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William C. Reeves New Investigator Award Competition - 2010

William C. Reeves New Investigator Award Competition – 2008

Symposium: Epidemiology and Control of West Nile Virus

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Surveillance

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Contributions of the University of California Mosquito Research Program to Problems in Mosquito and Vector Control in California

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ABSTRACT: The University of California Mosquito Research Program was established in 1972 and operated continuously until 2008. It faces an uncertain future. A brief history of the program is discussed, with an emphasis on the relationship of program goals with problems faced by California mosquito abatement agencies.

INTRODUCTION

When I was asked to give this talk, I first thought I would present a few examples of research results from projects funded by the UC Mosquito Research Program (MRP) that were provided to California mosquito abatement agencies and were adopted into their operational programs. However, as I read through 25 years of annual reports, I found so many examples of research highly relevant to problems with the control of California mosquitoes and the diseases caused by them, that I realized that this approach would be impractical. Projects supported totally, or in part, by the MRP were conducted by excellent researchers, resulting in literally thousands of refereed and non-refereed publications. To mention just a few that happened to be my favorites would be unfair to the many mosquito researchers whose excellent work would be overlooked. Instead, I decided to explain what the MRP was and how and why it was created, to outline the subject areas used to group the various projects and to discuss some of the types of projects in each grouping.

ORIGIN OF THE UC MOSQUITO RESEARCH PROGRAM

The MRP was established in 1972, but various events before that time, such as funds provided in connection with the California Water Project (thereafter known as the "water funds") and the Marler bills (SB 310, SB 311, Frederick W. Marler, February 5, 1970) were critical to the eventual establishment of the program. The program was originally funded with an annual appropriation of $300,000 that came from a re-direction of the water funds plus additional new funds provided by the California legislature. Over the years, no increases in the funding level occurred, but permanent salary range adjustments provided to personnel (mainly students and graduate research assistants) increased the budget over time. At some time after the original creation of the program, the annual appropriation from the legislature was discontinued, and the funds for the MRP became part of the University budget.

ADMINISTRATION OF THE PROGRAM

The program was established as a special program in the UC Division of Agriculture and Natural Resources (DANR). Between 1972 and 2008, there were five directors having responsibility for operation of the program: Carl Mitchell, 1972-1976; Russell Fontaine, 1976-1986; Bruce Eldridge, 1986-2001; James Lyons, 2001-2002; and Gregory Lanzaro, 2002-2008, With the suspension of program activities in 2008, DANR announced that MRP functions would be continued at some level as part of a new strategic initiatives program.

PROGRAM OPERATION

Formal procedures were established for solicitation of research ideas and proposals and for judging the quality and relevance of research projects submitted for funding. Evaluation of proposals submitted by UC faculty members was based on relevance to perceived problems in control of mosquitoes and mosquitoborne diseases and on scientific merit. Evaluation of the proposals was conducted by three committees: (1) A technical committee comprised of UC faculty researchers, (2) A mosquito research committee of the Mosquito and Vector Control Association of California (MVCAC), and (3) A public advisory committee with broad representation of outside agencies such as the California Department of Fish and Game. The pubic advisory committee did not judge individual projects, but did provide oversight of policies and operation of the program. During the life of the program, research was supported only for projects related to mosquitoes, and not other vectors. Attempts over the years to broaden the program to include other vectors were never successful, mainly because of budgetary restrictions.

PROGRAM AREAS

Research proposals were grouped into four areas for convenience in evaluation and reporting. In many cases the group assignment were arbitrary. The areas were: (1) Chemical control, (2) Biological control, (3) Biology and ecology of mosquitoes, and (4) Mosquitoes and public health.

CHEMICAL CONTROL

Studies included in this category sometimes involved searches for entirely new products, but more often focused on improving formulations of existing products. Over the life of the program, a number of promising new products were synthesized and existing

products were tested for their suitability as mosquito larvicides or adulticides. Sadly, for a variety of reasons, most of which are very familiar to all who work in some area of mosquito control, only a few of these products ever made it into the arsenal of California mosquito abatement agencies. Also included in this program area were many projects on the development of new ground and aerial pesticide applications, methods and equipment. An important research area was the discovery of physiological and biochemical systems of insecticide resistance by mosquitoes. Results of these studies often led to improved methods of detecting and managing resistance. The MRP also provided direct support to California mosquito abatement agencies to conduct detailed studies of pre- and post-treatment with adulticides. Many of these studies evaluated not only elimination of adult mosquitoes, but also the effects of mosquito dispersal from untreated areas and the impact of viral infections on the biology of mosquito vectors. Some of these studies have become classics.

BIOLOGICAL CONTROL

Biological control in the broad sense includes the introduction of micro- and macro-organisms, or their products, which parasitize, infect, poison or consume mosquitoes. As well, biological control studies encompass environmental management methods that favor naturally-occurring mosquito predators and parasites (i.e., natural mortality factors). Many macro-organisms were studied over the years as potential mosquito control agents. Not all of these agents have been widely incorporated into MAD programs, but some are used extensively (i.e., larvivorous fish), and others such as tadpole shrimp in special situations.

The mosquitocidal bacteria deserve a special place among MRP-funded research conducted at UC. Studies ranged from ecological and toxicological studies in a variety of California habitats to development of new strains of bacteria, using methods of genetic recombination resulting in new assortments of toxic spores. It is disappointing that few, if any, recombinant products have made their way to formulators and distributors, but there is hope that eventually new products based on modified bacteria will become available. Novel bacterial products are beginning to appear such as Valent's Vectomax which consists of a mixture of spores and toxins of both *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus* (Bs), and Clarke's Natular, whose active ingredient is spinosad, a naturally-derived active ingredient produced during fermentation by the soil organism, *Saccharopolyspora spinosa*.

The program also supported many studies of naturally occurring biological control agents. Over the years studies in a variety of habitats such as irrigated pastures, rice fields, created wetlands and salt marshes were conducted. Some of these showed that proper timing of pesticide applications could avoid harm to natural mortality agents and result in improved mosquito control using lower amounts of pesticides. In some instances this has led to changes in water management practices.

Although some believe that practical results resulting from biological control research have been rare, many scientists believe that biological control holds great promise for mosquito control in California and elsewhere, but that significant investments in research and development are needed for biological control to realize this promise.

BIOLOGY AND ECOLOGY OF MOSQUITOES

During the life of the program, more projects were funded in the biology and ecology of mosquitoes than in any other. Most of this research was aimed directly at California problems. The following references to specific types of studies represent just a few of the many types of projects supported by the MRP. Research on *Aedes albopictus* was conducted shortly after several detections of invasions of larvae of this species in imported nursery products. Results from these studies showed that permanent establishment of the species in California was very unlikely. In addition, many excellent studies were completed dealing with coastal marsh ecology and the development of long-term marsh management methods to eliminate mosquito problems.

MOSQUITOES AND PUBLIC HEALTH

Because of the great importance of mosquitoborne arbovirus diseases in California, research was directed primarily at detection, prediction and prevention of these viruses. This research was conducted in California almost continuously since the pioneering discoveries of the late William C. Reeves in the years just before World War II, extending to the present. During the last years of the program much of this support was directed at West Nile virus.

RELATED COMPONENTS OF THE PROGRAM

A student minigrant component of the program was only part of the contribution of MRP funds in attracting and educating graduate students. Many students received support for small research projects, some leading to advanced degrees and eventual entry of these students into the public health related areas after graduation.

From the beginning of the program, strong emphasis was placed on leveraging of MRP dollars to obtain extramural funding. In most instances, faculty used preliminary research results as proof of concept to obtain funding from federal agencies. For many years the UC Office of the President required reports of federal dollars received for various emphasis areas, including mosquito research. For some reason, this requirement was dropped in the mid-1990s. I was sorry to see this happen, because for most of the life of the program, the ratio of federal to state dollars was about 10 to 1 or greater.

Although the role of MRP in mosquito-related research conducted by UC faculty has been emphasized, mention must be made of support provided by California mosquito abatement agencies, either by direct cash contributions or by contributions

consisting of labor support, transportation, laboratory space and other items. The contributions greatly increased the amount of research conducted and also maintained the relevance of the research. UC faculty members involved in mosquito research activity were active participants in many activities of the MVCAC, including participation in continuing education programs and serving on MVCAC committees.

SUMMARY

Although the MRP is now gone, it is useful to consider what it was and what it did in terms of future needs for mosquito-related research in California. In this regard, the following points are offered as lessons learned from the more than 30 years the MRP existed:

- Research and development programs are expensive.
- Research programs cannot be conducted without dedicated scientists with appropriate training.
- Expectations of a one-to-one connection between every research project and a practical end product are unrealistic.
- The pay-offs to end users of research are many and varied and often unexpected.
- Scientifically-based programs (e.g., mosquito abatement programs) are highly dependent upon strong research and development programs.
- Credibility is a key issue in the justification and documentation of mosquito abatement programs.
- Mosquito abatement programs in California and elsewhere *must* find a means of promoting an effective research component for their operations.

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Evaluating Trap Bias in Bloodmeal Identification Studies

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ABSTRACT: *Culex* mosquitoes are the primary vectors of West Nile Virus (WNV) in California. Because these mosquitoes transmit WNV to a wide variety of competent and noncompetent hosts, understanding the bloodfeeding patterns of *Culex* may be important in understanding the differing patterns of WNV transmission throughout the state. Bloodfed mosquitoes can be collected in a variety of trap types, including resting boxes, gravid and CO_2 traps. These traps attract mosquitoes in differing reproductive stages, so sampling method may introduce biases into bloodmeal identification results. The current study explores this issue by identifying bloodmeals from *Culex* mosquitoes collected from different trap types at the same study site (a heronry north of Davis, CA) over the same period of time. No spatial differences were found in the proportion of bloodmeals from different hosts collected from resting sites at the study area. However, there was a significant difference in the apportionment of bloodmeals collected from resting sites and CO_2 traps. Additionally, this study explored the possibility of CO_2 trap competition from a large population of nesting ardeids. Dry ice-baited traps placed at the edge of the study area and away from the birds collected *Cx. tarsalis* females consistently over the summer, whereas those near the ardeids were not successful until the birds began to leave the area. Our results suggest that trap type may affect the results of bloodmeal identification studies and that CO_2 traps may be out competed by a large source of attractive host species.

INTRODUCTION

Culex tarsalis is an important vector of West Nile Virus (WNV) in California. By feeding on competent avian hosts, as well as mammals (Tempelis et al. 1965, Tempelis and Washino 1967, Reisen et al. 1990, Kent et al. 2009), *Cx. tarsalis* can maintain WNV in its avian zoonotic cycle as well as serve as a bridge vector transmitting WNV to horses and humans. Understanding specific bloodfeeding patterns of this mosquito could be important in understanding patterns of WNV transmission in differing environments. Feeding predominantly on noncompetent avian and mammal hosts could dampen transmission, whereas more frequent feeding on competent hosts could increase transmission in a given area (Keesing et al. 2006).

To understand the effects of host selection on WNV transmission, bias in estimating bloodfeeding patterns must be minimized. Previously, mosquitoes for bloodmeal identification studies have been collected by a variety of methods, including light traps, CO_2-baited traps, gravid traps and resting boxes (Apperson et al. 2004, Kilpatrick et al. 2006, Molaei et al. 2006, Savage et al. 2007, Kent et al. 2009). Because each of these methods attracts a different subset of the female mosquito population, trap type itself could affect the results of bloodmeal studies. For example, CO_2 traps attract host-seeking females, so bloodfed mosquitoes collected in these traps are likely to have a partial bloodmeal and may be biased toward highly defensive hosts.

Trap bias may also affect metrics of mosquito abundance, especially in cases where the environment is competing with the attractancy of a particular trap type. It is known that New Jersey light traps do not provide accurate abundance measures in highly lit urban areas (Milby and Reeves 1989), and that gravid traps are not effective in areas such as wetlands with numerous oviposition sites (Reisen and Pfuntner 1987). Because *Culex* mosquitoes have been shown to prefer traps baited with live birds or with bird odors compared with those baited with dry ice alone (Perez et al. 2007, Syed and Leal 2009), the effectiveness of CO_2 traps could be reduced in areas with a large number of competing hosts.

In the current study we addressed the hypotheses that: (1) The intermittent presence of competing hosts alters measures of host-seeking female abundance, and that (2) The apportionment of bloodmeals from different vertebrate hosts is influenced significantly by the collection method used. We tested these hypotheses at a farmstead near Davis, CA that supports communal nesting of four avian species in Ardiedae, a family which includes important amplifying hosts of viruses in the Japanese encephalitis serocomplex (Buescher et al. 1959) and competent hosts for WNV (Reisen et al. 2005).

