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PROCEEDINGS AND PAPERS

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The Cooperative Agreement Between the California Department of Health Services and Local Vector Control Agencies

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HISTORY OF THE COOPERATIVE AGREEMENT

The early 1960's were witness to a dramatic rise in popular and scientific concern about the effects that pesticides might have on human health and on the environment. Governmental regulations over the use of pesticides increased in response to these concerns. By the middle of the decade, it was becoming clear that the beneficial use of pesticides to control vectors was in jeopardy as local public health agencies were finding it difficult to comply with increasingly stringent government regulations. In protecting the public from the dangers of pesticides (both real and perceived), the public was being placed at increased risk from diseases transmitted by mosquitoes and other vectors.

During the mid-1960's, some California public health leaders began seeking a means to comply with state and federal regulations regarding the use of pesticides while protecting the judicial use of pesticides to control disease vectors and pests. In 1967, the California State Board of Health adopted a policy statement entitled "Recommended Standards Relating to the Use of Pesticides in Vector Control." This policy statement stressed that pesticide use should be limited to those vector populations which cannot be controlled practicably by other means. The State Board of Health clearly recognized the need for the evolution of vector control from a reliance on pesticide application to a program of integrated pest management (IPM) that included source reduction and public education in addition to the judicious use of pesticides. The standards presented in this policy statement were compatible with the pesticide use requirements of the California Department of Agriculture and were intended to serve as conditions for future cooperative agreements between the Department of Public Health and local vector control agencies.

On the recommendation of the State Board of Health, the Department of Public Health published a document entitled "Acceptable Pesticides and Their Use by California Mosquito Abatement Districts and Other Official Mosquito Control Agencies." This document included an "official list of pesticides" to be used for vector control in California and specified how these pesticides were to be used. Like the State Board of Health, the Department of Public Health emphasized the use of preventive measures directed toward the elimination of mosquito sources while also recognizing that the judicial use of pesticides was needed for mosquito control agencies to meet their legal requirement to protect the public from disease-transmitting mosquitoes and other vectors. This document also directed that agencies apply specific principles of pesticide use to protect the health of humans, domestic animals, wildlife and other non-target organisms. These principles included precision of targeting and timing to ensure pesticide application only to areas actually producing or harboring vectors, and the use of proper formulation and dosage of pesticides to protect public health and minimize non-target effects.

Concurrent with these publications, state and local public health leaders made convincing arguments to the Department of Agriculture that pesticides used for vector control were critical to protect the public from vector- transmitted diseases and furthermore, that these pesticides posed little or no significant risk to human health or the environment when properly used as per the product label at low dosage rates typical of vector control operations. The Department of Agriculture agreed and amended its regulations to allow local agencies working cooperatively with the Department of Public Health to apply pesticides for vector control that were defined as "injurious materials."

The first "Cooperative Agreement" between the California Department of Public Health and local vector control agencies was established in 1967. The purpose of this agreement was to:

"Provide for the protection of the public health and comfort through a coordinated program of safe, effective, and economical use of pesticides in the control of mosquitoes, by qualified local governmental mosquito control agencies organized and operated in accordance with provisions of the California Health and Safety Code."

By signing this cooperative agreement, local vector control agencies ("cooperating agencies") agreed to 1) use those pesticides listed on the Department of Public Health "official list of pesticides" only in the manner specified, 2) maintain pesticide use reports for review by appropriate governmental agencies, and 3) ensure that pesticide use did not result in harmful residues on agricultural products. In return, cooperating agencies were authorized to use pesticides listed on the Department of Public Health "official list of pesticides" even though these pesticides may be defined as "injurious materials" by the Department of Agriculture. Cooperating agencies were also granted significant exemptions from the legal requirements for property owner consent and notification of persons on property to be treated prior to a pesticide application.

During the first year of the cooperative agreement, 49 local vector control agencies signed this agreement with the Department of Public Health (Womeldorf 1976), and by 1969, nearly all local

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vector control agencies had signed a cooperative agreement. By 1974, the number of cooperating agencies had increased to 73 and the Department of Public Health included in the cooperative agreement a program to provide for training and certification of pesticide applicators as required by the 1972 amendments to the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA, as amended) (Womeldorf 1976).

From 1974 through 1991, revisions to the cooperative agreement were coordinated between the Department of (now) Health Services, the Department of (now) Food and Agriculture, the Department of Fish and Game, and cooperating agencies involved in vector control. In 1991, the California Department of Pesticide Regulation was formed and all pesticide-related statutory authority was transferred to this department from the Department of Food and Agriculture (DPR 2001). The local enforcement of pesticide use was retained by the County Agricultural Commissioners. To protect the benefits of the cooperative agreement, and in recognition of the shared responsibility of the Department of Health Services, the Department of Pesticide Regulation, and the County Agricultural Commissioners to protect human health, these three agencies signed a memorandum of understanding (MOU) to address the use of pesticides in vector control. The current MOU (Anon. 1995) assures that each agency is able to exercise appropriate legal authority to protect public health while eliminating any duplication of effort. Principles of agreement are established in the MOU that identify the roles of the three signatory agencies with respect to pesticide use and reporting, registration of public health pesticides, certification of public health pesticide applicators, and reporting of suspected adverse effects of pesticides on non-target organisms. This MOU essentially shifts some regulatory authority for pesticide use by vector control agencies from the Department of Pesticide Regulation to the Department of Health Services and serves as the basis for the cooperative agreement between the Department of Health Services and vector control agencies.

REQUIREMENTS OF THE COOPERATIVE AGREEMENT

Changes in Federal and State statutes coupled with changing departmental responsibilities and relationships have resulted in some significant changes from the first cooperative agreement. For example, the Department of Health Services no longer publishes an "official list of pesticides" for use in vector control. Pesticides used for vector control must now be labeled for this use and must be used in accordance with the product labeling.

During 2002, there were 73 vector control agencies that were signatory to the cooperative agreement. Signatory agencies must agree to:

1) Calibrate all application equipment and maintain all calibration records for review by the County Agricultural Commissioner.

2) Maintain pesticide application records for at least two years for review by the County Agricultural Commissioner.

3) Submit a monthly pesticide use report on Department of Pesticide Regulation form PR-ENF-060 to the County Agricultural Commissioner.

4) Report any conspicuous or suspected adverse effects upon humans, domestic animals or other non-target organisms to the County Agricultural Commissioner and the Department of Health Services.

5) Require employee certification by the Department of Health Services to verify employee competence to use pesticides in vector control operations and to maintain continuing education unit information for those employees participating in continuing education.

6) Be inspected by the County Agricultural Commissioner on a regular basis to ensure that the agency is in compliance with state and federal laws and regulations pertaining to the storage and use of pesticides.

The cooperative agreement includes the requirements listed above in order to meet the legislative intent in providing the many broad exemptions to California laws and regulations (described below) provided to vector control agencies and also to ensure that all state and federal pesticide use requirements are met.

BENEFITS OF THE COOPERATIVE AGREEMENT

Under the inter-departmental MOU, the Department of Health Services agreed to continue oversight of the examination, certification, and continuing education of employees of local vector control agencies who handle, use or supervise the use of pesticides in public health programs for the management of vectors. A local vector control agency may not enter into a cooperative agreement with the Department of Health Services pursuant to Health and Safety Code Section 116180 unless agency employees responsible for the application of pesticides have received pesticide applicator certification from the department. This program ensures that employees of cooperating agencies are properly trained in the safe application of pesticides and that they receive continuing education that meets all state and federal standards. Agency personnel benefit by receiving continuing education that is targeted toward the safe handling and application of pesticides used in public health vector control operations in California. In 2002, there were 1,143 employees at 103 agencies (73 of these agencies were signatory to the cooperative agreement) who held a pesticide applicator certification from the Department of Health Services.

In addition to the benefit of a targeted continuing education program, cooperating agencies also receive a number of significant exemptions to state laws and regulations that would apply to any other person or agency involved in the application of pesticides. These exemptions are granted due to the unique public health role of local vector control agencies. Furthermore, pesticides used by these agencies pose little or no significant risk to human health or the environment when properly used as per the product label in targeted control operations at low dosage rates typical of mosquito and vector control operations (Rose 2001).

Exemptions for cooperating agencies can be found in various statutory codes as well as in Title 3 of the California Code of Regulations (3CCR). The following are exemptions currently granted to cooperating agencies:

- **Education Code, Section 17613**. Cooperating agencies are exempted from the notification and posting requirements for pesticide applications at a school facility (section 17612). School

districts do not have to provide annual or pre-application notification to parents and staff of an intended pesticide application by a cooperating agency. School districts are also not required to post warning signs at school facilities that are treated by a cooperating agency.

· **Food and Agriculture Code, section 11408(e).** The use of pesticides by a cooperating agency is excluded from the definition of "agricultural use." As a result of this exclusion, cooperating agency personnel are not required to:

- Hold an agricultural pest control advisor license (section 11410, 12001)
- Register with the County Agricultural Commissioner (section 12002)
- Place pesticide use recommendations in writing (sections 11411, 12003)
- Obtain an operator identification number from the County Agricultural Commissioner (3CCR 6622)
- Maintain pesticide use records in accordance with 3CCR 6624 and submit a monthly summary of pesticide use report to the Agricultural Commissioner as per 3CCR 6627. Note: The cooperative agreement requires the maintenance of pesticide use records and the submission of monthly pesticide use reports to the County Agricultural Commissioner, thereby annulling this exemption.

· **Health and Safety Code, section 25174.7(a)(3).** Cooperating agencies are not required to pay a fee for any hazardous waste generated or disposed of as a result of their control or regulatory activities (sections 25174.1 and 25205.5).

· **3CCR 6400(c)(2)and 6400(e). Restricted Materials.** Exempts certain pesticides used by cooperating agencies from being designated as "restricted materials" by the Director of the Department of Pesticide Regulation. This exemption precludes the requirement to have a permit issued by the Agricultural Commissioner for each use of these pesticides (3CCR 6420).

· **3CCR 6620(a). Vector Control Exemption.** Exempts cooperating agencies from 3CCR 6614 (Protection of Persons, Animals, and Property), 6616 (Consent to Apply), and 6618 (Notice). Cooperating agencies may therefore apply pesticides registered for the purpose of vector control in residential areas even though there may be a reasonable possibility of contamination to non-target persons or property. In addition, cooperating agencies are not required to get property owner consent or provide notification to a property operator prior to a pesticide application. These exemptions are undoubtedly the most important benefit provided to vector control agencies that are bound by the cooperative agreement and reflect the general understanding that vector control operations protect public health and that rapid control or suppression of vectors over wide geographic areas is essential to achieve this protection. Cooperating agencies have neither the time nor the resources to provide notice or acquire consent prior to the application of a public health pesticide.

· **3CCR 6651. Vector Control Exemption.** Exempts cooperating agencies from 3CCR 6650-6656 (Article 3: Protection of Bees) when pesticides are diluted in one half gallon of water or more per acre treated. Cooperating agencies are not required to provide prior notification of pesticide application to beekeepers with apiaries within one mile of the application site. Cooperating agencies are also not required to provide notice to the Agricultural Commissioner prior to the application of pesticides in a legally defined citrus/bee protection area.

· **3CCR 6760. Employer Responsibility and Exceptions.** Cooperating agencies are exempted from 3CCR 6760-6776 (Article 3: Field Worker Safety) when conducting area-wide pesticide applications. Cooperating agencies are not required to:

- Provide hazard communication information to agricultural field workers (3CCR 6761)
- Ensure that persons are not present in areas to be treated (3CCR 6762)
- Provide training in the areas of pesticide exposure and personal rights to agricultural field workers (3CCR 6764)
- Identify a nearby emergency medical facility that will treat workers exposed to pesticides (3CCR 6766)
- Provide a decontamination facility for agricultural field workers that is within ¼ mile of the pesticide application area (3CCR 6768)
- Prevent re-entry of persons into a treated field (3CCR 6770, 6771, 6772, 6774)
- Post warning signs around treated fields (3CCR 6776)

In summary, the cooperative agreement between the Department of Health Services and local vector control agencies has provided cooperating agencies with the flexibility to perform their legally mandated role to control public health vectors while ensuring that all state and federal requirements regarding the application of pesticides are met.

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The authors would like to express their gratitude to Mac Takeda (California Department of Pesticide Regulation, Pest Management and Licensing Branch) for reviewing this article.

Investigation and Management of Epizootic Plague in the Truckee-Donner Area, California

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ABSTRACT: In September 2002, an epizootic of plague and die-off of chipmunks (*Tamias* spp.) were identified in Truckee, California, including Donner Memorial State Park. An investigation of the apparent die-off and subsequent suppression of rodent fleas was carried out in the park's campground and day-use areas to reduce the risk of human exposure to fleas carrying *Yersinia pestis*. Forty-nine sciurid rodents, representing six species, were captured and examined for fleas. An average of 10.1 fleas per animal was observed, indicating the need for flea reduction measures. Diazinon insecticide was applied to the campground and day-use area using hand dusting of rodent burrows and bait stations. Liquid deltamethrin was also used in carpeted bait stations near Donner Lake. Insecticide application was sustained for 14 days. Post-treatment evaluation indicated that the average flea index was reduced to 0.9 fleas per animal. Park personnel reopened the day-use area; however, park officials decided to keep the main campgrounds closed until the spring of 2003.

INTRODUCTION

Plague was introduced into California at the beginning of the Twentieth Century and became established in the state's sylvatic rodent populations by 1908 (Link 1955). Since its introduction, the causative agent of plague, the bacterium *Yersinia pestis,* has been identified in humans, wild carnivores, domestic pets, and commensal or wild rodents in 49 of California's 58 counties (California Department of Health Services [CDHS] Plague Records, 1909-2002).

Plague epizootics among sylvatic rodents resulted in human cases in the Lake Tahoe area and the Sierra Nevada Mountains as early as 1936 (Meyer 1942). Since the 1970s, increasing development and recreational activities have artificially enhanced rodent habitat and food availability and subsequently contributed to unnaturally dense populations of plague-susceptible rodents and a resurgence of plague activity in the Sierra Nevada (Smith and Lusk 1990). Plague records maintained by CDHS indicate that plague activity within the Truckee-Donner area is part of an apparent geographical plague focus that encompasses the Tahoe-Truckee and Tahoe Valley ecological subsections and much of the batholithic and volcanic flows that surround the area. Within this focus, a cyclical disease pattern exists that is characterized by periodic epizootic die-offs of susceptible rodent species, separated by inter-epizootic quiescent periods (Smith et al. 1994).

Epizootic plague among wild rodents in residential and recreational areas increases the risk of transmission to humans through the bites of infected rodent fleas and from plague-infected domestic cats. In these areas, epizootic plague necessitates a prompt response from local and state health officials. In accordance with California's statewide public health mandates, the CDHS Vector-Borne Disease Section (VBDS) oversees an integrated plague surveillance and control program (Smith et al. 1994) and may recommend control measures to the responsible agency when evidence of an epizootic is detected.

RECENT PLAGUE HISTORY IN THE TRUCKEE-DONNER AREA

Epizootic activity at several Tahoe-Truckee-Donner recreational areas during 1999-2001 prompted suppression measures to reduce plague vectors (fleas) and lower the public health risk. The affected recreational areas were temporarily closed and flea suppression measures were undertaken at Loggers campground (10 miles north of Truckee) in 1999, Martis Creek Lake campground (5 miles east of Truckee) in 2000, and the Tallac Visitor Center, South Lake Tahoe in 2001. In these areas, infected chipmunks (*Tamias amoenus* and *T. senex*) amplify plague transmission through their fleas (*Eumolpianus eumolpi* and *Ceratophyllus ciliatus*) to more susceptible ground squirrels (*Spermophilus beecheyi* and *S. lateralis*). The locally abundant ground squirrel fleas (*Oropsylla montana* and *O. idahoensis*) are competent plague vectors and pose an increased risk of transmission to humans, particularly when the fleas' natural hosts succumb to disease. This paper describes the most recent epizootic activity in the Truckee-Donner area and the plague suppression measures taken at Donner Memorial State Park (DMSP).

CHRONOLOGY OF EVENTS FOR EPIZOOTIC PLAGUE, 2002

The first evidence of plague activity in the Tahoe-Donner area during 2002 was a coyote (*Canis latrans*), sampled 10 miles northeast of Truckee, which tested serologically positive for plague antibody on April 30. On July 26, a black bear (*Ursus americanus*) captured from Homewood (Lake Tahoe, Placer County) tested serologically positive. Epizootic activity in the Truckee-Donner region became apparent when a *Y. pestis*-positive chipmunk (*T. amoenus*) was found along the park's western boundary in Truckee on August 9. On August 10, *Y. pestis* was identified in a lymph node aspirate from a hospitalized domestic cat that resided near the north shore of Donner Lake, adjacent to DMSP.

Based on direct evidence of plague activity adjacent to the park and circumstantial evidence from within the park (i.e., unconfirmed reports of dying rodents), park officials decided to close DMSP on August 27, to permit a comprehensive evaluation of the potential risk for plague transmission.

Additional bacteriologic evidence to support an extensive plague epizootic in the Truckee-Donner area continued after the park was closed. On August 28, *Y. pestis* was identified in the carcass of a *T. amoenus* found at a private park located about 2 miles north of Truckee. On September 2 and 3, two additional *Y. pestis*-positive *T. amoenus* (carcasses) were recovered from the day-use area of DMSP and the Tahoe-Donner subdivision just north of the park.

PLAGUE RISK ANALYSIS AND SURVEILLANCE AT DMSP

On September 11, 2002, VBDS biologists conducted surveillance at DMSP to determine the extent of the disease activity and to assess the public health risk. A standardized risk assessment, developed by CDHS, was used to rate various risk factors that are pertinent to plague exposure: the area's plague history, abundance of susceptible species, presence of reservoir species, flea index (average number of fleas/host), physical signs of an epizootic in progress (rodent die-off), and human exposure potential. Rating scores for these criteria, together with supporting laboratory evidence, can provide a sufficient indication of potential exposure risk to warrant vector suppression recommendations.

Eighty-five live animal traps (National and Sherman) were baited with oatmeal and peanut butter and set throughout high-use areas (campgrounds, day-use area, visitor center and staff housing) in the park. The traps were placed during the evening of September 10 and collected the morning of September 11. Rodents were abundant at all locations in the park. Forty-six rodents (54% trap success) representing six species were captured. All animals were lightly anesthetized and combed for ectoparasites. All fleas were collected and identified to species. Blood samples were taken from 7 *T. amoenus*, 1 *T. senex*, 3 *S. beecheyi*, 6 Douglas's squirrels (*Tamiasciurus douglasii*) and 4 northern flying squirrels (*Glaucomys sabrinus*). Twenty-five golden-mantled ground squirrels (*S. lateralis*) were captured and combed for ectoparasites; however, no blood samples were collected because historical evidence indicates this species is very susceptible to plague and live animals with plague antibodies are rarely found.

Serum antibody to *Y. pestis* was detected in one *T. senex* and one *T. amoenus* chipmunk from the park campground (antibody titers: 1:512 and 1:256, respectively). The percentage of rodents infested with fleas was high and varied by species from 83% to 100%. Chipmunks were typically infested with *E. eumolpi* and *C. ciliatus* fleas. Ground squirrels harbored *O. montana* and *O. idahoensis*. Fleas were pooled according to flea and host species, with fleas from serologically positive animals pooled separately. A total of nine pools were submitted for bacteriological testing; all were negative for *Y. pestis*.

The high pre-treatment host flea indices (Fig. 1) of chipmunks and ground squirrels, the presence of bacteriologically and serologically positive rodents within and around the park, and the park's high recreational use, contributed to a sufficiently high risk analysis score to indicate that flea suppression measures were necessary to mitigate the potential hazard of plague transmission to humans.

FLEA MANAGEMENT AT DMSP

On September 25, VBDS initiated flea suppression measures at DMSP, with assistance from park staff and Washoe Co., Nevada, Mosquito and Vector Control. Suppression procedures included dusting rodent burrows and deploying dust-bait stations (4" x 18" PVC tubes). One hundred forty lbs. of diazinon (Prentox, 2% dust) were applied into rodent burrows throughout DMSP. Additionally, 65 lbs. of diazinon were utilized in 181 dust-bait stations placed throughout the park's campgrounds.

Liquid deltamethrin (AgrEvo, Suspend SC), diluted to 0.06% in water, was used to treat 61 carpeted bait stations (PVC tubes lined with carpet) placed at the park's day-use area, along the southeastern shore of Donner Lake. The efficacy of this formulation was previously demonstrated at Martis Creek Lake campground where it provided good knockdown and effective flea suppression from one to six weeks after application (Bronson and Smith 2002).

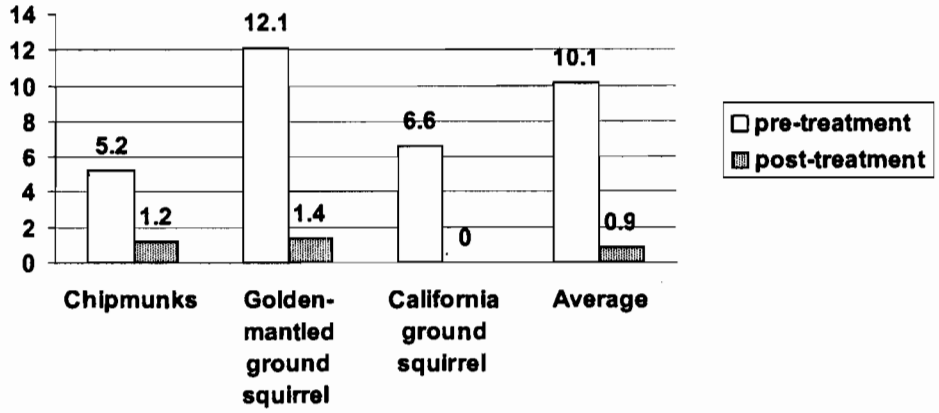

Figure 1. Pre-treatment (9/11/02) and post-treatment (10/8/02) flea indices of chipmunks and ground squirrels at DMSP, Truckee, California.

All bait stations were maintained on site for 14 days; DMSP staff re-baited and recharged the dust-bait stations as necessary.

Post-treatment rodent and flea surveillance was conducted on October 8. The post-treatment flea index was substantially lower than the pre-treatment flea index (Fig. 1), indicating effective flea suppression was achieved. Reduction in the overall flea index allowed DMSP personnel to reopen the day-use area; however, park officials decided to keep the main campgrounds closed until the spring of 2003.

DISCUSSION

Since the mid-1900s, insecticides have been applied through bait stations and hand-dusting of rodent burrows to effectively control plague during emergency epizootic situations (Kartman 1958, Barnes 1982). Murray (1964) and Murray and Barnes (1966) recognized that increasing sciurid rodent populations in recreational settings in the Sierra Nevada encourages epizootic activity. Frequent plague epizootics and associated human cases during the 1970s and 1980s within the region led to a cooperative plague prevention project with the California Department of Parks and Recreation (CDPR) that provided integrated plague management strategies for both Plumas-Eureka and Donner Memorial State Parks (CDHS status report to CDPR 1988). These strategies included monitoring of plague activity through surveillance, habitat modification and maintenance, control of flea vectors, rodent population management, and providing safety training and continuing education to park personnel on endemic plague.

The historical evidence of recurring plague activity within this geographical focus (CDHS plague records, 1909-2002) suggests that the Truckee-Donner area will presumably require continued attention and commitment from local and state officials. A reevaluation of the park's rodent populations and their flea indices is planned for the spring of 2003 to assess: 1) the residual efficacy of the fall flea suppression efforts, 2) the pre-season risk for human plague exposure, and 3) a rationale for continuing integrated plague management strategies.

Acknowledgements

Gratitude is extended to Norm Greenberg and the staff from Placer County Environmental Health Department, Truckee, for their cooperation and assistance. We thank Donner Memorial State Park superintendent Marilyn Murphy and her staff for their help with flea suppression measures. Recognition is extended to Dr. Bruno Chomel from the UC Davis Public Health Veterinary Laboratory, Al Hom from VBDS, and staff members of the CDHS Microbial Disease Laboratory, who were responsible for the testing of diagnostic specimens. Finally, special appreciation is given to Scott Monson and the staff of Washoe Co., Nevada, Mosquito and Vector Control, for their valuable assistance in the deployment of dust-bait stations.

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Feasibility of a Disposable Baited Trap for Eye Gnat Control

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ABSTRACT: Eye gnat control in the Coachella Valley has included use of a poison bait and attractant bait (Mulla and Axelrod 1977). In the last 10 years the Coachella Valley Mosquito and Vector Control District (the District), has used the "collar trap" in a unique program called "trapping out." The "collar trap" uses a liquid egg bait to attract and remove eye gnats from infested areas. The cost benefit analyses of the eye gnat control program indicated that the District needed to continue to maintain an effective control program, however the labor and expenses associated with the use of the collar trap needed to be reduced. The District staff looked into an alternative trap design and developed the disposable trap.

This paper presents a comparison between two trap designs regarding efficacy and cost of labor and material used.

EYE GNAT SURVEILLANCE

The eye gnats *(Hippelates spp.)* are shiny, black or dull gray stocky built small flies (1.5-2.5 mm) with yellow, orange, or dark-brown legs. Adult eye gnats feed on body secretions and excretions, sores, wounds and abrasions, humans and animals, plant juices and decaying organic matter (Mulla 1959). Eye gnats are mechanical vectors of human conjunctivitis, commonly known as "pinkeye." Since 1912, the correlation between outbreaks of pinkeye and abundance of eye gnats in southern California was documented especially by Tinkham and Mulla (James and Harwood 1969).

The CVMVCD has been using Tinkham traps for eye gnat surveillance for over 20 years. Abundance sampling is conducted weekly with 61 traps that are placed at 14 locations throughout the Coachella Valley. A noticeable reduction in the eye gnat population has been noticed in recent years. This reduction coincided with a drastic change in land use in the Coachella Valley, where large areas of agricultural land have been converted to residential developments where eye gnat production is lower compared to agricultural land.

TRAP DESIGN

Collar Trap

The collar trap is made of a two-quart clear plastic jar joined by a black collar with eight, 17 mm diameter holes, and a clear funnel in the upper jar. The trap is secured with a metal wire to wooden stakes or trees. The lower jar is filled with 700 ml of egg bait on a weekly basis (Axelrod et al. 1993). Both jars are replaced and washed at a 6-8 week interval. The traps require intensive labor, especially to set-up/disassemble and wash the jars. The trap patent right precludes its distribution to a third party.

Disposable Trap

The disposable trap is a 3-litter PETE soft drink bottle with the cap and eight 17 mm diameter holes that are made by using an aluminum sheet template and hot soldering iron with ¼ in. tip. The trap is secured to a tree with a ¼ inch nylon rope or metal wire. At country clubs, a tenite propionate plastic black tube with matching holes is used to obscure the trap content. The trap is filled with 1.5 liters of egg bait, and every 3-6 weeks, depending on the air temperature, the trap is replaced with a new one.

MATERIAL AND METHODS

For comparison, 25 disposable and 25 collar traps were set up in a drip irrigated citrus orchard with mid-size trees 3 m apart. Three tests were conducted: November 2000, May/June 2001 and June/July 2002. Each type of trap was set up in 4 positions in each test. In the first position, the traps were set in alternating order – 3 m apart. The second and third positions were alternating adjacent sides of the road – 3.5 m apart. In the fourth position, disposable and collar traps were placed at opposite sides of the orchard – 15-30 m apart to account for spatial effects.

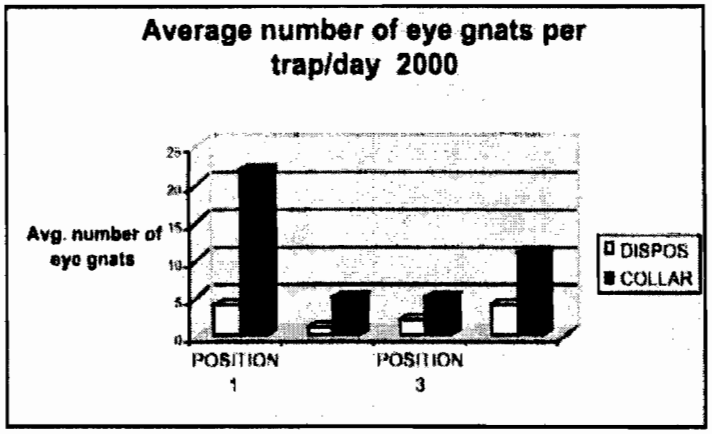
For three consecutive days, each trap was retrieved and replaced with a new one with fresh bait. The liquid egg bait from each trap was strained through several different sieve sizes and drained through a filter paper. The eye gnats and flies on each filter paper were counted and recorded.

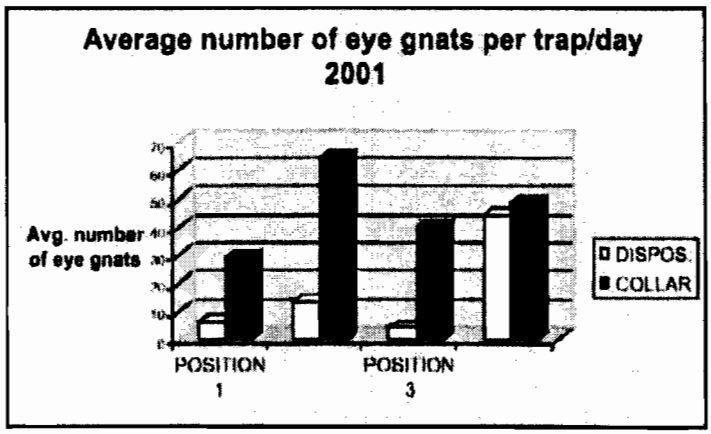
RESULTS AND DISCUSSION

Trapping Results

To compare the two types of traps we used the average number of eye gnats and flies/ trap day for all tests. There was no attempt to identify the species of collected eye gnats or flies. In 2001 and 2002, when the traps collected enough numbers of eye gnats/flies, separate multi-way classification analyses of variance (ANOVA – Microsoft Excel software version 6.0.22) were conducted on data from each of the tests, with trap type and position within the habitat as main effects.

Eye gnats – The average number of eye gnats in all 3-years tests and four positions was higher in the collar traps (Figure 1).





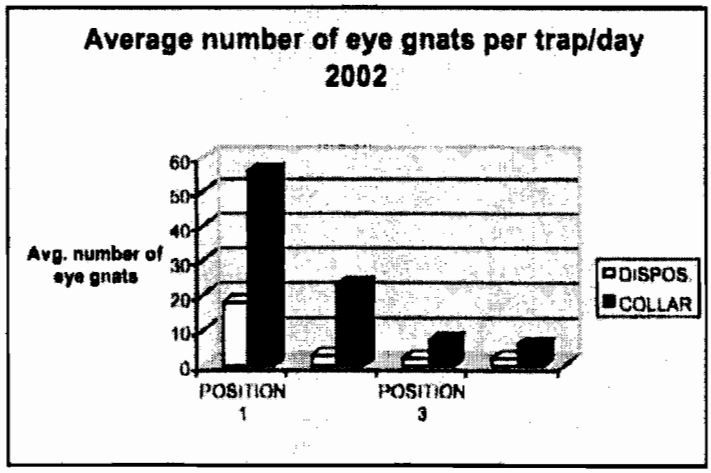
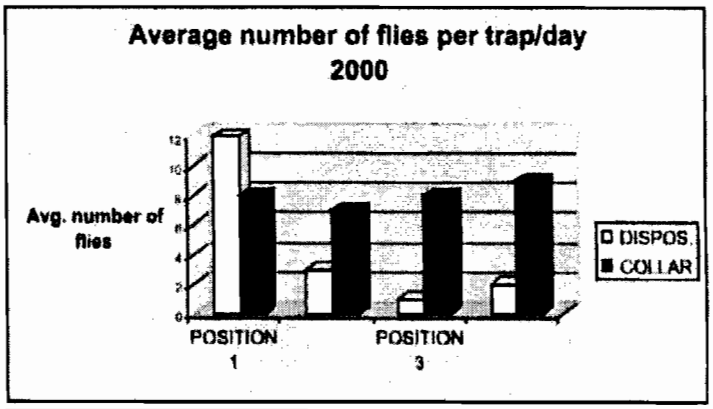
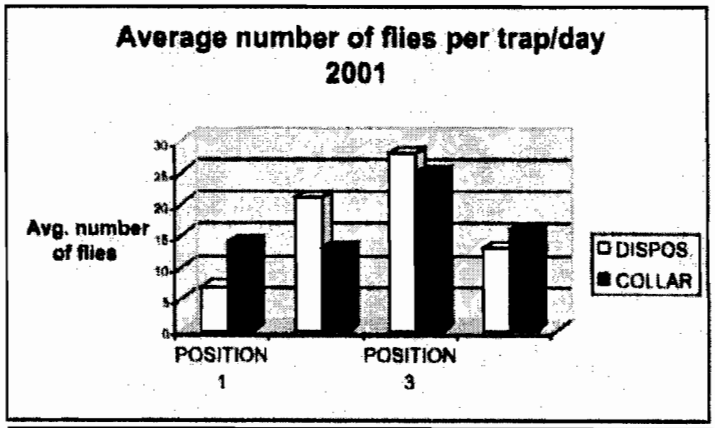


Figure 1. The average number of eye gnats per trap/day in 2000, 2001 and 2002.





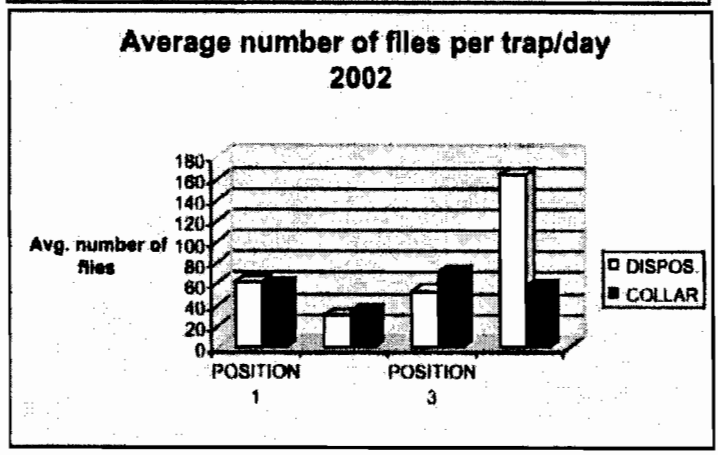


Figure 2. The average number of flies per trap/day in 2000, 2001 and 2002.

The ANOVA test for studies in 2001 indicated significantly higher numbers of eye gnats in the collar traps in positions 1, 2, and 3, while studies in 2002 indicated significantly higher numbers for positions 1 and 2.

Flies – The average number of flies was higher in collar traps in the year 2000, for the positions 2, 3 and 4, in the year 2001 and 2002 for the positions 1 and 4 (Figure 2.). The ANOVA test for studies in 2001 indicated significantly higher numbers of flies in the collar traps for position 1, while in 2002, the studies indicated significantly higher numbers of flies only in the disposable traps for position 4.

Cost Analysis

The cost analysis that included material for building the disposable/collar trap, labor required for building and servicing both trap types, is presented in Table 1. The cost analysis indicated

Table 1. The cost analysis includes material for building disposable/collar trap, labor involved with building and servicing traps and miscellanies.

	Collar	Disposable
$ Material/Trap	**$3.46**	**$0.48 + $ 2.08***
$ Labor/Trap	**$11.95**	**$0.73**
$ Total cost/trap	**$15.41**	**$1.21 / $3.29***
*** with black plastic tube**		

that the projected annual operational cost for the disposable trap was 50% lower compared to the collar trap.

RECOMMENDATIONS

The tests were performed over 3 consecutive years, encompassed 3 different breeding seasons, and 4 testing positions for each test. The results indicated that the collar trap was more effective for collecting eye gnats, and in certain circumstances for flies. However, projected annual operational cost of the disposable trap is less than 50% compared to the collar trap. To justify the use of the disposable trap, primarily in the agricultural section of the Valley, and have an effective eye gnat control program, the District needs to increase the effectiveness of the disposable trap. The first recommendation is to increase the number of disposable traps in the agricultural area, and place between 5,000 to 8,000 traps. The other factor that needs to be considered to increase the efficacy of the disposable traps is to insure replacement of the traps within 3-6 weeks depending on the season.

If the disposable trap is to be used in the country clubs, the same recommendations need to be followed. The number of the traps per 18-hole golf course needs to be increased from 54 to 62.

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Success in the Sink

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North of Sacramento, in the heart of the Sacramento Valley is the 1,200 square mile "rice bowl" of California. Inside this area, in Colusa, Glenn, Butte and Sutter Counties, 95% of the State's rice production takes place, nearly 415,000 acres. Also found here are 35,000 acres of national wildlife refuge, 18,000 acres of state wildlife areas, and nearly 40,000 acres of privately owned duck clubs. Nearly 40% of the "rice bowl" area is uncontrolled for mosquitoes. In the very center of this mosquito producing habitat, between Colusa and Gray Lodge, is the Butte Sink (Figure 1). Previously uncontrolled, this area was annexed into the Colusa Mosquito Abatement District (CLSA) in 1963.

This report describes the mosquito problems associated with the Butte Sink area of Colusa and Sutter Counties and a successful effort to obtain cooperation and funding for mosquito control. Primarily, the report describes the events related to CLSA, as they had 30 of the 36 clubs in their district. Each of thirty six duck clubs signed a cooperative agreement by the end of October 2002, agreeing to pay for adult mosquito control related to flooding of habitat for duck hunting. The payments supported applications of Naled (TRUMPET® EC) to successfully control production of adult *Ochlerotatus melanimom, Culex tarsalis, and Anopheles freeborni.* Adult mosquito was chosen over larval mosquito control with consideration to overall costs of products, the possibility of poor kill in deep water, and thick vegetation.

The Butte Sink is a 20,000 acre irrigated semi-natural wetland area that includes over 40 privately owned duck clubs (some are located in Butte County), an Audubon bird sanctuary, and a US Fish & Wildlife waterfowl refuge. In addition, several thousand acres of rice are produced in the area. After rice harvest, the fields are re-flooded for decomposition of the rice straw and duck hunting habitat. During the unsuccessful control of mosquitoes during the 2001 season, owners of farmland, orchards, and homes adjacent to the sink reported intolerable adult mosquito problems, thereby reducing their land values and quality of life. Over 32,000 adult mosquitoes were collected from one trap in a two night period during the time of highest production. Complaints from citizens were primarily directed towards Sutter-Yuba Mosquito and Vector Control District (SUYA). However, complaints and threats of class action lawsuits went to CLSA and the Department of Health Services (CDHS). With the approach of West Nile Virus into California, the managers of CLSA and the Sutter-Yuba Mosquito and Vector Control District (SUYA) were determined to take all necessary steps to minimize the problem and obtain mosquito control funding from the recreational hunting and birding areas.

In the early summer of 2002 some of the clubs irrigated to produce feed and habitat for ducks. All clubs flooded prior to the duck hunting season beginning the "fall flood-up" about mid-August. It took some clubs 21 days to flood, reaching "shooting level" and two months for the entire sink to reach "shooting level." The continuous production of *Oc. melanimon* was tremendous in these wetland areas. Counts of 500 larvae per dip were common. By the second week of flooding counts on *Cx. tarsalis* and *An. freeborni* were at 10 to 20 per dip over wide areas.

To complicate and increase the mosquito problem there has been a tendency for conversion of rice fields and other farmland to wetlands and duck hunting clubs. Mosquito production is much more extensive in summer and fall flooded wetlands than in row crops or rice. Rice fields produce unacceptable numbers of mosquitoes but mosquito numbers are moderated by buildup of mosquito eating fish and other aquatic predators. This conversion to wetlands has been accelerated because of the many state and federal and payment programs and private grants offered to the landowner. Often, the California Waterfowl Association (CWA) and other wetland support organizations offer free consulting and project management to landowners. Some of the incentive programs as of January 2003 are listed in Table 1.

Aware of the ever-increasing mosquito problem and the impact on local communities, the CLSA Board asked the District Manager what could be done. Following is a chronology of the major events at CLSA following the 2001 mosquito season:

2001, November - CDHS and the District Manager reviewed for the Board the district powers, laws, regulations, and Board responsibilities as found in the California Health and Safety Code (HS Code). The Colusa County District Attorney attended this meeting and gave support to the Board, affirming the strong abatement powers found in the HS Code.

2002, January - The Board adopted a resolution directing the manager to take all necessary steps, including legal abatement if necessary, to have the clubs pay for the mosquito control (the SUYA Board did the same).

January - The Board adopted a managerial proposal to enter into cooperative agreements with duck clubs assessing them $18.00/ acre up front, with any money not used for control returned to the club at the end of the year.

January - A letter was sent to all clubs asking them to sign an enclosed cooperative agreement (accepting responsibility to pay

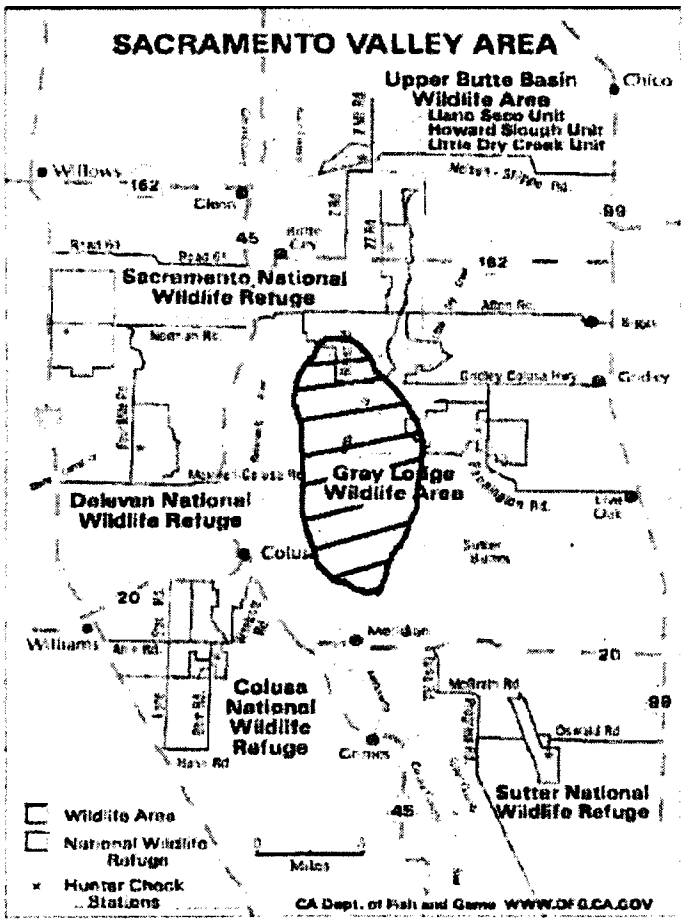

Figure1. Sacramento Valley area of California.

for the control provided by CLSA). The agreement was between the club, CLSA and SUYA. A copy of the HS Code was also included in the letter.

March - The Districts met with the Butte Sink Waterfowl Association, CWA, and Ducks Unlimited to discuss the problem, the issues, and the payment.

May - The Districts met with all of the duck club owners to discuss the agreement and mosquito production on their clubs.

May 28 - Intensive surveillance, sampling and documentation of mosquito production in the sink began.

June 3 – Date of the first mosquito control by ground ULV. Individual clubs become inaccessible with the fall flood-up but can be controlled by ground rig during the summer irrigation. Mosquito control was required because of the summer flooding requirements of some state and federal incentive programs. Billing statements were sent to clubs for the cost of control. All summer flooding related mosquito control was controlled by ground ULV, and eventually paid for by the clubs.

August 3 - A second meeting with club owners was held. Club owners proposed a "pay for control as needed" agreement. They would pay for control applications as they were made.

August 7 – A revised cooperative agreement, highlighting "pay for control as needed," was sent to all duck clubs by registered mail.

August 15 - The Audubon Society and one club adjacent to the Audubon were the first to sign the revised agreements. A majority of the clubs soon signed agreements.

Table 1. Incentive programs for landowners to convert farmland to wetlands.

Agency	Program	Description
US Fish and Wildlife Service	Conservation Easement Program	Pays landowners 40-60% of the fair market value of the land.
California Department of Fish and Game	Permanent Wetland Easement Program	Pays landowners 50-70% of fair market value of the land.
Natural Resources Conservation Service	Wetland Reserve Program	Pays land owner $2,000/acre to retire farmland. Pays 100% of the cost of restoring land to wetlands.
California Department of Fish and Game	California Waterfowl Habitat Program (Presley Program)	Pays landowners $20/acre/year for 10 years.
US Fish and Wildlife Service	Partners for Fish and Wildlife Programs	Pays 50% of restoration costs.
Natural Resources Conservation Service	Wildlife Habitat Incentive Program (WHIP)	Pays 75% of restoration costs.
Ducks Unlimited	Valley/Bay Program	Pays 50% of restoration costs.
Natural Resources Conservation Service	Conservation Reserve Program (CRP)	Pays $22/acre for 10 years for waterfowl enhancement.
United States Department of Agriculture	Conservation Reserve Enhancement Program (CREP)	Pays landowner of irrigated cropland $160/acre of rice or $100/ acre for other crops for 10 years to forego farming and establish upland habitat. Pays 50% to establish habitat.
Natural Resources Conservation Service	Water Bank Program	Financial incentives to preserve, restore, and improve wetland habitats. Ten year agreements.

August 20 - The fall flood-up in the Butte Sink began.

August 26 - Letters were sent to a number of clubs with notification of first larval production.

August 31 – The first aerial adulticide application of the insecticide TRUMPET® EC was made.

September 19 - Abatement notices were delivered to the seven that had not signed agreements. These clubs signed agreements before abatement hearings were held.

October 1 – By this date, 30 clubs in CLSA and 6 in SUYA had signed the agreements to pay for mosquito control on their properties.

During the 2001 mosquito season and following a multitude of phone calls, duck club meetings, registered mailings, intensive surveillance, and special board meetings, 36 duck clubs signed the cooperative agreements with CLSA and SUYA. No control costs were assessed the 6 duck clubs in the Sutter-Yuba District because the clubs opted not to flood until after the mosquito breeding season. The program and agreements resulted in the aerial application of TRUMPET® EC over 64,000 acres for control of adult mosquitoes. Some clubs were sprayed once and others up to 5 times, depending on the availability of water and flood-up practices. The total costs of $40,000 for aircraft and material was reimbursed to CLSA, almost doubling the chemical budget. Parameters of aircraft application are shown in Table 2.

Mosquito control during the 2002 season was considered the most successful ever achieved in the history of the Butte Sink mosquito control. Complaints and light trap counts were down. Land owners and duck club managers described the 2002 mosquito control as the best they had ever seen. Even a duck hunter was heard to say that this was the best opening of duck season ever, and there were no mosquitoes.

Indeed, there was "Success in the Sink!"

Acknowledgements

The authors wish to thank staff and biologists from the Colusa Mosquito Abatement District, the Sutter-Yuba Mosquito Abatement District and the California Department of Health Services. Special thanks to John Poyner, Colusa County District Attorney, for his support and attendance at various meetings.

Table 2. Aircraft and application information for mosquito control in the Butte Sink, 2002.

