PROCEEDINGS AND PAPERS

of the

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Mosquito and Vector Control Association of California

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Editor: Minoo B. Madon
Greater L.A. County VCD
12545 Florence Avenue
Santa Fe Springs, CA 90670
Phone: (562) 944-9656
Fax: (562) 944-7976
E-mail: mmadon@glacvector.org

Reviewers: Jeffrey Beehler, Northwest MVCD; Bruce F. Eldridge, UC Davis;
Lal S. Mian, California State University, San Bernardino;
William E. Walton, UC Riverside; James P. Webb, 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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EDITOR'S NOTE

During one of the quarterly board meetings in 1998, I was asked by Jack E. Hazelrigg, Chair of the MVCAC Publications Committee, whether I would accept being Editor for the Proceedings. I thought about it for a while, and with a certain amount of trepidation, I hesitantly accepted. Perhaps, if you even care to read this message, you may note that I have deliberately used two words: 'trepidation' and 'hesitantly.' My intent here is to address the status of the "Proceedings," as viewed by the scientific community. With this in mind, my first order of business was to review the existing "Guidelines for Publication." Didn't take me too long to decide that the existing Guidelines were in dire need of revision. Keeping well in mind that this being "just a 'Proceedings,'" the revised Guidelines were modified to suit the intended needs and requirements.

Traditionally, most "Proceedings" do not enjoy the same status or privilege shared by those publications entitled "Bulletin," "Journal," and so on. That is because, articles submitted for publication in a "Proceedings," traditionally, are not subjected to the highly regarded peer-review process employed by publications considered by the academic community as "valid publications." I specifically state this because of my personal experience. A few years ago, I corresponded with a senior author regarding a statement made in an article published in a peer-reviewed Journal. I mentioned that what was emphasized in a particular statement in their publication wasn't new information; since a similar statement had been co-authored by me several years ago, and published in a Proceedings (which I shall not name). To spare him the trouble, I included a copy of the article with the similar statement highlighted. The written response I received was something to the effect that the agency he represented (CDC), had a policy that forbade the use of citations from any Proceedings, as they were not considered "valid publications." I suppose that all the scientists in this agency have resigned to fervently adhering to this "policy" rather than evaluating the merits of the quality of an article, regardless of where it is published.

For the sake of discussion (I am well aware that this will likely elicit responses and arguments from my professional colleagues), if fellow scientists and the academic communities have such an abject view of a "Proceedings," then I ask ". . .why bother with all these meetings and conferences, why deliver the presentations, and why do these professional organizations bother to expend the time and money to publish Proceedings?"

Furthermore, one of the major viewpoints has traditionally been that ". . . the presentation was already delivered, you cannot change what had been already discussed . . ."

I beg to differ with those of you who subscribe to this, for the following reasons:

- Most of the speakers do not submit hard copies or diskettes of their presentations (as is usually required) at the meeting or conference. Moreover, following the many requests, pleadings, and other methods of persuasion, the contents of the articles when finally received do not always reflect (verbatim) what was delivered at the conference.

- Additionally, none of us professes to recall all the data presented by all the speakers (or at least those presentations you bothered to sit through) at all of these meetings and conferences! I recall reading somewhere that if one is capable of retaining ~5% of the technical material that comes across one's desk, you may be considered 'exceptionally gifted.'

- Moreover, when presentations end up being published either in Journals, Bulletins or Proceedings, they are permanently preserved, usually indefinitely. As is generally professed in the academic community "publish or perish" (if you wish to achieve 'immortality', then you must publish").

- Published material regardless of age, or their status as "valid publications," will usually be available for the thinkers and scientists of the future as reference materials to peruse through, and perhaps formulate opinions. 'Good research' doesn't necessarily mean that all the answers must be provided. Equal value should be given if the results of the research culminate in raising thoughtful questions for future researchers.

Okay, so I may be getting too verbose in this Editorial Note (that is, if you even bothered to read this far), and perhaps, your natural response will be ". . . so what are you trying to prove, Madon, what's your point"? There is no point, and I'm not trying to prove anything; I'm just casting some thoughts that have long been personally bothersome, with the hope that the reader(s) may either want to respond, prepare a rebuttal, or (if you prefer) tear me to ribbons. You are being offered this opportunity.

As part of his/her charge, an editor is responsible for setting standards and maintaining quality. Based on my experience[1], editors occasionally end up taking some degree of abuse. On occasion, authors regard edited comments as "criticisms;" editors are often accused of hurting an author's pride and feelings. Since the author/s prefer to be unique in delivering their presentations, their manuscript(s) usually are sent in a similar context, and they may not conform to the enclosed Guidelines for Contributors (as the proverbial saying goes "If all else fails, please read the enclosed Guidelines . . ."). Well, I attempted the bold move and tried to make some changes to some of the delivered presentations, and lo-and-behold, I was subjected to severe "verbal-bashing" by some of our notable scientists!

Then, even though the deadline dates are usually ignored, the familiar and ubiquitous and intimidating inquiry ensues ". . . why is it taking so long to get the publication out . . . what's holding it up...?," including a host of other complaints an editor has to contend with.

For instance, the Program Agenda for the Year 2000 MVCAC annual conference, lists ~72 presentations, of these, only 17 manuscripts, plus 2 submitted papers were published in this issue! Other speakers chose not to submit their presentations. This represents a rather weak response. If we are charged with the responsibility of maintaining the viability of this publication, it is imperative that the speakers cooperate by submitting the manuscripts of their presentations by the requested deadline date.

Articles, regardless of where published, ought to be given fair consideration. If one disagrees, there's always an opportunity for voicing disagreement, or providing valid rebuttals. In conclusion, I am appealing to some of you to shuck your elitist views about what constitutes a "valid publication." Let's begin with an attempt to improve the Association's Proceedings and set an example for others to follow.

In conclusion, I wish to extend my appreciation and gratitude to the reviewers, Jeffrey Beehler, Bruce F. Eldridge, Lal S. Mian, William E. Walton, James P. Webb and Glenn M. Yoshimura. They graciously donated their time and effort to make this issue possible.

Feedback is welcomed.

Minoo B. Madon

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[1] Over the number of years that I have had experience as an Editor-of-sorts, with humble beginnings way back in 1963 (working as an licensed Field Representative/Salesman/Entomologist/Training Instructor, at a major private pest control company in Los Angeles), including "other duties" as an "Editor" and compiler of the company Newsletter "The Chatterbug"; then moving on to being the first Editor of the Bulletin of the Society of Vector Ecologists (now "elevated" in status from Bulletin to Journal of Vector Ecology), various miscellaneous activities in the SOVE Newsletter, and presently as Editor of this Proceedings.

PROCEEDINGS AND PAPERS

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Conference Dedication
In Memoriam - H. B. Munns

David Brown, Manager
Sacramento-Yolo Mosquito and Vector Control District

Hilton B. Munns died Thursday, September 9, 1999 after a courageous battle against pancreatic cancer.

Munzy, as he was affectionately known, was born February 26, 1929 in Fennimore, Wisconsin. He was one of seven children to Rexford and Dorothy Munns. Munzy and his two brothers and four sisters helped his father run a dairy operation. When Munzy was of age he joined the U.S. Army. He played the trumpet in the Army band, and became an accomplished musician. Despite a broken jaw that ended his playing career, Munzy never lost his appreciation for jazz and big band music.

After his stint in the Army he attended Iowa College for two years and then went to work for a cheesemaker in Wisconsin. His employer immediately recognized his keen business sense and ability to work with people. When another factory was opened in Iowa, Munzy was asked to manage and run the operations. He did this until 1964, when he moved to Southern California and worked in sales for Wyandot (now a part of BASF) and Ralston Purina. He sold cleaning solvent for food processing machines, and it was during this time he honed his amazing people skills. He knew all of his customers by name; the names of their family members, where they lived, and could recount the last discussions he had with each and every one of them.

In 1971 he started Fennimore Chemicals. When Ralston Purina discovered this, they gave Munzy his notice, which Munzy later said "was the best thing the company ever did for me. It forced me to work solely on my business!"

Munzy was an active member of the Society of Vector Ecology, the Mosquito and Vector Control Association of California, the American Mosquito Control Association, and other regional vector control associations. He transcended what might be described as your typical salesman, instead becoming a true advocate of not only the vector control business, but of the people that made vector control their lives. He regularly brought people in the vector control business from Europe to see the United States and the vector control operations here, solely at his own expense. He never asked for anything in return, nor did he expect it.

His receptions were more like family get-togethers than business operations, as all that attended his SuperBowl parties will attest. His love of football, particularly the Green Bay Packers, was overshadowed only by his love of golf. His golf tournaments were always well attended, and though he had a slice that could cut bread, his enjoyment of the game never diminished.

Munzy's life was completed when he met his lovely wife Jessica, and he shared with close friends the contentment he enjoyed with her in his life. Jessica faithfully looked after Munzy during his last days of illness, and fulfilled his last wish of taking him home to his beloved Fennimore. The love they shared for each other was apparent to all who saw them together.

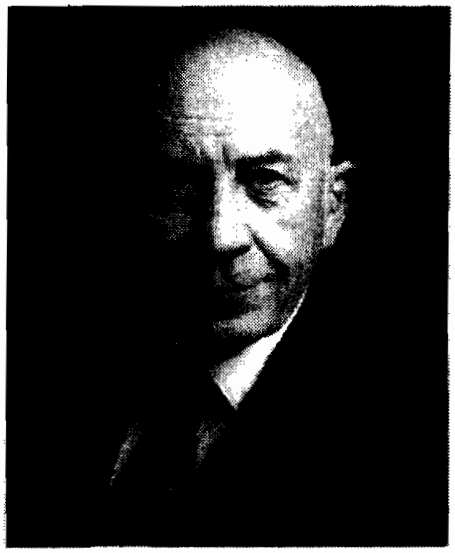
Munzy had three daughters from a previous marriage that he was extremely proud of, and never missed a chance to brag about their accomplishments. His genuine devotion to his wife, children and grandchildren were enormous.

I was honored to know Munzy as a person, and blessed to call him a friend. The professionalism, dignity, and class he brought to his industry will never be matched. He will be sorely missed by all he touched.

In Memoriam - Stephen M. Silveira

Marc R. Pittman

Mosquito Prevention Specialist, Turlock Mosquito Abatement District



Manager of the Turlock Mosquito Abatement District from August 1,1959 to April 30,1986.

Source Reduction Technician for the District from February 1,1954 to July 31,1959.

Steve was born May 10, 1921 in Newman, California. He grew up on a family dairy and attended Orestimba High School. While attending Modesto Junior College, he joined the Army Air Corps during World War II serving as a bomber pilot from 1941 to 1945.

Steve married his wife Lois in April of 1944 and after the war they farmed the land by growing walnuts and the family grew with the birth of a son, Steve Jr..

Prior to joining the Turlock Mosquito Abatement District, Steve worked at a Newman tractor company and was an adult agriculture instructor at Orestimba High School.

Steve started work with the District in 1954, and later on was promoted to District manager, replacing G. Edwin Washburn who had started in 1946, at the inception of the District.

Steve liked working in mosquito control and strived to make it the best mosquito control program in the state. He cooperated with the private industry; researchers in industry and at the University of California; Agricultural Extension; researchers from foreign countries and politicians to promote and improve mosquito control within the Turlock District and to help others elsewhere.

Steve loved working with people in mosquito control and hired the very best. His desire was that the District would have the best mosquito control program possible.

Steve was known as a peacemaker among the District personnel and received a plaque recognizing this role that proclaimed in part "Blessed are the peacemakers, for they shall be called sons of God. Mathew 5:9."

Steve served the District for 32 years, experiencing many changes taking place, from DDT and its resistance, to the beginnings of biological control organisms; from thermal foggers to cold foggers; from dairy drains to dairy ponds; from flood years to drought years; from low public profile to high public profile especially after passage of Proposition 13. Through all of this, Steve managed very well during these changes, keeping the District abreast on technology.

Steve loved his wife, son, daughter-in-law, granddaughter and was very devoted to his family. He loved to make furniture by hand, the old-fashioned way and even built the house his son lives in today.

Steve always took a great interest in whomsoever he met and wanted to learn more about the individual and was ready to help people if it was needed.

He was a special man and after working with and for him and the District a part of Steve goes with everyone he worked with and met, and will be passed on from generation to generation, he was a "neat boss" to work with and for, and you always came away a better person.

Steve was a member of the Turlock Rotary Club, which eventually helped form the Turlock Mosquito Abatement District back in 1946. He was President of the California Mosquito Control Association in 1967, and an Honorary Member of Mosquito and Vector Control Association of California since 1987.

Because of his flight experiences during World War II and his interest in airplanes, he became one of the founding members of the Castle Air Museum in Atwater, California.

Stephen M. Silveira left this world a better place May 26, 2000.

Submitted by Marc R. Pittman, Mosquito Prevention Specialist,Turlock Mosquito Abatement District.

West Nile Virus in New York: Issues of Concern for California

Introduction and background[1]

William K. Reisen

Arbovirus Field Station, Center for Vectorborne Diseases
School of Veterinary Medicine, University of California, Davis, California 95616

INTRODUCTION

In late summer 1999, an unusual cluster of encephalitis cases was recognized in New York. Relying on the collective wisdom that "if you hear hoof beats, think horses not zebras", initial sera from human patients reacting positively to St. Louis encephalitis virus (SLE) antigen were identified presumptively as SLE (CDC 1999). An emergency mosquito control program was initiated rapidly and most of the affected areas were treated by air with ultra low volume adulticides. Unexpected avian mortality, tests of necropsy specimens from birds dying at the Bronx Zoo, isolates from mosquito pools, and additional testing of human specimens identified the cause of the outbreak to be an exotic agent, West Nile (WN) virus (CDC 1999; Jia, et al. 1999; Lanciotti, et al. 1999). This was the first record of WN in the New World (Hayes 1989) and provided clear evidence that even in the modern era, the United States remains susceptible to the introduction and amplification of exotic arboviruses.

The introduction and subsequent outbreak of WN raised a number of questions, including:

- How did WN get to New York and where did it come from?
- What are the chances that this virus will overwinter and spread to other states?
- What is the risk of WN and other exotic viruses being introduced into California?
- Will the current California Encephalitis Virus Surveillance Program detect these introductions?
- What plans are in place to respond to such an introduction?

Today's symposium will address these and other issues as they relate to California. The speakers and the subject of their talks are listed below:

- B.F. Eldridge, U.C. Davis: Introduction
- N. Komar, CDC, Ft. Collins: West Nile outbreak in New York City
- W.C. Reeves, U.C., Berkeley: Threat of arboviral introductions into California
- C. Glaser, CDHS, Berkeley: Clinical aspects of infection and passive case detection
- L.D. Kramer, U.C., Davis: Diagnostics and surveillance
- V.L. Kramer, CDHS, Sacramento: Control and containment: California Department of Health Services perspective
- C. Beesley, Contra Costa MVCD: Control and containment: Mosquito and Vector Control District perspective

Before these presentations describing WN in the context of an invading virus, I thought that it would be useful to provide background information on its distribution and epidemiology.

[1] Paper, in part, was read by B. F. Eldridge, Department of Entomology, University of California, Davis.

BACKGROUND

West Nile is an arbovirus related closely to SLE and is classified within the Japanese encephalitis virus (JE) complex of the genus *Flavivirus* in the family Flaviviridae. In addition to JE found throughout Asia, other closely related viruses of public concern within this complex include Kunjin and Murray Valley encephalitis viruses that are endemic to Australia (Hayes 1989). There is considerable cross reactivity among viruses within this complex making diagnosis difficult where they are sympatric. West Nile is distributed from Europe south into much of Africa and east through the central highlands of India (Fig. 1). There are 2 main genetic lineages among strains that have been sequenced. Recent outbreaks of WN in Israel and temperate Europe including Romania and Russia (Hubalek and Halouzka 1999) have been attributed to viruses within lineage 1; isolates from New York fall into this lineage (Jia et al. 1999). The second major lineage includes strains found in endemic areas of Africa and Asia.

The ecology of WN is complex, with isolations recorded from a large number of mosquitoes and warm and cold-blooded vertebrates (Hayes 1989; Hubalek and Halouzka 1999). Ticks also have been found infected in nature, but their role in transmission has not been established firmly. The basic cycle seems to be similar to SLE, with *Culex* mosquitoes in both the *pipiens* and *sitiens* complexes as the primary vectors and birds in the order Passeriformes as the primary vertebrate hosts (Taylor et al. 1956; Hayes 1989). However, during outbreaks a variety of birds and mammals and mosquitoes become infected, although many may be dead end hosts. Mortality in birds has been reported, especially in corvids, columbiforms and anseriforms (Work et al. 1955; Hayes 1989). Because of similarities in the general ecology of the principal vector in Pakistan, *Culex tritaeniorhynchus* Giles, and the possible vector in California, *Culex tarsalis* Coquillett (Reisen 1981), the remainder of my presentation will focus on ecological studies done in the Changa Manga Forest near Lahore, Punjab Province, Pakistan (Hayes et al. 1980, 1982; Reisen et al. 1982) to provide background information on the epidemiology of this virus in an endemic rural setting.

Villages in Changa Manga Forest, Punjab Province, Pakistan, were surrounded by agricultural fields and an extensive planted mulberry forest that provided roosting and nesting habitat for a diverse avian fauna. One of our study villages was adjacent to a communal roosting area for crows. Surveys for human infection were conducted during 1968 and 1978 (Hayes et al. 1982) and showed a pattern consistent with a low annual infection rate (Fig. 2).

Mosquitoes were collected resting in the forest and village by power aspirator, biting/landing at human bait during late afternoon, and biting at buffalo bait in early evening (Reisen et al. 1982). Over 43,000 mosquitoes were enumerated to species and tested for virus

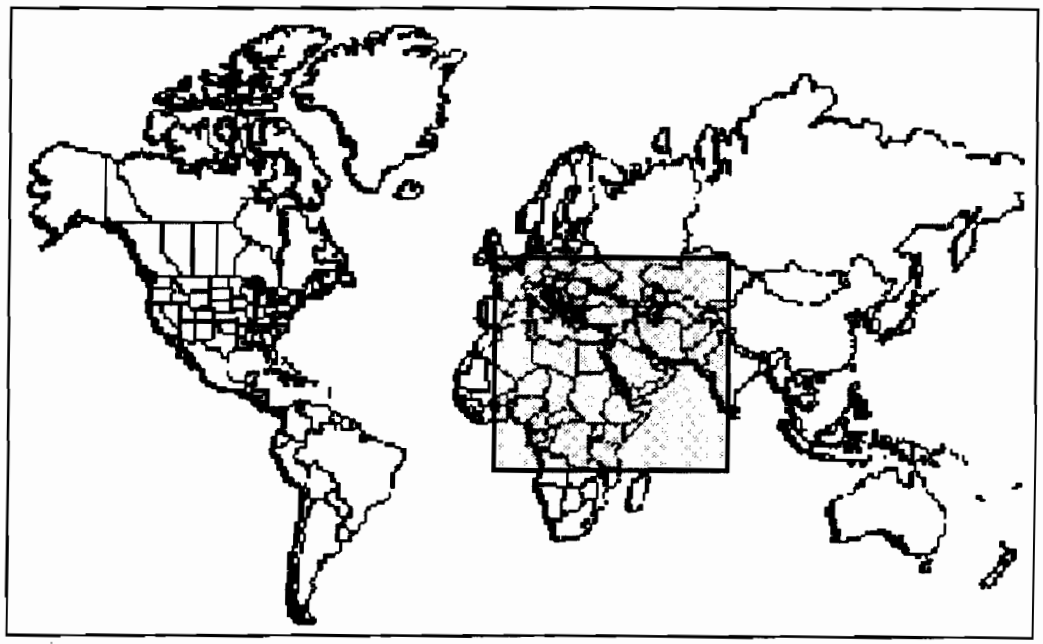


Figure 1. Map showing the approximate distribution of West Nile Virus.

infection by intracranial inoculation of suckling mice with negative results (Fig. 3). *Culex tritaeniorhynchus*, a member of the *sitiens* complex related to *Cx. tarsalis*, was the most abundant species collected, followed by *Culex pipiens quinquefasciatus* Say. Sampling and blood meal identification studies indicated that mosquitoes could be divided into four general categories:

- Rest in houses and feed on humans and birds at night: *Cx. quinquefasciatus*
- Rest in cattle sheds and feed on cattle at night: several *Anopheles*
- Rest in the forest and feed on humans during the day: several *Aedes*
- Rest in fields and forest and feed mostly on cattle at night: many *Aedes, Mansonia, Culex* and *Anopheles*

Abundant mosquito species as well as those implicated elsewhere in WN transmission were evaluated for their vector competence using the Egyptian 101 strain, a member of WN lineage 1(Akhter et al. 1982). Using a membrane feeding system, *Cx. tritaeniorhynchus* was the species most susceptible to oral infection, followed by *Cx. pseudovishnui* (also a member of the *sitiens* complex) and *Cx. univittatus* (Fig. 3). The rest of the mosquitoes including several *Aedes* and *Cx. quinquefasciatus* were relatively refractory to oral infection. Females then were infected by intrathoracic inoculation and evaluated for their ability to transmit virus by bite to suckling mice or to a blood/sugar mixture presented as a hanging drop. Once the gut barrier was by-passed by intrathoracic inoculation, most species were competent vectors, with *Cx. tritaeniorhynchus* and *Cx. univittatus* being the most efficient laboratory vectors (Fig. 3).

Wild birds were collected by mist netting near villages and within the forest (Hayes et al. 1982). A total of 317 wild birds were tested for previous WN infection by a neutralization test. Most bird species including crows exhibited antibody. The collection of antibody positive specimens indicated that most individuals probably did not succumb to infection and that the endemic lineage 2 strains endemic to this area of Asia might not be as pathogenic to birds as lineage 1 strains.

Our studies in Pakistan led to the following conclusions about WN relevant to the recent invasion in the New York area and possible introduction into California:

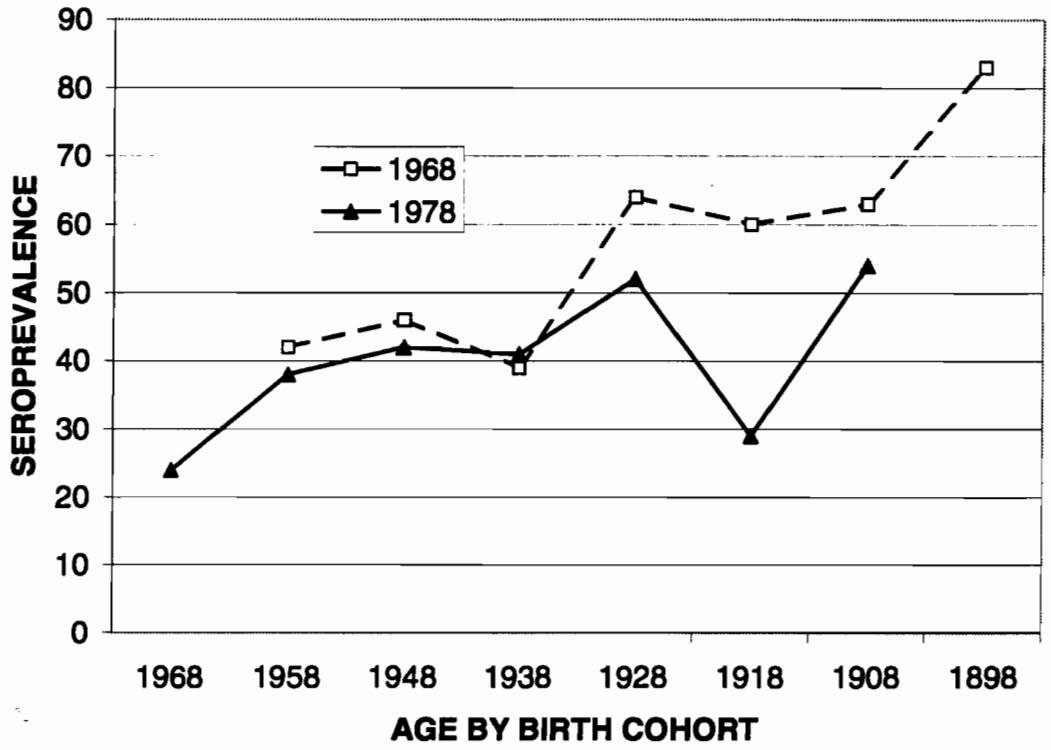


Figure 2. Prevalence of neutralizing antibodies against West Nile virus in villagers from Changa Manga Forest, Punjab Province, Pakistan [redrawn from Hayes, et al. 1982].

- West Nile frequently infected humans without noticeable morbidity or mortality. Most human infections in Pakistan must have been inapparent, because few central nervous system cases were noted or reported from these study villages which we visited weekly for a 2 year period. Lack of clinical illness in older age groups was attributed to elevated population immunity acquired naturally early in life when clinical illness is less frequent and severe. Populations in New York lacked this acquired resistance resulting in clinical illness, especially among the elderly.
- Infection of birds in an endemic area did not appear to cause extensive mortality, because most species sampled had some prevalence of neutralizing antibody. Based on extensive mortality among crows in New York, populations not exposed previously appear to be much more susceptible to infection.
- *Culex tritaeniorhynchus* was the most abundant mosquito and was the most efficient laboratory vector of WN; however, this species fed mostly on cattle and infrequently on humans and birds. In California, *Cx. tarsalis* probably would be a more effective vector than *Cx. tritaeniorhynchus*, because of it feeds frequently on both birds and mammals and may have comparable susceptibility to infection, based of vector competence studies with SLE.
- *Culex pipiens quinquefasciatus* was the only species that frequently blood fed on both humans and birds, but only was a moderately competent laboratory vector of WN. Similar results have been obtained with SLE and WN viruses infecting members of the *Cx. pipiens* complex in the United States; however, the invading lineage 1 virus may produce elevated viremias in acutely ill birds thereby facilitating horizontal transmission. Involvement of peridomestic *Cx. pipiens* complex populations may be critical for widespread transmission in urban situations.

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Species	Total(%)[1]	Infect(%)[2]	Trans(%)[3]
Culex tritaeniorhynchus	58	>90%	>80%
Cx. quinquefasciatus	21	7	52
Cx. pseudovishnui	3	73	56
Cx. univitattus	<0.1	56	92
Aedes lineatopennis	8	10	56
Ae. indicus	2	0	56
24 species	8		
Total	43,729		

[1]Collected by 7 methods during 58 collection trips over 18 mos
[2]Infected by membrane feeding on 3.4-4.7 log10 SMICLD50 of E101 strain
[3]Transmission to suckling mice or hanging drop after IT infection

Figure 3. Abundance and vector competence of mosquitoes from Changa Manga Forest, Pakistan, 1978-1979 [vector competence data from Akhter, et al. 1982.].

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Order	Group	%	Tested
Passeriformes	Shrikes	6	17
	Orioles	41	17
	Mynas	25	72
	Crows	20	10
	Bulbuls	27	26
	Babblers	28	57
	House sparrow	20	25
Columbiformes	Doves	22	9
Psittaciformes	Parakeets	56	16
Coraciiformes	Kingfishers	26	31
Others		24	37
	Total	27	317

Figure 4. Percent of wild bird sera positive for neutralizing antibodies against West Nile virus, Changa Manga Forest, Pakistan [data summarized from Hayes, et al. 1982].

West Nile Virus in the Northeast United States

Nicholas Komar
USPHS/CDC, Fort Collins, Colorado

Mosquito and Vector Control Association of California Annual Meeting
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SUMMARY

West Nile virus (WN) was identified as the etiologic agent in a cluster of 59 human cases of disease, characterized chiefly by aseptic meningitis and encephalitis. There were 7 deaths recorded during the outbreak, which occurred in August and September 1999 in and around New York City. Several isolates were made from *Culex pipiens* mosquitoes providing strong evidence that this abundant urban mosquito served as the vector. Morbidity and mortality associated with WN infection was observed in other non-human vertebrate populations including horses, a cat, and numerous wild and captive birds. Crows in particular were susceptible and succumbed to infection. Reporting of dead crows has served as a sensitive surveillance tool for determining the distribution of WN activity in 4 northeastern states: New York, Connecticut, New Jersey and Maryland. A serological survey of resident and migrant birds in the New York City area was conducted collaboratively by CDC and the USGS National Wildlife Health Center and indicated that 1. Avian transmission was widespread but was most intense in northern Queens where most human cases occurred. 2. Migrating birds were infected rarely, but an isolate from a healthy, migrant flycatcher on October 21 indicated a strong likelihood that southward-migrating birds could serve as transport hosts for WN, 3. Chickens develop strong antibody there after infection with WN and pre-existing flocks serve as good sentinnels for local transmission. 4. House sparrows and European doves are good wild bird sentinels for local transmission, 5. House sparrows, domestic pidgeons and mourning doves are good wild bird sentinels for local transmission, and 6. Low seropositivity in healthy crows, combined with >150 WN isolates from dead crows and reports of thousands of other dead crows, indicates a very high mortality rate in naturally infected crows. Reduction of mosquito populations by chemical means in New York proved to be an effective means of reducing risk of WN transmission once the epidemic was recognized. Active surveillance for mosquito-borne transmission to birds, promoting public awareness of personal protection from mosquitoes, and control of *Culex* mosquito populations, will be the best means of reducing public health, veterinary and wildlife impacts of WN in the U.S.

The Threat of Exotic Arbovirus Introductions into California

William C. Reeves

Division of Public Health Biology and Epidemiology, School of Public Health, University of California, Berkeley 94720

I found it a challenge to be asked to discuss the threat of arboviral introductions into California. As background, we already have identified 18 different arboviruses that occur in California and that seem to me to be plenty of resident viruses without adding more. However, we also know that over 500 other arboviruses exist in the world today (Karabatsos 1985) and some could be introduced into the USA and pose a real problem. I will discuss the history of the concern over such introductions and finally select 8 arboviruses that I believe are likely candidates to "invade" California. Other speakers on this panel will discuss the problems when West Nile virus appeared in New York in 1999.

My concern with exotic viruses began during the 1940s when World War II brought out that biological warfare was a new area for national concern and it was a new area for research of which some was done in California. This required the introduction of suspect viruses for study at several laboratories. Therefore it may be somewhat redundant to ask today if such viruses may be introduced because they already were and I recall 10 such viruses: Yellow Fever (YF), dengue-1 (Den-1), Venezuelan equine encephalomyelitis (VEE), Japanese encephalitis (JE), Chikungunya (CHKK), and Russian spring-summer encephalitis (RSSE). The research on these viruses was in support of a national defense program in the 1940s and 1950s and all possible care was taken to prevent the release of these agents into the environment.

One example of such studies ws the research on JE virus. This virus had been the cause of extensive epidemics in people and pigs in Japan. It was considered to be a likely biological warefare agent. We were directed by the Office of the Surgeon General of the U.S. Army to evaluate the ability of mosquitoes and birds of the Pacific coastal area to transmit and maintain such infection. We found that 7 of the 14 species of mosquitos tested from California could be vectors, 4 common wild bird species and chickens were susceptible and could propagate the virus. (Reeves & Hammon, 1946; Hammon et al. 1951, 1946).

It was important to demonstrate that such research could be done and that these viruses could be contained. Only RSSE virus posed a problem as it infected the scientist who brought it into his laboratory and he died from the infection. The study was terminated and I autoclaved the virus. There was no evidence that the virus had spread. Nine other viruses were evaluated for their ability to grow in a series of tissue cultures and antigens were prepared for the diagnosis of cases in international travelers including the military.

Another way in which exotic viruses were introduced into California is illustrated by 4 occasions when such viruses (YF, DEN, JE, VEE) were diagnosed as the cause of disease in residents of California. These people became ill shortly after their return from international travel into areas where the viruses prevailed. The YF case in 1999 was fatal. There was no evidence of secondary spread from any of these infections. All of these cases probably had passed the period when the viremia was sufficient to infect a vector. However, they had that potential. The modern era of increasing volume and rapid pace of international travel to California means that diagnosis of such cases and surveillance for their introductions must be maintained. Two of these 4 viruses are on my list of candidates for possible introduction into and establishment in California.

International movement of mosquitoes on airplanes and in used tires is another potential source for the introduction of viruses. Five species (*Aedes albopictus, Aedes japonicus, Aedes togoi, Aedes aegypti, Culex pipiens*) have been introduced historically; 3 of which were recent (Craven et al. 1988) and all have become established in the USA. Only *C. pipiens* currently is established in California. Each such introduction also was a potential source for the introduction of a virus. Four of these species were introduced recently from Japan in used tires. This brings me to consider the possible importance of transovarial transmission (TOT) of viruses in such mosquitoes, that is when infected females transmit infection to their progeny. Japanese encephalitis virus has been shown to have TOT in vectors such as those species that have been imported.

Although TOT has not been detected after any of the introductions I believe it is only a matter of time until such transmission will occur. Perhaps we have been lucky, but I don't like to rely on luck as a determinant of our future.

With this background I will now select 8 viruses (JE, WN, MVE, Rift Valley fever (RVF), Ross River (RR), VEE, La Crosse (LAC), Eastern equine encephalitis (EEE) from the over 500 that we know exist in the world and that I believe are likely candidates for introduction and establishment in California. Some of you would select other viruses for this list and with good reason. My selections are biased by my personal experience with them in the field and laboratory and by my knowledge of the vector and host populations for the 18 viruses we already know exist in California. I believe that we can match knowledge of our ecological conditions and the biological traits of our resident mosquitoes and vertebrate hosts with the cycles and ecological conditions that maintain the candidate viruses in their home environments. I also have considered the evidence that each of these viruses can be transovarily transmitted by their vectors and this was a major factor in their selection. Finally, the traits of native vectors and hosts of each of the viruses can be matched with the traits of abundant mosquitoes of the same genera and available hosts in California.

An additional factor for selection of these viruses was that each can cause epidemics in humans and or domestic animals. JE virus has caused major epidemics in areas of Asia and Pacific Islands and is rapidly approaching Australia. This virus also causes abortions in domestic pigs. You know the current experience with WN virus in New York. This virus also has recently caused epidemics in Africa and Europe. MVE virus has caused an epidemic and sporadic cases in a wide areas of Australia. RR virus, another arbovirus from Australia, causes a debilitating polyarthritis and already has spread to several Pacific Islands. RVF from Africa can cause epidemics and epizootics in humans and large mammals including cattle. VEE is a late addition to this list. This virus invaded Texas in 1971 where it caused 1,600 equine deaths and 80 cases in humans. (Anon. 1871). VEE virus continues to be widespread in Latin America and has multiple potential avenues for entry along our southern border. Two additional viruses we do not need in California are LAC and EEE. Both are enzootic and cause disease in humans east of the Mississippi River. So far they have not extended here.

In closing I will look at the ability we have to recognize and respond to an invasion. I believe the Arbovirus Surveillance Program that has been developed in California over the past 40 years will serve us well. The possibility of invasions by new viruses or the reemergence of our native viruses is considered in the planning of our program for the future. A sequence of detection and responses to such events is planned. This program represents a cooperative interaction of your community-based vector control programs, the California Department of Health Services, and the University of California. The California program is internationally considered to be a model for such activity.

