural locations in central California.

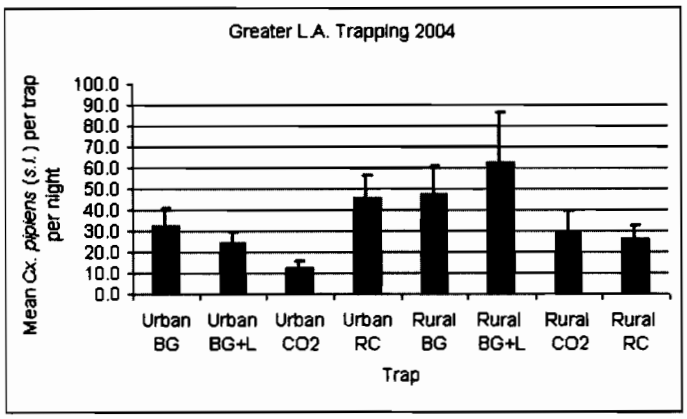

Figure 6. *Culex pipiens s.l.* collected from oviposition traps containing Bermuda grass infusion (BG), Bermuda grass + lactalbumen hydrolysate (BG+L), rabbit chow (RC), or CDC-style CO_2 baited light traps, in urban and rural locations in southern California.

These differences may be due simply to the variability of field studies or based on real genetic differences in chemical perception among members of the *Culex pipiens* complex with the latter option being tantalizingly interesting. The data so far suggests that *Cx. pipiens,* which occurs in northern California, is more rural and is more strongly attracted to water bodies containing infusions of bermuda grass. Conversely, *Cx. quinquefasciatus,* which predominates in the south is more urban, and is more likely to oviposit on water containing infusions of fermented processed rabbit food. A more mixed response in attractiveness of trap infusions occurs in the central valley where both subspecies and hybrids of the two all occur in sympatry in rural and urban settings.

Overall, Bermuda grass infusion seems to hold the most promise at this point for surveillance of West Nile virus in populations of *Cx. pipiens s.l.* as it collected the similar numbers of mosquitoes as the CDC-style CO_2-baited traps, yet with many more virus positive individuals. Surveillance using only the CDC-style CO_2-baitedtraps may greatly underestimate the amount of WN virus present in populations of *Cx. pipiens s.l.* Conversely, oviposition traps using these attractants are very poor tools for use in surveillance of *Cx. tarsalis.*

Acknowledgements

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Mosquito Surveillance and Control at an Urban Zoo

Chindi A. Peavey

San Mateo County Mosquito Abatement District, 1351 Rollins Rd. Burlingame, CA 94010

West Nile virus (WNV) presents a significant risk to animals held in zoos. The virus was first recognized at the Bronx Zoo in New York City in 1999. In 2002, the San Mateo County Mosquito Abatement District (SMCMAD) was asked by the San Francisco Zoo (the Zoo) to provide advice on mosquito control. The San Francisco Zoo is located in the city and county of San Francisco and is not covered by any established mosquito control district. It is owned and operated by a nonprofit organization, which is solely responsible for mosquito control on the property. Personnel at the zoo had begun developing a control program by identifying potential mosquito-breeding sites. The zoo had purchased 2 Mosquito Magnet® traps. The traps were placed near animals considered at special risk for WNV, such as flamingoes. Contents of the collecting bags were removed biweekly and stored for future identification. All animals found dead on zoo grounds, both collections and urban wildlife (such as birds or squirrels) were sent to Cornell University for virus testing.

In March 2002, SMCMAD staff conducted a site visit. To obtain preliminary information about the distribution and abundance of mosquitoes at the zoo, stored collections from the Mosquito Magnet® traps were counted and identified to species. The District then set 35 carbon dioxide-baited traps on zoo grounds. Traps were retrieved the following day. Further trapping was conducted in August, October and November.

The Zoo is located next to the Pacific Ocean just south of Golden Gate Park. It was built on beach dunes and has generally well-draining soils and a maritime climate. Cool damp air from the ocean strongly influence the climate. Precipitation averages 20 in / year. Fog envelopes the area every evening and often lingers for much of the following day. During winter, temperatures average about 50° F. The warmest weather usually occurs in September and October with average daily temperatures around 60° F. Lake Merced, a 50-acre reservoir fringed by tules is located ¼ mile southeast of the zoo (Fig. 1). A sewage treatment plant lies on the southern boundary of the zoo, across a parking lot. The plant is entirely underground and was completely renovated ~5 years ago.

West Nile virus was detected in wild bird carcasses for the first time in the San Francisco Bay area (Santa Clara County) in July of 2004. Virus was first detected in San Francisco County on August 17, 2004 and by the end of the year 14 wild bird carcasses

Figure 1. San Francisco Zoo

had tested positive in the County (Fig. 2). On November 2, 2004 a dead bird positive for WNV was collected 2 mi southeast of the zoo, near Lake Merced.

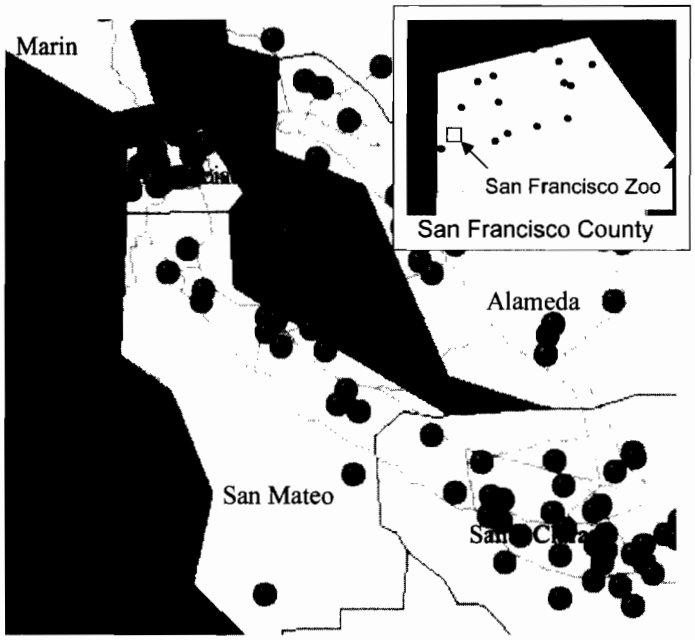

Figure 2. Geographic distribution of dead birds positive for West Nile Virus on the San Francisco Peninsula in 2004 (insert - San Francisco County).

MOSQUITO MAGNET COLLECTIONS

Collections from Mosquito Magnet® traps from August through December of 2002 were examined. Three species made up the bulk of mosquitoes collected during that period: *Culiseta incidens* (Thomson), *Culiseta particeps* (Adams), *Culex pipiens* L. (63%, 25%, and 12% of total, respectively). Figure 3 shows the seasonality and species distribution of mosquitoes in these collections. *Culiseta incidens* was the most prevalent mosquito in the traps during every month from August through December. The density of all 3 species reached its peak in September.

The Mosquito Magnet® traps had been shifted between specific locations at the zoo. Traps had been most consistently maintained in the flamingo enclosure and the penguin area, because these animals were considered particularly at risk. Cases of avian malaria occur regularly among the zoo's penguins. *Culex pipiens* is an efficient vector of this pathogen, which suggests that the penguins are regularly exposed to this species. However, mosquito density in Mosquito Magnet® traps in the penguin enclosure was consistently low. *Culex pipiens* was rare in these traps. The penguin enclosure and its surroundings are quite open. Winds sweep through the enclosure throughout the day in all seasons. However, the penguins sleep in alcoves set into an artificial hill made from concrete. These alcoves may also serve as shelters for host-seeking mosquitoes.

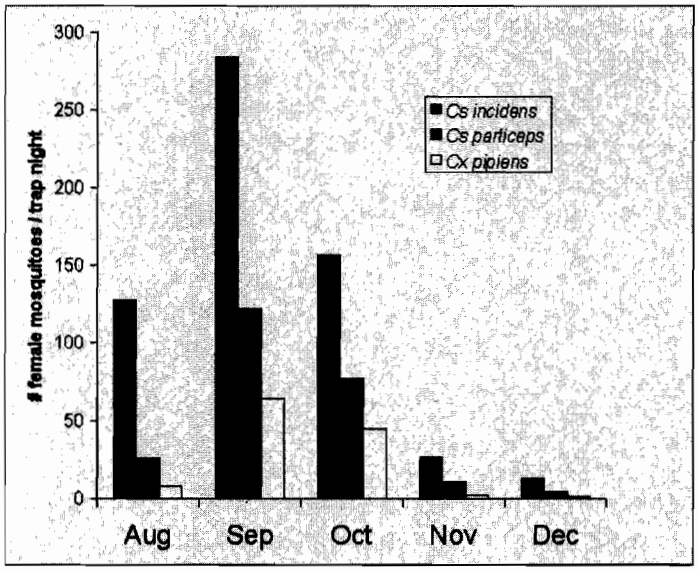

Figure 3. Species present in Mosquito Magnet traps at San Francisco Zoo in 2002.

CARBON DIOXIDE-BAITED TRAP COLLECTIONS

Carbon dioxide-baited traps were set on 4 occasions in 2003: March 22, August 1, October 10 and November 14. *Culiseta incidens* was the most commonly collected species during March and August, making up 50-90% of the mosquitoes collected (Fig. 4). However, in October and November, *Culex erythrothorax* became the most common species collected.

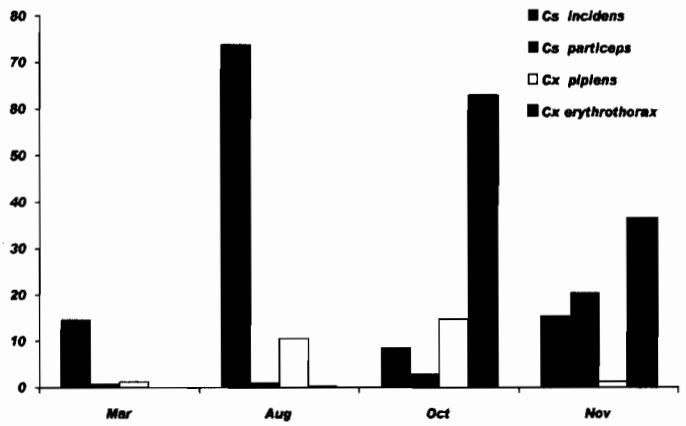

Figure 4. Species Distribution of female mosquitoes in carbon dioxide traps at San Francisco Zoo in 2003.

Figures 5 and 6 show the species distribution of mosquitoes collected in CO2-baited traps in March and October. In March, *Cs. incidens* was the most abundant mosquito in CO2-baited traps. It was collected throughout the zoo grounds. Density of *Cs. incidens* peaked in August. High densities at specific sites helped to focus inspections and identify problem areas. For example, containers stacked outdoors near the maintenance building were found infested with mosquito larvae. Water in the basement of an old pump house was another major source of mosquito development.

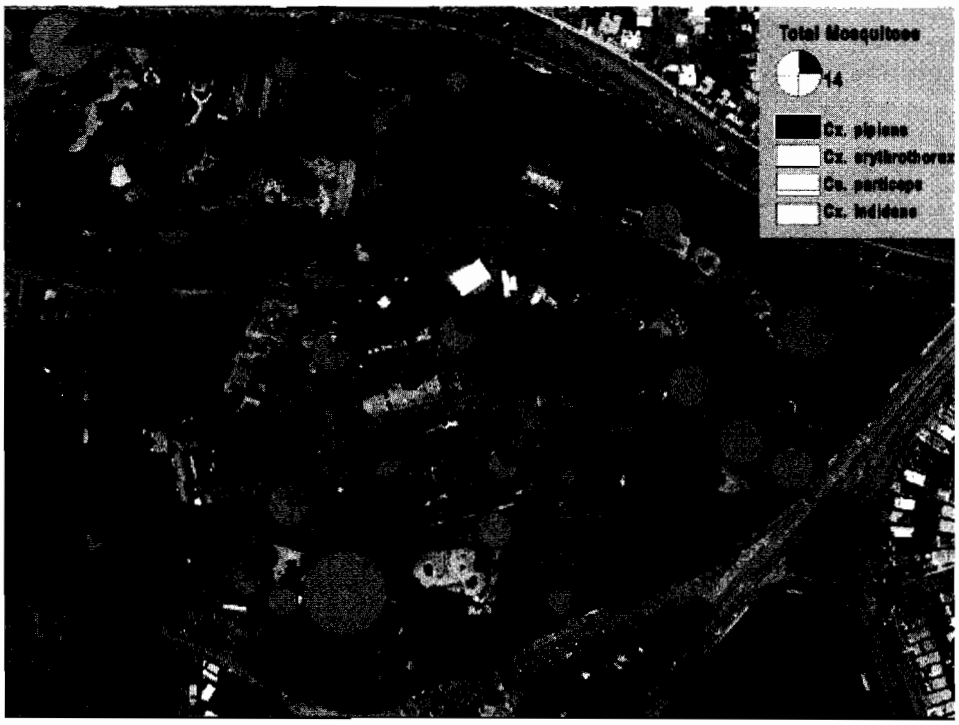

Figure 5. Species composition of mosquitoes collected in carbon dioxide-baited traps in March, 2003. Size of circle indicates total number of mosquitoes collected per trap night at each site.

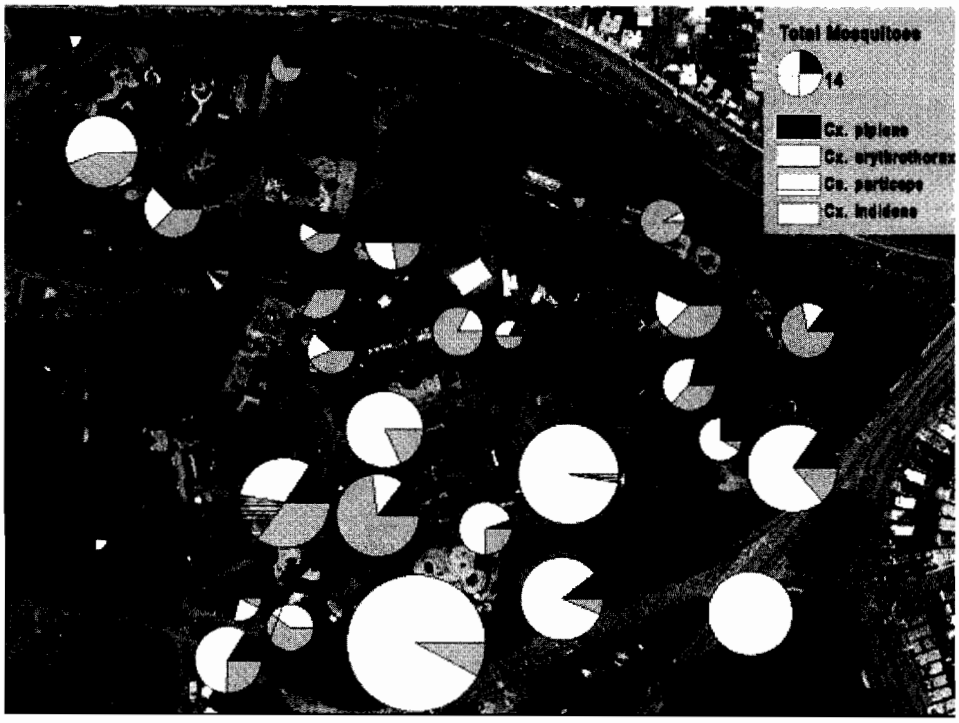

Figure 6. Distribution of mosquitoes in carbon dioxide-baited traps at San Francisco Zoo in October, 2003. Size of circle indicates total number of mosquitoes collected per trap night at each site.

Culex pipiens populations were low throughout the zoo. However, this species is a much more efficient vector of West Nile virus and presents a more significant health risk to humans and animals at the zoo.

In October, alarming numbers of *Cx. erythrothorax* Dyar began appearing in traps on the southeastern side of the zoo. *Culex erythrothorax* is also an efficient vector of West Nile virus. Larvae develop in tule marshes and do not generally travel far from the site of larval development. To determine whether these mosquitoes could be coming from tule marshes surrounding Lake Merced, additional traps were set in this area. These traps revealed very high populations of *Cx. erythrothorax* (up to 3,000 / trap night, Fig. 7). Collections of *Cx. erythrothorax* were greatest along the southeastern boundary of the zoo. *Culex erythrothorax* adults were also collected at a few sites near small ponds with patches of tule or cattails.

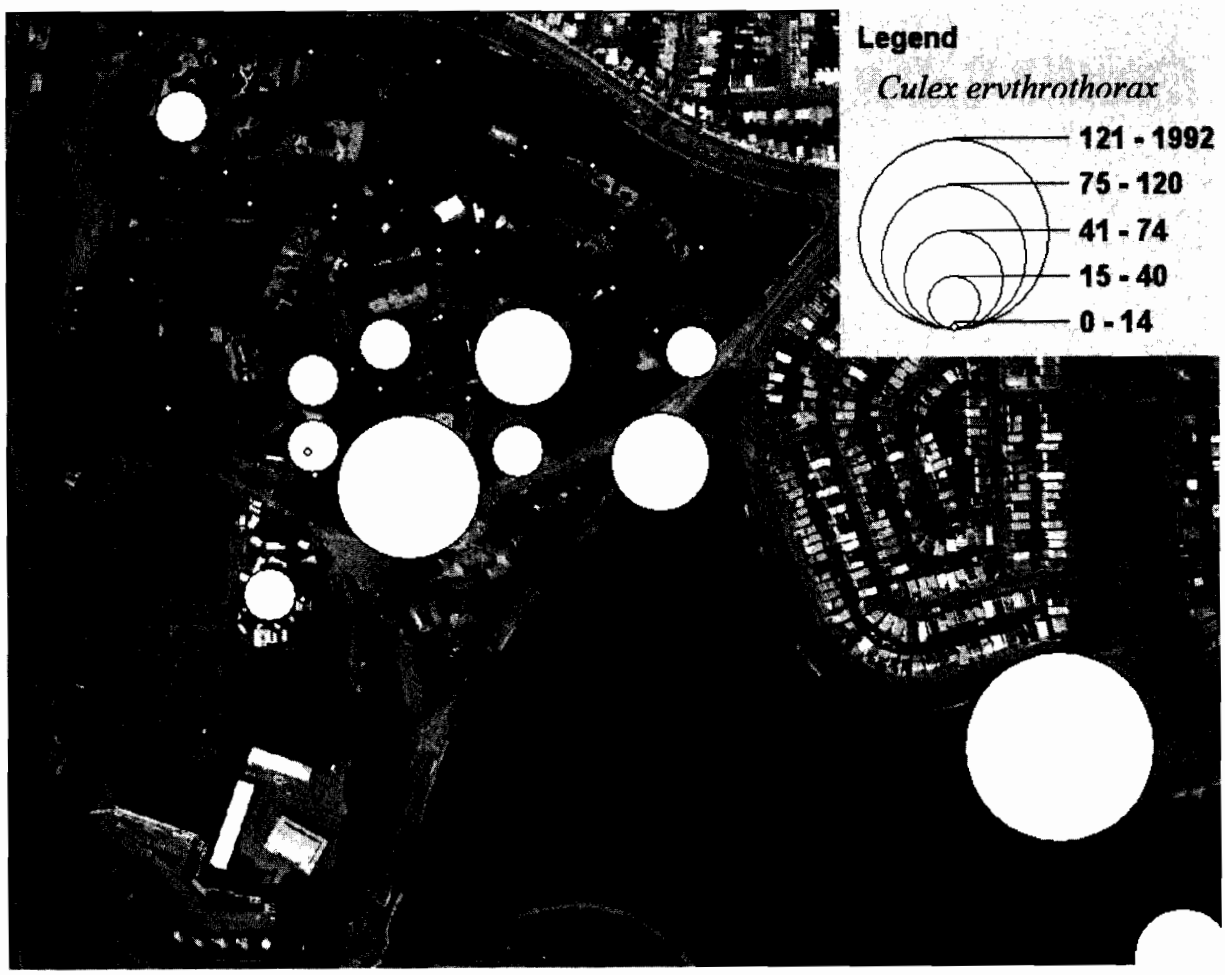

Figure 7. Number of host-seeking *Culex erythrothorax* females in carbon dioxide-baited traps in October, 2003. Size of circle indicates total number of mosquitoes per trap night at each site.

Evaluation of Mosquito and Arbovirus Activity in Orange County, California During 2004

Christoph C. Schwedes, Robert F. Cummings, Stephen G. Bennett, Carrie Fogarty, Ralph Havickhorst, Jim Francisco, Julisa Nevarez, Art Tilzer, and James P. Webb

Orange County Vector Control District, P.O. Box 87, Santa Ana, CA 92702

ABSTRACT: The Orange County Vector Control District continued its arbovirus surveillance program in 2004 by collecting and pooling mosquitoes, testing avian blood samples drawn from wild birds and sentinel chickens, as well as testing dead birds collected from various animal control agencies and the public. Evidence of West Nile virus (WNV) infection was detected in 180 wild birds, 253 dead birds, 164 mosquito pools, and 4 of 10 sentinel chickens in the County. By year's end, 64 confirmed human cases (3 fatal) and 2 dead horses were reported due to WNV. Overall, 4.6% of all wild bird samples and 8.3% of mosquito pools tested positive to either WNV antibodies or WN virus, respectively. *Culex quinquefasciatus* Say was the most abundantly trapped mosquito, accounting for the majority of submitted pools (1,246 of 1,963) and positive pools (153 of 164). House finches (*Carpodacus mexicanus* Say) and House sparrows (*Passer domesticus* L.) were the most frequently sampled wild birds (2,908 of 3,884), and together, had the highest WNV seropositive rate (159 of 180) during 2004. No St. Louis encephalitis (SLE) or western equine encephalomyelitis virus (WEE) activity was detected by any surveillance method.

INTRODUCTION

The 789 square miles of Orange County, with its human population of approximately 3 million, comprises of a variety of ecotypes, including urban, suburban, riparian flood channels, parkland, coastal mountains, and estuaries (US Census Bureau 2004). The Orange County Vector Control District (OCVCD) employed an integrated 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.

MOSQUITO SURVEILLANCE

Mosquitoes were collected weekly at 75 – 93 trapping locations throughout the County, combining CDC/CO_2-style host-seeking traps and Reiter/Cummings gravid female, ovipositional traps (Cummings and Meyer 1999). Blood-fed mosquitoes were also aspirated at known resting sites, and at locations of service requests. Trap number and distribution across the County was increased dramatically and focused in response to the activity levels of WNV in 2003 (Fig. 1). Mosquito pool submissions from OCVCD increased from 574 in 2003 to 1,963 in 2004. Of 1,963 mosquito pools submitted to the Center for Vectorborne Diseases (CVEC) at the University of California, Davis, 164 tested positive for WNV (Table 1). *Culex* mosquitoes were the only species found to be WNV-positive and of these, *Cx. quinquefasciatus* Say comprised the majority (153 of 164). WNV was found in mosquito pools throughout the county (Fig. 2).

Mosquito counts varied temporally throughout the year, peaking in June; however, WNV infection rates increased to their highest rates in late summer/early fall (August – October) as counts decreased (Fig. 3). Depending on the locality and time period, Minimum Likelihood Estimate (MLE) calculations for positive mosquito pools (Biggerstaff 2004) were found to range as high as 37.4 at one site in north Orange County (Fullerton) during July-August, while the yearly MLE of 2.5 was found for all species tested (Table 1).