MATERIAL & METHODS

Dry ice-baited (CO_2) traps and gravid mosquito traps were operated weekly from May through October 2008 at a four hectare farmstead located just north of Davis, CA. Throughout the summer months, this farmstead served as a rookery for four ardeid species, black-crowned night-herons (*Nycticorax nycticorax*), snowy egrets (*Egretta thula*), great egrets (*Ardea alba*) and cattle egrets (*Bubulcus ibis*). Mosquito abundance was monitored by placing ground-level CO_2 traps both at the base of trees containing ardeid nests and at the edge of the farmstead near surrounding agricultural fields. CO_2-baited traps were also operated in the tree canopy near ardeid nests in June when the birds were abundant, and in September when most birds had left the area. Additionally, resting mosquitoes were aspirated once or twice weekly from a walk-in red box and from the covered porch of the farmhouse. Mosquitoes from all traps were identified and enumerated by species and sex. Bloodfed *Culex tarsalis* females were scored as partially fed, freshly

fed, late fed, half gravid or sub gravid (WHO 1975) and were stored individually at -80°C for later bloodmeal identification.

For bloodmeal identification, the heads and thorax of individual females was removed, and DNA was extracted from the abdomens using DNeasy 96 Blood & Tissue Kit (Qiagen USA). The mitochondrial gene cytochrome c oxidase I *(COI)* was amplified using a nested PCR. First, an approximately 1600 base pair (bp) region was amplified using primers for the conserved tRNA-coding regions flanking the gene. Then, the 650bp 'barcoding' region of *COI* was amplified using vertebrate-specific degenerate primers (Cooper et al. 2007). Host DNA was identified either by using the Luminex® assay for *COI* developed in our laboratory (unpublished) or by sequencing the gene and using the 'Identify Specimen' feature of the Barcode of Life Data Systems (BOLD; www.barcodinglife.com).

RESULTS AND DISCUSSION

Carbon dioxide trap competition. CO_2 traps were operated weekly starting in late-May, and the first *Cx. tarsalis* were collected from a trap placed at the edge of the farmstead in early June. The trap at the edge showed two peaks in *Cx. tarsalis* abundance, one in mid-July and one starting in August and continuing through the end of September (Figure 1). The abundance pattern of this trap was similar to that at the nearby Yolo Bypass Wildlife Area (data not shown). A drop in mosquito numbers in the September 5 collection was likely due to a recent insecticide application in the area.

A different pattern emerged from the CO_2 traps set under the trees where the herons and egrets were nesting. No *Cx. tarsalis* were collected in those traps until late August (Figure 1). A few mammalophilic mosquitoes, mainly *Anopheles freeborni* and *Aedes melanimon*, were collected in the traps under the trees in early June (data not shown). This prompted the use of CO_2 traps in the canopy to see if the large number of ardeids (>10,000 individuals) were out-competing the CO_2 traps on the ground. Because the canopy traps were set adjacent to the nesting ardeids, it was presumed that the herons and egrets would serve as additional bait and that the canopy CO_2 traps would have higher collections than those set on the ground. However, no mosquitoes were collected in the canopy traps in June (Figure 1), so we initially concluded that that the lack of *Cx. tarsalis* in traps near the ardeids was likely due to preferential foraging at ecotones (Lothrop and Reisen 2001) rather than host competition.

This conclusion was revisited, however, as the ardeids began to leave the heronry. Starting in late August, the CO_2 traps placed under the trees began collecting *Cx. tarsalis*, and the abundance pattern of these traps began to emulate that of the trap placed at the farmstead's edge, reaching a peak of nearly 500 *Cx. tarsalis* per trap-night at the end of September (Figure 1). CO_2 traps placed in the canopy in September were also successful in collecting *Cx. tarsalis* females (~200 per trap night). Although neither the traps set under trees or in the canopy collected as many mosquitoes as the trap placed at the edge of the farmstead, it is clear that these traps collected more mosquitoes in late August and September after most ardeids had left than they did throughout June and July when the birds were nesting (Figure 1). It appears that the lack of

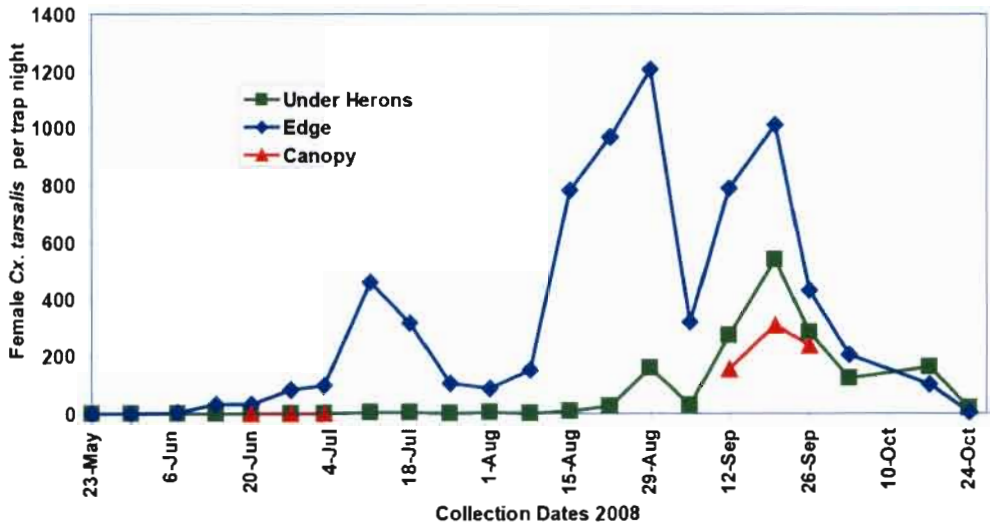

Figure 1. Average weekly counts of bloodfed and unfed female *Cx. tarsalis* from CO_2 traps placed at a farmstead heronry north of Davis, CA. "Under Trees" traps were placed at the base of trees containing heron/egret nests; "Canopy" traps were place in the tops of trees near heron/egret nests; and "Edge" traps were place at the boundary of the farmstead and surrounding agricultural fields.

mosquitoes from these traps in June and July was attributable not only to their placement away from the ecotonal boundary between agricultural fields and wooded farmstead but was also due to competition from the herons and egrets. These traps only began collecting mosquitoes as the population of ardeids diminished, and when the ardeids were present at their highest abundance, the dry ice-baited traps failed to capture any *Cx. tarsalis*, even when placed within one meter of the nests.

Bloodmeal trap bias. Bloodfed *Cx. tarsalis* were saved from all trap types throughout the summer. The majority of individuals from CO_2 traps were collected in early September, so this time period offered a large enough sample size to investigate potential trap biases. Species level bloodmeal identification was made for 145 engorged females (87% of total) collected from August 29 through September 12. Results are shown in Figure 2. Black-crowned night-heron was the most frequently fed upon host species from all trap types. This is important when considering the transmission of WNV, as these birds have been found with viremias as high as 9.8 $\log_{10}$ plaque forming units per mL of blood at this study site (Reisen et al. 2009).

For statistical analysis, the hosts were grouped as ardeids, other birds or mammals, and the proportions of each group were compared by chi-squared tests. There was no significant difference in the composition of bloodmeals from resting females collected from the red box or the porch ($\chi^2 = 0.66$; d.f. = 2, p = 0.7) which were approximately 100 m apart. Over 70% of these bloodmeals came from herons and egrets, and cow was the most predominant mammal (Figure 2). Overall, 14 host species were identified from the 112 resting *Cx. tarsalis* tested.

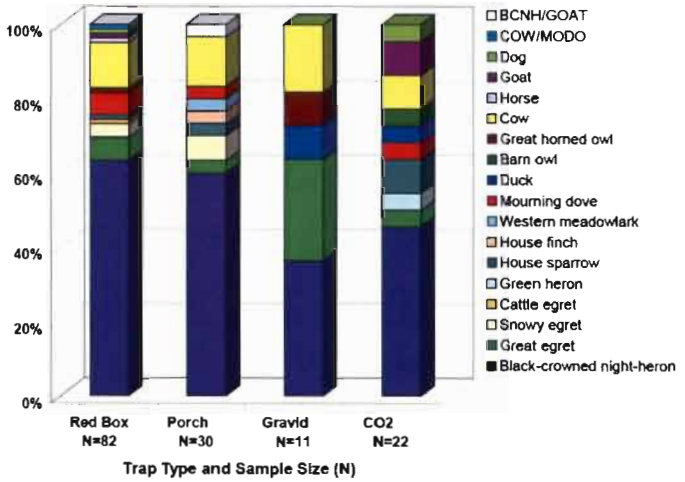

Figure 2. Percentage of host species identified from *Cx. tarsalis* bloodmeals collected in various trap types. All samples were collected at the same heronry study site from August 29 through September 12, 2008. The following are the scientific names of the host species: black-crowned night-heron (*Nycticorax nycticorax*, BCNH), great egret (*Ardea alba*), snowy egret (*Egretta thula*), cattle egret (*Bubulcus ibis*), green heron (*Butorides virescens*), house sparrow (*Passer domesticus*), house finch (*Carpodacus mexicanus*), western meadowlark (*Sturnella neglecta*), mourning dove (*Zenaida macroura*, MODO), duck (*Anas sp*?), barn owl (*Tyto alba*), great horned owl (*Bubo virginianus*), cow (*Bos Taurus*), horse (*Equus caballus*), goat (*Capra hircus*) and dog (*Canis familiaris*).

While no conclusions can be made about gravid traps due to the small sample size, there was a significant difference in bloodmeal composition between resting mosquitoes and those collected from CO_2 traps ($\chi^2 = 6.4$; d.f. = 2; p = 0.04). Significantly fewer black-crowned night-heron meals were identified from CO_2 traps; thus, a higher proportion of the bloodmeals were from non-ardeid birds and mammals. The bloodmeals from CO_2 traps were quite diverse, with 10 host species identified from only 22 mosquitoes.

Two avian species, green heron and barn owl, were identified only from mosquitoes collected in CO_2 traps, even though five times as many resting mosquitoes as host-seeking mosquitoes were tested. Several species (i.e., snowy egret, cattle egret, great horned owl, house finch, western meadowlark and horse) were identified from resting mosquitoes but not from mosqutioes collected in CO_2 traps. While this difference may, in part, be due to a larger sample size, it is clear that the composition and proportion of bloodmeals differed between resting and host-seeking collections. Any one sampling method would have resulted in different estimates of feeding indices as well as differing interpretations of *Cx. tarsalis* feeding patterns.

CONCLUSIONS

Our results suggest that trap bias may affect measures of *Cx. tarsalis* abundance as well as interpretation of *Cx. tarsalis* bloodfeeding patterns. CO_2 traps were found to be ineffective when placed near a large number of hosts, and bloodmeal apportionment differed between mosquitoes collected at resting sites and those captured in CO_2 traps. Future studies will assess trap biases in bloodmeal identification from other study areas and then focus on the bloodfeeding patterns of *Culex* mosquitoes around California.

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Distribution and Prevalence of novel flaviviruses in California

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ABSTRACT: Mosquito pools collected in California during 2007-2008 for arbovirus surveillance were subjected to additional testing to identify the prevalence and distribution of novel mosquito-specific flaviviruses not previously described in California. RNA extracted from mosquito pools was tested by real time RT-PCR using reagents designed from sequences from a virus described from *Culex tarsalis* in western Canada, tentatively designated Calbertado virus (CALBV). A two step RT–PCR using degenerate primers was used in mosquito pools with the highest Ct values to generate amplicons used for sequencing in order to establish phylogenetic relationships. In 2007, a total of 66 of 191 (34.6%) *Cx. tarsalis* mosquito pools tested positive for Calbertado viral RNA. In 2008, viral RNA from 4,755 mosquito pools (*Culex, Aedes, Culiseta* and *Anopheles* species) were tested, and a total of 542 pools (11.4%) were positive, of which 525 (11.0%) were *Cx tarsalis*. Infection rates for Calbertado in 2008 were highest for *Cx.tarsalis* (MLE= 9.76 per 1,000), followed by *Culex pipiens* (MLE= 0.35). In both years, the highest infection rates were within the Central Valley and the San Francisco Bay Area; the highest infection rates for *Cx. tarsalis* were in pools from Merced county (MLE = 24.53) in 2007 and San Joaquin county (MLE = 64.90) in 2008. For *Culex pipiens* in 2008, the highest infection rate was in Yolo county (MLE = 1.62). The highest seasonal infection rate (MLE = 15.82) was in May 2007 in *Cx. tarsalis*. Prevalence data indicated host preference of the newly described CALBV for *Cx. tarsalis*. Sequences analyzed from selected mosquito pools demonstrated a close genetic relationship among CALBV from California and other mosquito-specific flaviviral agents, Culex Flavivirus (CxFV), Kamiti River virus (KRV) and Cell fusing Agent virus (CFAV). Interestingly, results from our sequence data from California *Culex* spp. pools demonstrated the presence of both the novel CALBV and CxFVs, with CxFV being found predominantly in *Cx. pipiens quinquefascitus* pools. The effects of infection with heterologous flaviviruses on the transmission of alternative flaviviruses of human health significance such as West Nile Virus (WNV) and the effects of co-infection with this virus on fecundity or longevity of *Culex* vectors are not fully understood at this time. Further studies are needed, as these factors could alter vector competence of Californian *Culex* spp. for transmission of WNV.