Aircraft:	Cessna Ag Wagon
Speed:	120 mph
Nozzles:	2 Micronair AV-5000 Rotary atomizers
Swath:	500 ft.
Application rate:	121 acres per minute
Rate of Application:	1 oz/acre
Material:	Trumpet® EC (78% Naled)
Cost per acre:	$0.625

Evaluations of Barrier Spray Using Formulations of Pyrethrin and Pyrethroid Insecticides

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ABSTRACT: Experiments were conducted at a citrus orchard, duck club, and residential habitat to determine the ability of barrier pesticide treatments to disrupt mosquito dispersal. CO_2-baited CDC style and unbaited 20 cm diameter suction traps were used to measure changes in abundance and mark-release-recapture was used to measure dispersal patterns. Mortality of mosquitoes from exposure to treated vegetation was quantified using bioassay. In citrus trials using Pyrenone® CS at 1.5 and 4.6 l/ha, 57 and 84% control was achieved on the second day of treatment inside the perimeter barrier. At the duck club we used Demand® CS at a rate of 2.5 l/ha and achieved 90% control within treated vegetation. The experiment at habitats bordering a rural residential community was unsuccessful due to the collapse of regional mosquito abundance.

INTRODUCTION

Evidence from trapping studies (Lothrop and Reisen 2001, Lothrop et al. 2002) has indicated that *Cx. tarsalis* mosquitoes typically are distributed contagiously over the landscape, congregating at the ecotone of elevated vegetation. Aggregation of mosquitoes within the environment provides the opportunity to optimize control by focusing applications at distinct terrain features or vegetation types. For example, if 90% of the mosquitoes are aggregated within 10% of the environment, control could be optimized by directing 100% of control towards this segment of the population.

In the Coachella Valley, vernal amplification of virus has been focal, originating at marshes along the margin of the Salton Sea, with secondary late summer and autumnal amplification occurring near duck clubs located around the delta of the Whitewater Channel. Limitations of access into the marshes and of application of adulticides near fish habitat prevents the widespread use of ULV [ultralow volume] formulations for adult control within these habitats. These limitations frequently prevent adulticide applications intended to interrupt encephalitis virus transmission early in the season and thereby prevent its spread to populated upland areas. Low volume residual barrier treatments focus pesticide applications and avoid drift into sensitive wetland areas. The added benefit of barrier treatment is that it remains in place throughout the night, whereas ULV impacts only adults flying at the time of treatment.

METHODS

Field work was done at a citrus orchard, duck club, and residential habitats near the Salton Sea, Riverside County, California in collaboration with the Coachella Valley Mosquito and Vector Control District (CVMVCD).

TECHNIQUES

Mosquito collection. Host-seeking females were collected using dry ice-baited CDC-style traps operated without lights on metal standards at 1.5 m height (CO_2 traps). Flying mosquitoes were sampled without attractant by down-draft suction traps (30 cm diameter) that were positioned with the trap entrance at ca. 1 m height.

Mark-release-recapture. Adult mosquitoes for release were transferred to 5 gal cages where their number was estimated by 3 replicate counts using the strip method. Mosquitoes were offered wet toweling, but not sugar, and then transported to the field where they were marked with fluorescent dust and released within 24 hours of capture. Trapped mosquitoes were anesthetized with triethylamine and then examined under 10X magnification and ultraviolet light to identify marked individuals. Unmarked females were counted to determine the abundance pattern of the natural population.

Barrier spray. Adulticides were applied in accordance with label restrictions by certified personnel from the CVMVCD. Equipment included roller and diaphragm pumps that produced 250 psi and 350 psi, respectively, combined with a GunJet AA12 (Spraying Systems Co.) with a #6 orifice disk having an output of 2 gallons per minute. Water sensitive cards were clipped to the canopy of the vegetation to provide confirmation of coverage.

Bioassay. Mosquitocidal activity of treated vegetation was bioassayed by stapling treated leaves to 30 mm X 80 mm cards and placing them in 37 mm X 100 mm cardboard tubes with fiberglass window screen covers at both ends. Equal numbers of male and female *Cx. tarsalis* from a Coachella Valley colony were introduced into the containers and held overnight at 25° C after which mortality was recorded.

Analysis. Impact of treatment was measured by comparing counts pre and post spray using Mulla's formula (Mulla et al. 1971) that compares spray and control sites pre and post treatment.

Experimental design. Experiment 1 (Fig. 1) was done at a citrus orchard 500 m from the shore of the Salton Sea. Our objective was to interrupt the dispersal of mosquitoes from shoreline emergence sites into the orchard. Abundance was monitored using 18 CO_2 traps in each of 5 regions; shoreline, intermediate desert brush, orchard perimeter, orchard interior, and 1 km inland from the orchard (not shown in figure). Pyrenone® CS (6% Pyrethrin/ 60% Piperonyl Butoxide) Bayer Corp. was used because of its

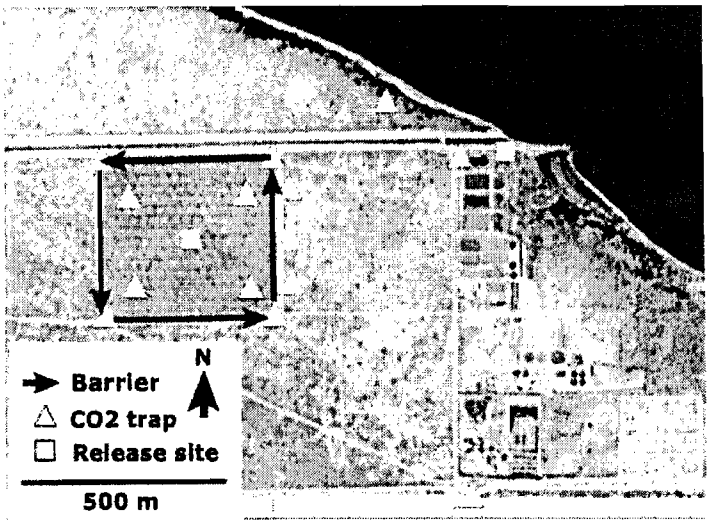

Figure 1. Map of study site for experiment 1, spray trials 1 and 2.

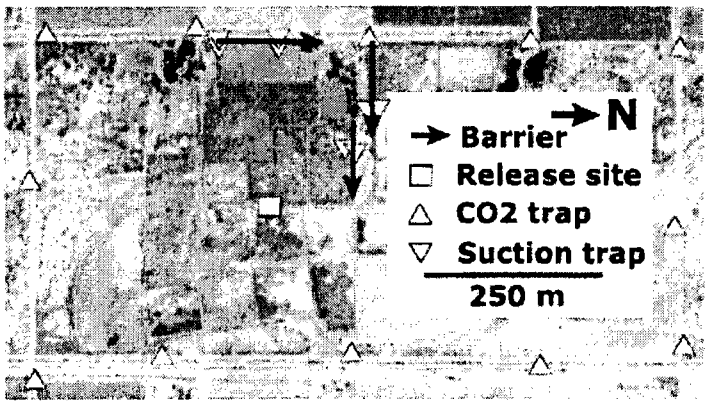

Figure 2. Map of study site for experiment 2.

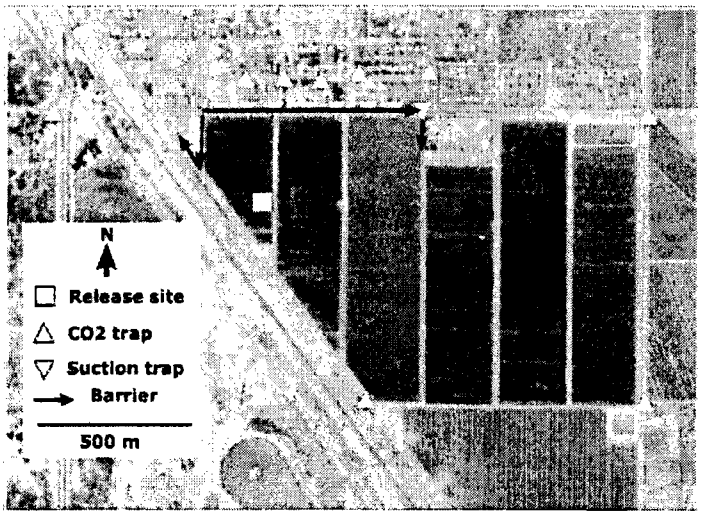

Figure 3. Map of study site for experiment 3.

regulated tolerance on citrus. Two experiments were conducted with 4 nights of trapping; 1 night of pretreatment sampling, followed by 2 days of perimeter barrier spray with release of marked mosquitoes, followed by one night of post treatment trapping. Application rates for experiments 1 and 2 were 1.5 and 4.6 l/ha, respectively. Bioassay was conducted after each treatment. To elucidate mortality patterns at the treated orchard ecotone, we conducted two tests along the east side of the orchard using suction traps to demonstrate focal changes in abundance. In the first test, traps were set 6 m apart at the end of 15 alternate rows with the central 5 traps used as controls. In the second test 5 control traps were set 30 m from the last treated trap.

Experiment 2 (Fig. 2) was done at a duck club near the community of Mecca. The target vegetation was *Tamarix* and *Pluchea* (non-crop) and was treated with Demand® CS (9.7% Lambda-Cyhalothrin) Syngenta Corp. at 2.5 l/ha with a recommended treatment cycle of 7 days. Our objective was to block movement of marked mosquitoes to traps placed in and beyond the treated barrier. We placed 12 CO_2 traps within a 0.8 by 1.6 km rectangular perimeter around the release site and 4 sets of paired suction traps on the front and back sides of the treated strips of vegetation. The CO_2 traps were intended to measure dispersal of marked mosquitoes and regional abundance. Marked mosquitoes were released between 1900 and 1930h on days 1 and 3, with an additional release on day 4 to supplement the low number released on day 3. The inside face of the vegetation was sprayed on day 3 at 1600h.

Experiment 3 (Fig. 3) was done on the south side of the community of Mecca. We used Demand® at 2.5 l/ha to treat a *Nerium oleander* (non-crop) wind break to block the movement of mosquitoes dispersing north from the duck clubs located south of Mecca, which were the only major breeding sources at that time of year. Marked mosquitoes were released 200 m south of town between 1830h and 1930h on day 1 and 3. Four CO_2 traps were placed outside the treated barrier, 0.6 to 1.1 km distant from the release site, and 5 were placed 1 block inside of town parallel to the treated barrier, 350 m to 580 m distant from the release site. Eight suction traps were placed along the treated side of the vegetation. Treatment was done on day 3 of 4 sample days.

RESULTS

BARRIER SPRAY EXPERIMENTS

Experiment 1. The two successive trials at the orchard showed similar patterns of reduction in *Cx. tarsalis* abundance (Fig. 4). We designated the shore (S) traps as controls, because they were furthest from the treated barrier and the primary source of mosquitoes. The greatest reduction in trial 1 was at traps in the interior (I) and in trial 2 in traps at the perimeter (P). There also was a comparative reduction in traps outside the barrier (O), perhaps because mosquitoes were moving among adjacent blocks of vegetation and coming in contact with the pesticide. The second trial, with an increased dosage showed an increased reduction in all traps within the treated area. Mark-release-recapture results were less clear in both trials, with marked mosquitoes moving through the barrier from both directions. The bioassay (Fig. 5) for

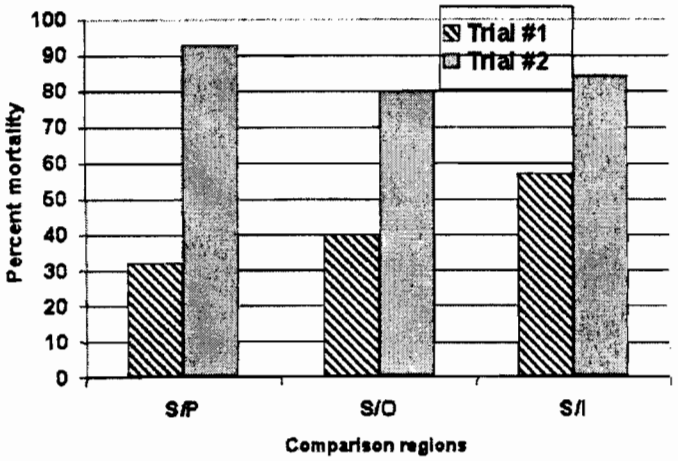

Figure 4. Experiment 1 percent mortality for trials 1 and 2. S = shore, O = outside barrier, I = inside barrier, P = perimeter.

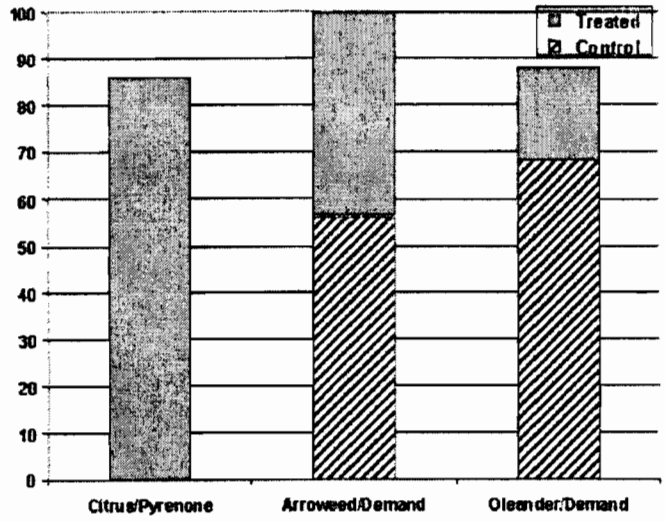

Figure 5. Bioassay results in percent mortality for experiments 1(trial 2), 2, and 3.

trial 2 treatment showed 86% mortality for mosquitoes held in proximity to treated leaves. In the two subsequent residual trials, the controls showed a parallel decrease in abundance indicating they were influenced by adjacent treated vegetation, as in the barrier trials. This confounded the abundance results, but the bioassay results were useful with 30% residual control.

Experiment 2. Our design did not create a completely protective barrier and consequently mosquito abundance was assessed primarily within the treated barrier using the 8 suction traps. Mosquito production was localized to the south and northwest of the study site, which resulted in similar pretreatment abundance among all 12 CO_2 traps. Dispersal of marked mosquitoes was not uniform, with traps to the south, east, and west recapturing the largest numbers of mosquitoes released pretreatment. Posttreatment releases followed the same pattern except for traps in the treated vegetation to the west, which had lower recapture. Control in the treated barrier compared to regional CO_2 trap average was 75% in CO_2 traps and 90% in suction traps. Abundance decreased by more than one natural log in suction traps after treatment for two successive nights (Fig. 6). The bioassay for this trial produced 100% mortality in treated cages. Interestingly, control mortality with untreated leaves was also elevated, perhaps indicating a fumigant effect from Arrowweed leaves.

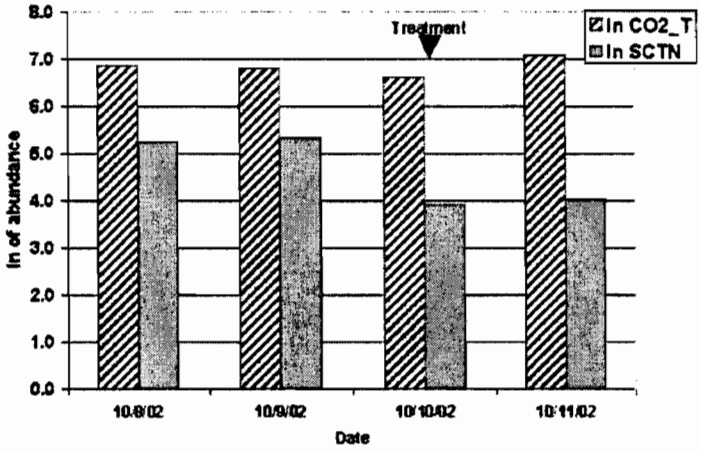

Figure 6. Experiment 2 reduction in natural log of abundance in suction traps compared to CO_2 trap controls.

Experiment 3. Using Mulla's formula control was 7% at CO_2 traps set one block north of the treated barrier. Suction traps did not collect sufficient numbers to measure abundance changes at the treated barrier. The bioassay in this trial produced 87.5% mortality adjusted to 19.5% when mortality related to oleander leaves in the control was subtracted.

DISCUSSION

Overall, barrier treatments seemed to disrupt the dispersal of mosquitoes resulting in localized reductions in host-seeking and flying female abundance. In each experiment, the limited number of sample days post treatment was intended to investigate immediate changes and not residual activity. Our protocols were directed toward selecting pesticides and methods that might interrupt mosquito dispersal, but substantial sustained reduction in regional abundance necessary to interrupt virus transmission will require the treatment of larger areas for longer periods. In experiment 1, abundance was reduced inside and at adjacent vegetation outside the barrier. It follows that reduction of a portion of a contiguous moving population will result in reduction in adjacent areas because of the loss of movement from the kill zone. In experiment 2, the reduction at the treated vegetation indicated that this method might produce regional reduction even without a continuous barrier. This is a critical finding for large area applications where elevated vegetation is discontinuous and may form ecological islands bringing mosquitoes and birds together to enhance virus transmission. Results at site 3 were confounded by the collapse of

regional mosquito abundance. In this experiment, the sparseness of the oleander along much of the barrier may have provided an inadequate landscape target for spray and may not have stimulated the congregation of mosquitoes. Additionally, it may be necessary to treat a more extensive barrier as mosquitoes could have entered town by an alternate route that did not take them through the barrier.

In future experiments we will refine and extend our methods to:

1) Increase pump pressure to improve spray penetration by producing uniform droplets small enough to increase the time they are suspended in the air.

2) Compare different pyrethroid formulations.

3) Increase the size of released cohorts to better describe interruption of dispersal by barriers.

4) Extend bioassays with positive and negative control using filter paper to discover any mortality caused by bruised plants.

Given the limited scale of our preliminary experiments and the positive results in Experiments 1 and 2, we feel there is justification to pursue this line of investigation with treatment areas on the order of 1 square mile over a 10-day duration. We expect to see more significant reduction in regional abundance as well as enhanced protection of designated core areas.

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Summary of Mosquito-borne Encephalitis Virus Surveillance in California: 1998-2002

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ABSTRACT: A five-year summary (1998-2002) of human, equid, sentinel chicken, mosquito, and wild bird surveillance for St. Louis encephalitis, western equine encephalomyelitis, California encephalitis, and West Nile virus is presented. This document extends the summary by Hui et al., (1999) for St. Louis encephalitis and western equine encephalomyelitis activity in California from 1969-1997, and adds maps of reported arboviral activity by county since 1969. A case of probable vaccine infection of a horse with eastern equine encephalomyelitis is detailed. The extension of the statewide surveillance system to include West Nile virus in California also is described. The data from 1998-2001 provides the background level of arboviral activity in California prior to the introduction of West Nile virus in 2002. The California Mosquito-borne Encephalitis Virus Surveillance Program combines information on climate variation, mosquito abundance and infection, transmission to sentinel chickens, and reports of horse and human cases to measure the risk of arbovirus transmission to humans (Kramer 2001). The purpose of the current paper is to summarize surveillance information gathered during the previous 5 years, thereby updating historical summaries by University of California, Berkeley from 1943 - 1987 (Reeves 1990) and by the Department of Health Services from 1969 - 1997 (Hui et al. 1999).

HUMAN CASE SURVEILLANCE

INTRODUCTION

Until the confirmed West Nile virus (WN) positive case in 2002, no human cases of arboviral infection had been reported in California since 1997 (Hui et al. 1999) (Figure 1, Table 1). Samples from suspect human cases of aseptic meningitis and encephalitis have been tested for decades by the California Department of Health Services (CDHS) Viral and Rickettsial Disease Laboratory (VRDL) for antibodies to St. Louis encephalitis (SLE) and western equine encephalomyelitis (WEE) viruses. In recent years, the Davis Arbovirus Research Unit (DARU) at the University of California, Davis (UCD) has tested samples from CDHS for a range of arboviruses. In 2000, CDHS initiated testing for WN after its introduction into the United States in 1999 (Husted et al., 2000).

1998

In 1998, 140 human sera and/or cerebrospinal fluid specimens were tested and none were positive for arboviral infection (Table 1). The California Encephalitis Project (CEP) was started in July 1998 by the VRDL through funding from the Centers for Disease Control and Prevention (CDC) to determine the etiology of unexplained encephalitis cases. Specifically, the CEP examined the patient demographics, exposure to arthropods, and expanded arboviral testing to include polymerase chain reaction (PCR), serology, and isolation for 15 viral agents (Kramer et al., 1999).

1999

In 1999, 127 patients were enrolled in the CEP; none were positive for arboviral infection (Table 1). Outside of the CEP program, there were 14 suspect cases of WEE and/or SLE from Imperial and Riverside counties (Husted et al., 2000). Although none of these was confirmed, this and previous studies on the seroprevalence of WEE and SLE in humans in Coachella Valley (Reisen et al. 1996) and Imperial Valley (Reisen and Chiles, 1997) prompted an additional survey in these counties. Seven hundred twenty-nine human sera were tested from four medical facilities; 15.9% of the samples tested had a SLE IgG enzyme linked immunoassay (EIA) index greater than one and 1.5% had a WEE IgG EIA index greater than one (Glaser & Kohlmeier, pers. comm.). These results were similar to Reisen et al.'s findings [19 (2.6%) WEE and 188 (16.4%) SLE] using the same technique and were remarkably similar to an early serosurvey in Imperial County (Jozan,

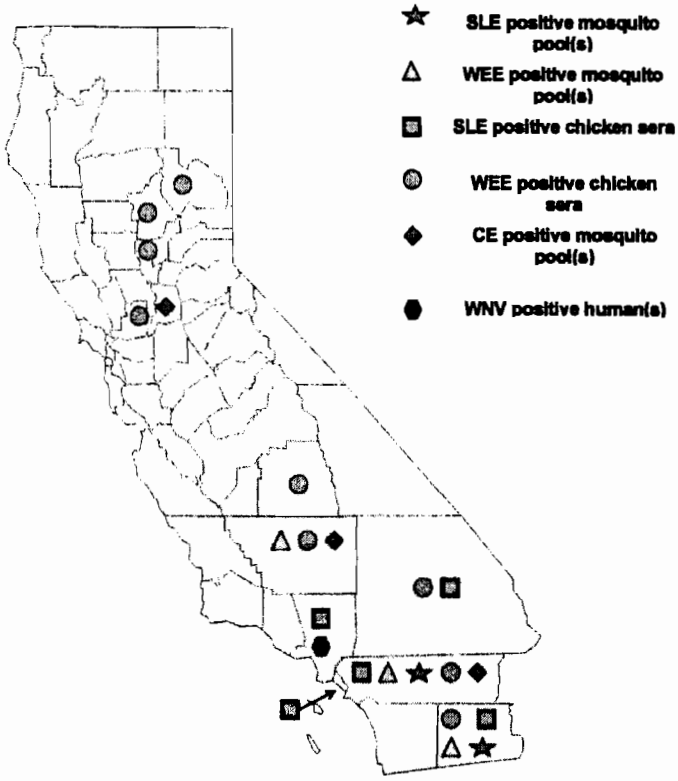

Figure 1. Locally Acquired Mosquito-borne Virus Activity in California, 1998-2002.

Table 1. Summary of Arbovirus Detection in California, 1998-2002.

Key to counties:

BU= BUTTE	CC= CONTRA COSTA	CO= COLUSA	FR= FRESNO	GL= GLENN	IM= IMPERIAL
KE= KERN	KI= KINGS	LA= LOS ANGELES	LK= LAKE	LS= LASSEN	MA= MADERA
ME= MERCED	MD= MODOC	MR= MARIN	OR= ORANGE	PL= PLACER	RI= RIVERSIDE
SA= SACRAMENTO	SB= SAN BERNARDINO	SD= SAN DIEGO	SJ= SAN JOAQUIN	SH= SHASTA	SI= SISKIYOU
SL= SOLANO	SM= SAN MATEO	SN= SONOMA	ST= STANISLAUS	SU= SUTTER	TE= TEHAMA
TU= TULARE	VE= VENTURA	YL= YOLO	YB= YUBA		

Year	Humans			Mosquitoes				Chickens			Horses			Dead Birds
	WEE	SLE	WNV	WEE	SLE	CE	WNV	WEE	SLE	WNV	WEE	EEE	WNV	WNV
1998	0	0	N/A	53 KE-42, RI-11	1 RI-1	0	N/A	101 BU-5, IM-26, KE-30, PL-4, RI-28, SB-6, SL-1, SU/YB-1	2 LA-1, OR-1	N/A	0	0	N/A	N/A
	140 tested			3557 pools				134 Flocks			5 tested			N/A
1999	0	0	N/A	0	0	0	N/A	3 RI-2, TU-1	25 IM-14, RI-8, SB-3	N/A	0	0	N/A	N/A
	141 tested			3566 Pools				190 Flocks			1 tested			N/A
2000	0	0	0	0	30 RI-30	0	0	0	49 IM-11, RI-36, SB-2	0	0	1 ** VE-1	0	0
	226 tested			3901 pools				170 Flocks			16 tested			20 tested
2001	0	0	0	0	70 RI-70	9 KE-8, SA-1	0	3 RI-3	62 IM-11, RI-51	0	0	0	0	0
	210 tested			3501 Pools				194 Flocks			15 tested			18 tested
2002	0	0	8 CC-1* LA-1 LA-2* OR-1* SF-1* SM-1* VE-1*	28 IM-19, RI-9	8 IM-8	17 RI-1	0	52 IM-40, RI-8, SB-4	45 IM-43, RI-2	0	0	0	1 LA-1*	0
	431 tested			4879 Pools				207 Flocks			83 tested			653 tested
Total	**0**	**0**	**8**	**81**	**109**	**10**	**0**	**159**	**183**	**0**	**0**	**1**	**1**	**0**

* Imported into California from other states
** Vaccine acquired, not natural transmission

1977). Collectively, these data indicate that humans are frequently infected with SLE in southeastern California, but few infections result in serious disease.

2000

In 2000, CEP enrollment increased to 370 patients who tested negative for WEE and SLE. When WN activity was detected in the eastern United States in 1999, testing was expanded to include antibody against WN. Of the 370 patients enrolled in the CEP in 2000, six had traveled to the eastern U.S. within the expected arbovirus incubation period and two patients reported mosquito bites. WN antibody was not detected in any of the patients (Husted et al., 2001).

VRDL also tested 226 serum and/or cerebrospinal fluid specimens (from both CEP and non-CEP patients) from patients exhibiting signs of aseptic meningitis or encephalitis for WEE and SLE. None showed evidence (Table 1) of recent arboviral infection (e.g. elevated IgM antibody response or a four-fold rise in antibody between paired sera) (Husted et al., 2001).

2001

In 2001, VRDL tested specimens from 210 patients, with negative findings for SLE and WEE. Of these, 167 were CEP patients (Table 1). All CEP patients were negative for antibodies for WN, even though six patients had traveled to the eastern United States within the WN incubation period and 28 reported recent mosquito exposure (Husted et al., 2002).

2002

For the first time in California, there was evidence of WN infection in humans. On August 10, 2002, a 31-year-old female from Los Angeles County became ill, was hospitalized, and later released. After specimens were screened by the LA County Health Department and VRDL, the patient was labeled a probable case of WN. Upon confirmation by DARU, she was diagnosed as a confirmed case of WN meningitis. The patient reported no travel history outside of Los Angeles County during the incubation period prior to onset of illness (Husted et al., 2003).

Other WN activity in California included seven imported cases: two cases of WN encephalitis (a Texas resident in Los Angeles County and a San Mateo County resident who traveled to Wisconsin), one case of WN meningitis (an Orange County resident who traveled to Nebraska), two cases of WN fever (a San Francisco County resident who traveled to Washington D.C. and a Ventura County resident who traveled to Florida and the Bahamas), and one case of WN associated acute flaccid paralysis (a Kansas resident who traveled to Los Angeles) (Table 1) (Husted et al., 2003).

MOSQUITO POOLS

INTRODUCTION

Every year since 1969, local agencies in California have submitted mosquito pools to CDHS for arboviral testing (for more detailed information on the advent of this program, see Reeves 1990). In 1969, the program became statewide, testing 3,591 mosquito pools. In the past 33 years, CDHS or the Center for Vector-borne Disease Research (CVBDR) at UCD have tested an average of 4,010 pools a year, ranging from a low of 1,801 pools in 1970 to a high of 7,818 pools in 1972. In 1998, CVBDR assumed responsibility from CDHS for statewide mosquito testing. Upon receipt at the CVBDR, mosquitoes were tested for arboviruses by an *in situ* enzyme immunoassay using Vero cell culture (Graham et al., 1986). Results were sent to CDHS and included in the weekly Arbovirus Surveillance Bulletin (ASB).

1998

In 1998, 3,557 pools comprised of 154,463 mosquitoes (usually 50 females per pool) from 25 agencies were tested for arboviruses by CVBDR. Of the pools tested, 42 pools of *Culex tarsalis* from Kern County and 11 pools of *Cx. tarsalis* from Riverside County (53 total) were positive for WEE (Figure 3, Table 1). One pool of *Cx. tarsalis* from Riverside County was positive for SLE (Figure 2, Table 1) (Kramer et al., 1999).

1999

In 1999, 3,556 pools comprised of 145,511 mosquitoes from 24 agencies were tested for arboviruses by DARU (a new unit within CVBDR). There were no positive mosquito pools (Table 1) (Husted et al., 2000).

2000

In 2000, there were 3,901 pools comprised of 160,947 mosquitoes submitted by 28 agencies for arboviral testing by DARU. Of these, 30 pools of *Cx. tarsalis* from Riverside County were positive for SLE (Figure 2, Table 1) (Husted et al., 2001).

2001

In 2001, there were 3,501 pools comprised of 145,338 mosquitoes submitted by 26 agencies for testing. There was an increase in arboviral activity from the previous three years with 70 pools positive for SLE (67 pools of *Cx. tarsalis* and 3 pools of *Culex quinquefasciatus*) from Riverside County (Figure 2, Table 1). Also, nine pools of *Ochlerotatus melanimon* from Kern (8 pools) and Sacramento (1 pool) counties were positive for California encephalitis (CE), (Figure 1, Table 1) (Husted et al., 2002).

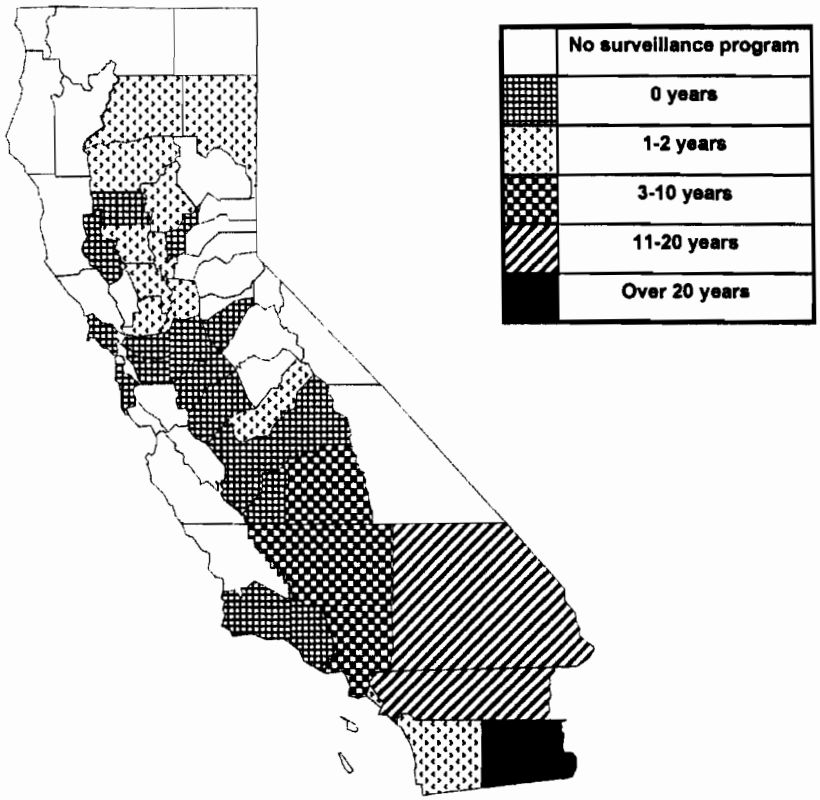

Figure 2. Number of Years with SLE-Positive Mosquito Pools in California, 1969-2002.

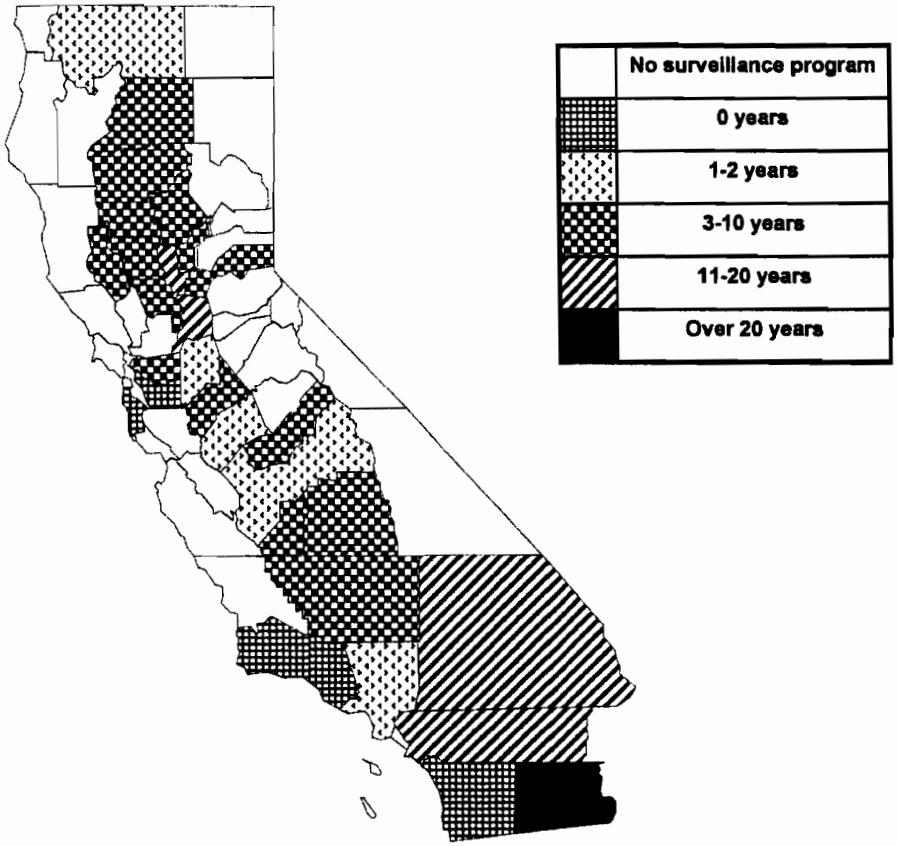

Figure 3. Number of Years with WEE-Positive Mosquito Pools in California 1969-2002.

California encephalitis is a rare human disease in California. The first isolation of CE was from humans in Kern County in 1943 (Hammon and Reeves, 1952). Since then, other closely related pathogens such as Jamestown Canyon and La Crosse viruses have been grouped with CE in the California serogroup. Taxonomically, the California serogroup lies within the genus *Bunyavirus*, in the family Bunyaviridae. Whereas other members of the California serogroup often cause human illness, cases due to infection by the prototype CE strain (last found in 1943) were not diagnosed again until 1996. In June of 1996, a Marin County man was the first person to be identified with disease associated with CE virus in 53 years (Eldridge et al. 1997) (Figure 1). Interestingly, this first human case reported since 1943 was followed five years later by the first mosquito pool isolations of CE in California since 1990 (Figure 1,Table 1) (Husted et al., 2002).

2002

In 2002, there were 4,879 pools comprised of 200,578 mosquitoes from 31 agencies submitted to DARU for arboviral testing. Of these, 28 pools were positive for WEE (Imperial County: 19; Riverside County: 9) (Figure 3, Table 1), 8 were positive for SLE (Imperial County), (Figure 2, Table 1), and 1 was positive for CE (Riverside County (Figure 1, Table 1) (Husted et al., 2003).

All but 4 WEE positive pools were comprised of *Cx. tarsalis*; the other 4 were *Cx. quinquefasciatus* pools from Imperial County. WEE positive pools were collected from June 5 to September 23 (Husted et al., 2003).

St. Louis encephalitis positive pools (8) were collected from Imperial County from July 9 to September 17; four of these pools were *Cx. tarsalis* and four were *Cx. quinquefasciatus*. One *Cx. quinquefasciatus* pool collected on August 20th from Riverside County was positive for CE (Table 1) (Husted et al., 2003).

SENTINEL CHICKENS

INTRODUCTION

Sentinel chicken flocks have been tested by CDHS since 1979 (for more detailed information on the advent of this program, see Reeves 1990). During the first year, there were 31 flocks. Over the past 23 years, the average number of flocks has been 109 and flock number has ranged from 22 in 1981 to a peak of 207 flocks in 2002. The number of hens per flock was reduced from 25 to 10 in 1991-92. Flocks were bled biweekly and sera were sent to the VRDL for testing by enzyme immunoassay (EIA) and indirect fluorescent antibody (IFA) tests. When needed, sera were sent to DARU for plaque reduction neutralization test (PRNT). Results were included in the weekly ASB.

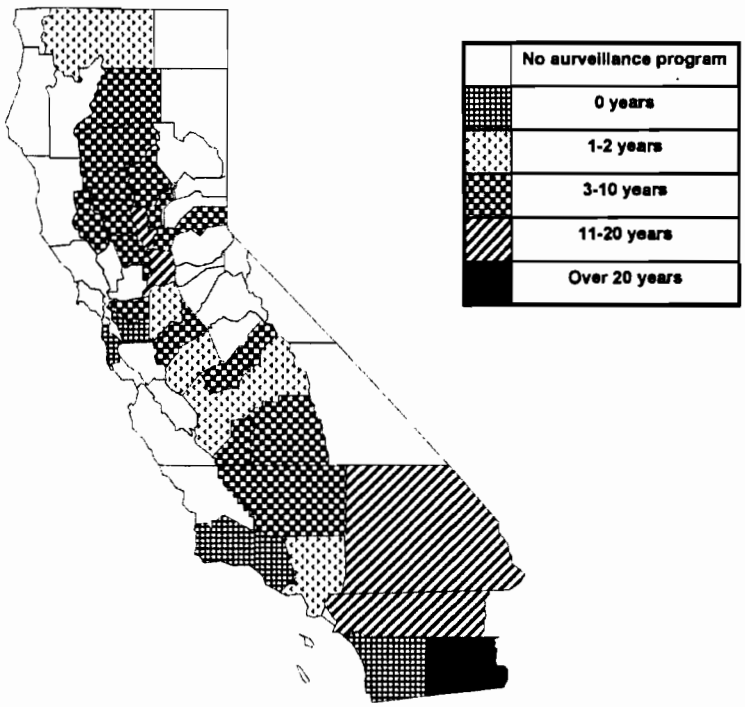

Figure 4. Number of Years with Sentinel Chicken Seroconversions to SLE in California, 1980-2002.

1998

In 1998, 19,840 chicken sera were tested from 134 sentinel flocks. Collections were made biweekly and sent to the VRDL for serology. One hundred-one chickens seroconverted to WEE in nine counties: Butte-5, Imperial-26, Kern-30, Placer-4, Riverside-28, San Bernardino-6, Solano-1, Sutter/Yuba-1 (Figure 5) (Table 1) and two chickens seroconverted to SLE (Los Angeles-1, Orange-1) (Figure 4, Table 1) (Kramer et al., 1999).

1999

In 1999, 19,978 chicken sera were tested from 190 sentinel flocks. Seroconversions to SLE were detected in 25 chickens from Imperial (14), Riverside (8), and San Bernardino (3) counties (Figure 4, Table 1). Three chickens seroconverted to WEE (Riverside County-2, Tulare County-1), (Figure 5, Table 1) (Husted et al., 2000).

2000

In 2000, 18,650 chicken sera were tested from 170 chicken flocks in California as well as 2,225 sera from Nevada, Oregon, Utah, Washington, and Arizona. There were 49 seroconversions to SLE from flocks found in Imperial (11), Riverside (36), and San Bernardino (2) counties (Figure 4, Table 1). There were no seroconversions to WEE (Figure 5, Table 1) (Husted et al., 2001).

2001

In 2001, 20,087 chicken sera were tested from 194 chicken flocks from California; 750 sera were tested from Nevada, Oregon, and Utah. There were 62 seroconversions to SLE in chickens from Imperial (11) and Riverside (51) counties (Figure 4, Table 1). Riverside County also had 3 chickens seroconvert to for WEE (Figure 5, Table 1) (Husted et al., 2002).

2002

In 2002, 20,087 chicken sera were tested from 207 chicken flocks. There were 45 seroconversions to SLE from Imperial (43) and Riverside (2) counties (Figure 4) (Table 1). Seroconversions occurred from July 8 to October 28.

There also were 52 seroconversions to WEE from Imperial (40), San Bernardino (4), and Riverside (8) counties (Figure 5) (Table 1). Seroconversions occurred from July 8 to November 15 (Husted et al., 2003).

NON-DHS SENTINEL CHICKEN TESTING

Three agencies performed the initial screening of their sentinel flock sera at their own laboratories. Sacramento –Yolo Mosquito and Vector Control District maintained sentinel flocks and tested the following: 1.) 1998- 1,780 sera samples from 8 flocks, 2.) 1999-1,961 sera samples from 9 flocks, 3.) 2000-1124 sera samples from

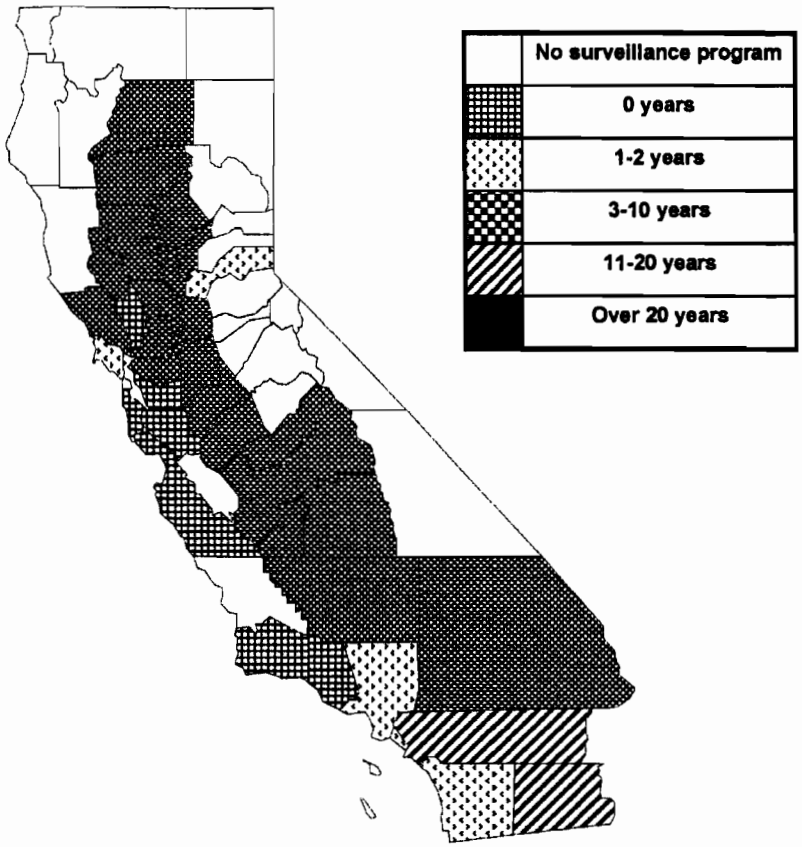

Figure 5. Number of Years with Sentinel Chicken Seroconversions to WEE in California, 1980-2002.

9 flocks, 4.) 2001- 1,221 sera samples from 9 flocks, and 5.) 2002- 1,490 sera samples from 10 flocks, with no seropositives. San Gabriel Valley MVCD tested 10-11 flocks that ranged from 43-65 birds total from 1998-2001 by EIA, none was found positive for SLE or WEE. In addition, Greater Los Angeles VCD did some of their own testing in 2001 and 2002.

FREE-RANGING BIRD TESTING

UCD ARBOVIRUS FIELD STATION

UCD researchers have live-trapped birds in Kern and Riverside Counties since 1996 (Reisen et al. 2000; Reisen et al. 2002). Birds were collected by mist netting or baited traps, bled, banded, and released. Sera were tested for WEE and SLE by a new EIA, with positives confirmed by PRNT (Chiles and Reisen 1998).

In 1998, 4,292 birds representing 65 species were caught in Kern County. Thirty-three birds in 9 species were positive for WEE (Table 2). In the same year in Riverside County, 2,846 birds representing 71 species were captured. Of these, 35 birds were SLE positive (7 species) and 11 birds (7 species) were WEE positive (Table 2).

In 1999, collections focused on baited traps and only 1,035 birds representing 24 species were tested in Kern County. Of these, California quail (*Callipepla californica*) was the only species positive for WEE (14 positives,173 samples); no birds were SLE positive (Table 2). In Riverside County, 1,462 birds representing 28 species were tested; only Gambel's quails (*Callipepla gambelii*) were positive for WEE (13 positives, 847 samples); none was positive for SLE. (Table 2).

In 2000, 1,878 birds representing 65 species were tested in Kern County. Of these, only the house finch (*Carpodacus mexicanus)* was positive for WEE (4 positives, 487 samples); none was SLE positive (Table 2). In Riverside County, 1,700 birds representing 73 species were tested. Two WEE positives were found in 2 species and 40 SLE positives were found in 9 species of birds (Table 2).

In 2001, 1,523 birds representing 51 species were tested in Kern County. Of these, only the house finch (2 positives, 339 samples) and the mourning dove (*Zenaida macroura*), (2 positives, 30 samples) were positive for WEE; no birds were SLE positive (Table 2). In Riverside County, 1,026 birds representing 47 species were tested. One WEE positive was found in Gambel's quail (297 samples) and 38 SLE positives were found in 9 species of birds (Table 2).

In 2002, 2,636 birds representing 53 species were tested in Kern County. None of these was positive for WEE or SLE. In Riverside County, 2,133 birds representing 57 species were tested. One WEE positive was found in Gambel's quail (445 samples) and 16 SLE positives were found in 5 species of birds (Table 2).

MOSQUITO AND VECTOR CONTROL DISTRICTS

Sacramento-Yolo MVCD tested wild birds from the Stone Lakes National Wildlife Refuge from 1998-2002 for SLE and WEE by EIA with the following results: 1998- 783 birds tested, no positives; 1999- 132 birds tested, 1 positive WEE; 2000- 828 birds, no positives; 2001- 1,466 birds, no positives; and 2002- 1105 birds, no positives. Also, 102 Canada geese (*Branta canadensis*) were screened for WEE and SLE in 2002; 1 was positive for WEE. All positives were confirmed at DARU by PRNT.

Orange County Vector Control District (OCVCD) performed wild bird testing from 1987-2002. Birds were captured using Australian crow traps and bled biweekly. Specimens were tested using the hemagglutination inhibition (HI) assay. Further details on bird capture, sample processing, and results from OCVCD's SLE live-bird surveillance program from 1987-1996 were published previously (Gruwell et al. 2000). In 1998, 2,277 samples (1,032 recaptures) were taken from house finches and 19 (0.83%) of these tested positive for SLE (Table 3). None was positive for WEE. Seropositive birds for SLE were trapped in all months except March, November, and December.

In 1999, wild bird testing continued with 3,528 house finch samples (1,681 recaptures) [8 (0.23%) were positive for SLE] and 728 house sparrow samples (515 recaptures) [1 (0.14%) was positive for SLE] (Table 3). Seropositive birds for SLE were trapped in May, June, October, and December.

In 2000, 3,149 house finches (1,564 recaptures) were tested and 3 (0.10%) were positive for SLE (Table 3). No samples were positive for WEE. Seropositive birds for SLE were trapped in February, May, and October.