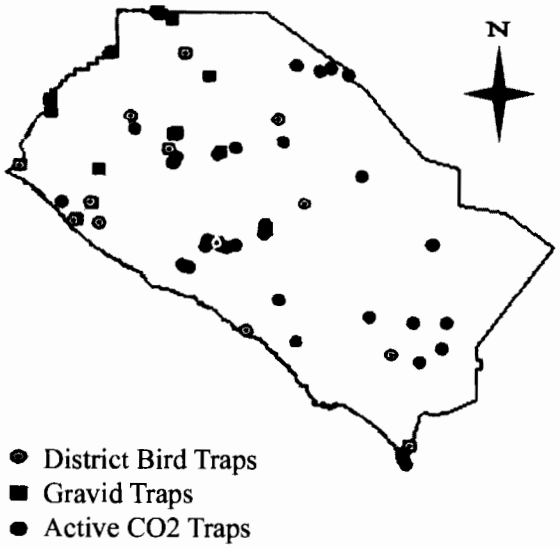

Figure 1. Map of Orange County bird and mosquito trap locations used in 2004.

Table 1. WNV-positive mosquito pools for 2004, by species.

Mosquito Species	Total Mosquitoes	Number Pools	Pools WNV Pos	Trap Type Gravid	Trap Type CO2	MLE
Cx. quinquefasciatus	41,858	1253	153	93.9%	6.1%	3.9
Cx. erythrothorax	10,320	264	1	0.1%	99.9%	0.1
Cx. tarsalis	5,315	162	4	2.5%	97.5%	0.8
Cx. stigmatasoma	927	40	6	95.1%	4.9%	6.9
Others *	5,529	244	0	20.1%	79.9%	0.0
Totals	**63,949**	**1963**	**164**	**4.9%**	**35.1%**	**2.5**

* Cs. *incidens*, Cs. *inornata*, Cs. *particeps*, An. *hermsi*, Oc. *squamiger*, Oc. *taeniorhynchus*, Oc. *washinoi*, and Ae. *albopictus*.

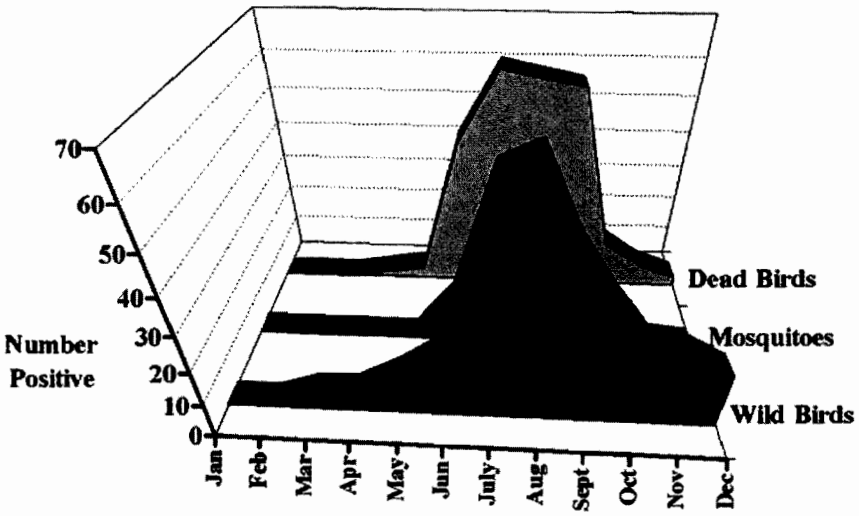

Figure 2. Comparison of sensitivity, strength, and seasonal effectiveness of 3 arboviral surveillance techniques. Data represents the number of first time WNV positive samples for each category per month.

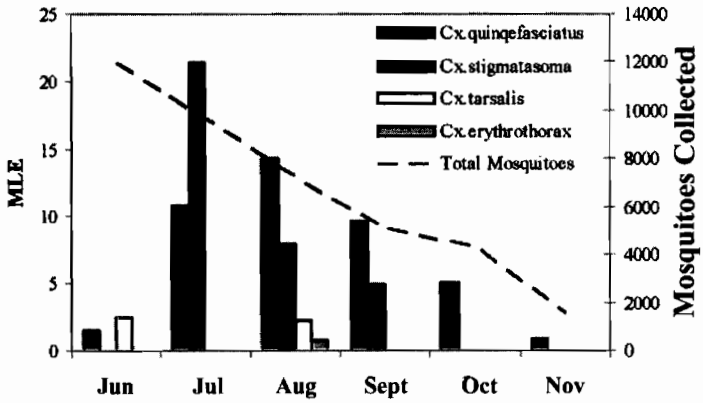

Figure 3. Mosquito population size by species, with corresponding MLE data from pooled samples.

SENTINEL CHICKENS

The only sentinel chicken flock (10 chickens) maintained by OCVCD was located in a riparian area of historically high mosquito, wild bird, and arbovirus activity along the San Diego Creek watershed in central Orange County. The chicken flock was bled bi-weekly throughout the year, and blood samples were tested for SLE, WEE, and WNV antibodies at the California Department of Health Services –Viral and Rickettsial Diseases Laboratory (CDHS-VRDL) and the OCVCD laboratory. No sentinel chickens seroconverted for WNV antibodies until August 10, well after detection of WNV in the county (Fig. 6). Three chickens tested seropositive by the CDHS—VRDL enzymatic immunoassay (EIA) (Reisen et. al. 1994) and by the OCVCD's blocking ELISA (Hall 1995, Jozan et. al. 2003). One additional chicken was found positive by blocking ELISA at the OCVCD that was never confirmed by the CDHS—VRDL test. No chickens tested seropositive for either SLE or WEE antibodies.

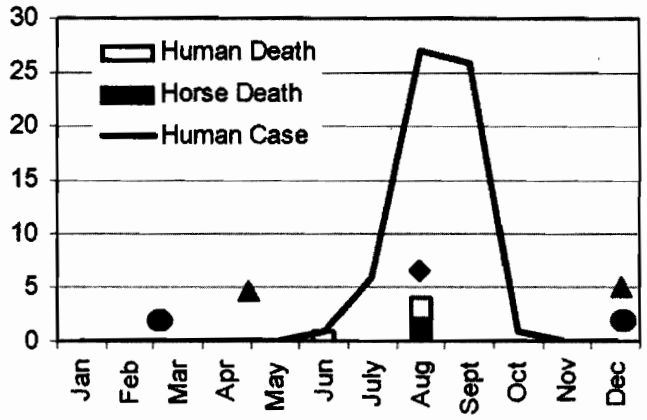

Figure 6. Timeline of arbovirus events by month, including first and last WNV positive wild birds (solid circle), dead birds (solid triangle), and initial sentinel chicken seroconversion (solid diamond).

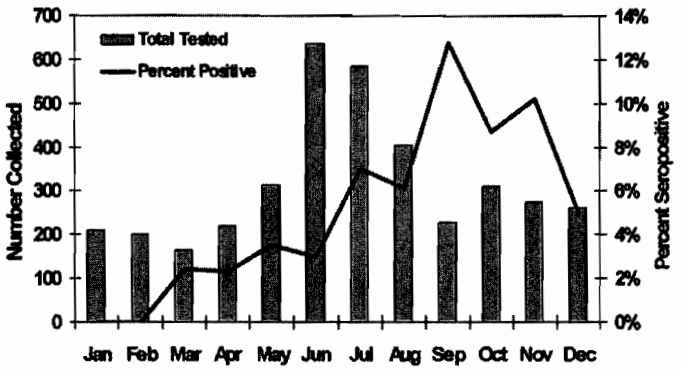

Figure 4. Graph of wild bird samples tested per month, with corresponding percentage positive for WNV.

WILD BIRD SEROSURVEILLANCE

Wild bird serosurveillance was intensified for 2004 by increasing the total number of active traps from 11 to 18 (Fig. 1). Wild birds were captured in modified Australian crow traps baited with fresh water and wild bird seed (McClure 1984), and each site was sampled biweekly. Birds were collected from each trap, banded, identified to species and sex, and bled by collecting 0.2 ml of blood. Samples were processed in the OCVCD laboratory using a hemagglutination inhibition assay (HAI) (Gruwell et al. 1988).

Serosurveillance focused mainly on two abundant passerine species, House sparrows (*Passer domesticus* L.) and House finches (*Carpodacus mexicanus* Say). These two species were the primary birds sampled (2,908 of 3,884), but a variety of other species were also trapped and tested (Table 2). WNV antibody-positive birds were detected from March through December 2004 (Figure 4).

The District's wild bird serosurveillance program was the first arbovirus surveillance method to detect WNV activity during 2004. Bird counts throughout the season remained sufficiently high for sensitive serosurveillance. Although the numbers of birds sampled per month peaked in June and July, the number of birds testing positive for WNV rose steadily, peaking in September at 12.8% (Figure 4). WNV antibodies were present in serum samples of eight bird species (Table 2). Positive samples were found from March 2 to the end of the year, making this method of surveillance the OCVCD's most sensitive and consistent arbovirus detection method (Fig. 2).

DEAD BIRD SURVEILLANCE

Dead Birds were collected from the public via dead bird call-ins and through cooperation with various animal control agencies. Of the 1,038 birds collected or submitted, 435 were fit for testing; of those, 253 turned up positive for WNV by VecTest (Stone et al. 2004), the OCVCD's immunohistochemistry (IHC) test (Jozan et. al. 2003), and/or polymerase chain reaction (PCR) by CVEC. WNV was found in 54 species of bird, with the American Crow

Table 2. Wild bird sera sample data from 2004, by bird species and number positive for WNV.

Bird Species	No. Blood Samples	Number Positive SLE	WEE	WNV (%)
House Finch	2296	0	0	119 (5.2%)
House Sparrow	612	0	0	40 (6.5%)
Brown-headed Cowbird	410	0	0	5 (1.2%)
California Towhee	113	0	0	2 (1.7%)
American Crow	23	0	0	5 (21.7%)
Other species *	430	0	0	9 (2.1%)
Totals	**3884**	**0**	**0**	**180 (4.6%)**

*** Positive Species**: Nutmeg Mannikin, White-crowned Sparrow, Green Heron,
Non Positive Species: Black-headed Grosbeak, Scrub Jay, Song sparrow, doves, hawks, warbler.

comprising 76% (192 of 253) of positive dead birds. Dead birds were found positive for WNV from the end of April through the end of December (Fig. 5). Collection in areas of continual positive submissions was aborted to allow for diversion of temporarily limited resources to allow for testing of samples from areas not yet confirmed for WNV activity.

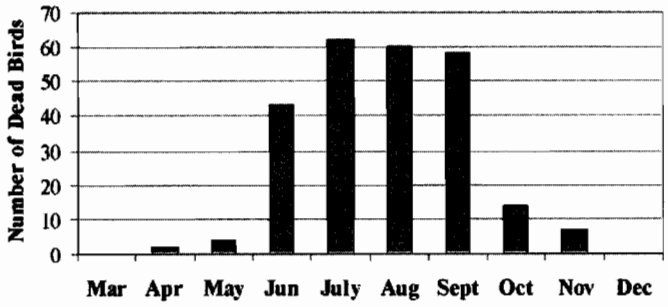

Figure 5. WNV positive dead birds by month (IHC and PCR).

FOCUS AND INTEGRATION

Craig Park in the city of Fullerton was one of the OCVCD's first monitoring sites to show WNV activity with all three detection methods (mosquitoes, wild birds, and dead birds). The MLE for virus-positive mosquitoes collected from this site averaged 6.8 for the entire year and peaked at 37.4 during the months of July and August. This locality continued to show evidence of arbovirus activity throughout the year after two WNV-positive wild birds were detected in March. Dead birds were also found in the area from May – November 2004. This site was an example of how the risk of human infection in a focal habitat could far exceed levels in other areas of the county.

Wild birds collected from sites along the Los Angles/Orange County border first showed signs of WNV activity in early March, and continued to show evidence of new infection into December. Areas with high rates of arbovirus activity coincided with riparian corridors, which were most likely acting as distribution systems for infestation to other urban areas. Mosquito pool data helped to provide a very detailed image of WNV virus activity, location and relative transmission prevalence. A detailed representation of arboviral activity is depicted in Fig. 7 by combining multiple data

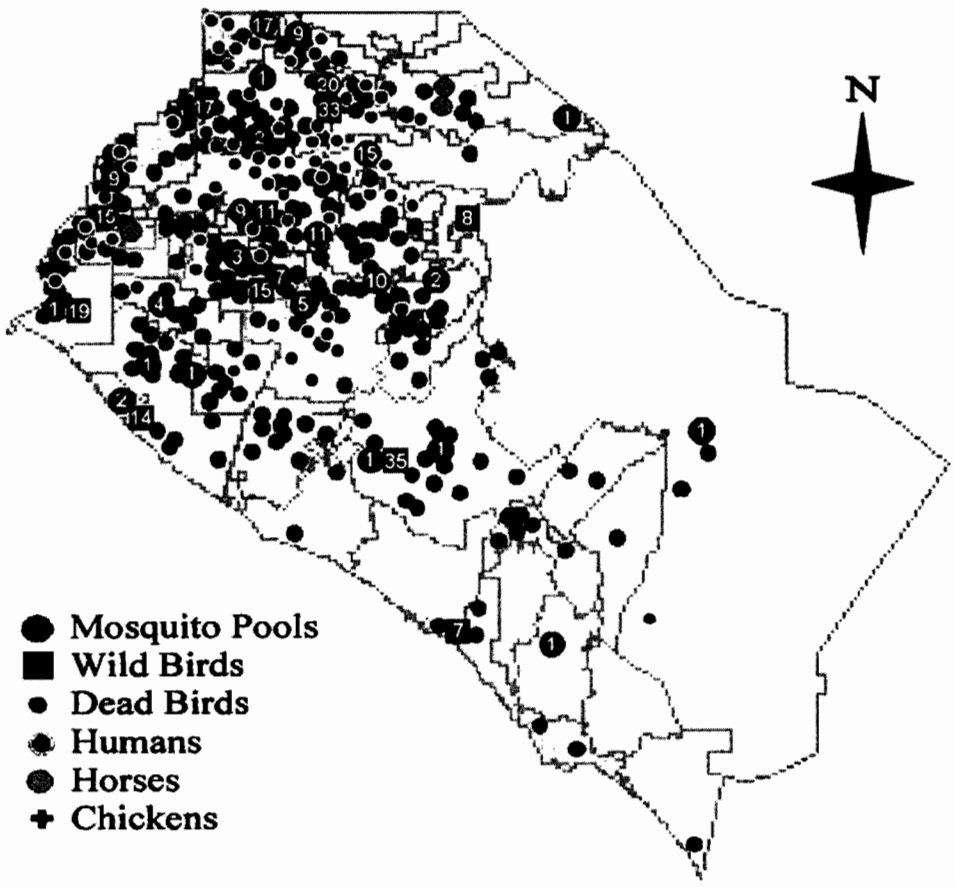

Figure 7. Map of Orange County, with distribution of WNV positive samples collected in 2004.

sources from the OCVCD's arbovirus surveillance project. This information was invaluable in determining areas of high infection risk, focusing mosquito control efforts, and increasing public awareness.

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West Nile Virus in the Moab Mosquito Abatement District, Grand County, Utah

Robert A. Phillips

Moab Mosquito Abatement District, P.O. Box 142, Moab, UT 84532

ABSTRACT: West Nile virus (WNV) was first detected in the Moab Mosquito Abatement District, Grand County, Utah, in a dead American crow, September 8, and a dead Black-billed magpie, October 7, of 2003. No other humans, animals, sentinel chickens, or mosquitoes were found positive that year. The first detection of WNV in 2004 was a positive *Culex tarsalis* Coquillett pool collected July 27. A dead Black-billed magpie July 30, 6 out of 9 sentinel chickens, 8 out of 40 *Cx. tarsalis* pools, and 125 out of 318 *Culex erythrothorax* Dyar pools tested positive for the virus in 2004. Probable infection rates peaked at 35/1000 for *Cx. tarsalis* on August 10 and 43/1000 for *Cx. erythrothorax* on October 19. The local biology of mosquito populations and disease transmission and the implications of very high infection rates in *Cx. erythrothorax* populations through October are discussed.

The California West Nile Virus Dead Bird Surveillance Program – Challenges and Solutions in 2004

Ryan M. Carney[1], Kerry Padgett[1], Barbara Cahoon-Young[2], Leslie Woods[3], Deryck Read[4], William Reisen[5], Vicki Kramer[6], and Stan Husted[1]

[1] *California Department of Health Services, Vector-Borne Disease Section, 850 Marina Bay Parkway, Richmond, CA 94804*
[2] *University of California, Center for Vectorborne Diseases, Old Davis Road, Davis, CA 95616*
[3] *CAHFS Davis Lab, University of California, West Health Science Drive, Davis, CA 95616*
[4] *CAHFS San Bernardino Lab, 105 West Central Avenue, San Bernardino, CA 92408-92408*
[5] *Arbovirus Field Station, 4705 Allen Road, Bakersfield, CA 93312*
[6] *California Department of Health Services, Vector-Borne Disease Section, 1616 Capitol Avenue, MS 7307, P.O. Box 997413, Sacramento, CA 95899-7413*

ABSTRACT: The 2004 was a watershed year for the establishment of West Nile virus (WNV) in California. Consequently, the California Department of Health Services (CDHS) responded by dramatically expanding and enhancing the Dead Bird Surveillance Program (DBSP) to facilitate the early detection of WNV and to monitor the ongoing transmission throughout the state. Over 90,000 dead birds were reported to the program in 2004 via the WNV website (www.westnile.ca.gov) and toll-free hotline (1-877-WNV-BIRD). Dead birds reported to the program were screened by condition, species, and location, and through coordination with the Center for Vectorborne Diseases (CVEC), California Animal Health and Food Safety laboratories (CAHFS), and over 130 local agencies, more than 5,000 birds were submitted, necropsied, and tested in 2004. The result was an over thirty-fold increase in the number of WNV positive dead birds from the previous year, and by October, WNV had been detected in over 3,000 dead birds from all 58 counties within the state. Reports of positive dead bird results were made on a weekly or biweekly basis by CDHS, which included immediate notification to all applicable local agencies. The DBSP also employed three novel methods in 2004 that successfully triaged the throughput of bird specimens and increased the sensitivity of the testing program. First, submissions were limited in zip codes with previously positive dead birds. Second, the list of acceptable species was expanded beyond corvids and raptors within targeted areas in order to increase surveillance sensitivity. Third, a commercial immunochromatic assay (VecTest) was integrated into the testing and reporting system. Other significant changes involve the restructuring of testing protocol and timetables to facilitate quicker turn-around times for test results and reporting, improved data management and communication with and between testing laboratories, biweekly updates on all WNV statistics on the CDHS website, and initiating limited testing on exotic bird species and tree squirrels. By creating a system that was responsive and dynamic in nature, the DBSP was able to accommodate the explosive nature of WNV activity and the rapidly changing needs of the public, local agencies, and testing laboratories in 2004. The DBSP was a key component in recognizing the presence and transmission of the virus throughout the state, and provided the first indication of the virus' presence in ninety percent of the counties in California. Utilizing data and information accumulated during 2004, developments and enhancements to the program in 2005 will be discussed.

Testing for West Nile Virus in California during the 2004 Surveillance Season

Barbara Cahoon-Young[1], Nicole Kahl[1], Marzieh Shafii[1], Siranoosh Ashtari[1], Sheryl Yamamoto[2], Emily Green[1], Aaron Brault[1], William Reisen[3]

[1] *Center for Vectorborne Disease Research, University of California, Davis, CA 95616*
[2] *Sacramento-Yolo Mosquito and Vector Control District, 8631 Bond Road, Elk Grove, CA 95624*
[3] *Arbovirus Field Station, University of California, Bakersfield, CA*

ABSTRACT: During the arbovirus surveillance season of 2004, the Center for Vector-borne Disease Laboratory tested 14,781 mosquito pools (1,132 WNV positive) and 5,149 birds (3,098 WNV positive); whereas during the 2003 surveillance season 10,114 mosquito pools (32 WNV positive) and 1,768 birds (96 WNV positive) were tested. Here we review the demands and challenges required to rapidly transition our lab from the use of more conventional and time-consuming assay techniques (e.g. virus isolation and enzyme immunoassays (in situ EIA) used during 2003 and previous years, to a more rapid, molecular basis {i.e. reverse transcription polymerase chain reaction (RT-PCR)} for virus detection; and to respond to the rapid increase in specimen volume and the greater need for quality control and test accuracy. Before transitioning solely to RT-OCR technology we performed numerous parallel assays that demonstrated the greater sensitivity of RT-PCR compared with in situ EIA (ca. 65% agreement). We further determined that the use of RT-PCR greatly decreased the time required for specimen reporting (turn-around-time), while it led to a substantial increase in our through-put. For tests of wild bird specimens we employed screening and confirmatory RT-PCR assays. For tests of mosquito pools we adapted the screening RT-PCR to a multiplex format that enabled the simultaneous assay of three different viruses (WNV, SLE, WEE); confirmations were performed by sigleplex RT-PCR. In other studies we used RT-PCR to assess the accuracy of two "rapid tests" (VecTest and RAMP) in the detection of WNV in oral swabs from corvids versus non-corvids. Additional studies of the use of "rapid tests" versus RT-PCR in the detection of WNV in mosquito pools are underway. Our overall experience attests to the superior performance of RT-PCR in accurate and high volume testing of arboviruses in mosquito and avian tissues.

West Nile Virus (a.k.a. Kunjin Virus) in Australia?

Richard C. Russell

University of Sydney and ICPMR, Westmead Hospital, Westmead, NSW 2145, Australia

ABSTRACT: Kunjin virus (KUNV) is an endemic flavivirus in Australia and is recognized as a subtype of West Nile virus (WNV), being closely related genetically and antigenically, but it is considered to be of minor medical and veterinary importance. Human infections are generally associated with mild febrile illness, rarely encephalitis, and no fatalities have been reported. Although also closely related to the medically more important Murray valley encephalitis virus (MVEV), KUNV appears to have a significantly different epidemiology in Australia. While there is evidence that KUNV and MVEV have vectors and vertebrate hosts in common, and both viruses are often involved together in disease outbreaks, KUNV is more active in apparently non-endemic regions.

KUNV has been isolated from mosquitoes trapped in most states of Australia, although it appears to be endemic only in the northern tropical regions. Activity un mire southern regions appears associated with heavy rainfall and flooding and it is thought the virus is introduced from northern endemic foci by movement of viraemic water birds, particularly of the Order Ciconiiformes, which are the likely avian hosts. *Culex* species, particularly *Cx. annulirostris* Skuse, are thought to be the principal vectors of endemic and epidemic activity, although there is evidence of vertical transmission in *Ochlerotatus* species that may indicate an important survival mechanism providing for reactivation of virus following flooding. Surveillance if KUNV activity in Australia is undertaken through chicken sentinel serosurveillance and virus isolation from mosquito collections, but variously according to state and region. Vector control is virtually non-existent in the dace of endemic or epidemic activity, with a reliance on governmental health warnings for public health protection.

Because KUNV is closely related to WVN, there has been concern within Australia that WNV-NY could exploit the same vectors and avian hosts if introduced to Australia, and as it appears to be considerably more virulent it may represent a greater medical and veterinary threat than KUNV. Although no imported human cases have been detected in Australia, and the prospect of the active virus arriving in a migrating bird after a transpacific journey is remote, the possibilities of introduction with traveling humans and imported animals do exist as they did for the USA in 1999. Indeed, a horse imported from North America (Canada via USA) in 2002 serconverted after arrival and while in quarantine confinement in Sydney. Less likely is the prospect of an infected mosquito being introduced, because of Australia's local quarantine restrictions, particularly the compulsory disinsection of aircraft.

Accordingly, the development of serological and molecular assays to differentiate WNV and KUNV, infection studies to determine the susceptibility of local birds, and vector competence investigations of potentially important mosquitoes are completed, underway or intended. The question of potential cross-protection between KUNV and WNV, by natural infection in vertebrate hosts or by immunization with vaccines derived from the viruses is entertaining the minds of those concerned for the introduction of WNV to the eastern seaboard of Australia where KUNV is seldom active and little protective immunity exists. The future awaits us!