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Arbovirus Surveillance: Detection and Diagnosis of Exotic Viruses

Laura D. Kramer

Arbovirus Research Laboratory, Center for Vector-borne Disease Research, University of California, Davis

Today I will discuss laboratory issues associated with the detection and diagnosis of viruses isolated from mosquito pools or vertebrate tissues, and of antibody from sentinel chickens. The procedures we have been following in the laboratory are not virus-specific, but rather are genus-specific assays. In other words, they only allow us to say we have isolated a flavi- or alphavirus, and we have detected flavi- or alpha- virus antibody. Historically this was economical and scientifically valid, because in California we were concerned with a single *Alphavirus*, western equine encephalomyelitis virus (WEE), and a single *Flavivirus*, St. Louis encephalitis virus (SLE). Further characterization is now needed to confirm the identity of virus isolates or antibodies. As the West Nile virus (WNV) outbreak in New York (NY) so clearly demonstrated, we are now a global community and need to take a proactive approach to surveillance in order to guarantee it is an early warning system. Arboviruses which have been isolated in the United States (U.S.) include toga-, flavi-, bunya-, reo- and rhabdoviruses. Because this symposium is on WNV, I will focus on the flaviviruses which include in the U.S., SLE, Powassen, dengue, and WNV. There are approximately 70 members of this genus in the world, half of which are associated with human disease. The virions are enveloped and spherical, 40-50 nm in diameter with 2 envelope proteins, E and M, and an icosahedral nucleocapsid, 25-30 nm in diameter. The genome is single-stranded positive sense RNA, approximately 11 kb in length. The flaviviruses are a diverse group, and may be transmitted by ticks and mosquitoes; some lack a known arthropod vector. Within the mosquito-borne group, the viruses of most importance to California are the Japanese encephalitis (JE) virus serogroup, including SLE and WNV, JE, Murray Valley encephalitis, Kunjin and Ilheus. The mosquito vector of dengue and urban yellow fever (YF) viruses, *Aedes aegypti*, is not a resident of California at this point. It may become established at any time, because it recently has colonized the Tuscon area of Arizona. Certainly, clinic and hospital physicians need to be on the alert for clinical cases of these infections in immigrants or travelers.

So what did we learn from N.Y., besides the fact that we cannot isolate ourselves from the rest of the world? The initial diagnosis of SLE virus was based on serologic findings using a flavivirus-specific antigen capture enzyme immunoassay. The final diagnosis of WNV was made by nucleic acid analysis and sequencing of the viral genome. Similarly, here in California we've demonstrated through nucleic acid sequencing that viruses circulating in California since 1952, and classified as WEE and SLE viruses, truly have been WEE and SLE. However, there are other laboratory procedures besides sequencing that allow us to identify virus to species.

First, let's look at serologic assays on human or other vertebrate sera. The sentinel chicken or wild bird serum samples, or human sera (preferably paired - acute and convalescent - and/or cerebral-spinal fluid, are tested by IgM or IgG enzyme immunoassay. Because there are significant cross-reactions among flaviviruses, serologically-positive samples must be confirmed by plaque reduction neutralization assay (PRNT) to determine the definitive etiology of the infection. End point titrations and cross-PRNT assays yield the most information, but diagnosis still may be difficult. It is important to note that when one is working with live virus, tests must be conducted in a Biosafety Level 3 (BSL-3) laboratory.

Antibody results may be difficult to interpret due to cross-reactions and past infections, therefore virus isolation is the gold standard for virus species confirmation. Mosquitoes captured in the field are identified, pooled in groups of 50 females, then frozen at –80°C for subsequent assay. In the BSL-3 laboratory, the pools are triturated in phosphate-buffered saline plus 20% fetal calf serum. Tissues such as brain, CSF, serum, etc., from moribund or dead vertebrates also can be tested in the same manner. After the mosquito pool or vertebrate tissue is triturated, it can be assayed by cell culture or nucleic acid techniques. The procedure we have been following in recent years is to inoculate 96-well cell cultures with the specimens, one plate for flaviviruses and one for alphaviruses, and depending on the mosquito species, one plate for bunyaviruses.

After 4-5 days for alphaviruses and 7 days for flaviviruses, the cells are fixed and an *in situ* EIA conducted. The results are read microscopically. If the cells in a well are positive, it previously has been accepted as SLE or WEE. But to confirm the results of these screening assays , we now should go back to the original pool and either do a PRNT using specific antibodies or inoculate fresh cell culture, and after an appropriate incubation period, harvest the cells to spot a slide. These cells then would be stained with virus-specific monoclonal antibody.

As an alternative, one could do a plaque assay on a 12-well plate, inoculate 2 wells per sample, and only add neutral red to one well. This would allow one to read plaques in one well, whereas the unstained well could be used to spot slides which then would be fixed and stained as above, or a PRNT could be conducted on the original pool as described above. The most efficient procedure would be to inoculate 96-well plates as with the *in situ* enzyme immunoassay, then after 4-5 days for alphaviruses and 7 days for flaviviruses, harvest half the media from the inoculated cells, continue incubating the plates, and do an antigen capture enzyme immunoassay with flavivirus- or alphavirus- specific antibody. If a specimen is positive, one would go back to the plate and spot slides with the cells to stain with virus-specific monoclonal antibody.

To conduct nucleic acid analysis, reverse transcriptase-polymerase chain reaction (RT-PCR) could be performed on total RNA extracted from the triturated mosquito pools or tissue samples. Flavivirus and alphavirus conserved primers could be used in a single multiplex reaction, followed by virus-specific primers if a positive is detected. Nucleic acid assays are very fast. But because of its sensitivity, has problems with contamination and requires technical know-how to perform properly. Either a standard RT-PCR or real time RT-PCR (TaqMan™, Applied Biosystems, CA) could be conducted. The latter being the most sensitive and fastest assay.

In California, we already have an excellent surveillance program that needs only minor modifications to strengthen it to detect exotic viruses. To expand the current surveillance program, I recommend maintaining a high level of mosquito surveillance to isolate viruses, and maintaining active sentinel chicken surveillance as the most sensitive method to detect the activity of arboviruses that have a bird-mosquito amplification cycle. In the laboratory, the existing diagnostic system should be strengthened by confirming the identity of viral isolates to species. Using the more sensitive testing methodology, confirmation of sentinel chicken seroconversions will require re-bleeding positive birds to collect whole sera that can be used in PRNT assays.

Roles and Responsibilities of Public Agencies in Emergency Situations

Vicki L. Kramer

Vector-Borne Disease Section
California Department of Health Services, 601 North 7th Street, Sacramento, CA 94234

California historically has experienced outbreaks of mosquito-borne diseases, such as St. Louis encephalitis (SLE) and western equine encephalomyelitis (WEE). It is critical that public agencies continue to monitor for and be prepared to respond to disease outbreaks caused by not only endemic agents, but also introduced pathogens such as West Nile virus. Many public agencies and officials are involved in responding to an epidemic. This report outlines key agency roles and responsibilities and provides background on the emergency conditions government officials respond to in California.

The Emergency Services Act of the California Government Code (GC § 8558) defines three conditions of emergency: 1) state of war emergency, 2) state of emergency, and 3) local emergency. A "state of war emergency" exists immediately, with or without a proclamation by the Governor, whenever this state or nation is attacked by an enemy of the United States. A "state of emergency" exists when there are conditions of disaster or extreme peril to the safety of persons and property within the state. These conditions typically are beyond the control of the services, personnel, equipment, and facilities of any single county or city and require the combined forces of a region or regions to combat. Examples of such conditions include fire, flood, storm, earthquake, epidemic, or riot.

A "local emergency" is declared when conditions of disaster exist within the territorial limits of a city or county, and these conditions require the combined forces of other political subdivisions to combat. A local emergency may be proclaimed only by the governing body of a city or county, or by an official, such as the local health officer, designated by ordinance adopted by that governing body (GC § 8630). A local emergency proclaimed by the county board of supervisors for a health-related reason would apply to all of the cities in the county, including those with a city health officer. A local emergency, as defined by the Government Code, includes such health-related conditions as air pollution, epidemics, and plant or animal infestation or disease. "Health emergency" is narrowly defined (HSC § 101080) as a spill or release of hazardous waste or medical waste that is determined by the California Department of Health Services (CDHS) Director or local health officer to be an immediate threat to public health.

At the state level, the Governor is the only official that can declare a state of emergency when conditions of disaster or extreme peril exist. The Governor declares a disaster when requested to do so by the appropriate official of the governing body, or finds that local authorities cannot cope with the emergency (GC § 8625). A request that the Governor proclaim a state of emergency can be made by the mayor or chief executive of the affected city, the chairman of the county board of supervisors, or the county administrative officer. A local health officer may not make such a request, nor may CDHS. However, county and city health officers may take any preventive measures necessary to protect and preserve the public health from any hazard during a local emergency or state of emergency within their jurisdiction (HSC § 101040, 101475). Preventive measures include abatement, correction, removal, or any other protective step required to protect public health.

According to the Health and Safety Code (HSC § 100170), CDHS may take any necessary action to protect and preserve the public health. The department may conduct studies, demonstrate innovative techniques, evaluate existing projects, provide training, and disseminate information. The department also may advise local health authorities, and, if the department determines that public health is compromised, it shall control and regulate the actions of the local health authorities.

During a state of emergency, the Governor may direct all state agencies to utilize state personnel, equipment, and facilities to the extent necessary for emergency response activities (GC § 8628). Emergency

expenditures by a state agency greater than $25,000 are subject to approval by the Department of Finance. The Department of Finance determines whether reimbursement is made to state agencies for disaster response expenditures (GC § 8649).

At the federal level, the President of the United States may declare a federal disaster at the request of a Governor. The Stafford Disaster Relief and Emergency Assistance Act allows state and local agencies to be considered as eligible applicants for federal emergency assistance. The Federal Emergency Management Agency (FEMA) administers the financial reimbursement program under the Act. To claim reimbursement of emergency related costs from FEMA, Damage Survey Reports are submitted to the California Office of Emergency Services (OES). OES forwards the reports to FEMA. If the emergency is related to public health, FEMA requests the consultation of the Centers for Disease Control and Prevention (CDC). CDC may tour disaster affected regions and prepare a report. Local mosquito and vector control agencies in California have historically been reimbursed for extraordinary mosquito control measures undertaken to protect the public health during disastrous floods.

In response to the West Nile virus outbreak in New York City in 1999, the State Health Commissioner declared a Public Health Threat for five counties and New York City. Usually such a declaration is initiated via a formal request from the County Health Commissioner. A Public Health Threat Declaration eases environmental restrictions on aerial adulticiding. Local agencies were subsequently eligible for reimbursement up to 50% of costs incurred.

Emergency situations may be declared at the local, state, or federal level. It is critical that public health agencies understand the roles and responsibilities of public agencies at all levels and be prepared to respond to a mosquito-borne disease outbreak.

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Preliminary Arboviral Response Plan (Checklist) Are We Prepared?

Charles Beesley

General Manager, Contra Costa Mosquito & Vector Control District
155 Mason Circle, Concord, CA 94520

ABSTRACT: This report presents discusses prior emergency response reports and explains why a new emergency response plan is needed. The report acknowledges the various public health partners, including the University of California and identifies their interdependencies, envisioned roles and responsibilities, strengths, weaknesses, uncertainties, public and legislative expectations and MVCAC's commitment to provide leadership and information to promote public health. A checklist of the different activities conducted by the involved partners is provided, entitled the Preliminary Response Plan.

BACKGROUND

This report represents the efforts of the Ad Hoc Epidemic Response Committee (Committee), formed by the Mosquito and Vector Control Association of California (MVCAC) in 1998. The Committee's charge was to develop an Arboviral Response Plan to address large-scale epidemic arbovirus activity in California. The Response Plan as presented today is still in the developmental phase, is formatted as a checklist of interagency activities and therefore is referred to as the Preliminary Response Plan or Checklist.

The Committee reviewed two prior statewide response plans: California's Mosquito-borne Encephalitis Virus Surveillance and Control Program,1987 (Control Program) (Walsh 1987) and the Interagency Guidelines for the Surveillance and Control of Selected Vector-borne Pathogens in California ,1995 (Interagency Guidelines)(Reisen 1995). These two documents established the interagency roles and responsibilities for local mosquito/vector surveillance and control agencies, the University of California Mosquito Research Program and the California Department of Health Services. Although the Interagency Guidelines replaced the Control Program, upon review, the Committee believed there were too many unanswered questions about actual interagency roles and responsibilities for us to simply update the Interagency Guidelines.

As a result, we formatted the proposed Checklist after the more pragmatic Control Program. The Checklist is intended to provide a template for a more formal response plan to be developed and adopted by MVCAC in 2000. The Checklist is based upon the anticipated chronology of events from routine surveillance during a "normal" season to the more intensive responses expected during an epidemic. The Committee recognizes that there are many questions outstanding that need to be resolved prior to the adoption of a final response plan, such as:

- Determine what are the parameters of a normal season - perhaps the University of California (UC) Model Surveillance Research Program(MSRP) can establish regional norms

- Agreements as to which agencies are going to do consistent, reliable, insecticide resistance testing to represent the current status of resistance throughout the state

- Establish thresholds for the different categories and levels of response. These thresholds could have sliding scales depending upon various factors, again perhaps the MSRP could be of assistance

- Agreement on what should be done in the uncontrolled areas, particularly whether or not to conduct surveillance, resistance testing etc., on a routine basis

- Agreement on recommended products for large scale emergency adulticiding

- Agreement by the identified lead agencies that they will accept their roles and responsibilities and perform their functions in a timely and effective manner

PARTNERS AND INTERDEPENDENCIES

Our current, and future abilities to respond effectively to arboviral emergencies is predicated upon a

considerable amount of cooperative interagency work. One has only to look at the Checklist to see that there is an array of agencies, from state to local, some with multiple departments having different and sometimes overlapping roles and responsibilities. These agencies are linked by historical events, interagency agreements, research projects and unwritten expectations. The three principle partners are: the California Department of Health Services/Vector-Borne Disease Section (CDHS/VBDS); local mosquito and vector control programs (MVCD's) through MVCAC; and the UC/MSRP (Eldridge, et.al. 1995). The partners were instrumental in developing the prior two response plans referenced earlier in this report. A list of the various agencies and departments is listed in Table 1.

ENVISIONED AGENCY ROLES AND RESPONSIBILITIES

The Checklist (Table 2) is based upon the following assumptions:

- Local MVCD's expand their normal surveillance operations when signs of arboviral transmission are detected in the early stages
- Local MVCD's accelerate their control efforts up to and including ULV efforts from the ground when early warning signs of arboviral transmission are detected
- VBDS will be responsible for initiating, and coordinating, surveillance and control efforts in geographic areas without organized MVCD's
- VBDS, in consultation with MVCAC and local health departments (HDs) makes the decision as to whether an emergency exists, and whether it is statewide, regional or local
- CDHS recommends to the Governor that a "state of emergency" be declared due to an arbovirus disease outbreak if a region cannot cope with the extent of the emergency and additional resources are needed
- At the local level, the county Board of Supervisors (BOS) (or in some instances, the Local Health Officer (LHO) of a county may declare a "local emergency" if additional resources are needed
- Local agencies and/or CDHS apply for federal emergency funding in consultation with CDC, if the President of the United States, at the request of the Governor, has declared a federal emergency
- The local agencies and/or the Executive Director of MVCAC solicit emergency State funding
- The Chief of VBDS makes the declaration of an emergency exemption from an Environmental Impact Report prior to implementing large scale ULV spraying
- The VBDS, in consultation with the UC Vector Control Advisory Committee (VCAC), UC Mosquito Research Program (UCMRP), California Department

Table 1: A list of state and local agencies which participate in arboviral reporting and control response.

ABBREVIATIONS

BOS	County Board of Supervisors
DPR	California Department of Pesticide Regulations
CDHS	California Department of Health Services
CDPH	County Department of Public Health
LHO	Local Health Officer
LEHD	Local Environmental Health Department
LMCA	Local mosquito control agency
MOU	Memorandum of understanding
MVCAC	Mosquito & Vector Control Association of California
MVCD	Mosquito and Vector Control District
PHCO	Primary Health Care Organization
UCVBCDR	University of California, Center for Vector-Borne Disease Research
UCMRP	University of California, Mosquito Research Program
UCMSP	University of California, Model Surveillance Program
VBDS	Vector-Borne Disease Section
VCAC	Vector Control Advisory Committee
VRDL	Viral and Rickettsial Disease Laboratory

STATUS

The following checkmarks are used in the Checklist to identify agency roles and responsibilities

✓ Standard procedure - routinely performed
✓✓ Not routinely performed but should be considered by the identified agency
✓✓✓ Expected to be done at appropriate time, as listed, by identified agencies

Table 2: **PRELIMINARY RESPONSE PLAN**

LEVEL I

Activity	Agency	Expected Response	Comment	Status
Monitor Rainfall Snow pack	VBDS LMCA's UCMSP	Annual review	UC Model Surveillance Research Program currently reviewing	✓✓
Review prior season	VCAC	Report to MVCAC at Spring BOD	Analyze prior season, suggest changes if needed	✓✓
Routine mosquito and virus surveillance activities	LMCA's VBDS UCMSP	LMCA's routinely perform	Establish normal versus emergency population and virus level thresholds for agencies region and the state in relation to statewide model surveillance program	✓✓
Maintain banks of viruses	UCVBDR	Resource bank available for statewide use	Includes maintenance of banks of viruses and newly emerged viruses in CA and that are vector-borne	✓✓
Inventories	MVCAC	Conduct annual inventories	Compile inventory of pesticides and equipment for large scale adulticide response. Verify available emergency reserve funds	✓✓✓
			Verify annual list of certified vector control operators.	✓✓
	VBDS			✓✓
MOU's	MVCAC VBDS	Interagency exchange of personnel and/or equipment	Develop regional memoranda of understanding for mutual exchange of personnel and/or equipment	✓✓✓

Table 2: **PRELIMINARY RESPONSE PLAN**

LEVEL II

Activity	Agency	Expected Response	Comment	Status
Monitor early season populations of vector species	VCAC UCMSP	Forecast > average early season vector populations	Analyze data to determine if existing surveillance efforts are sufficient	✓✓✓
Statewide aerial control response	VCAC MVCAC	Review plans for the upcoming year	Determine if plan etc. is still appropriate for the coming year	✓✓✓ ✓✓
Review and evaluate efficacy of labeled, available, candidate adulticides	MVCAC	Establish preferred materials list	Review annually for label changes, restrictions, operational, resistance limitations, etc.	✓✓✓
Review products of choice, availability and current registration	MVCAC UCMRP VBDS	Report on preferred materials list Review list	Prepared by Chemical Control Committee Objective review of pesticide efficacy Initiate procedures for waiver of application restrictions	✓✓ ✓✓✓ ✓✓
Determine inventories and availability	MVCAC	Maintain current list	Review annually	✓✓✓
Contact commercial, aerial applicators	MVCAC	Maintain current list Establish bid specifications	Contact when early indicators predict vector problems	✓✓✓

Table 2:	PRELIMINARY RESPONSE PLAN			
	LEVEL III			
Activity	**Agency**	**Expected Response**	**Comment**	**Status**
Isolation of virus from mosquito pools or antibodies from chicken flocks in *excess of expectancy*	UCD	Tests and reports to LMCA's etc.	As currently performed	✓
	VBDS	Sets the thresholds, makes the determinations	Statewide Control Preparation, whether off the shelf plan or developed during Level II phase	✓✓✓
Alert Local HD's notify physicians, veterinarians, etc.	VBDS	Have an established communication network in place	Activate during emergency planning phase	✓
Funding insufficient for comprehensive response	VBDS	To initiate control for those areas under local control and/or without local control	VBDS applies for Federal emergency funding, in consultation with CDC	✓
	MVCAC		MVCAC goes to State Legislature for additional funding	✓✓✓
> average populations of adult vector species	UCMSP	Determines if local, regional or statewide	Utilization of statewide population data	✓
	LMCA	Control response increased	Elevated local control response coordinated through VBDS	✓✓✓
	VBDS	If no organized control agency, contract for services		✓✓✓

of Pesticide Regulation (DPR), and United States Public Health Service Center for Disease Control and Prevention (CDC), recommends appropriate chemical control technology.

- The Chief of VBDS coordinates with federal agencies to procure emergency exemptions (40 CFR166) from FIFRA and emergency tolerance exemptions (40 CFR176) as a result of the emergency conditions.
- The CDHS/VBDS submits a proposed large scale ULV aerial program(s) to the Governor's office for approval, prior to implementation
- The Chief of VBDS is responsible for implementing and coordinating large scale ULV aerial adulticiding programs that are regional or statewide in nature
- The Executive Director of MVCAC is responsible for coordinating public information releases on behalf

of MVCAC, in conjunction with local MVCD's

- Ultra Low Volume (ULV) applications continue for a period of time determined by the VBDS and/or local agencies, until the density of vectors is reduced below a population threshold conducive to viral transmission

STRENGTHS

As longstanding partners we have many strengths to build upon. Local MVCD's have a strong foundation in disease surveillance through the use of mosquito population counts, sentinel chicken flocks and mosquito pools. The University has conducted extensive, local, and statewide research on arboviral transmission. The Center for Vector-Borne Disease Research at UC Davis (UCD) is capable of identifying new, exotic pathogens. Mosquitoes are collected by local MVCD's and submitted to UCD to test for viral infections. VBDS conducts statewide training and certification of MVCD personnel, coordinates the statewide surveillance program, and the Viral and Rickettsial Disease Laboratory (VRDL) conducts testing of the sentinel chicken flock sera to detect the presence of arbovirus antibodies. We also have mechanisms in place to exempt large aerial control spraying from environmental impact reports (EIR's).

WEAKNESSES

On the other hand, there are good reasons to be concerned about whether or not we can predict or respond effectively to a large scale epidemic. California has not had such an epidemic for over forty years, so we lack hands-on experience at this point. Recent studies have demonstrated that western equine encephalomyletis virus (WEE) is still virulent (Hardy et. al., 1997). Although WEE and Saint Louis encephalitis virus (SLE) are periodically detected from different regions of the State, there is reason to believe the general public has little, if any, immunity to these arboviruses should they become infected by a mosquito (Glaser pers. comm.). Regarding the control aspect, there is little agreement on what constitutes a local, regional, or state emergency, making it difficult to quickly activate a large-scale, coordinated, response. There is no agreed-upon adulticide of choice for large-scale aerial applications. As a consequence, contiguous MVCDs, or MVCD's within the same region could use different adulticides as part of a large-scale control response, giving an appearance of questionable operational coordination. Contrary to the past, when CDHS/VBDS conducted statewide pesticide resistance testing on a routine basis, we no longer consistently monitor levels, or distribution, of pesticide resistance. At present only a few MVCDs conduct pesticide resistance testing. California has experienced funding difficulties and cutbacks for the past twenty plus years, affecting the three key partners as well as the many other agencies listed in Table 1. There are also many areas of the State without organized mosquito control, leaving residents and visitors unprotected in the event of an arboviral emergency. We have no centralized, common data base on mosquito populations, insecticide resistance levels, or local control thresholds to assess statewide or regional trends, limiting our abilities for large scale coordination and oversight. Many agencies still rely on faxes and phone calls instead of web sites and electronic transmissions. Our overall, statewide, abilities no longer meet the high standards that California has long been known for.

UNCERTAINTIES

In addition to some inherent weaknesses, in the overall program, there are foreseeable operational and political uncertainties that would affect our overall response capabilities. For instance, by not having a recognized pesticide(s) of choice, we have no guaranties of availability and product delivery for large-scale operations. There are no advance agreements to deliver insecticide products within a certain period of time with agreed-upon price structures. Our existing plans have too many gaps in roles and responsibilities, severely limiting our ability to mobilize quickly and effectively. History also has shown that arboviral testing and reporting by physicians is not always consistent leaving us circumspect about the effectiveness of physician alerts. Because we have no tangible emergency plan, we would find very little initial support to approve a large-scale control operation, which essentially would have to be approved by the Governor's office. Given the public outcry in California to the Medfly aerial spray program, and persistent questioning in New York about the value of large scale spraying for mosquitoes, to suppress West Nile virus, there is little reason to expect the public to react any different in the event of large scale aerial spraying for arboviral control. Although a small percentage of the public will to be vocal against any large-scale use of pesticides, we can expect that a larger portion will be calling for legislative investigations if we do not respond in a timely, effective manner. In essence, we will be caught between the proverbial rock and a hard spot.

PUBLIC, LEGISLATURE EXPECTATIONS

In keeping with the above sentiment, we can anticipate that the public and Legislature expect that we, as public health officials, have emergency plans in place, conduct training sessions on emergency management under the California Emergency Services Act, such as the Standardized Emergency Management System (SEMS) under the Governor's Office of Emergency Services. Program. They expect our local programs to be linked, much like public safety agencies are (mutual exchange agreements, common databases, compatible communications equipment, etc.). They will assume we have established, or uniform, thresholds for emergency response as demonstrated by other local emergency and public safety agencies. Finally, given all of the legislative efforts over the past ten years to review and possibly restructure local government, they will expect that we are can deliver effective, efficient, accountable emergency response at the local, regional and state level. If we cannot, we can expect criticism at the least.

MVCAC's COMMITMENT

The Board of Directors of MVCAC recently adopted a vision, mission and principles for the MVCAC, all of which are relevant to the Committee's charge to develop a contemporary response plan. Specifically, the following was adopted by the Board of Directors on May 7, 1999.

Vision

Become the "default" experts in our field of public health - The MVCAC and its members should be the recognized experts on arthropod vectors, readily available as professional resources to be utilized by the Legislature and other blue ribbon commissions.

Initiate interactive planning between the MVCAC, CDHS and UC - Although all three agencies currently interact through efforts such as MosquitoNet, we should strive to achieve a higher level of interactive planning between the three groups, in effect, creating more of a partnership between existing and future programs. To do so will probably require more active planning and a greater commitment of financial, and related, resources, with the MVCAC providing the lead role.

Become a more sophisticated administrative machine - If the above actions occur, the end result should be a more clearly defined administrative operation which initiates activities, provides background reports, coordinates programs and services and administers a wider range of budgetary matters, all in the interest of providing more comprehensive services and benefits to the members.

Mission

The mission of the MVCAC is to provide leadership and information to promote public health through the delivery of mosquito and vector control services. The principles by which the MVCAC is guided are listed below.

Principles

Protect and enhance public health
Protect and improve the environment
Promote and implement research
Maintain and instill public confidence
Facilitate and enhance interagency programs
Inform and interact with policy makers
Maintain and improve professional standards

SUMMARY

California has a nationally recognized arbovirus surveillance system (System) based upon close cooperation between local agencies such as MVCD's, the CDHS and the UC. These cooperative efforts include a wide range of activities from field surveillance and control, laboratory diagnostic testing, to arboviral reporting. There have been many technological improvements since the Interagency Guidelines were written in 1995. Laboratory diagnostic techniques have improved, computer technology has advanced enormously, and communication systems have also improved greatly. We now have the statewide ability to collect, test, identify, and report results rapidly. In other words, we live in a more sophisticated, technology driven State with relatively high public expectations. Public health agencies are expected to keep up with the pace of technology and respond quickly to emergencies. However, our review of the existing emergency response system leads us to believe there is significant room for improvement and if we do not fill in these gaps to best respond to arboviral emergencies, we can expect public and Legislative criticism and/or scrutiny. Thus the development of the attached Checklist.

The Checklist is a transitional document, intended to provide a functional template until a new response plan is developed by the MVCAC. It is essentially a comprehensive list of the multiple roles and responsibilities between not only the three key partners but the many other agencies involved in monitoring and

responding to arboviral transmission. The Checklist was built upon prior reports on emergency preparedness, it identifies our strengths, weaknesses and foreseeable uncertainties in the event of an epidemic. The Checklist stresses the need for active, annual planning and preparation by the three key partners to ensure that each year we review our plans in light of recent experiences and foreseeable events. The Checklist is compatible with MVCAC's stated mission to provide leadership and information to promote public health through the delivery of mosquito and vector control services.

RECOMMENDATIONS

This report was presented to the Board of Directors at their annual meeting on January 26, 2000. The Board adopted the report with the following recommendations.

- Use the Checklist as a template for emergency response until a final plan is adopted
- Resolve the gaps in interagency roles and responsibilities
- Improve our analysis and predictive abilities by incorporating components of the Model Surveillance Program
- Develop a short list of preferred pesticides to be used in emergency applications
- Develop contractual agreements for products and services to be used in emergencies
- Develop an actual emergency response plan or document for Legislative approval
- Be prepared for local, regional, or statewide emergencies

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Beebe, Dennis, SCMAD
Meyer, Dick, Ph.D., OCVCD
Brown, Dave, SYMVCD
Mulligan, Steve, CMAD
Clausen, James, SYMVCD
Bass, Deborah, CCMVCD
Cornel, Anton, Ph.D., UCD
Pfuntner, Allan, WVVCD
Eldridge, Bruce, Ph.D., UCD
Reisen, Bill, Ph.D., UCD
Fujioka, Kenn, Ph.D., SGVVCD
Scott, Tom, Ph.D., UCD
Heim, Ralph, HNS
Sjogren, Bob, Ph.D., OCVCD
Husted, Stan, VBDS
Sullivan, Dave, Zanus Corp.
Inman, Allan, MCMAD
Thompson, Mac, VBDS
Townzen, Ken, VBDS

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A Model Surveillance Program for Vectorborne Diseases in California, 1999-2000

Bruce F. Eldridge[1], Anton Cornel[2], Carol Glaser[3], Candice Kohlmeier[3], William K. Reisen[4], Steven Schutz[5], and Thomas W. Scott[1]

[1]*Department of Entomology, University of California, Davis, CA 95616*
[2]*Mosquito Control Research Laboratory, Kearney Agricultural Center, Parlier, CA 93648*
[3]*Viral and Rickettsial Disease Laboratory, California Department of Health Services, Berkeley, CA 94704*
[4]*Arbovirus Field Station, Center for Vector-borne Disease Research, Bakersfield, CA 93312*
[5]*Contra Costa Mosquito and Vector Control District, Concord, CA 94520*

ABSTRACT: This report summarizes research during year 3 of a 5-year project to improve the efficiency of the California Vectorborne Disease Surveillance Program. Complete results are contained among the publications cited below. Enhanced surveillance for arboviral encephalitis in humans continued, but failed to detect any new cases. A study of grain-baited traps for surveillance of arboviral activity in wild birds concluded that wild birds are not suitable for rural surveillance because of lack of sensitivity and difficulties in interpretation of serological results. Further improvements were made to the surveillance website, including incorporation of automation for updating of databases and direct control of databases by testing laboratories. Future prospects for surveillance for vector-borne diseases in California are discussed.

INTRODUCTION

This report summarizes research during year 3 of a 5-year project to improve the efficiency of the California Vectorborne Disease Surveillance Program and is based on presentations at a surveillance symposium held at the Mosquito and Vector Control Association of California (MVCAC) annual conference in January, 2000 in Sacramento. Previous reports summarized the 5 interrelated objectives of the program. Included in this report are (1) the results of enhanced human surveillance for arbovirus infections in California, (2) an evaluation of the ability of grain-baited traps to focus on indicator bird species for surveillance, (3) the results of a mark-release-recapture experiment conducted in the San Joaquin River delta, (4) the development of new methods for the monitoring of insecticide resistance, (5) improvements made to the electronic reporting of results of arbovirus surveillance, and (6) an examination of the future of surveillance in California. Many people participated in the research presented herein. The individuals who made presentations at the symposium are listed as authors, and other participants are acknowledged at the end of the article.

1. Enhanced Human Arbovirus Surveillance

In 1999, attempts were made to detect acute human infections caused by St. Louis encephalitis (SLE) and western equine encephalomyelitis (WEE) viruses. One study, supported by special funds from MVCAC, focused on health care providers in Riverside and Imperial Counties. Results also were presented from an ongoing encephalitis project in California conducted by the California Department of Health Services (DHS), designated the California Encephalitis Project.

SLE and WEE in Imperial and Riverside Counties Human case detection

A goal of this study was to increase physician awareness of the potential for mosquitoborne viral infections in humans, and to encourage the submission of specimens from individuals presenting symptoms indicative of possible arbovirus infections. In addition, a background serosurvey based on convenience samples gathered data on previous infections. For case detection, physicians were alerted via a letter with an attached fact sheet on SLE and WEE infections, a case history form, and instructions for submitting specimens. Posters were placed in emergency rooms and clinics, and presentations were made to physicians.

Specimens from a total of 15 cases were submitted. Symptoms included febrile headache (11 cases), encephalitis (2) and vertigo (1). Eight of the specimens were from Riverside County, 7 were from Imperial County. Only one acute phase specimen showed IgG antibodies; none showed IgM antibodies.

No acute cases of St. Louis encephalitis or western equine encephalomyelitis were detected via this project. Unfortunately, physician compliance was low, resulting in few specimens for testing.

Serosurvey

Blinded samples of bloods drawn for other medical purposes were obtained from 4 different medical facilities (3 hospitals and 1 plasma center) and tested by EIA for IgG antibodies to SLE and WEE. A total of 729 bloods were collected from individuals ranging from 1 to 99 years of age (mean age 45). Males represented 56% of the specimens, females 44%.

Of the 729 samples tested, 116 showed IgG antibodies to SLE, 11 to WEE. In one specimen, SLE IgM was detected. As with all of these specimens, there is no associated clinical information. A follow-up study is in progress.

There was a high level of seropositivity to SLE among the 729 specimens, but there are problems with interpretation because dengue (DEN), West Nile (WN) and yellow fever (YF) viruses all cross-react with SLE. Cross neutralization tests on the IgM positive SLE will be conducted for DEN, SLE, YF and possibly WN, at the UC Davis Arbovirus Research Unit (ARU).

California Encephalitis Project

The goal of this long-term project is to establish the causes of encephalitis detected in California. The criteria for inclusion in this study are hospitalization with encephalopathy; depressed or altered level of consciousness for >24 h; lethargy or change in personality, and one or more of the following: fever, seizures, focal neurologic findings, CSF pleocytosis, EEG finding consistent with encephalitis, or abnormal neuroimaging. Case patients must be older than 6 months of age.

No cases of western equine encephalomyelitis were confirmed. Of 190 cases examined to date, 8 were positive for IgG antibodies. There have been no IgM positive specimens.

All cases with rural exposure or mosquito exposure tested were sent to the UC Davis ARU for further testing. Of 37 specimens tested, all were negative for California encephalitis virus, SLE and WEE.

2. Enzootic surveillance: ability of grain-baited traps to focus on indicator bird species

Introduction

The ARU is studying the role of wild birds in the maintenance and amplification of WEE and SLE in wetland habitats of California. These studies currently indicate that avian seroprevalence rates generally remain low and that most infections occur in relatively few species (Reisen et al. 2000); mostly house finches, house sparrows, California and Gambel's quail, and mourning and common ground doves that are sampled effectively by both mist nets and grain baited traps. The purpose of our research during 1999 was to convert our labor-intensive, mist net sampling into an efficient trapping program that mosquito control districts could use to augment encephalitis virus surveillance in rural areas. A similar transition in sampling was accomplished previously in suburban Orange County (Gruwell et al. 1988; McLean et al. 1988). Additional observations on antibody persistence were included because these data relate strongly to the interpretation of seroprevalence data, especially during the critical spring period.

Specifically, our objectives were to:

1. Measure enzootic virus activity in the Coachella and San Joaquin Valleys during 1998 and 1999.
2. Compare bird diversity and antibody rates between surveys with nets and grain-baited traps during 1996-1998 and sampling using grain-baited traps during 1999.
3. Describe field and experimental data on antibody persistence.

Virus Activity

Field research focused on the southern Coachella Valley near the Salton Sea in Riverside County and the Bakersfield area in the southern San Joaquin Valley in Kern County. One sentinel chicken flock, 1-3 grain-baited ground traps, a modified crow trap, and 1-3 dry ice-baited CDC style mosquito traps were positioned at each site. Sampling in Coachella Valley included 8 sites and was extended into the upper valley in an attempt to trap more house finches and mourning doves. In Kern County, sampling was limited to the Kern River and Tracy Ranch to focus on California quail and house finch populations, respectively. Sentinel chickens were bled from the comb, whereas wild birds were bled by jugular puncture. Birds were screened for antibody by appropriate enzyme immunoassays (EIA). Seropositive chickens were confirmed by an indirect fluorescent antibody test, whereas seropositive wild birds were confirmed by a plaque reduction neutralization test (PRNT). Mosquitoes were identified to species, enumerated, and *Cx. tarsalis* were pooled for virus isolation using Vero cell culture followed by an *in situ* EIA.