INTRODUCTION

The genus *Flavivirus* comprises viruses such as Dengue virus (1-4), Yellow Fever virus and West Nile Virus that are of health importance and involve transmission from an arthropod to a human. Some of the viruses grouped within the genus *Flavivirus* only replicate in mosquito cells and have been discovered around the world. The first description of a mosquito only flavivirus was published in 1975 when a novel virus named Cell Fusing Agent (CFAV) was discovered in *Aedes aegypti* mosquito cells (Cammisa-Parks et al. 1992, Crabtree MB 2003).

In 1999, a second mosquito-specific flavivirus was isolated for the first time from a natural population of *Aedes macintoshi*. This virus, named Kamiti River virus (KRV), was incapable of replicating in vertebrate cells (Cook et al. 2006, Cook et al. 2009). Subsequently, another mosquito only flavivirus, Culex Flavivirus (CxFV), was isolated in Japan in 2003 from wild caught *Culex* spp. (Hoshino et al. 2007). Recently, several publications have described the presence of CxFV from locations in the New World, including the Yucatan Peninsula in Mexico, Trinidad, and Guatemala and in the United States in Houston (Texas) and Iowa (Morales-Betoulle et al. 2008, Blitvich et al. 2009, Farfan-Ale et al. 2009, Kim et al. 2009).

In California, this is the first report of a mosquito-specific flavivirus, which we designate tentatively as Calbertado virus (CALBV). RNA from Calbertado virus was detected repeatedly in *Culex* spp. mosquitoes collected from various locations California during 2007 and 2008 as part of routine arbovirus surveillance.

MATERIALS AND METHODS

Collection Methods. During 2007 and 2008, mosquitoes were collected by Mosquito and Vector Control Districts throughout California with the use of CO_2 baited and gravid female traps. Mosquitoes were separated by species and sorted into pools of $\leq$ 50 female mosquitoes.

In 2007, *Cx. tarsalis* mosquito pools from March through June were collected from sites located in Kern, Stanislaus, Merced, Sutter and Yuba counties in the Central Valley of California. In 2008, pools from *Culex, Aedes, Culiseta* and *Anopheles* spp. were collected from July through October throughout the state. For both years, pools were sent to the Center for Vectorborne Diseases (CVEC) at the University of California, Davis, for testing as part of state-wide arbovirus surveillance program for WNV, St. Louis encephalitis virus (SLEV) and western equine encephalomyelitis virus (WEEV). Data collected (including sampling date, sample size, location and trap type) were provided by participating agencies and stored in the Surveillance Gateway database.

Flavivirus detection and sequencing. Total mosquito pool RNA was extracted with an ABI PRISM™ 6100 Nucleic Acid PrepStation (Applied Biosystems) and screened by real-time RT-PCR on an ABI Prism 7500 using previously described primers

and a probe capable of identifying CALBV (Armijos 2009).

Mosquito pools with the lowest real time RT-PCR Ct values were selected and a two-step RT-PCR performed. Initially, a Super Script II reverse transcription reaction was used following manufacturer recommendations (Invitrogen, Carlsbad, CA) followed by a single-plex (RT- PCR) using a forward primer and degenerate reverse primers designed to identify either CALBV or CxFV. Amplicons generated were visualized by electrophoresis on agarose gels; positive DNA fragments were extracted using the QUIAquick PCR purification kit (Qiagen, Valencia, CA)) and sequenced using an ABI 3730 DNA sequencer (Applied Biosystems). Mosquito pool infection rates were calculated using the Maximum likelihood of infection using Excel Add-Inn PooledInfRate (Biggerstaff 2003).

Nucleic acid sequences were screened against the GenBank database using the BLAST program and analyzed using Vector NTI software (Invitrogen).

RESULTS

Surveillance for CALBV in *Cx. tarsalis* pools in 2007 resulted in 66 positive pools (n = 191, 34.55%). Of the locations tested during 2007, Merced County pools demonstrated the highest infection rate (MLE= 24.53) (Table 1).

In 2008, a total of 4,755 pools were tested from *Culex, Aedes, Culiseta* and *Anopheles* spp. for which mosquito-specific flaviviral RNA was detected from 542 by real-time RT-PCR (11.40%); of these 525 (97%) were from *Cx. tarsalis* (Table 2). Maximum likelihood estimates of infection were highest for *Cx. tarsalis* (MLE=9.76) followed by *Cx pipiens pipiens* (MLE=0.35). Spatially, the highest infection rates in *Cx. tarsalis* were from San Joaquin County (MLE=64.90) and for *Cx. pipiens pipiens* Yolo County mosquito pools (MLE= 1.62) had the highest rate. Interestingly, the data for both years showed the highest infection rates to be within the Central Valley (Figure 1).

Data for *Cx. tarsalis* pools collected from March through June 2007 demonstrated the highest seasonal infection rate to be the month of May (MLE = 15.82). Data for pools collected from July through October 2008 showed the highest infection rate in *Cx. tarsalis* in August (MLE = 10.43). Prevalence data for both years indicated host preference for *Cx. tarsalis* with the newly described CALBV.

Additionally, data from 17 *Cx. tarsalis* mosquito pools selected for sequencing during 2007 identified CxFV in 3 pools (17.6). In 2008, of the total samples sequenced, 18 were identified to be dually infected with Calbertado and CxFV (n = 114, 15.7%); with the latter found in three *Cx. pipiens quinquefasciatus* mosquito pools.

County	Infection Rate	Lower Limit	Upper Limit	# Pools Tested	Total Positive Pools	# mosquito tested
Kern	7.03	2.97	14.48	40	6	911
Merced	24.53	6.33	82.52	7	3	176
Stanislaus	18.77	14.07	24.84	102	51	4048
Sutter	4.09	1.69	8.53	39	6	1581
Yuba	0.00	0.00	31.02	3	0	70
Total				191	66	6786

Table 1. – Infection Rate per 1,000 female *Cx. tarsalis* collected from March through June 2007.

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Yuba	0.00	0.00	31.02	3	0	70
Total				191	66	6786

Table 2. – Total number of mosquito pools tested and testing positive by real-time RT-PCR from in California from July through December 2008.

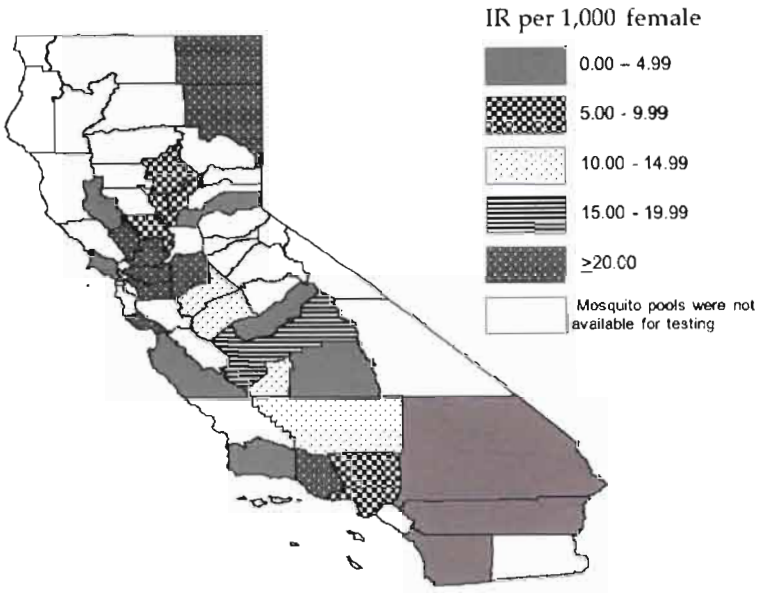

Figure 1. - Infection rates per 1,000 female mosquitoes collected in California from July through October 2008.

Sequences analyzed from selected mosquito pools demonstrated a close phylogenetic relationship between CALBV and CxFVs from California and other mosquito-only viral agents such as KRV, CFAV, and CxFVs isolated from Texas, Iowa, Guatemala, Mexico and Japan. Nucleotide identity comparison of our samples and other isolates show a 57% identity with KRV and 60% with CFAV for Calbertado virus isolated in California. For CxFV isolates the nucleotide identity is 100% with sequences generated in isolates from Japan.

Sequence generated from selected 2007 *Cx. tarsalis* pools, show a very close phylogenetic relationship with the nucleotide sequence isolated in mosquitoes collected from Canada (Pabbaraju et al. 2009) and the presence of two Calbertado and CxFV lineages in California.

DISCUSSION

The results generated from our study show that CALBV is found predominantly in *Cx. tarsalis* mosquito pools, while CxFV was more commonly associated with infection of *Cx. quinquefasciatus* mosquitoes. Interestingly, results from similar studies conducted in Canada and Colorado suggest the same host preference for both viruses (Bolling unpublished data) (Pabbaraju et al. 2009).

Initially in our study, a set of primers and a probe were used with a real-time RT-PCR assay with the goal of assessing the prevalence and distribution of the CALBV. Subsequent sequence data generated from selected positive pools showed that two viruses were present in a 15.7 % of the samples sequenced. The majority of positive samples for CALBV were found in *Cx. tarsalis* collected from locations along the Central Valley of California and the San Francisco Bay area. These data demonstrate differences in host preference, prevalence and distribution of mosquito-specific flaviviruses throughout the state. Given the mosquito-specific nature of these viral agents and the strong probability that they are maintained vertically, future studies are necessary to assess the specificity of the vector associations by performing vector competency studies for transovarial transmission of CALBV in *Cx. tarsalis* and CxFV in *Cx. pipiens* and *Cx. quinquefasciatus*.

Prior studies have indicated that previous infection with one virus can inhibit the efficiency of infection with closely related viruses in mosquito vectors (Eaton 1979, Newton et al. 1981). The effect of co-infection of these viruses on the transmissibility with heterologous flaviviruses such as WNV has yet to be fully assessed; however, the high infection rates in some areas of California with CALBV, identified in this study, indicate that this could potentially influence WNV transmissibility by *Culex* spp. vectors. Furthermore, the effects of mosquito-specific flaviviruses on *Culex* vector bionomics (including fecundity and longevity) could similarly reduce the force of WNV transmission or could be harnessed as a novel method to control pathogen transmission.

ACKNOWLEDGEMENTS

Funding for the genetic characterization of Calbertado Virus positive pools from Californian mosquito pools was provided through a contract by the Sacramento-Yolo County Vector Control Association and the University of California, Davis. We would like to thank all Mosquito control districts throughout the state that contributed mosquito pools to CVEC that were utilized in these studies.

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Effect of temperature on West Nile Virus replication in different host cell types: potential for altered transmission cycles in California

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ABSTRACT: The current dominant genotype of West Nile Virus (WNV) was first identified in 2002 (WN02; North American Genotype) and has displaced the founding North American strain (NY99; East Coast Genotype) in nature. NY99 is a highly avian-virulent strain that replicates proficiently in avian cell culture at temperatures simulating the avian febrile response. In the current study, replication phenotypes of two genetically distinct, naturally occurring North American strains of WNV (WN02 and NY99) were compared in *Culex tarsalis* cells at a range of biologically-relevant temperatures representing spring (22°C) to late summer (34°C) temperatures. The strains utilized were infectious-clone derived virus representing the well-characterized NY99 isolate and the founding Californian WN02 genotype (COAV997) isolated from a mosquito pool in Imperial Valley in 2003. In *Cx. tarsalis* cells, the California strain replicated to lower titers than NY99 at early time points when maintained at increased temperature, indicating the potential for longer *in vivo* extrinsic incubation periods and shorter transmission seasons.