Population Dynamics of *Culex* Mosquitoes and Adulticiding Spray Efficacy at Three Ecological Reserves in Orange County During 2002 - 2003

Robert F. Cummings, Greg Williams, Eric Weis, Matt Robinson, Amber Mills, Ralph Havickhorst, Tianyun Su, Richard Meyer, and James P. Webb

Orange County Vector Control District, P.O. Box 87, Santa Ana, CA 92702

ABSTRACT: The potential for mosquito-borne disease transmission to humans living near three artificial wetlands (University of California Ecological Reserve, Irvine Ranch Water District Marsh, and Bonita Creek Marsh) in Irvine, California, was assessed in 2002 and 2003. Four mark-release-recapture trials were conducted to examine the dispersal and population ecology of two mosquito species, *Culex erythrothorax* Dyar and *Culex tarsalis* Coquillet, at these wetlands. The effectiveness of the Orange County Vector Control District's adulticiding mosquito control program was evaluated at the Ecological Reserve, the only site under treatment. Mean dispersal and survivorship of unsprayed cohorts were 0.6 km and 0.82/day for *Cx. erythrothorax*, and 1.1 km and 0.95/day for *Cx. tarsalis*, respectively. Spraying reduced the numbers of each species in the Reserve by 95%, but counts returned to pre-control levels within three days post-treatment, supporting the hypothesis that artificial wetlands produce uncontrollable numbers of long-lived mosquitoes capable of vectoring arboviral diseases.

INTRODUCTION

As Reeves (1990) noted, urban expansion has not eliminated mosquito-breeding sites in California, but has actually placed more Californians in proximity to environments where there is a high probability for mosquito-borne disease transmission or periodic attack by large numbers of nuisance vectors. Throughout southern California, continued human population growth has created land usage conflicts between developers and environmentalists (Meyer 1992). Unfortunately, constructed wetlands located near housing developments and densely populated areas can produce large mosquito populations, which cause a nuisance and may pose a serious health hazard as vectors of pathogens causing diseases in humans (Walton et al. 1998, Russell 1999, Walton 2002).

Host-seeking *Culex erythrothorax* Dyar, *Cx. tarsalis* Coquillett, and the malaria vector, *Anopheles hermsi* Barr and Guptavanji, are routinely collected in large numbers at freshwater marshes in Orange County, with *Cx. erythrothorax* adults comprising > 90% of the wetland-sourced mosquitoes captured by the Orange County Vector Control District (OCVCD) over the years (Cope et al. 1986, Bennett et al. 1992, Cummings et al. 2002). These species are likely to become more abundant elsewhere in the county as more constructed wetlands are developed to impound and filter urban runoff.

The purpose of this study was to investigate the dispersal and the population characteristics of survivorship, reproductive success, blood feeding preferences, and efficacy of the OCVCD's mosquito control program on adult female mosquitoes associated with three wetlands (University of California, Irvine, (UCI) Ecological Reserve, Irvine Ranch Water District (IRWD) Marsh, and Bonita Creek Marsh) along the San Diego Creek Watershed near Upper Newport Back Bay during the spring and summer months of 2002 and 2003 (Fig. 1).

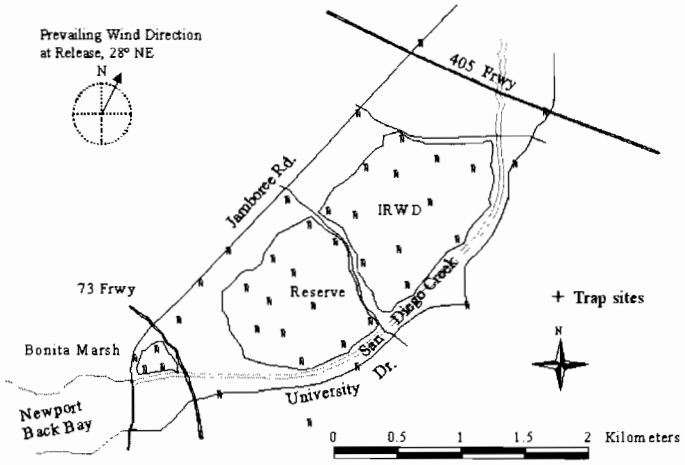

Figure 1. Trap Site Locations (Bonita Marsh: 4 traps; Reserve: 12 traps; IRWD: 12 traps; Jamboree Rd. and University Dr. Transects: 6 traps each).

METHODS

Four mark-release-recapture (MRR) trials were conducted in 2002 to estimate the dispersal pattern, distance traveled and survivorship of *Cx. erythrothorax* and *Cx. tarsalis* females at these wetlands. (For each trial, spray operations were halted in the study area for eight consecutive nights to allow enough time for the released mosquitoes to disperse). Host-seeking females were collected from each wetland in a total of 50 dry-ice baited traps (Sudia and Chamberlain 1962) on the nights prior to release. Mosquitoes were photographed in each net using a digital camera

and the pictures were downloaded into a computer, where they were counted later with the aid of Adobe Photoshop™ Version 5.0 software. The number of dead mosquitoes counted in the nets after each release was then subtracted from the computer-aided counts to determine the number of marked mosquitoes actually released in each trial.

Mosquitoes dispersing from the release areas were recaptured in 40 CO_2-baited suction traps at previously selected sites in the three wetlands and the outlying transects along Jamboree Road and University Drive (Fig. 1). Captured mosquitoes were returned to the laboratory, euthanized with carbon dioxide, identified to species, and counted at 40 x under a dissecting microscope. Marked specimens were identified under a dissecting microscope with an ultraviolet-B (UVB) fluorescent light (wavelength » 365 nm). Reproductive status (Detinova 1962) was determined to compare the age composition, and hence, breeding success of female mosquitoes in this study. Blood engorged females were separated and stored in individual vials in an - 80° C freezer. Their blood meals were later tested by a sandwich ELISA test (Chow et al. 1993) at the UC Davis Kearney Agricultural Center to determine the type of host fed upon by each female mosquito.

The effectiveness of the OCVCD's spray program on reducing mosquito numbers during routine adulticiding efforts at the UCI Reserve in 2002 and 2003 was measured in five adulticiding control trials. Traps were set up for eight consecutive nights at the same designated sites used in the MRR trials to assess the differences between the pre-spray counts and the post-spray numbers in the UCI and IRWD marshes and the two transects outside the wetlands.

Data adjustments were used according to the type of calculations needed (temporal or spatial), thereby accounting for sampling without replacement, trap failures and different trap densities (Brenner et al. 1984, Nelson et al. 1978). Daily survivorship was estimated using the regression method described in Milby and Reisen (1989), where longitudinal survivorship of a marked cohort of mosquitoes was calculated from the decline in the recapture rate and regressed as a function of cohort age in days. The percent reductions in mosquito numbers for each spray effectiveness trial were calculated using the method of Retnakaran (1980), the reciprocal of Abbott's Formula (Abbott 1925), where counts at the treated (sprayed) sites were referenced to changes at the experimental control (no treatment) Bonita Creek Wetland.

RESULTS

This study showed that the dispersal of *Cx. erythrothorax* females was generally limited to sites within their wetland habitat. For example, most females released at the UCI Reserve were recaptured within the Reserve (91.0%) and relatively few ventured into a non-wetland habitat (3.4%). Most (68.3%) flew less than 0.5 km and only 5.9% flew more than 1.0 km from their release point. The overall mean daily travel (MDT) by marked females in this study was 0.58 km, and once dispersed, they moved relatively little over time (0.032 km/day). The maximum distance any adult female of this species flew was 1.74 km.

Numbers declined at a constant rate and the relationship between log-transformed numbers and time was linear. Nearly half of all individuals were recovered within two days of release, and the longest duration between release and recovery was 22 days (1 specimen). The marked populations had a combined 7-day, mean daily survival rate of 0.82. The length of the gonotrophic cycle for *Cx. erythrothorax* was estimated to be 7 days.

Although substantially fewer *Cx. tarsalis* females were released than *Cx. erythrothorax* females (1,315 versus 34,159, estimated), they were found to have

dispersed throughout the recapture grid in comparatively equal proportions. About half (51.0 %) of the recaptured *Cx. tarsalis* specimens were recovered outside the UCI Reserve and at distances greater than 1.0 km from the release point. The MDT for *Cx. tarsalis* was estimated at 1.06 km, nearly twice the value obtained for *Cx. erythrothorax* (0.58 km). The longest distance traveled by one *Cx. tarsalis* female was 1.85 km, slightly more than the longest *Cx. erythrothorax* flight (1.74 km).

Population trends between these two species were markedly different. Although no *Cx. tarsalis* females were recovered after 10 days post-release; females of this species had a higher 7-day, mean daily survival rate compared to *Cx. erythrothorax* females (0.95 versus 0.82). The length of the gonotrophic cycle for the *Cx. tarsalis* population at the UCI Reserve was estimated to be 5 days, 2 days less than the length for *Cx. erythrothorax*.

Host-selection was also found to be significantly different. *Culex tarsalis* females were found to feed exclusively on birds. In contrast, mammals made up the majority of the identified blood meal sources for *Cx. erythrothorax* (65.0%), while the remaining 35.0% were found to have come from avian hosts. Humans made up 18.0% of the total blood meal sources for Cx. *erythrothorax* and none for *Cx. tarsalis*.

The effects of 4 sequential nights of adulticiding spray on a marked cohort of *Cx. erythrothorax* released in the UCI Reserve were found to have decreased the 7-day, mean daily survival by 41.5%, from 0.82 for the untreated groups to 0.48. The action of the spray on this cohort was such that only an estimated 0.59% of the original population remained alive at the end of 7 days, compared to an estimated 24.9% for the untreated populations. This is an example of how an adulticiding spray program can effectively disrupt a disease transmission cycle by reducing the vector population below sustainable levels for transmission to continue.

There was also a 4-day decline of > 95.0% in numbers of unmarked *Cx. erythrothorax* females and nearly a 90.0% reduction in counts of unmarked *Cx. tarsalis* from spraying during the same time period. Recruitment (additions of newly emerged females from the breeding source) quickly restored the unmarked populations in the Reserve to pre-spray levels within 3 days.

Barr et al. (1986) found parity rates in female *Cx. erythrothorax* collected at the UCI Reserve to be around 15.0%, many times higher than the 2.5% reported here. The OCVCD changed its method of mosquito control in the Reserve in 1991, from an unsuccessful larviciding effort to a more effective adulticiding program. The data from this study indicate that because of adulticiding, the reproductive success rate of female *Cx. erythrothorax* at the UCI Reserve has decreased substantially, as fewer adults have been able to survive long enough to reproduce.

Tempelis (1989), Reisen et al. (1992), and Walton et al. (1999) have reported mammalian hosts rates > 80.0% in blood meal

identification studies of *Cx. erythrothorax*. This study recorded a lower value (65.0%), but still confirmed a strong preference by *Cx. erythrothorax* females for mammalian hosts. One interesting finding in this study was the relatively large percentage (18.0%) of blood meals from humans. *Culex tarsalis* was again found to feed primarily on avian hosts, as documented by Reisen and Reeves (1990).

CONCLUSION

Multipurpose constructed wetlands may offer many potential benefits, including water quality improvements, wetland creation, wildlife conservation, and recreation. Properly designed artificial wetlands must emphasize minimizing mosquito breeding by including proper water and vegetation management strategies in their design. Currently, cost-benefit analyses for constructed wetlands do not include the costs associated with mosquito abatement, and vector control is often an afterthought. For example, annual costs for mosquito control at three constructed treatment wetlands in southern California and Arizona ranged between $5,250 and $6,665 per hectare in 1997 – 1999 (Walton 2002). Relying on adulticiding efforts is the last choice. Although highly successful at controlling mosquitoes when properly executed, routine adulticiding is expensive, stirs up environmental health issues, and must be done repeatedly. Mosquito numbers returned quickly to pre-spray levels once treatment was stopped.

Culex tarsalis adults were found in this study to be capable of biting more often than *Cx. erythrothorax* in a similar length of time. Additionally, their higher mean daily survival rate indicated that members of this species were long-lived and could potentially vector disease agents repeatedly within several weeks.

West Nile virus has been found at the IRWD Marsh in 1.25% (1 of 80) of the batches of *Cx. tarsalis* mosquitoes tested in 2004. Additionally, 11.5% (32 of 279) of the wild birds and 40% (4 of 10) of the sentinel chickens have tested antibody-positive to WN virus in 2004 at this wetland. The proximity of large numbers of wild birds, mosquitoes, and people makes the lower San Diego Creek Watershed area a potentially risky place for arboviral disease transmission to people.

The increased use of artificial wetlands for habitat mitigation and water reclamation purposes, combined with residential development close to these habitats, will pose a significant challenge to the OCVCD in its mosquito and arboviral disease control efforts in the coming years. Further studies will be needed to evaluate the disease risk at other artificial wetlands as more are built in Orange County.

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Current Status of Water Runoff Management and Mosquito Production in California

Marco E. Metzger

California Department of Health Services, Vector-Borne Disease Section, 2151 Convention Center Way, 218B, Ontario, CA 91764

ABSTRACT: Amendments to the federal Clean Water Act adopted in 1987 require states to develop and implement non-point source pollution management programs to abate pollutants carried by Stormwater and urban runoff. A principal component of these programs is the implementation of best management practices (BMPs), a term first adopted in the 1970s to represent actions, practices, or structures used to reduce the flow rates and/or constituent concentrations in runoff. Improving the quality of water runoff through use of BMPs, however, is technologically still in its infancy and is further complicated by stakeholder and activist groups with conflicting interests and priorities. Public health agencies comprise one such group who is concerned about possible deleterious effects on the public health resulting from BMP implementation. Because of their placement within urban and suburban areas, structural BMPs may increase the number of vector habitats in close proximity to humans. In 1998, the California Department of Health Services, Vector-Borne Disease Section, and several local vector control agencies began to assess public health implications of structural BMPs. The results of these efforts have contributed substantially to how subsequent BMPs have been designed, implemented, and maintained. The arrival and rapid spread of West Nile virus had underscored the importance of addressing the vector production problem in BMPs and has allowed this issue to gain nationwide recognition. However, many critical problems remain unresolved. In this paper, we will discuss the advances made by vector control within the California water runoff management community with emphasis on current BMP designs as well as existing and future challenges.

Mosquito Production in Stormwater Treatment Devices in South Lake Tahoe, California

Jonathan Kwan[1], Marco Metzger[1], Mitch Shindelbower[2], and Curtis Fritz[1]

[1] California Department of Health Services, Vector-Borne Disease Section, 1616 Capitol Ave, Sacramento, CA 95899
[2] El Dorado County Vector Control, 1170 Rufus Allen Blvd, South Lake Tahoe, CA 96150

ABSTRACT: In response to increasing evidence of mosquito production in stormwater best management practices (BMPs), a collaborative project was initiated in and around the city of South Lake Tahoe, California. The primary objective was to document mosquito production in selected BMP structures and to determine if these BMPs provide habitats suitable for extended mosquito breeding seasons of certain species. Mosquito production in selected natural sites in the surrounding area was used to gain insight on naturally occurring populations, species composition, and seasonal abundance. Thirty-two project sites were selected including 17 BMPs of three design types – dry systems; systems with sumps, vaults, or basins; and man-made vegetated treatment systems (VTS) – and 15 natural sites. Between December 2003 and October 2004, the percentages of weekly site visits in which mosquito production was observed were 1.7% in dry systems, 24.2% in sumps/vaults/basins, 10.4% in VTS, and 9.4% at natural sites. Natural sites were observed to hold water and breed mosquitoes most frequently during the colder months of early spring, whereas BMPs were more likely to hold water and breed mosquitoes during the warmer summer and fall months. The implications of mosquito production in urban and suburban BMPs, as well as possible extended mosquito breeding of certain species, for the risk of human infection with mosquito-borne diseases are discussed.

INTRODUCTION

In 1997, litigation between the California Department of Transportation (Caltrans) and several environmental organizations resulted in a requirement that Caltrans conduct an extensive benefit-cost study of stormwater treatment devices in southern California. This undertaking became known as the Best Management Practice (BMP) Retrofit Pilot Program. Stormwater treatment controls such as structural BMPs are mandated under Federal (Clean Water Act) and California State (Porter-Cologne Act) laws and are being implemented at an accelerated pace to comply with deadlines (Copeland 1999, 2003). Stormwater BMPs are designed to mitigate the harmful environmental impacts of urbanization on receiving waterways caused by both increased water runoff volume and the concomitant transport of pollutants. The majority of structures implemented by Caltrans control both water volume and pollutant discharges by temporarily detaining runoff and allowing passive treatment mechanisms such as trapping, settling, adhesion, and biological processes to improve water quality (Metzger et al. 2002, CDOT 2004).

In 1998, the California Department of Health Services-Vector-Borne Disease Section (CDHS-VBDS) raised concern that certain BMPs could impact public health by increasing available habitat for aquatic stages of disease vectors, particularly mosquitoes (CH2M Hill 1999, Chanda and Shisler 1980, Dorothy and Staker 1990, Florida Coordinating Council on Mosquito Control 1998, Kluh at al. 2002, McLean 2000, Metzger 2004, Metzger et al. 2003, 2002, O'Carroll 1978, Santana et al. 1994, Schimmenti 1979, Schmidt 1980, Smith and Shisler 1981). As a result, in 1999 CDHS-VBDS entered into a contractual agreement with Caltrans to provide technical expertise on vectors and vector-borne diseases potentially associated with BMPs. It was the intent of this agreement to protect public health by documenting and, where possible, mitigating vector production and harborage at these BMPs. In collaboration with several local southern California vector control agencies, CDHS-VBDS established a comprehensive mosquito surveillance and monitoring program, developed vector abatement protocols, and recommended design modifications to reduce or eliminate the potential for BMPs to produce or harbor vectors (CDHS 2002).

The Lake Tahoe Basin is of special concern with regard to the implementation of BMPs. Lake Tahoe is one of the three clearest alpine lakes in the world, but its clarity is threatened by both waterborne and airborne pollutants such as suspended solids, nutrients, and hydrocarbons. In particular, certain nutrients cause algal growth which decreases Lake Tahoe's clarity and interferes with natural ecosystems. Lake Tahoe has been losing approximately 0.5 m of clarity a year leading some researchers to believe it could become a turbid, ordinary lake within a single generation (Tahoe Regional Planning Agency 1980). In an effort to slow this degradation, the United States Federal Government and Tahoe Regional Planning Agency (TRPA) created strict constituent limitations for stormwater effluent that drains to the lake (Table 1) (Clinton 1997, Tahoe Regional Planning Agency 1980). State highways ring Lake Tahoe's 72 mile circumference, so roadside projects aimed at improving water quality are an important component of this comprehensive effort.

Table 1. National Pollutant Discharge Elimination System (NPDES) permit surface discharge limits for Lake Tahoe stormwater treatment.

Constituent	Max concentration allowed in surface H_2O discharges
Turbidity	20 NTU
Total Nitrogen	0.5 mg/L
Total Phosphorous	0.1 mg/L
Total Iron	0.5 mg/L
Oil and Grease	2.0 mg/L

NTU = nephelometric turbidity units
mg/L = milligrams per liter

VBDS currently recommends that stormwater BMPs hold water for less than 72 hours – the minimum time required for certain mosquito species to complete their lifecycle under optimum conditions (California Department of Health Services 2002; Metzger et al. 2003, 2002). However, many types of stormwater BMPs currently in use in the Tahoe Basin exceed this recommendation and can become highly conducive to mosquito production. In 2003, VBDS, Caltrans, and El Dorado County Vector Control (EDCVC) initiated a collaborative project to assess mosquito production in selected BMP structures in and around the city of South Lake Tahoe, California. The primary objective of this project was to document the presence, seasonality, and species composition of mosquitoes in BMPs compared to those present in natural sites in the surrounding area.

METHODS AND MATERIALS

Study area

The city of South Lake Tahoe, California, is located at approximately 1908 meters elevation in east-central California along the California-Nevada Border south of Lake Tahoe in the Sierra Mountain Range (latitude: N 38° 57' 9.180", longitude: W -120° 6' 24.228"). The city covers approximately 26 square kilometers. The city's topography varies from level to mountainous, with vegetation ranging from willows to manzanita shrubs to aspens to conifers. Average annual rainfall is approximately 66 cm, the majority of which accumulates between November and March. Average annual snowfall at lake level is 318 cm.

Mosquito sources

In this study, a mosquito source was identified as an area that had the potential to hold stagnant water sufficient to breed mosquitoes. A site was defined as a single area of standing water. Pools of standing water within one meter of each other were considered a single site. The exceptions to this rule were large wet basins, meadows, and marshes, which were considered one site.

Thirty-two sites in or around the city of South Lake Tahoe were selected for the project, consisting of 17 BMPs (man-made sites) and 15 natural sites. Five BMPs were built and maintained by Caltrans and the remaining 12 were randomly selected from a list provided by EDCVC of known mosquito sources. The natural sites were chosen at random from an EDCVC list of historically problematic sites.

The BMPs chosen were separated into three groups (types) based on design and function: dry systems; systems with sumps, vaults, or basins; and vegetated treatment systems (VTS). Dry systems are designed to drain completely following a storm event and to remain dry. Examples include detention basins, vegetated swales, infiltration devices, and media filters. Systems with sumps, vaults, or basins include those BMPs with features that hold permanent or semi-permanent standing water. Examples include above- and below-ground media filters, hydrodynamic separators, and vault-type devices. Vegetated Treatment Systems are wetlands that have been constructed or modified to receive and treat runoff (Metzger 2004).

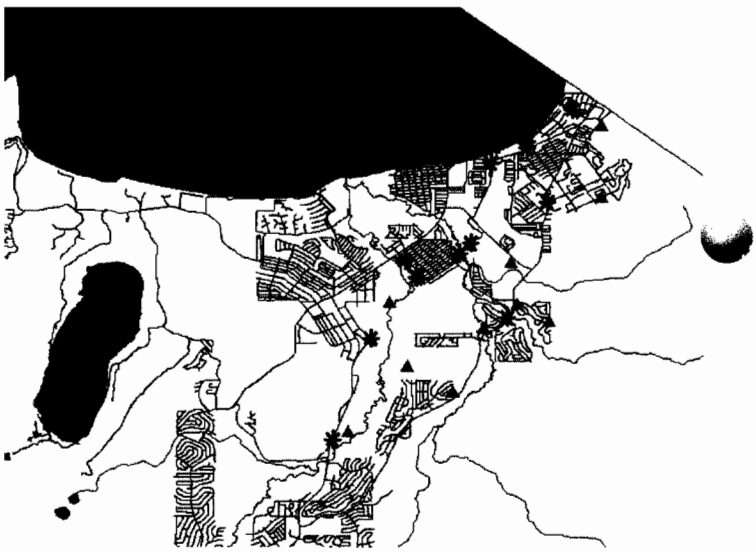

Figure 1. Digitized map of South Lake Tahoe, CA with geo-referenced project sites. black asterisk = BMP site; black triangle = natural site

All 32 sites in the project were geo-referenced (Figure 1) using a Trimble GeoExplorer 3 GPS device. Waypoint data was converted into Microsoft Access database files and shapefiles using Pathfinder Office software. The shapefiles were imported into ArcView 3.2a for GIS mapping. Digitized maps (e.g., street and topographical) of the Tahoe Basin area were provided by the El

Dorado County Surveyor's Office. Microsoft Excel and Microsoft Access were used to compile and analyze the data.

Data collection

Each study site was visited weekly from December 2003 to October 2004 (48 weeks). Data were collected at each site on each of the following features/variables: BMP type (i.e., dry system; sump, vault, or basin; VTS; natural); habitat type (e.g., pool, spring, meadow, marsh, ditch, etc.); water flow and turbidity; exposure to sun and/or shade; bottom type (e.g., cement, rocks, mud, etc.); vegetation present (e.g., cattails, marsh or meadow grass, algae, pine needles, etc.); mosquito presence (i.e., number/dip and species); larvicide use, if any (i.e., chemical used, quantity, rate and equipment used); daily high and low temperature and rainfall data from the National Oceanic and Atmospheric Administration weather station located at the South Lake Tahoe Airport.