In 2001, 2,818 house finches (1,191 recaptures) were tested and 4 (0.14%) were positive for SLE (Table 3). No samples were positive for WEE. Seropositive birds for SLE were trapped in February, May, and October.

In 2002, 2,588 house finches (1,159 recaptures) and 8 (0.31%) were positive for SLE (Table 3). In addition, 35 crows (1 recapture) were captured and 2 (5.7%) were positive for SLE. No samples were positive for WEE or WN. SLE seropositive birds for SLE were detected in March, April, and August.

San Gabriel County VCD tested wild birds by EIA from 1998-2000. In 1998, there was one SLE positive from 75 birds tested. This positive was confirmed by HI testing at Orange County VCD. In 1999 and 2000, 15 and 36 birds were tested respectively; none was positive for arboviruses.

EQUINE CASES

INTRODUCTION

Equine surveillance for arboviruses has been in place since 1954 (Reeves 1990). Horses showing neurological signs have sera and/ or brain tissue specimens submitted by veterinarians to VRDL for testing. In 2000, testing was shifted to DARU. Since 1969, there have been 69 reported cases of WEE in horses in California. A peak of 18 equine cases was reported in 1979. The last reported positive WEE horse case was in 1997.

1998

Specimens from 5 horses with neurological signs were submitted by veterinarians to VRDL. None of these were positive by serology or antigen testing (Table 1) (Kramer et al., 1999).

Table 2. Summary of Wild Bird Testing by U.C. Davis for SLE and WEE, 1998-2002.

Wild bird testing positives 1998-2002

County	Common Name	Scientific Name	Samples Total	SLE	Total % Positive	WEE	Total % Positive
1998							
Kern	American robin	*Turdus migratorius*	35	0	0	2	5.7
	Black-headed grosbeak	*Pheucticusmelanocephalus*	12	0	0	1	8.3
	Brewers blackbird	*Euphagus cyanocephalus*	39	0	0	1	2.6
	California quail	*Callipepla californica*	296	0	0	12	4.1
	California thrasher	*Toxostoma redivivum*	25	0	0	1	4.0
	House finch	*Carpodacus mexicanus*	911	0	0	9	1.0
	Mourning dove	*Zenaida macroura*	89	0	0	3	3.4
	Northern mockingbird	*Mimus polyglottos*	29	0	0	1	3.4
	Red-winged blackbird	*Agelaius phoeniceus*	246	0	0	1	0.4
	Song sparrow	*Melospiza melodia*	453	0	0	2	0.4
Riverside	Abert's towhee	*Pipilo aberti*	44	0	0	1	2.3
	Brown-headed cowbird	*Molothrus ater*	184	0	0	1	0.5
	Common ground dove	*Columbina passerine*	176	2	1.1	2	1.1
	Gambel's quail	*Callipepla gambelii*	459	23	5.0	2	0.4
	Great-tailed grackle	*Quiscalus mexicanus*	9	1	11.1	0	0
	House sparrow	*Passer domesticus*	239	6	2.5	3	1.3
	Least bittern	*Ixobyrchus exilis*	6	1	16.7	1	16.7
	Mourning dove	*Zenaida macroura*	22	1	4.5	0	0
	Purple finch	*Carpodacus purpureus*	4	1	25.0	0	0
	Snowy egret	*Egretta thula*	1	1	100	0	0
	Sora	*Porzana carolina*	4	0	0	1	25.0
1999							
Kern	California quail	*Callipepla californica*	173	0	0	14	8.1
Riverside	Gambel's quail	*Callipepla gambelii*	847	0	0	13	0
2000							
Kern	House finch	*Carpodacus mexicanus*	487	0	0	4	0.8
Riverside	Abert's towhee	*Pipilo aberti*	42	2	4.8	0	0
	American kestrel	*Falco sparverius*	1	1	100	0	0
	Common ground dove	*Columbina passerine*	90	8	8.9	0	0
	Gambel's quail	*Callipepla gambelii*	317	12	3.8	1	0.3
	House finch	*Carpodacus mexicanus*	50	3	6.0	0	0
	House sparrow	*Passer domesticus*	160	9	5.6	0	0
	Loggerhead shrike	*Lanius ludovicianus*	?	1			
	Mourning dove	*Zenaida macroura*	29	2	6.9	0	0
	Plain pigeon	*Columba livia*	13	2	15.4	0	0
	Warbling vireo	*Vireo gilvus*	50	0	0	1	2.0
2001							
Kern	House finch	*Carpodacus mexicanus*	339	0	0	2	0.6
	Mourning dove	*Zenaida macroura*	30	0	0	2	6.7
Riverside	Brown-headed cowbird	*Molothrus ater*	122	1	0.8	0	0
	Common ground dove	*Columbina passerine*	35	4	1.1	0	0
	Gambel's quail	*Callipepla gambelii*	297	122	41.1	1	0.3
	House sparrow	*Passer domesticus*	118	1	0.8	0	0
	Loggerhead shrike	*Lanius ludovicianus*	5	2	40.0	0	0
	Mourning dove	*Zenaida macroura*	55	5	9.1	0	0
	Northern mockingbird	*Mimus polyglottos*	4	1	25.0	0	0
	Red-winged blackbird	*Agelaius phoeniceus*	2	1	50.0	0	0
	Song sparrow	*Melospiza melodia*	43	1	2.3	0	0
2002							
Riverside	Common ground dove	*Columbina passerina*	88	3	3.4	0	0
	Gambel's quail	*Callipepla gambelii*	445	9	2.0	1	0.2
	Green-backed heron	*Butorides striatus*	2	1	50		
	Loggerhead shrike	*Lanius ludovicianus*	1	1	100	0	0
	Mourning dove	*Zenaida macroura*	332	2	0.6	0	0

Table 3. Summary of Wild Bird Testing by Orange County for SLE and WEE, 1998-2002.

Year	Common Name	Scientific Name	Samples Total	SLE	Total % Positive	WEE	Total % Positive
1998	House finch	*Carpodacus mexicanus*	2277	19	0.83	0	0
1999	House finch	*Carpodacus mexicanus*	3528	8	0.23	0	0
	House sparrow	*Passer domesticus*	728	1	0.14	0	0
2001	House finch	*Carpodacus mexicanus*	2818	4	0.1	0	0
2002	House finch	*Carpodacus mexicanus*	2588	8	0.3	0	0

1999

One specimen was submitted and tested negative for WEE (Table 1) (Husted et al., 2000).

2000

In 2000, 16 specimens were submitted for testing; of these, 15 specimens showed no sign of arboviral infection by WEE, EEE, or WN. The remaining sample tested positive for eastern equine encephalomyelitis (EEE), (Figure 1, Table 1) (Husted et al., 2001). Eastern equine encephalomyelitis virus and WEE are both members of the family Togaviridae, genus *Alphavirus*. Although related, EEE is much more virulent in horses when compared to WEE. In the eastern U.S., EEE is maintained and amplified in a transmission cycle involving *Culiseta melanura* mosquitoes and wild birds. Infections in equines and humans occur when infected birds are fed upon by mosquito species other than *Cs. melanura*, and then subsequently feed upon humans and/or equines (Morris 1998).

A 16-month-old horse from Ventura County was euthanized after exhibiting neurological signs (Franklin et al., 2001). Isolations of EEE were made from brain samples sent to the National Veterinary Service Laboratory, US Department of Agriculture in Ames, Iowa, VRDL, and DARU. Sequencing studies by several laboratories indicated that the isolate from this horse brain was similar to the vaccine strain used by Fort Dodge. The horse had a history of travel to Utah, southern California, and as far east as Texas for show purposes (all geographic areas west of the distribution of EEE), and no horses at these shows were from EEE-endemic areas or were known to have EEE. This horse and 27 others residing in the same barn had been vaccinated with a commercial four-way multi-dose vaccine for EEE, WEE, rhinopneumonitis, and tetanus one week before the horse showed the first sign of illness. Natural infection, bioterrorism, and importation were all excluded as possible causes of infection. Quite possibly, infection was from the four-way vaccine administered to the horse, which may have contained live EEE virus (Franklin et al., 2001).

2001

In 2001, 15 horses were tested for WN and WEE, none was positive (Table 1) (Husted et al., 2002).

2002

In 2002, 83 horse specimens were submitted to the VRDL for testing, 1 horse from Nebraska was found serologically positive (1:80) for WN. This specimen was submitted by the Los Angeles County Health Department, but was considered an imported case rather than locally acquired (Husted et al., 2003).

WEST NILE VIRUS SURVEILLANCE

INTRODUCTION

West Nile virus, a member of the Japanese encephalitis serogroup within the family Flaviviridae, was introduced into the United States in 1999. During the first year, there were 62 reported human cases of WN and 7 deaths from 4 states (Husted et al., 2000).

In response to the possible spread of WN to the west coast, California expanded its arbovirus testing program to include WN in 2000. CDHS initiated a dead bird surveillance and WN testing program (McCaughey et al, 2002). A letter was sent to over 600 agencies requesting bird carcass submissions. Furthermore, routine sentinel chicken serological testing was enhanced. Because SLE and WN are closely related, WN antibodies cross-react to the SLE antigen used in the screening EIA. Therefore, all specimens positive for SLE were also tested for WN and the viruses subsequently distinguished by PRNT. Routine mosquito pool, human, equine, and ratite encephalitis testing were also expanded to detect WN. Through 2002, no WN positive dead birds had been detected in California. In the first year (2000) of dead bird testing, 40 dead birds were reported from 10 counties and 20 met the criteria for testing (Husted et al., 2001). In 2001, the number of dead birds reported increased to 68 from 19 counties and 18 were tested (Table 1) (Husted et al., 2002). In 2002, WN virus surveillance in California was supported by a grant from the Centers for Disease Control and Prevention (CDC). Notifications were sent twice a year to approximately 600 agencies and groups explaining the program and requesting they contact CDHS when dead birds (especially crows) were found. Recipients of the mailing included California Department of Food and Agriculture, Department of Fish and Game, United States Fish and Wildlife Services, wildlife rehabilitation and refuge centers, National Audubon Society, local health

departments, mosquito and vector control districts, environmental health officers, veterinarians, and animal control. Necropsies of submitted carcasses were performed by the California Animal Health, Food, and Safety (CAHFS) lab. Specimens were forwarded to DARU for testing via plaque assay on Vero cell culture.

In 2002, the dead bird surveillance program significantly increased its reporting and testing abilities by establishing the West Nile Virus Hotline (1-877-WN-BIRD) and the California West Nile Virus Surveillance Information Center website (**http://www.westnile.ca.gov.**) On June 26, a CDHS news release entitled, "California Department of Health Seeks Public Help for West Nile Virus Surveillance" was released. Subsequently, there were several newspaper articles and radio interviews informing the public of the possible arrival of WN to California and providing the dead bird surveillance program's hotline. A total of 3,666 dead birds were reported from 56 counties; 653 birds from 45 counties tested negative for WN (Table 1) (Husted et al., 2003).

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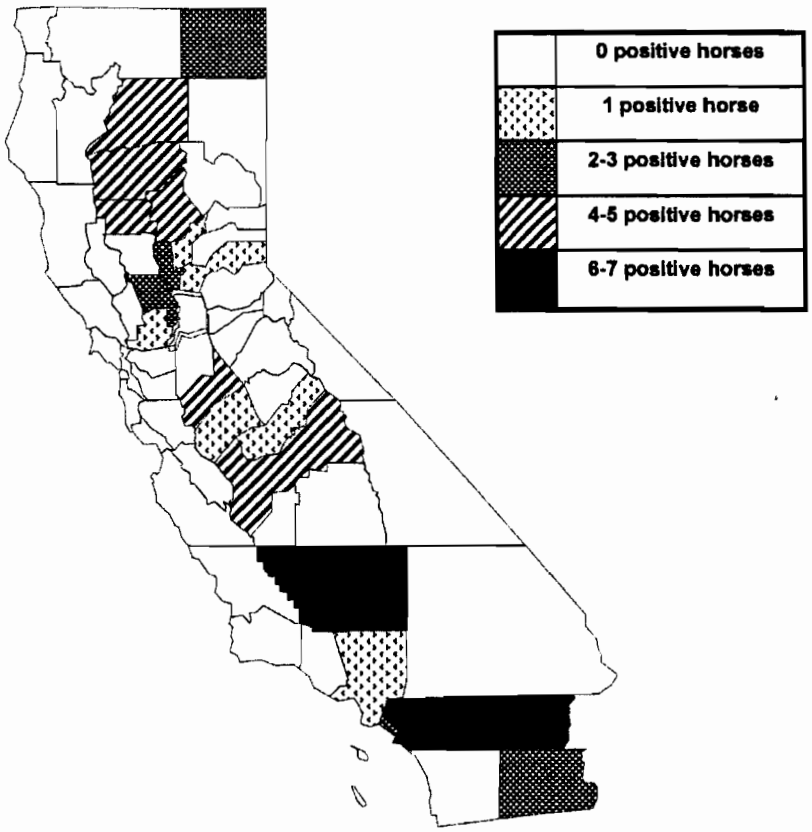

Figure 6. WEE-Equine Cases in California from 1969-2002.

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Surveillance for Mosquito-Borne Encephalitis Virus Activity and Human Disease in California, 2002

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ABSTRACT: In 2002, the California surveillance program for mosquito-borne encephalitis virus activity tested humans, equids, mosquitoes, sentinel chickens and dead birds to detect arbovirus activity. There were 431 suspected human cases of aseptic meningitis/encephalitis/atypical Guillain-Barre/acute flaccid paralysis/febrile illness. All were tested for St. Louis encephalitis (SLE), western equine encephalomyelitis (WEE), and West Nile virus (WN) infection. Eight were diagnosed to have WN infection. Seven were imported cases and one case was a resident of Los Angeles County. Adult mosquito abundance was monitored with New Jersey light trap collections and reported by local agencies to the California Department of Health Services (CDHS). Fifty local agencies maintained 207 sentinel chicken flocks. There were 45 chickens which seroconverted to SLE and 52 to WEE. Thirty-one agencies submitted 4,879 mosquito pools for virus testing. Twenty-eight pools were positive for WEE, eight were positive for SLE and one was positive for California encephalitis. Eighty-three equids with encephalitis were tested for WEE and WN infection. One was diagnosed as having WN infection. The source of infection was Nebraska. A total of 3,666 dead birds were reported to CDHS and 653 were tested and found negative for WN infection.

The California Mosquito-Borne Encephalitis Surveillance Program is a cooperative effort of the Division of Communicable Disease Control of the California Department of Health Services (CDHS), the University of California at Davis and Berkeley, the Mosquito and Vector Control Association of California, local mosquito and vector control agencies, local health departments, physicians, veterinarians, and other interested parties. Collaborating agencies in the West Nile virus (WN) surveillance program include the California Department of Food and Agriculture (CDFA), California Animal Health and Food Safety Laboratory (CAHFS), California Department of Fish and Game, the U.S. Fish and Wildlife Service, and the Centers for Disease Control and Prevention (CDC).

Program Components:

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

2) Enrollment of patients diagnosed with encephalitis into the CDHS California Encephalitis Project (CEP) which evaluates demographics, exposure to arthropods, and laboratory analyses to determine etiology.

3) Diagnostic testing of specimens from equids that exhibited clinical signs of viral neurologic disease compatible with arboviral infection of western equine encephalomyelitis (WEE), WN, and other arboviruses as appropriate.

4) Monitoring and testing of mosquitoes for the presence of St. Louis encephalitis (SLE) and WEE. Tests were also done for WN, California encephalitis (CE), dengue, and other arboviruses.

5) Serological monitoring of sentinel chickens for SLE and WEE antibodies in areas of California where encephalitis virus activity has occurred historically. Chicken sera from geographic areas where SLE seroconversions occurred and other selected areas of the state were also tested for WN.

6) Surveillance and diagnostic testing of dead birds, especially crows, for WN infection.

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

Arbovirus diagnostic procedures used in 2002 in California are summarized in Table 1.

HUMAN DISEASE SURVEILLANCE

The CDHS Viral and Rickettsial Disease Laboratory (VRDL) tested sera and/or cerebrospinal fluid specimens from 431 patients for antibodies to WN, SLE and WEE. Included in this series were 132 cases of aseptic meningitis, 251 cases of encephalitis, 11 cases of atypical Guillain-Barre/acute flaccid paralysis, and 37 cases of febrile illness.

Table 1. 2002 Arbovirus Diagnostic Procedures for California

	Criteria	Primary Test	Confirmatory Test	Virus Tested		
				SLE	WNV	WEE
Mosquito Pools	Collections by Local Agencies	*in-situ* EIA using vero cell culture (DARU)		X	X	X
Chicken Sera	Local Agency Sentinel Flocks	EIA (VRDL)	IFA (VRDL) PRNT as needed (DARU)	X	X	X
Equine Sera	Per request of the veterinarian	PRNT (DARU)		X	X	X
Equine Tissue	Screened by VPHS	Cell Culture (DARU)		X	X	X
Dead Birds	Screened by VBDS	Immunohistochemistry on heart, kidney, liver (CAHFS)	Cell Culture on kidney & lung (DARU)		X	
Other Animals	Screened by VPHS	PRNT for sera (DARU), Cell Culture for tissue (DARU)			X	
Human Sera	Screened by VRDL	EIA (for SLE and WEE), IgM-ELISA (for WNV) (VRDL)	PRNT (DARU/VRDL)	X	X	X
Human Spinal Fluid (if no serum available)	Screened by VRDL	EIA (for SLE and WEE), IgM-ELISA (for WNV) (VRDL)	PRNT (DARU/VRDL)	X	X	X

Source: California Department of Health Services

Key:

CAHFS: California Animal Health and Food Safety
DARU: Davis Arbovirus Research Unit
EIA: Enzyme Immunoassay
IFA: Indirect Fluorescent Antibody
IgM-ELISA: IgM-Enzyme-Linked Immunoabsorbent Assay
PRNT: Plaque Reduction Neutralization Test
VBDS: Vector-Borne Disease Section
VPHS: Veterinary Public Health Section
VRDL: Viral And Rickettsial Disease Laboratory

Of the 431 tested, 251 were enrolled in the CEP. For each patient enrolled, a battery of tests was conducted, including polymerase chain reaction, serology, and viral isolation for 15 agents. Testing for additional agents was pursued as clinical symptomology and exposure history warranted; extensive testing for arboviruses was conducted for cases with known mosquito exposure and those with a travel history to an area of WN activity.

One locally acquired human WN case was detected in 2002 in a resident of Los Angeles County and seven imported cases were identified. The locally acquired case, a 31 year-old female, was diagnosed with aseptic meningitis in August; she recovered fully. The patient reported no travel to areas where WN had been detected, nor had she received a blood transfusion or organ transplant.

Preliminary laboratory testing for infection was conducted by the Los Angeles County Health Department and VRDL. Further confirmatory testing was conducted at the UC Davis Arbovirus Research Unit (DARU) and CDC. Seven cases imported into California were identified. These included: (1) a 45 year-old male with WN meningitis from Houston, TX who visited Los Angeles, (2) a 57 year-old male with probable WN encephalitis resident in Contra Costa County who had traveled to Chicago, IL, (3) a 70 year-old male with WN meningitis resident in Orange County who had traveled to Nebraska, (4) a 20 year-old female with WN fever resident in San Francisco who had traveled to Washington, D.C., (5) a 66 year-old male (visiting San Mateo County) with WN encephalitis had just come from Milwaukee, WI, (6) a 54 year-old male resident of Kansas with acute flaccid paralysis who was visiting Los Angeles, and (7) a 50 year-old male resident of Ventura County with WN fever who had traveled to Florida and the Bahamas.

EQUINE SURVEILLANCE

Serum and brain tissue specimens from 83 equids displaying neurological signs were submitted for arboviral testing to DARU. One tested positive to WN which was imported from Nebraska with onset of illness in September; the equid recovered.

Table 2. Participation by local agencies in the statewide mosquito-borne encephalitis surveillance program, 2002.

County	Agency	Agency Code	New Jersey Light Trap	Mosquito Pools	No. Flocks	No. Chickens	No. Sera Samples Tested
Alameda	Alameda Co. MAD	ALCO	22	61	3	21	286
Butte	Butte Co. MVCD	BUCO	25	30	7	84	1,128
Colusa	Colusa MAD	CLSA	3		1	10	150
Contra Costa	Contra Costa MVCD	CNTR	18	70	4	40	559
Fresno	Consolidated MAD	CNSL	12	44	4	40	444
Fresno	Fresno MVCD	FRNO	9	51	2	20	212
Fresno	Fresno Westside MAD	FRWS	10	27	2	20	226
Glenn	Glenn Co. MVCD	GLEN	4	40	1	13	183
Imperial	Coachella Valley MVCD	IMPR		359	3	30	518
Imperial	Imperial Co. Environmental Health	IMPR			3	28	307
Imperial	Quechan Indian Reservation	IHSY			1	5	10
Inyo	Owens Valley MAP	OWVY	12		0	0	
Kern	Delano MAD	DLNO	8		2	16	161
Kern	Kern MVCD	KERN	20	507	9	90	1,247
Kern	West Side MVCD	WEST	12		3	30	336
Kings	Kings MAD	KNGS	9	8	3	30	341
Lake	Lake Co. VCD	LAKE		124	2	20	269
Los Angeles	Antelope Valley MVCD	ANTV	10		5	35	552
Los Angeles	Greater Los Angeles Co. VCD	GRLA	14	360	4	40	797
Los Angeles	Long Beach Environmental Health	LONG		346	4	40	581
Los Angeles	Los Angeles Co. West VCD	LACW		24	18	210	1,406
Los Angeles	San Gabriel Valley MVCD	SGVA		11	10	60	1,268
Madera	Madera Co. MVCD	MADR	5	12	2	20	200
Marin/Sonoma	Marin-Sonoma MVCD	MARN	21		7	75	983
Merced	Merced Co. MAD	MERC	18	16	6	35	453
Monterey	North Salinas MAD	NSAL	17		1	10	150
Napa	Napa MAD	NAPA			2	10	139
Orange	Orange Co. VCD	ORCO		49	1	10	135
Placer	Placer Co. VCD	PLCR	12		3	30	420
Riverside	Coachella Valley MVCD	COAV	24	1,014	9	80	1,511
Riverside	Northwest MVCD	NWST	12	310	6	60	817
Riverside	Riverside Co. Environmental Health	RIVR	13		6	66	996
Sacramento/Yolo	Sacramento-Yolo MVCD	SAYO	44	456	10	99	1,589
San Bernardino	San Bernardino Co. VCP	SANB	19	39	7	70	1,137
San Bernardino	West Valley MVCD	WVAL			3	30	457
San Diego	San Diego Co. Dept of Health	SAND	13	48	3	30	506
San Joaquin	San Joaquin Co. MVCD	SJCM	50	233	4	48	670
San Mateo	San Mateo Co. MAD	SANM	22	19	3	30	390
Santa Barbara	Santa Barbara Coastal VCD	SBCO		22	4	38	616
Santa Clara	Santa Clara Co. VCD	STCL	18		2	20	285
Santa Cruz	Santa Cruz Co. MVCD	SCRZ	7		1	10	139
Shasta	Burney Basin MAD	BURN	6		2	20	200
Shasta	Shasta MVCD	SHAS	18	76	5	55	741
Solano	Solano Co. MAD	SOLA	13		2	24	195
Stanislaus	East Side MAD	EAST			1	12	169
Stanislaus	Turlock MAD	TRLK	21	313	4	48	619
Sutter/Yuba	Sutter-Yuba MVCD	SUYA	38	143	7	70	971
Tehama	Tehama Co. MVCD	TEHA	9		2	20	220
Tulare	Delta VCD	DLTA	12	33	6	60	751
Tulare	Tulare MAD	TRLE	10		2	20	241
Ventura	City of Moorpark	MOOR	4	5	1	10	160
Ventura	Ventura Co. Environmental Health	VENT	19	29	4	40	498
Total			633	4,879	207	2,032	27,339

Source: California Department of Health Services

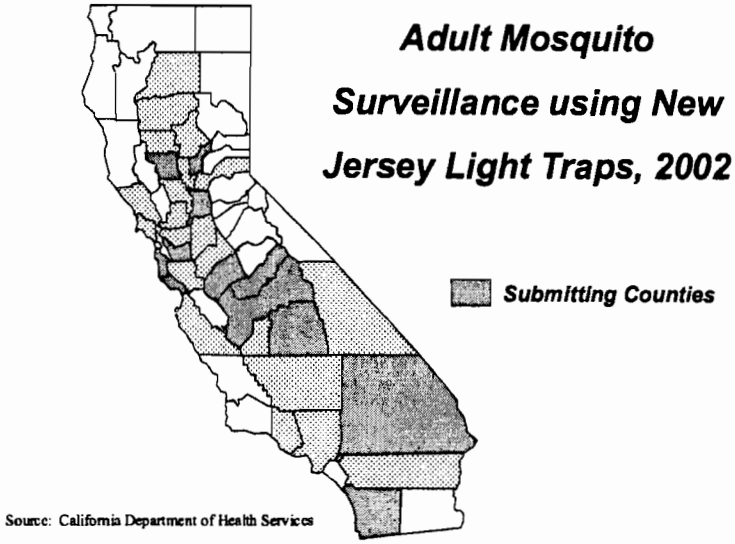


Figure 1. Counties that submitted weekly adult mosquito occurrence reports to CDHS.

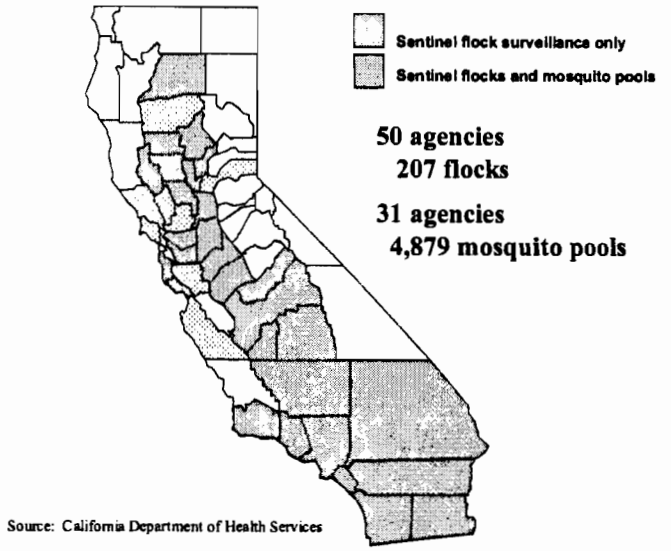


Figure 2. Counties which submitted chicken sera and/or mosquito pools for SLE, WEE, WN, and CE testing, California, 2002.

Table 3. Mosquitoes (*Culex* spp.) tested for WNV, WEE, and SLE by submitting county and agency, 2002.

		Cx erythrothorax		*Cx pipiens*		*Cx quinquefasciatus*		*Cx tarsalis*		Total	
County	Agency	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.
Alameda	ALCO							61	3,050	61	3,050
Butte	BUCO							11	579	11	579
Contra Costa	CNTR	11	550	9	450			50	2,500	70	3,500
Fresno	CNSL	2	27			11	411	30	1,110	43	1,548
Fresno	FRNO					20	827	31	1,200	51	2,027
Fresno	FRWS							27	1,309	27	1,309
Glenn	GLEN							33	1,650	33	1,650
Imperial	IMPR	28	1,235			51	2,059	208	9,312	287	12,606
Kern	KERN					21	471	335	10,675	356	11,146
Kings	KNGS							7	350	7	350
Lake	LAKE	1	17					92	4,408	93	4,425
Los Angeles	GRLA	43	1,762			236	8,655	63	2,097	342	12,514
Los Angeles	LACW					21	858			21	858
Los Angeles	LONG					189	6,162	145	5,711	334	11,873
Los Angeles	SGVA	1	31			10	348			11	379
Madera	MADR			11	550			1	50	12	600
Merced	MERC			3	115	3	150	9	391	15	656
Merced	TRLK							122	5,879	122	5,879
Orange	ORCO					36	723	13	300	49	1,023
Riverside	COAV	4	166			81	2,922	852	37,701	937	40,789
Riverside	NWST					148	6,375	111	4,183	259	10,558
Sacramento	SAYO			24	664			214	9,525	238	10,189
San Bernardino	SANB	4	192			18	437	16	378	38	1,007
San Diego	SAND	32	1,580					16	800	48	2,380
San Joaquin	SJCM			63	2,241			140	5,501	203	7,742
San Mateo	SANM			18	660			1	21	19	681
Santa Barbara	SBCO	3	100			8	261			11	361
Shasta	SHAS			17	900			59	3,192	76	4,092
Stanislaus	TRLK			2	65			152	7,197	154	7,262
Sutter	SUYA							96	4,316	96	4,316
Tulare	DLTA					6	245	27	1,099	33	1,344
Ventura	MOOR					5	32			5	32
Ventura	VENT	20	914	4	56			5	152	29	1,122
Yolo	SAYO			2	39			167	7,919	169	7,958
Yuba	SUYA			3	57			38	1,794	41	1,851
Total		**149**	**6,574**	**156**	**5,797**	**864**	**30,936**	**3,132**	**134,349**	**4,301**	**177,656**

Source: California Department of Health Services

Table 4. Mosquitoes (Other *Culex* spp.) tested for WNV, WEE, and SLE by submitting county and agency, 2002.

		Cx erraticus		*Cx restuans*		*Cx stigmatosoma*		Total	
County	Agency	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.
Fresno	CNSL					1	24	1	**24**
Imperial	IMPR	1	6					1	**6**
Lake	LAKE					7	200	7	**200**
Los Angeles	GRLA					2	45	2	**45**
Los Angeles	LONG			1	43	10	192	11	**235**
Merced	MERC					1	32	1	**32**
Riverside	NWST					48	1,761	48	**1,761**
Sacramento	SAYO					1	12	1	**12**
San Bernardino	SANB					1	10	1	**10**
Total		**1**	**6**	**1**	**43**	**71**	**2,276**	**73**	**2,325**

Source: California Department of Health Services

Table 5. Mosquitoes (*Culiseta* spp.) tested for WNV, WEE, and SLE by submitting county and agency, 2002.

		Cs incidens		*Cs inornata*		*Cs particeps*		Total	
County	Agency	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.
Imperial	IMPR			1	5			1	**5**
Los Angeles	GRLA	6	198			2	39	8	**237**
Los Angeles	LACW	1	19	2	89			3	**108**
Riverside	COAV			21	380			21	**380**
Santa Barbara	SBCO	3	115			1	10	4	**125**
Total		**10**	**332**	**24**	**474**	**3**	**49**	**37**	**855**

Source: California Department of Health Services

Table 6. Mosquitoes (*Ochlerotatus* spp.) tested for WNV, WEE, and SLE by submitting county and agency, 2002.

		Oc melanimon		*Oc taeniorhynchus*		*Oc washinoi*		Total	
County	Agency	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.
Butte	BUCO	19	990					19	**990**
Glenn	GLEN	7	350					7	**350**
Kern	KERN	151	6,594					151	**6,594**
Kings	KNGS	1	50					1	**50**
Lake	LAKE	24	1,196					24	**1,196**
Los Angeles	LONG	1	50					1	**50**
Merced	TRLK	32	1,548					32	**1,548**
Riverside	COAV	18	900					18	**900**
Sacramento	SAYO	15	390					15	**390**
San Joaquin	SJCM	30	1,111					30	**1,111**
Santa Barbara	SBCO			4	158	1	22	5	**180**
Stanislaus	TRLK	5	227					5	**227**
Sutter	SUYA	6	229					6	**229**
Yolo	SAYO	32	1,173					32	**1,173**
Total		**341**	**14,808**	**4**	**158**	**1**	**22**	**346**	**14,988**

Source: California Department of Health Services

Table 7. Mosquitoes (*Aedes* spp., *Anopheles hermsi*, and *Psorophora columbiae*) tested for WNV, WEE, and SLE by submitting county and agency, 2002.

		Ae albopictus		*Ae vexans*		*An hermsi*		*Ps columbiae*		Total	
County	Agency	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.
Imperial	IMPR			66	3,075			4	91	70	**3,166**
Los Angeles	GRLA	2	38			6	239			8	**277**
Riverside	COAV			34	1,087			4	71	38	**1,158**
Riverside	NWST					3	98			3	**98**
Sacramento	SAYO			1	23					1	**23**
Santa Barbara	SBCO					2	32			2	**32**
Total		**2**	**38**	**101**	**4,185**	**11**	**369**	**8**	**162**	**122**	**4,754**

Source: California Department of Health Services

ADULT MOSQUITO SURVEILLANCE

Forty local agencies from 33 counties conducted weekly adult mosquito collections from in 2002 using 633 New Jersey light traps distributed statewide (Table 2 and Figure 1). Data from these collections were forwarded to CDHS and collated weekly into the Adult Mosquito Occurrence Summary Report from April 3 – October 30.

MOSQUITO TESTING

Thirty-one local mosquito control agencies (Table 2, Figure 2) in California submitted a total of 200,578 mosquitoes (4,879 pools) (Tables 3-7) to be tested for arboviruses at DARU. Twenty-four pools of *Culex tarsalis* and 4 pools of *Culex quinquefasciatus* were positive for WEE. Four pools of *Culex tarsalis* and 4 pools of *Culex quinquefasciatus* were positive for SLE, and 1 pool of *Culex quinquefasciatus* was positive for CE (Table 8 and Figure 3). All positive mosquito pools were also tested for WN and none was positive. Negative pools were assumed to be negative for WN infection. WEE and SLE virus isolates from pooled mosquitoes over the past ten years are summarized in Figure 4.

CHICKEN SEROSURVEILLANCE

Fifty local mosquito and vector control agencies maintained 207 sentinel chicken flocks, an increase of 13 flocks from 2001 (Table 2 and Figure 2). Blood specimens from each flock were collected and tested biweekly. A total of 24,082 chicken sera from 48 agencies in California were tested for antibodies to SLE and WEE by VRDL. The Sacramento-Yolo Mosquito and Vector Control District (1,589 samples), the San Gabriel Valley Mosquito and Vector Control District (1,268) and the Greater Los Angeles

Table 8. WEE, SLE, and CE isolates from mosquito pools during 2002.

Mosquito species	Date collected	County	Agency	Virus Isolated					
				WEE		SLE		CE	
				pools	mosqs.	pools	mosqs.	pools	mosqs.
Culex tarsalis	5-Jun	Riverside	COAV	1	50	-	-	-	-
	12-Jun	Imperial	IMPR	6	300	-	-	-	-
	20-Jun	Riverside	COAV	1	50	-	-	-	-
	25-Jun	Imperial	IMPR	8	384	-	-	-	-
	10-Jul	Imperial	IMPR	1	50	-	-	-	-
	16-Jul	Riverside	COAV	1	30	-	-	-	-
	24-Jul	Imperial	IMPR	-	-	1	50	-	-
	12-Aug	Riverside	COAV	1	50	-	-	-	-
	20-Aug	Imperial	IMPR	-	-	1	15	-	-
	26-Aug	Riverside	COAV	1	50	-	-	-	-
	5-Sep	Imperial	IMPR	-	-	1	50	-	-
	9-Sep	Riverside	COAV	3	150	-	-	-	-
	17-Sep	Imperial	IMPR	-	-	1	50	-	-
	23-Sep	Riverside	COAV	1	26	-	-	-	-
Culex quinquefasciatus	11-Jun	Imperial	IMPR	3	150	-	-	-	-
	9-Jul	Imperial	IMPR	1	27	3	150	-	-
	24-Jul	Imperial	IMPR	-	-	1	50	-	-
	20-Aug	Riverside	NWST	-	-	-	-	1	50
Totals				28	1,317	8	365	1	50

Source: California Department of Health Services

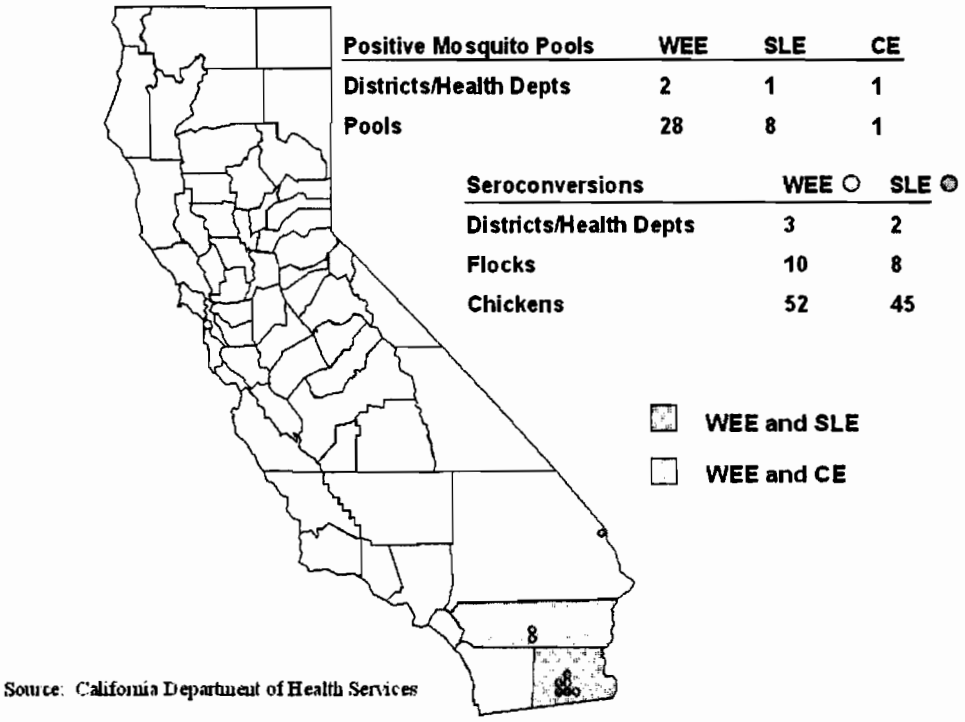
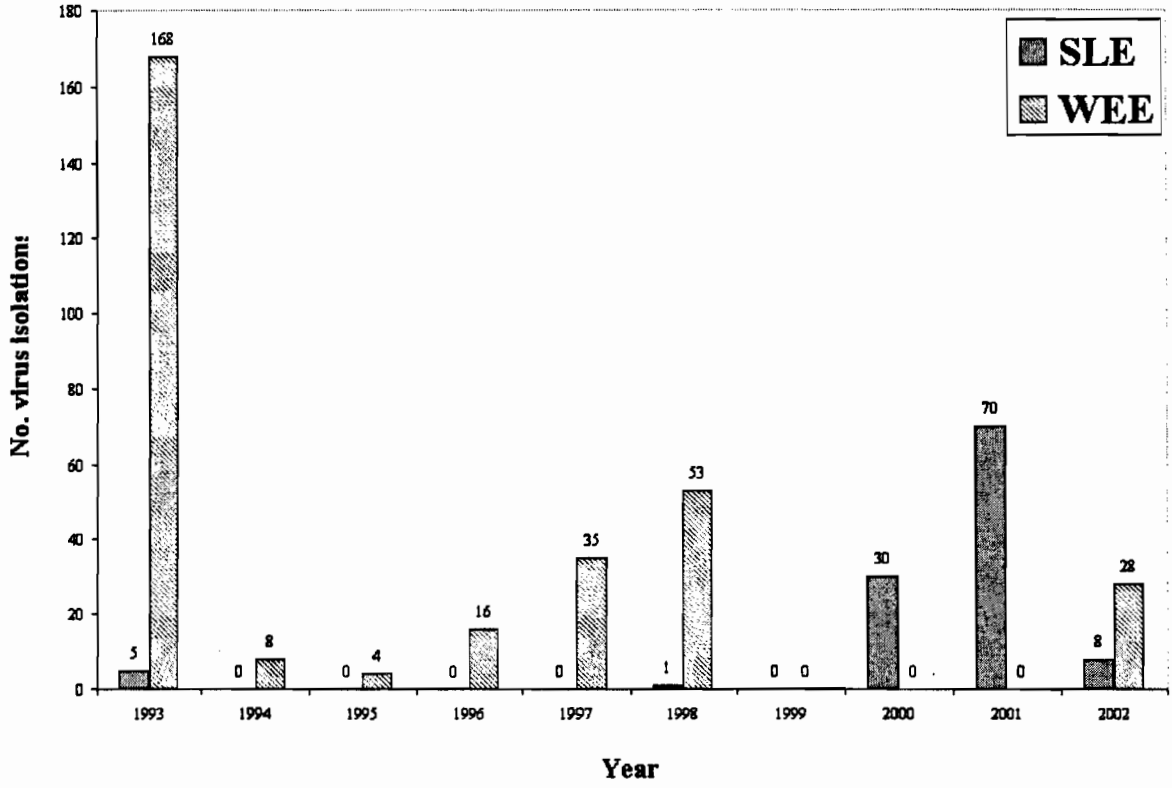


Figure 3. Collection sites of mosquito pools positive for WEE, SLE or CE, and location of sentinel chicken flocks with at least one or more seroconversions to WEE or SLE, California, 2002.



Source: California Department of Health Services

Figure 4. Isolations of SLE and WEE from pooled mosquitoes in California, 1993-2002.

Table 9. Chicken seroconversions to SLE and WEE by location and week (Monday of week shown below) bled, 2002

SLE

County	Agency	City	Location	7/8	7/29	8/5	8/12	8/19	9/2	9/16	10/14	10/28	11/11	Total
Imperial	IMPR	El Centro	Nichols						2					2
Imperial	IMPR	Holtville	Zenos		1		3		3	1				8
Imperial	IMPR	Niland	Bonowr				1	1	5	1		3	2	13
Imperial	IMPR	Niland	Wister						4	3				7
Imperial	IMPR	Seeley	Campbell	1	6			1						8
Imperial	IMPR	Westmoreland	West Mo			1		3	1					5
Riverside	COAV	Mecca	Gordon								1			1
Riverside	COAV	North Shore	Desert			1								1
			Total	1	7	2	4	5	15	5	1	3	2	**45**

WEE

County	Agency	City	Location	7/8	7/22	7/29	8/12	9/2	9/16	9/30	10/14	11/25	Total
Imperial	IMPR	El Centro	Nichols	1	3				1				5
Imperial	IMPR	Holtville	Zenos	1	2	2	1						6
Imperial	IMPR	Imperial	Quechan Reservation		3								3
Imperial	IMPR	Niland	Bonowr		5		4		1				10
Imperial	IMPR	Niland	Wister		1			1			1	1	4
Imperial	IMPR	Seeley	Campbell	1	3	1		1	1				7
Imperial	IMPR	Westmoreland	West Mo		4	1							5
Riverside	COAV	Mecca	Adohr						3		1		4
Riverside	COAV	Oasis	Jessup						3	1			4
San Bernardino	SANB	Needles	Treatment Plant						3		1		4
			Total	3	21	4	5	2	12	1	3	1	**52**

Source: California Department of Health Services

County Vector Control District (40) tested their own sentinel flocks for antibodies to SLE and WEE for a total of 3,257 additional serum samples which were all negative.

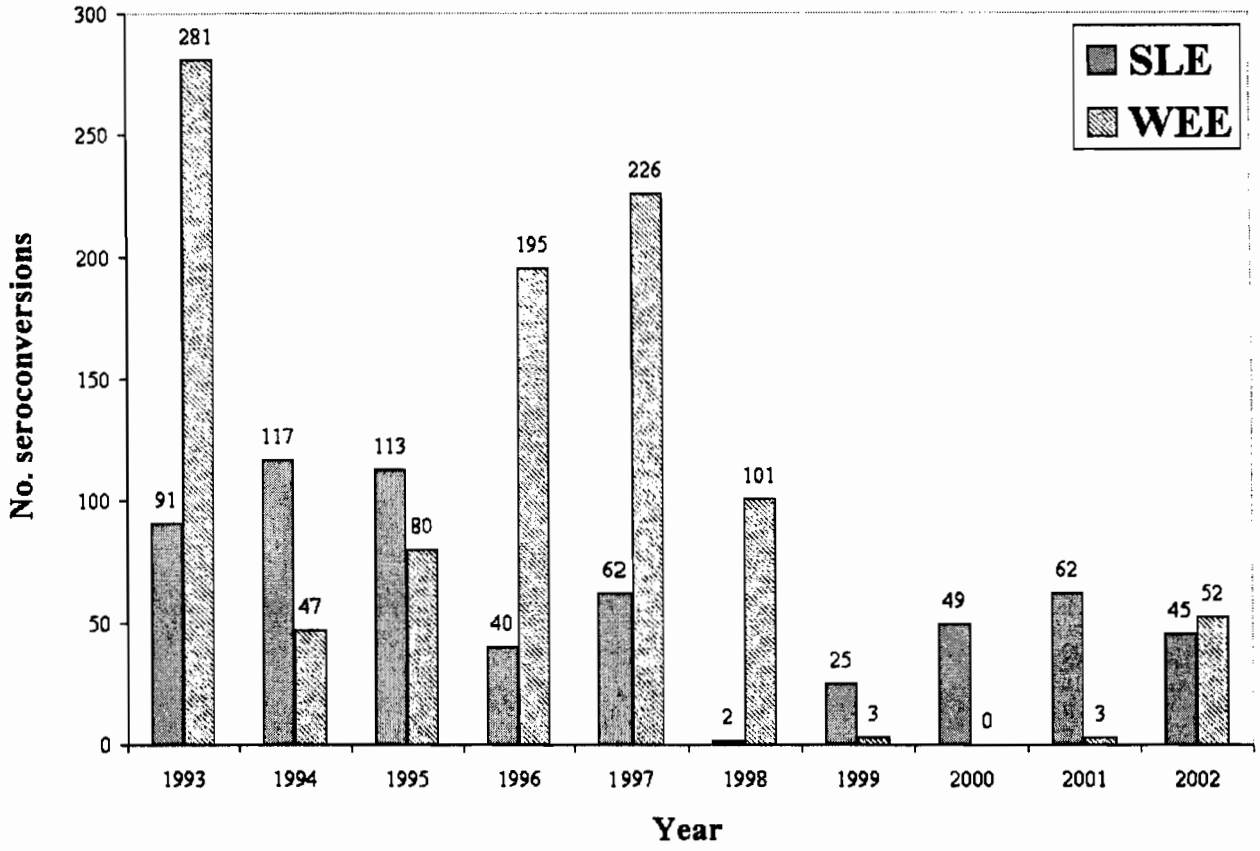
A total of 45 seroconversions to SLE were recorded in 2002 in sentinel chicken flocks in Imperial (43) and Riverside (2) counties (Table 9, Figure 3). The first SLE seroconversion was detected in one chicken bled on July 8 in Imperial County. The last seroconversions for 2002 were in Imperial County on November 12. None of the sera tested by VRDL or DARU was positive for WN. All specimens were screened for WN. VRDL also tested 686 chicken sera directly for WN using indirect fluorescent antibody test, and DARU conducted WN neutralization tests on 88 chicken sera. There were 52 seroconversions to WEE in Imperial (40), Riverside (8), and San Bernardino (4) counties (Table 9). The first WEE seroconversions were detected in 3 chickens bled on July 8 in Imperial County. The last seroconversion for 2002 was in Imperial County on November 25. No WEE seroconversions were found in samples from other counties. Seroconversions to SLE and WEE in sentinel chickens from 1993-2002 are summarized in Figure 5.

Exotic Newcastle Disease (END) infected thousands of chickens in southern California in 2002. CDHS cooperated with the CDFA by requesting that local agencies provide sera for testing of the sentinel flocks for END. Test kits were sent to each local agency so that a cloacal swab could be taken from 5 chickens per flock. The samples were then sent to CAHFS in San Bernardino for testing. All tests were negative.