Detection of virus activity was low in Coachella Valley and absent from the southern San Joaquin Valley during 1999 (Table 1). In the Coachella Valley, WEE infection was detected in a single sentinel chicken

seroconversion between November 1998 and March 1999 in a flock maintained to monitor winter virus activity and SLE was detected by 2 seroconversions during November 1999. In contrast, 13 after-hatching-year adult Gambel's quail had antibodies to WEE; no birds were positive for SLE. Standard surveillance (i.e., by sentinel chickens and mosquito pools) did not detect WEE in the southern San Joaquin Valley; however, 14 California quail were antibody positive for WEE. Quail presumably were infected during 1998, when WEE was active in both valleys.

Sampling emphasis on grain-baited traps focused testing on the 6 target bird species that comprised over 75% of the total WEE positives detected (Table 2). During 1996-1998 these 6 species comprised <40% of the total birds sampled, whereas during 1999 this percentage increased to >80%, although the number of sera tested declined sharply. Our trapping efforts did not collect sufficient numbers of house finches, and in Kern County catch was supplemented with birds trapped in vineyards for pest management. Likewise, our collections of mourning doves were irregular and insufficient for surveillance purposes.

Antibody persistence

Natural antibody persistence between seasons was verified by 4 quail that were positive during 1998 when WEE was active, banded, and recaptured again during the spring of 1999 (Table 3). Because the PRNT titers declined over time (one bird was negative, <1:20), we experimentally infected house finches to determine if residual antibody was protective from subsequent infection.

House finches were infected with WEE and SLE during the summer of 1998, held in a screened outdoor aviary, pre-bled the following summer and then rechallenged with the same virus. Similar to the quail, house finches retained low titered but protective antibody against WEE (Table 4). These birds produced a detectable anamnestic reaction after reinfection (i.e., an rapid immune response to a previously encountered antigen). In contrast to WEE, antibody in house finches infected with SLE during 1998 declined rapidly over the winter, and was not detectable when birds were bled the following summer. When rechallenged, residual antibody was not completely protective because 3 of 6 birds developed detectable viremia on day 1 post-infection. These birds then were negative on the following 3 days. The rapid decline in the viremia was attributed to the anamnestic response.

Summary

Overall, our data indicated that:

1. Grain-baited traps focused sampling on target bird species, but failed to collect sufficient numbers of house finches and mourning doves for effective surveillance.
2. Catch in traps was temporally variable and frequently hampered by predators.
3. Sensitivity to presence of arboviruses could not be compared effectively during 1999, because WEE and SLE were not sufficiently active in our study areas.
4. WEE antibody persisted over winter in recaptured quail, but titers decreased.
5. WEE antibody persisted over the winter in experimentally infected house finches and protected against reinfection, whereas SLE antibody decayed rapidly and some birds produced viremia upon rechallenge.
6. Based on the current and previous observations, we concluded that a wild bird surveillance program was not suitable for rural surveillance by mosquito and vector control districts, because:

a. Sensitivity. Temporal changes in wild bird seroprevalence rates did not provide early evidence of virus activity and did not detect low-level SLE activity in Coachella Valley during 1998 or 1999.

b. Interpretation. Variable rates of antibody decay, possible reinfection, and low antibody titers made it difficult to separate recent from previous infections testing for IgG. The only data suitable to detect early season virus activity was infection in hatching-year or recaptured birds. However, hatching-year birds were infected mostly during midsummer, and after virus amplification was detected by seroconversions among sentinel chickens or virus was isolated from mosquitoes. The numbers of birds that were collected antibody negative, banded and then recaptured antibody positive, was too low for sensitive surveillance.

c. Consistent data. We had problems with trap efficiency changing over time based on nesting behavior, presence of alternative food and predators.

3. Surveillance for Pesticide Resistance in California

The concept of vectorborne disease surveillance was broadened to include surveillance for pesticide resistance in response to guidelines of the Epidemic Arbovirus Response Working Group, a joint effort of MVCAC, DHS, and the University of California. The rationale for the inclusion of pesticide surveillance was the realization that presence of resistance in vector mosquitoes is dynamic, and must be studied in advance of potential outbreaks and for management of pesticide resistance.

First reports of apparent methoprene control failures against *Aedes nigromaculis* were noted in a pasture west of Fresno in September 1998. Methoprene failures had spread to an additional 10 pastures in Fresno County during the summer of 1999. In some of these pastures, methoprene, a juvenile hormone analog, had been used for at least 20 years as the primary insecticide to control this mosquito. Field trials, based on pupal counts and different methoprene application rates, showed that in some pastures, either no control, or only low levels of control, were achieved with Altosid Liquid Larvicide (ALL®) and Altosid® XR-G and 52-99% control with Altosid® Pellets (Cornel et al. 2000).

A methoprene bioassay for *Ae. nigromaculis* was developed. Preliminary results indicated that there was a methoprene tolerance difference of 50- to 100-fold between a methoprene-naive population and populations from pastures where methoprene had been used consistently for 6 to 20 years.

In preparation for bottle bioassay surveillance of *Cx. pipiens* complex and *Cx. tarsalis* mosquitoes, standard susceptible colonies of these mosquitoes were established. These colonies are susceptible to all of the chemical larvicides and adulticides registered for mosquito control in California.

4. Mark-release-recapture studies of *Culex tarsalis* in the San Joaquin delta

The objectives of this study were to investigate the flight range and distance, population dynamics and population density of the encephalitis mosquito, *Cx. tarsalis*, on islands in the San Joaquin River delta along the border between Contra Costa and San Joaquin Counties.

Adult female *Cx. tarsalis* were collected for release in dry ice-baited EVS traps at 2 locations on 3 consecutive nights in August 1999. Each of the 3 collections were transported to the release point at the southern end of Mandeville Island (San Joaquin County) on the evening after capture, were marked with 3 different colors of fluorescent powder (pink, green and blue on nights 1, 2, and 3, respectively) and released at dusk. Of a total of 57,247 released, 305 marked individuals were recaptured during the following 9 evenings in a total of 60 EVS (battery-operated CO_2-baited) traps located in roughly concentric rings within a 7 km radius from the release point. The predominant flight direction was west (upwind). The rate of dispersal was greater than had been anticipated based on previous studies, with recaptured mosquitoes moving an average of 3.6 km per night and a maximum of 5 km in a single night, easily crossing among islands. We dissected the ovaries of the majority of marked mosquitoes recaptured and found that less than 10% of individuals released successfully fed and reproduced during the study period. The parity rate of unmarked mosquitoes captured during the same period was slightly higher but did not exceed 28%. This low rate of feeding success suggests a lower risk of virus transmission at the time and place the study was conducted. Estimates of daily survivorship varied among release groups but were consistent with previous studies and suggested that some individuals could survive long enough to acquire and transmit encephalitis viruses. Population density estimates, calculated by the modified Lincoln Index method, averaged approximately 18,800/km^2. We concluded that mosquitoes produced at sources in the delta have the potential to rapidly impact nearby populated areas, and that successful control of *Cx. tarsalis* in this area, and reduction of disease risk, will require inter-agency cooperation, including sharing of pertinent data on a regional level.

Complete details will be published elsewhere (Schutz et al. 2000).

5. Analysis, prediction and reporting

A surveillance website is located at http://mosqnet.ucdavis.edu. Further improvements were made in 1999 in the electronic reporting of arbovirus surveillance information. A new concept for maintenance of database tables was incorporated in which the testing laboratory involved becomes the "owner" of the data, which are maintained on a central server. Testing laboratories are responsible for periodic updates to the data. Progress has been made on connecting laboratories directly to the server, but some problems remain. Much of the drudgery of updating maps and data surveillance summaries has been eliminated through installation of programs that automatically update the website once the data tables have been updated.

Financial support has been requested from the Centers for Disease Control and Prevention (CDC) to install a further extension of the system whereby mosquito agencies will have full access to those databases. By using specially designed client software, a full range of analysis options, including mapping, graphing and various types of reporting will be available. Under the present system, analysis of historical data is difficult; under the new concept, users could select any time frame desired. The implementation of this concept is being coordinated with Advance Computer Resources, Inc. A demonstration mapping server is planned for 2000.

6. The future of Surveillance for Arbovirus Diseases

The arbovirus surveillance system in California has 2 goals. First, to predict the threat of virus transmission to people, in other words, to serve as an early warning system. Second, to document local transmission; to

confirm that a given virus is present and is being transmitted. Monitoring of enzootic virus activity is the primary method used currently in California to estimate risk of human infection. However, the relationship between enzootic transmission of arboviruses and human infections is unclear. Consequently, this complicates predicting human infection based on enzootic transmission even though past studies in California have shown that critical decisions regarding risk of human infection can be based on the density of *Cx. tarsalis* (Reeves 1971).

Facts about arboviral encephalitis

The arboviral encephalitides continue to constitute a significant public health burden. Although the number of confirmed human cases of arboviral encephalitis has declined annually in recent years to just a few in California, the incidence nationwide continues to be 150–3,000 per year. Total cost association with these cases is considerable, and is currently estimated to be $150 million per year, including costs of vector control and surveillance activities. Cost of individual cases is also substantial. It has been suggested that costs for an individual case of eastern equine encephalomyelitis range from $21,000 for transiently infected individuals to $3 million for severely infected people (Villari et al. 1995).

The real threat to humans from arboviral diseases

The annual number of cases of western equine encephalomyelitis and St. Louis encephalitis occurring in California recently may suggest to some that the risk of human infection by these agents is virtually nonexistent (Reeves 1990). However, before concluding that there is little risk of future outbreaks of arboviral diseases, one should look at some disturbing trends for arboviral diseases worldwide. For example, the history of dengue in the Americas points out that we must not become complacent about sustaining California's outstanding arbovirus surveillance program.

In 1970, due to a Pan American Health Organization supported hemisphere-wide eradication campaign, the geographic range of *Aedes aegypti*, the primary vector of dengue virus, was restricted to just a few isolated areas in the Americas: the southeastern USA, the Caribbean region, and northeastern South America. By 1997, following relaxation of the eradication program, it has spread to most of Mexico, all of Central America, and parts of nearly every South American country. During roughly this same time period, dengue hemorrhagic fever, the cause of severe, often fatal, human disease, had gone from being absent to occurring in much of tropical America. The devastating pattern reported in the 1950s of dengue emerging as a major public health threat was repeating itself in tropical America, in part because efforts to control *Ae. aegypti* either ceased or were significantly reduced.

The recent appearance of West Nile virus (WN) in the northeastern USA similarly points out that we should not limit our vigilance only to viruses that we know are present in California. We no longer can limit our attention to activities within the boundaries of the state of California. We must be prepared to respond to vector-borne pathogens that may be introduced into California accidentally or on purpose (bioterrorism). This will require expertise and research on vector-borne pathogens that historically have not been detected in the state.

The WN outbreak in New York, which presumably resulted from the introduction of virus from the Old World, resulted in over 60 human cases and 7 deaths. Virus was isolated from 18 species of birds, mostly crows, within 50 miles of New York City. Virus was isolated from a dead crow in Baltimore, about 250 mi from New York City. Serosurveys produced evidence of infection in 75 horses and 15 dogs. There were 17 isolations of WN from mosquitoes, most from *Culex pipiens* and *Aedes vexans*.

Emerging diseases

In recent years there has been a fascination in the popular press, films, and in scientific circles with the so-called emerging diseases. Morse and Schluederberg (1990) provide a working definition for these kinds of pathogens. They explain that emerging diseases are caused by pathogens that either have newly appeared in a population or are rapidly expanding their range, with a corresponding increase in disease cases.

One could ask, does the introduction of vector-borne pathogens constitute a public hazard to California? We present 2 examples that indicate that introduced pathogens do constitute a threat for the citizens in our state. In fact, the WN epidemic in New York City points out that accidental or purposeful introduction of pathogens will happen. Therefore, vector control and public health officials need to be prepared to detect and respond to these situations when they occur.

According to Morbidity and Mortality Weekly Reports (MMWR), published by the CDC, each year from 1966 to 1999 a few hundred to over 3,000 cases of imported malaria were documented in the United States. In California alone, during 1998 and 1999, there were 217 and 208 reported imported malaria cases,

respectively. Anopheline vectors of malarial parasites occur in California, the parasites are being introduced into the state, and the threat of autochthonous transmission exists.

MMWR reports on imported dengue similarly support the notion that programs for detecting and responding to imported vector-borne pathogens need to be in place in California. In 1995, 86% of 441 people with dengue-like illness from 31 states and the District of Columbia were reported as having dengue. Of those people with confirmed dengue, 83 had recently traveled to the Caribbean (48), Mexico and Central America (24), Asia (5), and Africa (6). In 1996, 24% of 179 people with dengue-like symptoms from 32 states and DC were diagnosed as having dengue. Thirty-seven people had recent travel histories to the Caribbean (19), Asia (11), Africa (3), Pacific Islands (2) and Central and South America (2). Results from a study limited to the state of Florida during 1997-1998 indicate that of 83 people suspected of having dengue, the disease was confirmed for 36. All reported travel within 10 days of onset of illness to Haiti, Puerto Rico, Colombia, Venezuela, Barbados, Nicaragua or Thailand.

Malaria and dengue are reportable diseases; they are the disease agents that are accounted for by state and federal officials. But there is a wide variety of other vector-borne pathogens that could also be introduced into California and could go undetected. On a worldwide basis, there are hundreds of arboviruses that may pose a threat from introduction. Examples among the arboviral encephalitides include West Nile encephalitis, Venezuelan equine encephalomyelitis, tick-borne encephalitis, Japanese encephalitis, and Murray Valley encephalitis. In a recent talk to MVCAC, Dr. William C. Reeves highlighted a number of these agents that may be especially dangerous and constitute a significant threat if imported into California.

We should not limit our concern to pathogens, we must also consider and be prepared for the threat posed by imported vectors. During the past several decades, *Aedes albopictus, Aedes togoi*, and *Aedes japonicus* have been imported into other regions of the USA and all are known or potential vectors of human pathogens.

Elements of emerging-disease control plans

If we accept the premise that pathogen and vector introductions constitute potential threats to California, we should next ask what can we do to better prepare ourselves to detect and respond to an introduction? The following section is a series of suggestions, taken from a detailed report by Koplan and Hughes (1998), for fortifying California's current outstanding surveillance program.

Plans to provide adequate protection to citizens of California against the risk of infection with emerging arthropod-borne infectious agents should contain the following 6 components.

1) Two critical requirements for improved arbovirus surveillance are strengthening of networks (state, national, and international) to detect, control, and reduce emerging diseases and improvement of the capacity to respond to disease outbreaks, especially the improvement of medical and scientific expertise.
2) Surveillance networks should be improved so that they include better electronic communication to and from DHS and MVCAC with other states, local health departments, US quarantine stations, health care professionals, and veterinary and wildlife disease organizations. Improved networking is essential to the enhancement of California's capacity to respond to complex infectious disease threats, including bioterrorism.
3) There must also be a strengthening of disease surveillance and response plans and the development of improved standards and guidelines for such plans. An improvement of public health infrastructures, including laboratories, research facilities, technology, and communication links, are needed to implement these guidelines. Response plans must be developed to ensure prompt implementation of prevention strategies and enhanced communication of public health information about emerging diseases.
4) It is essential that we strengthen both applied and basic research. The investigation and monitoring of emerging pathogens, the diseases they cause, and the factors influencing their emergence is vital to our ability to respond to disease outbreaks. This research should include development of improved methods for gathering and evaluating surveillance data. Research should integrate laboratory science, epidemiology, and disease ecology to optimize the application of public health programs. Other needed research includes the development, evaluation, and dissemination of tools for the identification and understanding of emerging infectious diseases and their risk factors, and the development and evaluation of prevention and control strategies in agreed upon target areas.
5) We must focus attention and resources on training of medical and scientific personnel. Training is needed to enhance epidemiologic, laboratory, and research capacity. We should take advantage of training opportunities in infectious disease epidemiology, ecology, and diagnosis in

California, nationally, and internationally.

6) Finally, there should be encouragement for other organizations to improve their public health systems and to coordinate activities through national and international organizations.

If these issues can be addressed and solved, the result would be a statewide and national network for surveillance that should ensure prompt identification of infectious disease outbreaks. Equipment and personnel would be in place to provide appropriate and rapid public health responses to disease threats and bioterrorist incidents. Improved diagnostic methods will allow rapid detection of emerging diseases. Reference reagents would be available for public health and regional reference laboratories. The improved understanding of disease risk factors would lead to improved strategies for disease prevention and treatment. Among the most important outcomes would be that following generations of biologists, epidemiologists, laboratory scientists, and public health officials would be trained and prepared to respond to emerging disease threats.

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Table 1. Enzootic virus activity detected by 3 methods during 1998 and 1999.

		Coachella Valley			Kern County	
Year	Method	Number Tested	WEE+	SLE+	Number Tested	WEE+
1998	Mosquito pools	808	10	1	770	42
	Chicken flocks	10	26	0	9	27
	Wild birds	3,329	39	10	4,327	33
1999	Mosquito pools	470	0	0	149	0
	Chicken flocks	9	1*	2	9	0
	Wild birds	1,021	14	0	1,396	13

*Seroconversion occurred between Nov 1998 and Mar 1999

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*Schutz, S., T.W. Scott, B.F. Eldridge, K. Lorenzen and R. Swartzell. 2000. Flight range, dispersal and population dynamics of *Culex tarsalis* in the San Joaquin delta. J. Am. Mosq. Control Assoc. 17:Submitted.

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*Publication based on research presented in this paper.

Table 2. Percentage of total birds sampled and total infected with WEE of 6 key species collected in Coachella Valley and Kern County.

Species	Coachella Valley				Kern County			
	1996-98		1999		1997-98		1999	
	% total	% WEE	% total	% WEE	% total	% WEE	% total	% WEE
House sparrow	7.5	9.4	14.8	0.0	0.8	0.0	7.9	0.0
House finch	3.2	9.4	0.1	0.0	23.2	54.6	54.7	0.0
Gambel's quail	13.4	42.2	59.7	100.0	nc		nc	
California quail	nc		nc		6.6	18.2	16.9	100.0
Common ground dove	7.5	14.1	3.5	0.0	nc		nc	
mourning dove	4.4	1.6	8.0	0.0	1.4	4.6	1.9	0.0
other species	63.9	23.4	14.0	0.0	68.0	22.7	18.6	0.0
Total	10,945	64	1,396	13	8,021	66	1,021	14

Century. Centers for Disease Control and Prevention, US Dept. of Health and Human Services, Atlanta, GA.

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	Coachella Valley				Kern County			
	1996-98		1999		1997-98		1999	
Species	% total	% WEE	% total	% WEE	% total	% WEE	% total	% WEE
House sparrow	7.5	9.4	14.8	0.0	0.8	0.0	7.9	0.0
House finch	3.2	9.4	0.1	0.0	23.2	54.6	54.7	0.0
Gambel's quail	13.4	42.2	59.7	100.0	nc		nc	
California quail	nc		nc		6.6	18.2	16.9	100.0
Common ground dove	7.5	14.1	3.5	0.0	nc		nc	
mourning dove	4.4	1.6	8.0	0.0	1.4	4.6	1.9	0.0
other species	63.9	23.4	14.0	0.0	68.0	22.7	18.6	0.0
Total	10,945	64	1,396	13	8,021	66	1,021	14

Table 3. Antibody persistence between 1998 and 1999 in recaptured California and Gamble's quail.

	PRNT*		
	1998	1999	TIME(wks)**
California quail	160	20	28
	160	80	28
Gambel's quail	160	20	29
	320	0	21

Reciprocal titer of plaque reduction neutralization test

**Time between first positive bleed in 1998 and last recaptured in 1999.

Table 4. Reinfection experiment with house finches. V:V, infected during 1998 and then re-infected with same virus during 1999; V:S, infected during 1998 and then inoculated with saline during 1998; V and S, inoculated with virus or saline during 1998; n = sample sizes for each virus.

Time	TREATMENT			
25 wks PI	V:V	V:S	V	S
n	6	4	4	2
WEE				
EIA*	3.81	2.65	1.34	1.21
PRNT**	23	<20	<20	<20
Viremia***	0	0	>4.5(3)	0
SLE				
EIA*	2.73	2.33	1.06	1.2
PRNT**	<20	<20	<20	<20
Viremia***	2.9(3)	0	3.9(3)	0

*mean formula value = mean optical density of positive/negative wells.

**reciprocal of geometric mean titer

***$\log_{10}$ plague forming units

Surveillance for Mosquito-Borne Encephalitis Virus Activity and Human Disease in California, 1999

Stan Husted, Vicki L. Kramer, Michael S. Ascher, Christopher Rogers, William K. Reisen[1], Bruce F. Eldridge[2], Robert Chiles[1], and Donald A. Eliason[3]

Division of Communicable Disease Control
California Department of Health Services, 601 North 7th Street, M/S 486
P. O. Box 942732, Sacramento, CA 94234-7320

The California Mosquito-Borne Encephalitis Surveillance Program is a cooperative effort of the DHS Division of Communicable Disease Control, the University of California at Davis, the Mosquito and Vector Control Association of California, local mosquito and vector control agencies, local health departments, physicians, veterinarians, and other interested parties.

In 1999 the program included the following components:

1) Diagnostic testing of specimens from human patients exhibiting symptoms of viral meningitis or encephalitis.

2) Evaluating patients diagnosed with encephalitis by enrolling them in the California encephalitis project, which looks at demographics, exposure to arthropods, and includes a large number of laboratory tests to determine etiology.

3) Active surveillance for acute encephalitis and assessment of seroprevalence of exposure to WEE and SLE viruses in Imperial and Riverside counties.

4) Diagnostic testing of specimens from domestic animal species that exhibited clinical signs of neurologic disease compatible with SLE or WEE infection.

5) Monitoring and testing mosquitoes for St. Louis encephalitis (SLE) virus and western equine encephalomyelitis (WEE) virus infection.

6) Serological monitoring of sentinel chickens for SLE and WEE antibodies in areas of California where evidence of encephalitis virus activity has historically occurred.

Human Disease Surveillance

The Viral and Rickettsial Disease Laboratory (VRDL) tested 141 human serum and/or cerebrospinal fluid specimens from patients exhibiting symptoms of viral meningitis or encephalitis for antibodies to SLE and WEE viruses. Neither elevated IgM antibody nor a four-fold rise in total antibody between paired sera was observed in specimens from any of the suspect cases.

The DHS California Encephalitis Project enrolled 127 patients from June 1998 to August 1999. For each patient enrolled, a core battery of tests was conducted, including polymerase chain reaction, serology, and isolation for 15 agents. Testing for additional etiologic agents was pursued as clinical symptomatology and exposure history warranted; extensive testing for arboviruses was conducted for cases with known mosquito exposure. No cases of SLE or WEE were identified through the Encephalitis Project.

Active surveillance in Riverside and Imperial counties evaluated 14 suspect patients for WEE and SLE encephalitis; none was confirmed. Aliquots of serum from 729 patients, which were submitted to four different medical facilities for other purposes, were evaluated for presence of IgG antibodies to WEE or SLE to determine background seropositivity. Of the

1. Arbovirus Research Unit, Center for Vector-Borne Disease Research, University of California, Davis, CA 95616
2. Department of Entomology, University of California, Davis, CA 95616
3. Mosquito and Vector Control Association of California, Elk Grove, CA 95624

729, 116 (15.9%) had a SLE IgG enzyme linked immunoassay (EIA) index greater than one, and 11 (1.5%) had a WEE IgG EIA index greater than one. Even though there was a high level seropositivity to SLE, there were inherent problems with interpretation given cross reactivity with some other SLE, there were inherent problems with interpretation given cross reactivity with some other arboviruses.

Equine Surveillance

Serum and brain tissue specimens from one horse displaying neurological signs were submitted for arboviral testing at VRDL. Testing failed to detect antigen or antibody for WEE.

Mosquito Testing

Twenty-four local mosquito control agencies in California, and one agency each in New Mexico and Oregon, submitted a total of 145,992 mosquitoes (3,581 pools) for testing in 1999 (Tables 1a and 1b). Mosquito pools were also submitted by local vector control agencies in Washington and Oregon. Mosquitoes were tested for arboviruses at the Center for Vector-Borne Disease Research, University of California, Davis, by an in situ enzyme immunoassay using Vero cell culture. No pools were positive for WEE or SLE, but very few pools were collected in those areas, which had chicken seroconversions in 1999. WEE and SLE viruses detected in mosquitoes by the encephalitis virus surveillance program over the past ten years are summarized in Figure 1.

Chicken Serosurveillance

Sentinel chicken flocks were deployed and local agencies initiated mosquito collection in April 1999. Data from these sources were forwarded to DHS and collated weekly (May 13 to October 28) in the Adult Mosquito Occurrence Summary Report (AMOR) and arbovirus bulletins, which were distributed to all surveillance program participants. Positive serologies were communicated immediately by telephone to submitting agencies.

In 1999, 47 local mosquito and vector control agencies submitted sera from a total of 190 sentinel chicken flocks. Fourteen of these flocks were part of arbovirus research projects conducted by the Arbovirus Research in Riverside and Imperial counties. Blood specimens were collected and tested biweekly from each flock. Sera from over 19,978 chickens from California, Nevada, Oregon, and Utah were tested for antibody to WEE and SLE.

A total of 25 seroconversion to SLE were recorded among sentinel chickens in Imperial (14),

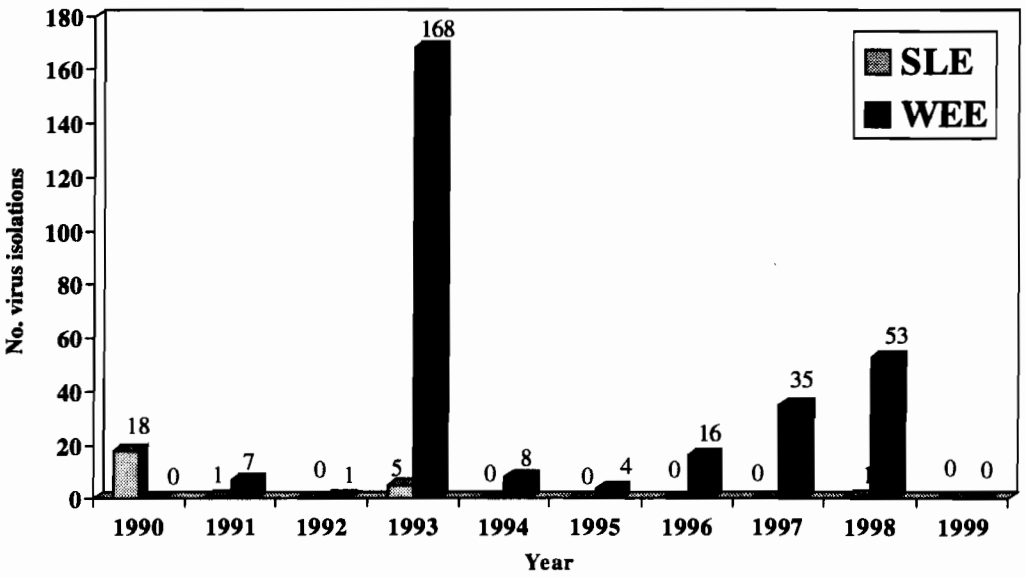

Figure 1. Isolations of St. Louis encephalitis (SLE) and Western equine encephalomyelitis (WEE) viruses from pooled *Culex tarslis* in California, 1990-1999.

Riverside (8), and San Bernardino (3) counties (Figure 2). The first SLE seroconversion was from a chicken bled on July 20 in Imperial County. The last seroconversions for 1999 were in Imperial County on November 8 and San Bernardino County on November 16. Table 2 lists the seroconversions to SLE by location and date bled.

A total of three seroconversions to WEE were recorded among sentinel chickens (Figure 2). The first seroconversions to WEE were detected in flocks from Riverside and Tulare Counties on July 28. The only other WEE seroconversion was another chicken on September 8 in the same Riverside County flock. Table 3 lists the serconversions to WEE by location and date bled.

Evidence of WEE activity in chicken flocks was considerably reduced in 1999 compared to previous years (Figure 3). In 1999, only two chickens from two flocks seroconverted to WEE compared to 101 chickens from 28 flocks in 1998. In contrast, 25 chickens from six flocks seroconverted to SLE in 1999, compared to only two chickens from two flocks in 1998. Figure 3 shows the SLE seroconversions for 1990-1999.

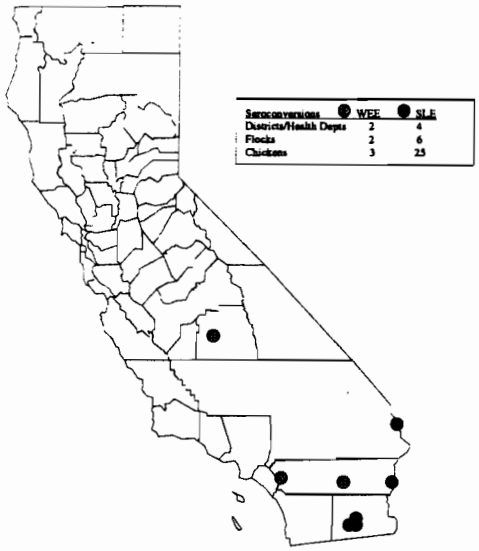

Figure 2. Sentinel chicken flocks with at least one serconversion to St. Louis encephalitis (SLE) or western equine encephalomyelitis (WEE) virus, California 1999.

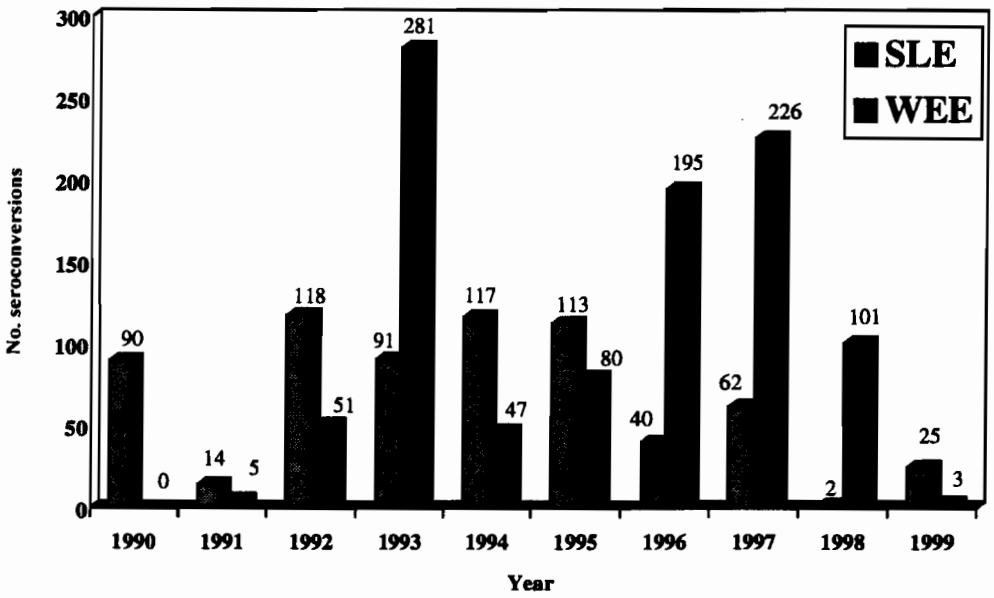

Figure 3. Seroconversions to St. Louis Encephalitis (SLE) and Western equine encephalomyelitis (WEE) viruses in sentinel chicken flocks in California, 1990-99.

Table 1a. Mosquitoes (*Culex* spp. and *Aedes melaninon*) tested for WEE and SLE viruses by submitting county, 1999.

		Ae. melanimon		*Cx. pipiens*		*Cx. quinquefasciatus*		*Cx. stigmatosoma*		*Cx. tarsalis*		Total	
County	Agency	Pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.
Contra Costa	CNTR	9	414							261	12931	270	**13,345**
Fresno	FRNO									15	721	15	**721**
Glenn	GLEN	15	750							44	2200	59	**2,950**
Kern	KERN	22	789							189	5596	211	**6,385**
Kings	KNGS									16	786	16	**786**
Lake	LAKE	44	2125					2	35	171	7573	217	**9,733**
Los Angeles	GRLA					277	12092	44	1259	108	4428	429	**17,779**
Los Angeles	LACW									103	4908	103	**4,908**
Los Angeles	LONG					64	2679			59	2127	123	**4,806**
Los Angeles	SGVA					2	47					2	**47**
Madera	MADR			3	150					8	400	11	**550**
Orange	ORCO					59	2074			5	157	64	**2,231**
Placer	PLCR									10	443	10	**443**
Riverside	COAV									373	18548	373	**18,548**
Riverside	NWST					244	7278	43	426	258	5608	545	**13,312**
Sacramento	SAYO	53	1142							354	15717	407	**16,859**
San Bernardino	SANB					32	1194	13	329	66	2768	111	**4,291**
San Joaquin	SJCM									90	4452	90	**4,452**
Santa Barbara	GLVY					8	244	2	28	9	300	19	**572**
Shasta	SHAS			2	102					13	578	15	**680**
Stanislaus	TRLK									62	2831	62	**2,831**
Sutter	SUYA									208	9740	208	**940**
Tulare	DLTA									11	252	11	252
Ventura	VENT									30	1430	30	**1,430**
Yolo	SAYO	4	175							137	6604	141	**6,779**
Yuba	SUYA									24	1081	24	**1,081**
Total		**147**	**5,395**	**5**	**252**	**686**	**25,608**	**104**	**2,077**	**2,624**	**112,179**	**3,566**	**145,511**

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

Table 1b. Mosquitoes (Other *Aedes* spp. and *Anopheles* spp. tested for WEE and SLE viruses by submitting agencies, 1999.

		Ae taeniorhynchus		*Ae washinoi*		*An franciscanus*		*An hermsi*		*An punctipennis*		**Total**	
County	Agency	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pool	mosqs.	pools	mosqs.
Santa Barbara	GLVY	6	236	1	19	2	38	5	166			14	**459**
Shasta	SHAS									1	22	1	**22**
Total		**6**	**236**	**1**	**19**	**2**	**38**	**5**	**166**	**1**	**22**	**15**	**481**

Table 2. Chicken seroconversions to SLE by location and date bled, 1999

County	Location	City	7/20	8/10	9/7-9	9/23	10/4	10/18-21	11/4-8	11/16	Total
Imperial	Nichols	El Centro	1	0	1	0	1	0	0	*	3
Imperial	Campbell	Seeley	0	3	3	0	1	0	1	*	8
Riverside	4th Avenue	Blythe	0	0	1	3	0	1	1	*	6
San Bernardino	Treatment Plant	Needles	0	0	1	0	0	2	0	*	3
Imperial	Cady	Brawley	0	0	0	0	3	0	0	*	3
Riverside	Adohr	Mecca	0	0	0	0	0	0	*	2	2
SLE Totals			**1**	**3**	**6**	**3**	**5**	**3**	**2**	**2**	**25**

*No chicken sera samples were taken this week

Table 3. Chicken seroconversions to WEE by location and date bled, 1999

County	Location	City	7/28	9/8	Total
Riverside	S. J. Wildlife Area	Moreno Valley	1	1	2
Tulare	Tulare	Tulare	1	0	1
WEE Totals			**2**	**1**	**3**

Evalution of Mosquito and Arbovirus Activity in Orange County During 1999

Robert Cummings, Carrie Fogarty, Stephen Bennett, Ralph Havickhorst, Lisa Opie, Keith Bow, Scott Phemister, 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 surveillance of mosquito and arbovirus activity throughout 1999 by collecting blood samples from wild birds and sentinel chickens, as well as collecting adult mosquitoes from ovipositional and CDC/CO_2 - baited traps. There were no positive mosquito pools, sentinel chickens or human cases in Orange County during 1999. Additionally, few SLE-positive wild birds were found in 1999. Only 8 (0.23%) of 3,528 sampled House Finches and 1 (0.14%) of 728 House Sparrow sera tested positive for SLE antibodies. *Culex quinquefasciatus* was the most commonly trapped mosquito throughout Orange County, except for a freshwater wetland area of Irvine, where *Cx. tarsalis* was predominant.