Immature mosquitoes were collected using a standard dipstick with a .47 liter (1 pint) cup. The number of dips for each site was dependent on area and vegetation and kept constant throughout the project (e.g., 2 dips for a catch basin; 1 dip every 2 meters for larger sites). Dips were taken at each site to determine whether immature mosquitoes were present. Immature mosquitoes (larvae and pupae) collected were counted and identified to species. To help protect public health in the Tahoe basin, BMPs that harbored immature mosquitoes were treated with methoprene (Altosid EC®) to prevent the successful development of adults.

RESULTS

A total of 1536 visits were made to the 32 study sites during the 48-week study period. Of 816 site visits to individual BMPs, immature mosquitoes were collected on 97 (11.9%) occasions. Separated by BMP type, immature mosquitoes were collected on four of 240 (1.7%) visits to dry systems, 58 of 240 (24.2%) visits to sumps and basins, and 35 of 336 (10.4%) visits to VTS. Immature mosquitoes were collected on 68 of 720 (9.4%) visits to natural sites (Table 2).

Table 2. Number of weeks (%) positive for mosquito breeding at project sites itemized into different BMP types in South Lake Tahoe, CA over the Winter months, Summer months, and over all 48 weeks (Dec 03 – Oct 04).

Mosquito Source	Winter months (Dec 03 - Apr 04)	Summer months (May 04 - Oct 04)	Total months (Dec 03 – Oct 04)
5 Dry Systems	0 (0%)	4 (3.08%)	4 (1.67%)
5 Sumps, Basins, Vaults	1 (0.91%)	57 (43.85%)	58 (24.17%)
7 VTS*	0 (0%)	35 (19.23%)	35 (10.42%)
15 Natural Sources	24 (7.27%)	44 (11.28%)	68 (9.44%)

* Vegetated Treatment System.

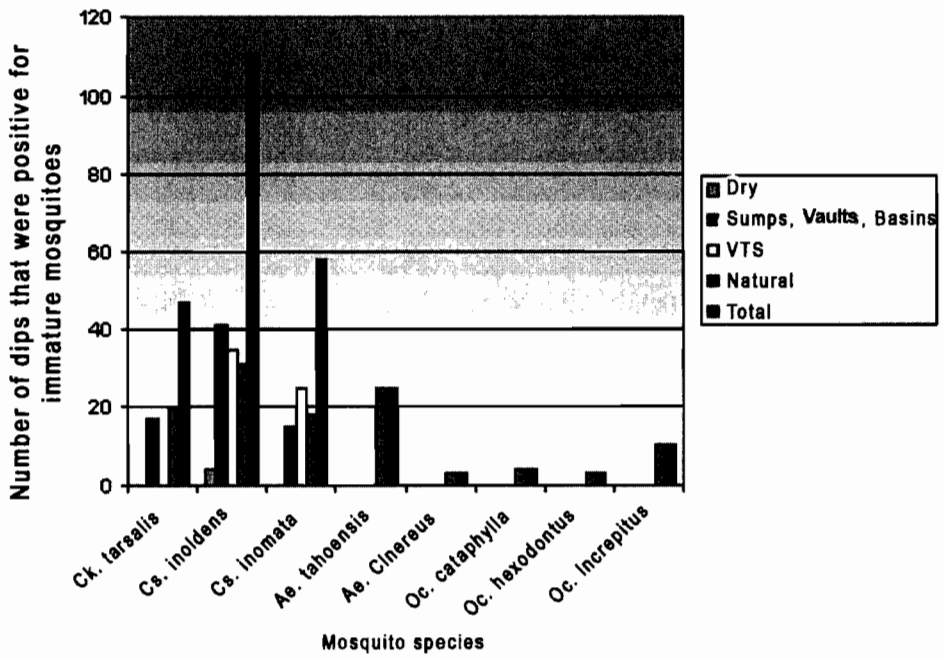

Figure 2. Number of positive dips where immature mosquitoes were observed during weekly project site visits. VTS = Vegetated Treatment System.

During the coldest months (average daily temperature = 33°F), between weeks 1 and 20 (December 2003 to April 2004), immature mosquitoes were collected from BMPs only once (i.e., underground vault) over the course of 110 (0.9%) visits. No immature mosquitoes were observed at dry systems and VTS during this period. In contrast, immature mosquitoes were collected during 24 of 330 (7.3%) visits to natural sites. After snow melt, between weeks 21 and 48 (May 2004 to October 2004) (average daily temperature = 53°F), immature mosquitoes were collected on 4 of 130 (3.1%) visits to dry systems, 57 of 130 (43.8%) visits to sumps and basins, and 35 of 182 (19.2%) visits to VTS. Immature mosquitoes were collected on 44 of 390 (11.3%) visits to natural sites during this period.

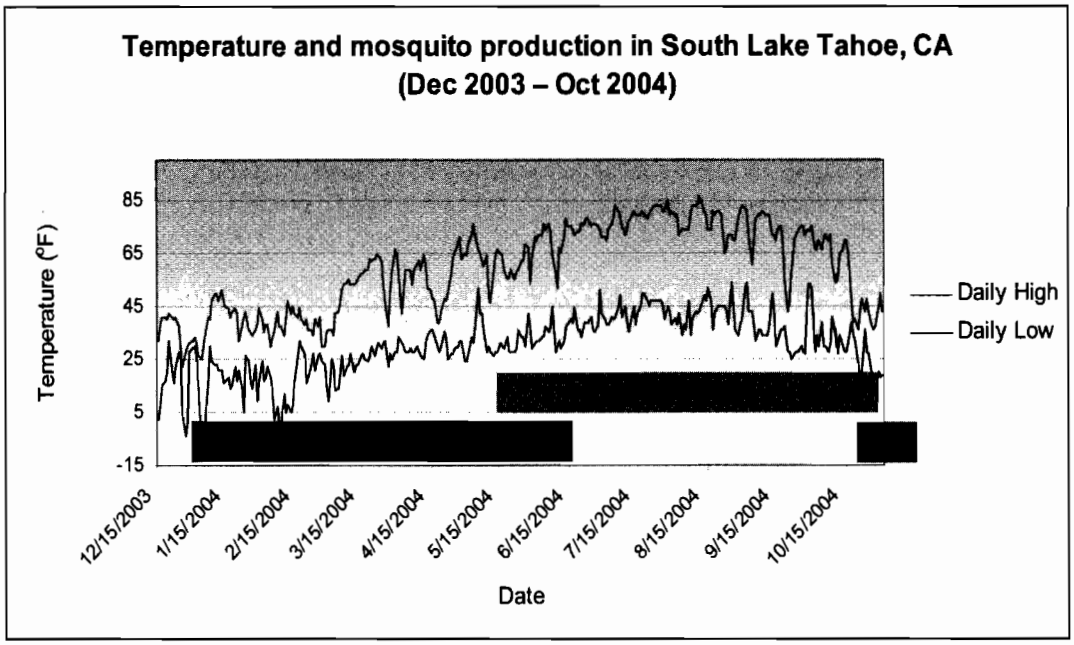

Figure 3. Daily temperature and mosquito production in South Lake Tahoe, CA from Dec 2003 to Oct 2004. Natural sites have a tendency to breed mosquitoes in the colder months, whereas the man-made sites have a tendency to breed mosquitoes more regularly in the warmer months.

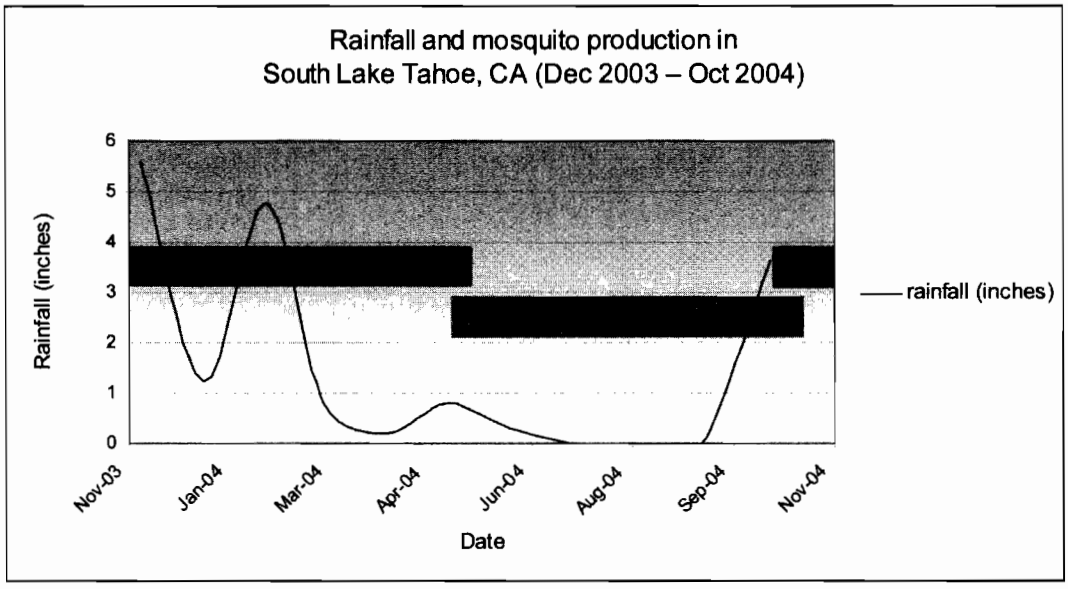

Figure 4. Monthly rainfall and mosquito production in South Lake Tahoe, CA from Dec 2003 to Oct 2004. Natural sites have a tendency to breed mosquitoes in the wetter months, whereas the man-made sites have a tendency to breed mosquitoes more regularly in the drier months.

Eight species of mosquito, representing four genera, were collected from study sites (Figure 2). The genus *Culex* was represented by a single species (*Cx. tarsalis*), the genus *Culiseta* by two species (*Cs. incidens*, *Cs. inornata*), the genus *Aedes* by two species (*Ae. tahoensis* and *Ae. cinereus*) and the genus *Ochlerotatus* by three species (*Oc. cataphylla, Oc. increpitus* and *Oc. hexodontus*). Overall, *Culex* and *Culiseta* mosquitoes were observed most often, especially in sumps, vaults and basins.

When average daily temperatures were low and monthly rainfall totals were high (average monthly rainfall greater than 2.54 cm) (weeks 1 to 20), immature mosquitoes were present more frequently in natural sites. When the average daily temperatures were warmer and monthly rainfall totals dropped, mosquito production was more frequent in BMPs (Figures 3 and 4).

DISCUSSION

This study provides preliminary evidence that certain BMPs in the Tahoe Basin increase available habitat to mosquitoes that may allow opportunistic species to extend their breeding season. BMPs that are poorly designed, improperly constructed, or inadequately maintained may retain water suitable for mosquitoes. Historically, the mosquito breeding season in South Lake Tahoe ended around the month of June; however, with the widespread deployment of BMPs, particularly below-ground devices protected from weather extremes, mosquitoes may be capable of breeding year round and over-winter as adults (CDHS, unpublished data). This potentially increases the risk of disease transmission to residents, companion animals, and wildlife. It may also create a

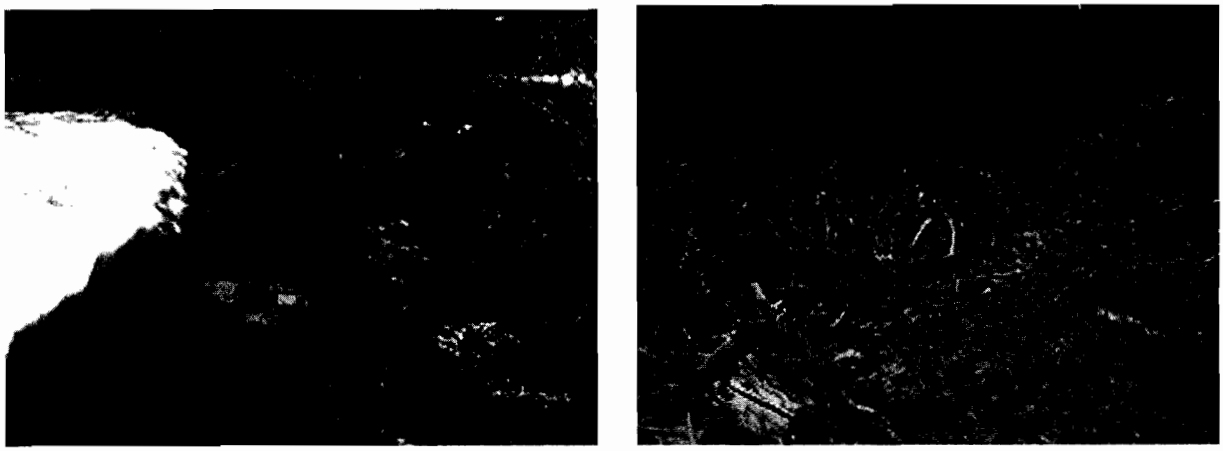

Figure 5. Natural site with stagnant snow melt water on the left April 1, 2004, and the same site dried up on June 10, 2004.

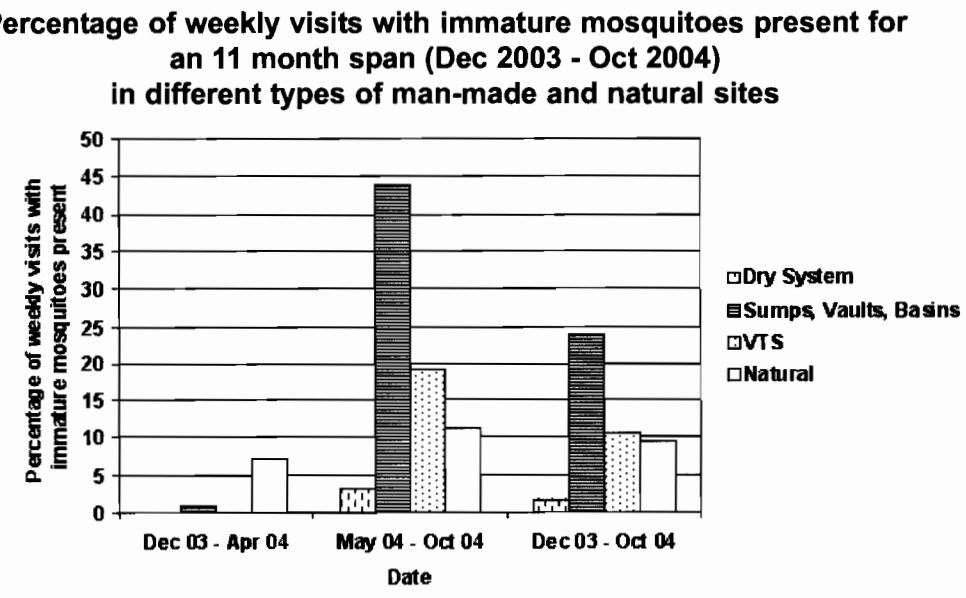

Figure 6. Number of weeks (%) positive for mosquito breeding at project sites itemized into different mosquito breeding site types in South Lake Tahoe, CA over the winter months, summer months and over all 48 weeks (Dec 03 – Oct 04). VTS = Vegetated Treatment System.

financial burden for the city by increasing yearly costs for vector control.

The extension of the mosquito breeding season can be attributed to seasonal temperature and rainfall. The fluctuations in temperature and rainfall appeared to be dependent variables of when and where mosquitoes would breed (Figure 3 and 4). During the colder months from December 2003 to April 2004, natural sites contained stagnant, cold, clear water, mostly from snow melt, preferred by the snow melt mosquito genera *Aedes* and *Ochlerotatus*. In contrast, during the warmer months of May 2004 to October 2004, the natural sites dried (Figure 5) while urban irrigation filled the BMPs, creating mosquito breeding habitats throughout the city (Fig. 6).

The mosquitoes identified at study sites (Table 3) correspond with species commonly found in South Lake Tahoe. These mosquito species differ in their preferred season and habitat for breeding. The majority of the *Culex* and *Culiseta* species in South Lake Tahoe breed in warm, murky waters typical of many BMPs. Two of these mosquito species, *Cx. tarsalis* and *Cs. inornata*, are directly involved with disease cycles that can be transmitted to humans. In California, *Cx. tarsalis*, a mosquito that prefers to breed in warmer weather, is the primary vector of St. Louis encephalitis (SLE) and western equine encephalomyelitis (WEE) and has proven itself to be an effective vector for West Nile virus (WNV) (Goddard et al. 2002, Reeves and Hammon 1962). *Culex tarsalis* is also capable of flying considerable distances (up to 26 km with estimates indicating they can fly 32-40 km if assisted by winds). This is an important variable in the distribution and transmission of mosquito-borne viruses (Durso 1996).

Table 3. Common mosquito species found in South Lake Tahoe

Genus	Species
Aedes	*ventrovittis*
Culiseta	*inornata** #
	incidens #
	impatiens
	particpeps
Culex	*tarsalis** #
	*pipiens (quinquefaciatus)**
	*stigmatosoma**
	*territans**
	*restuans**
Ochlerotatus	*cataphylla* #
	increpitus #
	hexodontus #
	tahoensis #

*These species are known potential vectors for West Nile virus
#These species were collected from the BMPs and natural sites during the project

Culiseta inornata is a mosquito that is also active during the colder months and has the potential to maintain WNV, SLE, and WEE transmission cycles when *Culex* mosquitoes are dormant (Goddard et al. 2002, Tempelis and Washino 1967, Anderson and Galloway 1987, Tempelis 1964). Another method of maintaining the mosquito-borne disease cycle when most mosquitoes are dormant is the mosquito's ability to search and over-winter in underground BMP devices. Three underground BMPs were checked during the winter months and one was found to house *Cx. tarsalis* and *Cs. incidens*.

The design of the dry systems allows for complete drainage within 72 hours following a storm event. A construction flaw with one of the dry system sites allowed it to hold stagnant water for mosquito breeding. The VTS had many cattails and willows in and around the edge of the pond that created ideal breeding habitat for mosquitoes. However, due to the low water table, many VTS became dry, like the natural sites, as the temperature increased and rainfall decreased in the summer. By design, water in sumps, basins, and vaults never fully drains. At these project sites there was always at least a few centimeters of water that mosquitoes could capitalize upon for breeding.

City planners, transportation agencies, and others should consider the potential for BMPs to support mosquito breeding habitat when designing and constructing these devices in areas where people live. BMPs are being installed at an accelerated rate in and around the city of South Lake Tahoe in an effort to comply with National Pollutant Discharge Elimination System (NPDES) runoff regulations (State Water Resources Control Board 1999). South Lake Tahoe is the most populous city around Lake Tahoe, and thus in theory has the potential to create the most runoff pollution into the lake. In order for South Lake Tahoe to accommodate an increasing number of residents and visitors, it will need to construct new neighborhoods, commercial property, and public use areas. Many of these development projects will require the installation of curbs and gutters that drain into catch basins and underground BMPs. If these BMPs fail to drain completely, their proximity to human residences may contribute to increased transmission of mosquito-borne disease agents, such as WNV. To help prevent any outbreaks of mosquito-borne diseases, vector control agencies should be consulted when these developments are in their blue print phase, as well as after they are in place and operational. These agencies can offer important perspectives that can curtail any threats and prevent unnecessary mitigation costs.

Mosquito production differed by season in man-made versus natural sites. Winter precipitation in South Lake Tahoe during the study period was below average for the preceding 9 years. Under normal conditions, natural sites may not have dried up as early in the year as they did for this project and would have continued breeding mosquitoes well into the summer. Further data collection over several years is necessary to better define the seasonal mosquito production in BMPs in South Lake Tahoe.

Acknowledgements

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Mosquito Occurrence in Underground Utility Enclosures; Past and Present

Charles M. Myers

California Department of Health Services/Vector-Borne Disease Section, 2151 Convention Center Way, Suite 218B, Ontario, CA 91764

ABSTRACT: Underground utility enclosures have been recognized for more than thirty years as potential breeding sources for vectors. In 1972 a cooperative study was carried out by the California Department of Health Services (CDHS), Pacific Gas and Electric Co., and Southern California Edison Co. (SCE) to determine the potential for mosquito production in subsurface electric transformer vaults. Following this study, recommendations for construction practices and management actions were agreed to by a number of electric utilities in the Western United States in order to prevent mosquito production. Due primarily to the passage of time and the relative inaccessibility of the transformer vaults it appears that little has been done to follow up on these earlier recommendations. Meanwhile, the number of transformer vaults alone has grown from a few tens of thousands to many hundreds of thousands in California. The author has also either observed or been informed by operational mosquito control personnel that other types of utility vaults utilized by cable television, telephone and other public utilities may also serve as breeding sources for various vector species. CDHS has contacted SCE staff and is in the process of setting up and coordinating studies to update the surveillance information and design and management recommendations agreed to earlier. The cooperation and input of other utilities and local vector control agencies will be sought during this process.

The Significance of Underground Storm Drains for Mosquito Control in Urban Los Angeles

Susanne Kluh, Jack E. Hazelrigg, Michael W. Shaw and Minoo B. Madon

Greater Los Angeles Vector Control District, 12545 Florence Ave, Santa Fe Springs, CA 90670

ABSTRACT: Underground storm drain systems (USDS) in Southern California have long been known to produce significant numbers of the southern house mosquito, *Culex quinquefasciatus* Say. However, a routine control program was not implemented until the impending introduction of West Nile virus (WNV). Surveillance data during the 2004 WNV epizootic and epidemic emphasizes the significance of *Cx. quinquefasciatus* in the urban transmission cycle and thus the importance of its control. Greater Los Angeles Vector Control District (GLACVCD) currently employs 10 fulltime vector control specialists to treat mosquito populations in the USDS. This paper discusses the importance of these treatment efforts in the struggle to prevent human disease as well as the need to identify all potential breeding sources in residential neighborhoods.

INTRODUCTION

The 1999-2001 re-assessment of mosquito occurrence and abundance in the Underground Storm Drain System (USDS) demonstrated again that these systems are capable of producing large numbers of *Cx. quinquefasciatus* Say. In response, the GLACVCD developed and implemented an USDS treatment program using the "LAvector/USDS Larvicide Applicator" (Kluh et al. 2001). In the spring of 2002, a crew of four vector control specialists (VCS) was initially assigned to the task of monitoring and controlling the District's extensive USDS, and in early 2003, anticipating the introduction of WNV in southern California, 6 additional VCS were added to the USDS staff to improve coverage. West Nile virus was first detected within GLACVCD boundaries on September 16, 2003, when a mosquito pool of *Cx. quinquefasciatus* tested positive for the virus. A total of four pools, all comprised of *Cx. quinquefasciatus* mosquitoes tested positive for WNV in September and October 2003 (Wilson et al. 2004). These early findings confirmed expectations that *Cx. quinquefasciatus*, would be a major factor in the urban transmission cycle of WNV in southern California, since the *Cx. pipiens L.*, the northern house mosquito was determined an important species in the amplification of WNV in the northeast (Nasci et al. 2001). Therefore evaluating the efficacy of USDS treatment efforts after the first complete mosquito season of WNv presence in the Los Angeles area in 2004 is of great importance to determine the future of the USDS program.

MATERIALS AND METHODS

Routine applications were being conducted using the "LAvector/USDS Larvicide Applicator", an Amflo Hydro-blast steam cleaning tool and siphon (Fig. 1). Truck mounted compressors (Fig. 2) provided 100 psi of pressure and at 1/8 of a turn nozzle opening, the droplet size varied between 20-40 µm. Each application of *Bacillus sphaericus (B.s.,* Vectolex WDG®) / *Bacillus thringiensis isralenesis* (*B.t.i.*, Vectobac 12AS®) through

Figure 1. LAvector/USDS Larvicide Applicator (Amflo Hydro-blast steam cleaning tool and siphon).