DEAD BIRD SURVEILLANCE FOR WEST NILE

The CDHS WN dead bird surveillance program was initiated in 2000 (supported by a CDC grant). In 2001, 68 dead birds from 19 counties were reported and 18 were tested and negative for WN. The program was expanded in 2002 (McCaughey et al., 2002). A toll-free hotline was created for dead bird reporting by the public. The California WN website was launched and featured an on-line dead bird reporting system. CDHS press releases provided information on the extent of the dead bird surveillance and encouraged the public to participate in the program by reporting dead birds. Contact information was gathered and compiled for each county in California. VBDS public health biologists gave presentations to local agencies to encourage participation in the program. The hotline received 3,666 dead birds reported from 56 counties in 2002. Six hundred fifty-three birds from 45 counties were tested for WN and all were negative.

Dead bird calls were screened to determine if the criteria for WN testing were met. The criteria were, the bird must have been dead for less than 24 hours at the time of the report and be a target species. From January to July, the target species were limited to raptors, crows, jays, magpies, and ravens. The target species were expanded in August to include finches, sparrows, blackbirds, and cowbirds. Subsequent to the detection of the human case in August in Los Angeles County, the target species list was expanded to include all birds except chickens and pigeons.



Source: California Department of Health Services

Figure 5. Seroconversions to SLE and WEE in sentinel chicken flocks in California, 1993-2002.

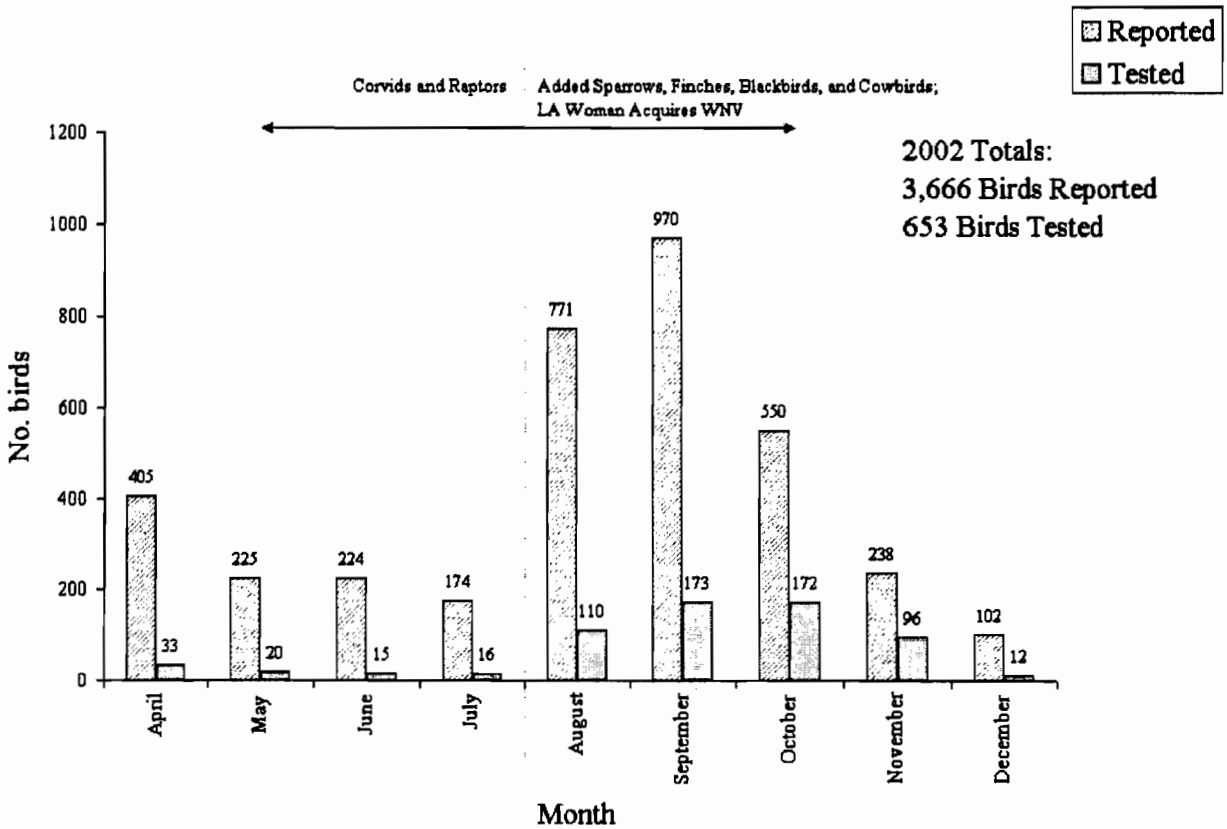


Figure 6. Dead birds reported and submitted by month to the CDHS WNV Hotline, California, 2002.

The number of calls to the hotline increased dramatically in August and September due to heightened public awareness (Figure 6) from the spread of WN across the United States. In 2002 there were 4,156 human cases from 39 states and the District of Columbia with 284 fatalities.

CDHS collaborated with over 130 local agencies for the collection of dead birds, including mosquito and vector control, animal control, veterinarians, zoo, and environmental health. Carcasses were submitted to CAHFS and selected tissues were tested for WN. VBDS staff notified reporting parties of the test results by mail. Statewide dead bird testing ended on November 30, 2002. Three agencies, Sacramento-Yolo MVCD, San Diego County Department of Health, and Santa Clara County Vector Control District, continued to fund dead bird testing during the winter season.

WEEKLY ARBOVIRUS SURVEILLANCE BULLETIN

CDHS published 33 weekly bulletins reporting arbovirus test results of humans, equids, mosquitoes, sentinel chickens, and dead birds. The bulletin provided updates on national WN activity. The bulletin was distributed from April 25 to December 20 to local, state, and federal public health and vector control agencies, universities in California, other state health departments, and the CDC.

CALIFORNIA WEST NILE VIRUS SURVEILLANCE WEBSITE

In January 2002, CDHS, in collaboration with CDFA, MVCAC, and the University of California at Davis, launched the California WN website (www.westnile.ca.gov). The website, entitled "California West Nile Virus Surveillance Information Center," included press releases, arbovirus surveillance bulletins, the California Mosquito-Borne Virus Surveillance and Response Plan, other educational and informational materials, and WN website links. The website also served as a means for the public to report dead birds using the online form.

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The California West Nile Virus Dead Bird Surveillance Program

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ABSTRACT: The California Department of Health Services (CDHS) West Nile virus (WN) dead bird surveillance (DBS) program was enhanced in 2002. A toll-free hotline and California WN website, featuring an online dead bird reporting system, were created for public reporting of dead birds. The CDHS received 3,666 reports of dead birds from 56 counties in 2002; 653 birds from 45 counties were tested for WN and found negative. After the one locally acquired human WN case in Los Angeles County, the avian target species list was expanded to increase surveillance efficacy. CDHS collaborated with over 130 local agencies, including mosquito and vector control agencies, animal control agencies, veterinarians, zoos, and environmental health agencies, for the collection of dead birds. This paper reviews the DBS program, addresses challenges for the program in 2002 and proposes measures to improve the program in the upcoming 2003 season. Anticipated challenges for the DBS program for the coming year will also be discussed. The change in laboratory methods in 2003 will extend the length of time that dead birds are testable, thereby increasing the submission rate and allowing more birds from rural counties to be tested.

AN OVERVIEW OF THE DEAD BIRD SURVEILLANCE (DBS) PROGRAM

The California Department of Health Services (CDHS) West Nile virus (WN) dead bird surveillance program was expanded in 2002. This program was supported largely by a grant from the Centers for Disease Control and Prevention. The WN hotline (877-WNV-BIRD) was created in April to increase the reporting of dead birds by the public, which numbered only 68 in 2001 (Husted et al., 2001); 18 of which were tested for WN. In 2002, the hotline received 3,666 reports of dead birds from 56 counties. A total of 653 birds from 45 counties were tested for WN with negative findings (Table 1, Figure 1).

CDHS biologists at the Vector-Borne Disease Section in Berkeley screened the public calls and coordinated the collection of carcasses by local agencies, which included mosquito and vector control districts, veterinary offices, environmental health, public health, animal control, and humane society agencies. The generalized submission criteria for WN testing in 2002 was that the bird had died within the last 24 hours at the time of the report and that the bird was one of the target groups (Table 1). Birds accepted for testing during the spring and early summer were raptors (predominately owls and hawks) and birds in the Family Corvidae due to their high mortality when infected with WN. The American crow (*Corvus brachyrhynchos*), the common raven (*Corvus corax*), the yellow-billed magpie (*Pica nuttalli*), the Western scrub jay (*Aphelocoma californica*), and the Steller's jay (*Cyanocitta stelleri*) are species from the Family Corvidae commonly found in California. Sparrows, finches, cowbirds, and blackbirds were added to target groups accepted for WN testing initiated in August of 2002.

The local agencies packaged the carcasses for shipping by an overnight courier or, in some cases, delivered the dead bird directly to one of four California Animal Health and Food Safety (CAHFS) Laboratories. Dead birds were tested for WN at CAHFS by immunohistochemistry performed on heart, kidney, and liver tissue. Kidney and lung tissue samples from each dead bird also were sent by CAHFS to the University of California Davis Arbovirus Research Unit (DARU) for WN isolation using Vero cell culture. The reporting party of each dead bird was notified of the test results by mail or phone within approximately three weeks from the time of their report.

ACCOMPLISHMENTS IN 2002

Increased dead bird reporting in 2002 was due in part to the following: the extensive media coverage generated by over 4,000 WN human cases throughout the United States, including Colorado; the California toll-free WN hotline (Figure 2); the creation of a California WN website (www.westnile.ca.gov), which featured an online dead bird reporting system in addition to information on WN; presentations by CDHS biologists on WN to many local agencies throughout California in an effort to increase agency participation and education; three CDHS press releases on WN, which included information on the WN hotline; the locally acquired human case in California; post card mailings by one mosquito and vector control district; and strong public education efforts by other

Table 1. Target dead bird groups reported to DHS and tested for WN by county, 2002.

	Crow		Hawk		Jay		Magpie		Owl		Raven		**Total**	
County	R	T	R	T	R	T	R	T	R	T	R	T	**R**	**T**
Alameda	11	5	2	2	5	2	1		2		1	1	**22**	**10**
Alpine*														
Butte	5		1		5		1		1				**13**	
Contra Costa	9	3	4		13	3	1		2	1	1		**30**	**7**
El Dorado	1	1	1		8	3	1	1					**11**	**5**
Fresno	15	5			28	6			1				**44**	**11**
Humboldt	13	1			2				1	1	2	1	**18**	**3**
Kings	4	1			3	2							**7**	**3**
Los Angeles	168	60	17	12	20	2	2		8	6	6	3	**221**	**83**
Marin	15	1	4	1	3								**22**	**2**
Mariposa*														
Merced	4				5	1	3		2				**14**	**1**
Napa	2		2	1	2	1			1	1			**7**	**3**
Orange	69	26	6		1	1			3		7	5	**86**	**32**
Placer	3	1	1		8	1	3				1	1	**16**	**3**
Riverside	53	15			6		2		3	1	5	1	**69**	**17**
Sacramento	156	27	11	2	164	33	106	24	7	4	8		**452**	**90**
San Bernardino	20	13	2	1			3		1		3	1	**29**	**15**
San Diego	34	15	7	3	4		1		8	2	4	1	**58**	**21**
San Francisco	4	1	4	2					1		1		**10**	**3**
San Joaquin	16	6	1		6	2	3	2	1	1			**27**	**11**
San Luis Obispo	5	2			3	1							**8**	**3**
San Mateo	4		1		4				2		3	1	**14**	**1**
Santa Barbara	11	2	3	1	2	1	1						**17**	**4**
Santa Clara	33	14	1		14	2	1				3		**52**	**16**
Santa Cruz	2	1			7	2					1		**10**	**3**
Shasta	11				13	2	2				1	1	**27**	**3**
Solano	14	2			5		1	1	3	1			**23**	**4**
Sonoma	13	2			4		1		1		1	1	**20**	**3**
Stanislaus	7	3			10	6	6	3	1	1			**24**	**13**
Sutter	8	1			3	2	1	1					**12**	**4**
Tehama	1		1	1	3		1	1					**6**	**2**
Tulare	12	2	1		7	1							**20**	**3**
Ventura	11	3	2	1	3				1	1	1		**18**	**5**
Yolo	44	7	5	2	38	12	14	2	3	1			**104**	**24**
Yuba	3	1			2	1	1						**6**	**2**
Other Counties**	12	4	9	7	16	2	1		4	1	1		**43**	**14**
Total	**793**	**225**	**86**	**36**	**417**	**89**	**157**	**35**	**57**	**22**	**50**	**17**	**1,560**	**424**
Other Species***													**2,106**	**229**
Grand Total													**3,666**	**653**

*Reported dead birds from non-target groups

**Other Counties that Reported (Tested) Target Dead Bird Groups (< 10 Reports)

Amador 3 jays (1); Calaveras 1 jay; Glenn 1 crow, 1 owl; Imperial 3 hawks (3); Inyo 1 hawk; Kern 1 crow, 1 hawk (1), 1 owl (1); Lake 1 crow (1); Lassen 1 jay; Madera 1 crow, 1 hawk, 2 jays, 1 owl; Mendicino 2 crow (1), 1 jay, 1 owl, 1 raven; Modoc 1 jay; Mono 1 jay; Monterey 2 crows (1), 2 hawk (2), 1 jay, 1 magpie; Nevada 1 jay; Plumas 1 crow (1), 1 jay; San Benito 1 crow; Sierra 1 jay (1); Siskiyou 1 crow; Trinity 1 crow, 1 hawk (1); Tuolumne 1 jay; Unknown 1 jay

***Other Dead Bird Groups Reported (Tested)

104 Blackbirds (11) 158 Finches (42) 434 Sparrows (114) 1,410 Other Species (62)

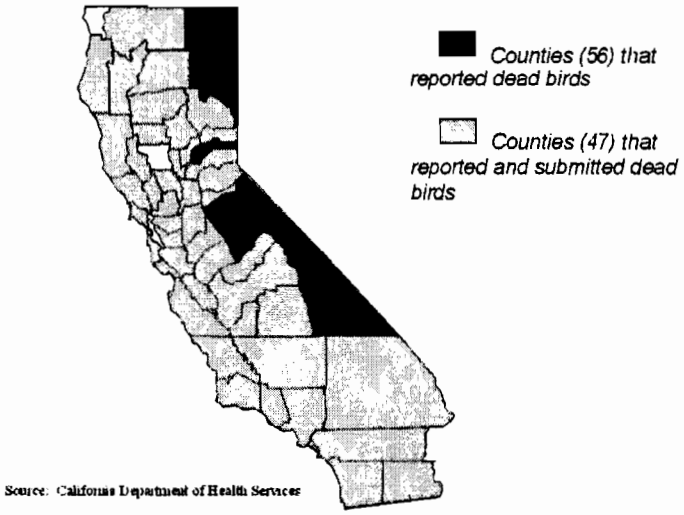

Figure 1. State map of dead birds reported and tested for WN by county, 2002.

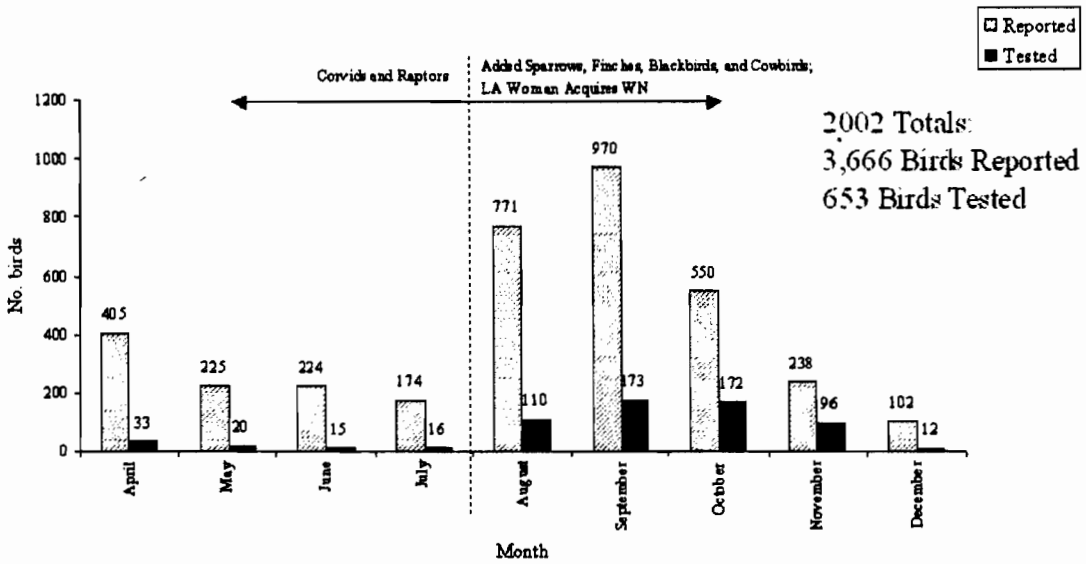

Figure 2. Dead birds reported to the hotline and submited for WN testing by month, 2002.

districts, which resulted in an increase in dead bird reports from the corresponding counties (Table 2).

Another accomplishment in 2002 was the increase in testing capability due to the strong commitment by the CAHFS and DARU laboratory personnel to coordinate and complete the testing of the 653 birds. Other highlights included CDHS staff working to compile contact information specific for each county in California and collaborating with over 130 local agencies to coordinate the collection and shipment of dead birds for WN testing.

2002 HOTLINE ACTIVITY

Several local agencies notified their constituents of the DBS program in early spring 2002, resulting in 405 dead bird reports to the CDHS hotline in April (Figure 2). However, the submission criteria limited birds accepted for testing in the spring to only raptors and corvids with few exceptions (Table 1).

Dead bird reports to CDHS decreased from May until a Los Angeles County resident was diagnosed with WN meningitis in August, causing increased public awareness and concern of acquiring WN. During the months of August and September, 771 and 970 dead bird reports were made, respectively, to CDHS (Figure 2). The species of birds accepted for WN testing were expanded in August to include sparrows, finches, cowbirds, and blackbirds, which increased the number of dead birds tested for WN. Additionally, in an effort to increase WN surveillance after the occurrence of the one locally acquired human case, all wild bird species, excluding pigeons, were accepted for testing from Los Angeles County.

Table 2. Target dead bird groups reported to DHS and tested for WN, 2002.

Bird Groups	Reported		Tested	
	#	%	#	%
Blackbird	104	2.84	11	1.68
Crow	793	21.63	225	34.46
Finch	157	4.28	42	6.43
Hawk	82	2.24	33	5.05
Jay	417	11.37	89	13.63
Magpie	157	4.28	35	5.36
Owl	57	1.55	22	3.37
Raven	50	1.36	17	2.60
Sparrow	434	11.84	114	17.46
Other	1415	38.6	65	9.95
Target Species Totals	**2251**	**61.40**	**588**	**90.05**
Grand Totals	**3666**	**100.00**	**653**	**100.00**

CHALLENGES FOR THE DEAD BIRD SURVEILLANCE PROGRAM IN 2002

STAFFING

A major challenge for the DBS program in 2002 was staffing of the CDHS hotline due to the unexpected high number of calls. One full-time staff position was added in 2002 to coordinate the DBS program, but the assistance of other staff biologists was required with the hotline during high volume calling periods. In 2003, the CDHS hotline will be managed by three staff devoted solely to the hotline during the summer months because the hotline is anticipated to receive ≥5,000 dead bird reports and ≥1,000 submissions.

Heightened staff efficiency was essential as the summer progressed. Staff members were required to directly answer a large volume of calls in order to minimize the amount of voice mail messages and also needed to promptly coordinate the collection and shipment of bird carcasses. An expanded Access® database will be created to eliminate repetition involved with filling out submission forms by hand thus expediting the dead bird reporting process. Additionally, a router on the phone system will be in place to better direct hotline calls. The call router will also include information in Spanish to better serve the diverse population of California. These measures to streamline hotline calls should allow the staff to handle a greater number of dead bird reports and submissions in the upcoming season.

An online dead bird reporting system was created on the California WN website in 2002. However, online reports required diligence on the behalf of the staff to check often for new reports, which tended to arrive sporadically. An improvement upon the online reporting system for 2003 will be an alert to staff via email when an online report has been made to ensure an immediate response. The California WN website will also be improved to become more user friendly and aesthetically appealing. Pictures of common bird species will be added to the website to aid in the identification of dead birds by the public.

FRIDAY AND WEEKEND REJECTIONS

A reduced submission rate of birds reported on Fridays and weekends was a significant problem for the DBS program. Many local agencies did not have access to a -70°C freezer or dry ice and therefore were unable to store bird carcasses over the weekend to ship on Monday. Additionally, the overnight courier would only pick up packages before noon in most rural areas and the only CAHFS laboratory that accepts deliveries on Saturdays is CAHFS Central in Davis. Even if a bird could have been collected on a Friday, it would not have been able to be shipped to a laboratory in a timely manner due to the complications listed previously. It was difficult for many rural counties to participate in the DBS program in 2002 because of the short time frame allowed for collection and shipping of dead birds.

There should be a decrease in Friday and weekend rejections in 2003 due to changes in laboratory testing methods. DARU will be testing for WN using a reverse transcriptase polymerase chain reaction (PCR) method, thus allowing virus to be detected in birds that have been dead for up to four days. Unlike previous years, rural counties will use blue ice instead of dry ice to submit dead birds for WN testing because of the change in testing methods, therefore allowing increased submissions from rural counties due to the extended time for shipping and the elimination of the need for dry ice.

NEED FOR ENHANCED LOCAL AGENCY SCREENING

Local agencies were encouraged to reject birds upon inspection that did not meet the criteria for WN testing, e.g. decomposed, wrong species, opening in chest cavity. CDHS plans to encourage enhanced screening by local agencies in 2003, thereby reducing the number of rejections by CAHFS and unnecessary shipping costs.

BIRD IDENTIFICATION

A key element in the DBS program was ensuring the proper identification of birds tested for WN. This will become very important once birds begin testing positive for WN. It was often difficult to accurately identify birds from public information provided through the hotline. There is a need for confirmation of bird identification by either local agencies or by CAHFS laboratory staff in 2003.

Posters with pictures of various common bird species will be distributed to the local agencies and to the CAHFS laboratories. The latter will bear the responsibility of confirming or correcting the identification of birds tested for WN. Also in 2003, CAHFS will fax back the dead bird submission form to hotline staff to signify receipt of the carcass and to confirm or correct the bird species, which will provide an easy way to ensure that dead birds were received and were correctly identified.

Other improvements that will be made by CDHS prior to the 2003 season include efforts to inform more local agencies at the county and city level of the DBS program and hotline number, because many callers complained about the number of calls made prior to receiving the hotline number. Radio and television public service announcements regarding WN and the DBS program also will be distributed for increased public awareness. Additionally, CDHS will gather information on wildlife rehabilitators to better serve the public reporting sick or injured birds which cannot be collected for WN testing due to California Fish and Game and U.S. Fish and Wildlife Service permit restrictions. In 2003, the zip codes where reported dead birds are found will be recorded in order to geocode database information.

POTENTIAL PROBLEMS IN 2003

FUNDING

Statewide WN dead bird testing ended for the season on November 30, 2002 and all allocated monies for the DBS program were utilized. Testing during the winter was conducted in four counties (Sacramento, San Diego, Santa Clara, and Yolo) through the support of local agencies. The Centers for Disease Control and Prevention grant for the DBS program will be available on April 1, 2003. In order to ensure funding for dead bird testing throughout the 2003 season, CDHS will limit testing to only corvids and raptors until mid-summer or the arrival of WN.

PRIORITIES OF VECTOR CONTROL AGENCIES

The DBS program greatly depends upon local vector control agencies to collect and ship dead birds, which could create a problem next season due to several factors. If the number of birds submitted for testing increases in 2003 as anticipated, some vector control agencies may not have sufficient staff to collect and ship all dead birds found in their district. Additionally, the priorities of the mosquito and vector control agencies may change once the first signs of WN are observed locally in California. There may be a shift in priorities to mosquito control by the local agencies and there may not be sufficient time for the collection of dead birds. CDHS and vector control agencies need to enlist the help of animal control agencies to provide back-up support to the vector control agencies in collecting and shipping birds for WN testing. A cover letter and questionnaire will be sent to all animal control and humane society agencies. The information gathered from the questionnaires will be distributed to the respective vector control agencies. Animal control agencies that express interest in participating in the DBS will be sent shipping boxes and submission instructions.

CAHFS LABORATORY WORKLOADS AND EXOTIC NEWCASTLE DISEASE (END)

As of January 8, 2003, the following counties were quarantined due to either confirmed END cases within the county or because of the geographic proximity to areas in which END has been confirmed: Imperial, Los Angeles, Orange, Riverside, San Bernardino, San Diego, Santa Barbara, and Ventura. The quarantine prohibits the movement of all birds and bird products from the quarantine area even though END almost exclusively affects domestic avian species. All END testing for California is conducted at CAHFS San Bernardino, thus increasing the laboratory's caseload.

Permits have been obtained by CDHS to ship bird tissues from CAHFS San Bernardino to CAHFS Central in Davis for WN testing. All birds within the quarantine zone are sent to the San Bernardino laboratory for tissue collection and are concurrently tested for END by PCR. Tissues are then forwarded by the San Bernardino laboratory to CAHFS Central and then delivered to DARU. For all counties outside the quarantine zone, local agencies will deliver dead birds to the nearest CAHFS laboratory and the carcasses will then be transported to CAHFS Central for WN testing or will ship the birds directly to CAHFS Central. The expected turnaround time for WN testing this coming season is expected to be approximately one week. The bottleneck in WN testing will probably be performing the necropsies.

CONCLUSIONS

The DBS program in 2002 was successful in increasing the amount of dead birds reported and tested. During the winter months, CDHS staff will make improvements to solve or lessen problems that occurred in 2002. The use of a computer database will increase hotline efficiency and there will be sufficient staffing to handle the increase in volume of dead bird reports. An improved system of reporting the receipt and identification of dead birds submitted to CAHFS for WN testing will be in place for the 2003 season. Animal control and humane society agencies will be contacted and encouraged to participate in the collection and shipping of dead birds to assist mosquito and vector control agencies.

Acknowledgements

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Enhanced Arbovirus Surveillance: Retrospective Evaluation of the California State Mosquito-borne Virus Surveillance and Response Plan*

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ABSTRACT: A previous retrospective evaluation of the California Mosquito-borne Virus Surveillance and Response Plan was extended by evaluating various methods for calculating risk for transmission of western equine encephalomyelitis virus (WEE) and St. Louis encephalitis virus (SLE) to humans. We evaluated the risk model using historical data from several areas of California to determine whether calculated risk levels would have approximated actual conditions during years with no, enzootic, and epidemic virus activity. Risk due to weather-related factors was assessed in two ways – the first, a biweekly method based solely on conditions during the previous half-month was compared to a cumulative method in which water and temperature-related risk was based on accumulated levels for the year prior to and including the previous half-month. For SLE, inversion of water-related risk so that drier conditions resulted in assessment of higher risk levels also was considered, and for WEE, the effect of treating horse and human cases as separate risk factors or combined as a single factor was evaluated. Generally, the cumulative method for assessment of weather-related risk equally or more accurately reflected actual conditions during the study periods and required fewer calculations and data manipulations than the biweekly method. Inversion of water-related risk factors for SLE resulted in a more accurate representation of actual virus activity during all study periods, including periods without virus activity as well as enzootic and epidemic periods. Treating horse and human cases as separate risk factors for WEE limited the early-season predictive ability of the model, but appropriately increased risk during epidemic periods when equine and human cases were occurring.

INTRODUCTION

The California Mosquito-borne Virus Surveillance and Response Plan was developed by the California Department of Health Services (CDHS), the Mosquito and Vector Control Association of California (MVCAC), and the University of California (UC) to provide a semi-quantitative assessment of risk for transmission of western equine encephalomyelitis virus (WEE) and St. Louis encephalitis virus (SLE) to humans within the state (CDHS et al. 2002). The risk model included in the plan incorporates environmental conditions, mosquito abundance, enzootic virus surveillance factors, human and equine case numbers, and the proximity of virus activity to the human population once the activity has been detected.

Overall risk, as defined by the response plan, is stratified into three levels: 1) normal conditions during which routine vector control and virus surveillance activities are conducted, 2) emergency planning conditions during which public education and vector control are intensified in response to escalating virus transmission risk, and 3) epidemic conditions during which human cases are occurring and vector management and virus surveillance efforts should be maximized.

This study extends a previous evaluation of the response plan (Barker et al. 2002) by evaluating the plan for additional years and locations and by considering multiple calculation methods for risk factors based on environmental conditions and horse and human cases.

MATERIALS AND METHODS

Study periods. The response plan was evaluated for years and locations with varying levels of virus activity to determine whether it would have provided early warning during years with impending virus activity and accurately reflected concurrent virus activity once it had been detected. Also, the plan's ability to represent low-risk conditions during years without virus activity was evaluated. The following years and locations were selected for evaluation: Coachella Valley (2000, 2001 SLE enzootic activity), Greater Los Angeles (1984 SLE epidemic), Kern County (1952 WEE/SLE epidemic; 1989 SLE epidemic; 1983, 1996, and 1998 WEE enzootic activity; 1989 no WEE activity; 1995 no WEE/SLE activity), Sacramento and Yolo Counties (1993 WEE enzootic activity), and Sutter and Yuba counties (1993 WEE enzootic activity).

* *An expanded description of this research has been published in the American Journal of Tropical Medicine and Hygiene (Barker et al. 2003).*

Response plan overview. The risk model developed as part of the response plan has been described previously (Barker et al. 2002, 2003; CDHS et al. 2002). Individual risk factors included in the model are: environmental conditions (temperature,[1] precipitation,[1] and runoff[2]), adult vector mosquito abundance,[3] vector infection rates,[4] sentinel chicken seroconversions,[4] equine cases (for WEE),[5] human cases,[5] and proximity of virus activity to the human population.[6] Each of these factors available at the time of assessment is assigned a value from 1—5, and all values are averaged to determine the overall risk level, which also is on a scale from 1—5. Overall risk for virus transmission falls into 1 of 3 categories: normal season (1.0—2.5), emergency planning (>2.5—4.0), or epidemic conditions (>4.0—5.0).

Environmental conditions. Risk based on environmental conditions was assessed in two ways. The first, described by Barker et al. (2002), was a biweekly method based solely on precipitation, runoff, and temperature levels during the previous half-month, and the second was a cumulative method based on accumulated water year precipitation and runoff (beginning October 1 of the previous year) and accumulated calendar year degree-days. For each half-month of each study period, cumulative precipitation, runoff, and degree-day values were reported as a percentage of their respective 30-year averages. For each study area, thresholds for risk assessment were determined by dividing the percentages for the second half of June into quintiles. The same thresholds also were applied to percentage values for all previous half-months, and higher risk values were assigned to wetter and warmer conditions. The risk value for environmental factors for the second half of June was applied to all subsequent half-months because, by that time, runoff has generally reached its annual peak, summer rainfall in most areas is negligible, and the accrual period for environmental factors includes the early season virus amplification period. Thus, the cumulative level for environmental factors in late June establishes risk for virus transmission thereafter.

For SLE, a third method for assessment of environmental risk was evaluated. This method was the same as the cumulative method described above, except that risk due to water-related factors was inverted so that drier conditions were assigned higher risk levels. The reason for considering this additional method was that SLE epidemics have been associated historically with hot, dry conditions (Monath 1980).

Other surveillance factors. Risk for mosquito and virus surveillance factors was assessed using the thresholds defined by the response plan (CDHS et al. 2002). For risk assessment, the average number of *Culex tarsalis* females/trap-night during each study period was expressed as a percentage of the previous 10-year average. If fewer than 10 years of collection records were available, all available years were included in the calculation of averages.

Overall risk for WEE was calculated with horse and human cases as separate factors or combined as a single factor to determine which method provided a more appropriate depiction of actual virus activity levels.

RESULTS AND DISCUSSION

WEE risk. For each year during which enzootic or epidemic WEE activity was detected, at least one method of risk assessment based on the model outlined in the response plan provided early-season warning of the pending virus activity, as well as some indication of concurrent virus activity once it had been detected (Table 1). The cumulative method of risk assessment for environmental conditions provided an equally or more accurate depiction than the biweekly method of actual WEE activity during most study periods and required fewer calculations for determining risk assessment thresholds. Also, because this method incorporated values for the entire year prior to the period of assessment, the levels for consecutive half-months generally were less prone to erratic fluctuations than levels based on the biweekly method.

Combining horse and human cases in the risk model would have elevated early-season risk levels for all study periods, usually resulting in appropriate indications of emergency planning conditions during years when subsequent virus activity was detected. However, the elevation of early-season risk assessments also resulted in inappropriately high risk levels in the emergency planning range during years in which WEE activity did not occur, such as 1989 and 1995 in Kern County (Table 1). Separation of the two factors would have caused overall risk to remain appropriately within the normal range during all of the half-months during 1989 and 1995, but also would have had the undesired effect of limiting risk levels during other years when WEE activity *was* detected (Table 1). For both the biweekly and cumulative methods,

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[1] Data for temperature, precipitation, and degree-days were obtained from the website of the University of California Statewide Integrated Pest Management Program at http://www.ipm.ucdavis.edu (last accessed 8/29/2003).

[2] Runoff data for all study areas except the Coachella Valley were obtained from the California Data Exchange Center, maintained by the California Department of Water Resources, at http://cdec.water.ca.gov. Data for the Coachella Valley were obtained from NWISWeb Data for the Nation, maintained by the United States Geological Survey at http://waterdata.usgs.gov/nwis/ (last accessed 8/29/2003).

[3] Adult *Cx. tarsalis* collection records were obtained from trapping data provided by Coachella Valley MVCD, Greater Los Angeles County VCD, Kern MVCD, Sacramento-Yolo MVCD, and Sutter-Yuba MVCD.

[4] Virus isolation rates for *Cx. tarsalis* and sentinel chicken seroconversion rates were collated from annual summaries in the Proceedings and Papers of the Mosquito and Vector Control Association of California, weekly Arbovirus Surveillance Bulletins published by the Vector-Borne Disease Section of the California Department of Health Services, data provided by individual MVCDs, and from Reeves and Hammon (1962).

[5] Information on equine and human cases was obtained from annual surveillance summaries in the Proceedings and Papers of the Mosquito and Vector Control Association of California.

[6] Human population densities for areas with detected virus activity were based on subjective determinations made by the staff of each individual MVCD.

Table 1. Percentages of half-months at or above the emergency planning range (overall risk >2.5) during periods preceding and immediately following detection of WEE activity for various risk calculation methods. Virus activity levels detected during the study period are indicated by the following colors: white (no activity), gray (enzootic activity), and black (epidemic activity).*

	Biweekly Method				Cumulative Method			
	Horse ←→ Human		Horse →← Human		Horse ←→ Human		Horse →← Human	
Study Period	% of HM in EP prior to WEE activity	% of HM in EP following WEE activity	% of HM in EP prior to WEE activity	% of HM in EP following WEE activity	% of HM in EP prior to WEE activity	% of HM in EP following WEE activity	% of HM in EP prior to WEE activity	% of HM in EP following WEE activity
KERN 1989	0	N/A	4	N/A	0	N/A	4	N/A
KERN 1995	0	N/A	13	N/A	0	N/A	30	N/A
KERN 1983	0	63	20	63	0	50	0	50
KERN 1996	0	14	27	43	9	29	82	43
KERN 1998	0	0	8	13	0	0	23	0
SAYO 1993	0	63	18	75	0	50	45	75
SUYA 1993	9	100	18	100	18	100	73	100
KERN 1952	0	100	11	100	0	90	22	100

*HM = half-month; EP = emergency planning conditions; N/A = not applicable.

treating horse and human cases as separate factors resulted in fewer half-months in the emergency planning range prior to the detection of WEE activity (Table 1). The only period when treating horse and human cases as separate factors would have increased the overall risk level was during the 1952 epidemic in Kern County, when both horse and human cases were occurring simultaneously.

SLE Risk. Each of the 3 risk calculation methods evaluated for SLE reached emergency planning conditions during some or all of the half-months following the detection of SLE activity (Table 2). At least one of the three methods also would have provided early warning of pending SLE activity during all study periods except 2001 in the Coachella Valley, when none of the methods of risk assessment would have provided an early warning of the enzootic SLE activity that occurred during the summer (Table 2).

The cumulative method with inverted risk assessment for water-related factors (drier conditions → higher risk) provided the best early warning of pending SLE activity, with the exception of the epidemic year of 1952 in Kern County (Table 2). During 1952, conditions differed markedly from the current situation in Kern County in terms of water management practices, mosquito control, and expected annual virus activity, so the model's ability to better reflect conditions during more recent periods was given greater consideration.

Table 2. Percentages of half-months at or above the emergency planning range (overal risk >2.5) during periods preceding and immediately following detection of SLE activity for various risk calculation methods. Virus activity levels detected during the study periods are indicated by the following colors: white (no activity), gray (enzootic activity), and black (epidemic activity).*

	Biweekly Method		Cumulative Method			
	Wetter = ↑ Risk		Wetter = ↑ Risk		Drier = ↑ Risk	
Study Period	% of HM in EP prior to SLE activity	% of HM in EP following SLE activity	% of HM in EP prior to SLE activity	% of HM in EP following SLE activity	% of HM in EP prior to SLE activity	% of HM in EP following SLE activity
KERN 1995	13	N/A	30	N/A	4	N/A
COAV 2000	8	13	8	13	33	63
COAV 2001	0	14	0	29	0	43
KERN 1952	29	100	29	100	0	100
GRLA 1984	29	100	36	100	64	100
KERN 1989	8	71	8	71	15	86

*HM = half-month; EP = emergency planning conditions; N/A = not applicable.

During all study periods, the cumulative method with inverted water risk provided the best indication of concurrent virus activity, as evidenced by higher percentages of half-months in the emergency planning range immediately following detected virus activity (Table 2). During 1995 in Kern County when virus activity was not detected, inversion of water-related risk again proved to be the best method of risk assessment, resulting in lower overall risk levels that reached emergency planning conditions during only a single half-month (Table 2).

Contributions of individual risk factors. The influence of individual risk factors on overall risk varies during a surveillance season depending on the number of factors being monitored at a particular time (Table 3). During a typical season, the only factors available during late winter and early spring are environmental conditions and passively monitored human and horse cases. Human and horse cases almost never are detected during this period, and as a result, early season risk levels are driven primarily by environmental conditions. During spring, additional virus surveillance is initiated, including mosquito trapping, sentinel chicken monitoring, and mosquito pool testing, and addition of these factors to risk calculations during the early periods when they are nearly always negative usually results in a reduction of risk until

Table 3. Contributions of individual risk factors in the response plan as a percentage of overall risk for WEE during a representative surveillance season in the Sacramento-Yolo MVCD during 1993.

Month	Half-month	Environmental conditions	Adult mosquito abundance	Mosquito infection rates	Chicken seroconversions	Equine cases	Human cases	Proximity of virus activity to populated areas	Percent contribution of each factor to overall risk
January	1								N/A*
	2	X				X	X		33 %
February	1	X				X	X		33 %
	2	X				X	X		33 %
March	1	X				X	X		33 %
	2	X				X	X		33 %
April	1	X				X	X		33 %
	2	X	X			X	X		25 %
May	1	X	X			X	X		25 %
	2	X	X		X	X	X		20 %
June	1	X	X		X	X	X		20 %
	2	X	X		X	X	X		20 %
July	1	X	X	X	X	X	X	X	14 %
	2	X	X	X	X	X	X	X	14 %
August	1	X	X	X	X	X	X	X	14 %
	2	X	X	X	X	X	X	X	14 %
September	1	X	X	X	X	X	X	X	14 %
	2	X	X	X	X	X	X	X	14 %
October	1	X	X	X	X	X	X	X	14 %
	2	X	X		X	X	X	X	17 %
November	1	X	X			X	X		25 %
	2	X				X	X		33 %
December	1	X				X	X		33 %
	2	X				X	X		33 %

* Because risk levels during the first half of January 1993 would have resulted from conditions during the second half of December 1992, these levels were not included in the study.

virus activity is detected. Also, as more factors are available for inclusion in risk calculations, the influence of individual factors is effectively reduced (Table 3).

Future considerations. Although the retrospective conditional simulations utilized in this study have provided some indication of the relevance of the response plan's risk model, the California Mosquito-borne Virus Surveillance and Response Plan will continue to be evaluated and modified prospectively during upcoming surveillance seasons. Also, the imminent arrival of West Nile virus (WN) in California will necessitate modification of the risk model to include dead bird surveillance (Eidson et al. 2001) and wild bird serosurveys as additional risk factors.

A central database currently is being developed to store historical and current mosquito collection and virus surveillance records from agencies throughout the state. Using these historical data with current data from the weekly electronic submissions of MVCAC member agencies, CDHS, and the UC-Davis Arbovirus Research Unit, it will become possible to integrate the response plan into California's surveillance website so that MVCAC member agencies can continuously monitor the overall risk for SLE, WEE, or WN activity within their local area and throughout California.

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We sincerely thank Coachella Valley MVCD, Greater Los Angeles County VCD, Kern MVCD, Sacramento-Yolo MVCD, and Sutter-Yuba MVCD for providing historical data. In particular, we are grateful to Debbie Lemenager, Richard Takahashi, Branka Lothrop, Arturo Gutierrez, Minoo Madon, Jacquie Spoehel, Jack Hazelrigg, Rhonda Laffey, Ken Boyce, and Deborah Dritz for assistance in obtaining the data used in this study. Funding was provided by a grant from the Office of Global Programs, National Oceanic and Atmospheric Administration.

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Wild Bird Arbovirus Surveillance in the Coachella Valley, California[1]

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A wild bird arbovirus surveillance program has been in place in the Coachella Valley, CA since 1996. In 2002, there were 8 trapping locations spread throughout the Coachella Valley (Fig. 1). Three different wild bird collecting devices were used at these 8 sites: ground traps, sparrow traps and mist nets. Ground and sparrow traps are grain-baited wire structures with ground level cone entrances that attract grainivores such as sparrows, doves, quail, blackbirds and finches. Mist nets are ten feet tall, 40 feet long and placed along bird flight paths. Birds either do not see or do not anticipate these nets and become entangled within them. Mist nets snare many different species of birds, and often catch those not attracted to grain baited traps, including but not limited to wrens, warblers, flycatchers, and other insectivorous birds. Each captured bird is fitted with the proper USGS band, its age and sex recorded, and a 0.1cc sample of blood is obtained by jugular puncture with a 28 g syringe and mixed with 0.9 cc of physiological saline. Blood samples are clarified by centrifugation and the sera is sent to the Davis Arbovirus Research Laboratory where they are screened for antibodies to West Nile [WN], western equine encephalomyelitis [WEE], and Saint Louis encephalitis [SLE] viruses using an enzyme immuno-assay (EIA) (Chiles and Reisen 1998). All positive EIAs are confirmed with a plaque reduction neutralization test [PRNT].

During 2002, 1,887 sera from 67 bird species were tested for antibody presence (Table 1). The species found positive for SLE in 2002 were a green heron (*Butorides striatus*), a loggerhead shrike (*Lanius ludovicianus*) and Gambel's quail (*Callipepla gambelii*). All of the positive samples for WEE were from Gambel's quail. Negative results from the large number of mourning doves (*Zenaida macroura*) and common ground doves (*Columbina passerina*) tested were unexpected.

Results during 2002 coincided with previous years (Reisen et al. 2000; Reisen et al. 2002), in that free-ranging avian seroprevalence patterns generally agreed with patterns of seroconversions by sentinel chickens in both frequency and

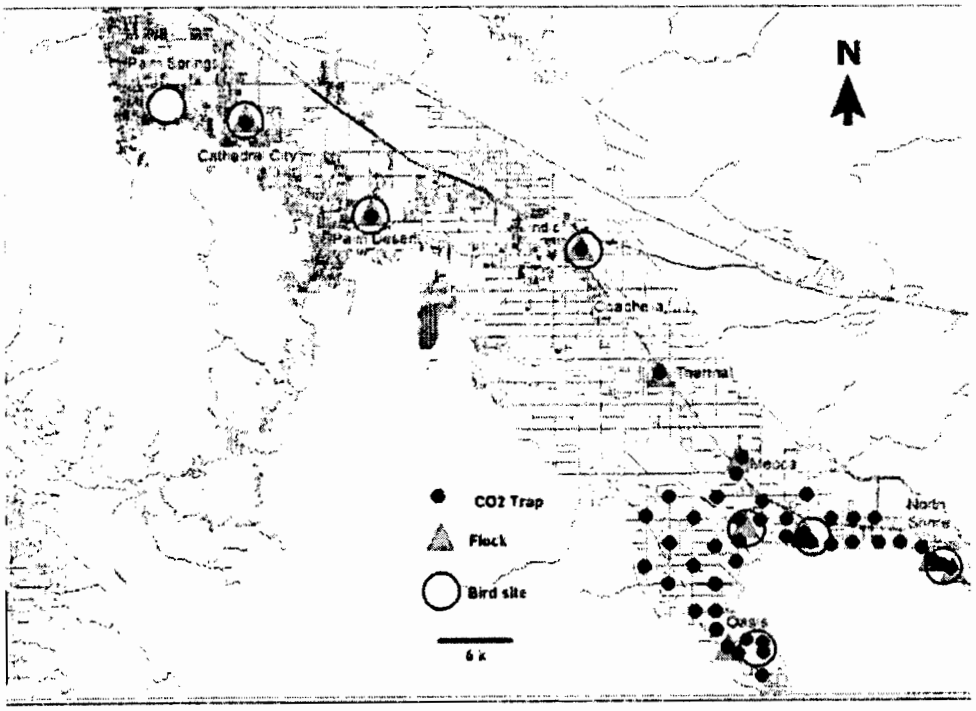

Figure 1. Map of study sites

[1] *The collection, banding and bleeding of wild birds were conducted under Protocol 8141 approved by the Animal Use and Care Administrative Advisory Committee of the University of California, Davis, California Resident Scientific Collection Permit 801049-02 by the State of California Department of Fish and Game, and Master Station Federal Bird Marking and Salvage Permit No. 21615 from the U.S. Geological Survey Bird Banding Laboratory.*

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Table 1. Surveillance results from 2001 and 2002.

	Number of Sera		
	Tested	WEE Pos.	SLE Pos.
2001			
Mosquito pools	800	0	70
Sentinel chicken sera	1,620	0	33
Wild bird sera	1,023	1	39
2002			
Mosquito pools	1,320	8	0
Sentinel chicken sera	2,160	7	2
Wild bird sera	1,887	7	3

distribution. Positive birds were limited in their distribution to sites near the Salton Sea and were not found north of Mecca. The results from quail were in agreement with previous years and indicated that this species is a good sentinel because it frequently is fed upon by infected host-seeking *Culex tarsalis* females. Shrikes frequently feed upon small birds, and it may be this route of infection that has led to the high seroprevalence in *L. ludovicianus* over the last several years [3 of 7 have tested positive]. We typically collect very few green herons, and this positive was a new record for our sampling program, although least bitterns (*Ixobrychus exilis*) from the same trapping site have been found infected.

The current results provide useful background information on infection frequency should WN invade California this coming summer. Sampling will continue at the current locations to monitor bird involvement in virus amplification at the Salton Sea, upland duck clubs, agricultural habitats and urban and suburban communities.