MOSQUITO SURVEILLANCE

The Orange County Vector Control District (OCVCD) continued its adult mosquito surveillance program throughout 1999 by collecting mosquitoes from a variety of residential and suburban wetland trapping sites. Mosquito collections were made at ten permanent sites in the county, using six gravid female ovipositional traps (Cummings 1992) and nineteen CDC/CO_2 - baited traps (Sudia and Chamberlain 1962). In addition, blood-fed female mosquitoes were collected from one modified Australian crow trap (McClure 1984) used to capture wild birds.

Suburban mosquito collections for all species (primarily *Culex quinquefasciatus* Say and *Culiseta incidens* (Thompson)) using CDC/CO_2 - baited traps in 1999 showed a decrease when compared to 1998 data, with the highest numbers peaking later and at lower levels in 1999 than the previous year (Fig. 1). In contrast to the suburban data, mosquito collections with CO_2-baited traps from a wetland habitat (San Joaquin Marsh, Fig. 2) consisted mostly of *Culex erythrothorax* Dyar and *Culex tarsalis* Coquillett. Numbers from this site showed an early season rise in 1999 compared to 1998, with the peak occurring in June, two months earlier than in 1998. In both years, *Culex erythrothorax* made up the majority of these collections from the marsh. However, counts of *Cx. tarsalis* from this same site were lower and peaked earlier in 1999 than in 1998 (Fig. 3), opposite to the trend seen for all other species from either the suburban locations or the San Joaquin marsh.

Gravid *Cx. quinquefasciatus* were collected in highest numbers from a suburban trap site in Garden Grove (Fig. 4). The highest collection, 205 gravid female mosquitoes, occurred in mid July (29th week of 1999) at this location.

A total of 2,990 mosquitoes in 102 pools were sent to the University of California-Davis, Center for Vector-borne Disease Research (UCD-CVDR), for testing (Table 1). Mosquito pools were obtained exclusively from ovipositional traps, and of these, *Cx. quinquefasciatus* composed all of the samples. Collections from the CO_2-baited traps were used primarily to monitor mosquito density and the effectiveness of the District's control program. No mosquito submissions were made from these collections. Of the pools from the gravid traps, none tested positive for either St. Louis encephalitis (SLE) virus or western equine encephalomyelitis (WEE) virus.

Orange County received an average total of only 7.2 inches of rainfall during the 1998-99 season (Annon., NOAA 1999), less than one-fourth of the 1997-98 season total (29.5 inches, Annon., NOAA 1998). As in most years, mosquito counts rarely correlate with rainfall. Most breeding sources in Orange County are man-made and need some sort of water supply from urban irrigation runoff during the dry summer months to support mosquito production. Year-to-year variations in numbers at the same sites are probably due to reasons other than the amount of rainfall.

ARBOVIRUS SEROSURVEILLANCE

Arbovirus serosurveillance consisted of biweekly testing of one flock of ten sentinel chickens and weekly

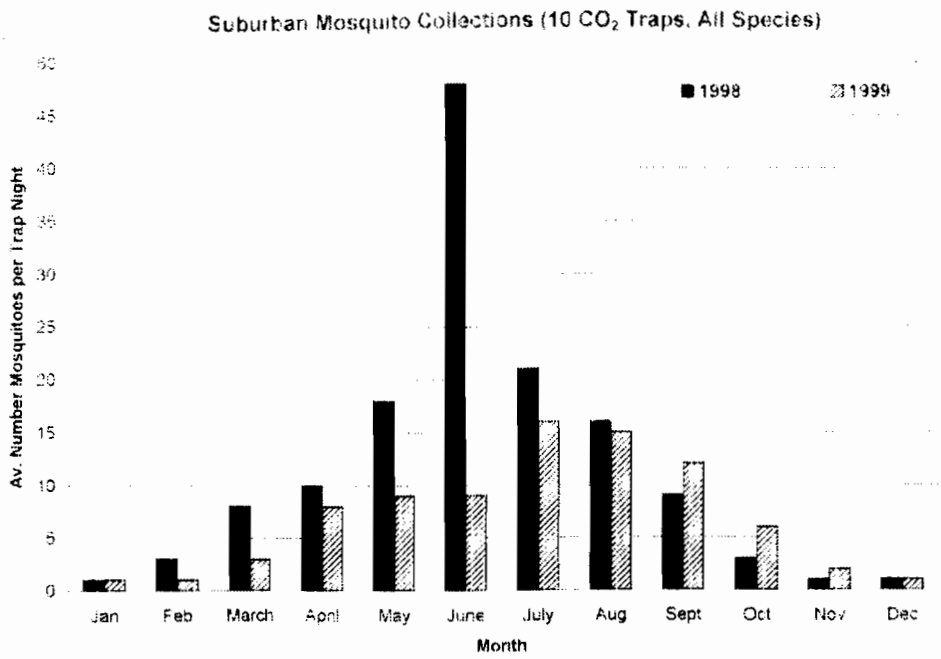

Figure 1. Host-seeking mosquito activity (all species, primarily *Cx. quinquefasciatus* and *Cu. incidens*) at 10 suburban mosquito collecting sites, Orange County, Calif. for 1998 and 1999.

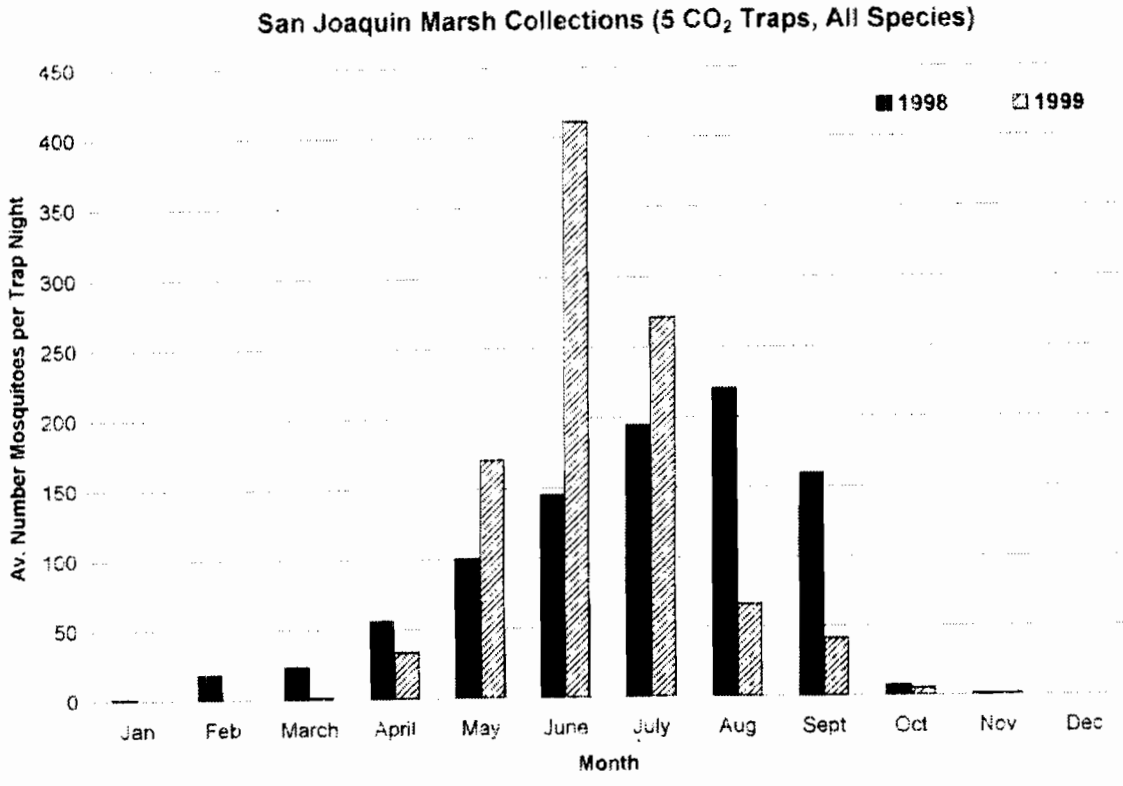

Figure 2. Host-seeking mosquito activity (all species, primarily *Cx. erythrothorax* and *Cx. tarsalis*) at the San Joaquin Marsh, Irvine, Calif. for 1998 and 1999.

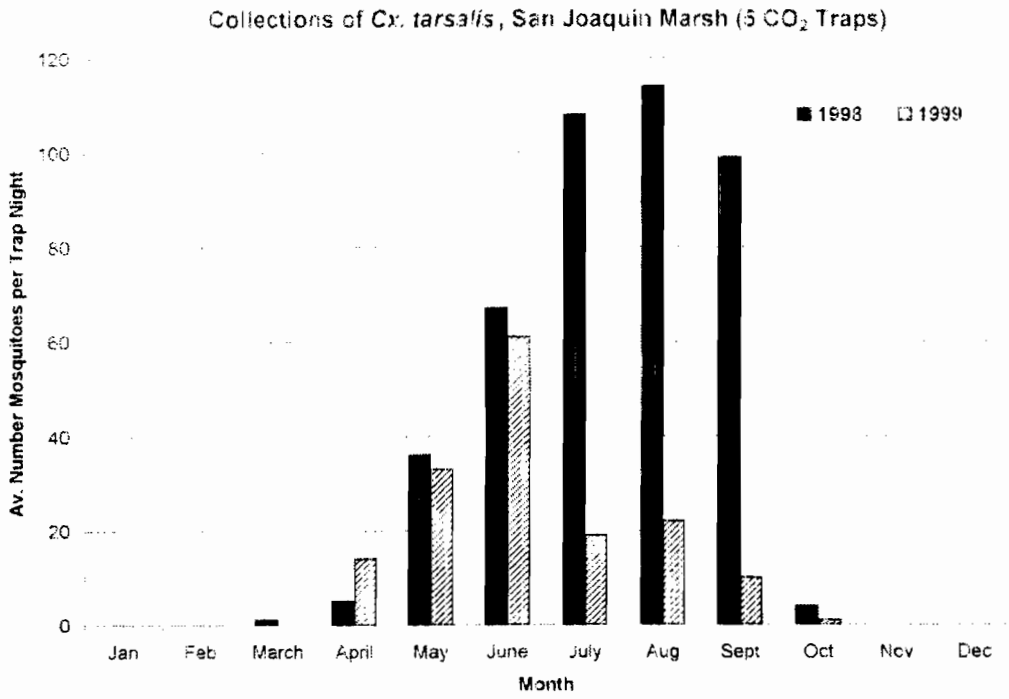

Figure 3. Host-seeking *Culex tarsalis* activity at the San Joaquin Marsh, Irvine, Calif. during 1998 and 1999.

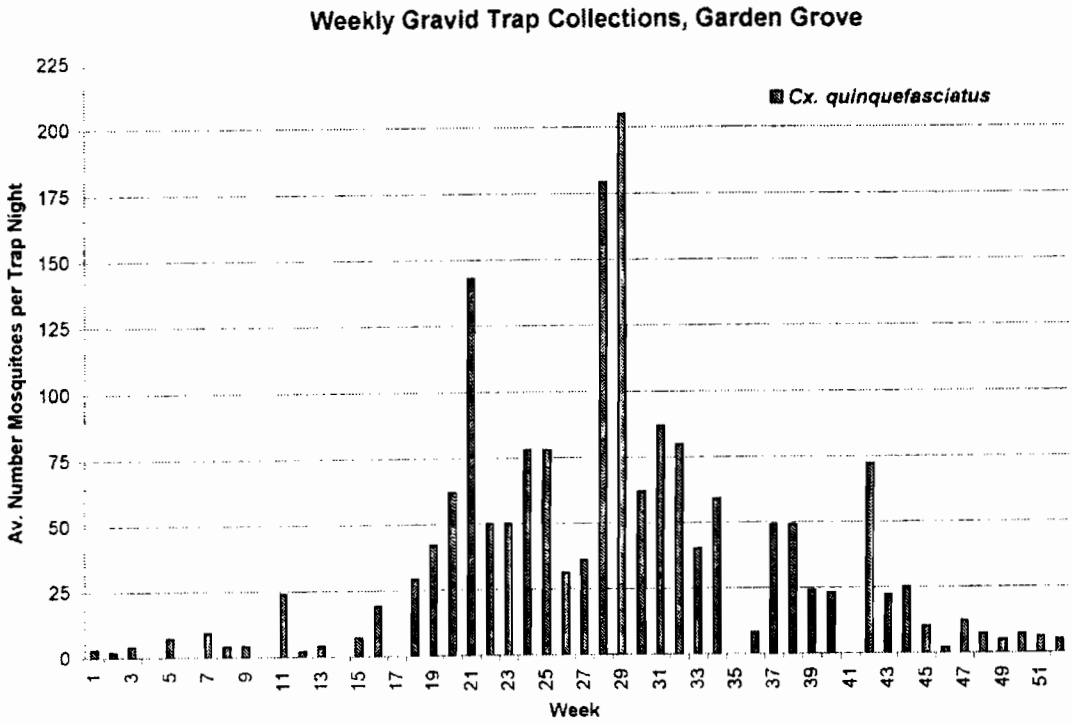

Figure 4. Weekly counts of gravid mosquitoes collected from ovipositional traps, OCVCD, Garden Grove, Calif. during 1998.

Table 1. Number of mosquitoes and mosquito pools submitted for SLE and WEE virus testing by species and trap type from Orange County during 1999.

Species	No. of Mosquitoes	Gravid Trap Pools	Stable Trap Pools	CO_2 Trap Pools	Total Pools
Culex quinquefasciatus	2,990	102	0	0	102
Culex tarsalis	0	0	0	0	0
Culex stigmatosoma	0	0	0	0	0
Totals	2,990	102	0	0	102

testing of wild birds, mostly House Finches (*Carpodacus mexicanus*) and House Sparrows (*Passer domesticus*) captured in ten modified Australian crow traps dispersed throughout Orange County. Sentinel chickens were tested for SLE and WEE antibodies by the CDHS/VRDL laboratory from April to October and the OCVCD laboratory every two weeks throughout the year. None of the sentinel chickens in Orange County or the Los Angeles basin tested positive for SLE or WEE antibodies during 1999.

A total of 4,308 wild bird blood samples was tested by hemagglutination inhibition (HAI) assay (Gruwell et al. 1988) at the OCVCD laboratory, resulting in 9 positive birds, eight of which were house finches (Table 2). The highest percent of positive birds occurred in June (0.5%) and October (1.3%) (Fig. 5). This was the only evidence of arbovirus activity in the coastal southern Californian region during the year.

Overall, 1999 was a year marked by low arboviral activity, as in 1998. Less than 1% of wild birds tested seropositive for SLE, and there were no birds positive for WEE.

Table 2. Small bird seroconversions for SLE and WEE antibodies in Orange County during 1999.

Species	No. Blood Samples	SLE Positive	WEE Positive	% SLE	% WEE
House Finch	3,528	8	0	0.23	0
House Sparrow	728	1	0	0.14	0
White-crowned Sparrow	15	0	0	0	0
Song Sparrow	37	0	0	0	0
Totals	4,308	9	0	0.21	0

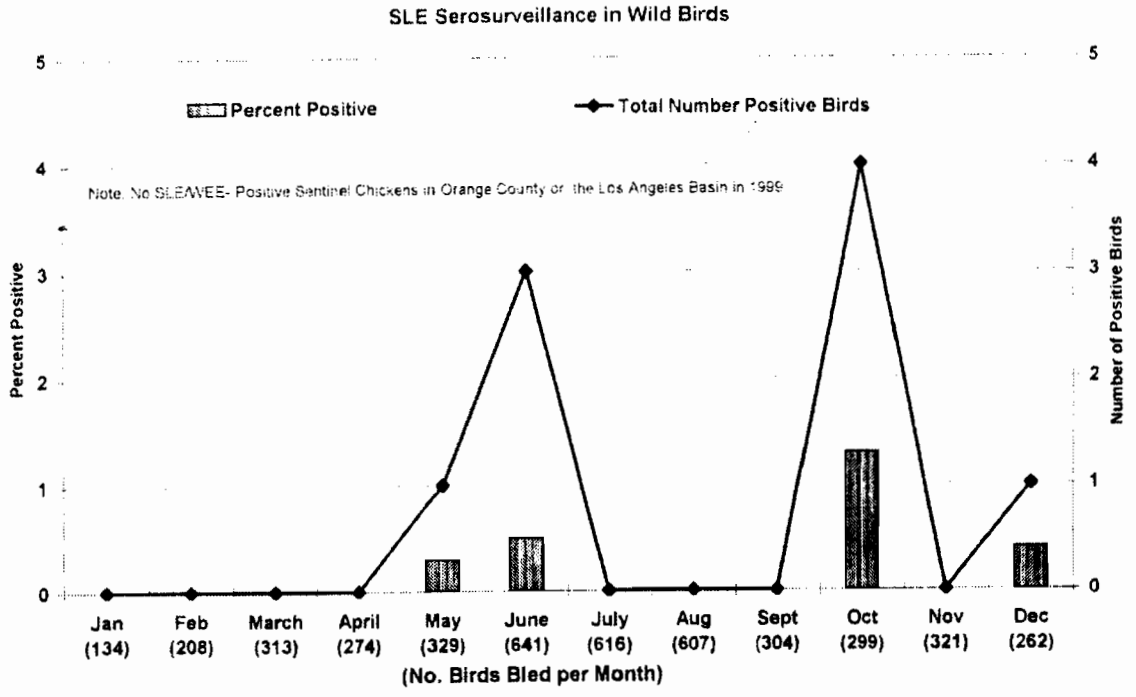

Figure 5. Arbovirus activity and seroprevalence in wild birds (House Finches and House Sparrows) from Orange County, Calif. during 1999.

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Arenavirus Antibody in Rodents from Southern California

Stephen G. Bennett,[1] Mary Louise Milazzo,[2] Charles F. Fulhorst,[2] and James P. Webb, Jr.[1]

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

ABSTRACT: The purpose of this study was to extend our knowledge of the geographic and natural rodent host ranges of New World arenaviruses in California. Sera from 1,573 rodents were tested for antibody against Whitewater Arroyo and Amapari viruses. Antibody was found in 71 (4.5%) of the 1,573 rodents: 4 from northwestern San Diego County, 3 from Los Angeles County, and 64 from Orange County. The antibody-positive rodents included 10 (7.1%) of 141 *Neotoma fuscipes*, 10 (3.3%) of 300 *Neotoma lepida*, 2 (3.5%) of 57 *Peromyscus boylii*, 12 (13.0%) of 92 *Peromyscus californicus*, 1 (0.63%) of 159 *Peromyscus eremicus*, 30 (8.4%) of 358 *Peromyscus maniculatus*, and 6 (2.2%) of 273 *Reithrodontomys megalotis*. Additional records of positive rodents were obtained from Environmental Health and Vector Control agencies in Los Angeles, Riverside, San Bernardino and San Diego Counties. This study provides the first evidence that New World arenaviruses occur in Los Angeles, Riverside and Orange Counties and northwestern San Diego County, and the first evidence that *Peromyscus* and *Reithrodontomys* species are naturally infected with New World arenaviruses.

INTRODUCTION

Small mammals (primarily rodents) appear to be the principal hosts of viruses belonging to the family Arenaviridae. The arenaviruses in North America include Tamiami (TAM) in southern Florida (Calisher et al.1970, Jennings et al. 1970), Whitewater Arroyo (WWA) in northwestern New Mexico (Fulhorst et al. 1996), and lymphocytic choriomeningitis (LCM) (Childs and Peters 1993), an Old World virus that probably was introduced into the Americas in recent times. *Sigmodon hispidus* (cotton rat) is the principal host of TAM virus, *Neotoma albigula* (the white-throated woodrat) is a natural host of WWA virus, and *Mus musculus* (house mouse) is the principal host of LCM virus.

The family Arenaviridae comprises two serocomplexes: the Old World (or LCM-Lassa) complex and the New World (or Tacaribe) complex (Buchmeier et al. 1995). Using the enzyme-linked immunosorbent assay (ELISA), the New World serocomplex viruses can be divided into two antigenic clusters (Childs and Peters 1993). The cluster "A"includes TAM and WWA viruses, and six South American viruses [Pichinde (PIC), Flexal, Pirital, Parana, Latino and Oliveros]; cluster "B" includes Amapari (AMA) virus and five other South American viruses (Tacaribe, Junin, Machupo, Guanarito and Sabia).

A recent study (Kosoy et al. 1996) provided the first evidence that rodents indigenous to California are naturally infected with New World arenaviruses. In that study, antibody against TAM and PIC viruses was found in 8 of 28 *Neotoma fuscipes* (the dusky-footed woodrat) collected from Ventura County, and 9 of 57 *N. fuscipes* and 1 of 37 *Neotoma lepida* (the desert woodrat) from southern San Diego County. All other California rodents examined were antibody-negative. Bennett et al. (in press) presented the first evidence of New World arenaviruses in Orange, Los Angeles and northwestern San Diego Counties and in rodent species other than *Neotoma* spp., including *Peromyscus maniculatus* and *Reithrodontomys megalotis*. This paper is a condensed version of Bennett et al. (2000)[1], updates the number of tested sera and further extends the geographic and natural host ranges of New World arenaviruses in California. This was accomplished by testing sera from wild-caught rodents for IgG against WWA or AMA virus, using an ELISA. It was expected that the use of the two antigens, 1 from each antigenic

[1] Orange County Vector Control District, Garden Grove, CA 92843
[2] Center for Tropical Diseases and Department of Pathology, University of Texas Medical Branch, Galveston, TX 77555-0609

cluster in the New World serocomplex, would provide a sensitive assay for antibody against a wide range of New World arenaviruses. Additional antibody-positive rodent data were obtained from vector control and environmental health agencies in Los Angeles, Riverside, San Bernardino and San Diego Counties. A summary distribution map of antibody-positive rodents is presented in Figure 1.

MATERIALS AND METHODS

Study area and collection of sera. A total of 1,573 sera was tested from rodents representing eight genera and thirteen species. Specimens were collected from 1988 through December 1999 at 189 sites in Orange County, 9 sites in northwestern San Diego County, and 8 sites on Santa Catalina Island, Los Angeles County, for a total of 206 collecting sites. Rodents were trapped in Sherman live traps (7.6 x 8.9 x 22.9 cm), baited with dry oats and placed during the early evening at sites considered to be suitable habitat. The traps were picked up early the next morning, placed inside plastic biohazard material bags and transported to a processing area. Handling and processing the rodents were done out-of-doors in the sunlight. The rodents were usually processed by a two-person team wearing currently approved safety gear. The rodents were placed individually (while still in the trap) into an ice chest containing dry ice until they were euthanized, then transferred onto a plastic tray for identification. Mice were bled infrasternally by cardiac puncture with 1/2-inch, 25 or 26 gauge needles and 1 cc tuberculin syringes. Rats were bled with 1- inch, 21 gauge needles and 3 cc syringes. Blood was ejected into a 25 ml plastic tube. The rodent was double-bagged in zip-lock freezer bags, each bag marked with the appropriate field number. The bagged rodent was immediately put into a dry ice container for temporary storage and later transferred to an ultracold freezer (-70 °C). The whole blood samples were stored in a refrigerator (4 °C) for no more than one hour before they were centrifuged, the sera transferred to 0.5 ml disposable microcentrifuge tubes and stored in a freezer at -20 °C prior to shipment. Three hundred and ninety-five of the 1,573 rodents were provided by LSA Associates, Inc. (Irvine, CA).

Assay for antibody. The sera were tested for antibody against WWA and AMA viruses using an ELISA described previously (Childs et al.1994). The antigens were sonicated, detergent extracts of Vero E6 cell monolayers. The test antigens were prepared from Vero E6 cell monolayers infected with AMA virus strain BeAn 70563 or the WWA virus prototype strain AV 9310135.3. The control (comparison) antigens were prepared from uninfected Vero E6 cell monolayers in a manner that was quantitatively identical to that used to prepare the test antigens. The working dilution of each test antigen was determined by box-titration against a homologous hyperimmune mouse ascitic fluid. The test and control antigens were diluted in 0.01 M phosphate buffered saline, pH 7.40, and coated onto U-bottom wells in 96-well polyvinyl chloride assay. Serial four-fold dilutions (from 1:80 through 1:5,120) of each serum were tested against the test antigens and corresponding control antigens. Bound antibody was detected by using a mixture of goat anti-rat IgG peroxidase conjugate and goat anti-*Peromyscus leucopus* IgG peroxidase conjugate in conjunction with the ABTS Microwell Peroxidase Substrate System. Optical densities (OD) at 410 nm (reference = 490 nm) were measured with a Dynatech MR 5000 microplate. The adjusted OD (ODadjusted) of a serum- antigen reaction was the optical density of the well coated with the test antigen less the OD of the corresponding well coated with the control antigen. A serum was considered to be positive to a test antigen if the ODadjusted at 1:80 and the ODadjusted at 1:320 both were > 0.200, and the sum of the ODadjusted for the series of four-fold dilutions (from 1:80 through 1:5,120) was > 0.750.

To improve the specificity of the ELISA results, endpoint titers against the WWA and AMA virus antigens and an LCM virus antigen were determined on all sera that screened positive against the WWA or AMA virus antigen. In this situation, serial fourfold dilutions (from 1:80 through 1:1,310,720) of a serum were tested against each of the three test antigens; the endpoint titer of a serum against a test antigen was the highest serum dilution for which the ODadjusted was > 0.200; and the homologous virus (i.e., the virus that had stimulated the production of antibody) was assumed to be that which was associated with the highest titer by >8-fold, when compared to the endpoint titers against the two other test antigens.

RESULTS

Antibody (IgG) against WWA virus and/or AMA virus was found in 71 (4.5%) of 1,573 rodent sera from 35 (17%) of 206 collecting sites (Figure 2). Serum samples from 1988 through December 1999 were tested retrospectively. Antibody-positives were detected every year since June 1991. A summary for the three counties included in this study is listed in Table 1.

The antibody-positive animals included: 10 (7.1%) of 141 *Neotoma fuscipes,* 10 (3.3%) of 300 *Neotoma lepida,* 2 (3.5%) of 57 *Peromyscus boylii* (brush mouse), 12 (13.0%) of 92 *Peromyscus californicus* (California

mouse), 1 (0.63%) of 159 *Peromyscus eremicus* (cactus mouse), 30 (8.4%) of 358 *Peromyscus maniculatus* (deer mouse), 6 (2.2%) of 273 *Reithrodontomys megalotis* (harvest mouse).

Titers against the WWA virus antigen in the antibody-positive animals ranged from less than 1:320 through 1:1,310,720, and the titers against the AMA and LCM virus antigens ranged from 320 to 1,280. Based on comparisons of the endpoint titers against the WWA, AMA and LCM virus antigens, the highest titers in antibody-positive animals were against WWA virus. High titers against AMA virus were found in three animals; one *N. lepida* (1:320) and two *P. maniculatus* (1:320 and 1:1,280). All sera were negative for LCM antibody.

Two or more rodent species were collected from each of 30 of the 35 antibody-positive sites. Antibody was found in only one species at 29 trapping sites; two species at five sites; and three species (*N. fuscipes, P. boylii* and *P. californicus*) at one site (Holy Jim Canyon in the Santa Ana Mountains). Antibody was found in 5 of 22 (22.7%) and 4 of 6 (66.7%) *P. californicus* collected in June 1998 and November 1999, respectively, from a site 4 km east of Holy Jim Canyon; and 1 (33%) of 3 *P. californicus* collected in June 1998 from a site 5 km northwest of Holy Jim Canyon. At Holy Jim Canyon, antibody was found in 3 of 9 *N. fuscipes*, 1 of 6 *P. boylii*, 1 of 6 *P. californicus*, and none of 2 *N. lepida* and 1 *P. eremicus*, all collected in a 1-week period in June 1996; and 1 *N. fuscipes* collected in July 1997. The rodents collected in 1996 were trapped in riparian and chaparral habitats; the antibody-positive *N. fuscipes* collected in July 1997 was trapped inside a vacation cabin. At another site in the Santa Ana Mountains (Lucas Canyon), 9 of 79 (11.4%) *Neotoma lepida* were antibody-positive during March-April, May, July, November-December of 1999. Three of these wood rats were captured inside storage trailers and heavy construction vehicles.

Arenavirus antibody was also detected in multiple rodent species from several sites in western Riverside County (pers. commun., Hugh Murray, Riverside Co. Environmental Health) and Los Angeles County (pers. commun., Michael Rood, L.A. Co. Dept. Health Services).

DISCUSSION

In the only previous study on the natural rodent host relationships of New World arenaviruses in the southwestern United States (Kosoy et al. 1996), antibody reactive with TAM and/or PIC virus was found in 51 (8.9%) of 574 wood rats (*Neotoma* spp.) and none of 1,940 other sigmodontine rodents (including 370 *P. boylii*, 21 *P. eremicus*, 821 *P. maniculatus*, and 44 *R. megalotis*) collected from Arizona, Colorado, Utah, New Mexico or California. The antibody-positive rodents from California in that study were collected from Ventura County and southern San Diego County. The present study thus provides the first evidence that species of *Peromyscus* and *Reithrodontomys megalotis* are naturally infected with New World arenaviruses, and the first evidence that rodents indigenous to Los Angeles, Orange, Riverside, San Bernardino and northern San Diego Counties are naturally associated with New World arenaviruses.

The family Arenaviridae includes 19 (5 Old World and 14 New World) serotypes. The results of a recent analysis of genetic data suggest that the diversity of the arenaviruses is the product of long-term coevolution with their principal hosts (Bowen et al. 1997). Presently, the WWA virus is known only from two strains recovered from *N. albigula* collected from Whitewater Arroyo in northwestern New Mexico. Assuming that the association between WWA virus and *N. albigula* represents a long-term, shared evolutionary relationship, then *Neotoma* species in California may be infected with viruses phylogenetically closely related to WWA virus.

Specific rodents (usually one or two closely related species) are the principal hosts of the arenaviruses for which natural host relationships have been well characterized. In the present study, arenavirus antibody was found in two or more species at six of the 35 antibody-positive sites. The antibody in multiple, sympatric species could represent horizontal (spillover) virus transmission from the principal host(s) to other species. The antibody in sympatric species also could represent the coexistence of multiple arenaviruses.

The dusky-footed woodrat (*N. fuscipes*) and desert woodrat (*N. lepida*) occur in most of the 35 antibody-positive collecting sites included in the present study, thus the finding of antibody in *Peromyscus* species and *Reithrodontomys megalotis* from these localities could represent horizontal virus transmission from *Neotoma*. However, antibody-positive *P. maniculatus* and/or *R. megalotis* were collected from 11 sites in the absence of *N. fuscipes* and *N. lepida*. These sites are unsuitable for woodrats (e.g., sparsely vegetated hillsides, grassy fields, flood plains, and weed-choked suburban drainage ditches, oil fields). In addition, temporal maintenance of arenavirus in *P. maniculatus* and *R. megalotis* populations was noted from one such habitat in San Clemente (southern Orange County) from 1994 through 1998, indicating horizontal or vertical transmission of virus in the absence of the suspected

reservoir hosts.

Perhaps the best indicator that arenavirus infection in *Peromyscus* can occur independently of infection in *Neotoma* spp. is the finding of antibody in *P. maniculatus* collected from Santa Catalina Island. There is no fossil or other historical evidence for the occurrence of *Neotoma* spp. on the island (personal commun., Paul Collins, Santa Barbara Museum of Natural History), yet *P. maniculatus* has existed on Santa Catalina Island since the late Cenozoic (6,000-9,000 years before the present) or longer (Collins and George 1990, Vedder and Howell 1980, Wenner and Johnson 1980). Whether the arenavirus associated with *P. maniculatus* on Santa Catalina Island is the same as that associated with *Peromyscus, Reithrodontomys* and/or *Neotoma* spp. in Orange and San Diego Counties remains to be determined.

Some arenaviruses cause severe febrile disease in human beings. The deer mouse, *Peromyscus maniculatus*, is a common rodent in sage-scrub plant communities and frequently invades human habitations. In southern California, residential and commercial developments increasingly encroach on sage-scrub habitats, thus increasing the potential for human contact with infected deer mice. In addition, *P. californicus, P. boylii, N. fuscipes* and *N. lepida* have been trapped on many occasions in and around vacation cabins, heavy equipment, stables, schools and other structures in the Santa Ana Mountains. They have also been found in attics and storage facilities of commercial buildings in the foothill region of Orange County. The human health significance of the arenavirus (es) associated with *Peromyscus, Neotoma* and other rodent species in southern California and elsewhere in the southwestern United States has not yet been investigated.

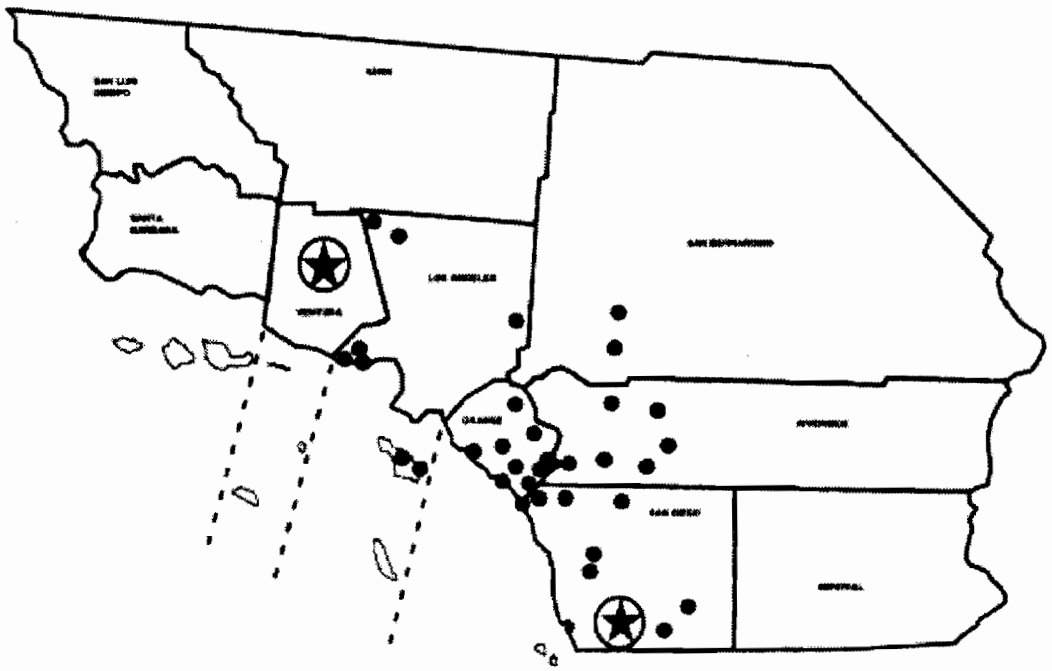

Figure 1. Map of southern California counties showing present (solid circles) and historical (stars) arenavirus antibody - positive localities. Historical records are from Kosoy et al. 1996.

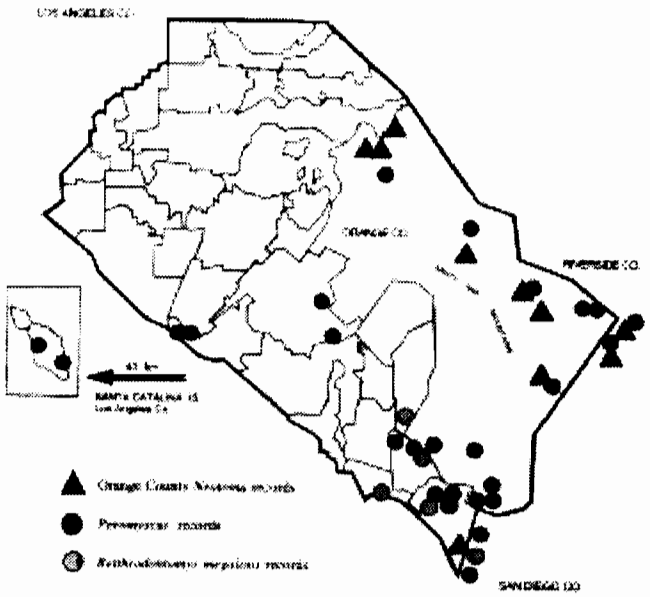

Figure 2. Distribution of arenavirus antibody-positive rodents in Orange County, California and adjacent areas.