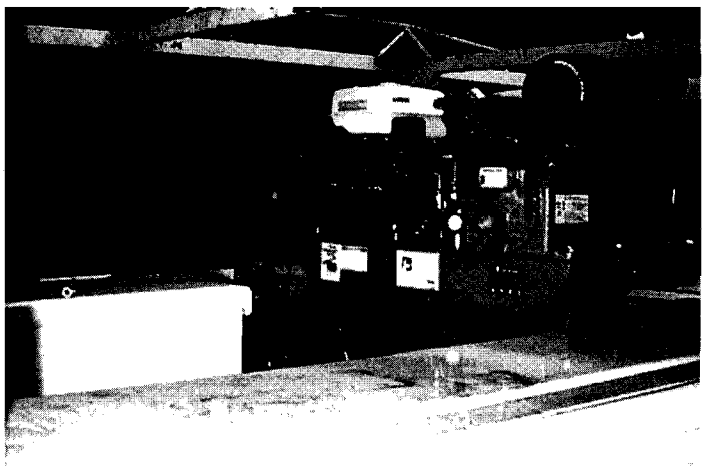

Figure 2. Truck mounted air compressor.

the opening in the manhole cover (Fig. 3) takes about 6 seconds and treatment was applied at every manhole cover of a breeding system (approximately every 158 m).

Figure 3. Treatment through manhole cover opening.

The impact the USDS treatment program has on mosquito populations emerging from these systems can best be evaluated in an area that has been monitored continuously prior to the establishment of a routine treatment program. For convenience reasons, monitoring sites (Fig. 4) were originally selected in relative proximity to the District headquarters in Santa Fe Springs, and these sites were used for various treatment trials and experiments during the developmental phase of the program, resulting in the collection of continuous surveillance data since 1999. Adult mosquito populations were monitored using encephalitis virus surveillance (EVS)/CO_2-baited traps (Newhouse et al. 1966), placed into each system just below the manhole cover, as well as above ground gravid traps (Reiter 1983, Cummings 1992). All adult mosquitoes were identified to species, counted and submitted to the university of California Davis—Center for Vectorborne Diseases (CVEC) for testing for WNV, SLE and WEE infections.

Mosquito surveillance and control activities were carried out in two areas of the District. In one community in san Fernando Valley that had a cluster of WNV human cases, a heavily mosquito breeding USDS was treated and evaluated , using both below ground EVS traps and above-ground EVS and gravid traps. In another community of with a cluster of human cases in the Pico Rivera/Whittier area of the Los Angeles Basin, both treatment (from *B.s./B.t.i.* to Bolden Bear 1111 oil) and treatment intervals from monthly to weekly were evaluated , using the aforementioned methodology.

Figure 4. Underground storm drain system (USDS) study trial area.

RESULTS AND DISCUSSION

Since establishing a routine treatment program, mean mosquito numbers/trap-night clearly demonstrate a drastic overall reduction of USDS mosquito occurrence in the surveillance area. In 2000 and 2001, mean mosquito numbers/trap-night were just below 250, but even after the initial implementation of the USDS treatment program (4 VCS in 2002), mean mosquito/trap-night counts were almost cut in half. Subsequently enhancing the program with additional manpower in the spring of 2003 and to enable staff to respond to known breeding sites in a more timely fashion, and mosquito numbers were reduced to just 8 adults/trap-night in 2004 (Fig. 5). The data thus indicate that GLACVCD's application method can provide adequate mosquito suppression when mosquito breeding USDS are identified and treated at appropriate intervals.

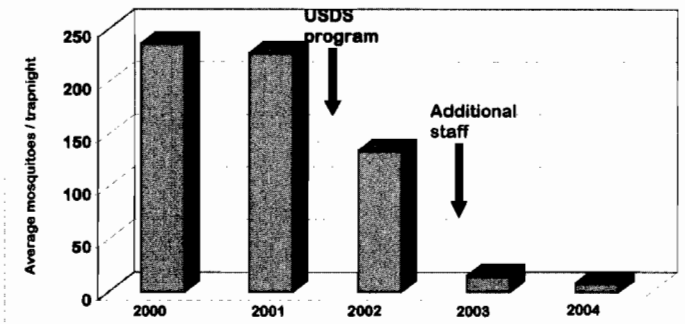

Figure 5. LA Basin USDS Surveillance Area (mosquito averages August – October).

During the significant WNV epizootic/epidemic in southern California in 2004, an area in the San Fernando Valley with a cluster of confirmed WNV positive human cases was found to have a heavily breeding USDS (Fig. 6). This system was trapped and the mosquitoes collected were submitted to CVEC for testing for virus isolation. Control was implemented immediately, and above and below ground trapping was continued to assess the efficacy of control measures. On the night of July 30, 2004, 1,097 *Cx. quinquefasciatus* were trapped in an EVS/CO_2 –baited trap set below ground within the breeding system, and 105 and 48 mosquitoes were collected in an above ground EVS/CO_2 –baited and a gravid trap respectively. Two weeks after the treatment, adult mosquito numbers below ground were substantially reduced to 6 mosquitoes/trap-night, and above ground EVS/CO_2-baited and gravid trap numbers decreased to 22 and 8 mosquitoes/trap-night, even though no additional above ground control measures had been taken. An area-wide survey of USDS revealed no additional breeding systems and the average number of mosquitoes/trap-night was reduced to 4. The USDS was included in the routine treatment schedule and mosquito numbers in the area have remained substantially lower (Fig. 7). During the entire surveillance period,

Figure 6. San Fernando Valley: WNV positive mosquito pools & human cases

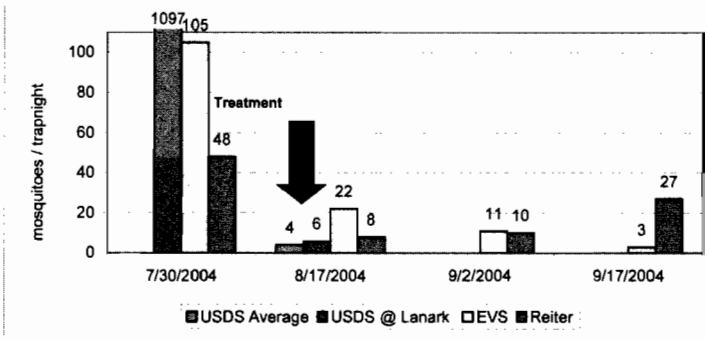

Figure 7. Mosquito occurrence at San Fernando Valley USDS before and after treatment.

mosquitoes were collected and sent for virus testing. When the problem was first discovered, a total of 18 pools were submitted (15 from below and 3 from above ground traps). Of these, a significant number (12 pools, 67%) tested positive for WN virus. Two weeks later, only 2 pools were submitted and both (100%) were WN positive. Notwithstanding the small number of mosquito pools, this increase may be due to the presence of older females in the area the interval short enough to notice the impact larvicidal operations in the area. Older female mosquitoes are of course more likely to have had blood meals and therefore the probability of infection is higher. However, adult mosquito abundance remained low during the next few weeks, and infection rates started to drop (Fig. 8). A faster drop in infection rates could only have been achieved by targeting persistant adult populations.

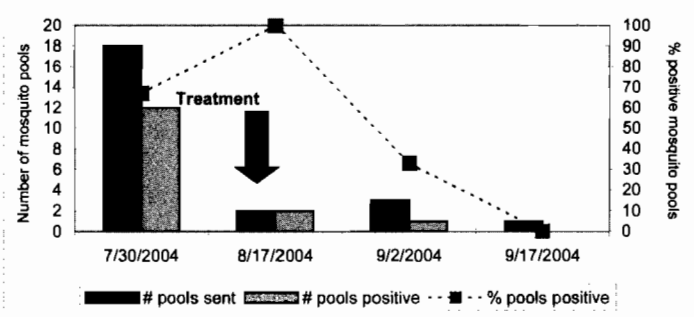

Figure 8. Mosquito pools submitted and WNV positive at San Fernando Valley USDS before and after treatment.

Figure 9. L.A. Basin area with clustering of WN+ human cases.

After experiencing the impact of a single heavily breading USDS on mosquito abundance and WNV occurrence in a San Fernando Valley neighborhood, similar clustering of human cases in the Pico Rivera/Whittier area in the Los Angeles Basin (Fig. 9) drew suspicions upon USDS in those neighborhoods. A thorough investigation of the USDS in the area showed that while a few systems were producing moderate numbers of mosquitoes, overall occurrence was low (8 mosquitoes/trap-night) (Fig. 10).

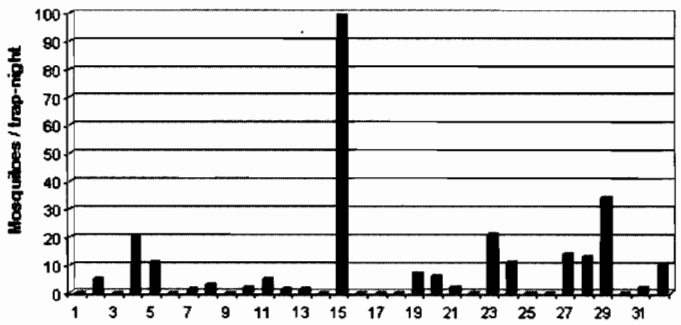

Figure 10. USDS mosquito surveillance in the Pico Rivera/Whittier area of the Los Angeles basin.

Nevertheless, treatment schedules were changed from monthly treatment with *B.s / B.t.i* based formulations to weekly treatment with petroleum oil (Golden Bear 1111®), in an attempt to further reduce mosquito numbers in the USDS. As a result of the intensified treatment effort, the USDS mosquito numbers in the trial area dropped to zero, however, average adult mosquito numbers in above ground gravid traps did not change significantly. The reduction in numbers of adults at the end of September was observed on a wider scale within the District and was probably weather-related (Fig. 11). The results of this trial prove that intensified efforts to reduce USDS mosquito populations even below a low threshold, do not further reduce mosquito abundance above-ground.

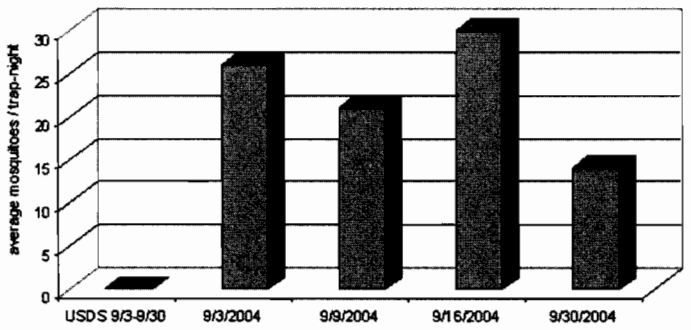

Figure 11. Average mosquitoes per trap-night in the L.A. Basin trial area above as well as below ground.

CONCLUSION

The data illustrate that GLACVCD's USDS larviciding program achieves an effective level of mosquito suppression in systems treated. Moreover, the results also demonstrate that due to the vastness and complexity of the USDS within the District boundaries, not all mosquito breeding systems have currently been identified and therefore, continued surveillance efforts in the USDS will have to be a long–term part of the overall program until all systems have been surveyed and evaluated. Identification and abatement of mosquito breeding within USDS (like the one in the San Fernando Valley) will drastically reduce overall mosquito occurrence and abundance in District neighborhoods. However, it will also continue to be of importance to identify and reduce above-ground breeding sources in order to ensure overall low mosquito abundance within the GLACVCD boundaries.

Acknowledgements

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Studies on Canyon Fly Biology and Ecology in Southern California

Alec C. Gerry and Bradley A. Mullens

University of California - Riverside, Department of Entomology

ABSTRACT: Canyon flies (*Fannia benjamini* complex) are small flies that are found throughout the western United States, the females of which exhibit a marked attraction to animals and people. These flies probably feed on body secretions (sweat, eye secretions, etc...) from which they obtain nutrients required to develop eggs. This attraction to animals and humans makes them a nuisance where they are present in particularly large numbers. Studies conducted in southern California to assess adult canyon fly activity showed a distinct seasonal and diurnal pattern with canyon fly abundance peaking in early summer and daily activity peaks in the early morning and late afternoon hours. Studies to determine response of canyon flies to a number of common attractants demonstrated a marked response to carbon dioxide which could be used in conjunction with suction traps to capture large numbers of the flies. Finally, it was found that at least one member of the *benjamini* complex (*Fannia conspicua*) was laying eggs in large numbers and developing on an exotic ground cover called red apple (*Aptenia cordifolia*).

Mosquito Control in a Subterranean Oasis in the San Francisco Bay Area

James H. Counts, Jr.

San Mateo County Mosquito Abatement District, 1351 Rollins Road, Burlingame, CA

ABSTRACT: The San Mateo County Mosquito Abatement District is located in the San Francisco Bay area. Of the 20 species of mosquitoes occurring in this county, *Culex pipiens* L. is the most abundant. It account for approximately 70% of time and resources devoted to mosquito control and surpasses *Cx. tarsalis* Coquillett as the most important potential local vector of West Nile virus. Larval development occurs primarily in underground sources such as storm drains, catch basins, utility vaults, sewer plants and voids under buildings. This presentation will describe the district's approach to controlling mosquitoes in underground sources. Specialized equipment developed for the diverse types of sources treated will be discussed.

Joint Code Enforcement Efforts

Gale Jirik and Craig Downs

Contra Costa Mosquito and Vector Control District, 155 Mason Circle, Concord, CA 94520

ABSTRACT: Greater demands are being placed on cities, counties, and special districts to protect the public by enforcing the California Health and Safety Code and local nuisance ordinances. At the same time, new storm water best management practice (BMP) requirement are being developed and implemented which include the element of enforcement. This report addresses the need to be involved in the process, what enforcement options are available to government agencies and districts, and the need for cooperation and coordination between agencies.

Aerial Surveillance as an Aid in Mosquito Abatement Program

Min-Lee Cheng, Ph.D[1], Jared Dever[2], Major Dhillon[2], Robert Real[1], Ramiro H. Salazar[1] and Ramiro N. Salazar[1]

[1] *West Valley Mosquito & Vector Control District, Chino, CA 91710*
[2] *Northwest Mosquito & Vector Control District, Corona, CA 92881*

ABSTRACT: The recent addition of aerial surveillance has enabled West Valley Mosquito and Vector Control District (WVMVCD) and Northwest MVCD to more effectively survey for backyard green pools and rural breeding sources. During 2004, the districts performed several helicopter surveillance flights, which resulted in the identification of hundreds of previously unknown breeding sites. All sources identified during the flights were photographed using a variety of photographic equipment, and several different methods were used to pinpoint sources in the photographs. In some cases it has been noted that the newly discovered sources were the leading cause of mosquito nuisance/ production in problematic areas. The resulting source control and elimination may have aided in the prevention of human West Nile virus cases in 2004.

An Alternative Larval Control Method for Cemeteries

Matthew C. Ball

Butte County Mosquito and vector Control District, 5117 Larkin Road, Oroville, CA 95965-9250

ABSTRACT: The need for an effective larval control method in cemetery urns was met using Agrosoke Watering Crystals. The size of cemeteries and the number of urns within them, made chemical control and/or physical control very time consuming. Lab experiments demonstrated the effectiveness and the length of control of Agrosoke Watering Crystals.

Effective Larval Control in a Waterfowl Habitat

Matthew C. Ball

Butte County Mosquito and vector Control District, 5117 Larkin Road, Oroville, CA 95965-9250

ABSTRACT: The need for an effective larvicide to control very high densities of *Ochlerotatus melanimon* Dyar in flooded waterfowl habitat was met using temephos (Abate 2-BG) applied by air. Post field checks and a significant reduction in dip counts demonstrated the effectiveness of temephos for this application. To find the most effective rate for this newly registered product seven trials were conducted.

Surveillance and Treatment of Mosquito Larvae on Flood-Irrigated Pastures, Bishop Paiute Reservation, Inyo County, CA

Brian Adkins and Thomas Gustie

Bishop Paiute Tribe, Environmental Management Office, 50 Tu Su Lane, Bishop, CA 93514

ABSTRACT: In 2004 the Bishop Paiute Tribe, located in Inyo County, CA, initiated a mosquito abatement program for their 350 ha (875 A) reservation. Approximately 0.6 ha (1.5 A) of mosquito habitat was documented, surveyed and treated last summer in the program's first year. The County of Inyo has provided training and assistance to the Tribe's Tribal Environmental Protection Agency. Together, we are working to minimize the threat of Wets Nile virus, first detected in Inyo County in the summer of 2004 (equine and bird cases). The threat of West Nile is expected to be greater in summer of 2005 with human infections likely.

Mosquito Surveillance and Control in the Log Decks in Lincoln, California

Jamesina J. Scott, Kelly Burchman, Ted Williams, and Kristal R. Brown

Placer Mosquito Abatement District, P.O. Box 216, 150 Waverly Drive, Lincoln, CA 95648

ABSTRACT: The Sierra Pacific lumber operation in Lincoln, CA includes 14.4 ha (36 acres) of space where logs are stacked in "decks" with individual decks covering 0.2 -).3 ha (0.5-0.75 A) and standing 18 m (60 ft) high. The decks are irrigated around the clock to prevent the logs from drying out and splitting which reduces their commercial value. The water that collects in the log decks and the associated ponds produces tremendous numbers of *Culex pipiens* L., *Cx. quinquefasciatus* Say, *Cx. stigmatosoma* Dyar, *Cx. tarsalis* Coquillett, and *Cx. erythrothorax* Dyar. Trapping data shows that these mosquitoes migrate into the adjacent residential neighborhoods. We have used chemigation with VectoBac AS-12 and aerial application of methoprene (Altosid pellets), and rarely, truck-mounted adulticiding. This paper will discuss our surveillance and control methods, and the challenges associated with working in this environment.

Building an Indoor Mosquitofish Aquaculture Facility

Noor Tietze

Santa Clara County Vector Control District, 976 Lenzen Avenue, San Jose, CA 95126

ABSTRACT: Santa Clara County Vector Control District constructed an indoor Mosquitofish aquaculture system. Facilities were designed to house 3 tanks or "raceways" for holding Mosquitofish used in the District's biological control program. Aquaneering, Inc. (San Diego, CA 92111) was contracted to build 3 marine-grade aluminum tanks, each 6.0x0.9x0.9 m (20x3x3 ft) (length, width and height), as well as all other operational components of the aquaculture system including: mechanical bead filter, a high rate fluidized bed biofilter, and in-line fluorescent ultra violet light sterilizer, 576.9 L (150 gallon) sump tank, two pumps and all necessary plumbing. Each "raceway" tank holds about 461.5 L (1200 gallons) of water and may be stocked with 4.5 – 6.75 kg (10-15 lb) of Mosquitofish without significant mortality. Grated trenches built into the foundation of the facility create access for a simple water delivery system where the plumbing is largely inconspicuous. One drawback has been electrolysis of the aluminum tanks that caused pitting and accretion formation along submerged portions of the tank walls and bottom.

Evaluation of a Successful Public Relations Campaign

Deborah Bass

Contra Costa MVCD, 155 Mason Circle, CA 94520

ABSTRACT: In 1999, the Contra Costa Mosquito and Vector Control District (District) surveyed their constituents to learn their perception, knowledge, and awareness of the District, local vectors, and vector borne diseases. Based on that survey, the District redirected their communication efforts and developed a five-year Public Relations Strategy and campaign to give meaningful direction to the districts communication efforts with their constituents. In 2004, a follow-up survey was conducted to evaluate the effectiveness of the campaign.

Mosquitofish Production in 2004

John Vignolo and Stacy Bearden

San Joaquin County Mosquito and Vector Control District, 7759 S. Airport Way, Stockton, 95206

ABSTRACT: San Joaquin County Mosquito and Vector Control District maintains a 5.2 ha (13 A) parcel of land which houses eleven 0.16 ha (0.4 A) ponds as well as ten trial-size ponds and four stock tanks. During the 2004 mosquito season, the district had a net gain of 1410.3 kg (3134 lb) of Mosquitofish. This equates to a gain of 128.25 kg (285 pounds)/pond. The district estimates a loss of 97.38 kg (216.41) pounds due to mortality in all ponds. This mortality is attributed to handling and high temperatures during transportation. A detailed pond by pond comparison will be made as well as suggestions for a successful rearing program.

West Nile Virus Surveillance in San Joaquin County in 2004

Stacy L. Bearden and Deanna Black

San Joaquin County Mosquito and Vector Control District, 7759 S. Airport Way, Stockton, 95206

ABSTRACT: During 2004, San Joaquin County Mosquito and Vector Control District (District) ramped up its surveillance system early in the year in an effort to intercept the vectors and show down the transmission of virus to our human populations. Our surveillance tools included chicken serology, mosquito pools, and dead bird pickups. The District ran 1041 mosquito pools in house using either RAMP or VecTest in addition to submitting 552 pools to CVEC for confirmation. The County also received 177 requests to pick up dead birds and submitted 120 birds for testing by RT-PCR. Sixty-two of these birds were tested in house using RAMP and 57 birds were tested using VecTest. The district extended its chickens surveillance into the month of November to ensure that no more seroconversion had taken place.

2004 WNV Public Outreach Campaign by San Joaquin County MVCD

Aaron Devencenzi

San Joaquin County Mosquito and Vector Control District, 7759 S. Airport Way, Stockton, 95206

ABSTRACT: The "2004 WNV Public Outreach Campaign by San Joaquin County MVCD" poster summarizes the efforts made by the San Joaquin County Mosquito and Vector Control District (SJCMVCD) and the SJC West Nile Virus Task Force to inform the public. The poster is reflective of a multi-agency approach to information distribution. High-risk groups were identified and specific materials were developed as communication tools. As the threat of the disease progressed and then became realized, the efforts to reach broader target groups' changed. A combination of posters, flyers, bookmarks, cards, letters, paid ads, news releases, public service announcements (PSA's), presentations, and informational booths were used as tools to provide public outreach. The poster has examples of these items and subsequently summarizes their use.

Improved Methods for Identifying Elevated Enzyme Activities in Pyrethroid-Resistant Mosquitoes

Troy D. Waite[1], Huazhang Huang[1], Bora Inceoglu[1], Julie A. Christiansen[2], Rory D. McAbee[2], Bruce D. Hammock[1], and Anthony J. Cornel[2]

[1]*Department of Entomology and Caner Research Center, University of California Davis, CA 95616*
[2]*Mosquito Control Research Laboratory, Department of Entomology, University of California at Davis, 9240 S Riverbend Avenue, Parlier, CA 93648*

ABSTRACT: The first wild population of pyrethroid-resistant mosquitoes was identified in the United States in Marin County, California. In order to identify the resistance mechanism(s) in this colony (Marin), activities of carboxylesterases (esterases), P450 monooxygenases (P450s), and glutathione-*S*-transferases (GSTs) were measured and compared to those in other pyrethroid-resistant colonies from Fresno, California (Bean), and Mozambique (Boane), and to a pyrethroid-susceptible colony (CQ1). Three novel fluorescent substrates were synthesized which were shown to be more specific for detecting elevated esterase activities potentially involved in pyrethroid resistance than the traditional commercial esterase substrate 1-naphthyl acetate. Partial purification of GSTs resulted in better detection of elevated GST activities than crude mosquito homogenates with the general GST substrate chlorodinitrobenzene. Promega P450-Glo™ Assay Systems, designed for detection of specific P450s isozymes in mammals, are reported for use in detecting elevated P450 levels associated with two separate catalytic pathways in mosquitoes. With the use of these methods, all three groups of enzymes were shown to be elevated in the resistant colonies versus the susceptible colony indicating the necessity to further investigate the potential effect of each enzyme in conferring resistance to pyrethroids.