Acknowledgements

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Surveillance for Adult Mosquito Populations in San Mateo County, California

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The San Mateo County Mosquito Abatement District (SMCMAD) encompasses 13 cities on the east side of the San Francisco Peninsula. The District has used New Jersey Light Traps (NJLTs) for over 30 years to monitor populations of adult mosquitoes. In recent years, mosquito collections in NJLTs have declined to very low levels. For the past 5 years only one of the traps has collected more than 1 mosquito/trap night. Most traps averaged less than 0.5 mosquitoes/trap night. Similar declines in the number of adult mosquitoes collected in NJLTs have been reported in other urban areas in California and may be due to the increase in competing light sources (Reeves and Milby 1989, Wegbreit and Reisen 2000). Due to the low numbers of mosquitoes collected, NJLTs have ceased to be a useful indicator of the success of larval control programs in San Mateo County. In particular, light trap collections reveal little information about distribution or density of *Culex pipiens* in the county. This species is a major focus of control efforts, accounting for 34% of mosquito-related service requests. It is commonly encountered in larval samples from catch basins and other sources. Because of its potential as a vector of West Nile virus, this species is a major public health concern and information on its density and distribution is vital to monitoring control. This and other species commonly encountered in larval samples (*Ochlerotatus washinoi*, *Oc. squamiger* and *Oc. sierrensis*) are only rarely encountered in light trap collections.

Carbon dioxide-baited traps can be useful in monitoring adult populations of a wide range of species. The advantages of these traps for monitoring adult mosquitoes in urban areas have been well documented (Reisen and Reeves 1990, Reisen et al. 1999, Reisen et al. 2001, Reisen et al. 2002). However, the greater investment required in staff time and materials (dry ice, batteries) has inhibited their widespread use by mosquito and vector control districts in the past. In 2002, the SMCMAD began investigating carbon dioxide-baited traps as a way to increase the sensitivity of sampling for adult mosquitoes. The following is a description of this district's experience with these traps.

Trap Construction

Traps were constructed by District staff following the design of the Orange County Vector Control District (Cummings and Meyer 1999). The design of the battery compartment was modified. The original design used a metal bracket to hold 3 batteries inside a plastic soap dish above the body of the trap. The new battery compartment is similar to a flashlight and consists of a 6" length of 1.5" diameter PVC pipe with threaded caps at each end. A metal bolt was inserted through a hole in the center of each end cap to form battery contact points. Traps were powered by rechargeable nickel-metal-hydride batteries (Radio Shack, Phoenix, AZ). A 1.5-liter insulated plastic thermos held the dry ice for each trap.

Traps were deployed every 2 weeks for 1 year. They were retrieved approximately 24 hours after deployment. Each trap was baited with 2.5-3 lbs of dry ice. Traps were powered with 3 "D" cell batteries, which were recharged after every use. Trap locations are shown in Figure 1.

Trap Results

Carbon dioxide-baited traps dramatically improved surveillance for adult mosquitoes. Collections averaged 7.8 mosquitoes/trap night (range 0-195) (Table 1). *Culex pipiens* was the most commonly collected species, followed by *Ochleratatus sierrensis*, *Culex tarsalis*, *Culiseta incidens*, *Cs inornata*, *Cx. particeps*, and *Cx erythrothorax*. During the same period, the district's 18 NJLTs collected an average of 0.07 female mosquitoes/ trap night (range 0-7). The species most frequently collected in NJLTs were *Cx. tarsalis*, *Cx. erythrothorax*, *Cs. incidens*, *Cs.*

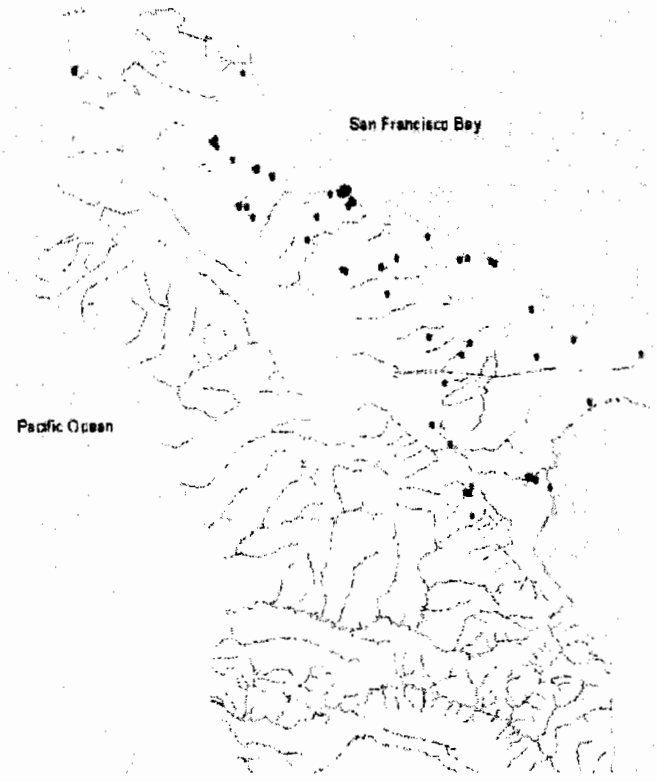

Figure 1. Location of Carbon Dioxide-baited Traps set in 2002.

Table 1. Comparison of numbers of mosquitoes collected in carbon dioxide-baited traps and New Jersey light traps in 2002.

	Carbon Dioxide-baited Traps			New Jersey Light Traps		
	Total	No. /		Total	No./	
Species	**Collected**	**Trap Night**	**Range**	**Collected**	**Trap Night**	**Range**
Culex pipiens[1]	2139	4.6	0-129	31	0.006	0-3
Oclerotatus sierrensis						
Males	962	2.1	0-195	2	0.0004	0-1
Females	309	0.7	0-204	2	.0004	0-1
Culiseta incidens	307	0.7	0-24	53	0.01	0-5
Culex tarsalis	260	0.6	0-21	58	0.01	0-7
Culiseta particeps	155	0.3	0-45	52	0.01	0-25
Culex erythrothorax	104	0.2	0-24	71	0.01	0-49
Culiseta inornata	62	0.1	0-7	45	0.009	0-5
All species	3625	7.8		331	0.065	

[1] All mosquitoes collected were females with the exception of *Ochlerotatus sierrensis*

inornata, and *Cs. particeps*. *Culex pipiens* was one of the least commonly collected species.

Initially, carbon dioxide-baited traps were placed at NJLT sites to determine whether simply changing the type of trap would be sufficient to improve adult surveillance. At most sites, carbon dioxide traps placed next to NJLTs did not collect significantly higher numbers of mosquitoes. However, when CO_2-baited traps were placed in trees or bushes near the NJLT, collections increased dramatically. The NJLTs were usually located adjacent to buildings, due to the need for electrical outlets. Such locations were often quite open and exposed. Carbon dioxide-baited traps can be placed in sheltered locations along tree lines or in bushes.

Mapping of CO_2-baited trap results revealed geographic patterns in mosquito abundance that were not evident from NJLT results (Figure 2). *Culiseta inornata* and *Cx. tarsalis* were found primarily along the shore of San Francisco Bay. *Culiseta incidens* was most prevalent in residential areas in the northern half of the district. *Culex pipiens* was found primarily in the developed flat lands east of the Coast Ranges.

The higher yield of mosquitoes in carbon dioxide-baited traps (compared to NJLTs) greatly increased the effectiveness of the District's monitoring system and allowed us to improve our control program. Deployment of CO_2 traps brought to light some larval sources that had previously been thought to be unimportant. For example, CO_2-baited traps at 2 sites collected large numbers of *Cx. pipiens* where NJLTs had collected no mosquitoes for the past several years. Extensive larval surveillance at one such site revealed mosquito larvae in tanks at a closed sewage treatment plant. At another site, larvae were found developing in catch basins in a nearby commercial development. Carbon dioxide-baited traps also provided more effective evaluation of control at previously identified larval sources. One of the NJLTs was stationed at a hotel with a long history of mosquito problems. Although there were several known sources of mosquito development at this site, NJLT collections were extremely low and did not vary significantly with season or timing of larval control. Service requests from hotel guests and management were more indicative of mosquito population fluctuations than NJLT yields. Placing a carbon dioxide-baited trap in this area allowed the district to monitor mosquito populations and led to the detection of new sources. Technicians

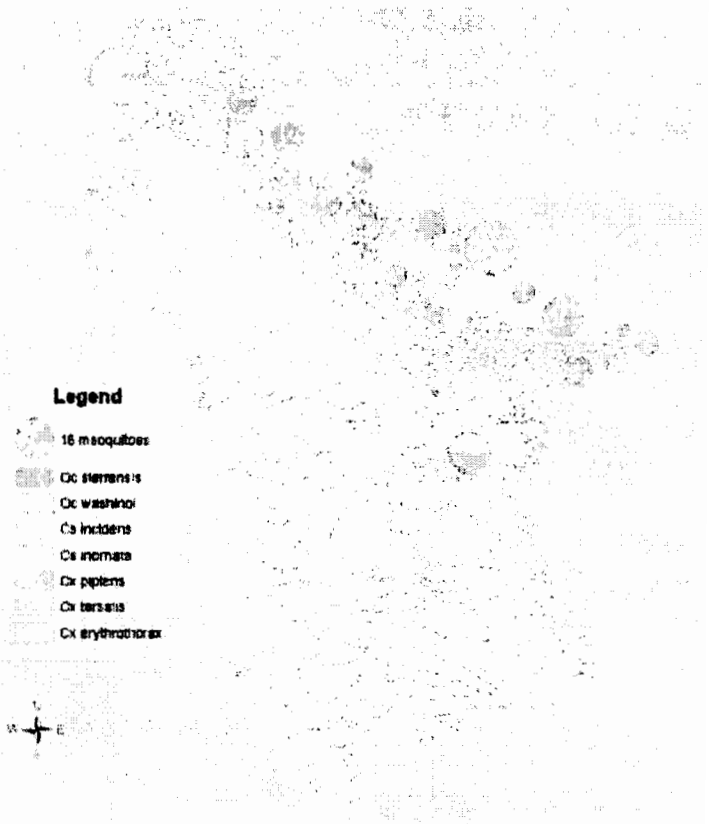

Figure 2. Mosquito Species Collected in Carbon Dioxide-baited Traps, 2002.

were able to detect mosquito breeding and respond before receiving service requests.

Cost of construction and deployment

Traps were constructed by district staff during winter months at a cost of ~$35.00 each. Most materials were readily available at local hardware stores. The Mabuchi motors for the traps were purchased from Peck Polymers (Santee, CA).

Batteries can be a significant operating expense and deterrent to use of carbon dioxide traps. Prior to 2002, the district tried using rechargeable alkaline batteries, which had to be replaced frequently when they failed to hold a charge. Nickel-metal hydride batteries were put into use in 2002. These batteries do not have "memory" and therefore do not need to be fully discharged before recharging. They can be recharged up to 2,000 times. Their higher initial cost (~ $10.00 each) is offset by greater durability and longevity.

Dry ice is purchased from a local company for approximately $0.90/trap. Seventy-five pounds of dry ice will fill approximately 30 traps. The District spends approximately $650/year on dry ice. The thermoses filled with dry ice are carried in cardboard boxes covered with a thick insulating blanket. This was found to significantly reduce the amount of ice lost to sublimation during transport while traps are distributed.

Carbon dioxide-baited traps required more staff time than NJLTs because they must be collected the day after they are set. To compensate for this, the traps were set every 2 weeks and technicians assisted in collecting traps within their zones.

Use of CO_2-baited traps requires a substantial investment of time and money, but has yielded far more useful results than NJLTs in this urban district. The dramatic difference in information gathered from these traps has more than made up for the extra effort involved. The staff is currently placing and collecting 30 traps every 2 weeks. Many of the district's NJLTs have now been removed (6 are still in operation). The NJLTs continue to be useful in rural areas. The district currently maintains NJLTs adjacent to marshes in Woodside and San Bruno, and at each of the district's 3 sentinel chicken coops.

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A Regional-based Study to Evaluate Lyme Disease Transmission Risk in Southern California

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ABSTRACT: Lyme disease (LD) is currently the most common vector-borne disease in the United States. In California, *Ixodes pacificus* Cooley and Kohls (the western black-legged tick) is the principal vector for transmission of the LD spirochete, *Borrelia burgdorferi* sensu lato. In the southern portion of the state, *I. pacificus* ticks have been found frequently in natural habitats in mountains and along the foothills; however, well-planned long-term studies on the seasonal activity of the tick and the transmission risk of LD are lacking. Here we provide details about a regional-based study plan. The objectives of this study are to elucidate the seasonality of *I. pacificus* under different environmental conditions, to detect and further identify the *Borrelia* spp. infecting the ticks, and ultimately to define the scope and transmission risk of LD in southern California.

INTRODUCTION

Lyme disease (LD) was first recognized as a new disease from Lyme, Connecticut in 1975 (Steere et al. 1977). In 1982, the Centers for Disease Control and Prevention (CDC) initiated nationwide LD surveillance and in January, 1991, the Council of State and Territorial Epidemiologists designated LD a nationally notifiable disease (CDC 1991). During 1991-2000, a total of 132,438 cases were reported from 49 states and the District of Columbia (CDC 2002). The highest number was in 2000 when 17,730 cases was reported. Montana is the only state from which no human infection has been reported. LD is currently the most common vector-borne disease in the United States. In California, the first human LD case was diagnosed in 1978 in a hiker from Sonoma County (Naversen and Gardner 1978). In 1989, passive surveillance for LD human cases was initiated by the California Department of Health Services (DHS). As of 2001, a total of 2,122 cases was reported from 54 out of 58 counties throughout the state.

Borrelia burgdorferi sensu lato is the etiologic agent of LD. Since the discovery of *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwalt, and Brenner in 1981 (Burgdorfer et al. 1982; Johnson et al. 1984), 10 different *Borrelia* spp. or genomic groups within the *B. burgdorferi* sensu lato complex have been identified (reviewed by Wang et al. 1999). Among those, however, only 3 appeared to be closely associated with Lyme borreliosis world-wide. *Borrelia burgdorferi* sensu stricto is associated with arthritis, whereas *B. garinii* and *B. afzelii* are mostly associated with neuroborreliosis and late cutaneous symptoms, respectively (Assous et al. 1993; Van Dam et al. 1993). *Borrelia burgdorferi* sensu stricto has been the only agent associated with human LD cases in the United States. *Borrelia garinii* and *B. afzelii* have been most common in European and Asian LD patients. Postic et al. (1998) reported the high level of diversity among nucleotide sequences of ribosomal gene *rrf-rrl* intergenic spacer region and the *rrs* gene from 18 atypical California isolates of *B. burgdorferi* senus lato. They also described a new species, *Borrelia bissettii*. It is possible that new or previously unknown species of *Borrelia* may contribute to LD.

Humans acquire LD spirochetes primarily through the bite of an infected tick. Several members of the *Ixodes ricinus* (Linnaeus) complex are proven competent vectors for transmission of LD spirochetes world-wide (Lane et al. 1991). In the United States, *Ixodes scapularis* Say (the black-legged tick) is the major vector for human LD in the Northeast and upper Midwest (Wallis et al. 1978; Spielman et al. 1985). In the West including California and Oregon, *Ixodes pacificus* Cooley and Kohls (the western black-legged tick) is the principal vector responsible for the transmission of *B. burgdorferi* from wild animals to humans (Burgdorfer and Keirans 1983; Burgdorfer et al. 1985).

Ixodes pacificus is widely distributed throughout California and has been collected from 55 out of 58 counties in the state. This tick is a three-host species and reportedly feeds on about 80 species of lizards, birds, and mammals (Arthur and Snow 1968; Furman and Loomis 1984). The immature stages of the tick (i.e., larva and nymph) feed primarily on lizards, small mammals, and birds, whereas the adult stage feeds on medium- to large-sized mammals such as jack rabbits and deer (Arthur and Snow 1968; Lane et al. 1981). Environmental parameters such as temperature, humidity, vegetation cover, and habitat type are important for the survival of *I. pacificus*. In addition, availability and species composition of wildlife hosts in the environment may contribute significantly to the life cycle of the tick.

Extensive studies on the relationships among *B. burgdorferi, I. pacificus*, and human LD exposure risk have been conducted in several areas of northern California. In the north coastal areas of California, *I. pacificus* nymphs are more important in transmitting *B. burgdorferi* to humans than adult ticks are (Clover and Lane 1995). Nymphal *I. pacificus* ticks are host-seeking during spring and summer months (March through July) when more people are outdoors in tick habitats, whereas adults are most active during the

winter months between November through March. Tallenklint-Eisen and Lane (1999) also reported that the *Borrelia* infection rate in nymphs is higher than that in adults. They found that in Mendocino County, the infection rate in the nymphs was as high as 41.3% in some localities, whereas only approximately 4% of adult ticks were found to be infected.

In southern California, human cases have been reported from every county. Our tick surveillance data have indicated that *I. pacificus* is frequently found in natural habitats in mountains and along the foothills throughout the region. Information about the seasonal activity of *I. pacificus* is, however, lacking. In 1992, Webb et al. reported the first discovery of *B. burgdorferi* in ticks in the southern portion of the state. The isolate was obtained from 1 of 20 *I. pacificus* ticks collected from Skeet Club Canyon, San Clemente on February 20, 1991. Others have also documented the detection of *B. burgdorferi* in ticks collected in the region (pers. comm. with J. Clover and D. Heft) even though the specific identity of these spirochetes requires further classification. It is apparent that a concerted effort to define the scope and transmission risk of LD in southern California is needed.

STUDY PLAN AND OBJECTIVES

In order to evaluate the transmission risks of LD in southern California, we developed a regional-based plan in collaboration with several county vector control agencies and research laboratories at the University of California system. The objectives of this study are to (1) elucidate the seasonal activity of *I. pacificus*, (2) detect and identify *Borrelia* spp. in *I. pacificus*, (3) determine the infection rate of *I. pacificus* with *Borrelia* infection, and (4) define the scope and transmission risk of LD.

TICK SAMPLING SITES

Survival and host-seeking activities of *I. pacificus* can be influenced by various environmental factors including different habitat types. During the summer of 2001, we conducted site evaluations in collaboration with Los Angeles County Department of Health Services, Los Angeles County West Vector Control District, and Riverside County Department of Environmental Health. Based on our field observations and the following considerations: (1) historical records of the presence of *I. pacificus*, (2) potential public health importance as demonstrated by their access to and known use by human populations, and (3) distinctively different ecosystems, we decided to choose 6 separate sites in 3 geographic locales in southern California for tick sampling (Figure 1). Locale 1, which includes Charmlee County Park and Tapia State Park, is located in Los Angeles County at the western end of the Santa Monica Mountains. Charmlee County Park is at the southern coastal side of the mountain range. Tapia State Park is at the northern inland valley side of the mountain range. Locale 2 is Griffith Park, Los Angeles County which is at the far eastern end of the Santa Monica Mountains. Locale 3 is located in Riverside County within San Bernardino National Forest including sites at

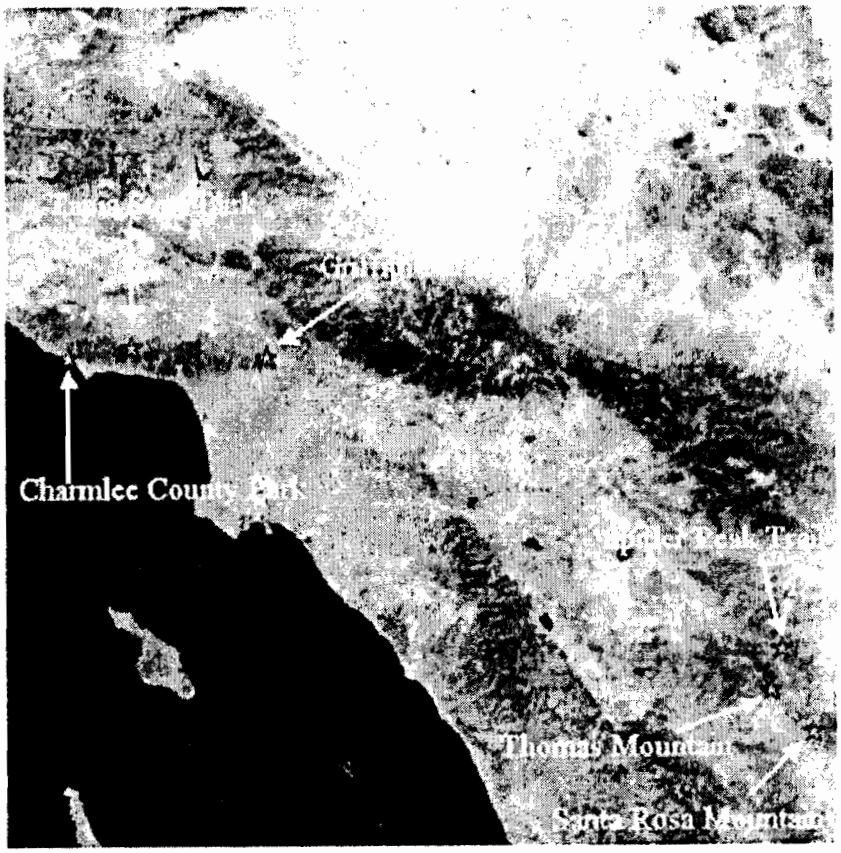

Figure 1. Geographical location of the study sites in Southern California.

the Santa Rosa Mountain, the Spitler Peak Trail, and the Thomas Mountain.

TICK SAMPLING METHODS

Tick sampling is being conducted at the chosen sites twice/ month starting in November, 2001. Collections are made by DHS staff and participating collaborators within their respective study sites. The same individuals conduct tick sampling on each succeeding collection date. Ticks are collected by using a standard flagging technique. This technique typically consists of a square meter flannel material that is dragged over low vegetation (brushy and grassy area) or leaf litter. For each site, one area is designated for a tick seasonal activity study and a "non-removal tick sampling method" is applied. Another area is designated for tick collection and a "removal sampling method" is used. There is no overlap between non-removal and removal sampling areas.

Non-Removal collections of ticks. Ixodes pacificus ticks are sampled along roads and trails for a minimum period of 1 person-hour of active flagging (i.e. one person for 60 minutes, two people for 30 minutes each, etc.). The flag is examined periodically (approximately 5-minute intervals). To minimize the direct impact of tick sampling procedure on the abundance assessment, ticks on the flag are identified to species, their developmental stages and sexes, counted, and are released at the site of collection. Tick abundance is expressed as the total number of ticks collected /person hour of active flagging. This will be used as quantitative data to determine the temporal distribution of ticks and will be compared with each site. In addition, meteorological and environmental data (ecological parameters) at all study sites will be analyzed. These include but are not limited to daily maximum and minimum temperature, humidity, and precipitation records. The correlation between these environmental factors and tick abundance will be established for a better understanding of the ecology of *I. pacificus* in southern California.

Removal collections of ticks. Ixodes pacificus ticks are collected and placed in labeled vials. These ticks are confirmed to species identification, and sent to our collaborators at the University of California system for *Borrelia* spp. detection and identification. For each site, the infection rate of the ticks with *Borrelia* spp. is determined. Furthermore, we will establish an Acarological Risk Index (ARI), which is the combination of tick abundance and infection rate. The ARI for each site can be compared to evaluate (rank) their respective LD transmission risks.

We anticipate that results derived from this multi-year study will provide much needed information about the geographical and temporal distribution of *I. pacificus* in southern California. The study may allow further molecular characterization of the *Borrelia* strains found in *I. pacificus* in the region. This information will enhance public health efforts to minimize Lyme disease transmission risk in southern California.

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A Survey of *Ixodes pacificus* on Bird and Lizard Hosts In Northern California

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ABSTRACT: Lizards and birds were surveyed for the tick, *Ixodes pacificus*, from five locations in three bioregions across northern California. Data on prevalence of infestation of host species, ratio of larvae to nymphs and estimates on proportions of the tick population supported by host species are offered. A new *I. pacificus* lizard host, the Gilbert's Skink, *Eumeces gilberti*, is identified in the Sierra Nevada and ten new avian hosts are recorded from across California. The Southern Alligator Lizard, *Elgaria multicarinata*, is identified as the principal host for immature stages of *I. pacificus* within all three bioregions. Lizards are parasitized by immature *I. pacificus* with a larval to nymphal ratio of 2:1, while jays and crows have a 1:1 ratio and sparrows and thrushes have greater than a 20:1 ratio. Birds were observed to be significant hosts for immature stages of *I. pacificus*, with some species infested at levels equal to lizards, within all three major bioregions.

INTRODUCTION

Some members of the *Ixodes ricinus* complex, particularly *I. ricinus, I. persulcatus, I. scapularis* and *I. pacificus* have low host specificity. The immature stages (larval and nymphal) of these species occur on lizards, ground-dwelling birds and small mammals (Furman and Loomis 1984, Lane and Loye 1989, Manweiler et al. 1990, Tälleklint and Jaenson 1994, Kollars et al. 1999). Importantly, host preference for the immature stages depends largely on host availability and other ecological factors of a given habitat (Oliver et al. 1996).

In western North America, *Ixodes pacificus* parasitizes a broad range of vertebrate hosts including reptiles, birds and mammals. Arthur and Snow (1968) identified 55 vertebrate hosts throughout the range of *I. pacificus* including 5 species of birds, 6 species of lizards and the Northwestern Garter Snake, *Thamnophis ordinoides*. Furman and Loomis (1984) identified 52 vertebrate host species in California that include 14 species of birds and 5 species of lizards; with immature stages occurring most commonly on alligator lizards, fence lizards and ground-inhabiting birds. Webb et al. 1990, reported *I. pacificus* larvae on 23 host species in California that includes 4 species of lizards and 2 species of birds.

The importance of lizards and birds as hosts for *I. pacificus* observed in these broad survey works is reflected in more focal investigations in the Sierra Nevada and Coastal Range of northern California. For example, a study site in the Sierra Nevada found lizards had significantly greater infestations of *I. pacificus* than did birds, and added 3 new avian hosts (Manweiler et al. 1990). A recent study in the Coastal Range noted again that lizards are the primary host for immature stages of *I. pacificus*, and added an additional 5 new avian hosts (Slowik and Lane 2001a). Finally, in the northern Sierra Nevada, Wright et al. (2000) identified 8 species of birds parasitized by *I. pacificus* immature stages, adding 4 new avian host records. These studies identified lizards as the primary hosts for the immature stages of *I. pacificus*, followed by birds and rodents.

The present report presents results of surveys conducted at five sites across northern California over five years to identify and quantify lizard and avian hosts for the larval and nymphal stages of *I. pacificus*. The report also presents data on the relative infestation of hosts, the larval-nymphal ratio for the principal host species and offers estimates of the *I. pacificus* population supported by lizards and birds for sites in the Sierra Nevada.

MATERIALS AND METHODS

Sampling Sites

The lizard and bird surveys were conducted over several years (1998 – 2002) during the spring (March through June), host-seeking period for immature stages of *I. pacificus*. Locations for the surveys were selected across northern California in Placer, Sacramento, Sutter, Yolo and Lake Counties. Five sites are located within 3 different bioregions in northern California that include the northern Sierra Nevada, the Sacramento Central Valley and the northern Coastal Range. Both lizards and birds were surveyed within the Auburn State Parks and Recreation District and the Folsom State Parks and Recreation District in the Sierra Nevada. Lizard and bird surveys were also conducted in Huff Canyon of the Sutter Buttes in the Central Valley and in Cache Creek canyon of the Coastal Range. Finally, a lizard-only survey was conducted at Putah Creek and a bird-only survey was conducted at Clear Lake State Park in the Coastal Range.

Each site was selected based on the following criteria: 1) A habitat dominated by oak, *Quercus* with substantial oak leaf litter middens, 2) specific location with mesic, sun protected topography or aspect, and finally, 3) each site previously established to have a population of *I. pacificus*, lizards and ground-foraging and nesting birds. A brief habitat and vegetation description of the survey sites follows. Drivers Flat and Orr Creek are located in the Sierra Nevada foothills at an approximate elevation of 610 m (2000 ft). The property, in part, belongs to the Auburn State Parks and Recreation District. The dominant vegetation is blue oak *Q. douglasii*, black oak *Q. kelloggii*, Ponderosa pine, *Pinus ponderosa*, Douglas fir, *Pseudosuga menziesii*, Pacific madrone, *Arbutus menziesii*, gray pine *P. sabiniana*, and poison oak *Toxicodendron diversilobum*.

Mississippi Bar and Willow Creek in the Sierra Nevada foothills have an elevation of approximately 66m (218 ft). The property is part of the Folsom State Parks and Recreation District. The dominant vegetation is blue oak, gray pine, interior live oak, *Q. wislizenii*, California buckeye, *Aesculus californica*, and poison oak. Near the river is Fremont cottonwood, *Populus fremontii*.

Huff Canyon in the Sutter Buttes has an elevation of 366 m (1200 ft) and is privately owned. The site is vegetated primarily with blue oak, valley oak, *Q. labata,* coyote brush, *Baccharis pilularis*, large manzanita, *Arctostaphylos manzanita*, California false indigo, *Amorpha californica*, California laurel (bay), *Umbellularia californica,* interior live oak and poison oak. The canyon has a north-south orientation with canyon walls at the base of which a seasonal creek flows. Some wet adapted vegetation occurs near the creek including Fremont cottonwood, various willow spp., California maidenhair ferns, *Adiantum jordanii* and Dutchman's pipe *Aristolochia californica*.

Cache Creek and Bear Creek in Capay Valley have an elevation of 366 m (1200 ft). The vegetation consists of a mixed blue oak and gray pine forest with a poison oak and California buckeye understory. Near the creek is Fremont cottonwood, willows, *Salix* species and blue elderberry, *Sambucus cerulea*. Steep east-west canyon walls protect the site from direct sun.

Clear Lake State Park has an elevation of 427m (1400 ft). The site is vegetated with blue oak, gray pine, California bay, *Umbellaria californica*, California buckeye and poison oak. The sample sites were on north and east facing slopes, well shaded, and covered with dense oak and pine leaf litter.

Bird Sampling

Birds were sampled at Drivers Flat every 2 weeks during spring (March through June) from 1997 to 2000. Birds were sampled at Cache Creek every 2 weeks from March through June 2000 and 2002. Sampling for birds at Mississippi Bar, Sutter Buttes, and Clear Lake was conducted every 2 weeks from March through June 2002.

Birds were captured using 3m x 12m mist-nets. Fourteen nets were placed at each location adjacent to vegetation and oak leaf litter. Nets were open for 4 hrs. each sample day beginning just after sunrise. Captured birds were banded on the right tarsus using USGS issued bands each with a unique number that permanently identifies the banded individual. Each bird was carefully inspected for ticks around the eyes, ears, bill and throat, back of head and legs and feet. Measurements were recorded on bill length, culmen length, wing chord and tail. Notes were made on fat deposits, feather molt and wear, age, sex, and sexual status. Each bird was weighed before release. These characters were used to accurately determine age, sex, status and health of birds (Pyle 1997).

A dissecting microscope 10X to 70X was used to locate and facilitate removal of ticks with forceps. Collected ticks were transferred to ethyl alcohol and stored in vials for later identification using keys (Furman and Loomis 1984, Webb et al. 1990 and Durden and Keirans 1996). After inspection the birds were released at the site of collection.

Lizard Sampling

Sampling for lizards was conducted during the spring (March to June) 1997 to 2002 at Drivers Flat and a near-by location, Orr Creek in Placer County, Willow Creek and adjacent Mississippi Bar (Sacramento Co.), Huff Canyon and adjacent North Butte in (Sutter Co.) and at Cache Creek and Putah Creek (Yolo Co.). Lizards were collected by hand or by noose as they foraged in leaf litter or from bark on trees, or as they rested under bark or rocks. Sampling for lizards was conducted from mid-morning to mid-afternoon on sunny days. Measurements were obtained from nose to vent and vent to tip of tail from each lizard. Sex was determined in *Sceloporus* spp. lizards using post-anal scales and in *Elgaria* sp. lizards when possible by everting the hemipenis (Stebbins 1985). Captured lizards were temporarily marked at Drivers Flat, Orr Creek and Willow Creek using liquid paper of various colors. Two types of marks were used. In the first series of captures, numbers were painted directly on the dorsal surface of the lizard making them easy to spot on trees or rocks using binoculars. In the event that the obvious marks may have increased predation on our lizards, we turned to a more obscure mark the second season. These captures had only one scale or later a combination of scales on the head (frontal, prefrontal, frontoparietal, parietal, interparietal or nuchal) marked with colored liquid paper. Records were kept to prevent duplicate markings.

The body of each captured lizard was carefully inspected using a hand lens or microscope (10X to 15X). Collected ticks were removed with forceps and transferred to ethyl alcohol and stored in glass vials for later identification. All ticks and mites were removed from each animal prior to release at the site of capture. Each sample trip returned to the same area, and the same trees, rocks and logs were inspected. Logs and rocks were turned and then replaced. Foraging lizards were replaced on the same tree or leaf litter at about the same location of capture.

Estimates of population sizes of lizards and birds were generated using the Schnable technique (Cox 1996) of repeated marking and recapture. It was expected that the population remained fairly constant within the sample periods that occurred during March to June.

RESULTS

We identify a new lizard host record for *I. pacificus*, the Gilbert's Skink, *Eumeces gilberti*, and several new avian hosts records including the Wild Turkey, *Meleagris gallopavo*, Acorn Woodpecker, *Melanerpes formicivorus*, Nuttall's Woodpecker, *Picoides nuttallii*, American Crow, *Corvus brachyrhynchos*, American Robin, *Turdus migratorius*, European Starling, *Sturnus vulgaris*, Wrentit, *Chamaea fasciata*, Brewer's Blackbird, *Euphagus cyanocephalus*, Bullock's Oriole, *Icterus bullockii*, and the House Finch, *Carpodacus mexicanus* (Table 1).

Based on the prevalence and intensity of infestation, lizards were observed to be the principal host for both larval and nymphal stages of *I. pacificus* across each of the three bioregions sampled. Five species of lizards were identified as hosts for immature stages of *I. pacificus* that include the Southern alligator lizard, *Elgaria multicarinata*, Western fence lizard, *Sceloporus occidentalis*, Sagebrush lizard, *S. graciosus*, Western skink, *Eumeces skiltonianus*, and the Gilbert's skink *E. gilberti*.

Elgaria multicarinata had the greatest prevalence and heaviest density of infestations of immature stages of *I. pacificus* in each of the three major bioregions surveyed. The mean density of infestations of *E. multicarinata* from the Coastal Range ranged from a high of 37 ticks/animal at the Cache Creek site to a low in the Sierra Nevada of 4 ticks/animal at the Folsom site. *Sceloporus occidentalis* ranged from a high in the Coastal Range of 8.3 ticks/animal from Cache Creek to a low of 2.4 ticks/animal at the Sierra Nevada Folsom site. *Eumeces* spp. lizards were very infrequently

Table 1. Mean density of *Ixodes pacificus* on lizards and birds in northern California

	Northern Sierra Nevada						Central Valley			Northern Coast Range					
	Placer			Sacramento			Sutter			Yolo			Lake		
Species	(n)	Range	Mean	(n)	Range	Mean	(n)	Range	Mean	(n)	Range	Mean	(n)	Range	Mean
Southern Alligator Lizard	120	(0-157)	5.76	2	(0-8)	4.00	2	(2-9)	5.50	7	(22-53)	37.10	0		
Western Fence Lizard	58	(0-20)	2.45	66	(0-30)	1.45	10	(1-11)	3.70	29	(0-29)	8.30	0		
Sagebrush Lizard	0			0			4	(0-4)	1.25	0			0		
Western Skink	0			0			2	0	0	10	(0-1)	0.10	0		
Gilbert's Skink	11	(0-6)	0.63	0			0			0			0		
California Quail	0			0			3	(0-2)	1.33	3	(0-2)	0.67	2	(0-12)	6.00
Wild Turkey	1	2	2.00	0			0			0			0		
Acorn Woodpecker	0			0			0			10	(0-3)	0.4	0		
Nuttall's Woodpecker	0			2	0	0	1	0	0	1	0	0	7	(0-1)	0.14
Steller's Jay	1	1	1.00	0			0			0			0		
Western Scrub-Jay	2	0	0	0			0			10	(0-7)	1.50	6	(1-8)	3.00
American Crow	0			0			0			0			1	4	4.00
Oak Titmouse	9	(0-1)	0.11	5	0	0	15	(0-12)	1.00	17	(0-1)	0.17	43	(0-10)	2.63
White-breasted Nuthatch	3	0	0	0			0			3	(0-1)	0.33	8	(0-5)	2.37
Bewick's Wren	17	(0-6)	1.41	8	(0-5)	1.75	4	(3-7)	4.25	1	4	4.00	1	0	0
House Wren	0			3	(0-1)	0.67	0			0			0		
Hermit Thrush	22	(0-20)	1.14	5	(0-2)	0.60	14	(0-12)	2.86	16	(0-12)	1.12	4	(0-13)	5.00
Swainson's Thrush	0			1	0	0	4	0	0	4	(0-2)	0.75	1	0	0
American Robin	1	0	0	0			6	(0-5)	1.67	2	0	0	0		
Wrentit	9	(0-1)	0.11	0			0			0			0		
European Starling	0			0			0			9	(0-7)	0.89	3	0	0
Orange-crowned Warbler	33	(0-2)	0.21	1	0	0	1	0	0	1	(0-1)	1.00	0		
Nashville Warbler	4	(0-5)	1.25	0			1	0	0	0			0		
Spotted Towhee	23	(0-5)	1.69	13	(0-5)	0.77	10	(0-9)	2.40	10	(0-22)	2.50	6	(0-5)	3.50
California Towhee	0			2	0	0	5	(0-14)	3.60	5	(0-4)	0.80	1	20	20.0
Fox Sparrow	4	0	0	0			2	(3-7)	5.00	0			0		
Song Sparrow	0			0			0			6	(0-4)	0.67	0		
White-crowned Sparrow	3	0	0	0			10	(0-5)	1.70	0			2	(1-2)	1.50
Golden-crowned Sparrow	16	(0-3)	0.25	0			6	(0-1)	0.17	16	(0-9)	1.87	14	(0-9)	1.21
Oregon Junco	7	0	0	0			1	1	1.00	46	(0-3)	0.06	0		
Black-headed Grosbeak	1	1	1.00	0			10	(0-3)	0.90	0			0		
Lazuli Bunting	2	0	0	2	0	0	14	(0-4)	0.64	0			0		
Brewer's Blackbird	0			0			0			14	(0-1)	0.07	1	0	0
Bullock's Oriole	0			0			1	0	0	12	(0-1)	0.08	2	0	0
Purple Finch	3	(0-1)	0.33	0			0			0			0		
House Finch	0			0			3	(0-3)	1.00	5	(0-2)	0.40	0		

parasitized with ranges from 0.1 to 0.6 ticks/animal from the Cache Creek site to the Sierra Nevada sites respectively (Table 1).

Ground-dwelling birds as a group were observed to be important secondary hosts for both larval and nymphal stages of *I. pacificus*. These birds represent a diverse group, including 31 species across 15 families. Five species were identified as significant hosts across all 3 bioregions, including the Spotted Towhee, *Pipilo maculates*, Hermit Thrush, *Catharus guttatus*, and the Bewick's Wren, *Thryomanes bewickii*. Additionally, the Oak Titmouse, *Baeolophus inornatus* was heavily infested in the Coastal Range, though only rarely infested in the Sierra Nevada.

The Hermit Thrush, *C. guttatus* had the greatest mean density of infestations of both stages of *I. pacificus* in each of the three major bioregions. The mean density infestations of *C. guttatus* from the Coast Range ranged from a high of 5 ticks/animal from the Lake County site to a low of 0.6 ticks/animal at the Folsom site and 1.1 at the Drivers Flat site in the Sierra Nevada. In the Sutter Buttes, *C. guttatus* had an infestation of 3 ticks/animal. For the Bewick's Wren, *T. bewickii*, infestation ranged from a high in the Sutter Buttes of 4.2 ticks/animal to a low of 1.4 in the Sierra Nevada. The Spotted Towhee, *Pipilo maculates* was more heavily infested in the Sutter Buttes and the Coastal Range, ranging from 2.4 ticks/animal and 3.5 ticks/animal respectively to a low in the Sierra Nevada of 0.7 to 1.7 ticks/animal (Table 1).

The ratio of larvae to nymphs on hosts was observed to vary among species but held constant from one bioregion to another. Both of the lizard species *E. multicarinata* and *S. occidentalis* had a ratio of 2 larvae to 1 nymph (2:1) in the Sierra Nevada and Coastal Range sites. Bird species such as White-breasted Nuthatch, *Sitta carolinensis* (1:2), Western Scrub Jay, *Aphelocoma californica* (1:1), and Oak Titmouse (1:1) had an either equal ratio of larvae to nymphs or were dominated by nymphs. Larval to nymphal ratios in bird species such as the White-crowned Sparrow, *Zonotrichia leucophrys* (16:1) Hermit Thrush (22:1) and Spotted Towhee (25:1) show infestations dominated by larvae.

Ixodes pacificus was the only species of tick collected from lizards sampled from all sites. The majority of ticks collected from birds were also *I. pacificus* (98 % excluding 5% identified only to genus), but other species removed from birds include *Haemaphysalis leporispalustris* from Lazuli Buntings, *Passerina amoena*, and White-crowned sparrows; *Argas brevipes* from Acorn Woodpeckers, *Melanerpes formicivorus*, and *Ixodes brunneus* from the Oak Titmouse. Rhinonyssidae mites were collected from the nasal cavity of one Western Scrub jay.

Repeated marking and recapturing of lizards from one site in the Sierra Nevada yielded an estimated population size for *S. occidentalis* of ca.160 animals within a roughly 2 acre area, and an estimated population size for *E. multicarinata* of ca.70 animals. Combining the two species of lizards yielded a rough approximation of 230 animals. Using an average infestation of any lizard captured during the sample window of 4 ticks/animal, we estimate that ca. 230 lizards may have supported ≈1000 immature ticks at this site at any one time during the sample period.

Repeated marking and recapturing of one resident bird species, the Spotted Towhee, *P. maculates*, with relatively heavy infestations, yielded an estimated population size of ca. 20 animals. Using an average infestation of 1.2 ticks/animal, we estimate that this one species of towhee approximately supports 24 immature ticks or about 2.5% of that supported by lizards at this site at any one time during the sample period. Populations of migrant birds such as the thrushes could not be estimated using Schnable technique, as recapture rates were too low.

DISCUSSION

This survey of lizard and bird hosts for immature stages of *Ixodes pacificus* across three major bioregions of northern California further establishes the very strong propensity of this tick species to parasitize the two most common lizard species, *Sceloporus occidentalis* and especially *Elgaria multicarinata*. Based on estimates of lizard population size, these 2 species appear to support the largest proportion, among small vertebrates, of the immature stages of *I. pacificus* in the Sierra Nevada.

This survey also establishes that birds, as a taxonomic unit, collectively contribute significantly as hosts for immature stages of *I. pacificus*. Individual bird species such as the Hermit Thrush, Bewick's Wren and the Spotted Towhee are parasitized by the immature stage of *I. pacificus* at nearly equivalent levels to that of *Sceloporus* lizards in the same habitats but support fewer ticks due to their smaller numbers in the area.

Two times as many larvae as nymphs of *I. pacificus* were collected from lizards in the Sierra Nevada and the Coastal Range sites. The larval/nymphal ratio on lizards (2:1) does not represent the ratio collected from flagging leaf litter where larval numbers far exceed (100s to1) those of nymphs. Fully engorged Ixodid females can lay thousands of eggs that hatch into larvae, but relatively few will survive and successfully find hosts, and molt to nymphs. Density of larvae in the litter is associated with locations where host deer drop engorged female ticks. We collected large numbers of larvae clumped in and below deer beds and even found engorged female *I. pacificus* within deer beds. Nymphs however, are collected, apart and individually, at distances down-slope from deer beds. It is therefore expected that significantly fewer nymphs exist in the habitat of these lizards. Yet ratios found on our lizards indicate that nymphs disproportionally parasitized them (2 to1 rather than 100s to1). These findings present either a preference by nymphs of *I. pacificus* for lizard hosts, lizard preference for nymph habitat, or other behaviors that place lizards in closer juxtaposition with nymphs than larvae.

Some bird species, including the Western Scrub-Jay and Oak Titmouse exhibited equal larval and nymphal infestations, while the Spotted Towhee and Hermit Thrush had 25 times the number of larvae as nymphs from the same sites. These differences may indicate varying host behaviors such as forage area, microhabitat target for foraging, and resting or nesting spots. The lizards and birds infested with immature stages of *I. pacificus* all depend on leaf litter middens for food and therefore have opportunities to be parasitized. The differences we observed in level of infestation and the ratio of immature stages may reflect different foraging strategies among these small vertebrates.

Both *Elgaria* and *Sceloporus* lizards forage in trees and on the ground but with different strategies. *Elgaria multicarinata*

actively seek prey, primarily insects, identifying them using chemical detection. They may cruise along the surface of the ground or climb trees and will readily enter burrows or burrow itself through leaf litter. *Sceloporus occidentalis* is primarily a sit-and-wait or ambush predator. *Sceloporus* also feed largely on insects, but may remain motionless on tree trunks for long periods of time or rest in crevices of bark. Unlike *Elgaria* sp., *Sceloporus* spp. regulates its body temperature by basking that requires periods of stasis, and males also devote much time to defending a territory. The behavioral differences of these 2 lizard species may contribute to the observed differences in levels of infestation with immature stages of *I. pacificus*.

All of the ground-dwelling birds we found infested with immature stages of *I. pacificus* are known to forage to some extent on or within leaf litter. The group infested with equal numbers of larvae and nymphs includes the jays and crows, which are known to store food within leaves or bury food below the soil surface. Members of the crow and jay family (Corvidae) forage and dig for insects in leaf litter by swinging their bills side to side to move leaves and middens, then pinching insects and even ticks with their bills (Isenhart and DeSante 1985 and Addison et al. 1989). The White-breasted Nuthatch and Oak Titmouse feed largely in trees, gleaning insects from crevasses in bark, but may also feed in leaf litter by probing with their bill to find invertebrates, seeds and nuts. Slowik and Lane (2001b) found *I. pacificus* nymphs host-seeking and resting in bark crevasses and moss growing on trees, primarily oaks, that had leaf litter at their bases. Since nymphs only were detected in trees, preferential foraging in trees by nuthatches and titmice could explain the high nymph to larval ratio on these and other tree-foraging hosts. These birds all nest within trees.

The group of ground feeding birds that were infested primarily with larvae includes the thrushes and sparrows. These birds forage for insects by hopping on the leaf litter and then vigorously scratching with both feet, digging into the litter and below the soil surface to expose insects that they will collect with their bills. Members of the thrush family, such as the Hermit Thrush and American Robin, feed mostly on insects and robins are known to be predators of ticks (Wilkinson 1970). This group of birds may actively seek deer beds as forage locations for ticks and other ectoparasites that may be associated with deer beds. Most sparrows, including Spotted Towhees, and also the Hermit Thrush nest on the ground, under thickets, often within leaf litter. This also may make them available to host-seeking ticks.

Finally, it is relevant to note that many of the birds infested with immature stages of *I. pacificus* are long-distance migrants. Species such as the Hermit Thrush, Swainson's Thrush, and American Robin are capable of transporting attached ticks considerable distances before detachment. These birds will seek out similar foraging sites, namely leaf litter patches, during their migrations that would be suitable for engorged ticks to drop and molt. Therefore, birds very likely have been and will continue to transport immature stages of *I. pacificus* to suitable habitats throughout the west.