Table 1. Prevalence of arenavirus antibody in rodents collected during 1988-1999, by county

Species	Los Angeles Co. (Sta. Catalina Is.)		Orange Co.		NW San Diego Co.		Total	
Chaetodipus californicus	-		0/14	(0.0%)	-		0/14	(0.0%)
Microtus californicus	-		0/13	(0.0%)	-		0/13	(0.0%)
Neotoma fuscipes	-		10/140	(7.1%)	0/1	(0.0%)	10/141	(7.1%)
Neotoma lepida	-		10/299	(3.3%)	0/1	(0.0%)	10/300	(3.3%)
Peromyscus boylii	-		2/57	(3.5%)	-		2/57	(3.5%)
Peromyscus californicus	-		12/82	(14.6%)	0/10	(0.0%)	12/92	(13.0%)
Peromyscus eremicus	-		1/124	(0.81%)	0/35	(0.0%)	1/159	(0.6%)
Peromyscus maniculatus	3/12	(25.0%)	24/283	(8.5%)	3/63	(4.8%)	30/358	(8.4%)
Reithrodontomys megalotis	0/5	(0.0%)	5/221	(2.3%)	1/47	(2.1%)	6/273	(2.2%)
Mus musculus	0/1	(0.0%)	0/73	(0.0%)	0/2	(0.0%)	0/76	(0.0%)
Rattus norvegicus	-		0/1	(0.0%)	-		0/1	(0.0%)
Rattus rattus	0/4	(0.0%)	0/31	(0.0%)	-		0/35	(0.0%)
Spermophilus beecheyi	-		0/54	(0.0%)	-		0/54	(0.0%)
Total	3/22	(13.6%)	64/1,392	(4.6%)	4/159	(2.5%)	71/1,573	(4.5%)

[1] Values are the number positive/number tested (% positive); "-" = none tested.

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Minoo B. Madon (Technical Director, Greater Los Angeles County Vector Control District) provided guidance and assisted in the collection of rodents; Michael Rood (Los Angeles County Dept. Health Services), Hugh Murray (Riverside County Dept. of Environmental Health), Jim Lang (San Diego County Dept. Health Services), and Lal Mian (California State University, San Bernardino) provided additional records of antibody-positive rodents; Richard Erickson and the staff at LSA Associates, Inc. (Irvine, CA) provided 395 rodents collected from Orange, San Bernardino, and San Diego Counties; Carrie L. Fogarty, Lisa C. Opie, and Keith L. Bow (Orange County Vector Control District) processed the blood (serum) samples; Misty Gay (Santa Catalina Island Conservancy), Mark Hoefs (Wrigley Botanical Garden, Avalon, CA), and the Catalina Island Marine Institute provided transportation and housing on Santa Catalina Island; Robert Sjogren (Manager, Orange County Vector Control District) provided administrative support. This research was supported in part by grant (C.F. Fulhorst) AI-41435 from the National Institutes of Health.

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History and Current Status of Plague Surveillance in San Mateo County, California

Chindi A. Peavey[1], Randy Blair[2], and Alexandra Porshnikoff[1]

[1]San Mateo County Mosquito Abatement District
1351 Rollins Rd. Burlingame, CA 94010
[2]San Mateo County Health Department

Plague, caused by the bacteria *Yersinia pestis* (Lehmann and Neumann) is a zoonotic infection carried in rodents and their fleas. There are 3 forms of the disease: bubonic, septicemic and pneumonic (Chin 2000). In bubonic plague, the lymph nodes draining the site of infection become swollen and painful forming a "bubo" (typically in the inguinal, axillary or cervical areas). If left untreated, the infection spreads to the circulatory system (septicemic plague) or to the lungs (pneumonic plague). Pneumonic plague can be spread directly between hosts by aerosol route. Urban plague, carried by commensal rats and their fleas, has caused epidemics in cities throughout the world since 542 AD (Pollitzer 1954). In the United States, enzootic plague is maintained among native mice and other rodents and periodically spills over into sciurids, which experience widespread die-offs or epizootics (Stark et al. 1966). Human cases in California are usually the result of exposure to infected fleas or sick animals during or immediately after such an epizootic (Nelson 1980).

The San Francisco Peninsula is noteworthy as the location of the first and largest outbreak of urban plague in the United States (Link 1955). San Bruno Mountain, located just south of San Francisco, is one of the most extensively surveyed foci of enzootic plague in the state. This paper will review the history of plague in San Mateo County, and present results of surveillance conducted by the San Mateo County Health Department (SMCHD) between 1975 and 1990.

The first recorded epidemic of plague in California began in the Chinatown District of San Francisco in 1900 (Meyer 1942, Link 1955). The outbreak continued for four years with 121 confirmed cases and 118 fatalities (Meyer 1942, Link 1955). Plague broke out again in San Francisco in 1907 with an additional 159 cases and 77 deaths (Meyer 1942). Unsanitary housing conditions following the 1906 earthquake and an explosion in the urban rat population were major contributing factors to the epidemic (Meyer 1942, Link 1955). Between 1903 and 1908, there were 19 human cases of plague on the east side of San Francisco Bay (Link 1955). At least 6 of these were associated with exposure to California ground squirrels *Spermophilus beecheyi* Cuvier (Meyer 1942). The importance of ground squirrels in plague ecology was demonstrated in 1908 when the bacteria was isolated from animals associated with human cases (McCoy 1908, Wherry 1908). These findings spurred an extensive campaign to test and eradicate ground squirrels throughout the state. Surveillance for plague in San Mateo County began in 1916 (Link 1955). By 1940, infected squirrels had been found at 8 locations in the county including sites in what are now the cities of Atherton, Menlo Park, Redwood City, San Mateo and Portola Valley (Anonymous 1955). In 1942, plague was isolated from fleas taken from ground squirrels on San Bruno Mountain. The presence of plague at this location was cause for concern because of its proximity to San Francisco. Public Health officials feared that the infection would spread to commensal rats resulting in another urban epidemic. Further surveillance focused on San Bruno Mountain and a number of studies were carried out to characterize the ecology of plague at this site (Miles et al. 1957, Kartman et al. 1958, Kartman et al. 1962, Hudson et al. 1964).

San Bruno Mountain is the northernmost extension of the Santa Clara Mountain Range. It is separated from the rest of the range by the San Bruno Fault (Hinds 1952). Elevations on the mountain vary from near sea level to 1,315 ft (Davis 1955). The cities of Colma, South San Francisco, Brisbane and Daly City lie around the base of the mountain. During the 1940-50s most of San Bruno Mountain was devoted to agriculture. Cattle grazed the slopes of the mountain and a series of hog ranches were situated around its base. Garbage from San Francisco was trucked in to feed the hogs and large populations of Norway rats, *Rattus norvegicus* Fischer, developed on these farms. Public Health officials conducted extensive rat and ground squirrel control on San Bruno Mountain to prevent plague from reaching the domestic rat population. The United States Public Health Service

(USPHS) was responsible for plague surveillance on San Bruno Mountain between 1953 and 1973. Their research helped identify indigenous mice (California meadow mice, *Microtus californicus* Peale and deer mice, *Peromyscus maniculatus* Baird) as reservoir hosts for the bacteria at this site (Kartman et al. 1962, Hudson et al. 1964, Hudson and Kartman 1967).

The San Francisco laboratory of the USPHS moved to Fort Collins, CO in 1973. The task of plague surveillance in San Mateo County was assumed by the County Health Department, their personnel conducting regular sampling from 1975 to 1990. The objective of these surveys was to monitor plague prevalence at known enzootic sites and investigate its distribution in the rest of the County.

MATERIALS AND METHODS

Trapping was conducted biweekly at various locations throughout the county. On each occasion, 25 Sherman live traps were set for 3 nights at each of 3 locations. Traps were set in the afternoon, baited with rolled oats, then checked the following morning. Captured animals were transported to the SMCHD laboratory for processing. At the laboratory, each animal was anesthetized, weighed, measured, and its reproductive status determined. Blood samples obtained by cardiac puncture were submitted to the California Department of Health Services (CDHS) which forwarded them to the Centers for Disease Control, Ft. Collins, Colorado for testing. The sera were tested for antibody against *Y. pestis* by a passive hemagglutination test (Hudson and Kartman 1967). Following guidelines of the Centers for Disease Control (CDC) an antibody titer of 1:16 or greater was considered evidence of infection.

RESULTS

Species captured

During the 16 years of this study, trapping was conducted at 252 sites and a total of 1,800 rodents were tested for plague. Two species, California meadow mice, *M. californicus*, and deer mice, *P. maniculatus* accounted for 90% of the animals tested (1083 and 537, respectively) (Figure 1). Other species captured included *Peromyscus californicus* (Gambel) (4% of captures, 76 animals) and *Peromyscus truei* (Schufeldt) (2% or 33 animals). The remaining 4% of captures consisted of 39 *Spermophilus beecheyi* Cuvier, 15 *Neotoma fuscipes* Baird, 11 *Rattus rattus* Fischer, and 6 *Reithrodontomys megalotis* Baird. Evidence of infection was detected only in *M. californicus* and *P. maniculatus*. The word "mice" in the remainder of this paper will refer only to these 2 species.

Plague prevalence

Evidence of infection with *Y. pestis* was detected in 3.5% of mice tested countywide (Table 1).

Figure 1. Proportion of animals of different species tested for plague in San Mateo County 1975-1990.

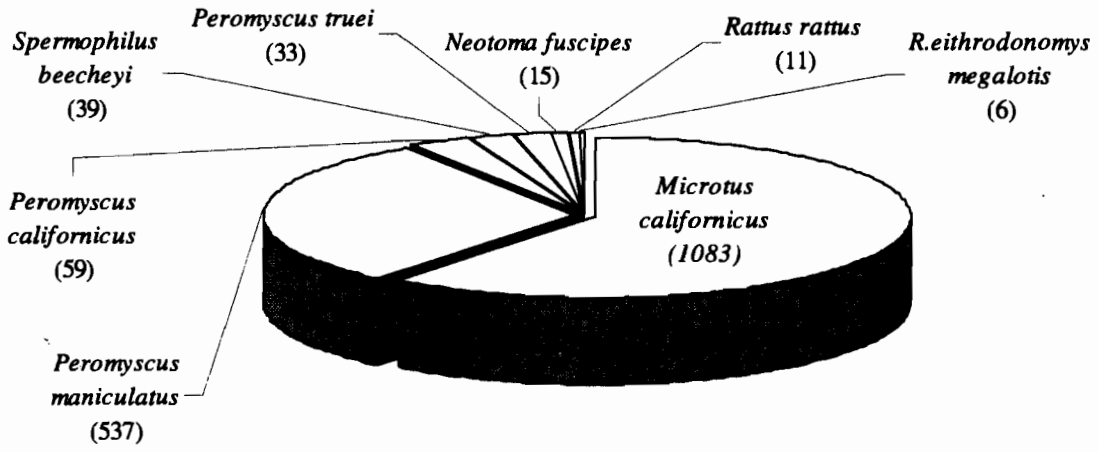


Table 1. Number of mice tested annually for plague (*Yersinia pestis*) in San Mateo County, 1975 - 1990.

Year	*Microtus californicus*			*Peromyscus manicu latus*			Total Mice	
	No. positive/No. tested	(%)	Titer [a]	No. positive/No. tested	(%)	Titer [a]	No. positive/No. tested	(%)
1975	1 / 8	(13)	16	1 / 12	(8)	32	2 / 20	(10)
1976	8 / 51	(16)	256	0 / 0			8 / 51	(16)
1977	0 / 84			0 / 136			0 / 220	
1978	0 / 178			3 / 136	(2)	128	3 / 314	(1)
1979	0 / 9			0 / 3			0 / 12	
1980	5 / 92	(5)	512	0 / 14			5 / 28	(5)
1981	0 / 16			0 / 12			0 / 28	
1982	16 / 131	(12)	412	5 / 24	(21)	194	21 / 155	(14)
1983	6 / 135	(4)	362	2 / 70	(3)	23	8 / 205	(4)
1984	5 / 75	(7)	223	0 / 29			5 / 104	(5)
1985	0 / 53			0 / 12			0 / 65	
1986	0 / 105			1 / 37	(2.7)	256	1 / 142	(1)
1987	2 / 28	(7.1)	16	0 / 17			2 / 45	(4)
1988	1 / 55	(1.8)	64	0 / 17			1 / 72	(1)
1989	0 / 31			0 / 11			0 / 42	
1990	0 / 26			0 / 7			0 / 33	
Totals	44 / 1083	(4.1)		12 / 537	(2.2)		56 / 1620	(3.5)

[a] Reciprocal geometric mean antibody titer

Prevalence at individual sites on particular dates ranged from 0-100% (Table 2). Small sample size precluded detailed comparisons of prevalence between sites or dates.

Positive sera were obtained from both *M. californicus* and *P. maniculatus*. The prevalence of infection in *M. californicus* (44 of 1083 tested countywide or 4.1%) was slightly higher than that in *P. maniculatus* (12 of 537 or 2.2%). However, this difference was not statistically significant ($X^2 = 3.595$, $v = 1$). Further, when one considers only mice from sites that yielded positive animals, the prevalence of infection in the 2 species is almost equal (44/141 = 31% for *M. californicus*, 12/41 = 29% for *P. maniculatus*) (Table 2).

Although the prevalence of plague did not differ between hosts, there were several occasions on which it was detected in only 1 host species at a particular site (Table 2). On 18 of the 28 occasions that plague was detected, positive titers were seen only in *M. californicus*. On 8 occasions it was detected only in *P. maniculatus*. *Peromyscus maniculatus* was the only animal found positive at Pescadero, Año Nuevo Reserve and Butano.

Temporal Distribution of Plague

An average of 101 animals were tested each year for evidence of infection (range 12-314) (Table 1). The number tested annually varied widely due to fluctuations in rodent population density and the number of personnel available to collect them. Plague activity was detected in 10 of the 16 years surveyed (Table 1). The lack of positive mice in some years may be a reflection of the number tested: less than 50 mice were tested in 4 of the 6 years with no positive animals. Conversely, positive serologies were seen in 8 of 10 years in which more than 50 mice were tested and in 6 of 7 years with over 100 tested

The prevalence of infection in mice collected countywide also varied widely from year to year (0 to 16 % for *M. californicus*, 0 to 21% in *P. maniculatus*)

Table 2. Sites in San Mateo County that yielded mice with positive serology to plague, 1975 - 1990.

Location	Date	*Microtus californicus* No. positive/ No. tested (%)		Titer[a]	*Peromyscus maniculatus* No. positive/ No. tested (%)		Titer[a]
1975							
Pescadero, Old Pescadero Dump	20-May	0 / 0			1 / 6	(17)	32
Half Moon Bay, Dunes Beach	19-Jun	1 / 8	(13)	16	0 / 0		
1976							
Pebble Beach	03-May	2 / 9	(22)	362	0 / 0		
San Bruno Mountain, Crocker Park	17-May	1 / 4	(25)	256	0 / 0		
San Bruno Mountain, NE Ridge	24-May	1 / 4	(25)	1024	0 / 0		
San Bruno Mountain, Crocker Park	07-Jun	4 / 5	(80)	152	0 / 0		
1978							
Pescadero, Old Pescadero Dump	20-Mar	0 / 3			1 / 5	(20)	512
San Bruno Mountain,Guadalupe Canyon Parkway	17-Apr	0 / 14			2 / 4	(50)	64
1980							
San Bruno Mountain, Saddleback	19-Nov	5 / 8	(63)	512	0 / 0		
1982							
San Bruno Mountain, Crocker Park	13-Jan	2 / 7	(29)	91	0 / 0		
San Bruno Mountain, Saddleback	13-Jan	2 / 4	(50)	512	0 / 0		
Montara, Hwy 1 & 2nd St.	28-Jan	0 / 1			1 / 1	(100)	64
Montara, George St.	24-Feb	0 / 0			1 / 3	(33)	64
Montara, George St.	12-May	1 / 4	(25)	32	2 / 5	(40)	128
Montara, Hwy 1 & 2nd St.	12-May	3 / 9	(33)	2580	0 / 0		
Montara, Martini Creek	12-May	1 / 4	(25)	512	0 / 0		
Montara, George St.	29-Dec	6 / 6	(100)	512	1 / 3	(33)	4096
Montara, Hwy 1 & 2nd St.	29-Dec	1 / 7	(14)	64	0 / 0		
1983							
San Bruno Mountain, Saddleback	12-Jan	2 / 6	(33)	724	0 / 0		
Montara, George St.	16-Feb	3 / 4	(75)	161	0 / 1		
Montara, Hwy 1 & 2nd St.	04-May	1 / 6	(17)	1024	0 / 0		
Año Nuevo State Reserve	17-Aug	0 / 7			1 / 4	(25)	16
Butano State Beach	17-Aug	0 / 2			1 / 6	(17)	32
1984							
San Bruno Mountain, Saddleback	28-Mar	5 / 7	(71)	223	0 / 0		
1986							
Año Nuevo State Reserve	11-Dec	0 / 4			1 / 2	(50)	256
1987							
Half Moon Bay, Miramontes Pt.	28-May	2 / 5	(40)	16	0 / 0		
1988							
San Bruno Mountain, Saddleback	08-Jan	1 / 3	(33)	64	0 / 1		
		44 / 141	(31)		12 / 41	(29)	

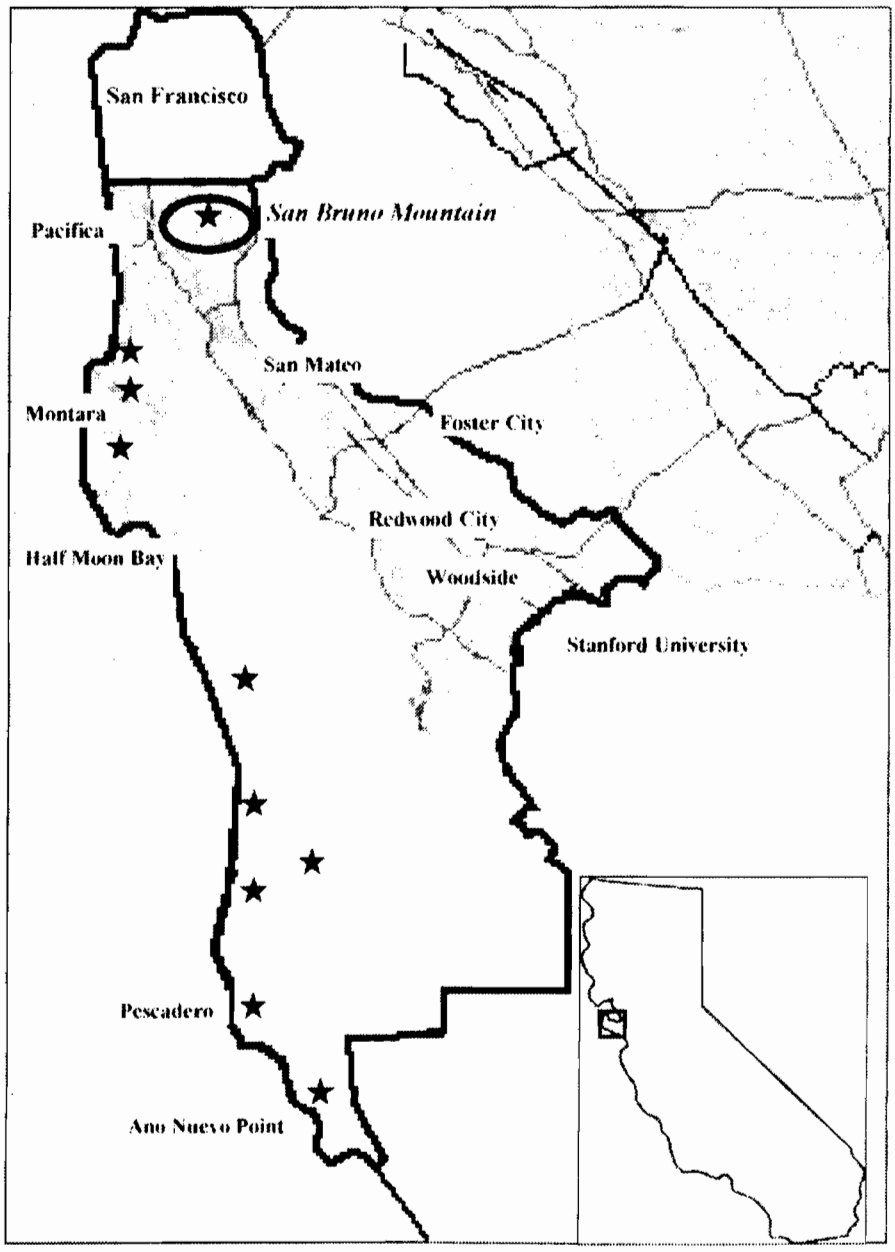
[a] Reciprocal geometric mean antibody titer

(Table 1). A high proportion of mice tested positive for plague in 1976 (16% or 8 of 51 tested), and 1982 (14% or 21 of 155 tested). A large number of mice tested positive in 1983, however, the proportion of mice with positive tests did not differ significantly from that of other years (4% or 8 of 205 tested). The prevalence of infection (20%) appeared high in 1975. However, the number of mice tested that year (n = 20) was low. The year 1977 was notable in that no positive results were obtained from the 220 mice tested. This number included 10 mice from San Bruno Mountain and 35 others from coastal sites that yielded positive animals in other years.

Spatial distribution of positive mice

Figure 2 shows the geographical distribution of serologically positive mice in the county between 1975 and 1990. Evidence of plague activity was detected in mice from San Bruno Mountain (29 mice from 4 sites), the coastal town of Montara (21 mice, 3 sites) and

Figure 2. Geographic distribution of mice with positive serology to *Yersinia pestis* in San Mateo County, California 1975-1990.



several other locations along the Pacific Coast (10 mice, 7 sites).

San Bruno Mountain was the most extensively sampled area in this study, accounting for nearly one third (29%) of the mice tested (472 animals). The mountain was surveyed in each of the 16 years between 1975 and 1990, and plague activity was detected in 7 years (Table 2). Of the 11 sites surveyed on San Bruno Mountain, infection was detected in 4 locations on the northern section.

Coastal sites accounted for an additional 58% of mice tested (939 animals). Trapping was conducted at 37 locations along the Pacific Coast between Pacifica and Año Nuevo Point. Montara, the northern-most location at which plague was detected on the coast, accounted for 8% of the mice tested (n = 132). Located between Pacifica and Half Moon Bay, Montara is about 10 miles south of San Bruno Mountain. Surveys were conducted annually in this area from 1982 to 1990. Evidence of infection was detected in mice collected in 1982 and 1983. Plague was detected in 3 locations north of town along the east side of Highway 1. The area encompasses a creek drainage containing riparian habitat, a seasonal marsh, chaparral, and open grassland habitats.

Trapping was also conducted near the towns of Half Moon Bay (65 mice tested), El Granada (31 mice), and Pescadero (138 mice) and in the vicinity of Dunes Beach (86 mice), Pebble Beach (103 mice), Butano (22 mice) and Año Nuevo State Reserve (41 mice). Information on dates that yielded positive mice is given in Table 3.

No evidence of plague was detected among rodents collected in the mountainous areas between Highway 280 and the Pacific coast or the rural areas of Woodside and Portola Valley in the southeast section of the county (Figure 2). Trapping was conducted in this area on several occasions between 1975 and 1990. However, the number of mice collected was low (194 mice, 11% of those tested countywide) in comparison to positive sites (Figure 3).

DISCUSSION

Plague has been monitored in wild rodents in San Mateo County since 1916, and was detected regularly

Figure 3. Proportion of mice tested for serological evidence of plague from different areas in San Mateo County, 1975 - 1990

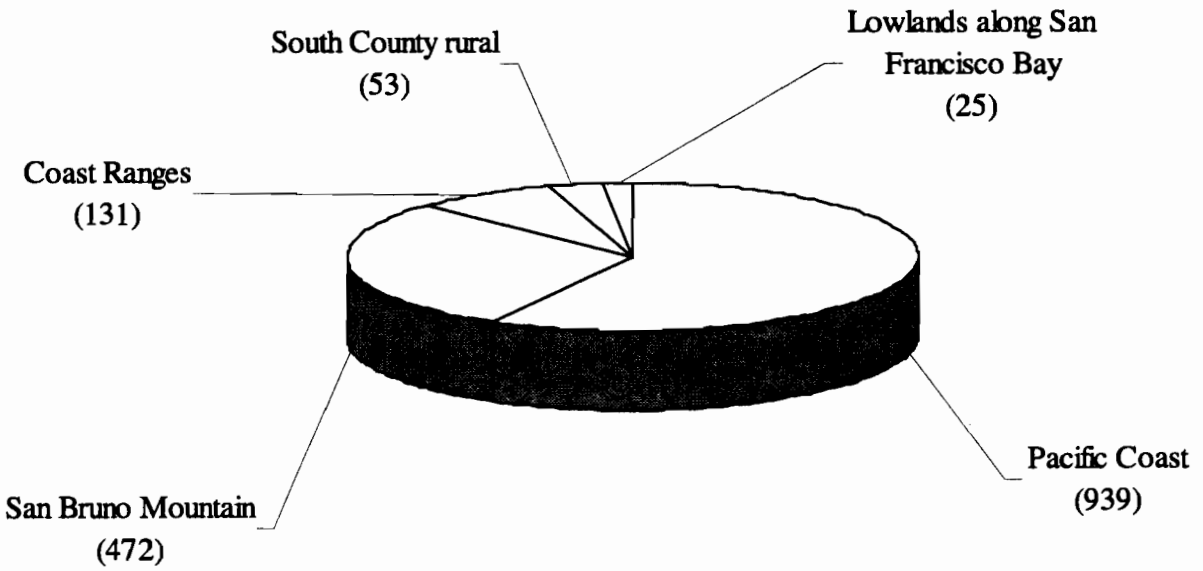


Table 3. Coastal sites in San Mateo County at which mice (*Peromyscus maniculatus* or *Microtus californicus*) seropositive for plague were collected, 1975-1990.

Location	No. times sampled	Years positive	No. positive[a] / No. tested[b]	Titer[c]
Dunes Beach	27	1975	1 / 86	16
Pebble Beach	16	1976	2 / 103	363 (64-2048)
Pescadero Dump	15	1975, 1978	2 / 96	128 (32-512)
Montara	30	1982, 1983	17/102	294 (32-8192)
Año Nuevo	4	1983, 1986	2 / 41	64 (64-64)
Butano	5	1983	1 / 22	32
Miramontes Pt.	2	1987	2 / 5	16 (16-16)

[a] Total number of mice tested between 1975 and 1990
[b] Total number of mice tested between 1975 and 1990
[c] Reciprocal geometric mean antibody titer (numbers in parentheses represent the range of positive titers)

during surveys conducted by the SMCHD between 1975 and 1990. Seropositive mice were collected in 10 of the 16 years surveyed. Failure to detect plague in some years during this period could be due to low sample size ($n < 50$) or may reflect the natural cyclical nature of the disease among the reservoir species sampled. The prevalence of infection among mice collected countywide varied between years with peak activity in 1975-76 and 1982-84. This paralleled the prevalence of infection seen in animals collected elsewhere in the state (CDHS records).

Evidence of infection was found in *P. maniculatus* and *M. californicus* and testing of both species is vital to the detection of plague in this area (Hudson and Kartman 1967, present study). Both species have been clearly shown to maintain plague in enzootic foci (Kartman et al. 1962, Quan and Kartman 1962). Although the prevalence of infection was similar in the 2 species, on several occasions plague was detected only in 1 of them. *Microtus californicus* and *P. maniculatus* differ somewhat in their ecology (Hooper 1944, Stark et al. 1966). *Peromyscus maniculatus* is a seedeater commonly found in chaparral. *Microtus californicus* frequents open meadows and habitats containing herbaceous vegetation. In addition, *M. californicus* populations tend to be much more cyclical than *P. maniculatus* and the number of animals collected at a particular site may fluctuate radically from year to year (Nelson 1980). Because of these ecological differences, it may not always be possible to collect sufficient numbers of *M. californicus* at a given site to thoroughly survey for presence of plague. Testing both species maximizes the possibility of detecting plague activity at a particular site.

The distribution of infected mice in the present study differs somewhat from that reported prior to 1950. In the surveys described here, plague was detected in rodents from 4 locations on the north side of San Bruno Mountain and a number of sites along the Pacific Coast. Earlier surveys reported positive animals collected from lowland areas along San Francisco Bay and on the eastern slopes of the Coastal mountains in addition to San Bruno Mountain (Anonymous 1955). This difference is probably due to historical changes in the distribution of rodent hosts and the locations at which trapping was conducted. Prior to 1940, surveys focused on rodents inhabiting ranches in low-lying areas adjacent to San Francisco Bay where high populations of California ground squirrels, were living in close association with humans. Development of this area since 1950 has drastically reduced habitat for meadow mice, deer mice and ground squirrels. In addition, decades of control programs aimed at ground squirrels had drastically decreased their numbers in lowland areas by 1960. During the present study, trapping concentrated on small towns along the Pacific Coast and on San Bruno Mountain because these were areas in which humans or commensal rodents might contact infected reservoir hosts. However, in 1968, a tree squirrel (*Sciurus niger*) was found dying from plague in the private housing areas immediately adjacent to the south side of Stanford University (Nelson 1980). This finding suggests that a focus of plague may still exist near the urbanized areas of Palo Alto and Menlo

Park.

Trapping was also conducted in the mountainous areas between Highway 280 and the coast between 1975 and 1990. However, the number of mice collected was low and no evidence of plague could be detected. Plague has been detected in these mountains in the past and further surveillance would be warranted. Plague was isolated in 1957 from mice and their fleas collected along San Andreas Lake in the Crystal Springs Watershed (Kartman et al. 1962). In 1942, plague-positive fleas were removed from California ground squirrels collected at the south end of the county near Skyline Blvd. (Anonymous 1955). Positive serological results have been obtained from wild carnivores collected in this area by the San Mateo County Agricultural Commissioner (for a description of California's carnivore serology program see Smith et al. 1984). Positive antibody titers were detected in a coyote collected 5 mi. southeast of San Gregorio and another from Tunitas Creek Rd. on the Western slopes of the Coast Ranges in 1978 (California Plague Reports 1978). In 1979, a seropositive coyote was collected in Woodside, on the eastern side of these mountains (California Plague Reports 1979). The distribution of plague in the mountainous areas of San Mateo County needs further investigation and will be the focus of future surveys.

Despite the continued presence of plague among wild rodents in San Mateo County, there have been very few human cases in this area since the San Francisco epidemics. The scarcity of human cases is probably due to the fact that they are rarely exposed to the disease even though it is present in the wild rodent population. There are 3 possible routes through which enzootic plague might reach humans in San Mateo County: 1) a buildup of ground squirrel populations near active plague foci 2) exposure of residents to plague-infected cats 3) transfer of infected fleas from wild mice to commensal rats resulting in another urban epidemic.

The California ground squirrel plays a key role plague epidemiology in California. Most human cases from sylvatic sources in California are associated with this rodent and its fleas (Nelson 1980, Nelson et al. 1986). The abundance of ground squirrels and the degree of human contact with them in San Mateo County has changed over time. Ground squirrels thrive in open grassland and disturbed landscapes. Prior to 1890, when most of the county was covered with chaparral and forest, ground squirrels were not numerous. In the late 1800s, large tracts of land were cleared for agriculture, creating ideal conditions for these animals. Ground squirrels were plentiful in San Mateo County in 1916-20,1929, 1936, and 1942 (Murray 1957). Awareness of the importance of ground squirrels in the exposure of humans to plague began in 1908, and vast resources were devoted to their eradication, including attempts to create a "squirrel-free zone" around the cities of the San Francisco Bay Area (Meyer 1942, Link 1955). Control efforts in San Mateo County began in 1915, focusing primarily on the ranches on San Bruno Mountain and in lowland areas along San Francisco Bay (Link 1955). By the 1940-50s ground squirrels were absent from most of the county due to suburban development and continuous rodent control. A survey of the county in 1981 noted that ground squirrels were restricted to the southern boundary of the county along San Francisquito Creek and on lands surrounding Stanford University. The current distribution of ground squirrels extends north from there along the shore of San Francisco Bay to Foster City. Although the SMCHD continues to monitor ground squirrel populations in this area, there is currently no program for their control. Control of the California ground squirrel in the county in earlier years is the most likely explanation for the absence of human cases.

San Bruno Mountain, in particular, possessed abundant ground squirrel habitat, but continued control has kept it largely free of ground squirrels since the 1940s. Most of the mountain remained open grassland through the 1950s and 60s due to the presence of livestock. The ranches had ceased operation by 1970 and vegetation has begun to revert to chaparral with riparian habitat in the canyons. During the 1980s housing was proposed for 1 of the sites that had consistently yielded plague positive mice (Northeast Ridge). To minimize the risk of exposure to plague, the developers were required control rodents in this area. This area has remained free of ground squirrels through the 1990s and contains very few mice due to the lack of dense vegetation. A 2nd long-term plague focus (Saddleback) was developed as a recreational site with picnic areas and a jogging trail in the late 1980s. Again, the development plans included rodent eradication prior to building and an agreement to allow continued surveillance of the rodent populations for evidence of plague. This area also remains free of ground squirrels, but contains significant populations of mice. Evidence of infection was detected in 1 *M. californicus* when the area was surveyed again in 1999. The nearest ground squirrels are found along dikes bordering San Francisco Bay in Foster City. This population has been expanding northward along the Bay shore and could eventually repopulate San Bruno Mountain if left unchecked.

In the absence of ground squirrels, cats remain

the most likely route of exposure to plague for residents of San Mateo County. Cats living near active plague foci can pick up the infection from wild rodent hosts. They frequently develop the pneumonic form of the disease and can spread the infection directly to humans through coughing and sneezing (Chin 2000). In the Pacific coastal region of San Mateo County, infected mice were frequently found in natural areas adjacent to housing tracts. In 1977, a veterinarian died of plague pneumonia that may have been acquired from a cat in this area. The patient had treated cats at a clinic in Half Moon Bay a week before the onset of his illness, then had moved on to a clinic for the Society for the Prevention of Cruelty to Animals in the Salinas/ Monterey area (R. Blair, unpublished data; Anonymous 1977). State and county public health personnel conducted follow-up investigations in both counties but were unable to pinpoint the source of infection. Examination of records at the clinic in Half Moon Bay showed the patient had treated several cats having symptoms consistent with feline plague. However, they were unable to obtain blood samples from any of these animals. Limited trapping in the vicinity of the patient's home, the clinics and the residences of animals he had treated was unproductive. The source of this patient's infection will never be known for certain. But the possibility that it was acquired in Half Moon Bay should not be overlooked. Plague positive mice have been collected in the past from areas around Half Moon Bay and in Monterey County.

The transfer of plague from wild rodents to commensal rodents is another way humans in San Mateo County might be exposed to plague. This is of particular concern on San Bruno Mountain because of its close proximity to San Francisco. The presence of wild rodent fleas on commensal rats was noted on numerous occasions in this area (Doane 1908; Fox 1909; McCoy and Mitzmain 1909; Eskey 1938; Miles et al. 1957, Eads and Barnes 1976). In addition, a Norway rat infected with plague was found on San Bruno Mountain in 1954, adjacent to one of the hog farms. Infected rats were also found within the city limits of San Francisco in 1941 and 1963 (Kartman et al 1958; Kartman 1963). Long-term control of commensal rodents on San Bruno Mountain prior to 1990 may have helped minimize contact between rats and infected mice. There is currently no program in the cities surrounding the mountain to monitor and control rats.

Enzootic plague is present in several locations in San Mateo County. The most well-characterized sites are on San Bruno Mountain and along the Pacific Coast. Evidence of infection has been detected in *P. maniculatus* as well as *M. californicus*. Detection of plague is best done by testing both species, particularly in areas where few *M. californicus* can be collected. At present, cats present the most serious potential risk of exposure to plague for residents of San Mateo. The re-establishment of ground squirrels near active plague foci would also present a public health risk. Therefore, the territorial expansion of ground squirrels in the county must be monitored and held in check. Finally, the transfer of infected fleas to commensal rodents remains a serious concern. Control of commensal rats in cities adjacent to known plague foci should be included in programs to protect the public's health.