INTRODUCTION

McAbee et al. (2004) described the first incidence of pyrethroid resistance in a wild population of mosquitoes (*Culex pipiens pipiens* var *molestus*) in the USA. Since then, several other pyrethroid-resistant populations of the *Culex pipiens sensu latu* have been identified in California (unpublished data). Members of the *Culex pipiens* complex are major vectors of the West Nile virus (WNV) in California (Goddard et al. 2002) and the USA (Turrell et al. 2000). The discovery of pyrethroid resistance in these mosquitoes is of concern because in the event of a WNV epidemic the effect of control measures directed against adult mosquitoes may be compromised and no alternative "adulticides" are currently under development.

In order to curtail the further spread of pyrethroid-resistant populations in the USA, improved methods of pyrethroid-resistance detection and monitoring must be developed so that effective resistance management strategies can be employed. Development of assays must proceed along the lines of designing mechanistic assays so that early stages of pyrethroid-resistance can be recognized. For example, elevated esterase activity that mediates organophosphate resistance in mosquitoes is currently monitored using simple, immunochemical assays and pyrethroid knock down resistance (*kdr*-type) can be detected by PCR specifically designed for *Cx. pipiens s.l.* (Martinez-Torres et al. 1999).

Major mechanisms of insecticide resistance involve either mutation within the target site of the insecticide, or an alteration in the rate of insecticide detoxification (Hemingway and Karunaratne 1998). Despite the presence of *kdr*-type resistance at low frequencies in Marin mosquitoes, partial and almost complete reversion to susceptibility as larvae was achieved with *S*, *S*, *S*-tributylphosphorortrithioate and piperonyl butoxide, respectively, suggesting the presence of esterase and P450 mediated resistance also (McAbee et al. 2004). Accordingly, we have started to compare the activity levels of these enzymes and of a potentially third system, glutathione-*S*-transferases (GSTs), to other pyrethroid-resistant (Bean and Boane) and susceptible (CQ1) colonies.

Since enzymes involved in insecticide detoxification may be qualitatively and/or quantitatively changed to confer resistance (Hemingway and Karunaratne 1998), it is necessary to use both general substrates and specific ones that mimic an insecticide. The former detect increases in total isozyme activity as compared to a susceptible colony, while the latter can detect specific isozymes possibly responsible for resistance. In this paper, we report the development of improved methods and novel substrates for detecting increased levels of enzyme activity in *Culex pipiens s.l.*

METHODS

Chemicals: Esterase substrates included the traditional substrate 1-naphthyl acetate (1-NA) and the novel fluorescent substrates *S*-acetate and *cis*- and *trans*-coumarin. GST assays were performed with the general substrate chlorodinitrobenzene (CDNB), while P450 substrates included the commercial substrates Luciferin 6' methyl ether (Luciferin-ME) and Deoxyluciferin (Luciferin-H). Components of buffers and solvents included dimethyl sulfoxide (DMSO), dithiothreitol (DTT), ethylenediaminetetraacetic acid (EDTA), phenylmethyl sulphonyl

fluoride (PMSF), and phenylthiourea (PTU).

Mosquitoes: Four colonies of mosquitoes, *Culex pipiens s.l.* were used in this study. CQ1 was used as a pyrethroid-susceptible colony that originated from Merced, California, in the early 1950s (McAbee et al. 2004). The three resistant colonies (Marin, Bean, and Boane) originated from field-collected specimens from Marin and Fresno Counties, California, and from the village of Boane, Mozambique. Marin, Bean, and Boane mosquitoes were resistant to permethrin, pyrethrum, and deltamethrin respectively. To maintain resistance in the Marin, Bean, and Boane mosquitoes, late 4th-instar larvae were exposed to a dose (approximately at LD_{50} levels) of permethrin, pyrethrum, or deltamethrin every 5 generations, respectively.

Preparation of mosquito extracts: Briefly, homogenates of whole 4th stage larval *Culex pipiens s.l.* (~ 50 individuals / colony) for GST activity assays were prepared by using a plastic mini pestle in 1.5 ml centrifuge tubes in 500 µl of an ice cold 0.1 M sodium phosphate buffer (pH 7.4; 10 mM DTT). The homogenate was momentarily centrifuged at 4°C to remove particulate material.

Cytosolic preparations were prepared according to Zhao et al. (1996). For esterase assays and GST partial purification assays, 4th-instars (~1000 individuals / colony) or adults (~200 individuals / colony) were homogenized in 24 mL 0.1 M ice-cold sodium phosphate buffer (pH 7.6; 0.1 mM PTU, 1 mM DTT and 1 mM EDTA) with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) at a speed of 4-7 for 20 s. Multiple homogenizations were carried out if needed. The homogenate was then centrifuged at 10000xg for 15 min and the supernatant was filtered through glass wool and centrifuged at 4°C at 100000xg for 1 h. The resulting pellet (microsomes) was suspended in 5 mL buffer. The supernatants (cytosols) and microsomes were stored at -80°C until used.

Preparation of mosquito microsomes for adult (~ 200 individuals / colony) P450 activity assays was done by removal of the heads to avoid enzyme inhibition from xanthommatin eye pigments (Schonbrod and Terriere 1971). Mosquitoes were treated with liquid nitrogen in a sieve with a 2-mm mesh. Small steel balls were gently shaken over the mosquitoes to fractionate the bodies and to separate the smaller heads (and wings and legs) from the joint abdominal-thoracic components. The abdomen-thorax complexes were then placed into 10 ml of a 0.1 M ice-cold sodium phosphate buffer (pH 7.6; 0.1 mM PTU, 1 mM DTT, 1 mM EDTA, and 1 mM PMSF) and homogenized in a 40-ml glass Dounce homogenizer with a loose B pestle (Wheaton Science, Millville, NJ). The separation of homogenate into cytosolic and microsomal extracts was done with centrifugation as described previously, but the pellet (microsomes) was placed into a 100- µl sodium phosphate buffer identical to the homogenization buffer without PTU and with 20% glycerol (v/v). Samples were stored at -80°C until used.

Protein concentrations of extracts were measured by the method of Bradford (1976) using bovine serum albumin as the standard.

GST partial purification: Glutathione sepharose affinity resin (Sigma-Aldrich, Co., St. Louis, MS) was used for partially purifying GST isozymes from cytosolic fractions. The resin was washed with 10 volumes of phosphate buffered saline (PBS) according to manufacturer's instructions. Cytosolic extracts were incubated with a ratio of 50:1 with glutathione affinity resin in batch format for 4 hours. After incubation, the mixture was centrifuged for 5 min at 2000xg to pellet the resin. The supernatant contained the unbound fraction. The resin was then washed three times with 10 volumes of PBS. Elution was accomplished by incubating the resin in 10 volumes of elution buffer (10 mM reduced glutathione; 50 mM Tris HCl, pH 8.0). The eluted proteins were concentrated using a 10000 Da cutoff ultrafilter (Centricon, Millipore, PA).

Enzyme assays: Colorimetric esterase activity assays with the general substrate 1-NA and cytosolic extracts were performed in 96-well microplates using a Spectramax microplate spectrophotometer (Molecular Devices, Sunnyvale, CA) by modifying the method of Gomori (1953). Reaction mixtures contained (final conc. in 250 µL): protein solution (20 µL), phosphate buffer (0.1 mM; pH 7.0) with 0.02% Triton X-100, and a solution containing Fast Blue B salt (1.2 mM) and substrate (2.15 mM final concentrations for 1-NA). Four replicates were assayed for each sample. Absorbance was measured at 450 nm during the first 5 min of the reaction, and rates were converted to nmol min^{-1} using the extinction coefficient 9.25 mM^{-1} 250 mL^{-1} for 1-naphthol (Grant et al. 1989). The amount of protein in each assay varied with substrates and was adjusted so that no more than 10% of the substrate was hydrolyzed over the reported time. Activities were corrected for non-enzymatic hydrolysis using reactions without protein as controls.

Fluorescent esterase activity assays with cytosolic extracts were performed by modifying the methods of Wheelock et al. (2003). In general, activities were measured in black 96-well polystyrene clear flat-bottom microtiter plates (Corning, Inc., New York, NY, USA) at 30°C for all hydrolases with a Spectrafluor Plus Fluorometer (Tecan, Research Triangle, NC). Substrates (Fig. 1) were prepared in ethanol (10 mM) for *S*-acetate or 10% DMSO in ethanol for *cis*- and *trans*-coumarin. Reaction mixtures contained (total volume 201 µL): 20 µL protein solution, 180 µL 20 mM Tris/HCl buffer (pH 8.0) for *S*-acetate or 180 µL 100 nM sodium phosphate buffer (pH 7.0) for *cis*- and *trans*-coumarin, and 1-µL substrate solution. Reactions were initiated by adding 1-µL substrate solution (final concentration 50 µM) followed by shaking for 5 s. Three replicates were performed for each substrate. Fluorescence was monitored with excitation at 330 nm and emission at 465 nm for *S*-acetate or excitation at 330 nm and emission at 450 for *cis*- and *trans*-coumarin. The standard curve of dependence of aldehyde or coumarin fluorescence response on protein concentration was generated by adding an equivalent amount of each protein sample to correct protein-induced aldehyde quenching.

GST activity assays with whole mosquito homogenates and partially-purified solutions were done by modifying the methods of Grant and Matsumura (1988) in a 0.1 M sodium phosphate buffer (pH 6.5). A 10 ì l of mosquito homogenate, 10ì l of CDNB, and 10 ì l of reduced glutathione (fin. conc. 5mM) were added to make a final assay volume of 300ì l/ well. Three replicates were done for each measurement; activities were corrected for non-enzymatic hydrolysis using reactions without protein as controls. The conjugation of CDNB to glutathione was monitored on a Spectramax microplate spectrophotometer (Molecular Devices, Sunnyvale, CA) by measuring absorbance at 340 nm at 30°C for

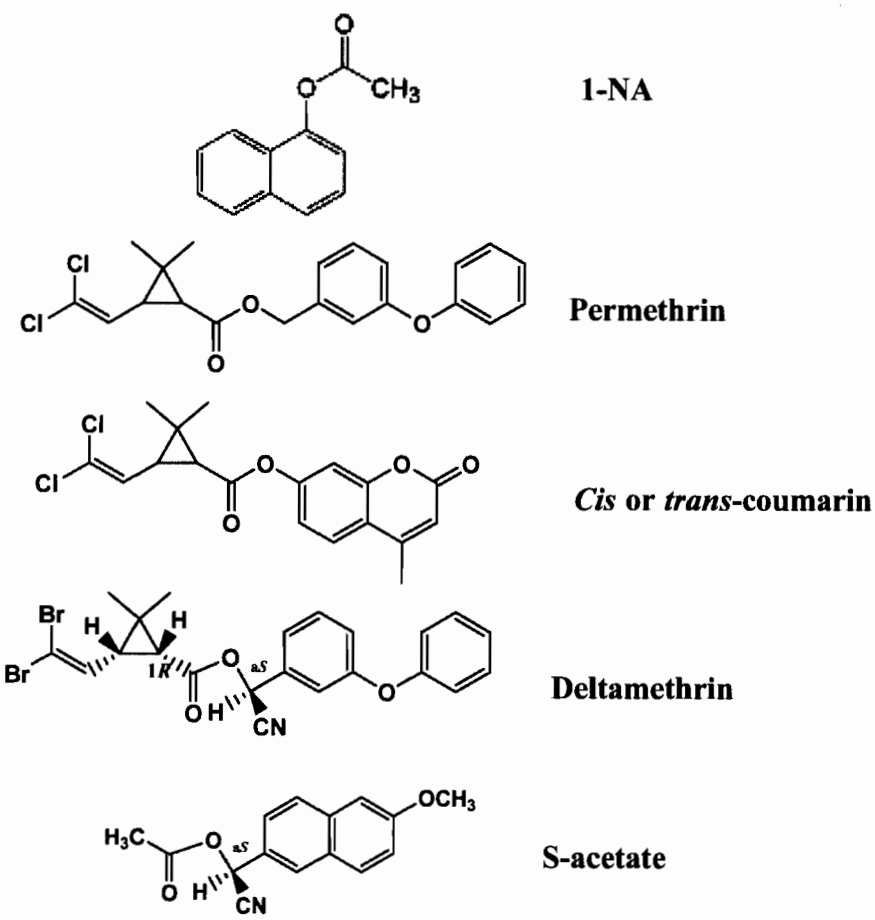

Figure 1. Structures of 1-NA, novel esterase substrates, and examples of Type I (Permethrin) and Type II (Deltamethrin) pyrethroids.

15 minutes after the addition of glutathione. Rates were converted to nmol min^{-1} using the extinction coefficients of 8.5 O.D. mM^{-1} 300 mL^{-1} for CDNB (Grant et al. 1989).

P450 activity was measured with the P450-Glo™ (Promega Corporation, Madison, WI) Assay System according to the manufacture's protocol. Two substrates, Luciferin-ME and Luciferin-H were used. When either substrate is metabolized by P450s, the product is luciferin, which is then reacted with beetle luciferase to produce chemical luminescence, the intensity of which can be correlated to the activity of the P450s.

RESULTS AND DISCUSSION

Activities of cytosolic esterases toward 1-NA and *S*-acetate varied greatly with substrates and colonies (Table 1). Compared with the pyrethroid-susceptible colony (CQ1), hydrolytic activities of cytosolic esterases toward 1-NA increased only in the adult stages of Marin (185% increase). In contrast, elevated activities of cytosolic esterases toward *S*-acetate were observed in the adult stages of all three pyrethroid-resistant colonies (211-277% increase) and in the larval stages of Marin and Boane (141 and 147% increase, respectively). Although *S*-acetate is a general substrate, it was synthesized to more closely resemble (Fig. 1) pyrethroids than 1-NA and seems to be more specific for detecting elevated esterase activities potentially involved in pyrethroid resistance.

When pyrethroid-like fluorescent substrates were used, results [Table 1] suggested that they were also possibly better indicators of pyrethroid resistance than 1-NA. Compared with CQ1, elevated esterase activities were present in the adult stages of all three pyrethroid-resistant colonies (133-161% increase) and in the larval stages of Marin and Boane (173 and 156% increase, respectively) with the substrate *trans*-coumarin. More than four times the activity of CQ1 was measured with *cis*-coumarin in adult Marin. This significant increase is expected because these fluorescent coumarin substrates are specific mimics of the type I pyrethroid, permethrin

Table 1. Specific activity of mosquito cytosolic esterases toward different substrates[a].

Colony	1-NA		*S*-acetate		*cis*-coumarin		*trans*-coumarin	
	Mean (±SD)	RR[b]	Mean (±SD)	RR[b]	Mean (±SD)	RR[b]	Mean (±SD)	RR[b]
Larvae								
CQ1[d]	22.10(0.14)	1.00	0.19(0.01)	1.00	NM[c]		26.64(1.50)	1.00
Marin[d]	25.17(2.14)	1.14	0.27(0.02)	1.41	7.90(0.63)		46.22(0.63)	1.73
Bean	13.25(1.26)	0.74	0.02(0.001)	0.10	1.10(0.33)		24.11(1.05)	0.91
Boane[d]	23.95(2.14)	1.08	0.28(0.01)	1.47	6.78(1.07)		41.18(2.54)	1.56
Adult								
CQ1[d]	93.82(1.64)	1.00	1.37(0.10)	1.00	88.27(15.64)	1.00	86.25(8.39)	1.00
Marin[d]	173(9)	1.85	2.89(0.32)	2.11	391(51)	4.43	258(22)	2.99
Bean	83.84(1.59)	0.89	3.52(0.15)	2.57	80.28(7.42)	0.91	115.11(9.82)	1.33
Boane[d]	89.50(3.10)	0.95	3.80(0.22	2.77	66.17(1.07)	0.75	138.6(5.4)	1.61

[a]Unit: present as nmol/min/mg for 1-NA and *S*-acetate, pmol/min/mg for *cis*- and *trans*-coumarin.
[b]RR: resistance ratio.
[c]NM: not measurable under a similar protein concentration.
[d]Data in column from Huang, H., B. Inceoglu, P.D. Jones, J.E. Stok, J.A. Christiansen, T.D. Waite, B.D. Hammock, and A.J. Cornell. Development of pyrethroid-like fluorescent substrates for esterases from pyrethroid-resistant mosquitoes. (unpublished).

(Fig. 1), to which Marin is known to be resistant.

In addition to esterases, elevation of GST activities in the resistant colonies as compared to the susceptible colony was found. This indicates that GSTs should be considered as a third complex of enzymes that are related to metabolism of pyrethroids in California *Cx. pipiens s.l.* Initially when whole insect homogenates were used, 2-fold or less increase in GST activity in resistant colonies compared to those in the susceptible colonies was detected (Fig. 2). The levels of GST in some colonies (Bean and Boane) were not elevated relative to CQ1. However, after affinity purification of GSTs, the activities increased in all resistant colonies by as much as 5-fold higher relative to CQ1. This brings into question the efficacy and sensitivity of measuring GST activities in crude, whole mosquito homogenates and hence the likelihood of missing important pyrethroid resistance mechanisms.

This is the first report of using the Promega P450-Glo™ Assay System for measuring P450 activity in insects. In mammals, these substrates have been used successfully to measure activities of individual isozymes which made them potentially useful in mosquitoes to identify the same. With Luciferin-ME, Boane p450s showed roughly a 50% increase in demethylation activity as compared to CQ1, Marin, and Bean. In contrast, hydroxylating activity of P450s with Luciferin-H, although about 10 times less that with Luciferin-ME, was increased by about 50% as compared to the other three colonies (Fig. 3). In this case, the assays most likely detected the elevation/existence of different P450 isozymes

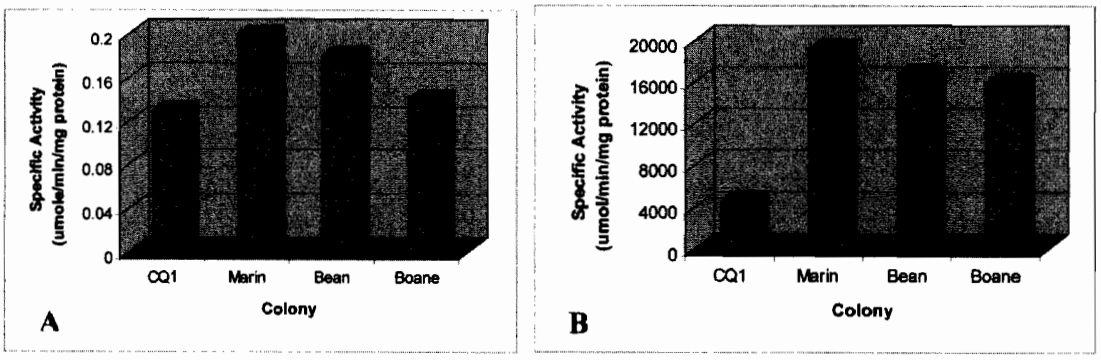

Figure 2. Comparison of larval glutathione-*S*-transferase activities of a pyrethroid-susceptible (CQ1) and three pyrethroid-resistant (Marin, Bean, Boane) *Culex pipiens s.l.* colonies with CDNB before (A) and after (B) affinity purification.

in the two pyrethroid-resistant mosquito colonies as compared to the susceptible one. Bean did not show increased levels of P450 activity with either substrate.

Caution must be taken in correlating elevated activity levels of enzymes with resistance in insecticide-resistant versus susceptible populations when using general or specific substrates until assays are run with the real insecticide and increased levels of catalysis are actually observed and measured. Assays with the actual insecticide may be difficult, expensive, and time-consuming as compared to biochemical assays. For this reason, biochemical assays with surrogate substrates are extremely important in targeting potential enzymes responsible for increased detoxification of pesticides. Thus, assays must be found that are sensitive enough to detect quantitative and qualitative changes in the enzyme profiles of mosquito populations. In this paper, we have introduced three new substrates for measuring elevated esterase enzyme levels in mosquitoes that seem to be more sensitive than the commonly-used general esterase substrate 1-NA. In addition, we have shown that partial purification of GSTs, assayed with the available general substrate CDNB, is more effective in detecting elevated activities than assays run with crude extracts. Finally, we have introduced the use of the Promega P450-Glo™ Assay System substrates, already known to be sensitive to certain P450 isozymes in mammals, for the use in insect systems and have shown that various substrates do seem to detect particular isozymes in them.

Interestingly, the levels of all three types of detoxification enzymes were found to be elevated in Marin and Boane. Bean was similar, but did not show an increase in P450 activity as compared to CQ1. We are now investigating all three enzyme responses more intensely, hoping to purify and characterize any isozymes directly responsible for pyrethroid cleavage.

Acknowledgements

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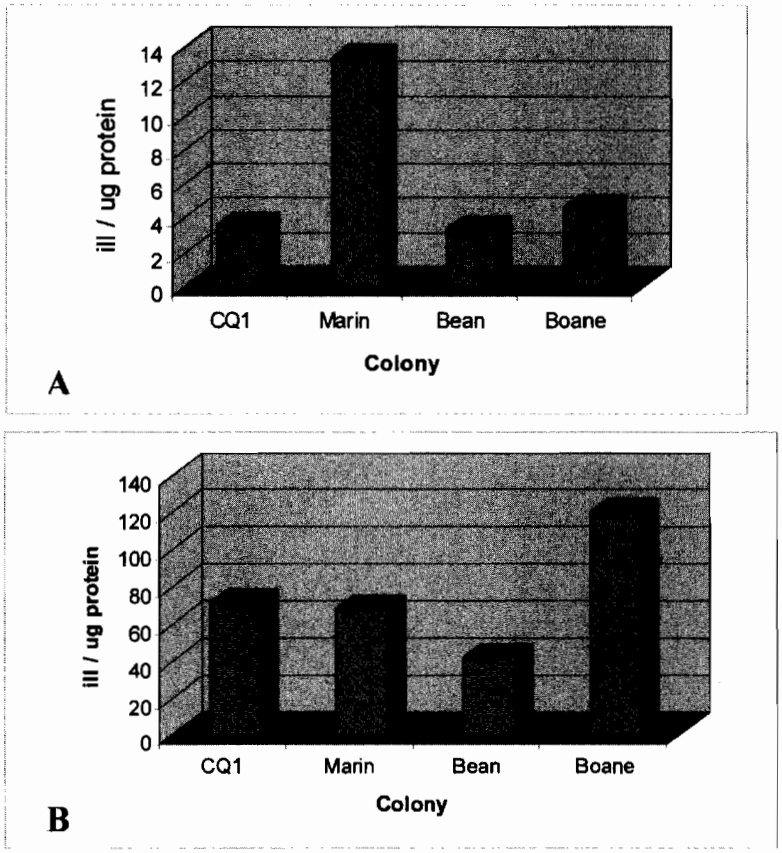

Figure 3. Comparison of adult microsomal P450 monooxegenase activities of a pyrethroid-susceptible (CQ1) and three pyrethroid-resistant (Marin, Bean, Boane) *Culex pipiens s.l.* colonies with two different Promega P450-Glo™ Assay System substrates. (A) Luciferin 6' methyl ether (B) Deoxyluciferin.

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West Nile Virus Surveillance and Testing Procedures for Dead Birds in Orange County, 2004

Karin De Collibus, Josie G. Weir, Seth Ruga, and Richard H. Evans

Orange County Vector Control District, P.O. Box 87, Santa Ana, CA 92702

ABSTRACT: With the emergence of West Nile virus in the U.S. in 1999, the Orange County Vector Control District initiated a surveillance program in 2000 to detect the virus when it arrived in California. Tests were developed to detect antibodies to WNV in trapped free-ranging wild birds, as well as virus in the tissues of dead birds (Jozan, Evans, et al 2003). In 2003, this program was initiated with a call for animal control agencies and the public to report and submit any dead birds. Birds were screened and necropsied to determine suitability for testing. Tissue samples were taken from suitable birds for the detection of viral RNA by reverse-transcriptase polymerase chain reaction (RT-PCR) and/or viral antigen via immunohistochemical staining (IHC). Additionally, on most corvids the VecTest® was performed on oropharyngeal swabs. In 2004, birds were received from 44 cities, totaling 1047 specimens, of which 435 were testable. By at least one testing method, 253 birds tested positive for WNV. Results for each test type were statistically evaluated and both IHC and PCR were found to be 94.6% accurate in determining the presence of WNV in dead birds.