Our observations of heavy infestation by *I. pacificus* on lizards in northern California argue that 2 species, *E. multicarinata* and *S. occidentalis*, contribute a substantial support for this parasite and may be critical principal hosts for larval and nymphal success. The inability of these lizards to reservoir *Borrelia burgdorferi* spirochetes, coupled with their ability to suppress infection in previously infected *I. pacificus* subsequently feeding on these lizards directs attention to other secondarily infested hosts for the maintenance of this disease in California (Lane and Quistad 1998, Wright et al. 1998).

Birds, especially ground-dwelling leaf litter foraging specialists, appear to play a significant role as hosts for both immature stages of *I. pacificus* in northern California. This avian contribution as hosts for *I. pacificus* is consequential in the distribution of this parasite throughout its range and in maintaining gene flow, including that to isolated populations (Kain et al. 1997). Birds should further be recognized as potential transporters and distributors of infected ticks throughout the range of this vector and may account for the apparent disjoint distribution of Lyme borreliosis (Webb et al. 1992) and ehrlichiosis (Vredevoe et al. 1999) in *I. pacificus* populations in California. Finally, bird species should not be ignored as potential reservoirs themselves, contributing to tick infections with spirochetes and rickettsiae in many of the varied habitats of California.

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Ecological Conditions Associated with Tick-borne Relapsing Fever in Inyo and Mono Counties, California, 2000-2002

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ABSTRACT: During 2000-2002, there was a total of 17 human tick-borne relapsing fever cases from 6 sites in Inyo and Mono Counties, California. Both of the cases reported from the Inyo County site were confirmed cases. There were 9 confirmed cases and 6 suspect cases from Mono County. The cases occurred at elevations ranging from 2134 to 2804 m. *Ornithodoros hermsi* ticks were collected from 3 of the 6 sites. Three ticks (1 nymph and 2 adults) were collected from 2 sites in Mono County, and 26 ticks (10 nymphs and 16 adults) were collected from the site in Inyo County. Carbon dioxide traps proved to be unsuccessful in collecting or attracting any ticks. The 27 *Ornithodoros hermsi* specimens from the Inyo and Mono County case-sites were tested by using *Borrelia hermsii* specific primers in a polymerase chain reaction, and 2 nymphal ticks from the Inyo County site tested positive.

INTRODUCTION

Tick-borne relapsing fever (TBRF) is a systemic disease caused by a spirochetal bacterium, *Borrelia hermsii* (Davis). At elevations above 1500 m. in the western United States, *B. hermsii* is transmitted by the bite of the argasid tick *Ornithodoros hermsi* Wheeler, Herms & Meyer (Herms and Wheeler 1936). These soft ticks and rodents, particularly chipmunks (*Tamias* spp.), act as reservoirs for *B. hermsii*. *Ornithodorus hermsi* may have a lifespan of several years and remain infective for life (Herms 1939, Pratt and Littig 1962). Transstadial transmission to subsequent tick stages and transovarial transmission to progeny are known to occur. Tick-borne relapsing fever also occurs in western Canada, Mexico, Central America, South America, eastern Europe, the Mediterranean, Africa, the Near East, and central Asia. The incubation period for the disease in humans is 5 to 15 days, with symptoms including cycles of fever lasting from 2 to 9 days followed by periods without fever lasting 2 to 4 days.

Tick-borne relapsing fever is a reportable disease in California. During 1991-2002, there were 98 human TBRF cases in California (California Department of Health Services [CDHS] 2001, and unpublished data). The number of cases ranged from 3 to 18/year with a median of 7 cases/year. The age of cases ranged from 2 to 85 years, with a median age of 34 years. Seventy-nine percent of the cases occurred from June to September. Although TBRF cases have been reported from 20 of the 58 counties in California, most cases during this time period were from Mono, Nevada, Placer, Siskiyou, and Fresno Counties. During 2000-2002, 17 confirmed and suspect TBRF cases were reported and investigated in Inyo and Mono Counties (CDHS 2002, and unpublished data).

BACKGROUND AND METHODS

Case interviews were conducted to determine the site where TBRF was most likely contracted. Sites were investigated approximately 30 days after cases potentially contracted TBRF, and data associated with the sites and the cases were obtained and analyzed to assist in the prevention and curtailment of TBRF transmission at that site. Buildings associated with the cases were investigated for the presence of *O. hermsi* and rodent activity. The location of sites was documented with Global Positioning System instruments and vegetation type determined using California Vegetation Classification Geographic Information System from the California Spatial Information Library (www.gis.ca.gov).

At 2 of the sites, CO_2 (dry ice) baited traps were used in an attempt to collect *O. hermsi* (Fig. 1). These traps consisted of a 2 liter, insulated dry ice container, supported 5.1 cm. above a sheet of white cloth approximately a meter square. Carpet tape was placed on the exposed surface of the cloth to capture any ticks that were attracted to the CO_2 source. The container was filled with ~ 1.8 kg. of dry ice. The container provided an approximate CO_2 flow rate of 500 ml./min.

Cases reported as confirmed were clinically diagnosed; while cases reported as suspect were not clinically diagnosed but had symptoms reported to investigators that were representative of those for TBRF. Some *O. hermsi* were tested with primers specific for *B. hermsii* by polymerase chain reaction (PCR) at the Department of Microbiology & Molecular Genetics, University of California, Irvine. Brief descriptions of the ecology associated with cases are discussed below:

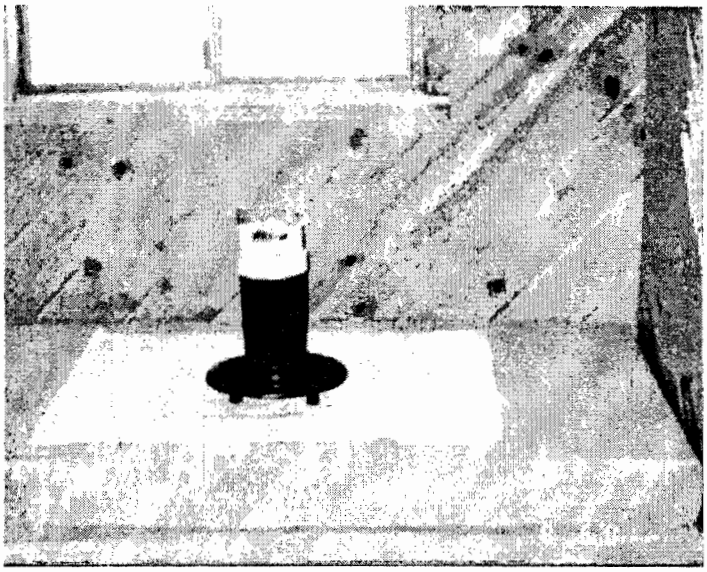

Figure 1. CO_2 trap designed to attract and capture ticks.

MONO COUNTY

Site # 1

A barracks at an Inyo National Forest facility has had a history of suspect and confirmed TBRF cases since 1986 (Fig. 2). The facility was located at an elevation of ~ 2320 m. The area associated with the site had a Jeffery Pine Vegetation type. The barracks, built in 1975, was a two-story wood structure with 5 wings and a total of 10 rooms. Each of the 5 wings had 1 room upstairs, 1 room downstairs and a bathroom at the stairs landing between the rooms. The 5 wings were accessed from a central, large, two-story open or common room.

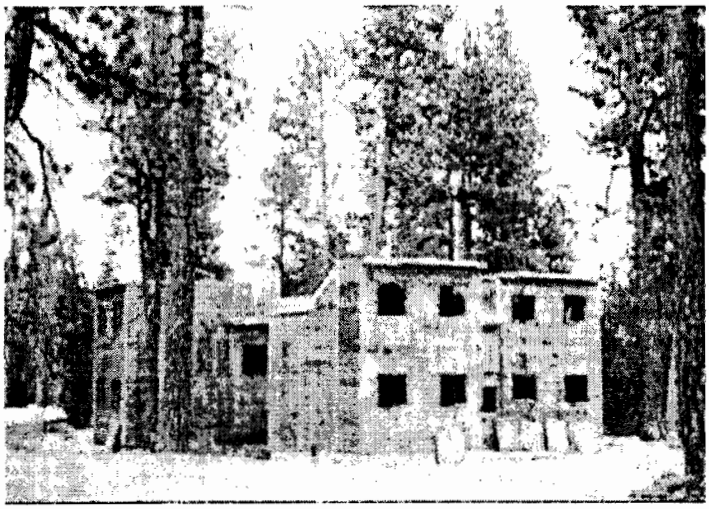

Figure 2. A barracks at a Forest Service facility, Mono County, California (Site #1). The site of several confirmed and suspect TBRF cases.

In 2000, 1 confirmed case and 1 suspected case were reported after a group of ornithologists stayed in the building. During July15-21, 2001, ~ 25 individuals conducting an archaeological dig within the area occupied the barracks or camped near the building. Four of the individuals who slept in the barracks developed a febrile illness. One of the individuals was diagnosed with TBRF; the other 3 were never diagnosed and considered suspect TBRF cases.

Attempts were made to collect *O. hermsi* from the barracks by searching in and under furniture, and by the use of CO_2 baited traps. Two traps were run from ~ 1500 to 0900 hrs. Although no ticks were recovered, there was considerable evidence of wild rodent activity in and around the barracks as well as throughout the facility. Numerous chipmunks (*Tamias* sp.) were observed at the facility.

Additional TBRF cases associated with the facility have been reported from sites other than the barracks. A suspect TBRF case occurred in the spring of 2001. The individual was a Forest Service employee who resided in a cabin with wood siding at the facility (Fig. 3). No ticks were recovered after a search of the cabin.

During 2002, 1 confirmed TBRF case was reported. The individual was a Forest Service employee who camped during

Figure 3. Tick-borne relapsing fever case cabin at a Forest Service facility, Mono County, California (Site #1).

August and September at a campground ~ 2.1 km. from the facility, and did not stay or sleep overnight in any of the facility's buildings. It was concluded that this case most likely contracted TBRF while camping near the facility.

Site #2

Two confirmed TBRF cases were reported from a one-story cabin with wood siding in the Inyo National Forest (Fig. 4). The cabin was located at an elevation of ~ 2134 m. The area associated with the site had a Singleleaf Pinyon Vegetation type. Both individuals stayed in the cabin from March 30-April 3, 2002. Access to the interior of the cabin was not available but observations indicated the structure was not rodent-proof; in addition, ideal rodent habitat existed proximal to the cabin.

Figure 4. Tick-borne relapsing fever case cabin, Mono County, California (Site #2).

Site #3

One confirmed TBRF case was reported from another one-story cabin with wood siding located in the vicinity of Site #2 (Fig. 5). The individual stayed in the cabin from June 22-23, 2002. There was no indication of rodent activity in the cabin, and the owner had rodent-proofed the cabin; however, ideal rodent habitat existed proximal to the cabin. The owner had collected 2 *O. hermsi* (adult) in the cabin (1 was dead). The ticks were found in the "open" in the living room and bathroom, and not associated with furniture, bedding, or a bedroom. The dead tick was kept as a voucher specimen, and the live, adult tick tested negative for the presence of *B. hermsii* by PCR. The owner reported that over the last 8 years several other guests that had stayed in the cabin exhibited TBRF symptoms.

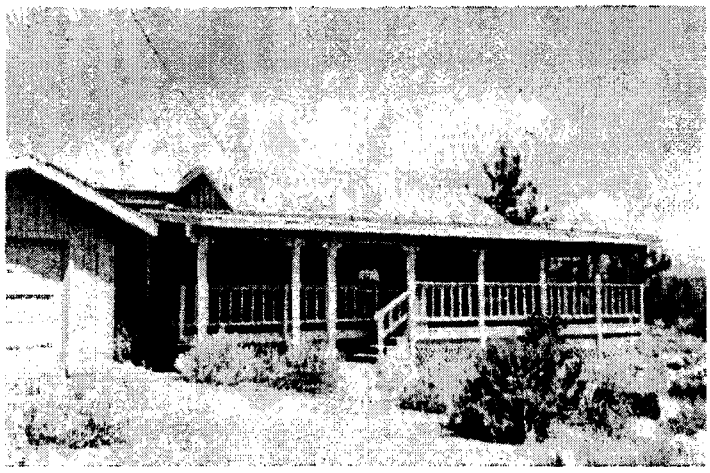

Figure 5. Tick-borne relapsing fever case cabin, Mono County, California (Site #3).

Site #4

Two confirmed cases and 1 suspect TBRF case were reported from near Mammoth Lakes in 2002, at an elevation of ~ 2210 m. The area associated with the site had a Jeffery Pine Vegetation type. Both confirmed cases were summer residents of a two-story cabin with wood siding (Fig. 6). The onset of the illness for the 2 confirmed cases was July 14 and August 2, 2002. On July 26, 2002, one *O. hermsi* (nymph) was collected from the bunk bed that the case individuals slept in. This specimen was not tested for *B. hermsii* but was kept as a voucher specimen. The suspect case camped in a tent near the cabin during the week of June 17, 2002.

Site #5

Figure 6. Tick-borne relapsing fever case cabin, Mono County, California (Site #4).

One confirmed TBRF case was reported from near Lake George. During the summer of 2002, the case stayed near the lake in a two-story cabin with wood siding (Fig. 7). During the stay, the individual took evening/night hikes and took naps in the woods during these hikes. The cabin was at an elevation of ~ 2804 m. The area associated with the site had a Lodgepole Pine Vegetation type. There was no observed indication of rodent activity in the areas of the cabin where access was obtained. The owner reported that mice were occasionally trapped in the cabin, but they were not a severe problem. The area around the cabin was landscaped and did provide ideal rodent habitat. After the investigation, it was concluded that the individual may have contracted TBRF while napping in the woods during one of the hikes.

Figure 7. Tick-borne relapsing fever case cabin, Mono County, California (Site #5).

INYO COUNTY

Site #6

Two confirmed cases of TBRF were reported from individuals occupying a cabin with wood siding, west of Bishop, at an elevation of ~ 2482 m. The area associated with the site had a Jeffery Pine Vegetation type. The cabin (Fig. 8) had 3 stories, an exposed basement, a living room/kitchen level, and an upstairs with 2 bedrooms. The cases stayed in the cabin for 1 night (June 22-23, 2002). The cabin had been unoccupied after June 23. On July 25, 18 *O. hermsi* (7 nymphs and 11 adults) were collected from the bed that the cases slept in. Some of the ticks were observed ovipositing on the bed linen. One adult tick was collected from a bed in the unused bedroom. There was evidence of rodent activity in and around the cabin. Subsequently, on the night of 30-31 July, CO_2 baited traps were set in both bedrooms. The following morning no ticks were captured in the traps, but 5 ticks (2 nymphs and 3 adults) were collected from the bed, mentioned above, where the cases had slept. Also, a single adult tick was collected from the carpet under the bed. One nymphal tick was collected from the bed in the other unused bedroom. A total of 26 ticks was collected from the cabin. Two of the nymphal ticks from the bedroom where the cases had slept tested positive for *B. hermsii* by PCR.

Figure 8. Tick-borne relapsing fever case cabin, Inyo County, California (Site #6).

DISCUSSION

Seventeen human TBRF cases from 6 sites were reported and investigated during 2000-2002 in Inyo and Mono Counties. Two confirmed cases were from Inyo County, and 9 confirmed and 6 suspect cases were from Mono County. Twenty-nine *O. hermsi* (11 nymphs and 18 adults) were collected from 3 sites in the 2 counties. Twenty-six *O. hermsi* (10 nymphs and 16 adults), including 2 *B. hermsii* infected nymphal ticks, were collected from the site in Inyo County.

All of the sites were at an elevation > 2100 m. Two of the cases had been camping with no association with a mountain cabin; another of the cases had stayed in a cabin but would take evening hikes and naps in the woods, where it is possible that the case could have contracted TBRF. The remaining 14 cases had slept in a mountain cabin or building with no other explanation of where they could have contracted TBRF. None of the structures were rustic, extremely run-down, or abandoned. All were furnished and had electricity, running water, and toilet facilities. None of the structures were utilized or occupied on a continuous basis. There was evidence of rodent activity in 3 of the 7 cabins or barracks associated with cases. Rodent activity could not be determined at 1 of the sites because of the lack of entry; though based on observations from the exterior of the structure, it was not rodent-proof and rodents were most likely inhabiting the cabin.

Ornithodoros spp. are long-lived and can survive for long periods without feeding. Once *O. hermsi* are established in a cabin they could continue to survive for months and even years; transmission of TBRF to an occasional human occupant could occur even though rodents no longer were active in the structure.

Recommendations about rodent-proofing structures and rodent management were provided to the Forest Service and the owners of the cabins. It was suggested that tick control would be difficult because of the reclusive nature of *O. hermsi* and to ensure safe and effective pesticide applications, commercial pest control professionals should be consulted and hired.

Follow-up investigations of these cases and sites will continue. The additional data obtained may aid public health efforts to reduce the occurrence of this disease in these 2 counties and other parts of the state.

Acknowledgements

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William C. Reeves
2003 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.

There were three competitors for the award at the 2003 Seventy-First Annual Conference, with first place awarded to Laura Goddard.

Previous William C. Reeves New Investigator Award Winners:

2002 - None

2001 - Christopher Barker

2000 - Jason Rasgon

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

Extrinsic Incubation Period of West Nile Virus in Four California *Culex* (Diptera: Culicidae) Species

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ABSTRACT: We compared the potential of four California *Culex* species as vectors of West Nile (WN) virus by examining their susceptibility to infection and extrinsic incubation period; i.e., time from infection until they can transmit virus. *Culex tarsalis, Cx. stigmatosoma, Cx. pipiens pipiens*, and two populations of *Cx. p. quinquefasciatus* were fed artificial viremic blood meals, held at 28°C and transmission was attempted daily for seven to10 females per group for 12 or more consecutive days. Species varied in their susceptibility to infection and ability to transmit virus. *Cx. tarsalis* and *Cx. stigmatosoma* were highly susceptible to infection, *Cx. p. pipiens* and *Cx. p. quinquefasciatus* (Bakersfield) were moderately susceptible, and *Cx. p. quinquefasciatus* (Coachella Valley) was refractory. WN virus transmission was first detected five to eight days post-exposure to virus. Transmission rates then increased to day 12 and 13 for all species tested, except *Cx. p. quinquefasciatus* (Coachella Valley) for which there was only one WN virus positive transmission on day six.

INTRODUCTION

The 1999 outbreak of West Nile (WN) virus in New York marked the first known transmission of this virus in the Western Hemisphere (Centers for Disease Control and Prevention 1999). Since its introduction, WN virus has spread across the United States at an alarming rate from New York to 43 other states and the District of Columbia. As of July 2003, over 4,100 human cases with 284 deaths were reported and more than 11,000 equine cases of WN were documented (Centers for Disease Control and Prevention 2003). The expanding range of WN and rising incidence of medical and veterinary cases indicate a need to understand patterns of WN transmission as fundamental information for the design and facilitation of strategies for mosquito control.

WN virus is a geographically widespread arbovirus in the family Flaviviridae, genus *Flavivirus* (Porterfield 1980). It is endemic to Africa, western Asia, the Middle East, and Europe. WN virus is maintained in an enzootic transmission cycle among infected wild birds and mosquitoes; a cycle that is conceptually similar to two viruses endemic to California—its close relative, St. Louis encephalitis (SLE) virus, and the *Alphavirus*, western equine encephalomyelitis (WEE) virus. Humans and horses are dead-end hosts for WN, but potentially may develop encephalitis and occasionally succumb (Hayes 1989).

As WN continues to expand its range toward the West Coast, it is important to evaluate the vector competence of California mosquitoes and determine their potential role in transmission. In the present study, we evaluated the vector potential for WN virus of four common California *Culex* species. By assaying daily infection and transmission, we determined the duration of the extrinsic incubation periods, or the time from virus exposure to the time when mosquitoes were able to transmit virus. We examined four species that are known competent laboratory vectors of WN virus: *Cx. tarsalis, Cx. stigmatosoma, Cx. pipiens pipiens,* and *Cx. p. quinquefasciatus* (Goddard et al. 2002). *Cx. tarsalis*, one of the most medically important mosquitoes in North America, is the primary vector of WEE and SLE viruses in California (Reeves and Hammon 1962) and is a competent vector of Japanese encephalitis virus; another flavivirus that is closely related to WN virus (Reeves and Hammon 1946). *Cx. stigmatosoma* is an abundant species in California that has been found naturally infected with WEE virus (Reeves and Milby 1990) and is a competent laboratory vector of SLE virus (Reeves et al. 1954). *Cx. pipiens* was identified as a major vector of WN virus during the 1999 New York outbreak (Turell et al. 2000) and may serve as a host for virus survival during the winter in temperate climates (Nasci et al. 2001). Members of the *Cx. pipiens* complex in California, *Cx. p. pipiens* and *Cx. p. quinquefasciatus,* potentially may fill the same role on the west coast as *Cx. pipiens* does on the east coast.

The extrinsic incubation period measures how rapidly virus can infect, replicate, and disseminate to the salivary glands, thereby indicating how quickly a mosquito can transmit virus. The extrinsic incubation period is an important factor in arbovirus epidemiology, because it determines how long a mosquito must survive after ingesting an infectious blood meal to become an effective vector. Defining patterns of infection and transmission of WN virus for competent vectors is necessary for predicting the potential roles of these mosquitoes in WN virus transmission in California. Details of vector-virus interactions are fundamental information for designing targeted vector and disease control programs.

METHODS

All experimental work was conducted in the biosafety level-3 containment facility at the Center for Vectorborne Disease, University of California, Davis.

Virus: WN virus strain 352611 AAF 9/23/99 isolated from a flamingo in New York and passaged twice in Vero (African Green Monkey kidney) cells was used to infect mosquitoes.

Mosquitoes: Four *Culex* species were collected from different locations in California. *Cx. tarsalis* were collected from Yolo County, *Cx. stigmatosoma* from Chino, San Bernardino Co., *Cx. p.*

pipiens from Shasta Co., and *Cx. p. quinquefasciatus* from Bakersfield, Kern Co., and Coachella Valley, Riverside Co. We defined members of the *Cx. pipiens* complex based on geographic location of collection and on previously described hybrid zones in California (Urbanelli et al. 1997). Mosquitoes collected from northern California were considered *Cx. p. pipiens* and those from the San Joaquin Valley and southeastern California were considered *Cx. p. quinquefasciatus*. The F_1 generation for each species, except *Cx. stigmatosoma,* was reared in the laboratory and used for experimental infection. *Cx. stigmatosoma* were wild-caught adults of unknown age.

Mosquito infection: Four- to five-day old mosquitoes (except *Cx. stigmatosoma* of unknown age) were fed on hanging blood droplets (defibrinated rabbit blood [Microbiological Media, San Ramon, CA]) containing 2.5% sucrose and $10^{7\pm0.1}$ plaque-forming units (PFU's) of WN virus/1.0 mL of blood. Engorged mosquitoes were incubated at 28°C, a photoperiod of 16 h light: 8 h dark, and provided a 10% sucrose solution in cotton wicks.

Experimental transmission: Every day post-infection, ten or fewer mosquitoes were starved 24 hours, immobilized by exposure to triethylamine, and their proboscis inserted into a capillary tube containing 1:1 fetal bovine serum (FBS) and 10% sucrose solution for ten minutes to assess their ability to transmit virus per os (Aitken 1977). Capillary tube contents were expelled into 250 ml of mosquito diluent (phosphate-buffered saline [PBS], 20% FBS, antibiotics) and frozen at -80°C until assayed. Individual mosquito bodies were frozen at -80°C prior to being assayed. The number of mosquitoes attempting virus transmission each day and the length of each experiment depended on the number of engorged mosquitoes surviving. Seven *Cx. tarsalis* were tested every day except day 12 (n=5); seven *Cx. stigmatosoma* were tested daily except day four (n=10), day six and eleven (n=8), day 13 (n=3), and day 14 (n=5); ten *Cx. p. pipiens* were tested daily except day 16 (n=6); ten *Cx. p. quinquefasciatus* from Bakersfield and Coachella Valley were tested daily.

Plaque assay: Saliva and body samples were assayed for virus by plaque assay in six-well tissue culture plastic plates containing Vero cells. Mosquito bodies were ground individually in 0.5 ml of mosquito diluent. Plaque assays were conducted by adding 100 ml of the ground mosquito suspension or saliva sample to confluent cell monolayers. Plates were incubated at 37°C for 1.5 h to allow for virus to attach and enter cells. Cell cultures were covered with a 2% agarose overlay containing 0.005% neutral red. After 96 h and 120 h of incubation at 37°C, in a 5% CO_2 atmosphere, plaques were counted.

Statistical Analysis: Infection and transmission rates were divided into two groups of day one through nine and day 10 through the last day. Each group was compared among all species by the Fisher Exact test using SAS 8.2 (SAS Institute, Inc., Cary, NC). Differences were considered statistically significant at alpha ≤ 0.05.

RESULTS

Transmission by *Cx. tarsalis* was first detected on day 5 post-exposure to virus [Fig. 1]. Transmission rates by *Cx. tarsalis* for the first nine days were significantly higher than rates for all species tested, except for *Cx. p. pipiens*, indicating *Cx. tarsalis* from Yolo County were able to transmit WN virus relatively soon after infection. *Cx. tarsalis* transmission rates >50% occurred from days ten to day 12. Infection rates for *Cx. tarsalis* during the first nine days were significantly higher than all other species, except *Cx. stigmatosoma*. Results could not be obtained beyond day 12, because of the synchronous death of experimental mosquitoes beginning at day ten. The Yolo Co. *Cx. tarsalis* may have been sensitive to the rearing temperatures or pathogenic effects of the virus.

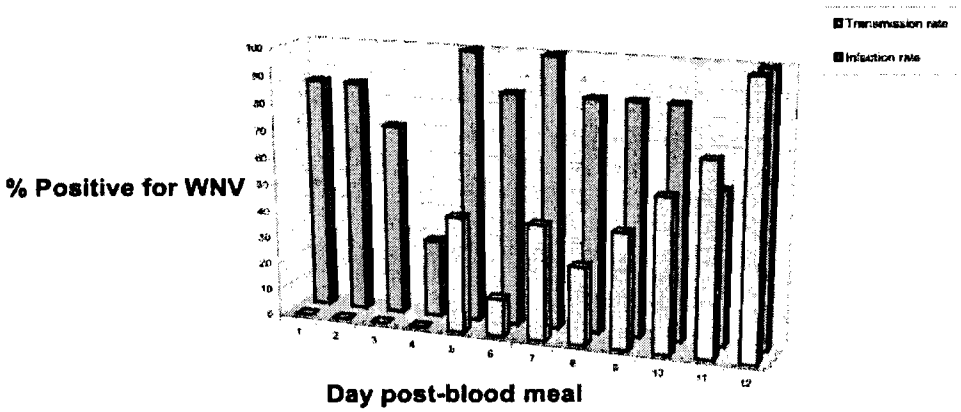

Figure 1. Daily infection and transmission rates for *Cx. tarsalis*.

Cx. stigmatosoma transmission was first detected at day eight and steadily increased to 100% by day 13 [Fig. 2]. Peak transmission rates occurred from day 12 to day 13. *Cx. stigmatosoma* infection rates for days one through nine and days ten through 14 were significantly higher than the rates of all other species tested. Infection rates were 100% for all 14 days, except for day one (57%) and day four (90%).

Cx. p. quinquefasciatus (Bakersfield) transmission rates for days ten through 15 were significantly lower than those of all other species tested except *Cx. p. quinquefasciatus* (Coachella Valley). *Cx. p. quinquefasciatus* (Coachella Valley) were refractory to WN virus infection. Transmission rates were significantly lower than those of all species tested for days one through nine and days ten

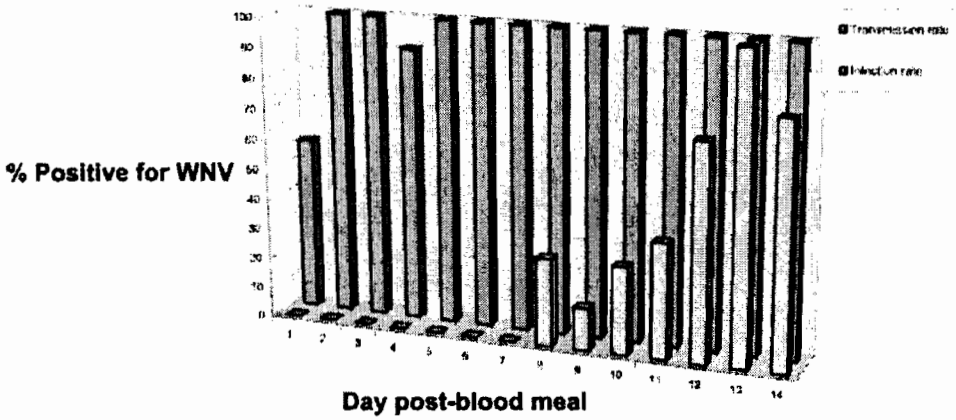

Figure 2. Daily infection and transmission rates for *Cx. stigmatosoma.*

Transmission was first detected at day six for *Cx. p. pipiens* with peak transmission occurring days nine through 12 [Fig. 3]. Transmission rates were significantly higher than rates for *Cx. p. quinquefasciatus* (Bakersfield) and *Cx. p. quinquefasciatus* (Coachella Valley) for days nine through 16. Infection rates for days one through nine and days ten through 16 were only significantly higher than *Cx. p. quinquefasciatus* (Coachella Valley). Transmission for *Cx. p. quinquefasciatus* (Bakersfield) was first detected on day seven, with peak transmission occurring on day 13 [Fig. 4]. One positive transmission was recorded on day three, which may have been the result of a "leaky midgut" (Houk and Hardy 1979) or experimental contamination of the transmission sample.

through 16 [Fig. 5]. Transmission was only detected on day six (10%) and this transmission was not associated with a positive body. *Cx. p. quinquefasciatus* (Coachella Valley) also had significantly lower infection rates compared to all species tested for all 16 days.

DISCUSSION

The susceptibility to WN virus infection and the duration of the extrinsic incubation periods varied among *Culex* species. Extrinsic incubation periods ranged from five to eight days. These relatively short periods may be attributed to several factors. One factor may be the relatively warm incubation temperature of 28°C.

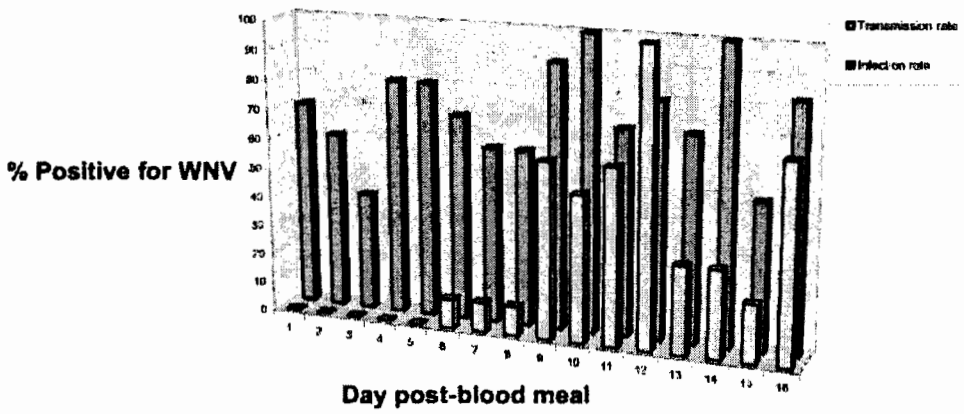

Figure 3. Daily infection and transmission rates for *Cx. p. pipiens.*

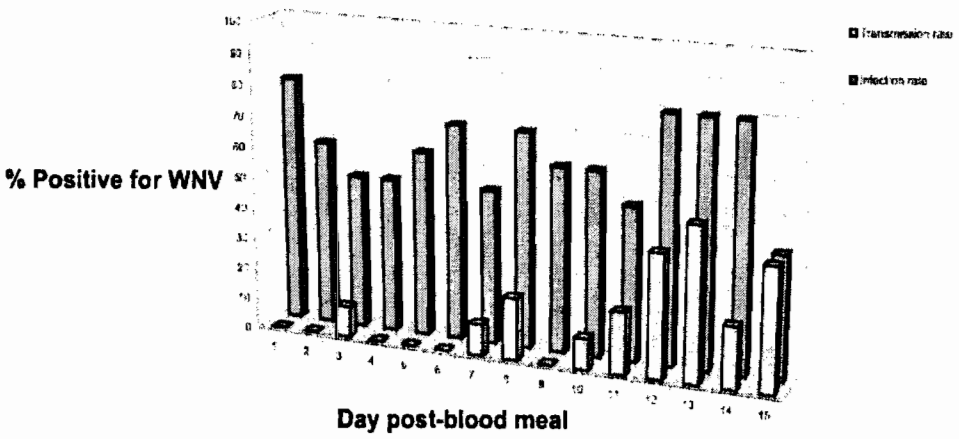

Figure 4. Daily infection and transmission rates for *Cx. p. quinquefasciatus* (Bakersfield).

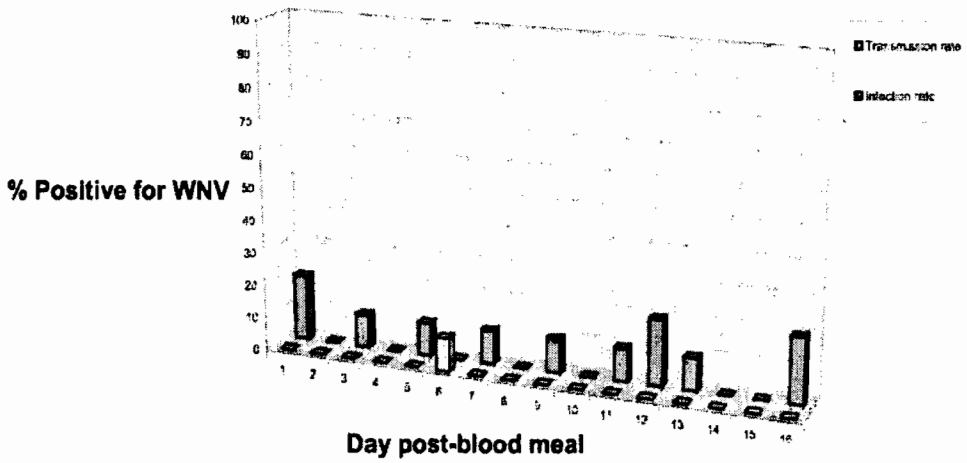

Figure 5. Daily infection and transmission rates for *Cx. p. quinquefasciatus* (Coachella Valley).

Increasing ambient temperature generally decreases the time required for WN virus replication (Cornel et al. 1993) and this varies markedly among viruses (Reisen et al. 1993). We expect that if mosquitoes were held at lower temperatures than we studied, that the duration of the extrinsic incubation period would be elongated (Chamberlain and Sudia 1961). Infection rates and extrinsic incubation periods also may be affected by virus dose, where a low infection dose can result in low infection and transmission rates and a long extrinsic incubation period (Kramer et al. 1981, Jupp 1974). We used a relatively high dose of $10^{7\pm0.1}$ PFU's of WN virus per mL because that titer is comparable to the viremia of an infectious avian host (Komar et al. 2003). Because we maintained a constant incubation temperature and infectious dose for all species tested in our study, we attribute differences in infection and transmission patterns to variation in susceptibility of mosquito species to virus infection (Hardy et al. 1983). Midgut and salivary gland barriers to infection and replication exist in mosquitoes that can affect their vector competence. After a mosquito ingests an infectious blood meal, virus enters and replicates in the midgut epithelial cells. Virus then disseminates to the hemocoel where it infects and replicates in various tissues including the salivary glands. Variation in these processes can affect the host competence of the vector species and therefore the epidemiology of an arbovirus. For example, mosquito species with low transmission rates and a long extrinsic incubation period must exist at high-density or have relatively reduced mortality to ensure that enough mosquitoes survive through the extrinsic incubation period to transmit virus.

Cx. tarsalis from Yolo County was the most efficient laboratory vector of WN virus in this study. It had the shortest extrinsic

incubation period of the four species tested and some of the highest transmission rates. This species' high susceptibility to WN virus infection indicates rapid viral entry and amplification in mosquito tissues and the absence of midgut and salivary gland infection barriers. *Cx. stigmatosoma* demonstrated high susceptibility to infection comparable to *Cx. tarsalis*, however, it was unable to transmit virus as quickly as *Cx. tarsalis*. After ten days of incubation, *Cx. stigmatosoma* transmission rates were comparable to those of *Cx. tarsalis*. The relatively late start in transmission for *Cx. stigmatosoma* despite significantly high infection rates indicates rapid viral entry and replication in the midgut, but slow escape and dissemination to the salivary glands. The long extrinsic incubation period means that *Cx. stigmatosoma* must survive at least eight days to be an effective laboratory vector of WN virus, three days more than the minimum for *Cx. tarsalis*.

Results for infection and transmission rates for mosquitoes in the *Cx. pipiens* complex were variable. Although all three groups initiated transmission on day six or seven, *Cx. p. pipiens* was more susceptible to WN virus infection with significantly higher transmission rates after ten days of incubation. *Cx. p. quinquefasciatus* from Coachella Valley demonstrated significant midgut infection and escape or salivary gland infection barriers. Differences in vector competence among mosquitoes within this complex indicate that there is epidemiologically relevant variation in the genetic structure of populations across California. Genetic components affecting midgut and salivary gland barriers to virus infection were evidenced by the significantly different transmission and infection patterns observed among *Cx. p. pipiens* and the two populations of *Cx. p. quinquefasciatus*. Additional studies are needed to determine the extent of genetic variation among mosquitoes in the *Cx. pipiens* complex and among populations of other species in California (Cornel et al. 2003). Understanding these genetic differences will help to better define entomological processes affecting vector competence and the epidemiology of WN virus.

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Although not collected in this study, the Asian Tiger mosquito (*Aedes albopictus*) was introduced into southern California (Madon et al., 2002). This species tends to breed in small containers, thus making it an ideal candidate for breeding in cemetery flower cups.

Acknowledgements

I thank the following people who have aided and directly supported this project: Dr. James Webb, who coined the title "Bone Yard Project," Dr. Richard Meyer, Robert Cummings, Amber Mills, Greg Williams, Lee Spath, Tawnia Pett, Jodie Stoddard, and Dr. Robert D. Sjogren who provided administrative support.

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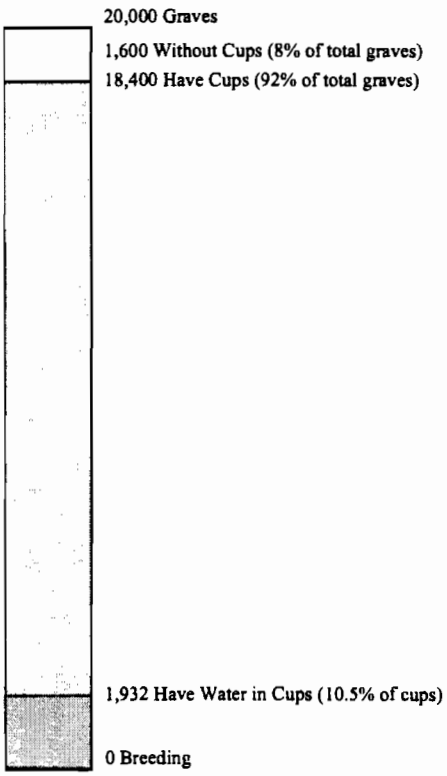


	Summer		Winter	
	Larvae	Adults	Larvae	Adults
Culex quinquefasciatus	0	0	0	0
Culex tarsalis	0	0	0	0
Culiseta incidens	0	0	0	0
Culiseta inornata	0	0	0	0

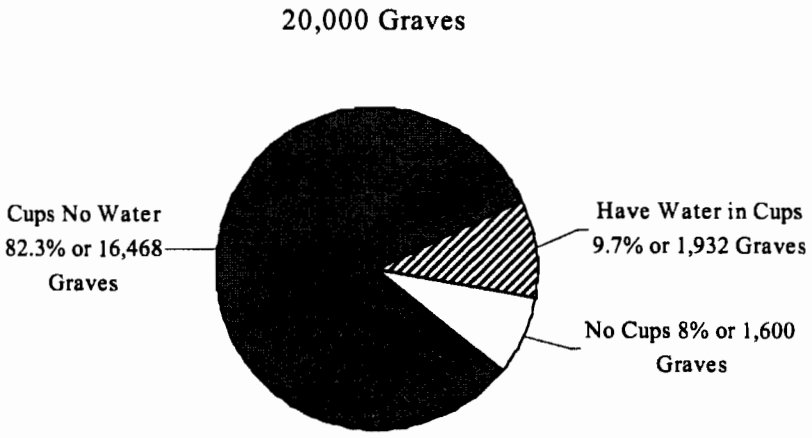
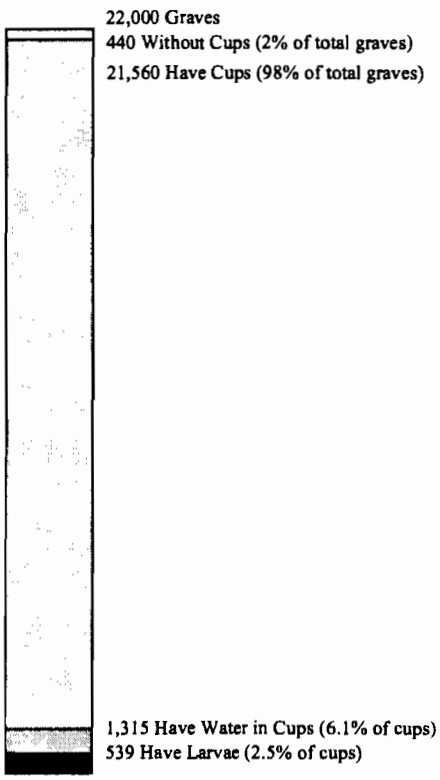


Figure 18. Corona Del Mar Pacific View



	Summer		Winter	
	Larvae	Adults	Larvae	Adults
Culex quinquefasciatus	55%	47%	25%	40%
Culex tarsalis	33%	43%	0	7%
Culiseta incidens	12%	10%	75%	53%
Culiseta inornata	0	0	0	0

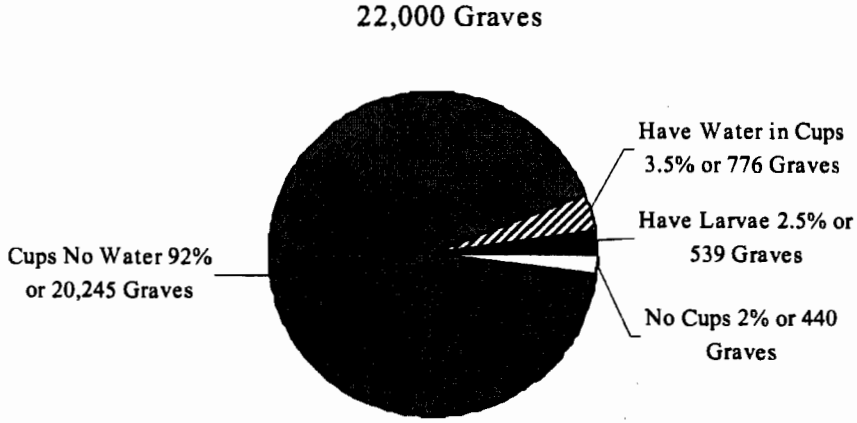
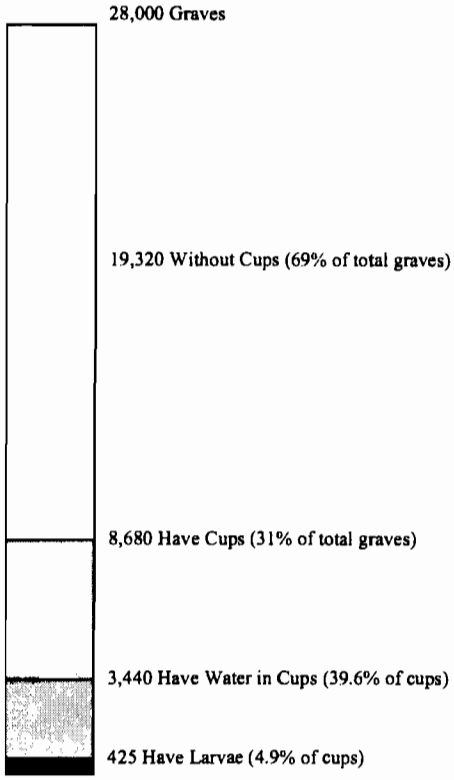


Figure 19. Huntington Beach Good Shepherd



	Summer		Winter	
	Larvae	Adults	Larvae	Adults
Culex quinquefasciatus	72%	54%	38%	22%
Culex tarsalis	4%	33%	0	0
Culiseta incidens	16%	13%	57%	74%
Culiseta inornata	8%	0	5%	4%

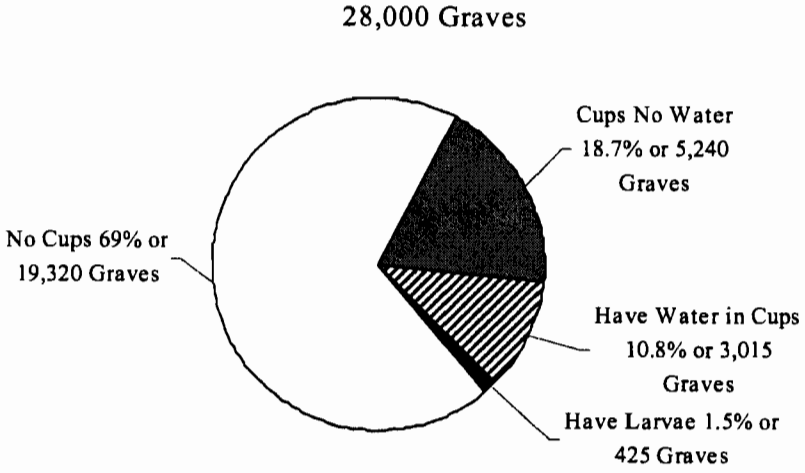
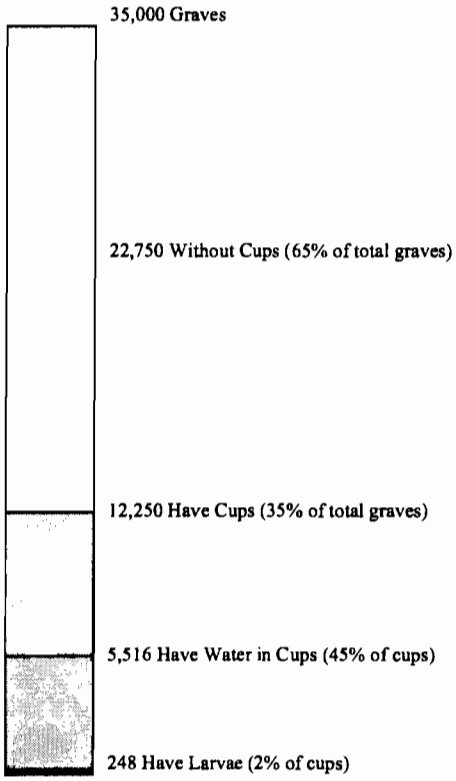


Figure 16. Fullerton - Loma Vista



	Summer		Winter	
	Larvae	Adults	Larvae	Adults
Culex quinquefasciatus	64%	55%	25%	27%
Culex tarsalis	5%	10%	0	0
Culiseta incidens	31%	35%	75%	73%
Culiseta inornata	0	0	0	0