INTRODUCTION

West Nile Virus (WNV) is maintained and amplified in a two-host transmission cycle between *Culex* mosquito vectors and avian hosts. When replicating in a two-host system, the virus is exposed to many host-dependent factors that can affect viral replication including disparate immune systems, host-cell receptors and body temperatures. The low genetic substitution rates observed in highly variable flaviviruses such as WNV have been attributed to the strong purifying selective effects of infection and replication within these disparate host systems (Scott et al. 1994, Coffey et al. 2008). Temperature plays an important role in WNV replication and transmission (Reisen et al. 2006, Kilpatrick et al. 2008) and delineates the seasonal phenology of arthropod vectors as well as their geographic distribution and the length of the extrinsic incubation (Reisen et al. 2006). West Nile Virus is capable of replication within a range of temperatures between 14°C in poikilothermic mosquito vectors during spring and fall to 44°C in febrile avian hosts (Kinney et al. 2006, Reisen et al. 2006). The requirement to replicate over such a wide range of temperatures may serve as an evolutionary constraint, resulting in compromised fitness in individual hosts to maintain eurythermic replicative capacity.

West Nile Virus was first introduced to North America in New York in 1999 (Davis et al. 2005) and the founding strain, NY99, is a representative of the early East Coast genotype. As WNV spread throughout the United States, a new genetic varaiant that was first identified in 2002 became the dominant North American genotype (WN02) (Davis et al. 2005). Full-length sequencing of the WN02 genotype revealed a non-synonymous mutation at nucleotide 1442, that results in an amino acid substitution in the envelope protein at position V159A (Davis et al. 2005). In order to understand how WN02 was able to replace NY99 in nature, *in vitro* and *in vivo* studies have been performed comparing the two virus genotypes. A study comparing WN02 and NY99 grown in chicken embryonic fibroblasts (DF-1) at 39°C, and *Aedes albopictus* cells (C6/36) at 28°C found no significant fitness differences *in vitro* (Moudy et al. 2007). *In vivo* studies of natural isolates of WN02 from New York state have shown that strains of WN02 were able to disseminate faster with a reduction in extrinsic incubation periods of two days as compared to NY99 in *Culex pipiens* (Ebel et al. 2004). Kilpatrick *et al.* demonstrated that increased temperature disproportionately increased the dissemination of WN02 compared to NY99, perhaps allowing WN02 be transmitted more rapidly than NY99 in nature resulting in the displacement of the founding genotype in North America (Kilpatrick et al. 2008). Previous *in vitro* studies have been performed with *Aedes albopictus*-derived cell lines (C6/36), and not cell lines derived from the natural *Culex* vectors and have not addressed the potential selective role of temperature.

The current study utilized a *Culex tarsalis* – derived cell line (Main et al. 1977) to analyze the WNV growth kinetics at biologically relevant temperatures ranging from spring/fall (22°C) to late summer (34°C). In addition to NY99, we utilized a natural isolate from California representative of the WN02 genotype (COAV997) that was obtained from a mosquito pool in Imperial Valley in 2003 (Reisen et al. 2004). Full-length sequencing revealed that COAV997 contains the conserved Env-V159A mutation that delineates the WN02 genotype as well as non-synonymous mutations in the NS1 gene (K110N) and NS4A gene (F92L) and two nucleotide substitutions in the 3' untranslated region (c10772t and a10851g). The current study investigates the replicative potential of a WN02 representative California strain at temperature extremes to further investigate if *in vitro* models can be used to identify temperature selective advantages of WN02 relative to NY99 to explain the displacement of the latter genotype.

MATERIALS METHODS

Cells and Viruses. *Culex tarsalis* D1 cells used for temperature sensitivity assays were derived from eggs from an autogenous colony (Chao and Ball 1973) and had been used to characterize arboviral growth patterns *in vitro* (Main et al. 1977). Cells were maintained with Schneider's *Drosophila* Medium (Gibco) supplemented with 10% Fetal Bovine Serum (FBS) and 5% penicillin/streptomycin. Cells were incubated at 28°C with 5% CO_2 and split 1:4 weekly. African green monkey kidney (Vero) cells were used for titrating viruses. Vero cells were maintained with Dubecco's Minimal Essential Medium (Gibco) supplemented with 10% FBS and 5% penicillin/streptomycin. Cells were incubated at 37°C with 5% CO_2 and split 1:10 biweekly. Virus derived from a NY99 infectious-clone was utilized as the East Coast genotype for this study. The construction of the clone and virus rescue protocols have been described previously (Beasley et al. 2004, Kinney et al. 2006). The WN02-representative California strain (COAV997) was isolated from a pool of *Cx. tarsalis* collected in Imperial Valley during 2003 (Reisen et al. 2004).

Temperature sensitivity assay. To assess differences in viral replication in response to temperature, *Cx. tarsalis* (D1) cells were used to generate viral growth curves at 22, 28 and 34°C. D1 cells were seeded in 25cm^2 vent/close flasks at a dilution of 1:4 and allowed to grow for 2-3 days until they were approximately 40-50% confluent. Cell counts were determined in order to calculate a multiplicity of infection (MOI) of 0.1. Nine flasks of D1 cells were inoculated per virus to assay each of the three temperatures in triplicate. D1 cells were inoculated with 300µL of diluted virus that was adsorbed for 90 minutes at 28°C with 5% CO_2. Following this incubation period, unabsorbed virus was removed, cells were washed twice with 1 mL of PBS (-Mg_2/-Ca_2) (Gibco), and 6mL of Schneider's *Drosophila* medium was added to each flask (supplemented as described above). Flasks were completely closed (as opposed to vented) to prevent the presence of CO_2. Inoculated flasks were incubated in triplicate at 22, 28 and 34°C for a total of 240 hours. Every 24 hours, 50 uL of supernatant was collected, diluted 1:10 in Schneider's *Drosophila* medium containing 20% FBS and 5% penicillin/streptomycin and frozen at – 80°C. Viral titer was estimated for duplicate samples from each replicate for collections taken at 24, 72, 120 and 168 hours post inoculation (hpi) by plaque assay on Vero cells. Briefly, a series of ten-fold dilutions was allowed to adsorb on a confluent monolayer of Vero cells for one hour at 37°C with 5% CO_2. An initial agarose overlay was added immediately after the adsorption, and a secondary agarose overlay containing 3% neutral red was applied two days later. Plaque forming units (PFU) were enumerated on day three, multiplied by the dilution factor and converted to logarithmic scale to determine the $\log_{10}$ PFU/mL.

STATISTICAL METHODS

To determine if the overall replication phenotypes were different between the two viral strains, a repeated measures statistical approach was used. The statistical program, Minitab, was used to determine statistical significance using a general linear model based on the logarithmic data at a 95% confidence interval. A two-sample t-test was used to determine statistical significance between viral titer of the two strains at specific time points using a 95% confidence interval.

RESULTS

Viral growth kinetics were determined for NY99 and COV997 in *Cx. tarsalis* cells maintained at 22, 28 and 34°C. NY99 and COAV997 showed similar replication phenotypes at 22°C with mean titers of 6.4 (± 0.1) $\log_{10}$ PFU/mL and 6.2 (± 0.1) $\log_{10}$ PFU/mL, respectively, occurring at 168 hours post inoculation (hpi) (Figure 1A). At 28°C, the mean titers at 168 hpi for NY99 and COAV997 were 7.7 (± 0.1) and 7.2 (± 0.1) $\log_{10}$ PFU/mL, respectively (Figure 1B). The mean peak titer (34°C) for NY99 was 7.3 (± 0.1) $\log_{10}$ PFU/Ml, and COAV997 replicated to a comparable mean peak titer of 7.0 (± 0.3) $\log_{10}$ PFU/mL (Figure 1C). Using a general linear model based on the logarithmic data that compared the viral titers at all collected time points, the replication phenotypes were not significantly different at 22°C ($p = 0.8$), 28°C ($p = 0.6$) or 34°C ($p = 0.5$). To determine if there were significant differences between the two viruses at individual time points, a two-sample t-test was used. When NY99 and COAV997 were maintained at 22°C, there were no significant differences at any time point. When the comparison was made for the 28°C growth kinetics, NY99 replicated to statistically higher titer than COAV997 at 24hpi ($p = 0.01$) and 168hpi ($p = 0.02$) samplings. NY99 also replicated to significantly higher titers until 72hpi at 34°C [24hpi ($p = 0.02$) and 72hpi ($p = 0.01$)].

DISCUSSION

Temperature plays an important role in WNV replication and transmission. The ability to replicate at temperature extremes in both avian and mosquitoes could play a selective role in WNV evolution. Efforts have been made to understand the evolutionary factors that enabled WN02 genotype to displace the founding East Coast Genotype. Our findings indicate that the California WN02 strain is as tolerant of temperature extremes (22°C and 34°C) as NY99 is in *Cx. tarsalis* cells, although NY99 maintained a higher titer than COAV997 for the first 72 hours of the inoculation at 34°C. These data differ from the *in vivo* study in *Culex pipiens* in which a WN02 representative strain naturally isolated in New York replicated to higher titers and disseminated faster at elevated temperatures (32°C) as compared to NY99 (Kilpatrick et al. 2008). Moudy et al. also compared New York strains of WN02 to NY99 in an *in vivo Cx. tarsalis* study that did not take temperature extremes into account and they found that WN02 had a shorter

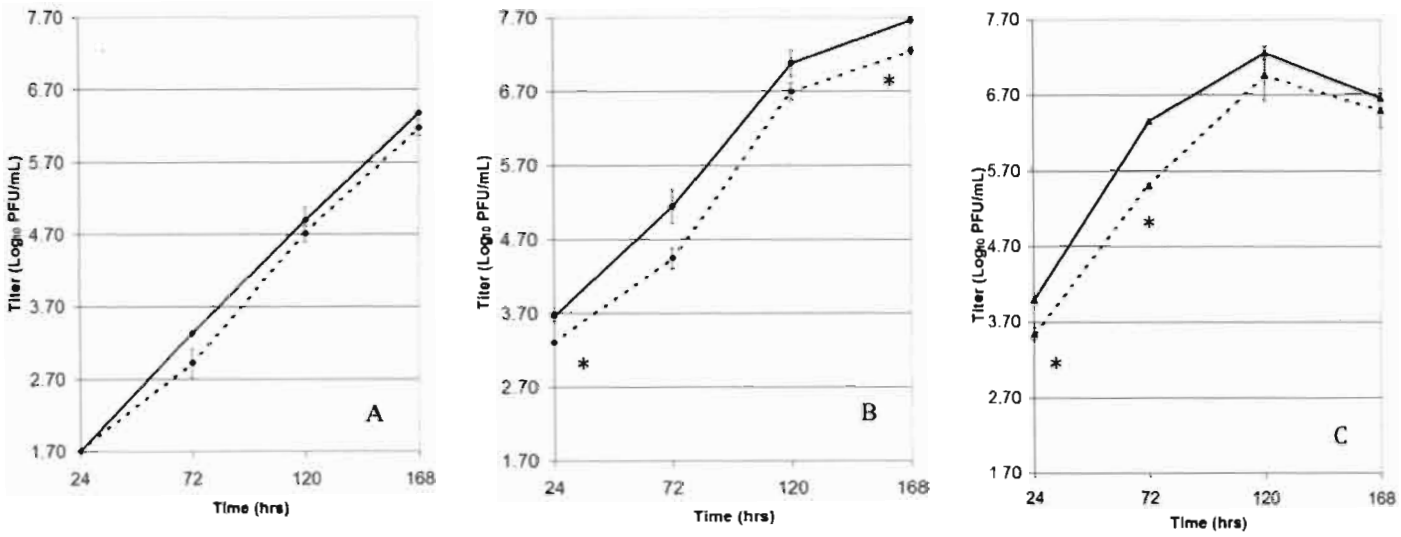

Figure 1. Viral growth kinetics comparing NY99 (solid line) and COAV997 (dashed line) in *Culex tarsalis* D1 cells maintained at 22°C (A), 28°C (B), and 34°C (C). Viral titers were determined by ten-fold serial dilution plaque assay in Vero cells. Limit of detection is 1.7 Log_{10} PFU/mL. Statistical significance was determined by a two-sample t-test with 95% confidence interval indicated by asterisk (*).

extrinsic incubation period than NY99 at 27°C (Moudy et al. 2007). Potentially, the genetic variation between the New York strains used in other studies could have a replicative advantage as compared to the California strains. Since *in vitro* models are not always indicative of *in vivo* results, comparison of the California strain to NY99 in an *in vivo* temperature study is warranted.