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The Africanized Honey Bee Program in San Bernardino County, 1999

Lal S. Mian1/, Chris N. Nwadike and Mehenna Yakhou2/

San Bernardino County Vector Control Program,
Division of Environmental Health Services, Department of Public Health
2355 East 5th Street, San Bernardino, CA 92415-0064

ABSTRACT: During 1999, the San Bernardino County Vector Control Program (SBCVCP) responded to 662 bee swarms or colonies. About half (332) were Africanized honey bees (AHBs). The distribution of AHBs by region was 28.6% in the desert, 1.2% in the mountains and 70.2% from the valley area. In comparison with the European honey bees (EHBs), the AHBs accounted for 88% in the desert, 38% in the mountains and 35% in the valley. AHB activity peaked in April with a smaller surge late in September. Bee distribution by habitat was 60% in/on vegetation, 17% in/on structures, 6% in utility boxes and 17% miscellaneous habitats. There were two animal and two human bee stinging incidents reported in 1999. Training and public education efforts were carried out as needed.

The cost on AHB services amounted to $104,650, including $3,600 used on pesticides and $101,050 (4,042 man-hours x $25 average hourly rate) on labor. The per parcel cost was $0.25 and per capita cost $0.10.

INTRODUCTION

The first finding of the Africanized honey bees (AHBs), *Apis mellifera scutellata* Lepeletier, was confirmed on April 2, 1998 in the Joshua Tree area of San Bernardino County. The El-Nino rains resulted in abundant vegetation and bloom and the spread of AHBs was so fast that on December 3, 1998 the entire county was declared by the County Agricultural Commissioner as colonized by these bees. During 1998, three multiple stinging incidents were reported. The first incident occurred on July 28 in Newberry Springs, the second on October 22 involving 6 people in Big River, and the third on December 15 in San Bernardino (Mian and Yakhou 1999).

The entire 1998 data on AHB migration and colonization, pre-arrival phase surveillance, personnel training, public education and incurring cost, are given by Mian and Yakhou (1999). This paper presents the post-arrival first year data on the AHB service response, spatial and temporal distribution, human/animal stinging episodes, training, public education efforts and incurring cost.

MATERIALS AND METHODS

The response protocol for 1999 was almost similar to the one used during the1998 operations (Mian and Yakhou 1999). A call about nuisance bees in a residential neighborhood was promptly responded by a bee team. The team consisted of two AHB trained technicians in a vehicle equipped with a mounted sprayer carrying the M-Pede insecticide registered for AHB control in California. After controlling the suspect colony, a sample of 50 bees collected in 70% ethanol, was brought to the laboratory for identification, using the fast AHB identification system (FABIS). In the case of a human or animal stinging incident, the sample was forwarded to the California Department of Food and Agriculture Laboratory in Sacramento for DNA confirmation. Moreover, multiple stinging victims were routinely advised to have follow-up medical attention to monitor any delayed toxicity.

RESULTS AND DISCUSSION

During the 1999 season, SBCVCP received 1,405

1/ Department of Health Science, California State University, San Bernardino, CA 92407-2397
2/ Department of Accounting, Georgia College and State University, Milledgeville, GA 31061-0490

bee-related calls. Out of these, 662 appeared to be actual bee swarms or colonies. Of the total bee samples collected during the 662 responses, 332 (50%) were AHBs and 330 (50%) were the European honey bees (EHBs), *Apis mellifera ligustica* Spin. For bee distribution purpose, the county was divided into three biotopes, namely desert, mountains and valley. The area on the east side of the mountains up to the Colorado river was the desert region. The area to the west of the mountains was called the valley region. Within each biotope, the proportion of AHBs to EHBs was quite different. The overall bee samples tested by region were 28.6% from the desert, 1.2% from the mountains and 70.2% from the valley. In the desert, the ratio of AHBs:EHBs was 88%:12%. In the mountains it was 38%:62% and in valley 35%:65% (Table1). Seasonally, bees remained active throughout the year with peaks in April in the Spring and September in the Fall (Fig. 1). In spatial distribution, 60% of the swarms/colonies were from vegetation—tree canopy, branches and tree holes, followed by structures both inside and outside (17%), utility boxes - water meter boxes, sprinkler valve boxes, mailboxes (6 %), and miscellaneous habitats (17%) (Table 2).

During this period, one animal multiple stinging incident occurred on February 20 in San Bernardino when two pitbulls were attacked by provoked bees, killing one of the dogs. A human stinging incident occurred at a sanitary landfill in Rialto on June 29 where one person was stung 26 times by bees. A sample of the bees was later confirmed as Egyptian honey bees, *Apis mellifera lamarckii* Cockerell. In another incident in Lucerne Valley on July 4, one of the three stung horses was so severely affected that the attending veterinarian euthanized her the following morning. Five people, a male and four female residents, also received multiple bee stings in the incident. The bees were confirmed as AHBs.

In training and public education, the SBCVCP staff gave10 training sessions to various civil and governmental agency personnel. As part of the public education and awareness program, the program staff gave 17 newspaper articles, 2 radio interviews and 4 television.

The bee control program used 200 gallons of M-pede. At a cost of $18.00/gallon, the cost of pesticide alone was $3,600.00. In bee service responses including lab time, the program spent 4,042 man-hours. At an average hourly rate of $25.00, the incurring labor cost was $101,050.00. The total program cost (time and pesticide) amounted to $104,650.00. With a parcel count of 416,988 in the program territory, the cost per parcel was $0.25. Similarly, with a population of 1,057,055, the per capita cost was $0.10.

TABLE 1. Distribution of bee samples by region in San Bernardino County, 1999.

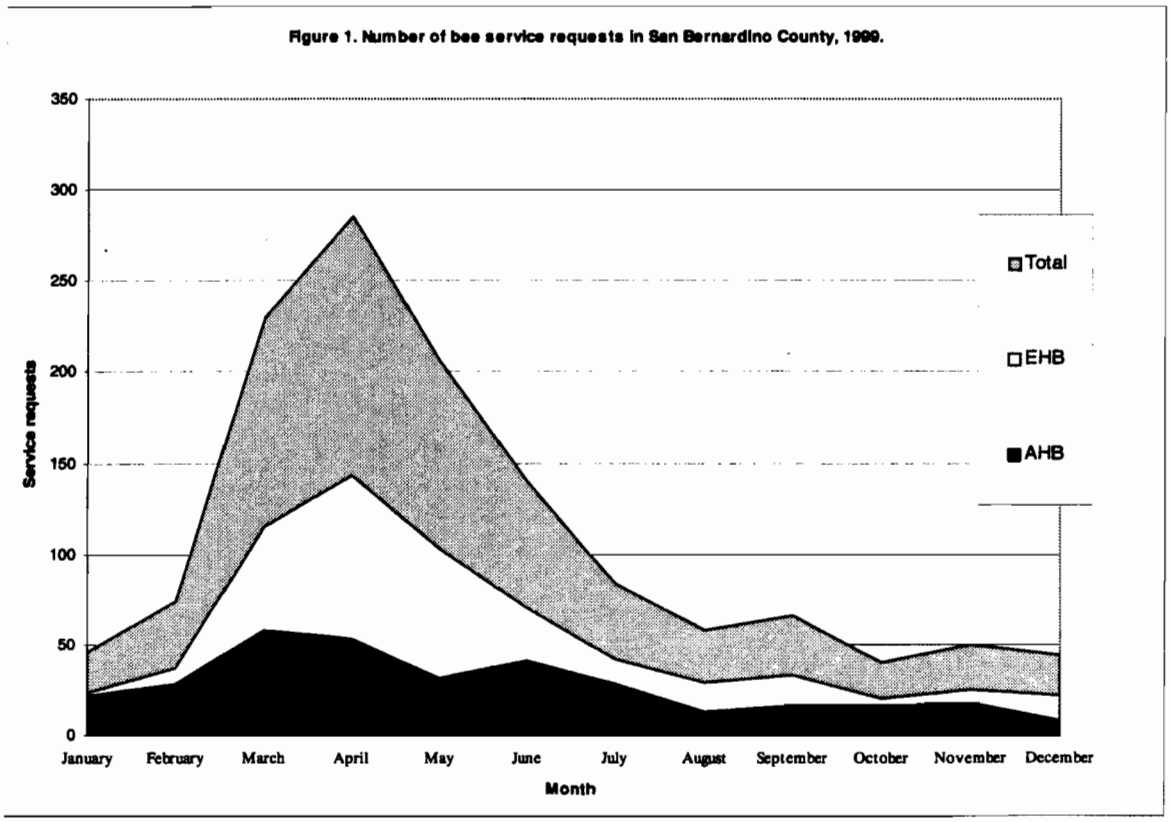
	Number and (%) of samples identified by region		
Region	AHB (%)	EHB (%)	Total (%)
Desert	167 (88)	22 (12)	189 (28.6)
Mountains	3 (38)	5 (62)	8 (1.2)
Valley	162 (35)*	303 (65)	465 (70.2)
Total	332 (59)	330 (50)	662 (100)

*includes one sample confirmed as Egyptian honey bees, *Apis mellifera lamarckii.*

Table 2. Distribution of bee samples by habitat in San Bernardino County, 1999.

Habitat	Number and (%) of samples identified by habitat		
	AHB (%)	EHB (%)	Total (%)
Vegetation 1/	152 (39)	242 (61)	394 (60)
Structures 2/	70 (63)	46 (37)	116 (17)
Utility boxes 3/	28 (79)	8 (22)	36 (6)
Others 4/	82 (71)	34 (29)	116 (17)

1/ All plant parts, including tree holes.
2/ Out/inside of walls, eaves, etc.
3/ Water meter boxes, sprinkler valve boxes, mailboxes and similar other containers.
4/ Included hay stack, bird house, boat/trailer, bulldozer, cable spool, railroad tracks, equipment, fence post, flag pole, furniture, garden tool box, gas tank, junk pile, light pole, love seat, lumber/wood pile, metal drum, milk crate, pallet, parking sign, plant stand, play set, shopping cart, solar reflector, street sign, table, telephone pole, truck, waste tire, water tank, and wooden wagon, each with one or more swarms.



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History of Pesticide Dispersal Equipment Used to Control *Aedes squamiger* on Bair Island San Mateo County, California

James H. Counts, Dennis Jewell, and Chindi A. Peavey

San Mateo County Mosquito Abatement District
1351 Rollins Rd, Burlingame, California

ABSTRACT: Bair Island is a series of artificial and natural islands (approximately 3,000 acres) located midway between the cities of San Francisco and San Jose on the east side of the San Francisco Peninsula, San Mateo County, California. The islands are located in proximity to the cities of Belmont, San Carlos, and Redwood City with combined populations of 150,000 persons. This paper is an overview of the problems, equipment and control strategies employed to combat the winter salt marsh mosquito *Aedes squamiger* (Coquillett) on these islands.

INTRODUCTION

The winter salt marsh mosquito, *Aedes squamiger*, is a major pest species in the San Mateo County Mosquito Abatement District. Its control accounts for a large proportion of the District's resources ($30,000-80,000 annually). Larvae of this species develop in impounded water in salt marshes of San Francisco Bay during winter and spring. Adults emerging from March through May fly up to 15 miles inland in search of a blood meal before returning to the salt marsh to lay eggs (Bohart and Washino 1978). This is a particularly pestiferous mosquito because it is a relentless day-biter that readily feeds on humans. Bair Island is the largest larval habitat for salt marsh mosquitoes in San Mateo County. If left unchecked, this source results in enormous numbers of service requests from residents of the cities of Redwood City, San Carlos and Belmont. Mosquito control at Bair Island has evolved as conditions have changed and the District has learned new ways to meet the challenges. This paper will review changes in the distribution of salt marsh mosquitoes in San Mateo County over time and describe some of the equipment that has been used to control them.

Historical Changes in the Distribution and Abundance of *Ae. squamiger*

Winter salt marsh mosquitoes were abundant throughout San Francisco Bay at the turn of the century and the need for their control was the impetus for the formation of a mosquito abatement district on the San Francisco Peninsula in the early 1900's (Quayle 1906). Throughout most of the 1950's and 60's, however, *Ae. squamiger* was virtually absent from San Mateo County and other areas in the southern arm of San Francisco Bay. Their absence during this time has been attributed to conversion of most tidal wetlands in the area to salt ponds and the use of organochlorine pesticides for mosquito control. In 1967, *Ae. squamiger* commenced a recrudescence in the District after a period of nearly 15 years. They were first observed in a quarter-acre site located at the freeway interchange of US 101 and Highway 92 in the city of San Mateo. Larval sources followed a definable pattern of expansion from this site in succeeding seasons. By the 1971-72 season, larval sources were found 10 miles south and 7.5 miles to the north of the original site. In April 1971, the District began receiving calls from residents of the city of Belmont for day-biting mosquitoes. By the end of May that year, 150 service requests had been received. A half-acre parcel of land at the interchange of Highway 101 and Ralston Ave. (now Marine World Parkway) was treated to curb this outbreak. In 1972, the surveillance program was intensified and 2 additional breeding sites were located. That season we received 31 service requests. By 1973, *Ae. squamiger* populations had become established on Bair Island.

Bair Island is a 3,000-acre parcel encompassing four land masses separated from the mainland and each other by sloughs (Figure 1). The area was developed into a series of salt evaporation ponds in 1946. While the salt ponds were in operation and for several years afterward, this area did not support mosquito larvae. The ponds contained large stretches of open water with a high salt content and lacked emergent vegetation. The

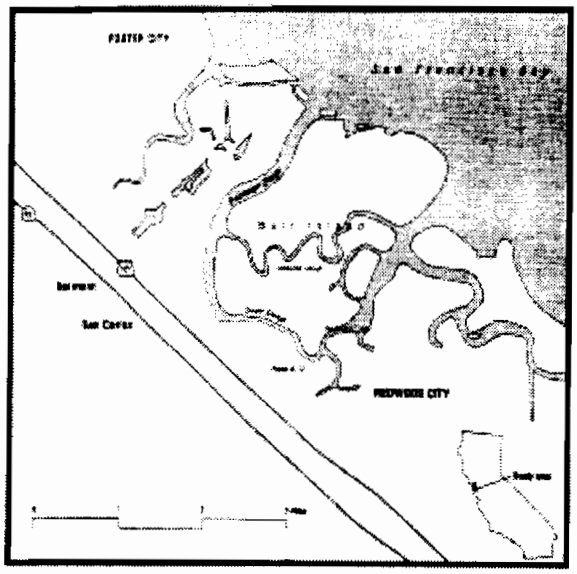

Figure 1. Map of Bair Island, San Mateo County, California.

ponds were drained in 1965 but continued to fill with rainwater each winter. Repeated seasonal flooding and drying caused the formation of deep cracks on the interior of the ponds. By 1970, pickleweed, *Salicornia virginica* Linneaus, had recolonized the cracked ground and Bair Island had become ideal habitat for salt marsh mosquito larvae. California experienced a number of drought years in the mid 1970's with a resultant 5-year period of inactivity. In 1978, near normal rainfall returned to California. Rainwater once again filled the interior of the ponds, creating thousands of acres of habitat for *Ae. squamiger* mosquitoes within 2 miles of 150,000 residents. It was estimated that there were 3.65 trillion mosquitoes developing in water impounded on Bair Island. That year, we received a record 2,039 service requests. The 1981-82 season was also a year of heavy rainfall and the District employed a helicopter for the first time to successfully control *Ae. squamiger.* Drought conditions returned in the mid-80's and District personnel were able to control the mosquitoes at Bair Island with hand cans and 4-wheel drive equipment. However, the early 1990's showed another increase in mosquito populations and the District once again called for aerial insecticide applications. Aerial applications have now become the standard for pesticide applications in this area.

Historical Review of Equipment Used by the District

Fluctuations in the abundance and distribution of salt marsh mosquitoes in San Mateo County over time have resulted in changes in the equipment used to control them. During the 1950's and 60's, the Distri. used a pre-1950 4-wheel drive Dodge Power Wagon and an Oliver tractor for mosquito control in the saltmarshes. The tractor was used for both larviciding and adulticiding. A 5 hp Potts mist blower or 50-gal power sprayer unit was mounted on a rear platform and the marsh was treated by driving along the dikes. In the late 1960's and early 1970's, the District began purchasing International Harvester Scout™ 4-wheel drive vehicles. These were equipped with 50-gallon power spray units or could be used with the Potts mist blower. Large areas could be treated much faster than with other vehicles, but the Scouts suffered from clutch and transmission problems at vehicle spraying speeds. In 1974, we purchased a Coot™ ATV and the following year a second one was obtained. These vehicles were able to traverse areas that were difficult to cross on foot and accommodated 2 technicians, plus a power spray unit for remote areas. The Coots had several disadvantages, they required frequent maintenance and parts were difficult to obtain. In addition, their wheels left deep ruts that later had to be treated for mosquitoes themselves. Shortly after Coots were introduced, the manufacturer ceased operations and parts became unavailable. The District sold the vehicles at auction and in 1981 purchased a military surplus Mighty Mite™ All Wheel Drive vehicle. This vehicle was built for the US Army by Chrysler Corp™. Once again, replacement parts became a problem and the vehicle was sold. The District now uses 2 Argo™ ATV's in marshes. Both vehicles are equipped with power sprayers, cluster nozzles, and telescoping arm extensions for application of liquid larvicides. A herd seeder can be installed for granular or pellet formulations. These vehicles can be used with or without tracks depending on the terrain. Maintenance support is readily available and the vehicles have the added advantage of very low ground pressure. Their impact on marsh vegetation is minimal.

The aforementioned equipment was useful for mosquito control in salt marshes bordering East Palo Alto and East Menlo Park and a 300-acre section of Bair Island adjoining Highway 101. However, the majority of Bair Island is separated from the shore by sloughs and can only be reached by boat. In 1970, the only boat owned by the District was a 12-foot wooden craft with a 7 hp outboard motor. This had been adequate for the limited inspection that had been required up to that time, but not for the regular visits that were now needed for surveillance of the area colonized by salt marsh mosquitoes. In 1971, the District purchased secondhand a 14-ft wooden boat with a 50 hp motor. This was used to transport personnel to the islands for inspection and hand treatment work. However, this boat

had a deep draft and could only be used at high tide. A 5 hp airboat was purchased with a very shallow draft to be used at lower tide cycles. Unfortunately, it proved to be wholly inadequate for our needs and was soon abandoned. We now have a modified 14 ft Klamath aluminum boat with a 25 hp outboard motor for transporting personnel and equipment. This boat has become the workhorse of the District. It is used during favorable tide cycles in the fall, winter and spring for marsh inspection and spot treatment. It can carry up to 800 lbs, yet is light enough and small enough to travel through many of the sloughs at low tide. Hand supports have been added to allow personnel to stand in the bow during spray applications. This boat is also currently being used to assist the US Fish & Wildlife Service in its eradication program for non-native cord grass (*Spartina alterniflora* Loisel).

In 1996, the District purchased a hovercraft as a means of accessing the outer islands during unfavorable tide cycles. The vehicle accommodates 5 persons and is primarily used for inspection, but can also be used for light treatment work as well as rescue operations if called on by other government agencies. There is a good deal of maintenance involved with this vehicle and a number of problems occurred early on. The technical and maintenance support by the dealer has been outstanding and the initial problems have been resolved. This vehicle does not actually fly into the pickle weed marshes but is used to sidle up to the dike margins over water or mud. It is able to lift off the bay mud with 5 passengers aboard and allows for inspections without the constraint of tides.

Over the last several years, the District has devoted vast amounts of time and resources to the control *Ae. squamiger* in salt marshes at Bair Island. Trial and error has led to the acquisition of better equipment allowing us to inspect and treat the islands in a timely fashion, preventing most major fly-offs. With the present boat and hovercraft, all parts of the island can be accessed under a wide range of conditions. The quantity of pesticides applied is minimized through early detection of larval sources and careful delineation of their distribution. The amount of time required for treatment has been cut drastically through the use of aerial spraying. For example, in 1978, 6 technicians spent the better part of 13 days to treat the islands with little success. At present, the District employs a helicopter service on contract to treat the entire 3,000 acres in 6-7 hours. This combination of better surveillance and more efficient methods of pesticide application over large areas has maximized the efficacy of our control program.

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Source Reduction Methods Used to Control *Aedes Squamiger* (Coquillett) on Bair Island, San Mateo County, California

Stanley B. Kamiya and Chindi A. Peavey

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

ABSTRACT: Bair Island is a 3,000-acre parcel on San Francisco Bay located one mile from the highly urbanized areas of Redwood City and San Carlos. This area consists of seven abandoned salt evaporation ponds, breeding large populations of the salt marsh mosquito, *Aedes squamiger* Coquillett. This paper reviews source reduction methods that have been used at the site including disking to eliminate cracks, and siphons to remove rainwater that accumulates behind the dikes each winter.

INTRODUCTION

Abandoned salt ponds present a significant breeding source for salt marsh mosquitoes in south San Francisco Bay. Once drained, these ponds become seasonal wetlands, collecting rainwater during winter months and drying up each summer. This repeated seasonal drying results in the formation of deep cracks, conducive for the development of salt marsh mosquitoes. Over time, pickleweed, *Salicornia virginica* L., becomes established in the interior of the ponds, further promoting mosquito development. Bair Island is an example of the mosquito control problems presented by former salt ponds. It is the largest single mosquito-breeding source in San Mateo County, $30,000 – $80,000 is expended annually at this site towards control efforts. This paper will briefly review the history of Bair Island and describe source reduction methods that have been employed there.

Bair Island is located on the western edge of San Francisco Bay adjacent to Redwood City and San Carlos. The property is roughly rectangular, 1.5 mi across at its widest point and approximately 3 mi long. It covers approximately 3,000 acres, 1,600 of which are potential mosquito-breeding habitat. Four major waterways run through the property (Figure 1). Steinberger Slough separates the property from Redwood Shores on the north, Redwood Creek runs along its southern boundary. Corkscrew and Smith Sloughs separate the parcel into three sections. Two of these sections are islands, entirely surrounded by water. The remaining 328-acre piece is the only area accessible by land.

Historically, most of Bair Island was tidal salt marsh. In 1946, the Leslie Salt Co. constructed dikes to develop the area into seven salt evaporation ponds. When salt production was curtailed in 1965, the ponds were drained, leaving the dikes intact. Mobil Oil Corporation acquired the property in 1973, with the intent to build a major housing development. Plans for the development were eventually dropped and after several changes in ownership, the land was acquired by Peninsula Open Space Trust. The property is currently owned by California Department of Fish & Game and the US Fish & Wildlife Service. It will be restored to tidal salt marsh to enhance populations of the Salt Marsh Harvest Mouse, *Reithrodontomys flaviventris* Dixon, and the California Clapper Rail, *Rallus longirostris obsoletus* Oberholser. This is the largest piece of salt marsh remaining in San Mateo County. It supports large and diverse populations of ducks, geese and shorebirds in addition to the two endangered species mentioned above (Goals Project 1999).

While the salt ponds were in operation and for several years afterward, Bair Island did not produce mosquitoes. Although the interior of the pans held water, they had a large surface area and lacked emergent vegetation. Mosquito problems developed in the early 1980s following the formation of cracks in the sediment on the floor of the pans. In 1983, the District conducted disking at one of the pans (pond A12 - the only area accessible by land). This method of source reduction breaks up the top layer of soil (4"-8") to eliminate cracks and improve drainage (Herms and Gray 1944). Disking for mosquito control grew out of its use for agriculture in reclaimed salt marsh along San Francisco Bay. It stimulated plant growth by allowing water to penetrate

the top layer of soil. Disking for source reduction has been used routinely in the past in diked, reclaimed salt marsh along the Bay (San Mateo County Mosquito Control District Annual Report, 1950). Other districts in the Coastal Region area have employed the same method to reduce cracked ground in dredge disposal sites (R. Keith, Marin-Sonoma MVCD, personal communication). In the 1960s and 70s, this District used disking to control biting black gnat, *Leptoconops torrens* Townsend developing in deep cracks in adobe soils of the coastal foothills (Whitsel and Schoeppner 1966).

The 1983 disking at Bair Island resulted in a dramatic reduction in mosquito production for 7 years. Permission was received from the Army Corps of Engineers to disk the area again in 1991. Restrictions on the permit included a provision that no more than 100 acres of ground be disturbed at a given time and that no changes were made in existing waterways. At that time, the interior of the pan contained little or no vegetation and was therefore not considered habitat for Salt Marsh Harvest Mice. Most of Bair Island contains extensive stands of pickleweed and cannot be disked again due to its potential impact on the mice.

Another method of source reduction at Bair Island is the use of siphons. Siphons were initially installed in 1973 by South Shore Properties, a successor to Mobil Oil Corporation. Five of the seven ponds lacked any type of drainage structures such as tide gates. The siphons served to prevent the accumulation of rainwater behind the dikes in preparation for construction of the housing development. Two to three siphons were installed in each pond. Each siphon consisted of a pipe extending from the interior of each salt pan, over the top of the dike, and emptying into the slough. They were constructed from schedule 40 2/0 PVC piping (8" diameter) in 20' sections, cemented together with Weld-On 725 Wet 'R' Dry PVC Plastic Pipe Cement (IPS Corp., Gardena, CA). The siphons were started at low tide by attaching a vacuum pump to a valve on each pipe at its highest point (the top of the dike). Once the water had been pulled up to this point, it flowed into the slough by gravity. Valves were installed on the outboard side of each siphon to prevent backflushing at high tide. Early valves were fashioned by attaching a length of inner tube onto the end of each siphon. These had a tendency to get pulled into the siphons at high tide, allowing the water to reverse flow. In 1999, the District purchased 2 Tide-Flex Check Valves (Red Valve Co. Inc., Carnegie, Pa.) at a cost of approximately $1,000 each. These were sections of rubber, 18" long, attached to the end of the pipes and contoured down to a duckbill to allow passage of water out but prevent backflow (Fig. 2). Later, a low-cost valve of the same type was fabricated from a 24" piece of rubber inner tube and a piece of wood 13" x 2" x 1". One end of the inner tube was attached to the outboard end of the siphon pipe. The wooden piece was anchored inside the inner tube so that it formed a duckbill. The resulting valve looked similar to the one pictured in Figure 2. This arrangement closed the siphon when the tide rose in the channel and prevented the valve from being pushed back into the pipe.

When first installed, the siphons were effective at removing water from the interior of the ponds. Their usefulness declined in later years because subsidence within the pans and silt deposition in the slough dramatically decreased the elevational differences between the inside and outside of the pans. At present, the siphons function efficiently at low tide but shut off when the tide rises. Draining the pans by this means requires that the siphons be restarted at successive low tides over several days and is very labor intensive.

Bair Island is an example of the development of mosquito control problems following man-made changes to the salt marshes of San Francisco Bay. By 1988, 34,455 acres of former tidal salt marsh in San Francisco Bay had been developed for salt production (Goals Project, 1999). Land use practices on some of this land has shifted away from salt production with the subsequent development of mosquito control problems such as those encountered on Bair Island. Twenty-five thousand acres of salt evaporation ponds remain in active use in the south Bay today and these have the potential to breed large numbers of salt marsh mosquitoes in the future. The best solution to mosquito control in such areas is the restoration of tidal flow. However, breaching dikes is often unfeasible or requires an extended period of review by various regulatory agencies and the public. Disking and use of siphons are two relatively low cost alternatives for temporary source reduction to reduce the acreage requiring pesticide treatment.

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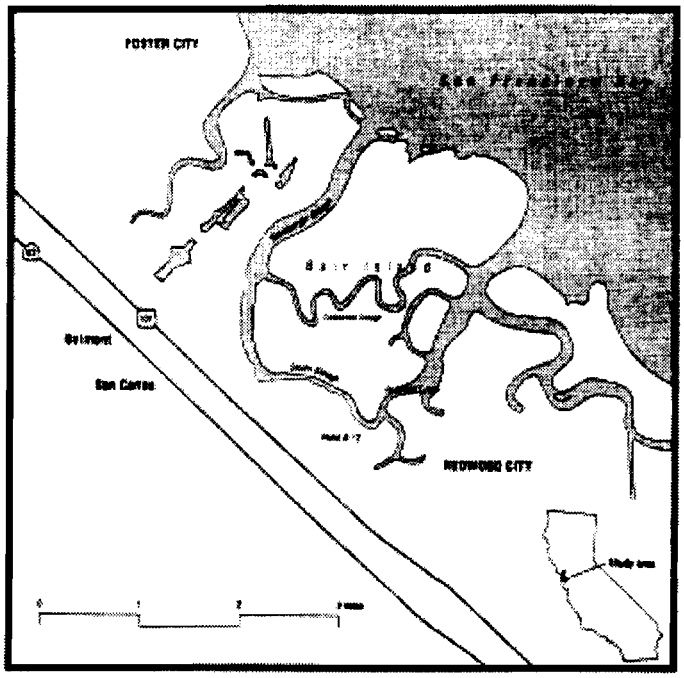

Figure 1. Map of Bair Island, San Mateo County, California.

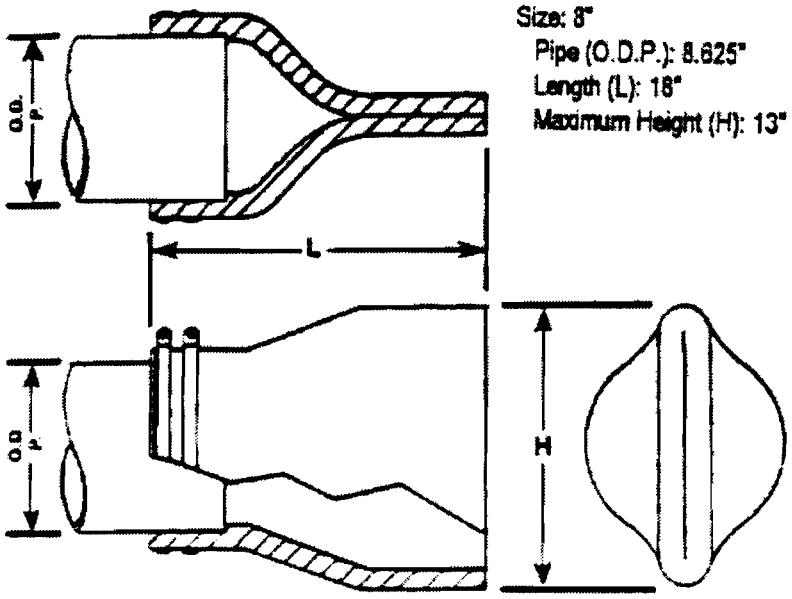

Figure 2. Diagram of a commercially available valve used on siphons at Bair Island to prevent backflushing (Tide-Flex Check Valve, Red Valve Co. Inc., Carnegie, Pa.).

William C. Reeves New Investigator Award

The William C. Reeves New Investigator Award is given annually by the Mosquito and Vector Control Association of California in honor of the long and productive scientific career of Dr. William C. Reeves, Professor Emeritus, School of Public Health, University of California at Berkeley.

The Award is presented to the outstanding research paper delivered by a new investigator based on quality of the study, the written report, and presentation at the annual conference.

Jason Rasgon was the recipient of the 2000 award at the 68th Annual Conference held in Sacramento, California. The other finalists were Keith Bow, Beth May, and Linda Styer. Available finalists' papers are printed on pages 68-84.

Previous William C. Reeves New Investigator Award Winners:

1999 - Parker D. Workman
1998 - Yvonne Ann Offill
1997 - John Gimnig
1996 - none
1995 - Margaret C. Wirth
1994 - Merry L. Holliday-Hanson
1993 - Jeffrey W. Beehler
1992 - Darold P. Batzer
1991 - David R. Mercer
1990 - Gary N. Fritz
1989 - Truls Jensen
1988 - Vicki L. Kramer

Estimating the Efficacy of *Lagenidium giganteum* Liquid Cultures

Beth May and Jean S. VanderGheynst

Department of Biological and Agricultural Engineering
University of California, Davis

ABSTRACT:: *Lagenidium giganteum*, an EPA approved biological control agent of mosquito larvae was grown in shake flask culture and its efficacy tested against second instar *Aedes aegypti* mosquito larvae. Bioassays were set up based upon the respiratory activity, or CO_2 evolution rate (CER), of the liquid culture. Logistic regression was completed on the bioassay results using CER as a potential predictor of efficacy and it was determined that CER was not correlated with the probability of attaining at least 95% infection (p=0.26). One possible explanation is that the dynamic response of the liquid culture to ambient atmospheric conditions biased the CER measurement.

INTRODUCTION

The fungus *Lagenidium giganteum* (Couch) (Lagenidiales: Pythiaceae), a facultative parasite of mosquito larvae, is among the more promising biological control agents for mosquito control. Due to both its effectiveness and host specificity, it has been approved for use by the US and California EPA. When applied in the field, it is capable of establishing itself and providing mosquito control both initially and over time (Guzman, et al. 1987,Jaronski, et al. 1983,Kerwin, et al. 1986,Kerwin, et al. 1987). Additionally, it has been found to survive the winter in some sites and provide control during the next mosquito breeding season (Jaronski, et al. 1983). The ability of *L. giganteum* to effectively control mosquitoes depends on water conditions such as temperature (Jaronski, et al. 1983,Jaronski, et al. 1983), salinity, and pH (Lord, et al. 1985), as well as the condition of the organism itself.

A factor in the adoption of biological control agents for mosquito control is the ability of the end user to predict resulting control from a given application rate. Previously field application rates of *L. giganteum* have been based on number of cells (Kerwin, et al. 1986,Kerwin, et al. 1987), volume of liquid growth media (Kerwin, et al. 1986), number of petri dishes (Guzman, et al. 1987,Jaronski, et al. 1983,Jaronski, et al. 1982) and number of infected, fragmented mosquito cadavers (McCray, et al. 1973). Although infection was monitored over a period of time in each case, none of these methods attempted to predict observed control. Therefore, it is advantageous to find an indirect method to evaluate the efficacy of a biological control product at any given time during either the cultivation process or its storage.

Specific carbon dioxide evolution rate (μmol CO_2/ min/ g) and oxygen consumption rate have been used as indirect measures of active fungal biomass in both solid-state cultivation (Sato, et al. 1983,Sato, et al. 1988) and liquid fermentation studies (Shuler, et al. 1992). It is active biomass of *L. giganteum* that is responsible for the asexual production of infective zoospores. Thus, measurement of respiration rate may offer a means of predicting efficacy of *L. giganteum* produced in liquid culture or other cultivation processes. The goals of the experiments presented in this paper were to determine if *L. giganteum* efficacy could be correlated to CO_2 evolution rate and to develop a method to predict efficacy from application rate and CO_2 evolution rate.

MATERIALS AND METHODS

Source and maintenance of the fungus: *L. giganteum* was obtained from AgraQuest (Davis, CA). The fungus was maintained in both liquid and agar cultures consisting of 0.025 g cholesterol, 1.25 g peptone, 1.25 g Ardamine pH (autolyzed yeast extract), 3 g glucose, 0.075 g $CaCl_2$, 0.075 g $MgCl_2$, 3 g corn oil, and 0.1 g soybean lecithin in one liter of distilled deionized water (PYGLC). Agar cultures contained 15 g agar per liter in addition to the PYGLC liquid described above. Corn oil was added aseptically to the autoclaved media to prevent thermal degradation. Liquid cultures were maintained in an incubated shaker at 30° C and 150 rpm. Agar cultures were maintained at 26° C.