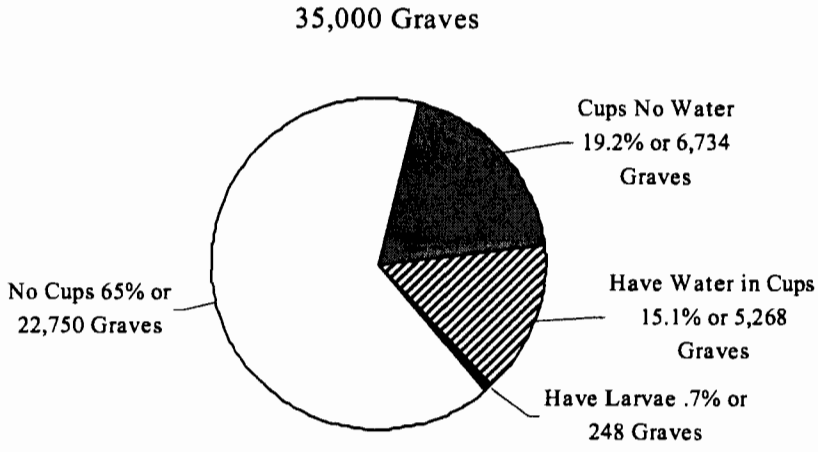
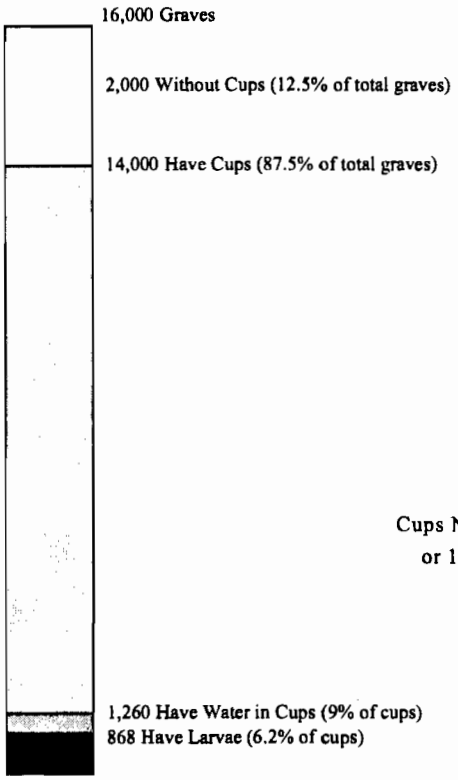


Figure 17. Cypress Forest Lawn



	Summer		Winter	
	Larvae	Adults	Larvae	Adults
Culex quinquefasciatus	50%	59%	33%	34%
Culex tarsalis	17%	26%	0	0
Culiseta incidens	33%	11%	67%	66%
Culiseta inornata	0	4%	0	0

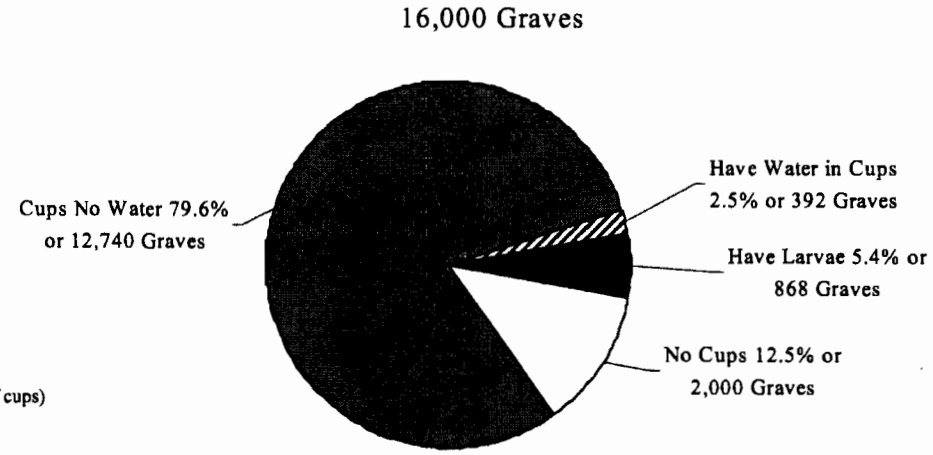
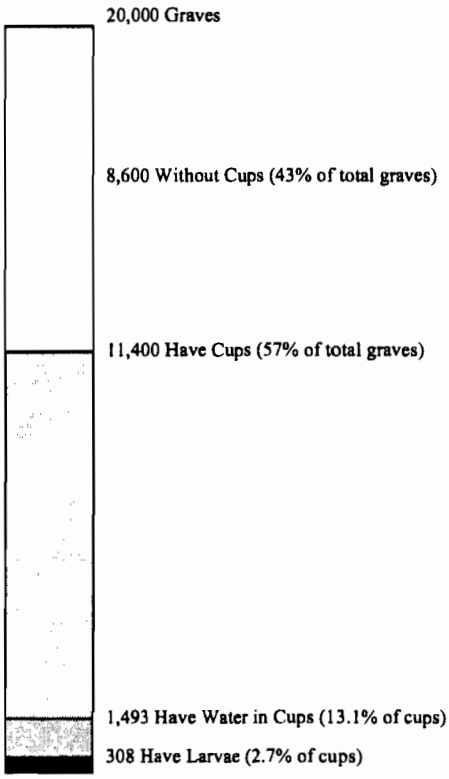


Figure 14. Brea Memory Gardens



	Summer		Winter	
	Larvae	Adults	Larvae	Adults
Culex quinquefasciatus	90%	88%	12%	50%
Culex tarsalis	10%	12%	0	15%
Culiseta incidens	0	0	78%	35%
Culiseta inornata	0	0	0	0

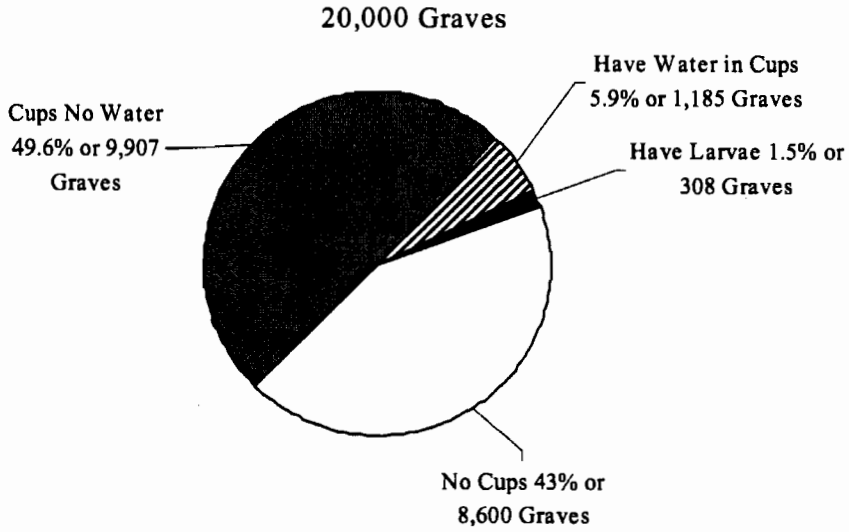
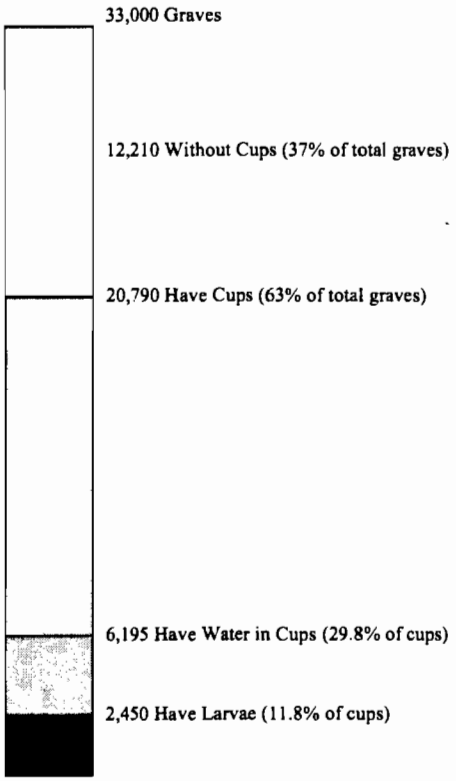


Figure 15. Costa Mesa – Harbor Lawn – Mt. Olive



	Summer		Winter	
	Larvae	Adults	Larvae	Adults
Culex quinquefasciatus	97%	69%	33%	25%
Culex tarsalis	0	6%	0	0
Culiseta incidens	3%	25%	67%	75%
Culiseta inornata	0	0	0	0

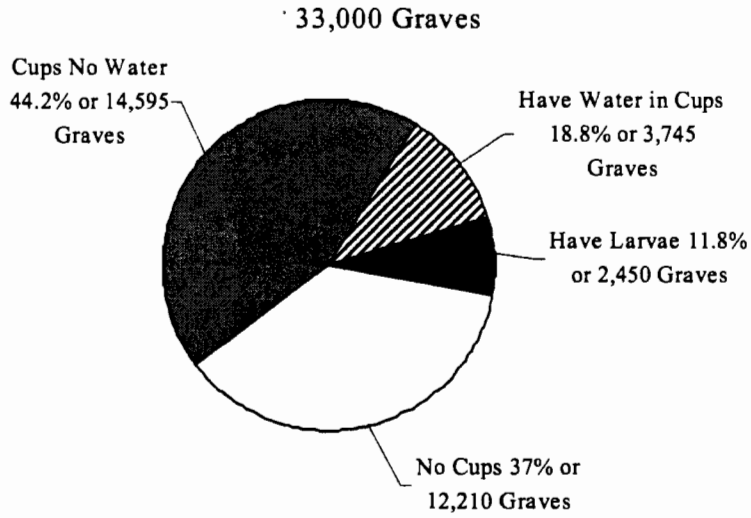
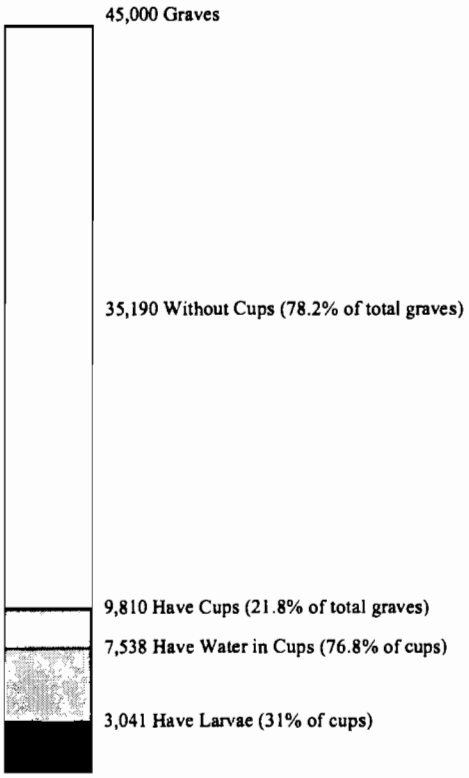


Figure 12. Orange Holy Sepulcher



	Summer		Winter	
	Larvae	Adults	Larvae	Adults
Culex quinquefasciatus	67%	70%	25%	12%
Culex tarsalis	11%	10%	0	0
Culiseta incidens	22%	20%	75%	69%
Culiseta inornata	0	0	0	19%

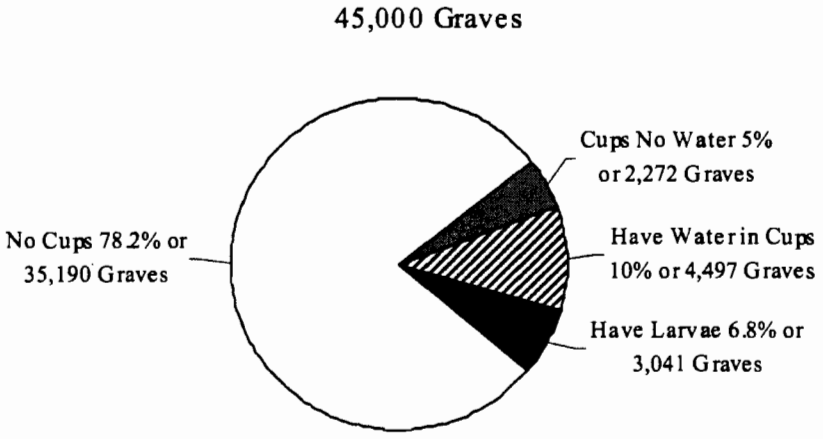
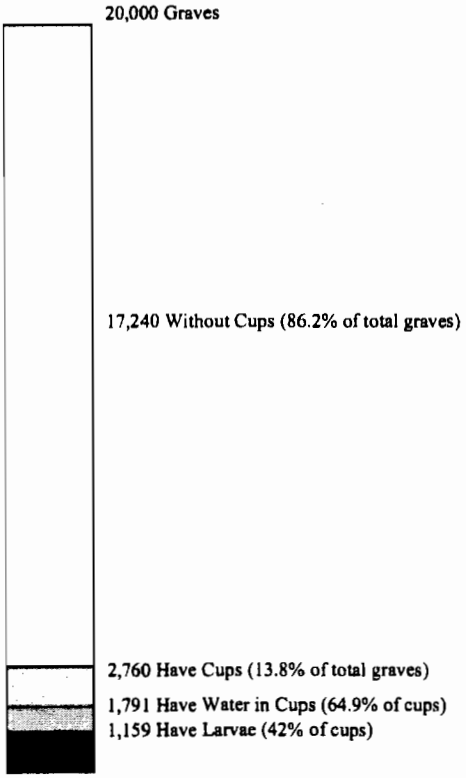


Figure 13. Fairhaven Memorial Park



	Summer		Winter	
	Larvae	Adults	Larvae	Adults
Culex quinquefasciatus	50%	83%	36%	15%
Culex tarsalis	13%	3%	5%	0
Culiseta incidens	30%	7%	59%	80%
Culiseta inornata	7%	7%	0	5%

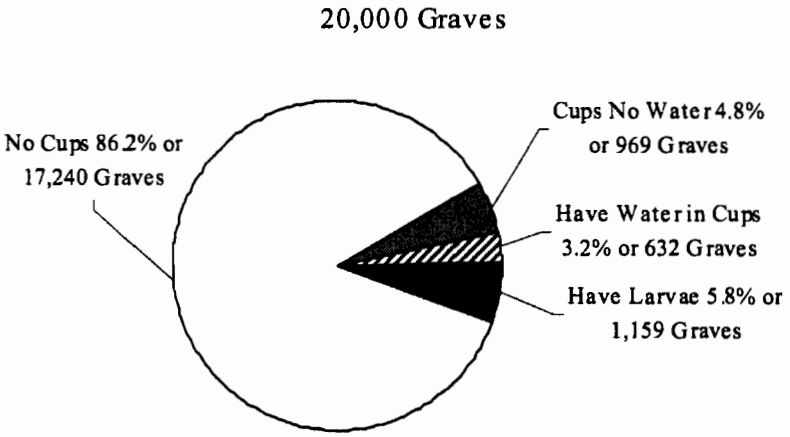
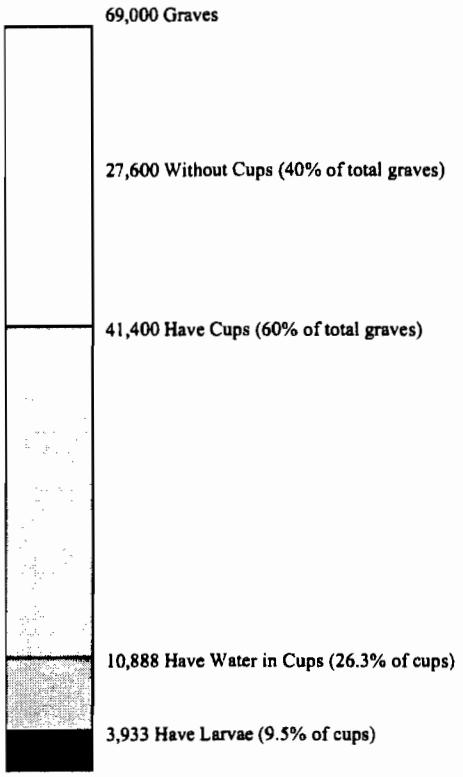


Figure 10. Melrose Abbey Memorial Park



	Summer		Winter	
	Larvae	Adults	Larvae	Adults
Culex quinquefasciatus	50%	83%	36%	15%
Culex tarsalis	13%	3%	5%	0
Culiseta incidens	30%	7%	59%	80%
Culiseta inornata	7%	7%	0	5%

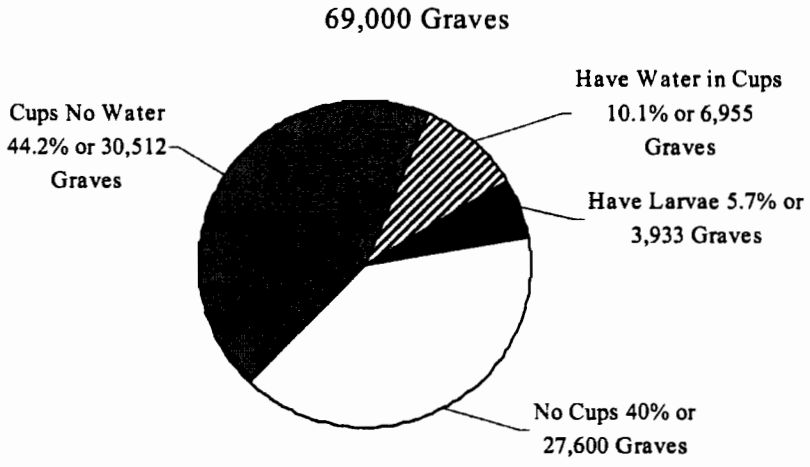
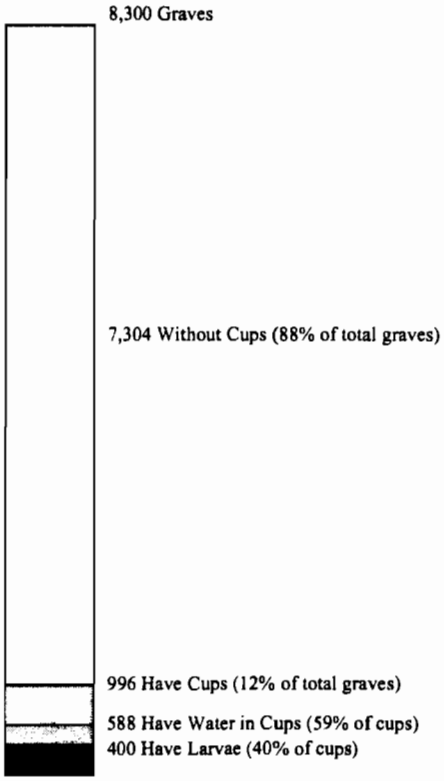


Figure 11. Westminster Memorial Park



	Summer		Winter	
	Larvae	Adults	Larvae	Adults
Culex quinquefasciatus	57%	62%	22%	25%
Culex tarsalis	43%	32%	0	0
Culiseta incidens	0	6%	78%	75%
Culiseta inornata	0	0	0	0

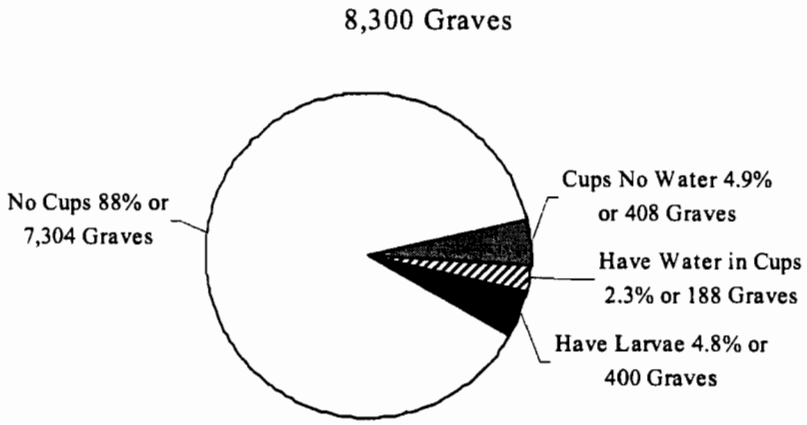
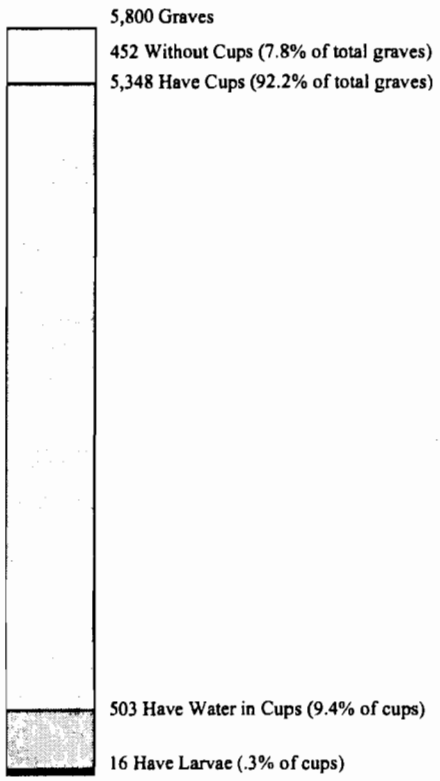


Figure 8. El Toro Cemetery



	Summer		Winter	
	Larvae	Adults	Larvae	Adults
Culex quinquefasciatus	100%	100%	100%	100%
Culex tarsalis	0	0	0	0
Culiseta incidens	0	0	0	0
Culiseta inornata	0	0	0	0

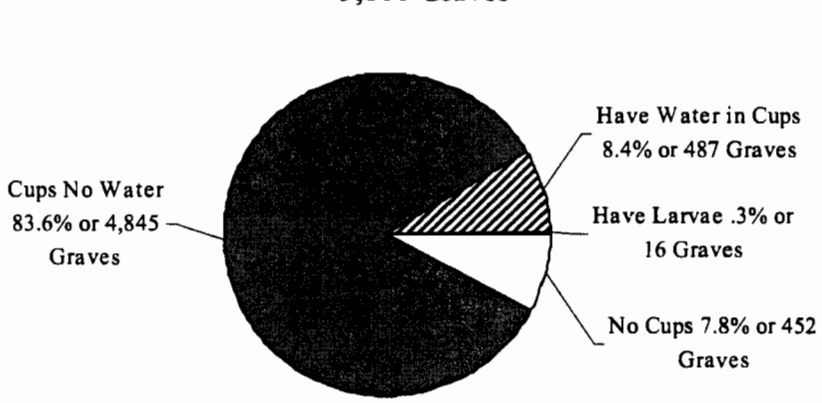
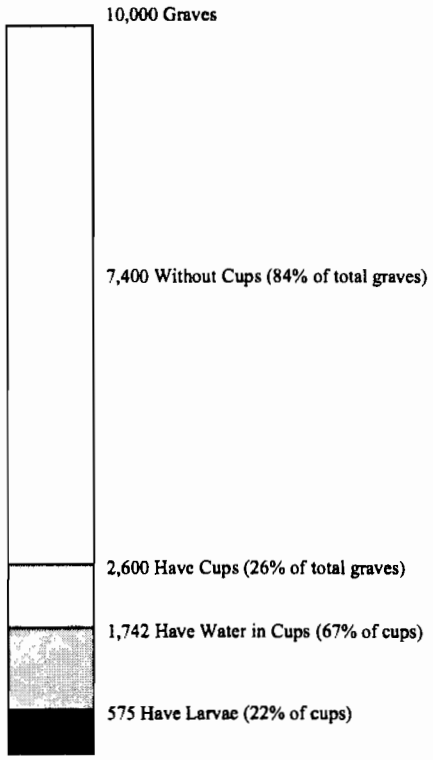


Figure 9. Lake Forest Ascension



	Summer		Winter	
	Larvae	Adults	Larvae	Adults
Culex quinquefasciatus	55%	63%	40%	20%
Culex tarsalis	9%	21%	0	0
Culiseta incidens	36%	16%	60%	80%
Culiseta inornata	0	0	0	0

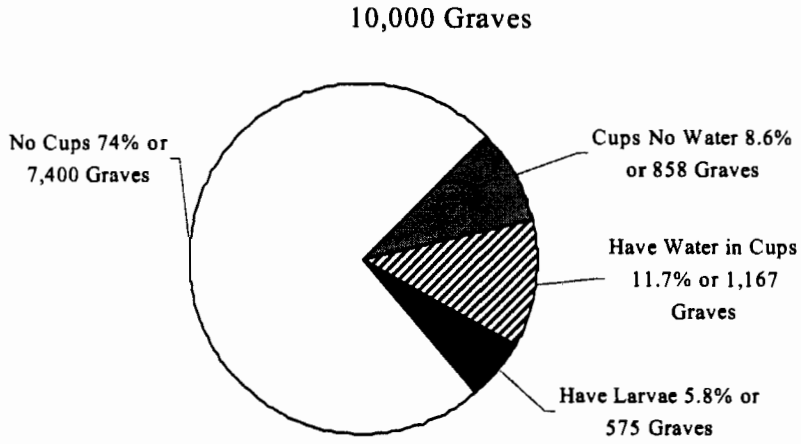
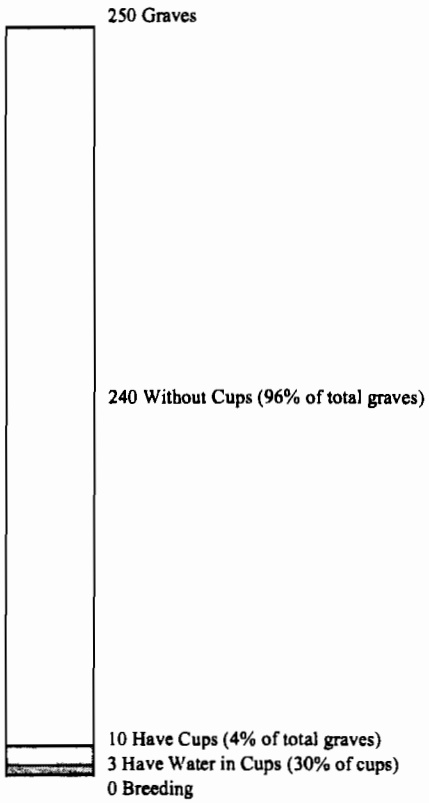


Figure 6. Anaheim Cemetery



	Summer		Winter	
	Larvae	Adults	Larvae	Adults
Culex quinquefasciatus	0	0	0	0
Culex tarsalis	0	0	0	0
Culiseta incidens	0	0	0	0
Culiseta inornata	0	0	0	0

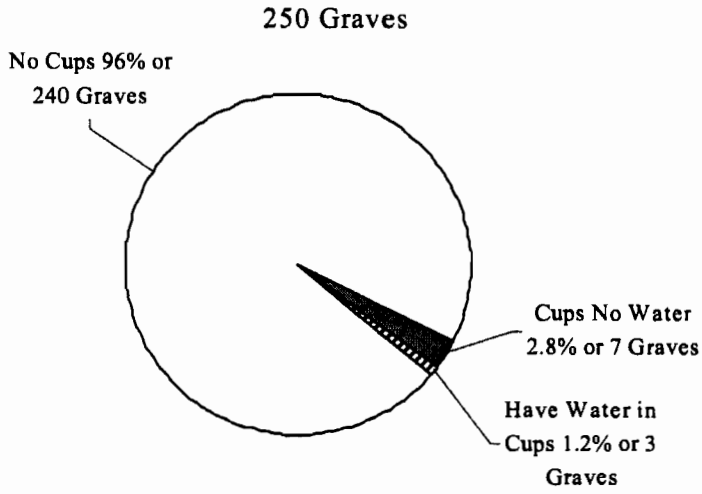


Figure 7. Holy Cross

cups with those that contain breeding and non-breeding. The results are shown in Table 1.

From the very small differences of the measured quality features between the BC and NBC data for each factor, other factors not known must be important for cup selection.

The grading scale (Table 2) was an attempt to compare each of the cemeteries with the amount of mosquito breeding caused by their maintenance practices (i.e., not emptying cups with water on a weekly basis). This scale compares the total number of graves, the number of graves that have cups, cups with water and cups that have larvae.

The species of mosquitoes collected in the larval stage and as adults are depicted in Table 3.

Figures 6-19 compare the percent of each type of mosquito larvae and adults found at each cemetery in summer and winter. The data in these figures are based on projection of graves sampled. A bar graph comparing the following data was made for each of the cemeteries in the County: Number of total graves in each cemetery; number of graves that have cups; number of cups that have water; number of cups that have larvae present; and status of all graves surveyed.

Table 1. A comparison of averages of 68 samples taken from cups with breeding and non-breeding for pH, temperature, conductivity, and dissolved oxygen.

	pH	Temperature Celsius (°C)	Conductivity	Dissolved O_2 (Mg./Liter)
Breeding Cups (68)	7.67	17.3	0.35	1.01
Non-breeding Cups (68)	7.73	16.9	0.30	1.02
Difference	0.06	0.4	0.05	0.01

Table 2. The Cemetery Grade Card.

Location	Total Number	Number of of Graves with Cups	Number of Graves with Water	Number of Cups Breeding	Cups with Grade*
Anaheim Cemetery	10,000	2,600	1,742	575 (22%)	D
Holly Cross, Anaheim	250	10	3	0 (0%)	A
Melrose Abbey Memorial Park, Anaheim	20,000	2,760	1,791	1,159 (42%)	F
Westminster Memorial Park	69,000	41,400	10,880	3,933 (9.5%)	C
El Toro Cemetery	8,300	996	588	400 (40%)	F
Lake Forest Ascension	5,800	5,348	503	16 (0.3%)	A
Orange Holy Sepulcher	33,000	20,790	6,195	2,450 (11.8%)	C
Fairhaven Memorial Park, Santa Ana	45,000	9,810	7,538	3,041 (31%)	D
Brea Memory Gardens	16,000	14,000	1,260	868 (6.2%)	C
Costa Mesa Harbor Lawn - Mt. Olive	20,000	11,400	1,493	308 (2.7%)	B
Fullerton Loma Vista	28,000	8,680	3,440	425 (4.9%)	C
Cypress Forest Lawn	35,000	12,250	5,516	248 (2%)	B
Corona Del Mar Pacific View	20,000	18,400	1,932	0 (0%)	A
Huntington Beach Good Shepherd	22,000	21,560	1,315	539 (2.5%)	B

* 0% - 1.9% = A, 2.0% - 3.9% = B, 4% - 19% = C, 20% - 38% = D, 39% - 100% = F

Table 3. The percentage of larvae (cups) and adults (CDC/CO_2 traps) collected for each species.

Species	Larvae	Adults	Time
Culiseta incidens	75%	80%	Nov.-Mar. (2000-2001)
Culiseta inornata	5%	0%	
Culex quinquefasciatus	20%	20%	
Culiseta incidens	5%	5%	Apr.-Oct. (2001)
Culex tarsalis	5%	15%	
Culex quinquefasciatus	90%	80%	

MATERIALS AND METHODS

Sampling was done by counting equal numbers of grave sites at the four corners and center of each of 14 cemeteries. Larvae were sampled with a modified turkey baster and a white long handled cup (Fig. 3). Adults were sampled using a CDC/CO_2 trap (Fig. 4). The larvae and adults were sampled every two weeks over a two year period. See GIS map of cemetery locations (Fig. 5). Using two (2) meters, the YSI-550-DO (dissolved oxygen) and model YSI-63 (pH, temperature, conductivity), 68 breeding cups (BC) were sampled and 68 nonbreeding cups (NBC) were sampled. The sampling using the two (2) meters was done every month over a period of one year.

RESULTS AND DISCUSSION

It was found that there are major differences in each cemetery's procedure for flower removal and the emptying of water from the cups. These procedures often have a direct effect on the numbers of mosquitoes produced (i.e., if the cups were emptied once each week, there was no mosquito production).

There was a great difference in the number of mosquito larvae found in the cups sampled. This may be due to: temperature of the water, predators, chemicals from flowers, herbicides, bacteria, blue-green algae, and water quality which was determined by comparing the pH, dissolved oxygen, conductivity, and temperature of water in

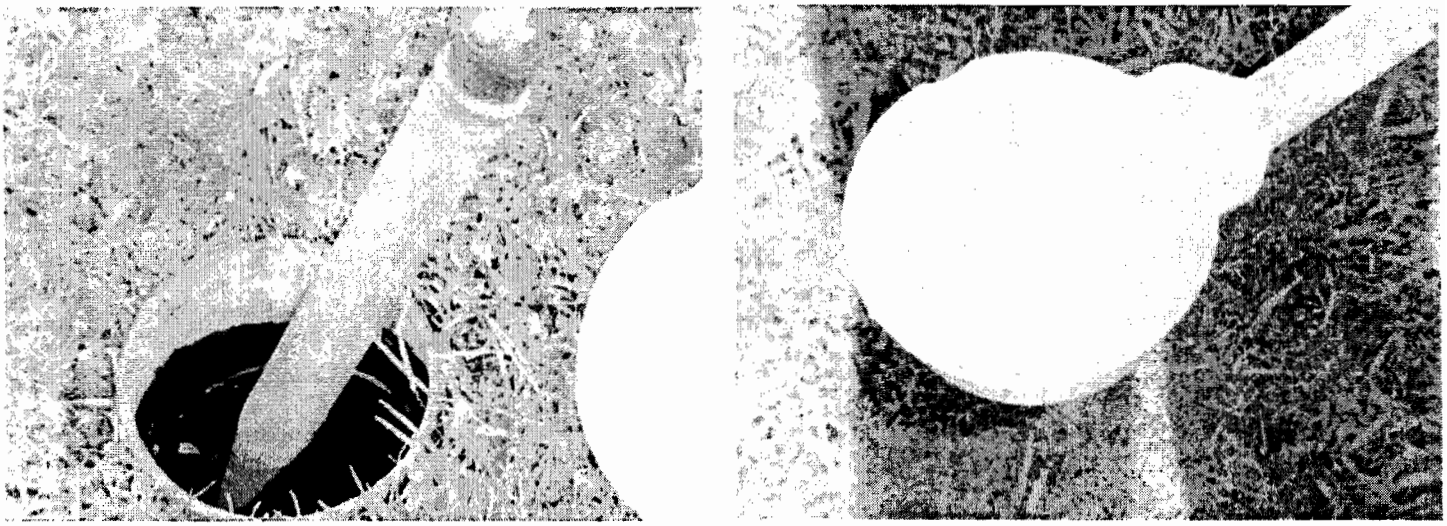


Figure 3. Mosquito larvae collected with a modified turkey baster and then placed in a standard dipper.

Figure 4. CDC/CO_2 baited mosquito trap.

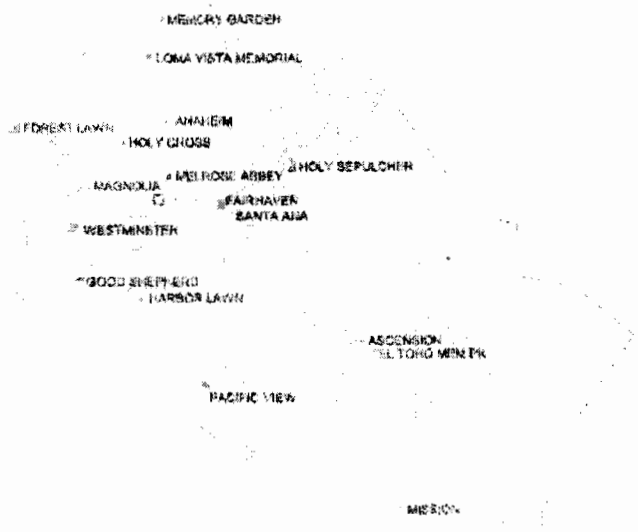


Figure 5. Orange County cemetery locations.

Cemeteries as a Source for Mosquito Breeding in Orange County, California

Ralph Havickhorst

Orange County Vector Control District, 13001 Garden Grove Boulevard, Garden Grove, CA 92843-2102

INTRODUCTION

Orange County has changed from a citrus orchard, vegetable, and dairy farm environment to one of business and urban development. The cemeteries that were here and the more recent ones are now surrounded by housing. This makes those people living in proximity to the cemeteries more vulnerable to the nuisance and diseases that may result from mosquito bites.

The older cemeteries usually have a single galvanized cup at the end of the grave site, set directly into the soil, with the top flush or slightly below the earth's surface. If the cup is used only two to three times per year, it often becomes overgrown with the grass, which makes it difficult to find and to empty the water. Most cemeteries use an overhead irrigation system that continues to resupply the cups with water. Mosquitoes that find these cups are protected by the overgrowth of grasses and are able to complete their reproductive cycle (Fig. 1).

The newer cemeteries use a different method, or cup within a cup. One is sunk into the soil as a permanent fixture, and the second cup fits into it like a sleeve. This cup can easily be picked up and turned upside down and emptied, which most cemeteries now do on a weekly basis (Fig. 2).

In the late 1950s, I conducted a survey of Orange County cemeteries for mosquito breeding because we were receiving an excessive number of service requests from homes in proximity to cemeteries. This survey was published in 1970 (Shanafelt, 1970). Previous mosquito studies at cemeteries in California include those by Aarons (1948), Dhillon (1980), Dhillon and Mulla (1982), Kelly (1941), and Mulla et al. (1978).

Most of the cemeteries in the 1950s used galvanized cups set directly in the ground and usually could not be easily emptied. The mosquito production as a result was excessive enough that we had to send a team of at least 10 operators walking among the rows and spraying each cup with diesel oil, Flit-MLO®, or weed oil. This significantly reduced the number of service requests in proximity to the cemeteries and was reduced to almost nothing within three weeks. We recommended that the cemeteries' employees turn cups over at least once a week.

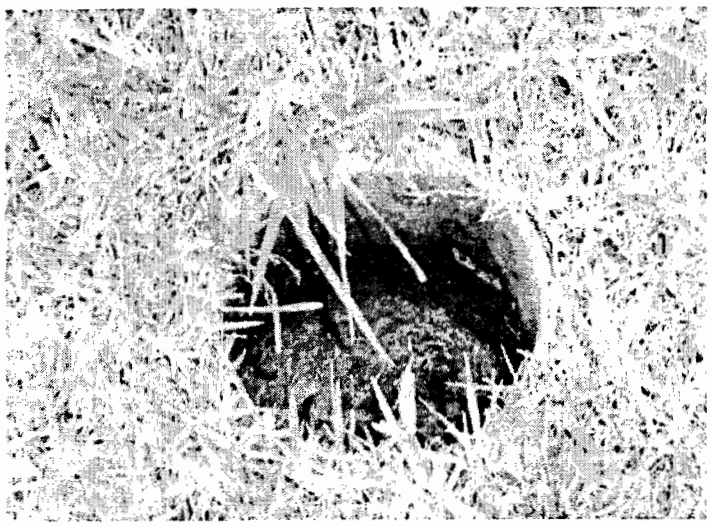


Figure 1. Galvanized cups that are not dumped on a regular basis may be continuous mosquito producers.

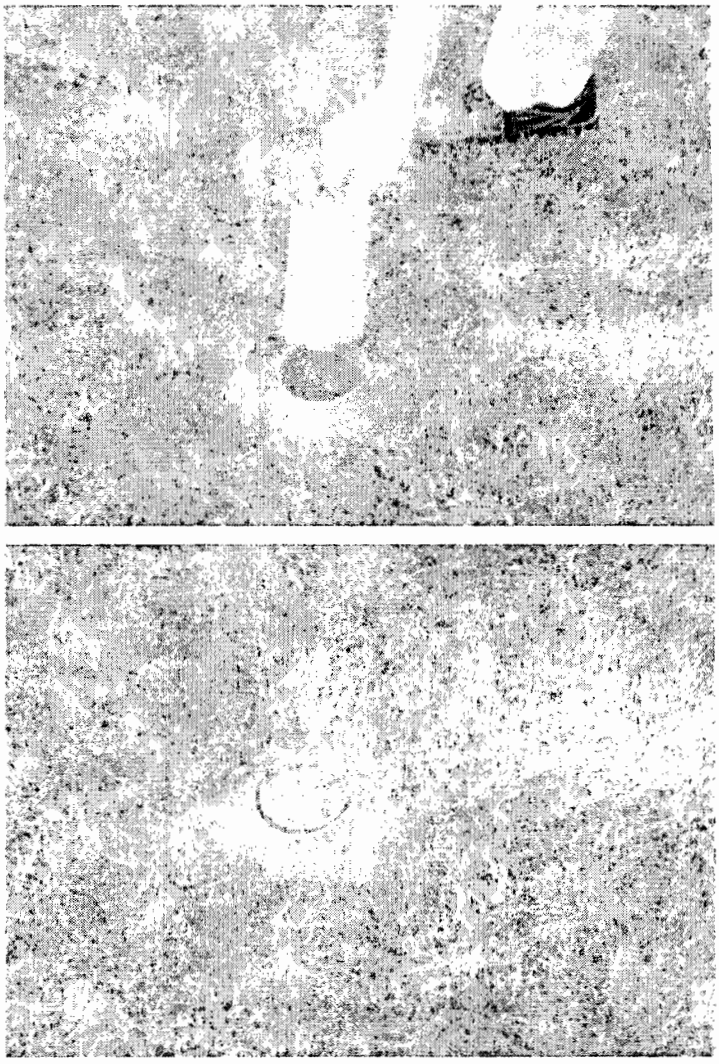


Figure 2. Examples of the newer cups that many cemeteries use that are easily emptied, resulting in reduced mosquito production.

Aedes albopictus Recovered in Nursery Near Gilroy, California

Noor S. Tietze, Ph.D.

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ABSTRACT: During the summer of 2001, immature and adult *Aedes albopictus* were discovered at a nursery near Gilroy, California (following the initial discovery of *Ae. albopictus* introduction in shipments of "lucky bamboo" at the Los Angeles and Long Beach harbors, Madon et al. In Press, J. Vector Ecology). The nursery specializing in orchids and lucky bamboo (*Dracaena*), had recently obtained shipments of the latter from Taiwan and mainland China that were found to contain live larval, pupal and adult specimens. Third and 4th instar larvae and pupae were pipetted from Styrofoam shipping containers and transported to the laboratory where they were identified as *Ae. albopictus*. Specimens were provided to the CDC via CDHS and to Tom Zavortink at the University of San Francisco for taxonomic confirmation. Control efforts were made to eliminate standing water developmental sites, and pyrethrin-based adulticide was applied within a week of the discovery. Due to the presence of orchids in the nursery, a Pyrenone crop spray was selected to avoid injury to the costly plants. Post-treatment sampling using ovijars (based on a design by Eric T. Schreiber) and attempts using carbon dioxide-baited traps recovered only *Culex pipiens*. Since further attempts failed to recover larvae or pupae from shipping containers and other container habitats at the nursery the control efforts were deemed a success. It is unlikely that this tropical mosquito species would survive and reproduce in the vicinity of the nursery due to the dry environmental conditions and lack of potential developmental sites.

Editor's Note: Abstract was submitted in March 2002.

year will demonstrate the effectiveness of the VM in the field at elementary schools, with a $150,000 budget. For the third year, the VM will continue school visitations with the curriculum adjusted to a one-hour program reaching more classrooms per day. Staffing includes the VM Coordinator/Grant Administrator, Education Program Assistant, and a part time staff member to assist in the *Exploritorium Resource Center*. The program is restricted to fifth-graders for ease of evaluation purposes. The statistical analysis is uniform by not comparing different age groups and grade levels. If the program is permanently accepted, 4-6th graders will also be included. After the first academic year of experience in the field, the District's Board of Trustees will evaluate the VM effectiveness, and decide to either accept or not accept it as a permanent addition to the District's Education and Community Outreach Program.

This innovative mobile science program will increase the number of elementary students participating without a commensurate increase in costs. The program will increase the number of students reached annually from the current 3,567 to ~15,000. For the second year, the curriculum will be reduced to a one-hour program, attaining 30,000 students, thereby reaching a majority of the 141,000 fifth-grade students in 34 cities within the District boundary. Forty-one superintendents of schools have approved the applied science-based program and have committed their school districts and respective elementary schools. A total of 82 schools, 286 classrooms and 9,159 students will be reached from September 2002 through April 2003. The VM will provide better utilization of District staff and taxpayer money for community outreach.

The VM is made possible thanks to the generous support from Mediamobiles, Inc., Merv Griffin Productions, Accurate Image, Inc., Wells Fargo Foundation, Valent BioSciences, Rohm and Haas, Wellmark International, Aventis Environmental Science, Handicaps, Inc., Sam's Club Foundation, Wal*Mart Foundation, Fennimore Chemicals, Vopak, Carolina Biological Supply Company, and Mercury Messenger Service.

The VECMobile's Three-Year Pilot Program

Stephanie Miladin

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The Mobile Education Unit (MEU), known as the VECMobile (VM), is the first of its kind in the nation, aimed at teaching fifth-grade elementary school students about vectors and vector- borne diseases. This unique 35-foot traveling Recreation Vehicle (RV) classroom makes science come alive for youngsters. Students explore the world of vectors as they navigate through the mobile vehicle's simulated estuary, learning about mosquito, bee, and fish biology, neighborhood sources, vector-borne disease transmission, control methods and surveillance efforts. Interactive computer games, live specimens, basic scientific equipment, and targeted hands-on projects encourage scientific thinking as students develop theories about vectors. Implementation of this three-year pilot program began January of 2000.

To augment the current educational program, the VM was created and is sponsored through the Greater Los Angeles County Vector Control District, and operated/subsidized by the Greater Los Angeles Mosquito and Vector Control Public Health and Educational Foundation, a non-profit, public benefit corporation. This Foundation's 501(c)(3) tax-exempt status allows tax-deductible contributions from private industry to help fund this project. To date, the Foundation has received $23,825 in grant funding and $133,728 of in-kind support. Starting February 2003, bi-annual statistical analysis will be provided to grantors, demonstrating how the VM curriculum actively engages students' learning.

The main objectives of this program are to reach under-represented, disenfranchised, inner-city youngsters in need of interactive science programs; to supplement the individual classroom visitations by the Education Program Specialist; and to raise public awareness of GLACVCD. The VM will empower youngsters and their families to detect and remove vector sources around their homes, significantly reducing the chance of disease transmission by mosquitoes and other vectors and strengthen existing and create new community partnerships.

One month prior to the VM arrival, teachers and students receive a program orientation package that familiarizes and prepares the classroom teacher and students for the upcoming VM experience. The following materials are included: a pre-test and post-test sheet for each student comparing the two scores and measuring the effectiveness of all lessons within the program; a 28-page "Vector Inspector Activity Book" for each student; a 22-page "Teachers Guide to the Vector Inspector Activity Book; an 18-page overview of the actual VM curriculum; a short teacher questionnaire requesting feedback from the classroom teacher on the VM program's effectiveness; and an addressed, postage-paid envelope to mail pre- and post-test sheets and the teacher questionnaire back to the District. The VM curriculum fulfills the California's State Board of Education science content guidelines.

One fifth-grade class, approximately 40 students and one teacher spend two hours visiting the VM and the outdoor *"Exploratorium Research Center."* Twenty students circulate through four different stations within the VM. Station 1: What's An Adult Mosquito? allows students to construct a 3-D female mosquito by correctly choosing body parts that relate to a mosquito's physical and behavioral requirements. Station 2: Where Do Mosquitoes Come From? allows students to identify the actual stages of the mosquito life cycle under a macrovideo and also relate habitat changes to physical control methods. Station 3: Why Are Mosquitoes Dangerous? allows students to interact with a programmed (CD-ROM) computer, learning the functions of a female mosquito proboscis and the transmission cycle of St. Louis encephalitis. Station 4: What Is Biological Control? allows students to observe mosquito larvae behavior and equate fish predator morphology and prey behavior. Students make observations and formulate conclusions while experiencing biological control of mosquitoes (5 larvae are added to a fish tank containing mosquitofish, goldfish, and catfish).