Moudy et al. studied the potential fitness differences between NY99 and WN02 in a mosquito cell line (C6/36) at 28°C and an avian cell line (DF-1) at 39°C, but found no significant fitness differences between the two strains *in vitro* (Moudy et al. 2007). C6/36 is an *Ae. albopictus* derived cell line that is well characterized and permissive to WNV replication; however, *Culex* spp. mosquitoes are more relevant WNV vectors and using a *Cx. tarsalis*-derived cell line potentially provides a more biologically relevant *in vitro* model; however, only modest differences were identified between the two viral strains in this study. The previous *in vitro* study with an avian cell line was performed at 39°C, but the role of febrile temperatures in an avian system was not addressed and could potentially further delineate fitness differences or virulence determinants between NY99 and WN02. In previous WNV temperature sensitivity studies, NY99 was able to replicate efficiently at high temperatures (44-45°C) in duck embryonic fibroblast (DEF) cells that approximated the body temperature of infected American crows (AMCRs), while a WNV strain that demonstrated low-virulence in AMCRs (Kenyan-3829) had compromised replication relative to increased temperatures (Kinney et al. 2006). Future studies will focus on determining potential fitness differences between the California WN02 strain and NY99 in response to temperature extremes in an avian cell line as well.

ACKNOWLEDGEMENTS

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Effects of Larval Diet on Life History Traits of *Culex* Mosquitoes

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ABSTRACT: The responses of life history characters to changes in abundance of larval diet were examined for *Culex quinquefasciatus*, *Culex stigmatosoma* and *Culex tarsalis*. Differences in larval habitat preferences were reflected in laboratory studies that evaluated development and survivorship across ten food concentration treatments. Survivorship for *C. tarsalis* larvae rapidly declined at high food concentrations. Immature developmental times of *C. quinquefasciatus* and *C. stigmatosoma*, species commonly found in hypereutrophic waters, were significantly shorter than for *C. tarsalis*. Despite similar larval habitat preferences, *C. quinquefasciatus* and *C. stigmatosoma* had comparatively small and large adult body sizes, respectively. *Culex tarsalis* adults were noticeably larger and heavier than *C. quinquefasciatus* and, occasionally, smaller and lighter than *C. stigmatosoma*.

INTRODUCTION

Mosquitoes serve as vectors of arboviruses and filarial worms and transmit these pathogens and parasites to other animals, resulting in diseases such as avian malaria, human filariases, encephalitis and West Nile Virus (Goddard 2003). To reduce the threat to human health and level of nuisance, the life cycle and potential mosquito production from different habitat types must be well understood. Food levels for mosquito larvae often differ appreciably among potential developmental sites, resulting in differences in survivorship and adult size. Three *Culex* species are commonly found in California: *Culex quinquefasciatus*, *Culex tarsalis* and *Culex stigmatosoma* (Bohart and Washino 1978). Larval habitats of *C. quinquefasciatus* generally include artificial containers, water barrels, basins and ground pools with minimal plant shelter (Laird 1988). *Culex tarsalis* is generally found in water associated with agricultural usages, and *C. tarsalis* larvae are less tolerant of water containing high concentrations of suspended matter than are *C. stigmatosoma* and *C. quinquefasciatus* larvae (Reisen et al. 1992). *Culex stigmatosoma* larvae are generally found in highly enriched waters such as sewage drains, cesspools, secondary treatment sewage ponds and dairy pastures, but are capable of living in waters with low concentrations of suspended matter.

The objective of this study was to examine the responses of three life history traits of the three *Culex* species across a range of food resource levels that are characteristic of larval mosquito developmental sites in nature. The effects of food availability on larval survivorship, developmental time and body size (wing length and mass) at eclosion were examined. Food levels were chosen to represent a range of food quantity across aquatic environments where larvae of the three species are naturally found, ranging from the comparatively low food availability characteristic of wetlands to the high food levels characteristic of ponds containing large amounts of organic matter.

METHODS AND MATERIALS

Culex stigmatosoma and *C. tarsalis* colonies were established from egg rafts collected from the UCR Aquatic Research Facility (Riverside, CA) in 2006. *Culex quinquefasciatus* used in these experiments were from a laboratory colony (BtSyn strain) derived from several populations in southern California in 1990 (Georghiou and Wirth 1997). The larval diet consisted of three parts mouse chow to one part Brewer's yeast. Ten food treatment levels ranged from 25 µl to 2000 µl of a stock solution of 1.5 mg of larval diet per 50 µl administered every other day. Within 24 h of hatching, larvae were placed individually in 10 ml of deionized water and maintained in a temperature-controlled environment (23 ± 1 °C) with a 16:8 hr L:D cycle. Larval rearing containers were scanned, at minimum, thrice daily for water level maintenance, death, pupation and emergence. Sample size consisted of 30 larvae for each treatment per species per gender (repeated 3-10x). *Culex quinquefasciatus* were reared at only three food levels. After a successful emergence, each adult specimen was anesthetized and frozen (-20 °C) until further analysis. Each adult mosquito was dried overnight at 43 °C, weighed to the nearest microgram three times using a Sartorius M2P electronic microbalance, and a mean mass was calculated. Wing length was measured from the distal end of the alula to the end of the wing (along the R3 vein), minus the fringes at 10x using a stereodissecting microscope.

We analyzed life history data (by sex) using multiple analysis of variance (MANOVA; SAS Institute 1999) with wing length, dry mass and developmental time as dependent variables, and species, food concentration and interaction as the independent variables. Standardized canonical coefficients were used to interpret the contribution of each dependent variable to any significant effects. When significant effects were found, multiple post-hoc comparisons were carried out using Tukey's HSD. All results are given as mean ± 1 SE.

RESULTS

Immature developmental time and body size (wing length and adult dry mass) of the three mosquito species did not change similarly across the gradient of food concentration examined in this study; the species by food concentration interaction was significant for each of the three *Culex* life history characters (♂: $F_{54,1776}$ = 6.10, p < 0.0001; ♀: $F_{54,1614}$ = 4.87, p < 0.0001). The standardized canonical coefficients for the species by food concentration interaction effect for males indicated that dry mass contributed most to species-specific differences among food levels on axis 1 and interspecific differences of developmental time across food levels contributed highly to axis 2 (Figure 1). For females, interspecific differences of body size were important determinants of the significant interaction effect across food levels. Species-specific differences of wing length across food concentrations contributed the most to discriminant axis 1 and dry mass contributed most to interspecific differences of females responding across food levels along axis 2 (Figure 2). Increasing food concentration had a comparatively greater direct effect on adult dry mass than on the other life history characters. Regardless of food concentration, *C. tarsalis* required more time to complete immature development than did either *C. quinquefasciatus* or *C. stigmatosoma*. Wing length also contributed significantly to the differences among species in both sexes.

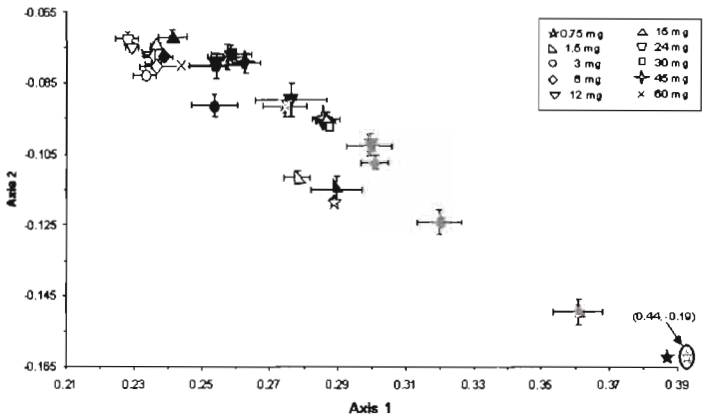

Figure 1. Bivariate means (± 1 SE) for standardized canonical coefficients for life history traits of males of three *Culex* species reared at ten food concentrations (0.75 – 60.00 mg/d). White, black and gray symbols represent *C. quinquefasciatus*, *C. stigmatosoma* and *C . tarsalis*, respectively. The response of *C. tarsalis* to the lowest food level (point surrounded by a circle) is off the scale illustrated.

Survivorship to eclosion differed significantly between *C. stigmatosoma and C. tarsalis* and food concentrations (species: $F_{1,77}$ = 69.56, p < 0.0001; food concentration: $F_{9,77}$ = 10.68, p < 0.0001).

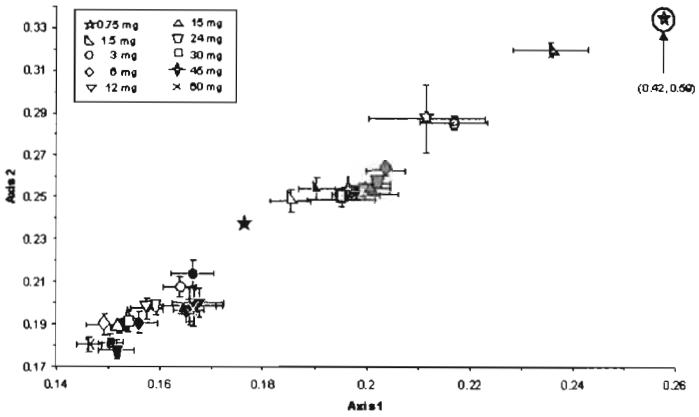

Figure 2. Bivariate means (± 1 SE) for standardized canonical coefficients for life history traits of females of three *Culex* species reared at ten food concentrations (0.75 – 60.00 mg/d). White, black and gray symbols represent *C. quinquefasciatus*, *C. stigmatosoma* and *C . tarsalis*, respectively. The response of *C. tarsalis* to the lowest food level (point surrounded by a circle) is off the scale illustrated.

The interaction of the two factors was not statistically significant ($F_{9,77}$ = 1.29, p = 0.257). Survivorship to eclosion of *C. tarsalis* increased directly within the lower food concentrations (0.75 – 3 mg/d), averaged > 60% at intermediate food concentrations (6 – 18 mg/d) and then declined appreciably at food levels > 18 mg/d . Survivorship to eclosion of *C. stigmatosoma* also increased directly across the three lowest food levels, but unlike *C. tarsalis* did not decline markedly at the four highest food levels tested. Across food concentration, *C. tarsalis* survivorship (38.11 ± 2.38%) was significantly less than *C. stigmatosoma* survivorship (73.94 ± 3.58%, p < 0.0001). For the three food treatment levels (1.5, 6 and 24 mg/d) where all species were reared, there was a significant difference for survivorship across species ($F_{2,35}$ = 10.88, p = 0.0002). *Culex tarsalis* survivorship (48.56 ± 4.17%) was significantly lower than both *C. quinquefasciatus* (81.11 ± 7.05%, p = 0.001) and *C. stigmatosoma* (76.30 ± 7.05%, p < 0.005). Survivorship significantly differed across species at medium and high food levels (Tukey tests, p < 0.05). Survival of *C. quinquefasciatus* and *C. stigmatosoma* was similar at each of the three food levels examined (Tukey tests, p > 0.05).

There were significant species, concentration and species by concentration effects for wing length, adult dry mass and developmental time (all p < 0.0001). Wing lengths of *C. stigmatosoma* adults were significantly longer (♂/♀: p < 0.0001) than the other two species. The same pattern was observed for adult dry mass. Individuals reared at low food concentrations (< 3 mg/d) were significantly smaller (♂/♀: Tukey tests, p < 0.0001) and less massive (♂/ ♀: Tukey tests, p < 0.01) than individuals at food concentrations > 3 mg/d. At the low food concentrations, differences across species were marginally significant (p≤ 0.05). As food resources became more readily available, differences

across species became more significant as maximum male wing lengths [2.90 ± 0.03 mm (24 mg/d), 3.59 ± 0.09 mm (18 mg/d), 3.08 ± 0.04 mm (12 mg/d)] and maximum male dry mass [0.549 ± 0.010 mg (24 mg/d), 0.713 ± 0.019 mg (12 mg/d) and 0.610 ± 0.011 mg (18 mg/d)] were obtained for *C. quinquefasciatus*, *C. stigmatosoma* and *C. tarsalis*, respectively. Females exhibited a similar pattern but were significantly larger and more massive than their male counterparts for a given food concentration (all $p < 0.05$) except for the lowest food concentration.

Unlike wing length and adult dry mass, developmental time significantly decreased with increasing food concentration. Across the gradient of food concentration examined here, immature developmental times for *C. tarsalis* in nearly all food treatments were significantly longer than for *C. quinquefasciatus* and *C. stigmatosoma* (♂/♀: Tukey tests, $p < 0.0001$).

DISCUSSION

The comparatively lower survival of *C. tarsalis* larvae in the high food environments at the upper end of the food gradient indicates that poor larval survival is an important factor limiting its occurrence in the hypereutrophic developmental sites used by *C. stigmatosoma* and *C. quinquefasciatus*. Moreover, the low food conditions in these experiments did not seem to favor *C. tarsalis* larvae relative to the other species. *Culex tarsalis* immature development time was also longer at a particular food concentration than for the other species, and surprisingly low food levels characteristic of its larval developmental sites (e.g., wetlands, sumps in irrigated pastures) increased the developmental times of *C. tarsalis* more than for the other species. Because water in the rearing vessels was not changed on a daily basis, waste products presumably increased as larval diet increased. It is possible that *C. tarsalis* larvae are less capable of tolerating waste product buildup than are the larvae of the two species common in highly enriched habitats in nature. This might explain the severe reduction of survivorship at high food concentrations and the comparatively lower survivorship and longer developmental times of *C. tarsalis* larvae at all food concentrations.