Respiration rate: Liquid cultures were harvested and sampled for specific CO_2 evolution rate after varying cultivation periods, ranging from 1 to 9 days. Bioassays were prepared by fixing the amount of material needed per 100 ml bioassay to deliver a specified amount of CO_2 evolution rate. Specific CO_2 evolution rate was estimated from a CO_2 mass balance on 50 g of the liquid culture (equation 1). This was accomplished by transferring 50 g of the liquid culture to a flask with a constant air flow (20 or 40 ml/min) and stirred via a stir bar. Carbon dioxide levels at both the inlet and outlet of the flask were measured using an infrared CO_2 sensor (Telaire, Goleta, CA) after 45 min.

$$\text{Specific } CO_2 \text{ evolution rate} = \frac{\frac{\rho \upsilon}{MW}(CO_{2_{outlet}} - CO_{2_{inlet}})}{W} \quad (1)$$

where:

ρ= density of air ($\mu g/cm^3$)

υ= volumetric flow rate of air (cm^3/min)

MW= molecular weight of air ($\mu g/ \mu mol$ air)

$CO_{2\ outlet}$= molar fraction CO_2 in outlet air ($\mu mol\ CO_2/ \mu mol$ air)

$CO_{2\ inlet}$= molar fraction CO_2 in inlet air ($\mu mol\ CO_2/ \mu mol$ air)

W= weight of liquid media in flask (g)

Bioassays: Mosquito bioassays were conducted using a modification of the procedure described by Kerwin and co-workers (Kerwin, et al. 1986). All bioassays were performed in triplicate and consisted of exposing 10 second instar *Aedes aegypti* mosquito larvae to aliquots of *L. giganteum* PYGLC liquid cultures diluted to 100 ml with distilled and deionized (DI) water for 7 days. Controls were set up as described without media addition. Dead mosquitoes were removed from the bioassay daily and investigated microscopically for the presence of *L. giganteum* hyphae. Bioassays were conducted at various CO_2 evolution rates ($\mu mol\ CO_2$/ min/ 100 ml). The amount of media added to bioassays was determined by dividing the desired CO_2 evolution rate by the specific CO_2 evolution rate of the liquid culture.

Statistical analysis

Statistical analyses were conducted on each of the potential indicators of efficacy (amount of media, CER and number of cells) and bioassay results to determine if they could predict when 95 % infection would occur. The data was separated into those bioassays that achieved at least 95% infection (success) and those that didn't (failure). Logistic regressions were completed with JMP IN v3.2.6 (Cary, NC).

The logistic regression was done using the following equation:

$$p = \frac{1}{1 + 10^{-[(b_0 + b_1 x)]}} \quad (2)$$

Where p= probability of attaining at least 95% infection

b_0,b_1= empirical coefficients determined from logistic regression

x= potential predictor variable (CER, cells or media)

Suspended and dissolved solids: Bioassay liquids were analyzed for both total and volatile solids (suspended and dissolved). Five grams of liquid culture were shaken with 20 ml DI water for 10 min. The resulting solution was filtered through a 47 mm glass fiber filter (Environmental Express, Mt. Pleasant, SC) and washed with an additional 20 ml DI water. The retained solids were dried at 101° C to yield total suspended solids and then combusted at 550° C to obtain volatile suspended solids. Total dissolved solids were determined by drying 20 ml of the filtrate at 101° C. Volatile dissolved solids were determined by combusting the resulting dry solids at 550° C.

CER and pH monitoring: To assess the potential effect of the carbonate system on CER measurements, *L. giganteum* cultures were harvested and pH and CER were monitored for two hours. Two 75 ml liquid cultures, six days old, were opened, mixed together and then separated into two identical 50 gram samples. Both samples were placed on the same shaker and aerated at 20 ml/min. CER was monitored on one flask and pH on the other.

RESULTS AND DISCUSSION

Figure 1 shows the effect of media addition on level of infection observed in the liquid culture bioassays. In general, infection decreased as the amount of media increased above 3 g. At loading levels above 3 g, infection levels dropped below 50%. It was observed that water clarity was reduced when media addition rose above 3 g. The decrease in infection at higher loading rates might have been due to the inhibition of zoospore production by *L. giganteum* as a result of overloading the bioassays with suspended and dissolved organic material. Decreased infection has been observed at high COD loading rates (Jaronski, et al. 1982) and zoospore production has been shown to be inhibited by solutes associated with growth media (Lord, et al. 1985).

Figure 2 shows the pupation rate at various loading levels. Generally, as more media was added to the bioassays, the pupation rate increased. The higher levels of pupation may have been due to the reduction in zoosporogenesis by *L. giganteum* as loading rate increased and/or an accelerated developmental rate due to the excessive media addition acting as a food source.

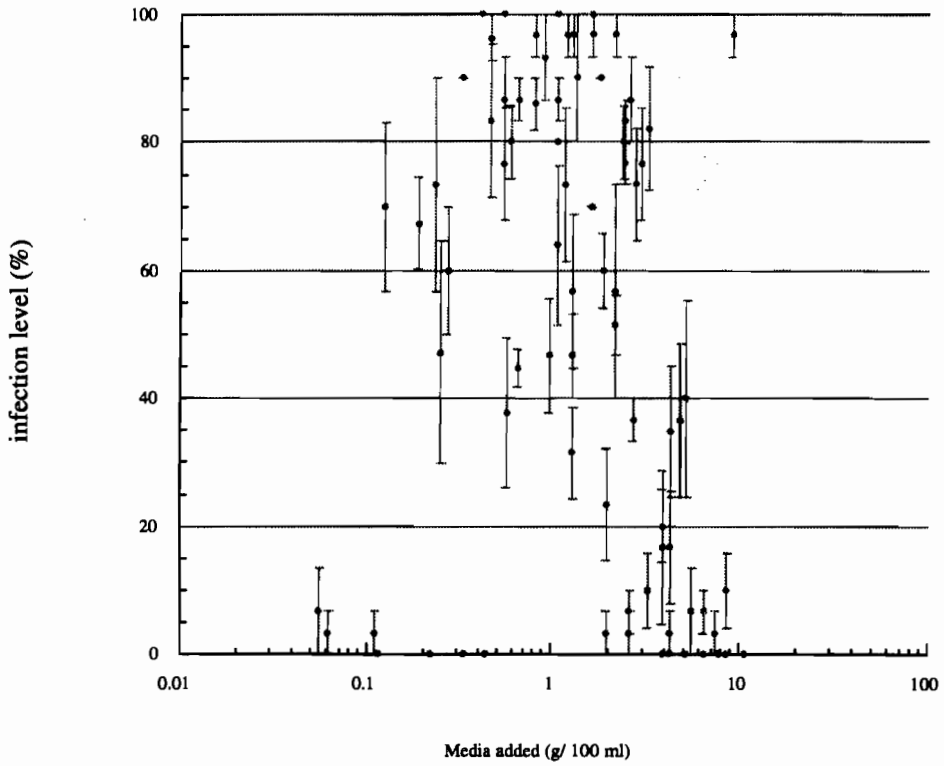

Figure 1. Effect of media addition on infection level in 100 ml bioassays.

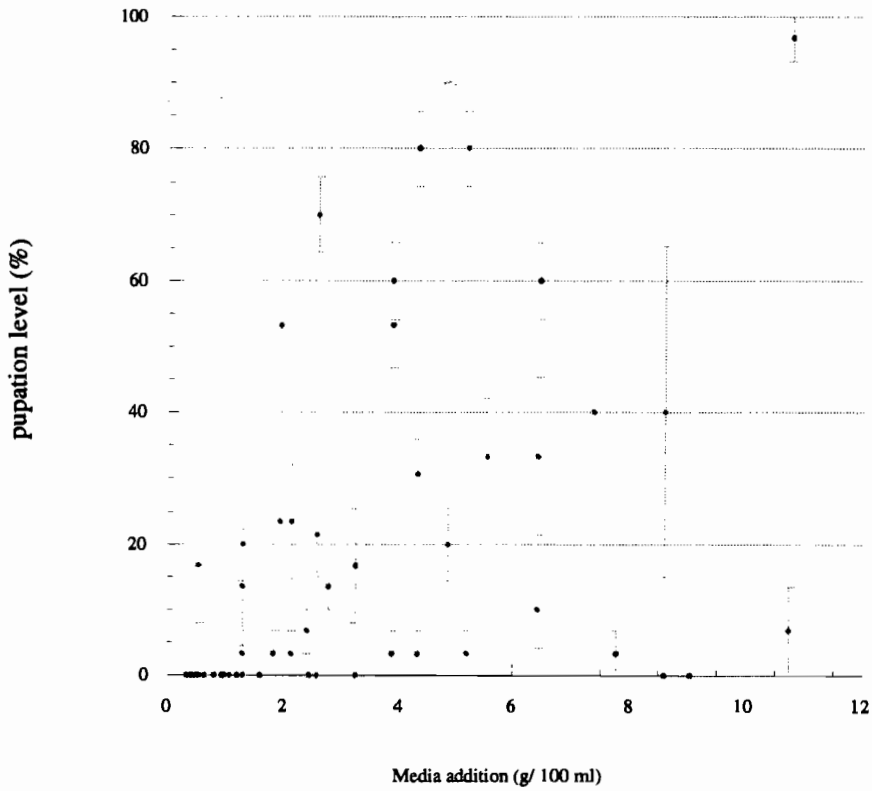

Figure 2. Effect of media addition on pupation level in 100 ml bioassays.

Pupation was not observed in the controls.

Bioassay liquids were assessed for total solids content. Table 1 shows the results of the solids analysis for liquid cultures after 0, 4 and 8 days of cultivation. Each gram of liquid culture provided approximately 5 mg of total solids. The total solids within the liquid did not change over time even though the fractions of dissolved and suspended solids did fluctuate. Initially, almost all solids were dissolved, but by day 4 most of the solids were suspended. Solids were evenly distributed by day 8. Since high loading rates resulted in low levels of infection, only those bioassays containing less than 3 g (15 mg total solids) were considered further.

Table 1. Total solids content of liquid culture during cultivation.

Days of cultivation	Total solids (dry gram/wet gram)	Suspended solids (% of total dry solids)	Dissolved solids (% of total dry solids)
0	0.004	5	95
4	0.004	92	8
8	0.005	50	50

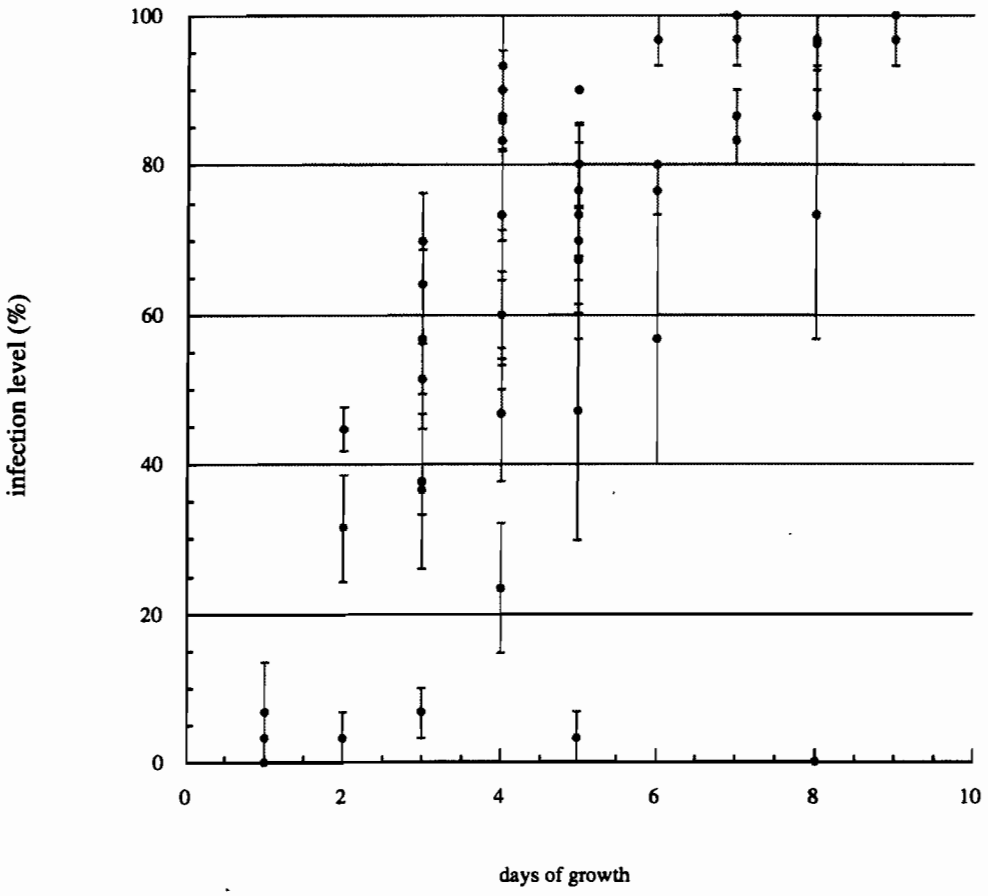

Figure 3. Influence of cultivation time on level of infection of *Aedes aegypti* larvae in 100 ml bioassays.

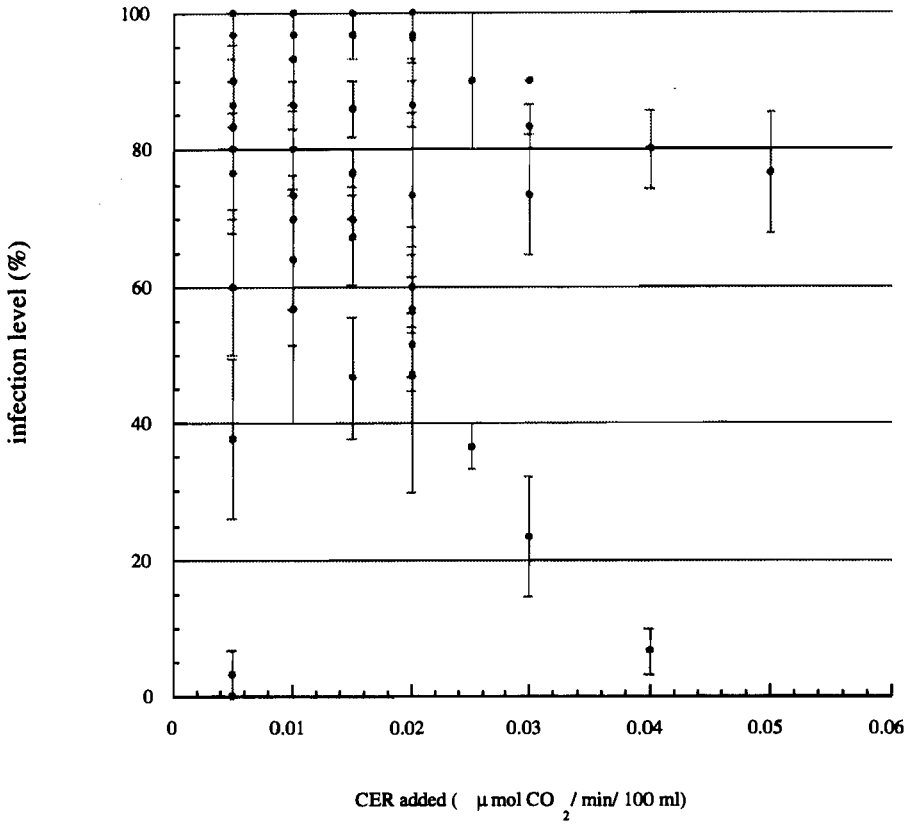

Figure 4. Effect of CO_2 evolution rate addition (µmol CO_2/ min/ 100 ml) on infection level in bioassays.

Figure 3 shows the infection levels for those bioassays containing media levels below 3 g of media as a function of the age of the liquid culture. Percent infection increased with length of cultivation regardless of loading rate. Cultures younger than 4 days did not induce infection at levels higher than 80%. Perhaps these younger cultures had not yet developed the presporangial structures necessary for zoosporogenesis to occur upon dilution. Therefore, it is recommended that cultures younger than 4 days not be used for operational mosquito control.

Figure 4 shows the level of infection at varying amounts of fungal respiratory activity added for only those bioassays containing less than 3 g of media from cultures greater than 3 days old. There was no correlation between CER and the probability of achieving greater than 95% infection as determined by logistic regression (p=0.26).

Figure 5 shows the pH and CER profiles for a 6-day-old liquid culture. Time is measured in minutes from when aeration begins. CER was observed to increase rapidly in the first five minutes and then drop to a steady state value of 0.7 µmol CO_2/ min after 60 minutes of aeration. This pulse may have been a direct result of CO_2 mass transfer from the liquid to the gas phase. Prior to harvesting, liquid culture shake flasks were covered with aluminum foil with only diffusive gas transfer. The gas phase above the liquid had a CO_2 concentration of about 2%. Once flasks were opened and aerated, dissolved CO_2 quickly left the liquid phase in an attempt to come to equilibrium with the new CO_2 concentration in the ambient air (0.3% CO_2) supplied to the flask. As a result the total dissolved CO_2 in the liquid decreased causing the pH to increase (Figure 3.3) (Sawyer, et al. 1978).

A steady state value for CER was observed after one hour of aerating. Our methods measured CER after only 45 min of aeration. This may explain why CER, as measured, could not be correlated to efficacy.

CONCLUSION

Liquid cultures of *Lagenidium giganteum* were tested for specific respiration rate and diluted to supply a known CO_2 evolution rate (µmol CO_2/ min/ 100 ml) to bioassays containing 10 second instar *Aedes aegypti*

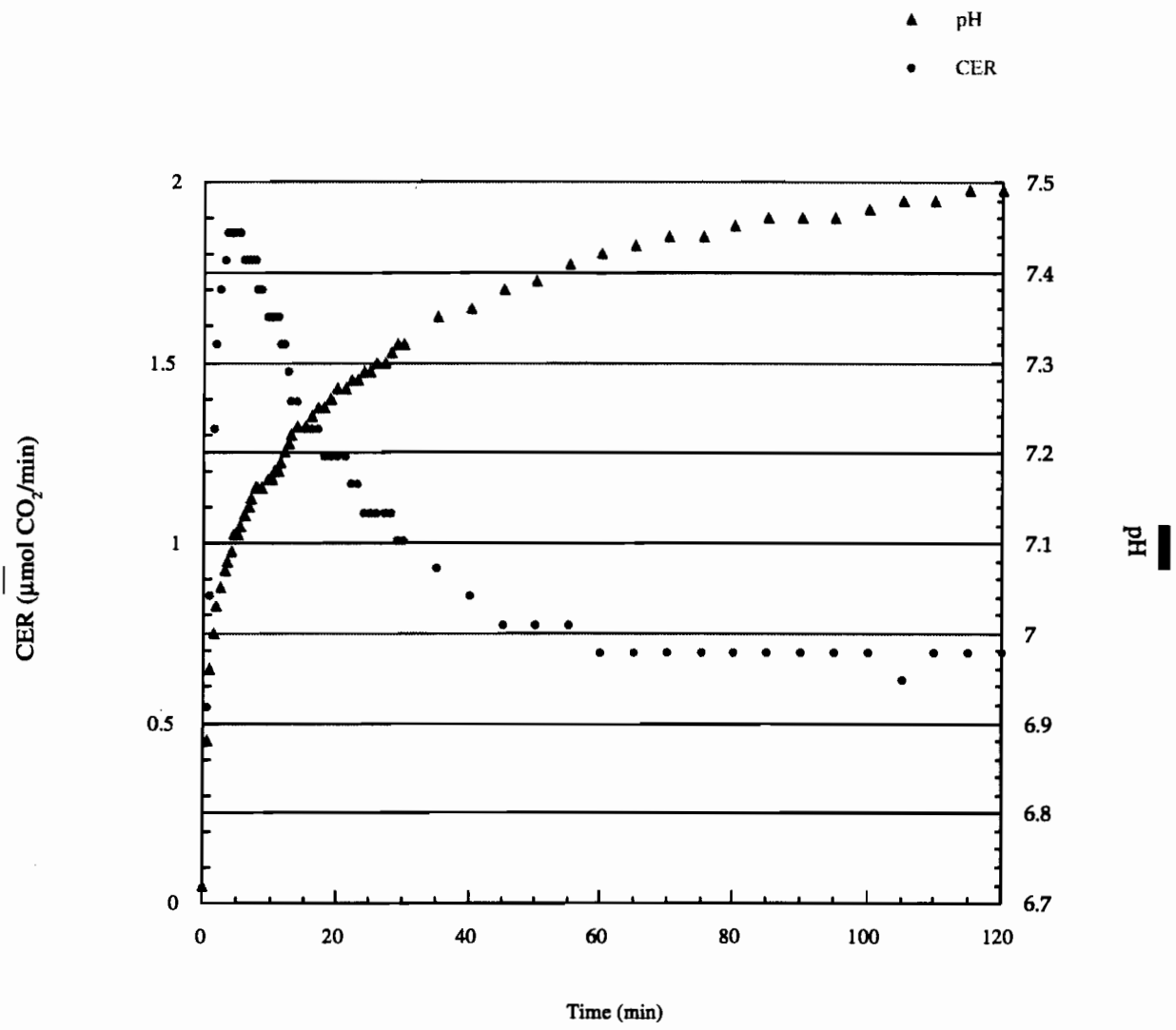

Figure 5. Measured carbon dioxide evolution rate (CER) and pH from time of culture transfer.

mosquito larvae. Analysis of the bioassay data showed that solids overloading, likely leading to a decrease in zoospore production and subsequent reduction in infection, occurred when more than 15 mg total solids was added to the 100 ml bioassays. Additionally, cultures less than 3 days old had lower infection levels than older cultures.

Statistical analysis revealed that there was no correlation between CER and the probability of achieving greater than 95% infection ($p=0.26$). However, subsequent studies in which CER was measured over time have suggested that the system may not have been at steady state at the time of CER measurement. Additionally, lower CER dilutions in bioassays may have improved the statistics.

One potential advantage of using CO_2 evolution rate as a measure of efficacy is that an estimate of a product's potential for control could be made within a few hours. Alternatively, mosquito bioassays require several days to complete. Thus, more efficient delivery of *L. giganteum* for mosquito control may be made possible by measurement of CO_2 evolution rate. However, it should be noted that the optimal CO_2 evolution rate addition range might change with media makeup and/or particular strain used. Therefore, the method provided here may need to be verified to find the optimum range for the *L. giganteum* culture of interest.

Acknowledgements

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Geographic Distribution of *Wolbachia* in the California *Culex pipiens* Complex: Infection Frequencies in Natural Populations

Jason L. Rasgon

Department of Entomology, University of California Davis, Davis CA 95616

ABSTRACT: *Wolbachia* is a bacterial endosymbiont associated with the phenomenon of cytoplasmic incompatibility (CI). In the last 10 years genetic manipulation of mosquitoes has been explored as a way to make them refractory to and/or unable to transmit pathogens. In order for this strategy to be successful, desirable traits must be spread through vector populations. CI has shown theoretical promise as a mechanism for driving genes through mosquito populations. I am currently determining the utility of mathematical models that predict *Wolbachia* equilibrium infection frequencies in natural *Culex pipiens* complex populations throughout California. To be valid, the predicted output of these models must be compared to and agree with observed infection frequencies in natural mosquito populations. Using the polymerase chain reaction (PCR) I determined infection frequencies and the geographic distribution of *Wolbachia* in California populations of *Culex pipiens* complex mosquitoes. Populations from 14 sites across California were sampled, and 10 populations have been examined for *Wolbachia* frequency at this time. Infection frequencies were found to be approaching fixation at all 10 sites. These high infection frequencies indicate that *Wolbachia*-induced CI is indeed a promising candidate for a transgenic driver mechanism in mosquitoes.

BACKGROUND AND OBJECTIVES

The maternally inherited bacterial endosymbiont *Wolbachia* is found in a wide variety of arthropods, infecting 15-20% of all known insect taxa (Werren et al. 1995). In many taxa *Wolbachia* infection is associated with the phenomenon of cytoplasmic incompatibility (CI) (Yen & Barr 1973). When there is a single strain of *Wolbachia* present in the population, infected males are reproductively incompatible with uninfected females (no viable offspring result from the mating). Infected females are reproductively compatible with both infected and uninfected males (Figure 1) (Hoffmann et al. 1990, O'Neill et al. 1992, Turelli & Hoffmann 1991; 1995; 1999). The phenomenon of CI gives infected females a reproductive advantage and allows *Wolbachia* to drive rapidly through populations (Turelli & Hoffmann 1991; 1995; 1999).

Recently, considerable attention has been placed on using *Wolbachia* as part of a strategy to genetically transform vectors in order to make them incapable of transmitting pathogens (Beard et al 1998, Pettigrew & O'Neill 1997). Because genetically transformed insects are often less fit than their wild-type counterparts, a

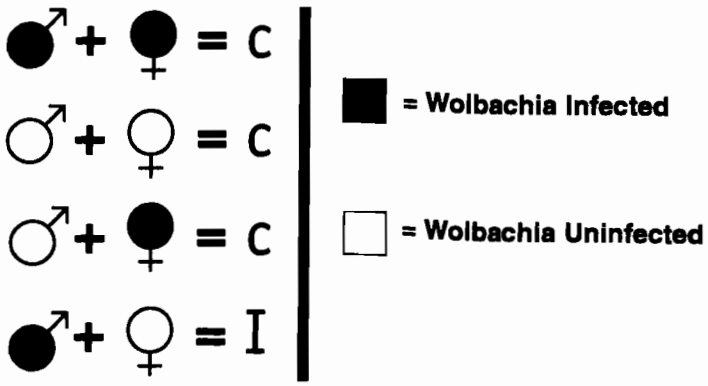

Figure 1. Mating outcomes from all 4 possible crosses between individuals infected with a single strain of *Wolbachia* (simplest case). (C = compatible cross, I = incompatible cross).

driving mechanism is needed to push engineered refractory genes through vector populations; a process known as population gene replacement. The phenomenon of CI is receiving serious consideration as just such a drive mechanism (Beard et al. 1998, Pettigrew & O'Neill 1997, Sinkins et al. 1997, Turelli & Hoffmann 1999).

In order to use CI for vector control it is essential that we understand the population dynamics of *Wolbachia* infection in mosquito populations. To date, all studies of the population dynamics of *Wolbachia* infection in natural arthropod populations have been limited to *Drosophila* (Turelli & Hoffmann 1991, 1995). Mathematical models have been developed to explain and predict equilibrium infection frequencies and the spread of *Wolbachia* in *Drosophila* (Hoffmann et al 1990, Turelli & Hoffmann 1995). The objective of this study was to determine the infection frequencies and geographic distribution of *Wolbachia* in the California *Culex pipiens* complex. This was a necessary first step toward my ultimate goal of modeling the infection dynamics of *Wolbachia* in California *Culex pipiens spp.*

PROCEDURES

Experimental set-up: Specimens of the *Culex pipiens* species complex (*Cx. pipiens*, *Cx. quinquefasciatus* and hybrids) were collected from natural populations across California and assayed for *Wolbachia* infection by a polymerase chain reaction (PCR) based assay. This assay has been validated in other studies (Turelli and Hoffmann 1991, 1995). PCR assay of field collected specimens revealed the geographic distribution and infection frequencies of *Wolbachia* infected *Cx. pipiens* complex mosquitoes throughout the state.

Field Collections: Except for specimens collected from Anderson, mosquitoes were collected as larvae and pupae and shipped live in water from their collection sites to UC Davis. Specimens from Anderson were collected as adults using a CDC-miniature light trap and shipped on dry ice to UC Davis. Immatures were placed in 500-ml plastic cups and ~0.5 g larval food [1 part flake fish food, 2 parts ground rabbit pellets, 2 parts bovine liver powder (ICM Biomedicals, Inc., Aurora, OH)] was added if necessary. Specimen cups were then placed in 3.8 liter emergence cages and maintained in an environmental chamber set at 27 °C and 90% relative humidity. Cages were checked daily for emergence of teneral adults. Adults were removed by aspiration and stored at -80 °C until processed for DNA extraction.

DNA Extraction: DNA was extracted from individual adults by a modified protocol of Black and DuTeau (1997). Individual whole adults were placed in 1.5 ml microcentrifuge tubes, ground in 100 µl extraction buffer [0.08M NaCl, 0.16M sucrose, 0.06M EDTA (pH 8.0), 0.1M Tris-HCl (pH 8.0), 5% SDS] and incubated at 65 °C for 20 min. Twenty-five µλ 4.4M KOac was then added to each tube. Homogenized specimens were placed on ice for 35 min and centrifuged at 15,000 G for 20 min. Supernatants were transferred to new tubes and cellular debris discarded. Two hundred µl ice-cold 100% EtOH was added to each tube, tubes were gently mixed by overturning twice, and stored at -20 °C for 24-48 hours to precipitate DNA. Following DNA precipitation, tubes were centrifuged at 15,000 G for 25 minutes to pellet DNA. Supernatants were carefully decanted to avoid disturbing DNA pellets and discarded. Pellets were washed once with ice-cold 70% EtOH and twice with ice-cold 100% EtOH. Pellets were dried at 37 °C for 1 hr and resuspended in 100 µl autoclaved deionized water. DNA samples were stored at -20 °C until used for PCR (-80 °C for long-term storage). One µl of DNA solution was used as template DNA for each PCR reaction.

PCR Procedure: PCR was conducted using a primer multiplex system, which consisted of two primer sets amplified simultaneously. Primer set 1 amplified a 0.9kb fragment from *Wolbachia* 16S rDNA and is specific to *Wolbachia* of all strains (forward: 5'-TTG TAG CCT ATG GTA TAA CT-3', reverse:5'-GAA TAG GTA TGA TTT TCA TGT-3') (O'Neill et al. 1992). Primer set 2 amplified a 0.4kb fragment from insect mitochondrial 12S rDNA (forward: 5'-CTA GGA TTA GAT ACC CTA TT-3', reverse: 5'AAG AGC GAC GGG CGA TG-3') (O'Neill et al. 1993). The second primer set is universal for insect mitochondrial DNA and was used as a control to verify the quality of the DNA extraction. Samples were amplified on a Perkin-Elmer GeneAmp PCR System 9700 thermocycler (PE Applied Biosystems, Foster City, California) using a protocol of 95 °C for 5 min followed by 30 cycles of: 95 °C / 1 min, 54 °C / 1 min, 72 °C / 1 min. After amplification samples were held at 72 °C for 5 min and stored at 4 °C until visualized. Ready-to-go (RTG) PCR beads (Amersham-Pharmacia Biotech, Piscataway, NJ) were used for PCR reactions to ensure standardized amplification. Each 25 µl PCR reaction contained: 20 ng of each *Wolbachia*-specific primer, 10 ng of each insect 12S rDNA primer, 1 µl template DNA and 1 RTG-PCR bead. PCR products were separated in a 1% agarose gel by electrophoresis, stained with ethidium bromide and visualized on a UV transilluminator. Gels were photographed for a permanent record of results.

RESULTS AND DISCUSSION

Cx. pipiens complex mosquitoes were collected from 14 different locations in California during the summer of 1999. At present, mosquitoes from 10 of the populations have been assayed by PCR for *Wolbachia* infection (Figure 2). The geographic distribution of populations sampled extended from Riverside County in the south to Shasta County in the north, a range of >1,000 km.

Individuals positive for *Wolbachia* infection exhibited two amplified bands; the *Wolbachia*-specific fragment at 0.9kb and the insect mtDNA control fragment at 0.4kb (Figure 3). For *Wolbachia*-negative individuals, only the control band was detected. Individuals missing both bands were excluded from the analysis due to presumed failed PCR. Previously confirmed positive and negative specimens and/or known *Wolbachia* infected and uninfected *Drosophila simulans* were used as positive and negative controls, respectively.

Infection frequencies at all 10 sites were extremely high, ranging from 96% to 100% (Figure 2). Out of 537 total individuals assayed statewide, 533 were infected (>99%). Uninfected specimens were confirmed twice by PCR. One hundred percent of females (265) and 99% of males (268/272) tested positive for *Wolbachia*. Infection frequencies were not significantly different between sites ($\chi^2 = 0.096$, df = 9) or by sex ($\chi^2 = 0.059$, df = 1).

The concept of population replacement with transgenic insects is currently hampered by the lack of a suitable drive mechanism to spread transgenic factors through natural insect populations of interest. *Wolbachia*-associated cytoplasmic incompatibility is being closely examined as one such possible transgenic drive mechanism. In theory, CI can spread traits that are very closely linked with *Wolbachia* into the population along with the symbionts. For population replacement with transgenic insects to be successful, transgenes of interest must be driven to high frequencies throughout natural populations. In natural *Cx. pipiens* complex populations in California, *Wolbachia* infection levels are extremely high; 100% for epidemiologically important females and nearly 100% for both sexes combined statewide. This is encouraging, because it indicates that a transgenic drive mechanism in mosquitoes based on *Wolbachia*-induced CI may spread introduced transgenes to high enough levels to interrupt the disease cycle.

In order to use CI efficiently as a transgenic drive mechanism it is crucial to understand which parameters

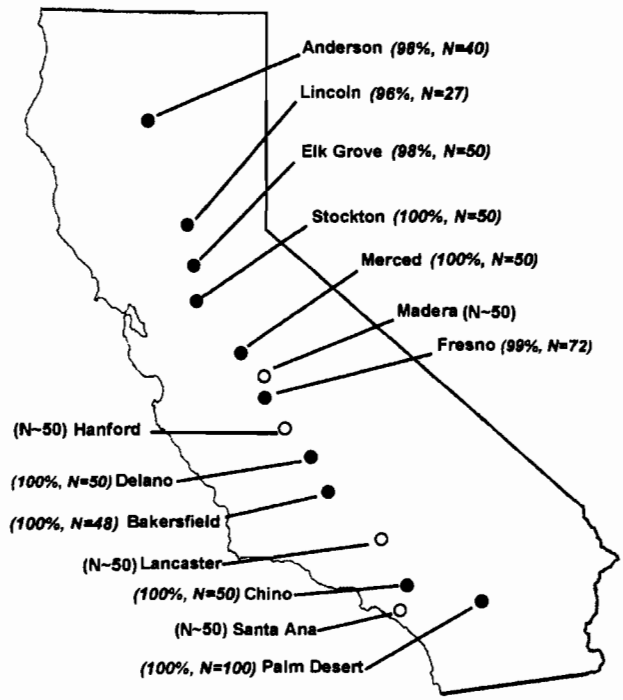

Figure 2. *Culex pipiens* complex collection sites in California and *Wolbachia* infection frequencies. Solid circles denote locations where *Wolbachia* infection frequency was determined. Open circles denote locations where specimens were collected but have not been tested (~50 specimens from each site will be tested).

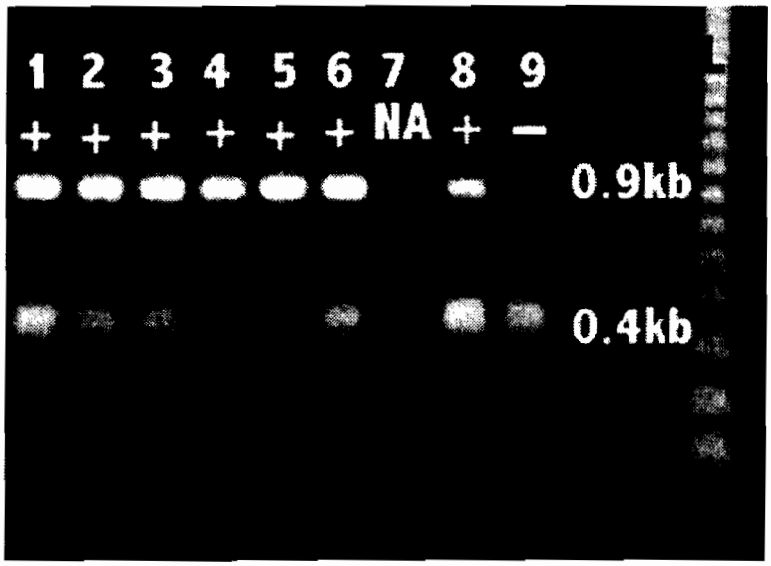

Figure 3. PCR products separated on a 1% agarose gel, stained with ethidium bromide, and visualized by UV light. *Wolbachia*-specific bands are visible at 0.9kb and insect mtDNA control bands are visible at 0.4kb. Specimens 1-6 and 8 = positive for *Wolbachia*. Specimen 9 is negative for *Wolbachia*. Specimen 7 is an example of failed PCR. L denotes 123bp ladder and NA denotes no amplification.