INTRODUCTION

West Nile Virus (WNV) first appeared in New York, during the summer of 1999. Since that time it has steadily spread westward across the United States, causing widespread mortality in corvid populations. In response to the inevitable arrival of WNV in California, the Orange County Vector Control District (OCVCD) initiated a surveillance program in 2000 to detect the virus in dead birds, in addition to the ongoing mosquito and wild bird surveillance used to monitor native arboviruses (St. Louis encephalitis and Western equine encephalomyelitis).

In dead birds, 3 methods are commonly used to determine whether a bird has been infected with WNV; immunohistochemical staining (IHC), RT-PCR, and VecTest® (Gibbs and Mead. 2002, Ellis et al. 2002). The IHC and PCR methods differ in their sensitivities with IHC detecting being about to 10^2 and PCR theoretically 10^1 particles per sample. Because a virus is composed of both protein and nucleic acid components it would be expected that a viremic bird would test positive by both IHC and PCR. The VecTest® is a commercial dip-stick test to detect WNV in mosquitoes. Since corvids are known to shed virus in oropharyngeal secretions, the VecTest® has been adapted to test these secretions for WNV. However its utility in this regard has been brought into serious question by the fact that ≥15-20% false negatives occur routinely.

METHODS AND MATERIALS

Bird Surveillance: In response to anticipated WNV activity, a request was made to the public to report any dead birds. Birds were collected from 44 cities by the staff of OCVCD, with the help of the public, animal rehabilitation groups and animal control agencies. Many of the birds were determined to be unsuitable for testing based on telephone conversations as a result of carcass deterioration, i.e. greater than 24 h. If a bird appeared to be suitable, an OCVCD technician or a lab assistant was dispatched to retrieve the bird. The exact address and Thomas Guide^R map coordinates of each positive bird were plotted on a large county map ("War Map").

Bird Necropsy: Each bird was necropsied by a veterinary pathologist to obtain tissues for immunohistochemisty (IHC) and RT-PCR. Additionally, on most corvids the VecTest® was performed on oropharyngeal swabs. In practice, spleen, kidney, liver, heart, and brain (cerebellum, cerebrum, optic tectum) were sampled for IHC. However, many birds that were brought in were found to be in varying states of postmortem degeneration, thus preventing complete organ sampling of all birds. Additionally, a kidney sample was placed in lysis buffer, frozen, and stored at -70° C prior to shipment to the Center for Vectorborne Diseases (CVEC), University of California, Davis for RT-PCR testing.

Immunohistochemistry: Tissues were fixed in 10% formalin solution and then cut into cassettes before being sent to a commercial histopathology service for processing, paraffin embedding, sectioning (5 um) and mounting on charged microslides. Slides were then stained according to the procedure described by Steele et al. (2000).

RESULTS AND DISCUSSION

Of the 1047 birds collected, only 435 were testable and 253 of these were found positive for WNV antigen/nucleic acid by IHC, PCR, and/or VecTest. Birds were collected from January through December with the first positive occurring in April and the last in December (Fig. 1). Both IHC and PCR were performed on 242 of the 435 birds found acceptable for testing. The two testing methods agreed for 216 (89.4%) of these birds. IHC was negative and PCR positive in 13 birds, while in an additional 13 birds PCR was negative and IHC positive. These data correspond to each test being 94.6% accurate in determining the presence of WNV in a specimen. Based on the test results, WNV activity occurred predominantly in the northern part of the county (Fig. 2). However, some infected birds were found south of this line, possibly

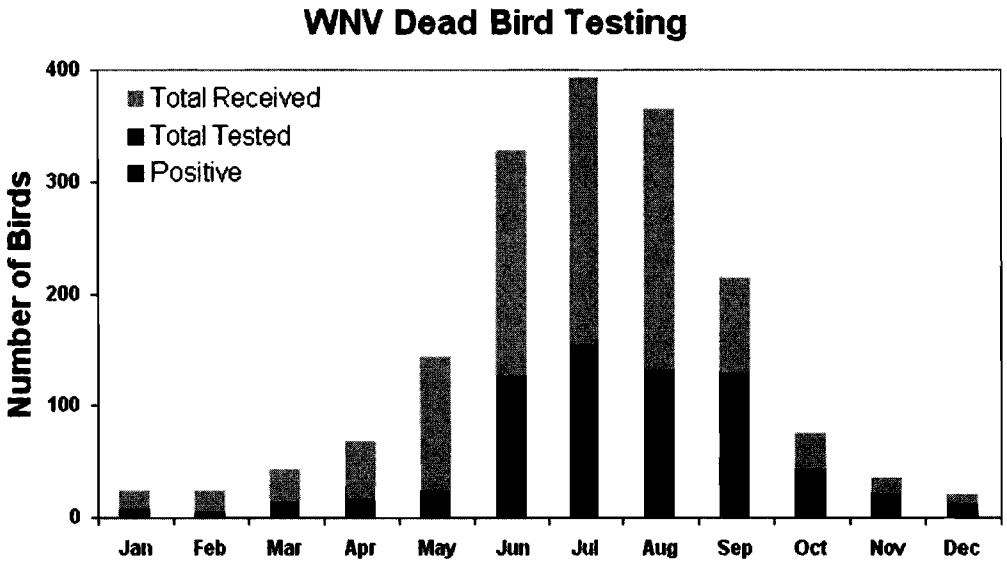

Figure1. Numbers of dead birds collected, tested, and positive by month. Positives were found from April through December, 2004.

as a result of normal flight activity. A small focus of WNV-positive hummingbirds, comprising 5 of the 7 positive hummingbirds reported in California in 2004, was found in the Laguna Woods area in the southern portion of Orange County (Fig. 2).

Both IHC and PCR proved to be highly accurate (94.6%) in detecting the presence of WNV in dead birds, despite their vastly differing methodologies. The inherent sensitivity difference of an order of magnitude is offset by the reliable, cell specific results obtained via IHC. Staining of tissue allows a precise analysis of the cell and tissue types being infected by the virus (Figure 3). Furthermore the lower sensitivity of IHC may screen out specimens that have or do not have a patent viral infection, but perhaps died of other causes. IHC also preserves a stable specimen that can be re-examined if there is any doubt as to the test results, allowing for ease of confirmation.

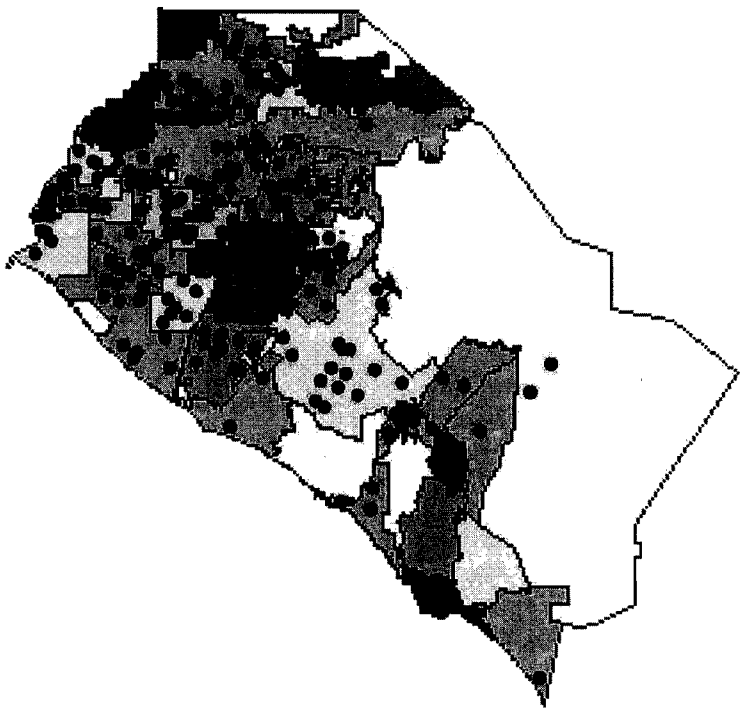

Figure 2. Birds tested positive by IHC, RT-PCR, and Vectest^{R}, Orange

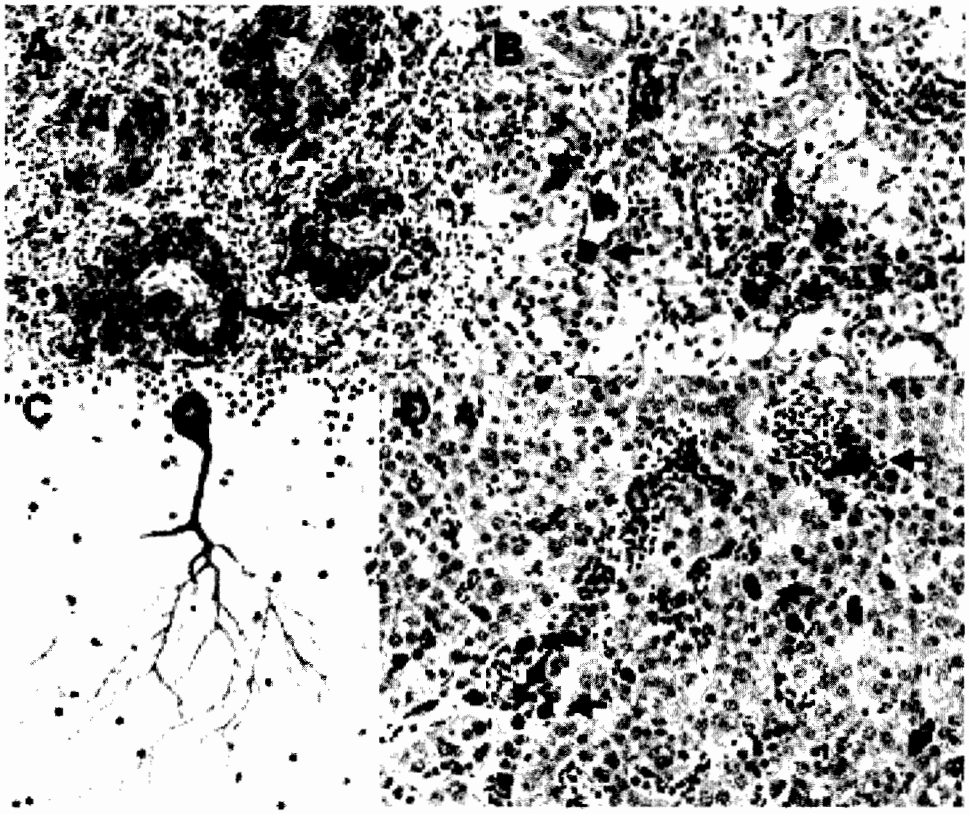

Figure 3. WNV positive crow tissue sections: A) Spleen showing WNV positive macrophages surrounding arterioles. B) Kidney macrophages positively stained for WNV antigen. C) Purkinje Cell in the cerebellum. D) Liver macrophages.

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Distribution of Adult Mosquitoes Trapped at Various Heights in the Prado Wetlands, Riverside County, CA in 2003-04

Lal S. Mian[1/] and Greg A. Williams[2/]

[1/]*Department of Health Science, California State University, San Bernardino, CA 92407-2397*
[2/]*Northwest Mosquito and Vector Control District, 1966 Compton Avenue, Corona, CA 92822*

ABSTRACT: Of the 6447 mosquitoes collected in CO_2-baited traps over five nights during September 2003 through January 2004, the two predominant species were *Culex tarsalis* (39.0%) and *Cx. quinquefasciatus* (35.5%), followed by *Cx. erythrothorax* (16.5%), *Cx. stigmatosoma* (4.4%), *Anopheles hermsi* (2.8%), *Culiseta particeps* (1.4%) and *Cs. inornata* and *Ochlerotatus washino* (0.4%). Traps were hung from trees at heights of 0.6, 2 and 6 m. *Anopheles hermsi*, *Cx. erythrothorax* and *Cs. particeps* were collected in higher numbers at lower levels, whereas Cx. *quinquefasciatus*, *Cx. stigmatosoma* and *Cx. tarsalis* varied as the season progressed. The latter three species had a bimodal distribution at the lowest and highest levels during September; later the pattern changed with more mosquitoes (>50%) being trapped at the lower levels.

INTRODUCTION

With the recent introduction, establishment and potential threat of West Nile virus in California, collection of baseline data on the ecology and behavior of known and potential vector species becomes an important aspect in the modification of mosquito surveillance and control strategies. The use of CO_2 –baited traps in arbovirus surveillance has been extensively practiced in potentially known breeding as well as exposure risk areas. The age and flight patterns of mosquitoes are key determinants of host seeking behavior and pathogen transmission to wildlife, avian, or a human host. Physiological age composition of female mosquitoes in a sample may vary based on location, method and time of capture and the age status of individuals (Gillies 1974). Whereas some studies show a greater proportion of nulliparous mosquitoes in CO_2-baited traps than CDC miniture light traps (Magnarelli 1975, Feldhaufer and Crans 1979), a high proportion of parous individuals may be collected in CO_2-baited traps than in resting shelters (Milby et al. 1983). Variability in parity rate can be found in different species caught in miniture light and CO_2-baited traps (Morris and Defoliart 1969). According to Myer et al. (1984), parity rates for female *Culex tarsalis* Coquillett caught in CO_2-baited traps were higher than those captured in New Jersey light traps or resting boxes. Based on these studies, host-seeking parous females caught in CO_2-baited traps would represent epidemiologically important samples in arbovirus surveillance. The traps are placed at eye level (~2m) above the ground level. Older and ornithophagous individuals may be found even at higher levels. In Gambia, South Africa parous *Cx. thalassius* Theobald were collected at 9.15 m, whereas nulliparous individuals appeared to remain at 0.91 m (Snow and Wilke 1977). The occurrence of nulliparous mosquitoes was attributed to incomplete development of wings and flight muscles. Pfuntner et al. (1988) reported higher parity rates for *Cx. quinquefasciatus* Say at 10 m than at the lower trap heights in rural areas of Chino. They also found *Cx. stigmatosoma* Dyar and *Cx. tarsalis* more at the 5-m than at 1-m height. In subsequent studies conducted near a dairy by the Prado Basin wetlands, *Cx. quiquefasciatus* and *Cx. tarsalis* were reported to exhibit a bimodal distribution with peaks at 0.6- and 6-m levels (Mian et al. 1990, Mian 2003).

The area of Prado Wetlands that is close to dairies and human habitation, provides ideal breeding habitats to a variety of mosquito species (Mian et al. 1990). It also harbors a diverse group of wildlife including mammals and especially birds that play a major role in the transmission of encephalitis viruses. The present study, an extension of 2002 trials carried out from August to November, was undertaken to determine the distribution of host-seeking or feeding zones of adult mosquitoes at 3 different heights from September 2003 to January 2004.

MATERIALS AND METHODS

The study area situated in the northeastern part of the Prado Wetlands has coordinates of 33° 53' 70" N and 117° 36' 21" W. At this site, 4 willows, *Salix* sp., approximately 18 m high and 70-80 m apart, were selected along the north bank of northeastern diversion of the Santa Ana River. The trees were partially surrounded by dense vegetation. A nylon rope (18 m long, 2.1 cm thick) was made into a loop around a strong branch (~7 m high) on each tree. Three CO_2-baited traps were hung at 0.6, 2 and 6 m level from the rope. The traps were set up in the evening and picked up the following morning. The tests were run over five nights—September 10, 19, November 3, December 22, 2003 and January 14, 2004.

In the laboratory, field collected mosquitoes were anesthetized with triethylamine and then identified to sex and species (Myer and Durso 1993). Statistical analysis (*P* values) was done using PSI (1993).

RESULTS AND DISCUSSION

A total of 6447mosquitoes was collected during the 5 nights of trapping. The most abundant species was *Cx. tarsalis* (39.0%), followed closely by *Cx. quinquefasciatus* (35.5%). Other species collected were *Cx. erythrothorax* Dyar (16.5%), *Cx. stigmatosoma* (4.4%), *Anopheles hermsi* Barr & Guptavanji (2.8%), *Culiseta particeps* Adams (1.4%) and *Cs. inornata* Williston, and *Ochlerotatus washinoi* Lanzaro & Eldridge (0.4%). Data on mosquito distribution by trap height showed that *An. hermsi*, *Cx. erythrothorax,* and *Cs. particeps* were found in significantly larger numbers at lower levels, whereas *Cx. quinquefasciatus*, *Cx. stigmatosoma,* and *Cx. tarsalis* levels varied as the season passed on (Table 1). Variations in data from tree to tree made statistical analysis difficult for some species. However, based on numerical data, the latter three species clearly showed a bimodal distribution earlier during the study in September. This finding appears to be in agreement with earlier studies that focused on summer and fall populations in this area (Pfuntner et al. 1988, Mian et al. 1990). In a previous study carried out in the same area, summer and fall mosquito numbers were positively correlated with increasing trap heights (Mian 2003). The lower numbers at near ground level could have been due to the lack of younger females.

Table 1. Distribution of adult mosquitoes trapped at various tree heights in the Prado Wetlands in 2003-04.

Date	Height (m)	Mean[1/] number of mosquitoes/trap-night (Total # collected)							
		Anh	*Cxe*	*Cxq*	*Cxs*	*Cxt*	*Csp*	*Other*	Total #
09/11/03	0.6	11.3(34)	37.7(113)	121.0(363)	13.0(39)	235.3(706)	13.0(39)	3.3(10)*	1304
	2.0	0.7(2)	51.0(153)	77.7(233)	5.7(17)	29.0(87)	0(0)	2.0(6)*	498
	6.0	0(0)	16.0(48)	155.0(465)	24.3(73)	121(363)	0.7(2)	0(0)	951
	P-value	0.0319	0.2420	0.6419	0.5101	0.6200	0.1771		
09/19/03	0.6	8.5(34)	48.3(193)	56.8(227)	8.3(33)	129.3(517)	5.3(21)	0(0)	1025
	2.0	1.8(7)	40.5(162)	71.5(286)	9.3(37)	63.0(252)	0(0)	0(0)	744
	6.0	0(0)	11.3(45)	105.3(421)	18.0(72)	138.3(553)	0(0)	0(0)	1091
	P-value	0.0365	0.6510	0.4184	0.2220	0.5571	0.0026		
11/03/03	0.6	2.0 (8)	12.0 (48)	0.5 (2)	0 (0)	0 (0)	0 (0)	0 (0)	58
	2.0	2.5 (10)	6.3 (25)	3.8 (15)	0.3 (1)	0 (0)	0 (0)	0 (0)	51
	6.0	1.8 (7)	5.3 (21)	9.8 (39)	0.3 (1)	0 (0)	0 (0)	0 (0)	68
	P-value	0.8956	0.5675	0.1621	0	0	0		
12/22/03	0.6	3.0 (12)	21.3 (85)	27.3 (109)	1.5 (6)	3.3 (13)	3.5 (14)	0 (0)	239
	2.0	0.8 (3)	5.8 (23)	17.0 (68)	0.8 (3)	2.0 (8)	1.3 (5)	0.3 (1)**	111
	6.0	0.5 (2)	7.8 (31)	8.8 (35)	0.3 (1)	1.3 (5)	0.3 (1)	0 (0)	75
	P-value	0.0934	0.2616	0.3251	0.0322	0.6803	0.1259		
01/14/04	0.6	7.8 (31)	12.5 (50)	2.3 (9)	0 (0)	0.5 (2)	2.8 (11)	0.3 (1)**	104
	2.0	7.8 (31)	10.5 (42)	1.8 (7)	0 (0)	0.5 (2)	0.3 (1)	0 (0)	83
	6.0	0 (0)	7.3 (29)	2.8 (11)	0 (0)	1.3 (5)	0 (0)	0 (0)	45
	P-value	0.1092	0.5997	0.8362	0	0.5731	0.0336		
Total mosquitoes		181	1068	2290	283	2513	94	18	6447
%		2.8	16.5	35.5	4.4	39.0	1.4	0.4	100

[1/] Mean of 4 replicates (trees). Mosquitoes by species were: *Anh—Anopheles hermsi, Cxe—Culex erythrothorax, Cxq—Cx. quinquefasciatus, Cxs—Cx. stigmatosoma, Cxt—Cx. tarsalis. Csp—Culiseta particeps.*

**Culiseta inornata.*

***Aedes washinoi.*

Percent distribution profiles of the six species and their host-seeking/feeding zones clearly support the preferences of each species (Fig. 1). For example, *An. hermsi*, *Cx. erythrothorax,* and *Cs. particeps* with a preference for lower, near ground level, might be feeding on small hosts such as rodents and lagomorphs. Higher numbers at the lower levels might also include nulliparous and younger females with wings and flight musculature not fully developed enough to fly at higher levels (Snow and Wilke 1977). Species preferring host feeding at higher levels may epidemiologically increase the chances of feeding on avian hosts and the likelihood of encephalitis virus transmission. The data on *Cx. quinquefasciatus*, *Cx. stigmatosoma,* and *Cx. tarsalis* in this

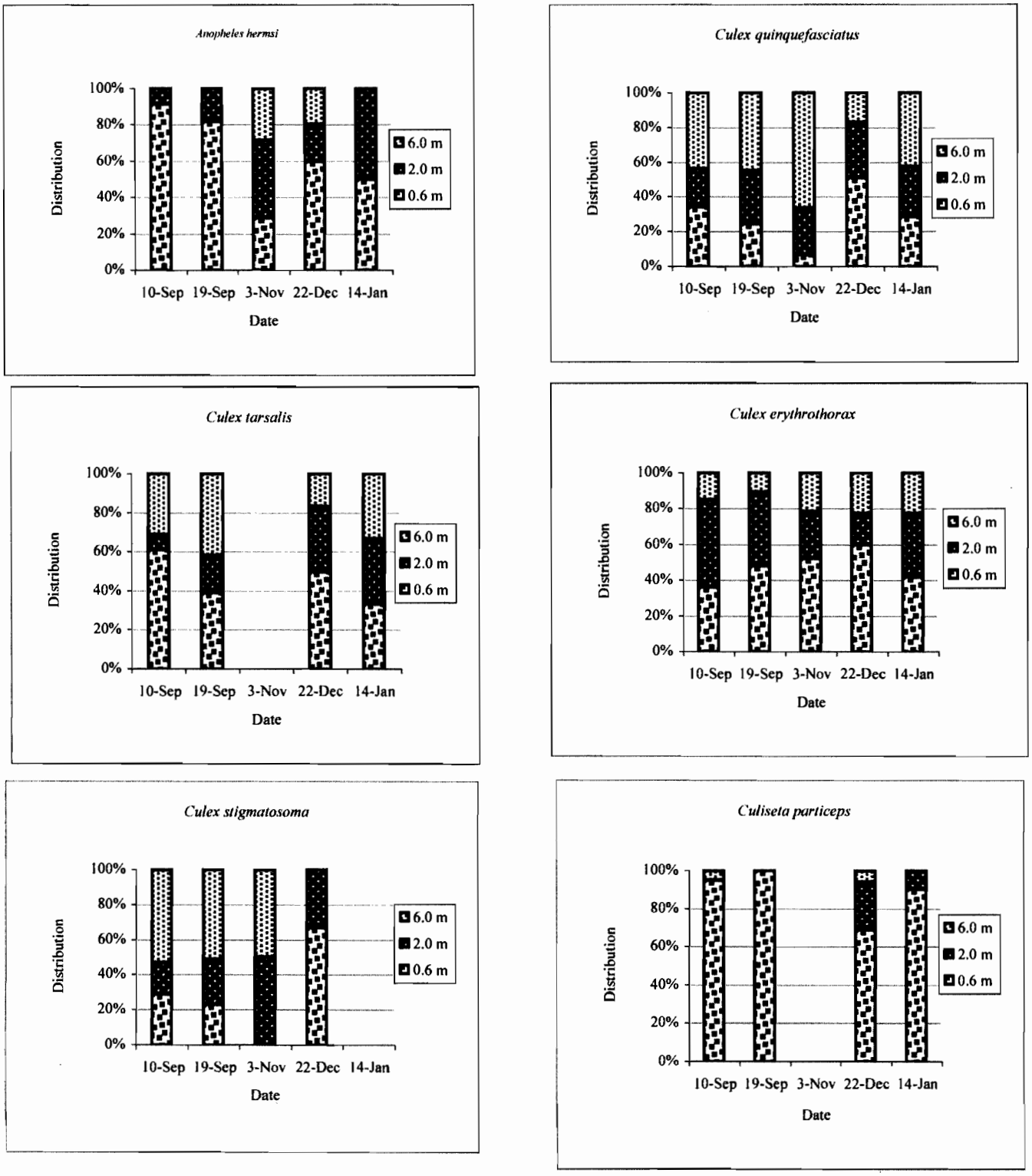

Figure 1. Percent distribution of adult mosquitoes by species collected at various heights in the Prado Wetlands in 2003-04.

study showed significantly higher numbers at the tree canopy level (6 m) than at the 2 m level during September (Fig. 1). Earlier studies also reported higher proportions of parous females than nulliparous individuals of these species caught in CO^2-baited traps (Myer et al. 1984, Mian et al. 1990). Therefore, trapping for parous, older females, using gravid traps (Reiter 1987) may further improve trap efficiency in arbovirus surveillance. This further necessitates to evaluate CO_2–baited traps and to virtually include sentinel chickens at the higher levels in order to determine if seroconversion can be detected earlier in the season in a wetland habitat.