While the four teams are on-board the VM, the other 20 students are outside, under the mobile vehicle awning, which shelters the *"Exploratorium Research Center."* Students can investigate specific scientific and entomological topics and interactive exhibits in detail at the following five displays: "What's A Vector?" allows students to manipulate doors and switches on a four-sectioned interactive display, instructing students how to answer questions and provides a survey of the world's vectors. "Not In Your Neighborhood" allows students to view pictures of mosquito breeding sources and provides a form to complete after they look for sources in their own backyard and neighborhood. Students can earn a "Certificate of Recognition" if they complete and return their "Vector Inspector Mosquito Breeding Source Hunt" sheet to the District. The "World of Insects" allows students to view several insect collections from around the world with magnifying glasses. Collection instructions will also be provided for students who want to attempt actual insect collecting. "How We Look For Diseases" allows students to observe and examine actual insect traps in operation and explains how vector-borne diseases can be monitored and/or predicted. "How Do Bees Communicate?" allows students to watch live European honeybees inside a Plexiglass beehive.

The first year of this pilot program involved establishing the Greater Los Angeles Mosquito and Vector Control Public Health and Education Foundation, researching and developing program materials and the curriculum. The first year budget was $130,000. For the second year, the Foundation obtained grant funding and outfitted the donated 35-foot VM to sustain the program. The second

Acknowledgements

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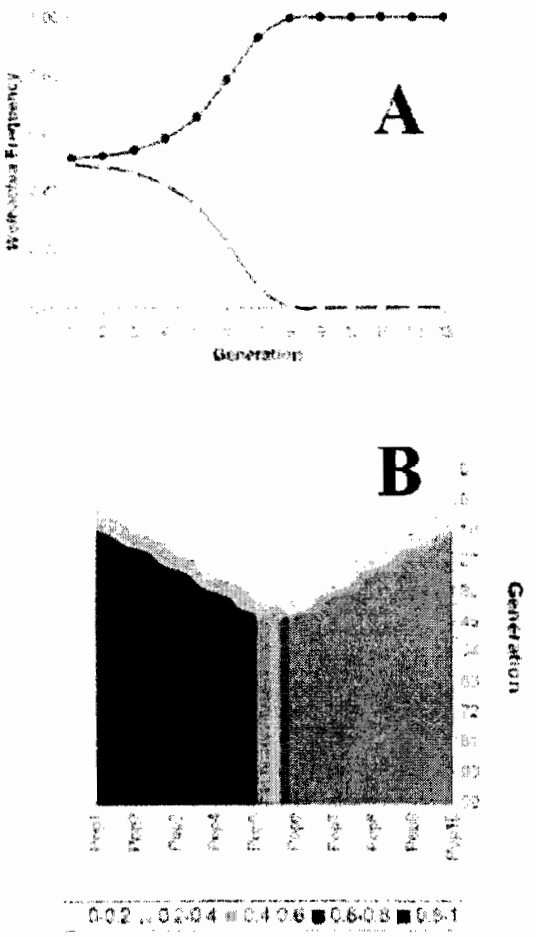

Figure 7. Stability of 2 mutually incompatible *Wolbachia* strains in a population. Both strains have no fitness effects ($F = 1.0$), cause 100% CI ($H = 0$), and are transmitted 100% ($\mu = 0$). A: Random mating model. Black circles denote strain A, white circles denote strain B. Strain A is initially slightly more frequent than strain B. Strain A rapidly eliminates strain B in 8 generations. B: metapopulation model. Parameters are the same as in 7A. Black denotes strain A, gray denotes strain B. Increasing darkness corresponds to increasing frequency. Both strains are introduced into opposite subpopulations at an initial frequency of 10% (at the subpopulation level). Both strains spread through migration until they come into contact. CI prevents the spread further spread, but the boundary zone between the strains is stable.

in natural populations for several species of *Drosophila* (Turelli and Hoffmann 1995, Hoffmann et al. 1998), and for *Culex pipiens* complex mosquitoes (Rasgon and Scott, In press).

Estimates of *Wolbachia* introduction thresholds, however, are affected by population subdivision, and simple models may not be adequate for determining this value. Metapopulation dynamics can be advantageous for disease control efforts. In a sub-divided population, the *Wolbachia* introduction threshold can be effectively reduced compared to that predicted by a random mating model, as infection can become established locally with a relatively small initial introduction and then spread into adjacent subpopulations by migration.

Metapopulation dynamics can also be disadvantageous. If the migration rate between subpopulations is too low, the spread of infection into adjacent subpopulations can be very slow or even completely stopped; infection may become fixed locally but may not spread to all epidemiologically significant individuals, or may take an unacceptably long time to spread. If the migration rate is too high, movement of uninfected individuals into the subpopulation can swamp out and eliminate infection. This critical migration rate (m_{Crit}) varies according to *Wolbachia* vertical transmission, CI and fitness effects, as well as on the magnitude of the introduction. To counteract the "swamp-out" effect, it may be necessary for the initial introduction to be many times larger than that predicted by the random mating model, thus making disease control efforts more difficult.

Population subdivision can explain apparent co-existence of multiple mutually incompatible strains in a population, which is impossible according to the assumptions of the random mating model, and may help to explain field observations of bi-directional incompatibility as seen in European *Culex pipiens* complex mosquitoes (Laven 1957, Guillemaud et al. 1997). This information must be taken into account in applied *Wolbachia*-based disease control strategies, as the stable boundary zone between 2 incompatible cytotypes can act as a barrier to gene flow and keep transgenes from spreading throughout the entire population.

The results of this study indicate that a thorough understanding of vector metapopulation dynamics is critical for designing *Wolbachia*-based strategies for vector-borne disease control. Critical areas of future study include quantifying migration and gene flow in natural vector populations, defining genetic and geographic boundaries of individual subpopulations, and determining at what geographic scale *Wolbachia* releases must be attempted. It is critical to remember that vector population subdivision is just one ecologically complex factor that will affect the success of *Wolbachia* introductions into vector populations to control disease. Other factors of importance include, but are not limited to, vector population regulation (Dobson et al 2002), vector population age structure (Rasgon et al 2001, Rasgon et al 2003), assortative mating, stability of transgene constructs in the field, and many others. Now that the molecular science underpinning transgenic disease control is becoming mature, the future success of these types of strategies will rely on a thorough understanding of vector insect ecology (Scott et al 2002). Combining molecular science with ecological studies has the potential to result in the development of novel, cost-effective, and efficient vector-borne disease control strategies for the twenty-first century.

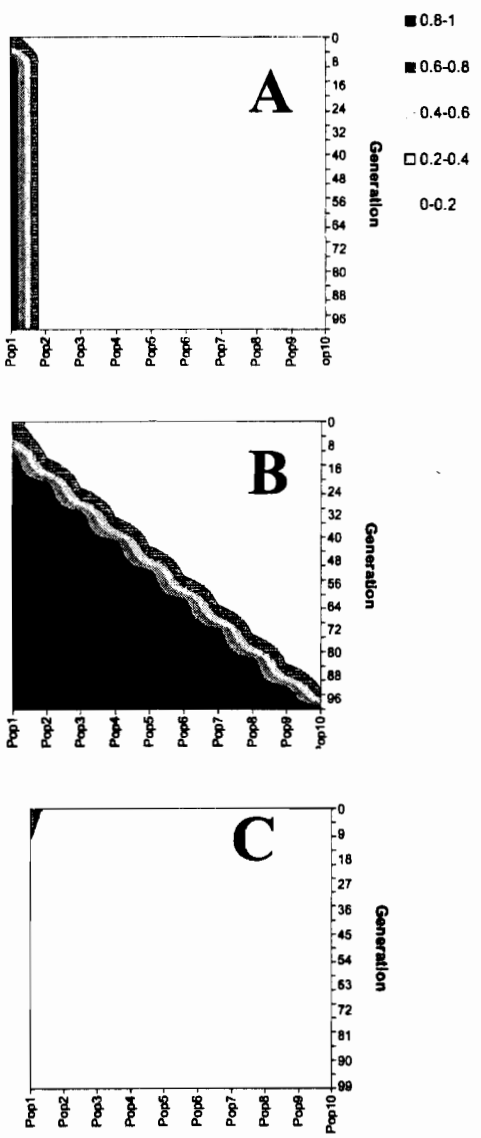

Figure 5. Effect of migration rate on *Wolbachia* metapopulation spread. μ, H and F are as stated in Figure 3. Increasing dark color denotes increasing *Wolbachia* frequency. All initial introductions were made by introducing infected individuals into subpopulation at 30% (relative to the subpopulation). 1A: low migration, m = 0.001. Infection becomes established in the subpopulation, but migration is too low to spread into adjacent areas. B: Moderate migration, m = 0.1. Infection becomes established in subpopulation 1 and spreads in a wave of advance into adjacent areas, ultimately spreading throughout the entire population. C. High migration, m = 0.22. The migration rate has passed a critical threshold and infection is swamped out by uninfected individuals migrating into the subpopulation and eliminated.

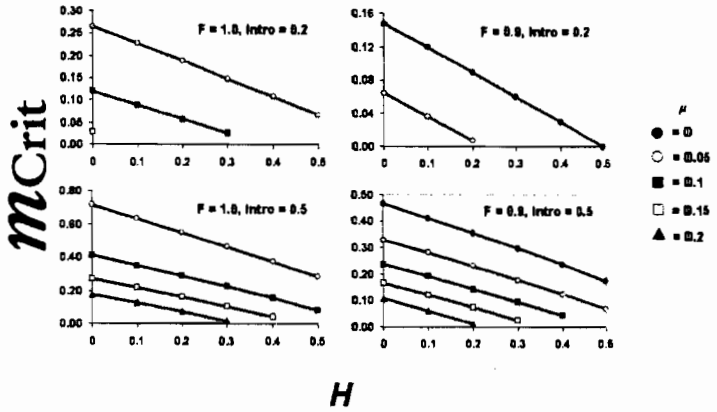

Figure 6. Critical migration rate (m_{Crit}) above which infection is swamped out and eliminated. m_{Crit} varies according to *Wolbachia* parameters μ, H and F, and with the magnitude of the initial introduction. Figure 6 shows how m_{Crit} changes for parameter values in the range of: $0 \leq \mu \leq 0.2$, $0 \leq H \leq 0.5$, and $0.9 \leq F \leq 1.0$ (in 0.1 increments), and with initial introductions at 20% and 50% at the subpopulation level. Missing data points denote locations in the parameter space where infection will always be lost. In the condition where $F = 1.0$ and $\mu = 0$ for any value $H < 1.0$ over the introduction magnitudes simulated here, there is no m_{Crit} value < 1.

Wolbachia *strain co-existence:*

Current theory states that in a randomly mating population, 2 mutually incompatible *Wolbachia* strains cannot co-exist – one strain will always rapidly out-compete and eliminate the other (Hoffmann and Turelli 1997) (Figure 7A). However, multiple bi-directionally incompatible crossing types have been observed in natural populations of European *Culex pipiens* complex mosquitoes on a small geographic scale (Laven 1957, Guillemaud et al. 1997). Population subdivision can help to explain this apparent paradox. On a local scale it is true that 2 mutually incompatible strains cannot co-exist; however, they can potentially co-exist in adjacent subpopulations. Figure 7B shows the boundary zone where 2 mutually incompatible *Wolbachia* invasion fronts meet. This boundary zone between the 2 strains is stable over time, and is artificially maintained by migration of infected individuals into the zone from either invasion front. CI will prevent the spread of different strains into areas infected with the opposite cytotype, but samples drawn from around the zone of contact will contain individuals infected with either the A or B strain. This gives the appearance that 2 incompatible strains co-exist in the same area.

DISCUSSION

Population subdivision does not affect the frequency that infection will reach in the population if *Wolbachia* successfully invades. If one's study goal is merely to determine this stable equilibrium frequency for a particular *Wolbachia* strain, simple models are adequate to the task. This approach has been validated

$$\Pi X^i_{B,t+1} = [(F_B X^i_{B,t+0.5}(1-\mu_B) + F_{AB} X^i_{AB,t+0.5}\mu_{AB,B}]$$
$$[X^i_{AB,t+0.5} H_{B,AB} + X^i_{B,t+0.5} + X^i_{A,t+0.5} H_{B,A} + X^i_{W,t+0.5}]$$
(10)

and

$$\Pi X^i_{W,t+1} = [(X^i_{W,t+0.5} + F_A X^i_{A,t+0.5}\mu_A + F_B X^i_{B,t+0.5}\mu_B) + F_{AB} X^i_{AB,t+0.5}\mu_{AB,W}] +$$
$$F_{AB} X^i_{AB,t+0.5}\mu_{AB,W}]$$
$$* \ (X^i_{AB,t+0.5} H_{W,AB} + X^i_{A,t+0.5} H_{W,A} + X^i_{B,t+0.5} H_{W,B} + X^i_{W,t+0.5})$$
. (11)

Π denotes the sum of the terms on the right-hand side of equations 8 – 11.

RESULTS

Stable equilibrium levels:

In all cases, population subdivision has no effect on *Wolbachia* stable equilibrium levels. Simple models thus are adequate for predicting the frequency that infection will ultimately reach in the population following a successful invasion.

Wolbachia *introduction threshold levels:*

Depending on the values for vertical transmission, CI and fitness effects, *Wolbachia* infection must surpass a certain threshold level for infection to successfully invade the population. If the initial introduction is below this level, infection will be lost from the population (Figure 3). Population subdivision may allow introductions below the threshold level to be successful because introductions that are below threshold as calculated for the entire population may be (locally) above threshold in an individual subpopulation (Figure 4). If the initial introduction is concentrated in a single subpopulation, infection can become established locally, and then spread into adjacent subpopulations by migration of infected individuals. This effectively lowers the introduction threshold for the entire population (Figure 4B).

This effect is highly dependent on the effective migration rate between subpopulations. If the migration rate is too low, infection may become locally established in an individual subpopulation, but will not spread (Figure 5A). If the migration rate is increased, infection can disperse throughout the entire population (Figure 5B). However, if migration becomes too great and passes a critical value, rather then spreading, infection will be swamped out by uninfected mosquitoes migrating into the subpopulation and be lost (Figure 5C). This critical value for migration (m_{Crit}) depends both on *Wolbachia* parameters (μ, H and F) and on the magnitude of the initial introduction (Figure 6).

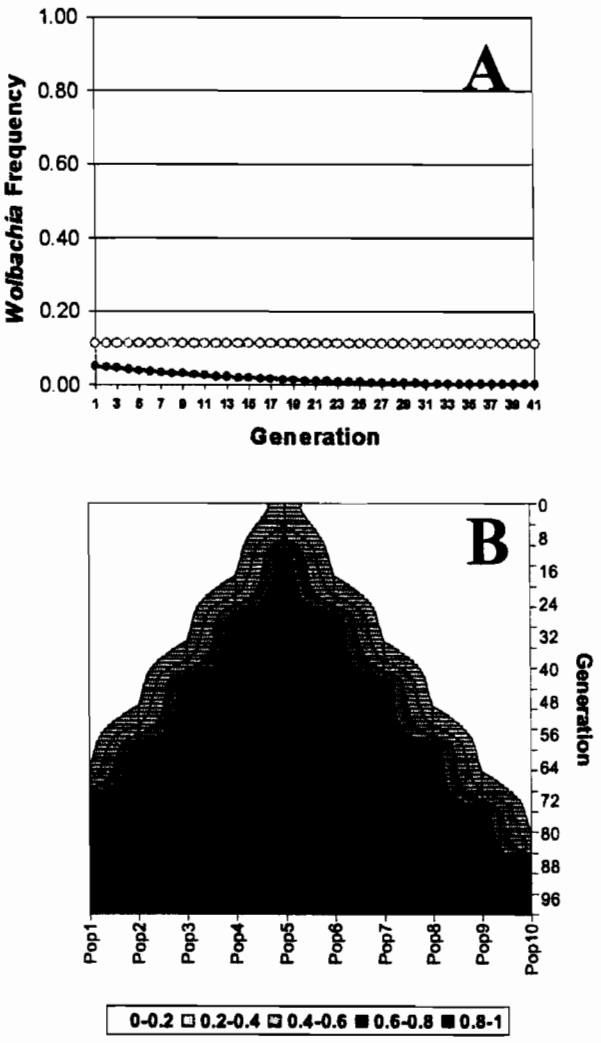

Figure 4. Comparison between random mating and metapopulation dynamics. *Wolbachia* parameters in A and B (μ, H and F) are as stated in Figure 3. A: Random mating dynamics. White circles denote introduction threshold 11.45%. Black circles denote dynamics when infected individuals are released below this threshold point at 5%, resulting in infection loss. B: Metapopulation dynamics. Increasing dark color denotes increasing *Wolbachia* frequency. A release magnitude equivalent to 5% of the total population (similar to 4A) is conducted, but release is concentrated in subpopulation 5. While a release at this level is not sufficient for infection invasion as calculated across the total population, it exceeds the introduction threshold for an individual subpopulation. Infection reaches stable equilibrium of 99.37% in subpopulation 5, then spreads by migration to adjacent subpopulations in a "wave of advance" ($m = 0.05$).

P_S is globally stable, and represents the equilibrium frequency infection will reach in the population following a successful invasion. P_U is unstable, and represents the frequency infection must exceed for invasion to occur (introduction threshold). If the initial introduction is less than P_U, infection will be lost (Figure 3).

Metapopulation model:

The model can simulate the dynamics of up to 2 different *Wolbachia* strains (A and B) and is based on theory developed by Hoffmann and Turelli (1997). An individual mosquito can be uninfected (W), infected with a single *Wolbachia* strain (A or B), or be infected with both *Wolbachia* strains (AB), for a total of 4 distinct cytotypes. For *Wolbachia* infection parameters, the terminology is similar to the previous section, with some modifications. Table 1 shows the parameters and simplifying assumptions used in the model. The metapopulation is set up as a linear array of subpopulations. I assumed that in a given generation, migration can take place in nearest-neighbor fashion (i.e., individuals can move into immediately adjacent subpopulations, but not further). The model assumes random mating within a single subpopulation. $N^i_{Tot,t}$ represents the total number of mosquitoes of all cytotypes at generation t in subpopulation *i*. The frequency of each cytotype *C* (where *C* = A, B, AB, or W) in subpopulation *i* at generation t is denoted as $X^i_{C,t}$. The number of mosquitoes in subpopulation *i* of cytotype *C* at generation t is denoted $N^i_{C,t}$, and is calculated as

$$N^i_{C,t} = X^i_{C,t} N^i_{Tot,t} \qquad (4)$$

It is now necessary to take into account migration into and out of each subpopulation. Let *m* equal the rate of migration where $m = 0$ equals no migration, and $m = 1$ equals complete random mating (panmixia). Let M equal the net number of migrants of cytotype *C* into subpopulation *i* at generation t+0.5 such that

$$M^i_{C,t+0.5} = m\frac{(N^{i-1}_{C,t} + N^{i+1}_{C,t})}{2} - m(N^i_{C,t}) \qquad (5)$$

By combining equations 4 and 5, the number of individuals of cytotype *C* in subpopulation *i* at generation t+0.5 can be calculated as

$$N^i_{C,t+0.5} = N^i_{C,t} + M^i_{C,t+0.5} \quad (6)$$

The total number of mosquitoes of all cytotypes *C* in subpopulation *i* ($N^i_{Tot,t+0.5}$) can be found by simply summing the numbers of mosquitoes of each cytotype. The frequency of each cytotype *C* at generation t+0.5 is then calculated as

$$X^i_{C,t+0.5} = \frac{N^i_{C,t+0.5}}{N^i_{Tot,t+0.5}} \quad (7)$$

Finally, the frequencies of each cytotype due to *Wolbachia* dynamics in subpopulation *i* at generation t+1 are calculated as

$$\Pi X^i_{AB,t+1} = F_{AB} X^i_{AB,t+0.5}(1-\mu_{AB}) \quad (8)$$

$$\Pi X^i_{A,t+1} = [(F_A X^i_{A,t+0.5}(1-\mu_A) + F_{AB} X^i_{AB,t+0.5}\mu_{AB,A}]$$
$$[X^i_{A,t+0.5} H_{A,AB} + X^i_{A,t+0.5} + X^i_{B,t+0.5} H_{A,B} + X^i_{W,t+0.5}] \quad (9)$$

Table 1. Parameters for the metapopulation *Wolbachia* dynamics model. If each infection type behaves independently of the other, then the model can be simplified accordingly: $F_{AB} = F_A F_B$; $\mu_{AB,A} = \mu_B(1-\mu_A)$, $\mu_{AB,B} = \mu_A(1-\mu_B)$, and $\mu_{AB,W} = \mu_A\mu_B$; $H_{A,AB} = H_{A,B} = H_{W,B}$, $H_{B,AB} = H_{B,A} = H_{W,A}$, and $H_{W,AB} = H_{W,A}H_{W,B}$.

Parameter	Definition
$\mu_{AB,A}$	Frequency of A ova produced from AB females
$\mu_{AB,B}$	Frequency of B ova produced from AB females
$\mu_{AB,W}$	Frequency of W ova produced from AB females
μ_A	Frequency of W ova produced from A females
μ_B	Frequency of W ova produced from B females
$H_{A,AB}$	Hatch rate of embryos produced from A ova fertilized by AB sperm
$H_{B,AB}$	Hatch rate of embryos produced from B ova fertilized by AB sperm
$H_{A,B}$	Hatch rate of embryos produced from A ova fertilized by B sperm
$H_{B,A}$	Hatch rate of embryos produced from B ova fertilized by A sperm
$H_{W,A}$	Hatch rate of embryos produced from W ova fertilized by A sperm
$H_{W,B}$	Hatch rate of embryos produced from W ova fertilized by B sperm
$H_{W,AB}$	Hatch rate of embryos produced from W ova fertilized by AB sperm
F_A	Fecundity of A females relative to W females
F_B	Fecundity of B females relative to W females
F_{AB}	Fecundity of AB females relative to W females

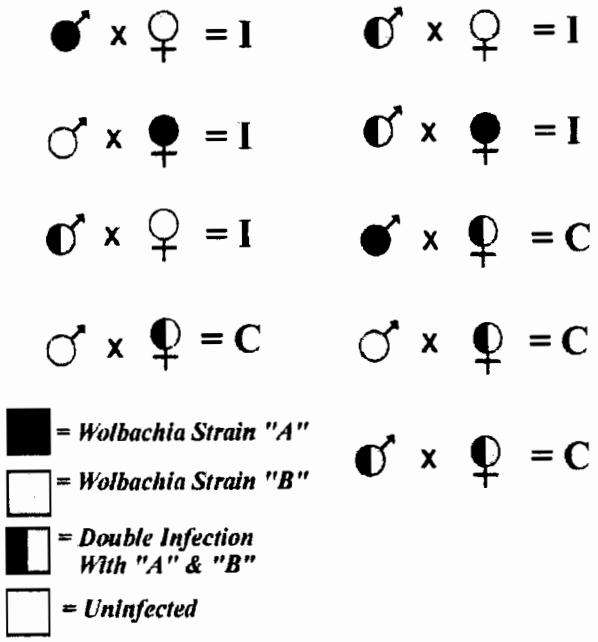

Figure 2. Mating outcomes from crosses in which mosquitoes are infected with 2 incompatible *Wolbachia* strains (C = compatible cross, I = incompatible cross). Offspring cytotype from compatible crosses are similar to the maternal cytotype.

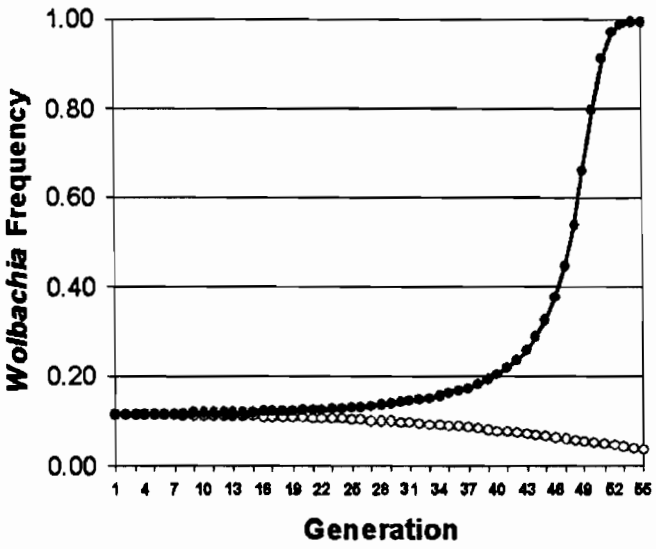

Figure 3. The predicted dynamics of *Wolbachia* spread according to the random mating (Turelli-Hoffmann) model where $\mu = 0.05$, $H = 0.1$ and $F = 0.95$. Black circles denote dynamics where infected individuals are introduced just above the predicted threshold point of 11.45%. Infection is predicted to reach a stable equilibrium of 99.37%. White circles denote dynamics where infected individuals are introduced just below this threshold point, resulting in loss of the infection in the population.

introduced infections may behave. Current available models of *Wolbachia* dynamics in natural populations predict 3 kinds of information that are critical for using *Wolbachia* in an applied manner to control disease: 1) the unstable equilibrium; i.e., the introduction threshold of infected individuals that must be released for infection to become established in the population, 2) the stable equilibrium frequency that infection will ultimately reach, and 3) how long (in generations) this invasion will take from a given introduction level (Turelli and Hoffmann 1999) (Figure 3).

For ease of calculation and analysis, these models make numerous simplifying assumptions that may not be ecologically realistic. One important assumption is that the insect population is panmictic, or randomly mating (Turelli and Hoffmann 1999). While this assumption may reasonably hold in natural populations on a local spatial scale, it is unlikely to hold true over a larger geographic area. Over a large geographic area, natural vector populations can be more realistically described as metapopulations, or a series of separate subpopulations connected by varying degrees of migration (Urbanelli et al. 1995, 1997). To assess the impact of vector population subdivision on *Wolbachia* population dynamics, a spatially-explicit metapopulation model of *Wolbachia* spread was developed. I compared the predictions of this model to those of the current random mating model, and discussed the significance of population subdivision for applied *Wolbachia*-based disease control strategies. Vector population subdivision can make applied *Wolbachia* introductions into vector populations easier or more difficult, depending on conditions.

PROCEDURES

Random mating model:

The dynamics of *Wolbachia* in a randomly mating insect population have been modeled extensively (Turelli and Hoffmann 1999). Infection parameters are defined as: μ = % uninfected offspring from an infected female ($\mu = 0$ if transmission is 100%), H = relative hatch rate of an incompatible vs. compatible cross ($H = 0$ if CI is 100%), F = relative fecundity of an infected vs. uninfected female ($F = 1$ if there is no effect on fecundity), $s_F = (1-F)$, and $s_H = (1-H)$. Assuming discrete generations, the frequency of infected adults (p) at generation t+1 has been shown to be

$$p_{t+1} = \frac{p_t(1-\mu)F}{1 - s_F p_t - s_H p_t(1-p_t) - \mu s_H p_t^2 F} \quad (1)$$

Equation 1 predicts two equilibrium values:

$$p_S = \frac{s_F + s_H + \sqrt{(s_F + s_H)^2 - 4(s_F + \mu F)s_H(1-\mu F)}}{2s_H(1-\mu F)} \quad (2)$$

and

$$p_U = \frac{s_F + s_H - \sqrt{(s_F + s_H)^2 - 4(s_F + \mu F)s_H(1-\mu F)}}{2s_H(1-\mu F)} \quad (3)$$

Population Subdivision Can Help or Hinder *Wolbachia* Introductions Into Vector Populations

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ABSTRACT: *Wolbachia* spp. are maternally inherited endosymbionts associated with cytoplasmic incompatibility (CI) i.e., reduced egg hatch when an infected male mates with an uninfected female. *Wolbachia*-induced CI is of interest as a potential mechanism to drive transgenic traits into vector populations to control vector-borne diseases. For simplicity, current models of *Wolbachia* spread assume a randomly mating, panmictic vector population. Natural vector populations can be more realistically described by metapopulation dynamics; i.e., multiple subpopulations connected by some degree of migration. A spatially-explicit metapopulation model of *Wolbachia* spread was developed to assess the impact of population subdivision on *Wolbachia* dynamics. Regardless of the type of model used, *Wolbachia* frequency must exceed a threshold point for invasion to take place. Introduction levels that would be insufficient for *Wolbachia* invasion in a randomly mating population can be sufficient for invasion in a sub-divided population because infection can become fixed locally, then spread to adjacent areas by migration. However, this effect is highly dependent on the migration rate between subpopulations. If migration is too low, infection may become fixed in a local subpopulation but will not spread into adjacent areas. If migration is too high, infection can be swamped out by uninfected individuals entering the area. Theory suggests that in a randomly mating population, one *Wolbachia* strain will always out-compete and eliminate the other. However, at the boundary zone of 2 *Wolbachia* invasion fronts, population subdivision can artificially maintain 2 incompatible strains in a subpopulation by migrants entering the subpopulation from each invasion front. These results indicate that an understanding of metapopulation dynamics is critical for designing *Wolbachia*-based strategies for vector-borne disease control.

BACKGROUND AND OBJECTIVES

Wolbachia spp. are maternally inherited bacterial endosymbionts that infect a wide variety of invertebrate taxa. *Wolbachia* infection is associated with a variety of host reproductive alterations including parthenogenesis, male killing, feminization of males, and cytoplasmic incompatibility (CI) (Stouthamer et al. 1999). CI completely or partially sterilizes matings between infected males and uninfected females. Matings between infected females and infected or uninfected males are fertile (Figure 1). Infected females, therefore, have a reproductive advantage, allowing *Wolbachia* to spread rapidly through host populations (Turelli and Hoffmann 1999). Crossing patterns can become more complex with multiple *Wolbachia* strains (Figure 2).

The spread of *Wolbachia* has applied interest for the control of vector-borne diseases and pest insect populations (Pettigrew and O'Neill 1997). The evolution of insecticide resistance in important vector species is becoming an increasing problem (Hemingway and Ranson 2000), and there are no vaccines available for important vector-borne diseases such as malaria and dengue (Beaty 2000). To address these concerns, research is now underway to create genetically modified vector arthropods that are unable to transmit pathogens (Pettigrew and O'Neill 1997). However, there is as yet no feasible method to spread or "drive" engineered genetic traits into vector populations to a high enough frequency to interrupt pathogen transmission cycles. Because of the ability for *Wolbachia*

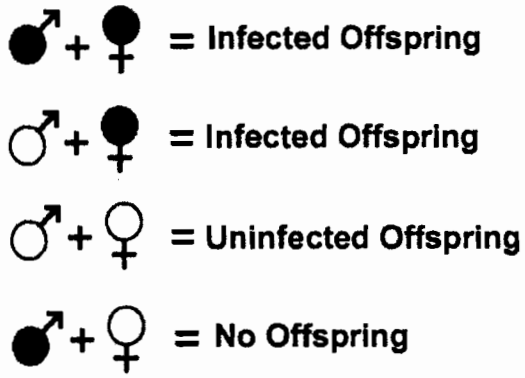

Figure 1. Mating outcomes from all 4 possible crosses between mosquitoes infected with a single strain of *Wolbachia*. Black = infected, white = uninfected.

infection to spread rapidly through populations, *Wolbachia* may be useful as a mechanism to drive introduced transgenic traits into vector populations to control disease (Turelli and Hoffmann 1999).

Before *Wolbachia* can be utilized in any vector-borne disease control strategy, it is essential to understand the dynamics of infection in natural vector populations in order to predict how

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A negative EIA result for SGA does not necessarily reflect a lack of *Cx. tarsalis* exposure. Under experimental conditions, a minimum threshold of approximately 200 bites was found necessary for seroconversion. Sentinel chickens that were sampled in October were in all likelihood subjected to at least some degree of *Cx. tarsalis* exposure during the season. It is possible that antibody levels waned by the end of the season among chickens that seroconverted around the time of peak mosquito abundance. Other possible reasons for the lack of antibodies to *Cx. tarsalis* SGA among some sentinels include diversion of mosquitoes by competing attractants in the vicinity, such as mosquito traps, the presence of large populations of other domestic animals, or placement of the coop in a manner that may partially block mosquito access, such as placement up against a wall. All of these factors were found to be present to varying degrees at many of the study flock sites, and warrant further analysis.

Sera from *Cx. tarsalis*-exposed chickens cross-reacted with *Cx. pipiens* SGA, although the reactivity was less than that observed with homologous SGA. Sera from the *Cx. pipiens*-exposed chicken was more cross-reactive with *Cx. tarsalis* SGA than sera from the *Ae. aegypti*-exposed chicken. Sera collected from the former chicken $\geq$11 weeks after the initial mosquito exposure exceeded the positive cutoff value when tested against *Cx. tarsalis* SGA, while all sera from the latter chicken tested negative. The significance of these findings when testing sentinel chickens that are presumably exposed to a variety of mosquito species remains to be determined. In the present study, serologic evidence of exposure to *Cx. tarsalis* SGA among sentinel chickens from Coachella Valley MVCD served as an indicator of risk for SLE virus exposure. With the anticipated establishment of West Nile virus on the West Coast, it is important that arboviral surveillance be operated at high levels of sensitivity. This new test provides a tool for evaluating the sensitivity of flock sites for detection of circulating arboviruses and hopefully will lead to identification of a standardized set of criteria that maximize a flock's mosquito exposure. Ideally, a recombinant salivary gland protein that is specific for *Cx. tarsalis* could be developed to ensure a readily available supply of antigen for the EIA. To provide a highly sensitive surveillance system using sentinel chickens the goal should be to provide maximum exposure of sentinel chickens to attract potential arboviral vectors.

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Twenty sites (53%) had an electric light source within 50 ft. of the coop. This could be a potentially competing mosquito attractant, although most of these light sources were not regularly used. Only 20 of 38 (53%) flocks had mosquito traps within 100 ft. of the coop. This precluded attempts to correlate mosquito abundance at the flock sites with seroconversion to SGA or arboviruses at these sites. Accessibility of the interior nest and roosting area of the coop to mosquitoes, defined as the approximate percentage of the perimeter without barriers to mosquito entrance (i.e. a solid wall) ranged from 5-100% (mean 65%). For 12 of 38 (32%) coops, only ≤50% of the perimeter of the nesting and roosting area was mosquito-accessible. A similar estimate was made of the accessibility of CO_2 or NJ light traps to mosquitoes at sites where traps were located within 50 ft. of the coop. Of the 18 sites with a trap present, 8 (44%) traps were accessible only through ≤50% of their perimeter, usually as a result of their proximity to a wall blocking the radius of light visibility or CO_2 diffusion.

The EIA was used to measure the antibody response to *Cx. tarsalis* SGA among sentinel chickens in order to estimate their relative exposure to *Cx. tarsalis*. Ninety eight percent of sentinel chickens in Sacramento-Yolo MVCD were seronegative for *Cx. tarsalis* SGA in April 2002, compared to 45% in October 2002. The October 2002 seroprevalence varied widely among flocks in Marin-Sonoma, Sacramento-Yolo and Sutter-Yuba MVCDs (Table 1). The correlation between year-end seroprevalence to *Cx. tarsalis* SGA and some of the flock site characteristics was examined. There was essentially no correlation between the percentage of the coop perimeter that afforded access to coop interior and *Cx. tarsalis* SGA seroprevalence. It was not possible to evaluate the effect of mosquito abundance and trap distance at each MVCD due to the lack of traps at some flock sites. At the one MVCD where data were available, there was a small positive correlation between *Cx. tarsalis* SGA seroprevalence and distance from the flock site to the nearest mosquito trap (correlation coefficient = 0.535; *p*-value = 0.172), indicating that sentinel chickens with traps in the immediate vicinity received less mosquito exposure. The correlation between seroconversion to *Cx. tarsalis* SGA and mosquito abundance at this same MVCD was negligible. Variation in trap distance from the flock sites and in the type of traps used by MVCDs makes uniform interpretation of the abundance data with respect to potential flock exposure difficult.

A high number of sentinel chickens in Coachella Valley MVCD seroconverted to SLE during 2001, which made it possible to evaluate the association between SLE and *Cx. tarsalis* SGA seroconversions. Serum specimens were available from 47 of the 51 (92%) sentinel chickens from this district that seroconverted to SLE in 2001. Of the 71 chickens that were seropositive for *Cx. tarsalis* SGA, 43 (61%) were SLE-positive, compared to 4 of 68 (6%) that were seronegative for *Cx. tarsalis* SGA. Chickens that were seropositive for *Cx. tarsalis* SGA were significantly more likely to be SLE-positive compared to chickens that were seronegative for *Cx. tarsalis* SGA (Table 2).

Table 1. Antibody Seroprevalence to *Cx. tarsalis* Salivary Gland Antigen at Sentinel Flock Study Sites, 2002.

District	**# Positive/# Tested (%)**	
	April	**October**
Sacramento-Yolo MVCD	2/100 (2)	48/88 (55)
Sutter-Yuba MVCD	N/A*	29/70 (41)
Marin-Sonoma MVCD	N/A*	14/63 (22)

*Specimens were not available for testing

Table 2: Association between Antibody to St. Louis Encephalitis Virus and *Culex tarsalis* Salivary Gland Antigen among Sentinel Chickens in Coachella Valley MVCD, 2001.

Status	**SGA+**	**SGA-**	**Total**
SLE+	43	4	47
SLE-	28	64	92
Total	71	68	139

Odds Ratio=24.6; 95% Confidence Interval 7.5, 89.7; *p*-value<0.0001

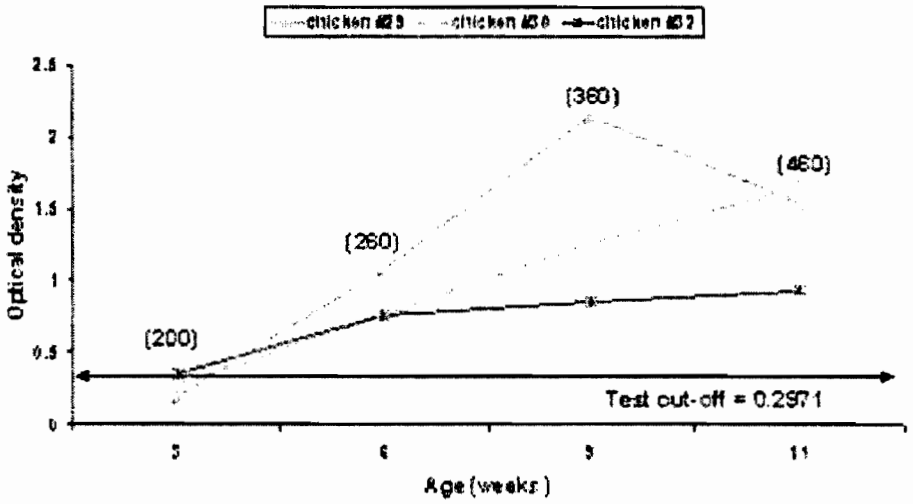
Note: Of the 47 specimens from SLE positive chickens, 46 were obtained within 14 days of the SLE seroconversion date, and 1 was obtained 42 days prior to the SLE seroconversion date.

Serum specimens from laboratory-housed chickens with no mosquito exposure and those with known exposure to only *Cx. tarsalis* were used to validate the assay. Serum specimens were also obtained from two chickens exposed exclusively to either *Culex pipiens* or *Aedes aegypti*. These sera were used to evaluate cross-reactivity with *Cx. tarsalis* SGA. Sera from chickens exposed to *Cx. tarsalis* only were also tested against *Cx. pipiens* SGA extract to evaluate the potential for cross-reactivity.

RESULTS AND DISCUSSION

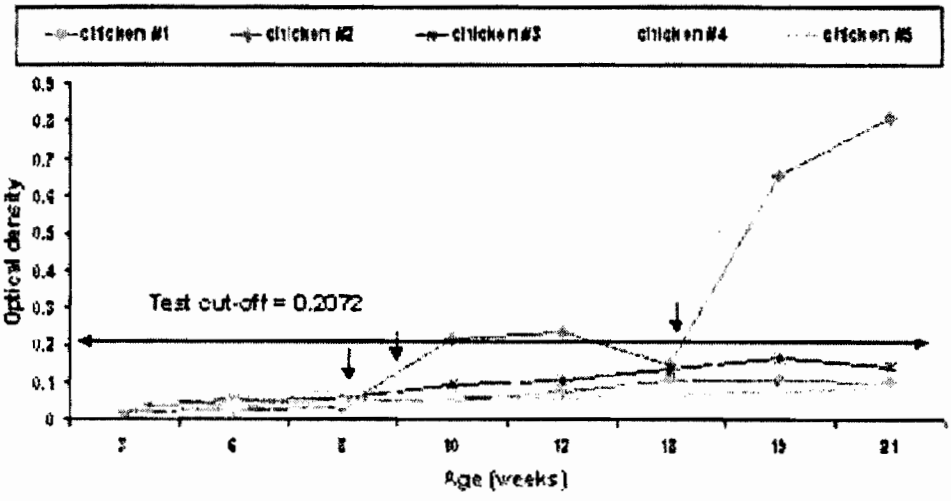
Three *Cx. tarsalis*-exposed chickens developed a strong antibody response to SGA by 6 weeks after being fed on by an average of 260 *Cx. tarsalis* each. (Figure 1). In a separate feeding trial, two chickens seroconverted after being fed on by approximately 180 *Cx. tarsalis* each, while three unexposed chickens did not (Figure 2).

On-site surveys were conducted at all flock sites in Coachella Valley (9), Marin-Sonoma (7), Sacramento Yolo (9), Sutter-Yuba (8), and Shasta (5) MVCDs. The mean number of chickens per site was 9.7. Approximately half of the flocks were located in agricultural habitat (20), which consisted primarily of row crops, rice, and pasture. Other habitat types included suburban (7), riparian (3), marshland (2), and others (6), such as a seasonal wetland and desert. Foliage was present at almost all sites, with trees at 30 (79%) of sites. Trees, or other foliage, provided shade to 26 (68%) of sites. Permanent water sources, other than the chickens' water source, were uncommon. Examples of permanent water sources included ponds (4) and cattle troughs (2). One or more non-permanent water sources, such as ditches, containers, and flooded agricultural fields were observed at nearly every site. Domestic animals, such as cows, horses, sheep, pigs, dogs, cats, and poultry, other than sentinel chickens, were present within 50 ft. of the sentinel flocks at 18 (47%) of the sites.



Note: Chickens were exposed to Cx. *tarsalis* from 3 days of age to 10 weeks of age; numbers in parentheses indicate average cumulative number of bites per chicken

Figure 1. Chicken Antibody Response to *Culex tarsalis* Salivary Gland Antigen.



Note: Arrows indicate time of Cx. *tarsalis* exposure; #2 received total of 185 bites and #4 175 bites; #1-3 were not exposed to Cx. *tarsalis*.

Figure 2. Chicken Antibody Response to *Culex tarsalis* Salivary Gland Antigen.

Sentinel Chicken Immune Response to *Culex tarsalis* Salivary Gland Antigen: A Marker for Risk of Arboviral Infection

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ABSTRACT: Approximately 2,000 sentinel chickens are deployed annually throughout California by Mosquito and Vector Control Districts (MVCD) for surveillance of western equine encephalomyelitis and St. Louis encephalitis (SLE) virus activity. The sensitivity of this surveillance system and its public health value could be increased by development of objective criteria to assist in positioning flocks where exposure to mosquito vectors is greatest. An enzyme immunoassay (EIA) to detect antibodies to *Culex tarsalis* salivary gland antigen (SGA) in chickens was used to measure mosquito exposure among flocks in northern and southern California. The seroprevalence to *Cx. tarsalis* SGA was approximately twice as high in flocks in the Sacramento Valley Region as in flocks in the Coastal Region. The seroprevalence also varied among flocks within MVCDs. There was a positive correlation between seroprevalence to *Cx. tarsalis* SGA and mosquito trap distance from the coops (correlation coefficient = 0.535; *p*-value = 0.172), indicating that sentinel chickens with traps in the immediate vicinity received less mosquito exposure. Sentinel chickens from southern California that were seropositive for *Cx. tarsalis* SGA were significantly more likely to be SLE-positive compared to chickens that were seronegative for *Cx. tarsalis* SGA. This new test can be used to evaluate the sensitivity of flock sites for detection of arbovirus activity and to identify factors that maximize a flock's exposure to mosquitoes.

INTRODUCTION

In California, sentinel chickens are an important part of the surveillance system to detect western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) virus activity. These viruses are transmitted mainly through the bite of infected *Culex tarsalis*, although other mosquito species play a secondary role (Reeves and Milby 1990).

Previous studies have used similar methodologies to study the human antibody response to the SGA of various biting arthropods. Persons with hypersensitivity to mosquito bites have been shown to have elevated IgE and IgG antibodies, although evidence of an antibody response was nearly ubiquitous in those over 2 years of age (Konishi 1990, Peng et al. 1995). A dose-response relationship was found between the tick engorgement index, a surrogate for tick saliva dose, and anti-tick saliva antibody levels among persons bitten by ticks in a highly Lyme disease-endemic area of New York (Schwartz et al. 1993). Antibody levels to *Ixodes pacificus* were elevated among persons residing in Lyme disease-endemic areas in northern California and were correlated with seropositivity to *Borrelia burgdorferi* (Lane et al. 1999). A recombinant salivary protein from the sand fly, *Lutzomyia longipalpis*, was developed to conduct serologic testing to identity populations at risk for leishmaniasis (Barral et al. 2000).

The objectives of this study were to: 1) measure the antibody response of sentinel chickens to *Cx. tarsalis* salivary gland antigen (SGA); 2) determine if SGA exposure is associated with risk of arboviral infection, and; 3) identify physical characteristics at flock sites that contribute to SGA exposure. It was equally important to identify sites with poor SGA exposure, as such sites are likely to be less sensitive for detection of arbovirus activity. This information could be useful in allocating resources to maintain and improve the public health value of sentinel chicken flocks. Evidence of low SGA exposure could demonstrate an effective mosquito control program.

PROCEDURES

Study sites were selected in northern and southern California. Blood samples were collected biweekly from flocks in Sacramento-Yolo Mosquito and Vector Control District (MVCD) in 2001 and 2002 and from selected Coachella Valley MVCD flocks in 2001. End-of-the-season specimens were collected from flocks in Sutter-Yuba MVCD in 2001 and 2002 and Marin-Sonoma MVCD in 2002. These sentinel flock sites were visited at least once to complete a questionnaire on environmental characteristics, mosquito abundance, and the history of flock seroconversions to WEE and SLE viruses.

Serum specimens were tested by an indirect enzyme immunoassay (EIA) in 96-well plates coated with *Cx. tarsalis* SGA extract. Wells were blocked with 2% casein in phosphate buffered saline with 0.05% Tween 20 (PBS-T). Sera were diluted in PBS-T with 0.5% bovine albumin (BA). Biotinylated goat anti-chicken IgG (heavy and light chain) conjugate diluted in PBS-T with 0.1% BA was used with a peroxidase-labeled avidin-biotin complex (Vector Laboratories, Burlingame, CA) to amplify antibody detection. After a 20 min incubation with the ABTS substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD), the plates were read at 405 nm. Specimens were tested in triplicate, with two wells coated with SGA extract and a third SGA-free well. The recorded value for each specimen was the average of the optical densities for the two SGA-containing wells, minus the value of the SGA-free well. Specimens were considered positive when they exceeded the mean plus 3 standard deviations of the negative control serum wells on the plate.

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