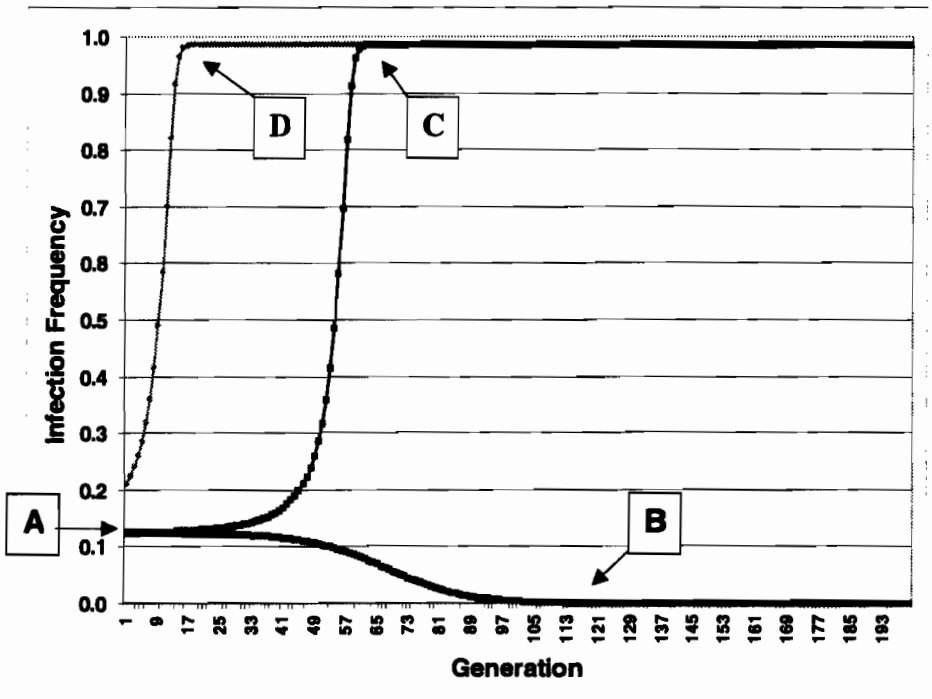

Figure 4. An example of the dynamics of *Wolbachia* spread based on the Turelli and Hoffmann mathematical model where vertical transmission = 90%, incompatibility = 90%, and there are no fecundity effects due to infection. Minimum frequency of infected individuals that must be exceeded to establish stable infection spread is 12.5% (A). Below this frequency, infection will not become established and will be lost in approximately 120 generations (B). If the minimum establishment threshold is exceeded, the infection rate is predicted to reach a stable equilibrium of 98.6 % in approximately 60 generations (C). If initial introduction is higher than the minimum establishment threshold, stable equilibrium will be reached in a correspondingly shorter time period. With an initial introduction of 20%, stable equilibrium of 98.6% will be reached in 18 generations (D).

govern *Wolbachia* spread in natural insect populations. The following parameters have been identified and a mathematical model describing their interactions has been validated for *Wolbachia* spread in natural *Drosophila simulans* populations. If one knows the vertical transmission efficiency, level of incompatibility, and fecundity load of infection, the model can be used to predict (1) whether *Wolbachia* infection will become established in the population and, if so, at what frequency, (2) how many generations establishment will take, and (3), perhaps the most important from a vector modification perspective, the minimum frequency of infected individuals that must be released in order to establish a stable infection in a population of wild mosquitoes (Figure 4).

Vertical transmission, CI, and fecundity load are currently under investigation in both laboratory colonies and field populations of *Cx. pipiens* complex mosquitoes. The extremely high infection frequencies detected in natural California *Cx. pipiens spp.* leads to the following 3 predictions currently being tested: (1) vertical transmission of *Wolbachia* in California *Cx. pipiens spp.* is essentially perfect (i.e., 100% of an infected female's offspring will be infected), (2) the level of *Wolbachia*-induced CI expressed in California *Cx. pipiens spp.* is perfect (i.e., a cross between an infected male and an uninfected female will result in no viable offspring), and (3) there are no major fitness effects (fecundity and survival loads) associated with *Wolbachia* infection in California *Cx. pipiens spp.*

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Epidemiologic Implications of Dynamic Mortality in the Yellow Fever Mosquito

Linda M. Styer

Department of Entomology, University of California, Davis, CA 95616

ABSTRACT: The yellow fever mosquito, *Aedes aegypti*, affects the health of millions of people each year by transmitting the virus that causes dengue fever, one of the most important arthropod-borne viral diseases in the world. Because no licensed vaccine exists to prevent dengue and no clinical cure has been found, disease suppression depends entirely on control of mosquito vector populations. Since the 1950s, medical entomologists have recognized the importance of adult mosquito survival in determining the extent of pathogen transmission and this insight led to the extensive use of adulticides to reduce the daily survival of adult mosquitoes. Models of vector-borne disease spread, such as vectorial capacity, use entomological factors to predict the number of new human infections resulting from a single infected person entering a fully susceptible population. Mosquito survival rate is the most sensitive component of these pathogen transmission models because of the extended incubation period of pathogens in their mosquito hosts. A key assumption of a majority of these models, however, is that mosquitoes experience a constant mortality rate at all ages. I tested this assumption of age-independent mortality by examining the mortality patterns of a large population (>100,000) of *Ae. aegypti*. The resulting daily mortality curves refuted the assumption that mortality is age-independent and revealed distinct age and sex-specific mortality patterns. To determine the effects of dynamic mortality patterns on values of vectorial capacity, I modified the vectorial capacity equation to account for age-dependent mosquito mortality. The resulting estimates of pathogen transmission varied significantly when two different mortality patterns were assumed. A better understanding of the mortality patterns of *Ae. aegypti* will contribute to improved control measures of this and other important vectors of arthropod-borne pathogens.

INTRODUCTION

Female *Aedes aegypti* are the most important vectors of dengue virus, a virus that greatly impacts human health worldwide. Because no licensed vaccine exists to prevent dengue and no clinical cure has been found, disease suppression depends entirely on control of mosquito vector populations. Since the 1950s, medical entomologists have recognized that adult mosquito survival is the most sensitive component of pathogen spread models such as vectorial capacity due to the extended incubation period of the pathogen in the mosquito vector (MacDonald 1952, Garret-Jones 1964, Dye 1992). This insight has led to the extensive use of adulticides to reduce the daily survival of adult mosquitoes.

Considering the importance of mosquito mortality for predicting pathogen transmission, many studies have attempted to quantify the patterns of mortality seen in field and laboratory populations of vector mosquitoes. Using results of previous laboratory and field studies, MacDonald (1952) concluded that most mosquitoes do not survive long enough to experience senescence and therefore mosquito mortality patterns are dictated by age-independent factors such as predation. Clements and Patterson (1981) countered this notion of a constant mortality rate by re-analyzing survival data for wild and laboratory-reared populations of mosquitoes. They found that the Gompertz function (exponentially increasing mortality with age) best described the pattern of mortality for most mosquito species, although for *Ae. aegypti* they got mixed results in which the mortality of a cohort of blood and sugar-fed females was best described by the Gompertz model, whereas the sugar-fed cohort exhibited increasing mortality with age, but was not adequately described by the Gompertz model.

Recently, a third pattern of mortality was detected in mortality studies of large cohorts of two other dipteran species, Mediterranean fruit flies, *Ceratitis capitata* (Carey et al. 1992) and *Drosophila melanogaster* (Curtsinger et al. 1992), in which after an initial increase in fly mortality, the rate of death leveled off and then declined. This mortality pattern,

called the logistic pattern, was detected in these studies due to the large sample sizes (>10,000 *D. melanogaster*, 1.2 million *C. capitata*). Because of the sheer numbers of flies, the researchers were able to capture the deaths of flies that lived to the oldest ages and had increased resolution of the mortality pattern, especially at very young and very old ages (Promislow et al. 1999).

Given the importance of mosquito mortality and the large numbers of studies undertaken to better understand mosquito mortality, few have used population sizes of greater than 1,000 mosquitoes. Therefore, much of the dynamics of mosquito mortality patterns have gone undetected, dynamics which may explain how pathogen transmission can still exist even when vector population levels are very low, or could help identify the most critical period in the adult life of a mosquito that should be targeted by control efforts. Additionally, the effect of dynamic mortality patterns on models of vector-borne pathogen transmission that assume age-independent mortality (Garret-Jones 1964, Focks et al. 1995) is largely unknown, as changes in the predictions of these models have the capacity to greatly alter estimates of entomological thresholds.

Accordingly, the aims of this study were to (1) use a large population of captive *Aedes aegypti* to test the hypothesis that mortality rates change with age and (2) determine if dynamic mortality rates enhance estimates of pathogen transmission from the vectorial capacity model. A better understanding of mortality patterns of this important vector species will increase our understanding of mosquito mortality in general, information that can be used to improve control strategies for a variety of mosquito-borne pathogens.

PROCEDURES

Immature and adult mosquitoes were maintained in a 12 h light-12 h dark photoperiod under controlled environmental conditions (26-29 °C, 25-50% RH). To ensure synchronous emergence, immature mosquitoes were fed a standardized diet and maintained at a constant density. Pupae were collected, placed into 15 cm x 60 cm x 90 cm screened adult experimental cages, and allowed to emerge for a 24-h period. Adult mosquitoes were provided with water and 10% sucrose solution from cotton wicks introduced through ports in the side of each cage.

Each experimental cage was inspected daily at the same time (± 1 h), dead mosquitoes were removed by mouth aspiration and recorded by date and sex. Mortality data from each cage were continuously tabulated in a Microsoft Excel database, which was utilized to construct a cohort life table (Carey 1993). Life table parameters were determined for number living (N_x), fraction surviving (l_x), age-specific survival (p_x), age-specific mortality (q_x), frequency of death (d_x), and expectation of life (e_x).

The effect of dynamic mortality on the vectorial capacity model was determined by modifying the original Garret-Jones (1964) model to include age-specific parameters for daily survival and life expectancy. The original equation describes the number of new inoculations/infective person/day (C):

$$C = \frac{ma^2 p^n}{-\ln p}$$

and is described by the following terms: m = mosquito density, a = mosquito biting rate, p = mosquito daily survival rate, n = extrinsic incubation period, and 1/-ln p = expectation of infective life. The modified equation describes vectorial capacity for the age at which a mosquito first bites an infective person (x):

$$C_x = ma^2 \prod_{i=x}^{x+n} p_i * e_{x+n}$$

The two modified terms in this equation are

$\prod_{i=x}^{x+n} p_i$ = product of the survival rates observed for caged mosquitoes from age x to age $x+n$ and e_{x+n} = expectation of infective life at age x+n when a mosquito is infectious. Mosquito density (m), biting rate (a) and extrinsic incubation period (n) were held constant for all ages in both models.

RESULTS AND DISCUSSION

The deaths of >100,000 mosquitoes (55,997 males and 45,111 females) from 29 experimental cages were recorded. Mean and maximum life spans differed between males (mean = 16 days, max = 71 days) and females (mean = 32 days, max = 92 days). Moreover, there were distinct sex-specific mortality patterns. The mortality rate of male mosquitoes increased rapidly for the first 20 days, after which it leveled off and declined. Females, on the other hand, exhibited a lower initial mortality rate that steadily increased with age.

The observed sex-specific mortality difference could be due to the different dietary requirements of male and female mosquitoes. Male *Ae. aegypti* feed

exclusively on carbohydrates while females feed primarily on human blood, but can also utilize carbohydrates for energy (Edman et al. 1992). In this study, males received their complete diet while females received a diet restricted to carbohydrates that lacked blood. Large-scale mortality studies of female Mediterranean fruit flies demonstrated that flies fed a restricted diet lived longer than those fed a full protein diet (Carey et al. 1998). The restricted diet of female mosquitoes in this study may have placed them into a non-reproductive "waiting mode" which may explain their longer lifespan compared to males.

Mortality patterns of both male and female mosquitoes were dynamic with respect to age, refuting the assumption that mortality is age-independent. This result has important implications for vector-borne disease epidemiology because current models of vector-borne pathogen transmission assume that mosquito mortality is constant at all ages. Considering that vector mortality is the most sensitive component of the vector-borne disease transmission cycle (Dye 1992) the current use of an average or constant mortality rate without an attempt to identify the underlying pattern of mortality results in vectorial capacity estimates that underestimate the complexity of the arbovirus transmission cycle.

Results from this analysis indicate that, using laboratory results, the pattern of vector mortality can cause estimates of vectorial capacity to vary significantly. Fig. 1 shows two hypothetical types of dynamic mortality patterns, a quickly accelerating mortality rate that levels off and declines, and an initially low but steadily increasing pattern of mortality. These patterns have the same average daily mortality of 0.10. When values from these mortality patterns are used in the modified vectorial capacity model (Eqn. 2), the resulting estimates of pathogen transmission are very different (Fig. 2). Low initial mortality (<0.01) leading to a peak mortality of 0.15 by day 30 (A, Fig. 1) allows mosquitoes that bite an infected person early in their life to survive through the extrinsic incubation period. These infectious mosquitoes, however, have a very short life expectancy because of high mortality early in life. The reduction in pathogen transmission efficiency with this type of mortality pattern is apparent in that after day 15, vectorial capacity values are no longer high enough (vectorial capacity <1) to sustain continued pathogen transmission (A, Fig. 2). Moderately low mortality (<0.1) for the first 30 days (B, Fig. 1) results in a larger percentage of mosquitoes that live through the extrinsic incubation period and results in a relatively long expectation of infective life. This pattern of mortality translates into initially high vectorial capacity values that would support continued pathogen transmission (vectorial capacity >1) in mosquitoes that bite an infected person up to 23 days of age (B, Fig. 2).

If these mortality patterns characterized two populations of competent mosquitoes, their estimates of vectorial capacity would be identical under the assumption of age-independent mortality (Eqn. 1). However, considering the dynamic nature of mortality rates, the potential for these two populations to spread pathogens is very different. Having this type of detailed knowledge of mosquito mortality patterns would allow control personnel to direct adulticides at the population with the highest potential for pathogen spread, leading to better control of vector-borne diseases.

From this type of analysis, it is clear that a more thorough understanding of the patterns of mortality experienced by mosquitoes is necessary for accurate predictions of pathogen transmission. Age-specific patterns of mortality determine the age structure of mosquito populations and consequently the proportion of the population that is old enough to potentially be infectious. In addition, these patterns determine the life expectancy of infectious mosquitoes and thus the number of new humans inoculated with the pathogen. There is a need to determine if mosquitoes that are fed blood and are infected with dengue virus will exhibit mortality patterns that are similar to the ones in this study. In future studies, I plan to investigate the effects of blood-feeding and dengue infection on mortality rates of laboratory reared mosquitoes.

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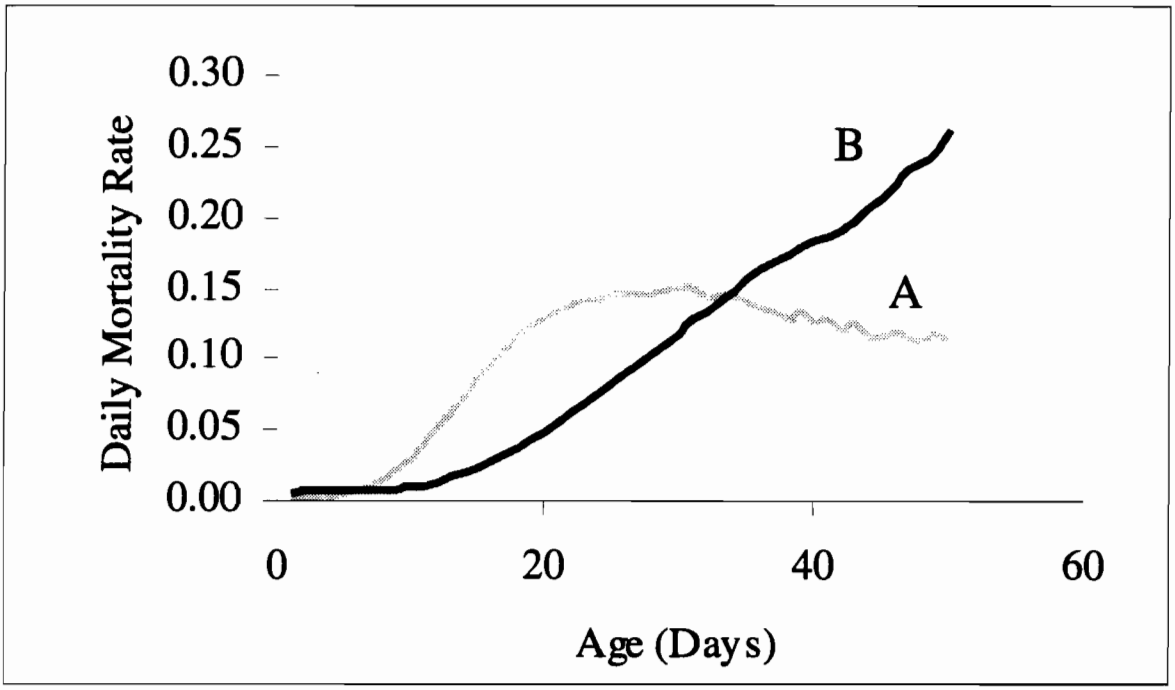

FIGURE 1. Two hypothetical patterns of age-specific mortality with the same average mortality rate of 0.10, a quickly accelerating mortality rate that levels off and declines (A), and a steadily increasing mortality rate (B).

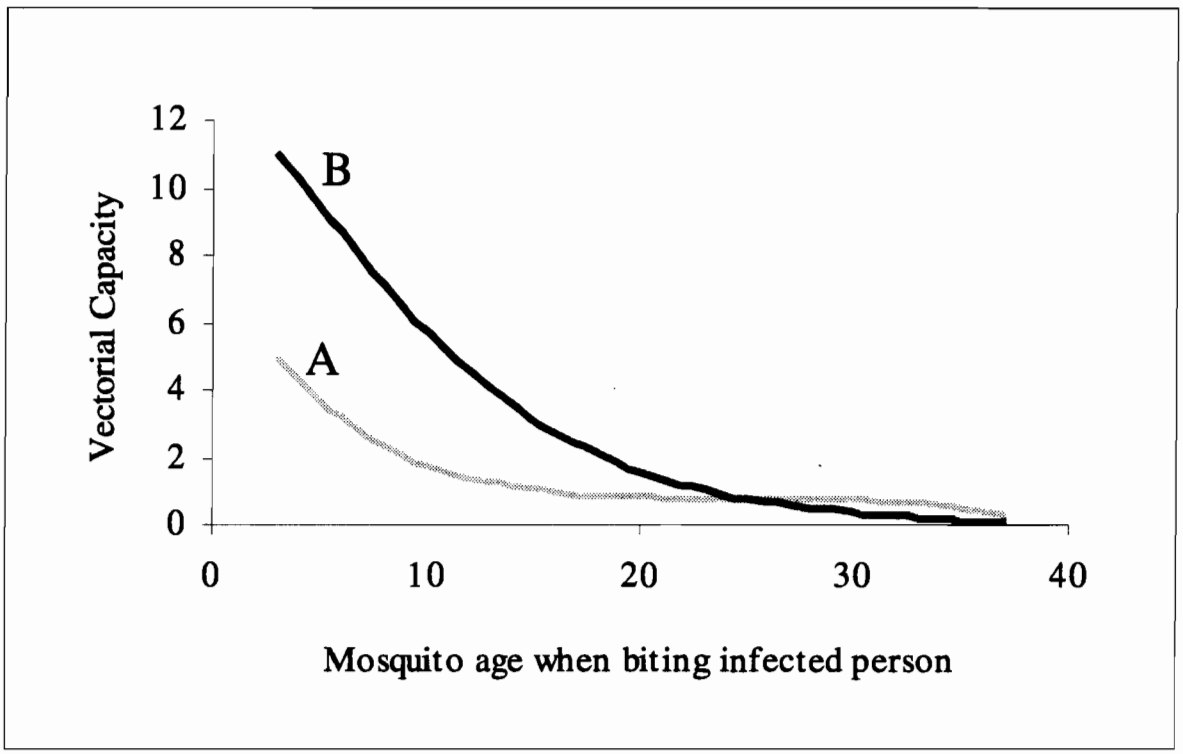

FIGURE 2. Dynamic estimates of vectorial capacity for the ages at which a mosquito first bites an infected person for two mortality patterns shown in Figure 1 using the modified vectorial capacity equation (Eqn. 2). The static parameters used in the equation were $m = 1.75$, $a = 0.75$, and $n = 13$.

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Mosquito Abundance and Arboviral Activity in San Bernardino County During 1999

Lal S. Mian1/, Chris N. Nwadike and Lesli Allsup

San Bernardino County Vector Control Program, Environmental Health Services/Public Health
2355 East Fifth Street, San Bernardino, CA 92415-0064

ABSTRACT: Of the 8,079 mosquitoes collected in New Jersey light traps and CO2-baited CDC type traps in San Bernardino County in 1999, the most abundant mosquito was *Culex tarsalis* (53.9%), followed by *Culex quinquefasciatus* (16.9%), *Culex stigmatosoma* (13.5%), *Culiseta incidens* (9.7%) and Culex *erythrothorax* (2.5%). Other species found in small numbers included *Aedes sierrensis*, *Aedes vexans*, *Anopheles franciscanus* and *Anopheles feeborni*. Species abundance and composition between regions varied significantly. All 110 pools of culicine species submitted for testing were found negative for both Saint Louis encephalitis (SLE) and western equine encephalomyelitis (WEE) viruses. The Needles chicken flock showed two seroconversions to SLE in September and October.

INTRODUCTION

The San Bernardino County Vector Control Program (SBCVCP) conducts encephalitis virus surveillance (EVS) and mosquito control operations in all areas of San Bernardino County except for areas served by West Valley Vector Control District and some non-member cities. The county with three distinct biotopes— the desert, mountain and valley, has a majority (>80%) of its >1.6 million human population residing in the valley area. The remaining population is scattered over various parts of the desert and mountain regions. Historically, cases of Saint Louis encephalitis and western equine encephalomyelitis have been reported in the desert and valley regions from time to time. During the past 13 years, there were five confirmed human cases of Saint Louis encephalitis in the county (Emmons et al. 1988, 1989, 1994; Reilly et al. 1995). During the same period, activities of both SLE (Saint Louis encephalitis), and WEE (western equine encephalomyelitis) viruses were reported in the desert region—Needles and adjoining areas along the Colorado River.

Data generated in EVS activities in 1999 are presented here in relation to mosquito abundance and arboviral activity.

MATERIALS AND METHODS

EVS procedures described by Mian and Prochaska (1990) were continued as follows:

Adult Mosquito Population Dynamics

The abundance of various mosquito species was monitored weekly by the use of New Jersey light traps. The number and locations of these traps along with procedures for identification and processing were the same as last year (Mian et al. 1999).

Arboviral Activity in Female Mosquitoes

The procedure and frequency of mosquito collection in the desert and valley areas were similar to those reported by Mian et al. (1999). Female mosquitoes collected overnight were processed in the same manner as done in 1997 (Mian et al. 1998). Mosquito testing for virus activity was done at the Center for Vector-Borne Disease Research, University of California (U.C.), Davis.

Arboviral Activity in Sentinel Chickens

The number and locations of sentinel flocks and blood sampling and testing protocol were a repeat of last year (Mian et al. 1999).

1/ Department of Health Science, California State University, San Bernardino, CA 92407-2397

RESULTS AND DISCUSSION

Data on mosquitoes collected in New Jersey light traps and CO2-baited CDC traps were used to calculate the faunal species composition in 1999 (Table 1). Of the total 8.079 adult mosquitoes collected, 56.3% were from the former type of traps and 43.7% from the latter. The most abundant mosquito was *Culex tarsalis* Coquillet (53.9%), followed in descending order by *Culex quinquefasciatus* Say (16.9%), *Culex stigmatosoma* Dyar (13.5%), *Culiseta incidens* (Thompson) (9.7%), and *Culex erythrothorax* Dyar (2.5%). Other species found in small numbers included *Aedes* and *Anopheles* species. *Anopheles freeborni* made 0.4%, *Anopheles franciscanus* McCracken 0.3%, *Aedes vexans* 0.4% and *Aedes sierrensis* (Ludlow) 0.2% of the total mosquitoes captured. *Culex. tarsalis*, *Cx. erythrothorax*, *Cs. inornata*, *Ae. vexans* and *An. franciscanus* were found in significantly higher numbers in the desert than in the mountains and valley areas. Adversely, *Cx. quinquefasciatus*, *Cx. stigmatosoma*, *Cs. incidens*, *Ae. sierrensis* and *An. freeborni* were higher in the valley and mountains than in the desert region.

A total of 110 pools of culicine mosquitoes (*Cx. tarsalis, Cx. quinquefasciatus* and *Cx. stigmatosoma*) collected in CO2-baited CDC type traps in the desert and valley areas, tested negative for both SLE and WEE viruses.

The results on chicken serology showed two seroconversions to SLE virus in the Needles flock only. The first seroconversion was reported on September 17 (bled Sep. 7, 1999), and the second on October 18 (bled Oct. 8, 1999). Last year's flock at this site had two sero-conversions to WEE and none to SLE.

Upon receipt of confirmation of seroconversions in the desert region, the areas were posted with "Encephalitis Warning" signs followed by press releases to local newspapers advising residents to take necessary precautions to avoid mosquito bites, during outdoor activities especially at dusk and dawn in the affected areas. In the wake of virus activity, mosquito source reduction and control activities were intensified in the area. During the 1999 EVS season (May through

Table 1. Percent species composition of adult mosquitoes caught in different traps in San Bernardino County during 1999.

	Mosquito composition by area					
	CO2-baited traps		NJ light traps		Total	
	Number	%	Number	%	Number	%
Aedes sierrensis	3	<0.1	11	0.3	14	0.2
Aedes vexans	0	0	33	0.9	33	0.4
Anopheles franciscanus	3	<0.1	25	0.7	28	0.3
Anopheles freeborni	29	0.6	0	0	29	0.4
Culex erythrothorax	8	0.2	197	5.6	205	2.5
Culex quinquefasciatus	1,222	26.9	146	4.1	1,368	16.9
Culex stigmatosoma	434	9.5	657	8.6	1,091	13.5
Culex tarsalis	2,735	60.1	1,616	45.8	4,351	53.9
Culiseta incidens	114	2.5	669	19.0	783	9.7
Culiseta inornata	0	0	177	5.0	177	2.2
Total (A)	**4,548**	**100**	**3531**	**100**	**8,079**	**100**
% of Total	**56.3**		**43.7**			
Trap-nights (B)	**194**		**3402**			
Mosquito Index (A/B)	**23.4**		**1.0**			

October), no human case of mosquito-borne encephalitis occurred in the SBCVCP territory.

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Surveillance for Rodent-Borne Pathogens in San Bernardino County During 1999

Lal S. Mian[1], Chris N. Nwadike and Charles Fulhorst[2]

San Bernardino County Vector Control Program, Division of Environmental Health services
Department of Public Health, 2355 East 5th Street, San Bernardino, CA 92415-0064

ABSTRACT: The San Bernardino County Vector Control Program carried out routine surveys for bacterial and viral pathogens in local rodent populations during 1999. Of 13 plague surveys involving 137 rodents (97% California ground squirrel, *Spermophilus beecheyi*), none of the sera tested positive for *Yersinia pestis* antibody.

In 16 Hantavirus surveys, 5.3% of the total animals (212) tested sero-positive for the Sin Nombre virus (SNV). SNV- positive animals included 5 deer mice, *Peromyscus maniculatus*, 4 western harvest mice, *Reithrodontomys megalotis*, 2 California voles, *Microtus californicus*, and one each of California mice, *Peromyscus californicus*, pinyon mice, *Peromyscus truei* and dusky-footed wood rats, *Neotoma fuscipes*.

In Arenavirus surveys including 143 small rodents, 5 (3.5%) tested seropositive for arenaviruses. Of the five animals, two *P. maniculatus*, one *Chaetodipus formosus*, and one *N. fuscipes*, were positive for the Amapari virus, and one *P. maniculatus* for the Whitewater Arroyo virus.

INTRODUCTION

Rodent-borne diseases have been found in San Bernardino County from time to time. For decades, enzootic and occasionally epizootic plague activity in rodent populations have been reported in the foothills and mountain habitats, necessitating regular surveillance efforts during the summer months (Mian and Hitchcock 1998). Of the Hantaviruses, the Sin Nombre virus (SNV) activity was first detected in San Bernardino County in deer mice, *Peromyscus maniculatus*, and cactus mice, *Peromyscus eremicus*, in the Needles area along the Colorado river in 1996 (Mian 1997). Similarly, activity of the little known Arenavirus, Whitewater Arroyo virus (WWA), was first detected in a deer mouse in the Devil's Canyon area, San Bernardino, in 1998 (Mian, unpublished data). WWA was first isolated and characterized from the southwestern U.S. by Fulhorst et al.(1996). Detailed information including serological grouping of arenaviruses has been described in the literature (Fulhorst et al. 1996, Kosoy et al. 1996).

The San Bernardino County Vector Control Program (SBCVCP) conducts surveillance programs for plague, Hantavirus and Arenaviruses in selected areas of the county. Data generated in the surveillance of rodent-borne pathogens in 1999 are discussed here.

MATERIALS AND METHODS

The general methodology used in the surveillance was similar to Mian et al.(1999). In plague surveillance, national traps were used to collect diurnal rodents such as ground squirrels and chipmunks.

In both Hantavirus and Arenavirus surveys, Sherman traps were used to trap nocturnal mice. Blood samples collected from ground squirrels and chipmunks on nobuto strips, were sent to the California Department

[1] Department of Health Science, California State University, San Bernardino, CA 92407-2397.
[2] Department of Pathology, Medical Branch, University of Texas, Galveston, TX 77555-0609

of Health Services for plague testing at the University of California, Davis. Mice sera for SNV testing were mailed to the Viral and Rickettsial Disease Laboratory in Berkeley and those for Arenavirus to the University of Texas Medical Branch at Galveston.

For plague and Hantavirus activity, the protocol used for public education was similar to that described by Mian and Hitchcock (1998).

RESULTS AND DISCUSSION

In plague surveillance, 13 surveys were carried out from May through October (Table 1). A total of 187 animals, including 183 (97%) *Spermophilus beecheyi*, 3 (2%) *Ammospermophilus leucurus* and one (<1%) *Tamias merriami*, were tested. None of the animals tested positive for the *Yersinia pestis* antibody. The flea index ranged from <1 to 4.8. Plague activity in wild carnivores was reported by the California Departmenet of Health Services (Charles Myers, personal communication). Two coyotes sampled on August 17 and 18 at the Green Valley Lake and one on August 25 at San Antonio Heights, were sero-positive for plague.

Earlier in the season, the Serrano campground in the Big Bear area, which was closed down due to plague activity late in 1998, was treated in mid-May, using Diazinon 2D in bait stations, to control fleas. The campground was reopened in time for the Labor day weekend after a post-treatment evaluation for the flea index was performed.

In Hantavirus surveillance, 16 surveys were carried out during 1999. Based a total of 212 rodents trapped, the percent faunal composition in these surveys was *Peromyscus maniculatus* (25.0%), *Reithrodontomys megalotis* (14.6%), *Permyscus boylii* (11.3%), *Peromyscus truei* (11.3%), *Neotoma fuscipes* (9.0%), *Mus musculus* (5.7%), *Peromyscus californicus* (5.7%), *Peromyscus eremicus* (5.7%), *Chaetodipus formosus* (2.8%), *Peromyscus crinitis* (2.8%), *Microtus californicus* (2.3%), *Neotoma lepida* (1.9%), *T. merriami* (1.4%) and *Rattus rattus* (0.5%). As shown in Table 2, chronological SNV activity was detected at different sites—Colton, Highland, San Bernardino, Devore, Fontana and Barton Flats area, demonstrating a wide distribution of viral activity in the areas surveyed so far. SNV activity was found in samples from *P. maniculatus*, R. *megalotis*, *M. californicus* and *N. fuscipes*. Each SNV activity prompted press releases to the local news media, advising residents to take precautionary measures in the affected areas.

Surveillance for Arenaviruses began in late1998 when one *P. maniculatus* out of 25 different rodents was found positive for WWA in the Devil's Canyon area, San Bernardino. Based on a total of 143 animals collected in 1999, *P. maniculatus* was the most abundant species (28.7%), followed by *R. megalotis* (23.0%), *P. californicus* (20.3%), *P. eremicus* (6.3%), *P. boylii* (5.6%), *N. fuscipes* (4.9%), *C. formosus* (3.5%), *P. truei* (2.8%), *N. lepida* (2.1%), *P. crinitis* (1.4%), and *M. californicus* (1.4%). As shown in Table 3, one *P. maniculatus* was positive for WWA in the Forest Falls area. From two separate sites in Redlands, four rodents (two *P. maniculatus*, one *C. formosus* and one *N. fuscipes*, tested sero-positive for the Amapari virus, serologically quite different from WWA. Efforts to isolate viruses from the tissues did not yield positive results. More surveillance over a larger area is required in order to get a better understanding of the distribution and prevalence of these viruses in natural rodent populations in San Bernardino County.

Table 1. Plague surveillance in San Bernardino County, 1999.

Date	Location	Animals tested a/	Ectoparasites	Flea index:
05/13	Serrano CG*	12	1	<1.0
06/22	San Gorgonio CG	19	60	3.2
06/28	Apple White CG	23	69	3.0
08/17	Green Valley Lake CG	21	55	2.6
08/25	Goffs Station/I-40	3b/	0	0
08/30	Rio Barranca GC**	16	77	4.8
08/31	Crab Flat CG	7	19	2.7
08/31	Childrens Forest CG	4c/	6	1.5
09/27	Big Falls CG	7	14	2.0
09/28	Heart Bar CG	14	5	0.4
10/12	El-Ranch Verde Ctry/Club	2	6	3.0
10/25	Littlefield S. Park	8	10	1.2
10/27	Miller Canyon GC	1	2	2.0

*CG-Campground, **GC-Group Camp

a/ *Spermophilus beecheyi*

b/ All *Ammospermophilus leucurus*

c/ one *Tamias merriami*, three *S. beecheyi*

Table 2. Distribution of Sin Nombre virus activity in San Bernardino County, 1999.

Date	Location	# animals tested	# sero-positive
02/04	Flood Channel, Colton	26	1
02/18	Shelton Trails, Highland	40	2
03/31	2N49/E. Palm, San Bernardino	28	1
04/16	Cajon Blvd/FS, Devore	34	2
05/06	4470 Lytle Creek Rd, Lytle Creek	8	0
05/12	N. Cherry/Coyote Cr., Fontana	18	7
07/08	N. Mountain Dam, Upland	9	0
07/14	Flood Channel, Upland	4	0
08/04	1N69, Angeles Oaks	15	0
08/06	Redford Camp Rd, Barton Flats	23	1
08/13	Trona	6	0
08/18	Green Valley Lake Campground	15	0
08/19	Crab Flat Campground	3	0
08/25	Sweeny Granite MT. Center	12	0
09/09	10,000 Blk Edison Ave., Chino	7	0
11/03	Devil's Canyon, San Bernardino	17	0
	Total	265	14 (5.3%)

Table 3. Distribution of arenaviruses in San Bernardino County, 1999.

#Positive Date	Location	# Animals Tested	# Positive APV*	WWA**
01/08	Alabama St., Redlands	9	1	0
01/15	Thurman Flats, Forest Falls	10	0	1
01/29	Pet Cemetery, Devore	28	0	0
03/04	Kilkare Road Picnic Park	23	0	0
03/12	Oak Glen Park	7	0	0
07/20	Kilkare Picnic Site	8	0	0
07/29	Green Spot Dam, Mentone	5	0	0
07/30	2N56, Upper Lytle Creek	30	0	0
09/17	Alessandro FC, Redlands	10	3	0
12/09	Cleghorn Rd., Cajon Pass	13	0	0
	Total	**143**	**4**	**1**

*APV-Amapari virus
**WWA-Whitewater Arroyo virus

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