The reason to extend this study into the winter was to ascertain if decreasing mosquito populations would show the same pattern as observed during the fall season. The data indicated a slightly different pattern in *Cx. quinquefasciatus*, *Cx. stigmatosoma,* and *Cx. tarsalis* in December than in September. However, the small sample size does not allow making any generalized conclusions.

Acknowledgements

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Operational Benefits from Longitudinal Sampling of *Culex pipiens* and *Culex tarsalis* at a Sewage Treatment Plant in San José, California

Daniel Strickman

Santa Clara County Vector Control District, 976 Lenzen Ave., San José, CA 95126

ABSTRACT: Mosquitoes were sampled weekly with dry-ice baited EVS traps from 22 June 2004 through 20 January 2005 at the San José Water Quality Control Plant. Ten traps were used in the same locations each week, attempting to represent all areas of the 800-acre plant. A technician applied comprehensive larval control measures throughout the plant until the end of September. Results showed a number of trends: *Culex pipiens* were periodic with a three-week cycle; *Cx. pipiens* populations were high between late July and mid September; *Cx. tarsalis* had a dramatic peak in numbers in early September; and trap collections doubled each week for three weeks following cessation of control measures. Among the conclusions of operational significance were the need for control measures during the cool season, the need to intensify larval surveillance for *Cx. tarsalis* in late August, and the location of larval sites correlated to adult collections in particular traps. The exponential increase during three weeks following cessation of control activities suggests that control had depleted the reserve of female mosquitoes. High numbers of mosquitoes along one side of the plant was the first indication of a major sewage leak, resulting in a $15,000,000 contract to fix the problem.

INTRODUCTION

Sewage treatment plants are a perennial problem for mosquito abatement districts (Bickley and Mallack 1961, Gophen and Gophen 1986, Mian et al. 1986). Regardless of the method of treatment, the plants usually provide many large habitats for mosquitoes. These habitats are the direct result of the basic mission of sewage treatment: disposal of large quantities of water laden with organic waste. To handle this waste, quantities of water are put through various processes that create tanks, troughs, above- and below-ground waterways, and earthen lagoons. Some of the processes inhibit mosquitoes, but operational needs inevitably put these structures out of service from time to time, creating huge accumulations of stagnant water. Although management by capping structures, careful drainage, and partnership with mosquito abatement can reduce the problem, sewage treatment plants usually produce mosquitoes at some time during the season.

Santa Clara County is served by four sewage treatment plants, the largest of which provides water treatment for the cities of San José, Santa Clara, Milpitas, Campbell, Cupertino, Los Gatos, Saratoga, and Monte Sereno. The San José Water Quality Control Plant (SJWQCP) serves 300 square miles and 1.5 million people with a capacity to treat 167 million gallons of waste per day. The plant is a state-of-the-art facility, the end product of which is essentially clean fresh water that is either dumped into San Francisco Bay or recirculated into the community as recycled water for irrigation.

In 2004, the Santa Clara County Vector Control District (SCCVCD) and the SJWQCP entered into an agreement for mosquito abatement at the plant. This agreement included access and funding, but it also required that SCCVCD provide metrics on the mosquito population under control. This paper reports some of the results of that effort, documenting the seasonality of the most abundant mosquito species, providing biological notes on the mosquito fauna, and showing how a systematic trapping effort can have specific operational benefits.

MATERIALS AND METHODS

The SJWQCP (37.43°N, 121.95°W) is a large complex consisting of a central plant and large acreages of lagoons (Fig. 1). The plant is intentionally sited at a distance from residential areas, the closest being the community of Alviso approximately 3 km away. The buffer around the plant consists of an old salt pond (Pond A18), a mitigation site formed from the floodplain of Coyote Creek, a reach of Coyote Creek, the McCarthy Ranch industrial area, large tracts of fallow land retained as a buffer, Arzino Horse Ranch (a boarding facility), diked salt marshes, and Zanker Landfill. The plant itself is 900 m north to south and 1,200 m east to west with structures, roads, or landscaping on the entire area. Some of the lagoons have clean edges, but others support growth of various native (tule, *Schoenoplectus californicus*; alkali bulrush, *Bulboschoenus maritimus*; pickle weed, *Salicornia* spp.; salt grass, *Distichlis spicata*, and cattail, *Typha latifolia*) and introduced (brass buttons, *Cotula coronopifolia*; Australian salt bush, *Atriplex semibaccata*) wetland plants.

The mosquito population within the plant was sampled weekly from June 22, 2004 to January 21, 2005 by placing 10 encephalitis virus survey (EVS) traps (Rohe and Fall 1979) in locations intended to capture mosquitoes at the perimeter and interior of the facility (Fig. 2). Traps were baited with 2 kg of dry ice as a carbon-dioxide source and no lights were used. Using insulated buckets, the dry ice was always sufficient to produce carbon dioxide from before sunset to after dawn. All traps were approximately 0.5 m above the ground in places sheltered by vegetation or structures. Female mosquitoes were identified to species using Bohart and Washino (1978), Darsie and Ward (1981), and Meyer and Durso (1993).

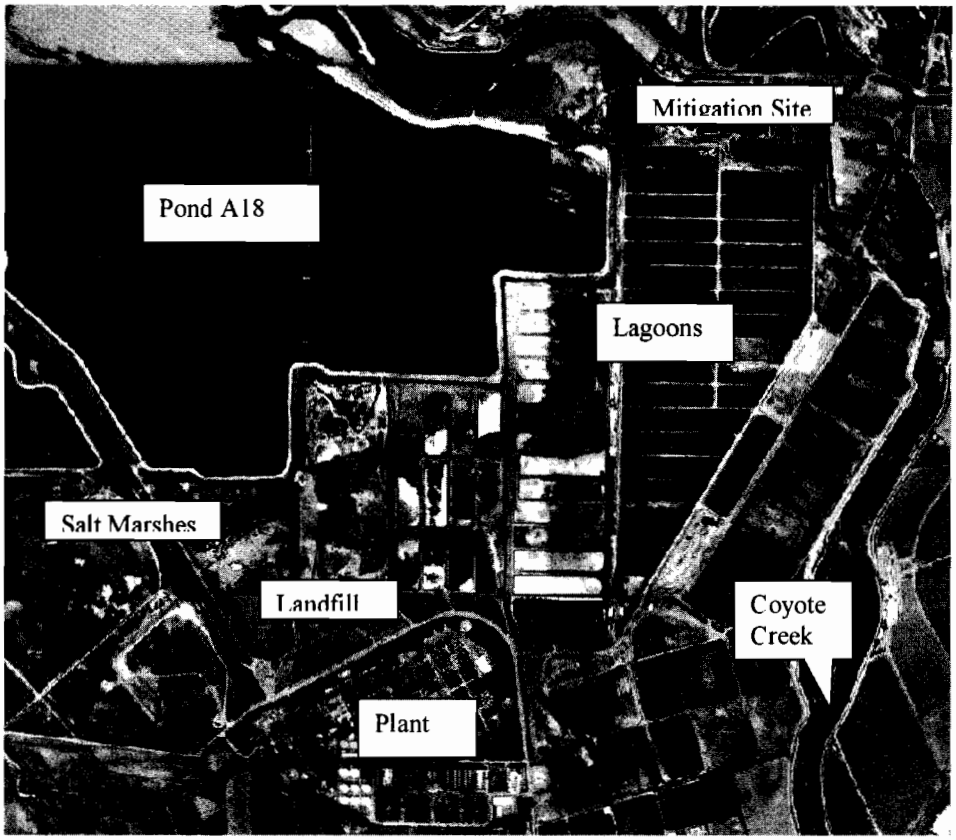

Figure 1. Aerial photograph of the San José Water Quality Control Plant and its environs, Santa Clara County, California.

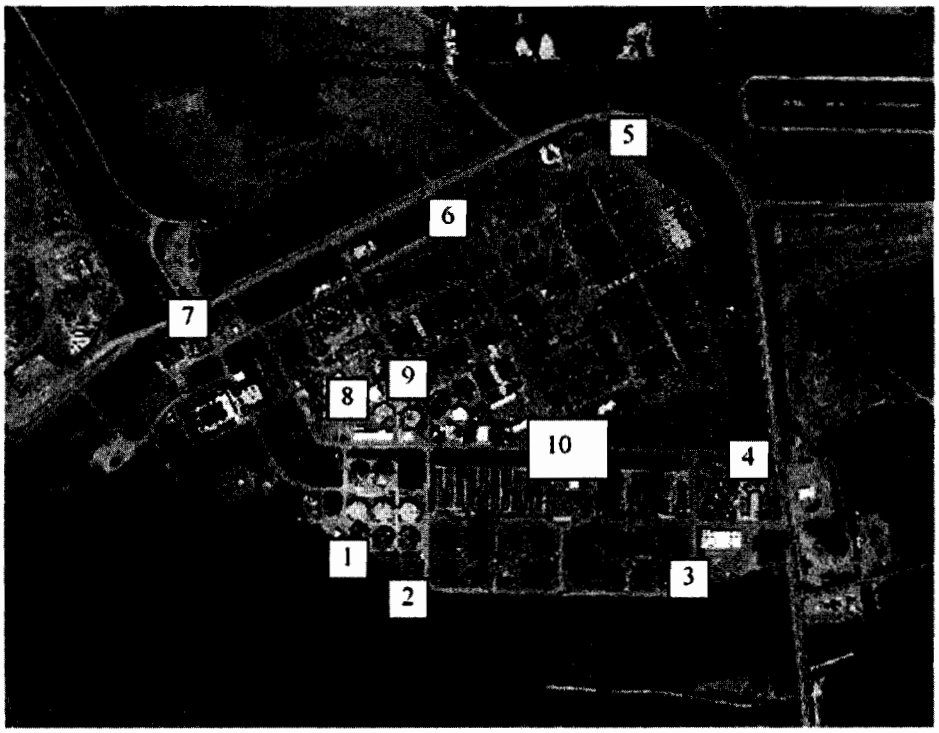

Figure 2. Aerial photograph of the San José Water Quality Control Plant showing the location of ten EVS traps operated June 2004 through January 2005.

RESULTS

Although not quantified, operational experience clearly demonstrated that the SJWQCP plant produced mainly *Culex* (*Culex*) *pipiens* L. The lagoon area often contained larval *Cx.* (*Cux.*) *tarsalis* Coquillett, as well as *Cx. pipiens*. During the winter, some of the lagoons supported populations of larval *Aedes* (*Ochlerotatus*) *squamiger* (Coquillett)[1] and *Culiseta* (*Culiseta*) *inornata* (Williston). Presumably, other mosquitoes (*Ae.* (*Och.*) *dorsalis* (Meigen), *Ae.* (*Och.*) *melanimon* Dyar, *Ae.* (*Och.*) *washinoi* Lanzaro and Eldridge, *Cx.* (*Cux.*) *erythrothorax* Dyar, and *Cx.* (*Cux.*) *stigmatosoma* Dyar) collected in the EVS traps developed somewhere else in the surrounding area.

The traps were distributed within a relatively small area, but the relative number of mosquitoes captured in each trap varied greatly (Fig. 3). In fact, Traps 1 and 2 captured almost half of the *Cx. pipiens* and more than half of the *Cx. tarsalis*. Catches in traps on the interior of the plant (Traps 8 and 9) were particularly low. Trap 10 captured a significant number of *Cx. pipiens*, but it was located close to an underground source that was not discovered and treated until late in the season.

The seasonal distribution of mosquitoes was prominent, in spite of the fact that a full time effort was applied to control at the plant (Fig. 4). *Culex pipiens* declined in numbers as minimum temperatures dropped below 12°C and daylengths shortened to less than 12 h in early October. Regular peaks of *Cx. pipiens* occurred with a periodicity of three weeks during the time of its highest numbers in July through August. *Culex tarsalis* increased in abundance during September, a phenomenon noted in other parts of the county and by other mosquito abatement districts in the region (John Rusmisel, Alameda County Mosquito Abatement District, personal communication).

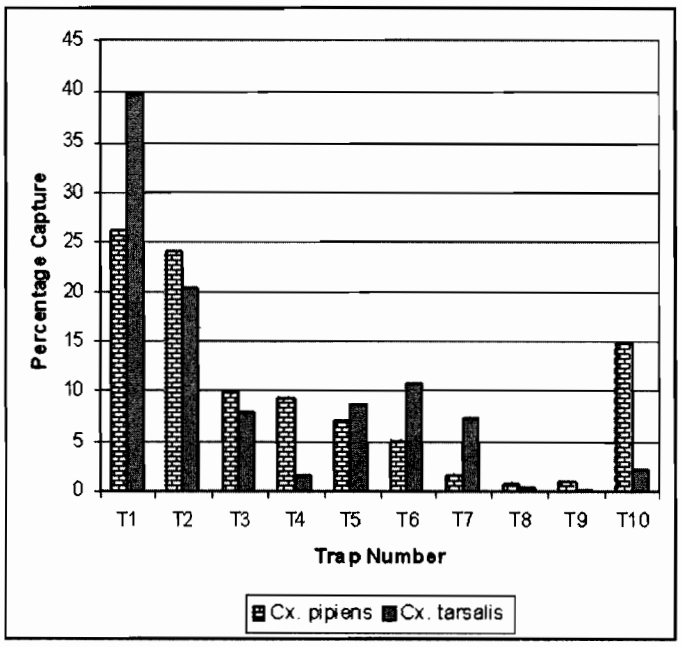

Figure 3. Percentage of *Cx. pipiens* and *Cx. tarsalis* captured by each of the ten traps operated for six months at the San José Water Quality Control Plant.

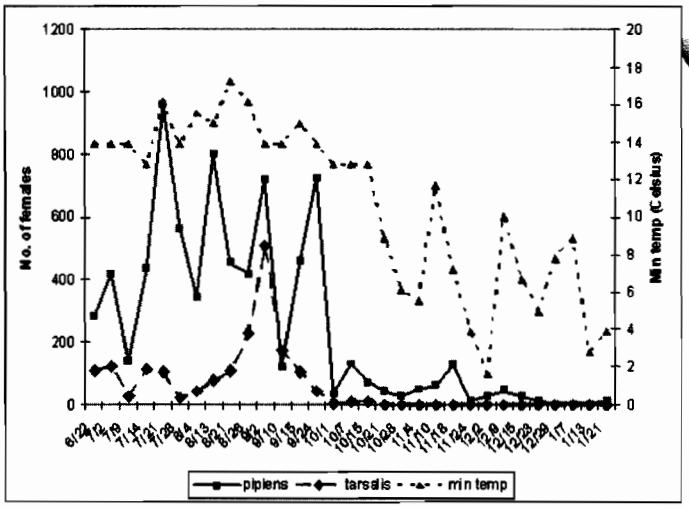

Figure 4. Seasonal distribution of *Cx. pipiens* and *Cx. tarsalis* captured at the San José Water Quality Control Plant.

Traps 1-3 captured many more *Culex* than the other traps (Figs. 3 and 5). When this trend was noticed, technicians began a search for the source of the mosquitoes and found a trench extending from the plant for about 1 km to the south. The plant manager had not been aware of the site because it was located in the seldom visited buffer zone beyond the boundary of the plant. When the manager saw the site, he realized that it was a major problem for the operation and he initiated a $15 million program to correct it. Considering Traps 4-10 separately also indicates that control efforts were relatively effective.

DISCUSSION

Systematic trapping at a particularly problematic site was useful in a number of ways. Most dramatically, a major source of mosquitoes was discovered, leading to a large civil engineering project and a permanent solution. The experience also shows that replication in trapping is necessary to get reliable numbers. Although the SJWQCP was a small area, ten traps produced very different results from one another.

The data showed the seasonality of *Cx. tarsalis* and *Cx. pipiens* adult females at the southern shore of San Francisco Bay. *Culex tarsalis* was most abundant during a peak in the early fall, almost as though females had been holding their egg rafts and then

[1] The author chooses to retain Edward 's generic designation of *Aedes* (Savage and Strickman 2004), especially considering recent analyses of *Ochlerotatus* indicating its polyphyly as a genus (Reinert et al. 2004).

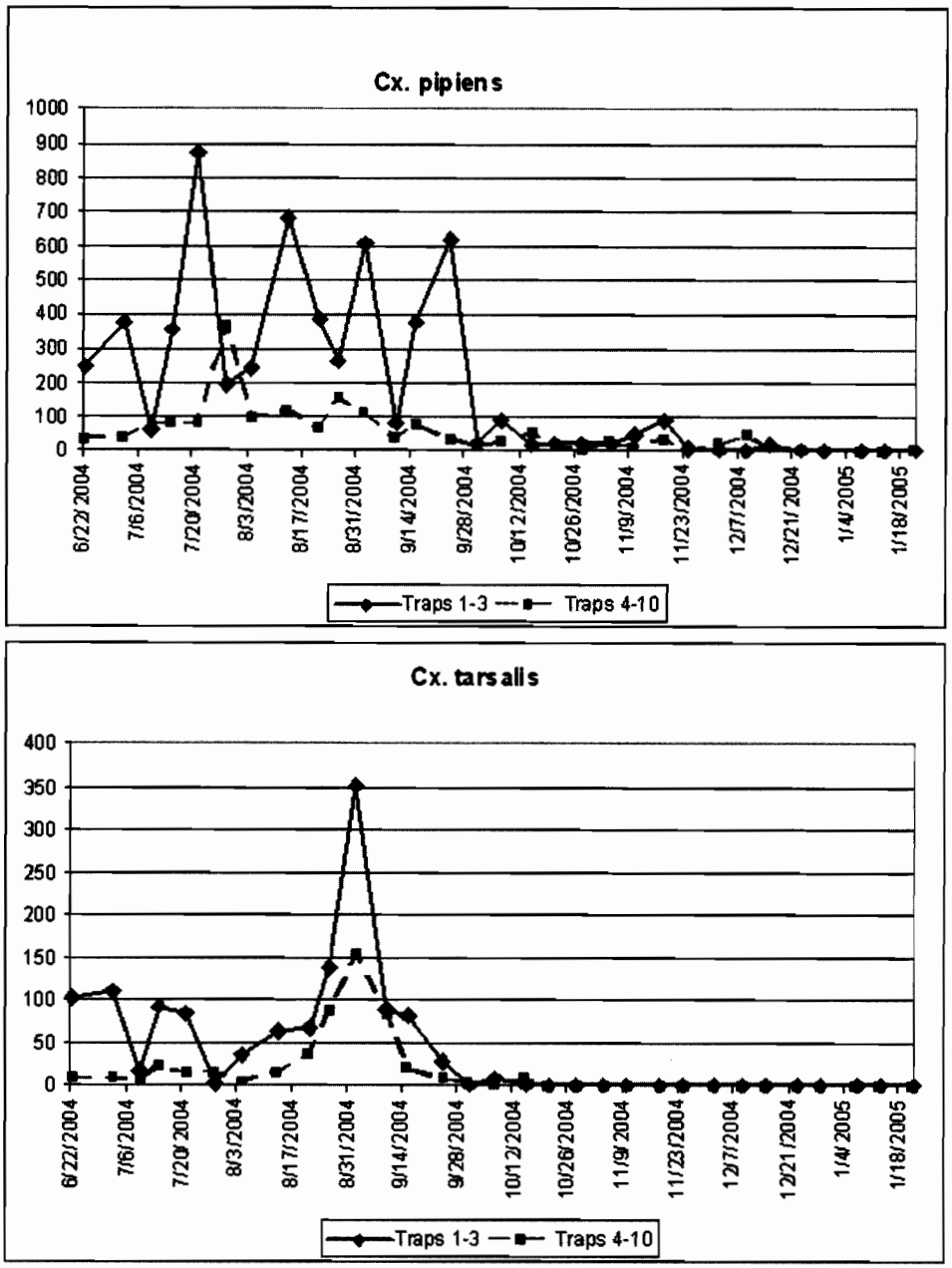

Figure 5. Mosquitoes captured by traps located along the southern edge of the San José Water Quality Control Plant (Traps 1-3) and in other parts of the plant (Traps 4-10).

concentrated their oviposition efforts during a short period. This behavior was not observed in Imperial County in southern California (Walters et al. 1980, Bown and Work 1973), where this species was most abundant in June and August or September, avoiding the hottest part of the summer. In a southern California Constructed wetland, *Cx. tarsalis* is most abundant in early August (Jiannino and Walton 2004) when the weather is hottest for that area. Apparently, *Cx. tarsalis* abundance is affected by local weather conditions that can vary greatly in different parts of the state.

Culex pipiens was most abundant in July through September, corresponding to the warmest part of the year in this part of Santa Clara County. In hotter regions, like Egypt (Meegan et al. 1980), this species also declines in numbers during the hottest part of the year with peak abundance in April-May and again in October-November. Where weather is more moderate, *Cx. pipiens* is most abundant during the warmest part of the summer (August in Ohio, Mans et al. 2004; July in South Korea, Kim et al. 1999). The apparent periodicity of *Cx. pipiens* at the SJWQCP seems surprising, considering that the species develops in permanent sites and the eggs have no mechanism for delaying their hatch. A similar periodicity was observed in *Cx.* (*Cux.*) *quinquefasciatus* Say by counting the number of egg rafts deposited in ovibuckets (Strickman 1988).

The importance of mosquito control at the SJWQCP can be viewed in a number of ways. On the one hand, the site is isolated and few mosquitoes produced within it seem to travel to residential areas of the city. On the other hand, the workers at the industrial sites nearby also deserve protection. Perhaps more important, this highly productive site may serve as an important reservoir for the emergence of overwintering *Cx. pipiens* and as a focus for transmission of West Nile virus. *Culex pipiens* is no stranger to sewage treatment plants (Ishii and Sohn 1987, Nasci et al. 2001) and better methods for its control will always be welcomed by mosquito abatement districts.

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Mosquito and Vector Control Association of California (MVCAC)
Attention: Emily Young
660 J Street, Suite 480
Sacramento, CA 95814
(916) 440-0826 • (916) 442-4182 FAX
E-mail: eyoung@mvcac.org

Editor: Lal S. Mian, Ph.D.
Department of Health Science & Human Ecology
California State University,
San Bernardino, CA 92407-2397
Phone: (909) 537-7409 Fax: (909) 537-7037
E-mail: lmian@csusb.edu