As expected, females of each three *Culex* species were noticeably larger and heavier than their male counterparts with one exception; at the lowest food concentration, individuals had a mean dry mass of 0.20 mg, regardless of sex or species. As food became more available, the differences between the sexes became more apparent. The differences of body size at the low vs. high food levels were caused in part by a requirement for the minimum amount of larval nutritional resources necessary for basic metabolic processes and successful development. The second lowest food concentration administered to the larvae represents an important transition point for the three *Culex* species. Above this food concentration, survivorship approximates 50%, and species differences became more apparent with *C. stigmatosoma* adults growing significantly larger than either *C. tarsalis* or *C. quinquefasciatus* adults.

In contrast to body size, developmental time decreased with increasing food availability. Prolonging immature development, especially at the lower food concentrations, allowed for the accumulation of necessary resources for successful adult emergence, especially for females who require additional reserve for egg production. Because *C. tarsalis* larvae are generally found in low nutrient waters that can vary spatially and temporally (Walton et al. 1990), it is possible to encounter unsuitable habitats from time to time, selecting for a slower growth rate in this species. It is also possible that the observed patterns in developmental time (as well as survivorship) for *C. tarsalis* could be explained by species differences in larval feeding rate and particle size intake of *C. tarsalis* compared to either *C. quinquefasciatus* or *C. stigmatosoma*.

In summary, the observed patterns for life history traits indicate that nutrient resources were critical during larval development for these three *Culex* species at the lowest food treatment levels. Future research will focus on this narrower range, taking into account how differences in feeding rate, particle size intake and nutrient quality may impact the life history traits of *Culex* mosquitoes.

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An Analysis of Sentinel Chicken Utility for Surveillance in Urban Environments

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ABSTRACT: West Nile Virus (family Flaviviridae, genus *Flavivirus*, WNV) invaded Los Angeles, California in September 2003, and since then has followed a pattern of emergence, amplification, subsidence and resurgence across this urban landscape as previously reported. A time-series analysis of human case counts and sentinel chicken seroconversions revealed temporal concordance, suggesting both were associated with concurrent tangential transmission from the basic passeriform-*Culex* amplification cycle. Sentinel chickens provided the advantage of knowing the exact location and an approximate time frame for transmission, as opposed to human cases which frequently were reported late and were assumed to be acquired 2 -14 days prior to onset at their place of residence. Time series analyses suggest that in Los Angeles, sentinel chicken seroconversions provided a useful surrogate for human cases of West Nile disease, which frequently have delayed reporting, thereby allowing a real time warning for intervention.

INTRODUCTION

Historically, sentinel chicken flocks in Los Angeles were a part of the statewide St. Louis encephalitis virus (family Flaviviridae, genus *Flavivirus*, SLEV) monitoring program, but were limited to three flocks of ten birds each. Sentinel chickens have the advantages of providing a history of transmission events at a specific location and are more cost-effective than other arboviral indicators (Scott et al. 2001). Sentinel chickens can produce WNV-specific antibody as early as 5 days post-infection, and enzyme immunoassay (EIA) can detect seroconversion as early as 7 - 10 days post-infection (Senne et al. 2000). Therefore, when bled at regular intervals, chickens also delineate the approximate time of infection. An additional advantage of using sentinel chickens in an urban area is that their low viremia is unlikely to infect the *Culex* mosquito vectors (Reisen et al. 2005, Bowen and Nemeth 2007, Reisen et al. 2008) reducing the ethical dilemma of placing potentially competent avian host species near residences. In this paper we sought to determine the efficacy of sentinel chickens in predicting human cases of West Nile Fever and West Nile Neuroinvasive Disease.

METHODS

Description of Sites. Six sites within or near parkland embedded within the Los Angeles urban continuum were selected because of urban zoning regulations, security, spatial coverage and the abundance of peridomestic birds (Figure 1). Sites were selected to cover the gradient from cool maritime habitats (Machado Lake) to hot and dry inland valleys (Santa Clarita).

Sentinel chickens. Flocks of ten White Leghorn hens were maintained and sampled at each of the six core sites described above. Sentinel chickens develop WNV antibody approximately 7 - 10 days after experimental infection (Langevin et al. 2001, Patiris et al. 2008, Senne et al. 2000) but are bled in a biweekly

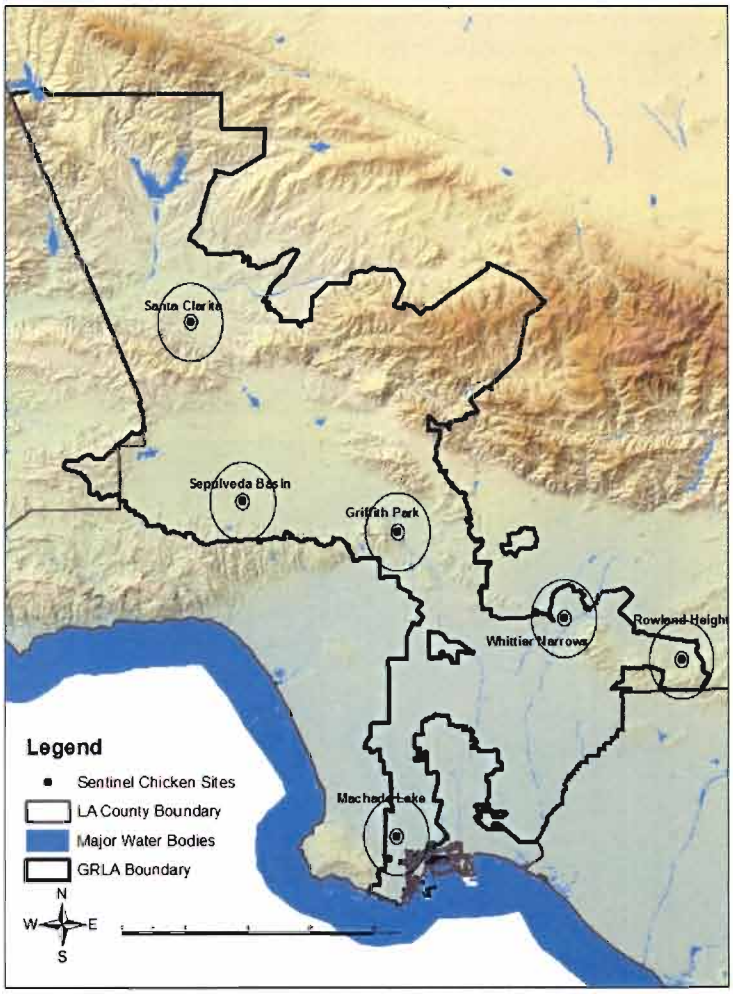

Figure 1: Sentinel chicken sites located within the Greater Los Angeles County Vector Control District.

(i.e. two-week) cycle in California, which confounded the temporal detection of seroconversion events.

Human Case Reports. Human cases of West Nile fever and West Nile neuroinvasive disease were monitored by the Los Angeles County Department of Public Health, Acute Communicable Disease Control Program, through passive case detection and reporting. Cases were limited to those that matched the CDC definition for WNV-associated neuroinvasive or febrile illness and had been laboratory-confirmed, typically by demonstration of IgM antibody in sera or spinal fluid by enzyme immunoassay (EIA) (CDC 2009). Additional human infections were discovered through blood donor programs and noted as asymptomatic blood donors (ASD) unless symptoms developed after donation, at which time the individual was included as a case. The association between sentinel chicken seroconversions and human case counts was evaluated by time series graphs and cross-correlation analysis.

RESULTS

A time-series graph of human cases and sentinel chicken seroconversions revealed apparent temporal concordance (Figure 2), although the amplitude for seroconversions was lower due to the limited population of chickens monitored. This concordance was evaluated by cross-correlation analysis holding human case counts constant and shifting the sentinel chicken conversion counts progressively earlier or later in time. Due to the seasonal trend in both data sets, the time period was restricted to the months of June - November, when there were non-zero values in one of the datasets. The cross-correlation coefficients with 95% confidence intervals revealed that sentinel chicken seroconversions were marginally associated with human cases four weeks prior to their occurrence ($r = 0.22$, $p = 0.056$), significantly associated two weeks prior to case occurrence ($r = 0.47$, $p < 0.001$) and highly correlated during the same time period ($r = 0.74$, $p < 0.001$). Chicken seroconversions continued to be significantly correlated with human cases up to six weeks after human case occurrence ($r = 0.34$, $p = 0.005$).

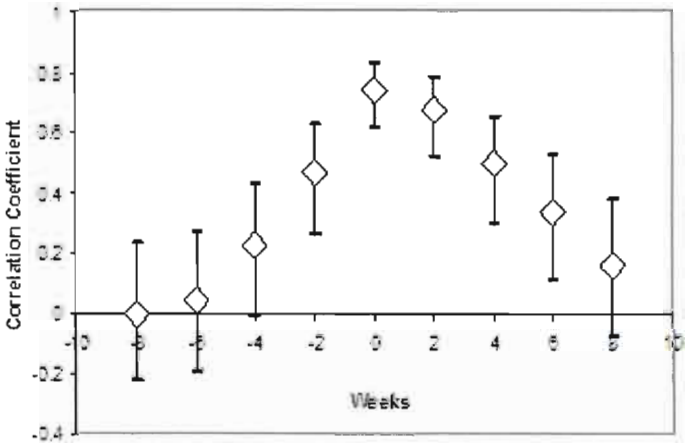

Figure 3: Correlation coefficients for human case counts with lagged sentinel chicken seroconversion counts (lag is represented on x axis), with 95% confidence intervals. Human case counts were held constant, while sentinel chicken seroconversion counts were lagged both forward and backward in time as indicated on the x axis.

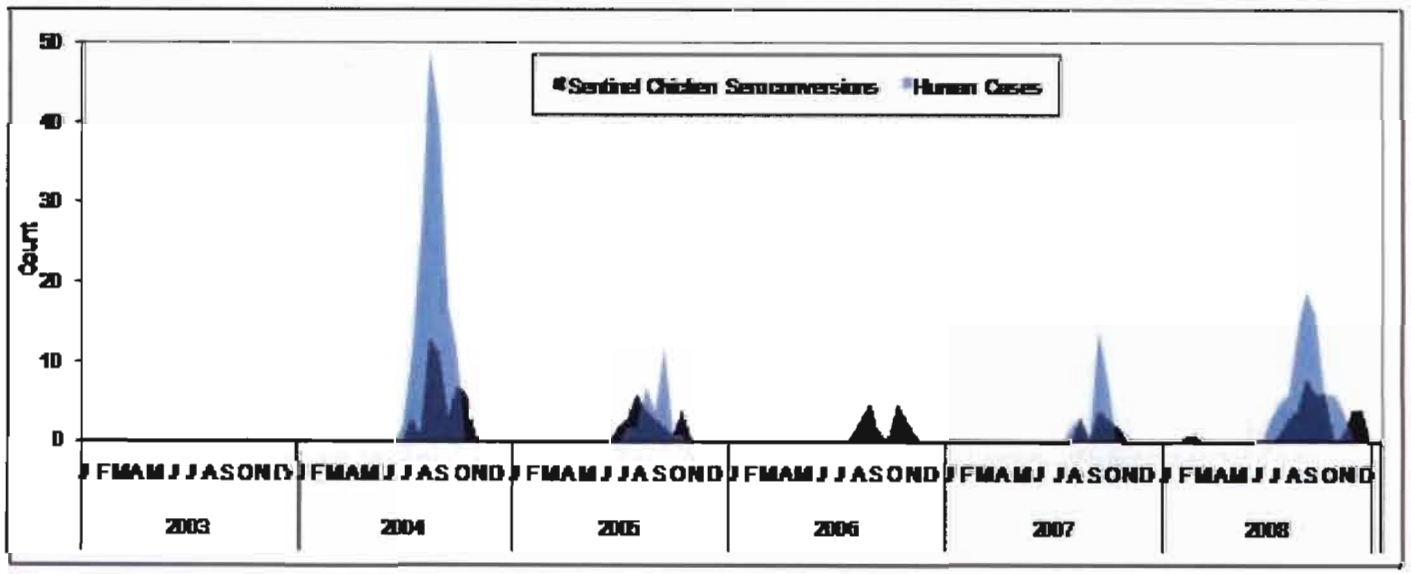

Figure 2: Biweekly sentinel chicken seroconversions and human cases for 2003-2008, showing concordance in chicken seroconversion and human case occurrence.

DISCUSSION

Sentinel chicken seroconversions provided evidence of WNV invasion and resurgence outbreaks in Los Angeles during the 2004 and 2008 epidemics. Furthermore, sentinel chicken seroconversions and human case occurrence had high temporal concordance, even with incubation periods and sampling intervals confounding the dates of detection. Detection of seroconversions could be improved by shortening the intervals between bleeding from two to one week. Our current analysis indicated that although sentinel chickens were not a valuable early predictor of human cases (Kwan 2009), they provided a highly significant indication of concurrent WNV tangential transmission to humans. Concordance of human cases and sentinel chicken seroconversion has been noted in other areas with WNV activity (Gleiser et al. 2007), including areas where *Cx. p. quinquefasciatus* is the primary tangential vector (Palmisano et al. 2005, Unlu et al. 2009), although in the later paper concordance was only observed in the third year of WNV activity.

The passive surveillance system for human WNV infection in California often had delays in reporting of cases to mosquito control agencies. This was compounded in Los Angeles where an ethnically diverse population of more than ten million people sought care from a mosaic of medical providers and diagnostic laboratories, creating geographic, jurisdictional and linguistic challenges for epidemiologic surveillance. Therefore, knowledge of the risk for tangential virus transmission using a sentinel chicken surrogate for human cases could be very useful for the implementation of emergency control and public education. Furthermore, if we are able to predict tangential transmission with an adequate antecedent time lag, this may improve the ability of mosquito control to interrupt transmission.

ACKNOWLEDGEMENTS

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Symposium 1. Epidemiology and Control of West Nile virus: Collaborative projects among the Center for Vectorborne Diseases, the Mosquito and Vector Control Association of California districts and the California Department of Public Health

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Our symposium this year focused on mosquito and arbovirus surveillance sampling, diagnostics and interpretation [Table 1]. Chris Barker described derivation of a human population based trap and sentinel chicken flock allocation scheme that will be evaluated against district surveillance sampling programs as part of a collaborative research project with the CDC in Ft. Collins and the California Department of Public Health. Forrest Melton, NASA Ames, presented an update on the development of a remotely sensed, unsupervised classification system for detecting clean and neglected swimming pools to facilitate inspection and treatment. Ying Fang evaluated the new MagMax RNA extraction system for mosquito pools and dead bird tissues. Her results indicated that both acute and previous WNV infections are detected in bird tissues, necessitating the separation of results for operational purposes into acute infections that require an operational response and chronic infections that are of informational importance but probably do not warrant a control response. Jennifer Kwan showed that although sentinel chicken seroconversions failed to provide an early warning of enzootic activity, they did provide a useful and accurate surrogate for spill-over transmission to humans, thereby allowing districts in urban areas to respond operationally without having to wait for notification of human cases. Tara Thiemann summarized in-progress studies on *Culex* blood meal host identification within five biomes of California that depicted opportunistic host selection patterns enabling *Culex* to function as both amplification vectors of WNV among avian hosts and bridge vectors of WNV to horses and humans. Stan Wright, Sacramento-Yolo MVCD, reviewed the impact of WNV introduction on the avian abundance and herd immunity at the Stone Lakes NWR. These surveillance and ecological studies are only useful if they lead to successful intervention. Sarah Wheeler evaluated the utility of three WNV vaccines in protecting Western Scrub-jays, a model corvid, from WNV. Her results demonstrated immune priming and some protection, but not the same level of sterilizing immunity produced by natural infection. Hugh Lothrop evaluated EcoExempt products for adult mosquito control over organic crops and concluded that these products will require considerable modification and reformulation before effective results can be realized. These research projects would not have been possible without the excellent logistical and fiscal support of collaborating mosquito control districts, especially Coachella Valley, Greater Los Angeles, Kern, Sacramento-Yolo and Sutter-Yuba MVCDs.

Table 1. Listing of the speakers and presentations in the Symposium on the Epidemiology and Control of West Nile Virus

- Reisen, W. K.: Introduction
- Ying, Y.: Comparative Sensitivity of RNA Extraction methods: What it Means in Wild Bird and Mosquito Surveillance
- Barker, C. M: Population-based Strategies for Surveillance Site Allocation
- Thiemann, T.: Survey of *Culex* Host Selection Patterns in California
- Wright, S.: Avian Seroprevalence in Sacramento County, 2004 – 09
- Wheeler, S. S.: Evaluation of three West Nile Virus Vaccines in Western Scrub Jays
- Kwan, J: An Analysis of Sentinel Chicken Utility for Surveillance in Urban Environments
- Melton, F.: Towards an Unsupervised Classification Scheme for Detecting Clean and Neglected Swimming Pools from Satellite Data
- Lothrop, H. D.: ULV Efficacy Trials of EPA Exempt Adulticides

Enhanced sensitivity of RNA extraction: What it means for wild bird and mosquito surveillance

Ying Fang, Maureen Dannen, Sandra Garcia, Helen Lu and William K. Reisen

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ABSTRACT: Sensitivity of the new MagMAX™-Express-96 Magnetic Particle Processor system was compared to the ABI Prism® 6100 PrepStation by concurrently testing positive mosquito pools and avian tissues using both systems and examining RT-PCR cycle threshold [Ct] scores using manufacturer chemistry and protocols. The MagMAX system was easier to use, faster and more sensitive than the ABI 6100 system. Enhanced sensitivity testing tissues from birds submitted through the Dead Bird Program prompted us to report RT-PCR results as negative [Ct > 40], recent infection [Ct < 30 or Ct > 30 and confirmed by 2nd primer set] or chronic infection [Ct > 30 and not confirmed by WN2]. Recent infection indicates that the bird most likely died of West Nile Virus and should prompt extended surveillance and intervention responses by control or public health agencies. Chronic infection indicates that the bird most likely was infected some time in the past and probably died of other causes.

INTRODUCTION

The California State Mosquito-borne Virus Surveillance and Response Plan serves as the principle decision support tool for mosquito control and includes tables to calculate relative risk (Kramer 2009). Risk calculations are distributed to each participating agency weekly during the surveillance season and can be calculated directly through data managed by the California Surveillance Gateway. Key variables within these calculations are measures of mosquito infection calculated using the maximum likelihood estimate excel add-in (Biggerstaff 2003) and the number of dead birds in each area confirmed to be West Nile Virus [WNV] positive at necropsy. Both variables rely on the detection and identification of virus infection, and these estimates can vary due to sampling design and effort as well as assay sensitivity and specificity. A random sampling design, for example, provides an overall estimate of infection for a general area, whereas repeated focal sampling may provide unduly inflated or 'best estimates' of enzootic activity (Reisen and Lothrop 1999).

Testing methodology has evolved rapidly since WNV invaded North America, and the numbers of tests required by surveillance in California has increased by almost an order of magnitude, from 3,000 – 4,000 mosquito pools per year to almost 30,000 mosquito pools and dead bird tissues per year. Originally, mosquito pools at CVEC were tested using an in situ-enzyme immunoassay, but this was replaced by more rapid and specific molecular methods (Chiles et al. 2004). Currently, mosquito pools and dead bird tissues are tested for WNV as well as western equine encephalomyelitis [WEEV] and St. Louis encephalitis [SLEV] viruses at the Center for Vectorborne Diseases [CVEC] by real-time [TaqMan] reverse transcriptase-polymerase chain reaction [qRT-PCR] using an ABI [Applied Biosystems Inc, Foster City, CA] 7900 platform and published (Lanciotti et al. 2000, Shi et al. 2001) and unpublished primers. Originally, RNA was extracted using a *ABI* PRISM™ *6700* Automated Nucleic Acid Workstation; however, although fully automated, this system was slow, required frequent maintenance and repair and was replaced by a manual vacuum manifold system [ABI Prism® 6100 PrepStation] using basically the same chemistry as the ABI6700.

Recently, ABI replaced the ABI6100 RNA extraction system and chemistry with new magnetic bead 96-well technology called the MagMAX™-Express-96 Magnetic Particle Processor system [<http://www.ambion.com/catalog/CatNum.php?1837>]. Magnetic bead technology binds RNA more efficiently than previous glass fiber filter column methods resulting in higher RNA yields and eliminating of filter clogging due to cellular particulates/debris. The current research compares the sensitivity of the ABI 6100 and MagMAX methods and describes the impact of enhanced sensitivity on the California surveillance program.

MATERIALS AND METHODS

All RNA extractions were done using proprietary Applied Biosystems Inc. [ABI] protocols, chemistry and equipment as set-up and recommended by the manufacturer. qRT-PCR used the same ABI chemistry and equipment throughout, and primers labeled with VIC, FAM and TET reporter dyes. Standard curves were generated from stock WNV grown in Vero cell culture. The sensitivity of RNA extraction using the MagMAX system was compared to the ABI 6100 system by concurrent testing of mosquito pools submitted for surveillance to CVEC and of kidney tissues collected from birds reported dead to the California Dead Bird Program (McCaughey et al. 2003) and necropsied by the California Animal Health and Food Safety Laboratory. For mosquitoes, 21 pools reported positive during 2009 were retested using the MagMax system. Originally pools of 50 mosquitoes were shipped to CVEC on dry ice, thawed, supplemented with 3 ml of mosquito diluent [Dulbecco's Modified Eagle Medium, 10% fetal bovine serum, antibiotics], triturated with glass beads on a Spex mixer-mill, clarified by centrifugation and extracted for RNA using the ABI6100 protocol. For the current evaluation, pools were thawed and RNA was extracted from 300 or 50 uL aliquots

using either ABI 6100 or MagMAX protocols, respectively. For avian tissues, 23 birds reported as positive using MagMax during the 2009 season were retested. Originally duplicate kidney tissue samples were sent to CVEC; one in ABI lysis buffer and one frozen dry on dry ice. Specimens in lysis buffer were triturated on a Spex mill, clarified and then the RNA was extracted using the MagMax system. For the current evaluation, kidney samples were thawed and two glass beads and 1 ml of mosquito diluent were added; the samples were then triturated on a Spex mill, clarified by centrifugation and the RNA extracted from 300 or 50 uL aliquots using both ABI6100 and MagMAX protocols, respectively.

RESULTS

Standard curve. Standard curves for RNA extracted from cell culture using the MagMAX system and tested by our singleplex [WNV only] and multiplex [WNV plus WEEV and SLEV] protocols were generated for qRT-PCR Ct scores plotted as a function of WNV PFU equivalents [Fig. 1]. Curves were linear within the 1 to 6 $\log_{10}$ PFU/0.1 mL titer range, with Ct [cycle threshold] scores ranging from 12 – 31. Based on WNV growth curves in infected mosquitoes (Reisen et al. 2006b), our assay should detect all infected mosquitoes at all time points post infection.

Mosquito pools. When ABI 6100 and MagMAX RNA extraction protocols were compared, all mosquito pools tested positive, and Ct the scores were significantly correlated [r = 0.68, P < 0.01] [Fig. 2]. However, on average Ct scores following MagMAX RNA extraction [mean = 25.8, SE = 3.5] were significantly lower [paired t-Text = 6.9, df = 21, P<0.001] than following ABI 6100 RNA extraction [mean = 30.3, SE = 3.9], implying greater RNA yields and increased sensitivity. The difference of 4.45 was considered to be > 10 fold difference based on the curve in Fig. 1.

Avian tissues. RNA extracted from kidney tissue samples sent to our laboratory in MagMAX lysis buffer and tested using the MagMax protocol had Ct values ranging from 9.8 to 34.5 using a primer set from the envelope gene of the virus [Table 1]. Interestingly, birds in this study with Ct scores > 30 were all non-corvids and, with the exception of the lesser goldfinch, were all species that are known, in part, to survive infection with WNV (Komar et al. 2003, Komar et al. 2005, Padgett et al. 2007, Reisen et al. 2005). Of the 11 samples with Ct scores > 30, only two were confirmed by WN2 [primer set from the non-structural portion of the virus genome]; the remaining birds were again positive by WN1 upon re-extraction. When kidney tissue was titurated in mosquito diluent and RNA extracted using the MagMAX protocol, 11 [48%] of these 23 birds had Ct scores > 40 and would have been considered WNV negative. Interestingly, although only one of the 12 positive birds [number 09-2660] had a Ct score > 40 using the ABI 6100 protocol, mean Ct scores for birds positive using the MagMAX RNA extraction [Ct = 17.3] were significantly lower [paired t-Test = 16.2, df = 10, P < 0.001] than those using the ABI6100 protocol [Ct = 26.5].

DISCUSSION

There are major advantages for using the new MagMAX system over the previous ABI6100 system. The ABI6100 system required high sample volume input [300 uL], needed pre-filtration to eliminate cellular debris that would clog the columns, relied on a vacuum manifold to pull samples through a glass fiber column, required a technician to be present to add reagents according to protocols and took ca. 40 min for each 96 sample extraction. In comparison, the MagMAX system requires low volume input [50 uL], does not require pre-filtration, uses a more sensitive magnetic bead-based technology, is semi-automated in that plates

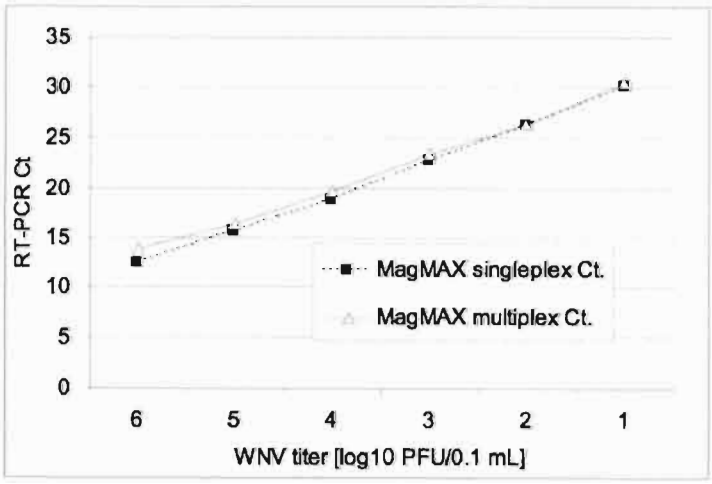

Figure 1. Standard curve of RT-PCR Ct score for single- and multi-plex assays plotted as a function of WNV titer.

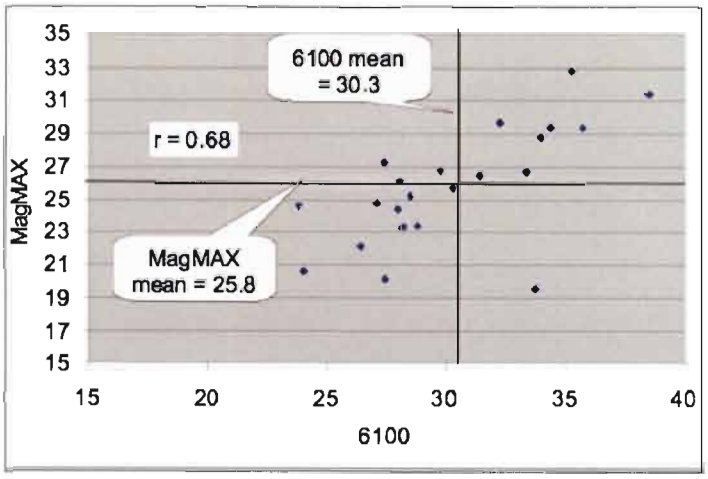

Figure 2. RT-PCR Ct scores for 21 mosquito pools tested for WNV after RNA was extracted concurrently by either the MagMAX or ABI 6100 systems.

Vertebrate Tissue Samples				
		in Lysis Buffer	in Mosquito Diluent	
		MagMax	MagMax	6100
DHS Bird #	Species/Comments	WN1 Ct	WN1 Ct	WN1 Ct
09-2583	House Finch	25.2	28.1	35.9
09-2640	Western Scrub Jay	16	15.9	28
09-2651	Western Scrub Jay	15.7	16.8	24.5
09-2664	Western Scrub Jay	12.6	17	28.1
09-2702	House Sparrow	31.1	und	und
09-2706	MacGillivray's Warbler	31	und	und
09-2707	Western Scrub Jay	14	15.8	25.4
09-2660	House Sparrow	30.9	33.9	und
09-2695	House Finch	30	und	und
09-2692	House Finch	28.9	und	und
09-2657	House Sparrow	31.1	und	und
09-2719	Mourning Dove	31.3	und	und
09-2629	Northern Mockingbird	31.9	und	und
09-2716	Lesser Goldfinch	34.5	und	und
09-2711	House Finch	16.5	18.8	29.4
09-2734	Western Scrub Jay	15.9	18.1	28.9
09-2713	Western Bluebird	33.8	und	und
09-2665	Western Scrub Jay	19.7	18.5	26
09-2699	Western Scrub Jay	14.1	12.9	21
09-2766	House Sparrow	32.5	und	und
09-2741	Yellow -billed Magpie	9.8	11.3	17.2
09-2687	Yellow -billed Magpie	15.8	17.6	27
Q9-91	Eastern Gray Squirrel	34.6	und	und

Table 1. Kidney tissues from different vertebrate species submitted to CVEC in lysis buffer or frozen. Samples then were triturated in lysis buffer or mosquito diluent, the RNA extracted using the MagMAX or ABI 6100 systems and the RNA tested for WNV by singleplex qRT-PCR. und = undetermined, Ct score ≥40.

can be preloaded and placed into the machine allowing automatic processing and takes only 15 min for 96-well RNA extraction. In addition to rapid and high throughput, the MagMAX system provides greater RNA yields in comparison to the ABI6100 system, thereby enhancing sensitivity. Because infected mosquitoes usually are collected by gravid or CO_2–baited traps > 3 days after their infectious blood meal, females should have replicated virus to > 2 $\log_{10}$ PFU (Reisen et al. 2006b), and these virus levels would be detectable by both the ABI 6100 and the MagMAX systems. Therefore, data generated using the more sensitive MagMAX RNA extraction system should not change estimates of mosquito infection rates. This was demonstrated by the data in Fig. 2 where all samples were positive [i.e., Ct < 40].

The 2010 avian tissue testing paradigm includes shipment of tissues from CAHFS in 1 mL of MagMAX BI lysis buffer and then RNA extraction using the MagMAX system that uses an additional solution consisting of lysis binding solution with propanol plus carrier RNA. In our evaluation herein, this MagMAX system detected 11 birds not positive when tissue samples were shipped frozen, triturated in mosquito diluent [same as virus transport medium], and then RNA extracted using the MagMAX or ABI6100 protocols. Interestingly, 9 of these 11 positive birds with Ct scores > 30 could not be confirmed using WN2, a second primer set from the non-structural region of the WNV genome, but were again WN1 positive upon sample re-extraction. Recent studies have shown that some birds surviving WNV infection develop infections in tissues such as the kidney and spleen that may be detectable by RT-PCR as long as 6 – 8 months post infection (Nemeth et al. 2009a, Wheeler and Reisen 2009). Although these tissues were repeatedly positive by primer sets from the envelope gene [WN1], they could not be confirmed by primer sets from the non-structural region [WN2] or by virus culture after blind passage through mosquito cells. Interestingly, these birds frequently retained elevated neutralizing antibody titers, perhaps indicating that infectious virus was intermittently released into the blood stream, thereby 'boosting' the humoral immune system. Attempts to detect virus within blood samples from these birds were repeatedly unsuccessful after the initial acute viremia subsided, although infectious WNV was recovered from RT-PCR positive tissues from house finches upon necropsy as long as 6 wks post infection (Reisen et al. 2006a).

In previous years, RT-PCR results with Ct scores < 30 were considered to be positive and > 40 negative or undetermined. We attempted to confirm birds with WN1 Ct scores > 30 but < 40 by retesting the RNA using WN2. If confirmed, the bird was reported to be WNV positive. If negative by WN2, we then re-extracted the RNA and tested again by WN1. If positive by re-extraction, we reported the bird as positive. We have noticed that many of the birds in the 30 – 40 Ct range that could not be confirmed by WN2 were species that usually did not die after experimental infection; e.g., quail or mourning doves (Reisen et al. 2005), or survived and were known to develop chronic infections (Reisen et al. 2006a, Wheeler and Reisen 2009). Birds with Ct values < 30 were species such as American crows and western scrub jays known to die with high viremia during acute infection. Enhanced sensitivity due to improved RNA extraction has further complicated the interpretation of positive RT-PCR results from tests on avian kidney tissues as shown in Table 1. Therefore, beginning in 2010, we will continue to report birds with Ct > 40 as negative. However, birds with Ct scores < 30 or with scores > 30 and confirmed by WN2 will be reported as "recent infection", whereas birds with Ct scores > 30 and not confirmed by WN2 will be reported as "chronic infection". We feel strongly that birds scored as "chronic infection" did not die from WNV infection and probably did not represent a recent transmission event. Early in the season when there are no other indicators of virus activity, we presume that these birds were infected sometime during the previous transmission season (Reisen et al. 2006a). We currently are exploring the significance of these long term infections and events that may trigger recrudescence. However, to date we have no data to indicate that these birds ever develop a viremia sufficient to infect blood feeding mosquitoes and feel that long term persistent immunity provides permanent protection from re-infection (Fang and Reisen 2006, Nemeth et al. 2009b, Wheeler and Reisen 2009).

ACKNOWLEDGEMENTS

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Population-based Strategies for Surveillance Site Allocation

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INTRODUCTION

The goals of arboviral surveillance programs differ from those of objective sampling schemes such as population censuses because the goal is not to obtain an unbiased representation of an entire area's average virus activity. Rather, the goals are to provide early, sensitive detection of enzootic virus activity and to provide an indication of the risk of spillover viral transmission to humans and other animals outside the enzootic transmission cycle. To accomplish these goals, many surveillance programs with knowledge of past mosquito and arboviral activity conduct routine sampling approximately evenly over the urban and suburban areas within a district and target rural trapping in smaller population centers or areas with a history of virus activity.

In collaboration with the Arboviral Diseases Branch of the Centers for Disease Control and Prevention and the Vector-Borne Disease Section and Viral and Rickettsial Diseases Laboratory of the California Department of Public Health, we have initiated a project to develop and evaluate a framework for surveillance sampling that could be implemented in areas without a surveillance history. Here, we present the development of a sampling design that is flexible enough to allow for variation in available human and financial resources and could be implemented for any area in the western USA using freely available data to maximize the coverage of the human population to be protected by the program.

METHODS

Study areas and data sets. Three mosquito control districts (Coachella Valley MVCD, Kern MVCD and Sacramento-Yolo MVCD) have agreed to participate in the study, and all have well-established histories of multi-faceted arboviral surveillance and detectable virus activity. The districts differ in latitude, climate and land use, but each includes a range of habitat types from rural agriculture to densely populated urban areas.

For each district, human population data were obtained from the U.S. Census Bureau's 2000 estimates of total population and area by census block, which was the finest scale for which data were available. These data were related to U.S. Census TIGER/Line shapefiles for the 2000 census blocks using ArcGIS 9.3 (ESRI, Redlands, CA) before import into PostgreSQL for spatial calculations.

Sampling grid. At the start of this project, Sacramento-Yolo MVCD suggested using the Public Land Survey System (PLSS) of townships as a spatial framework to guide placement of surveillance sites. We adopted this 6 x 6 mi (36 mi^2) gridding system because it: 1) Provided adequate spatial resolution; 2) Was already in use by or familiar to many agencies; 3) Allowed for practical considerations, including a manageable total number of traps per agency and grid cells large enough to provide freedom for local decisions on trap placement within each cell; and 4) Could be applied in other agencies outside of California. The resources required for a sentinel chicken flock are greater than those needed for a mosquito trap, so we decided that flocks would be distributed at a density approximately ¼ that of mosquito traps. Therefore the 6 x 6 mi grid cells were aggregated into groups of 4 for flocks, resulting in a second, coarser 12 x 12 mi grid (144 mi^2) for each agency (Fig. 1). After the grid cells were defined, the human population was calculated for each cell in PostgreSQL by totaling the population from all blocks within the cell. For blocks that overlapped cell boundaries, the human population was divided between multiple cells in proportion to the area contained within each cell.

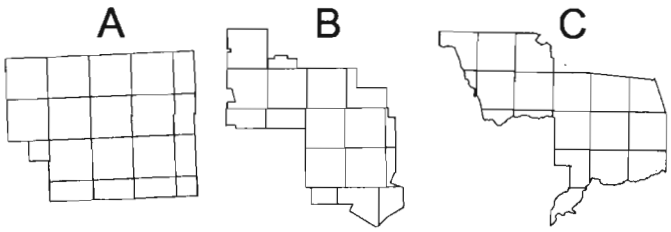

Figure 1. Maps showing the 6x6-mi (gray lines) and 12x12-mi (bold black lines) grid cells used for mosquito trap and chicken flock allocation, respectively, within the Coachella Valley MVCD (A), Kern MVCD (B), and Sacramento-Yolo MVCD (C).

Allocation of sites. Initially, sites were allocated among the grid cells in each agency using one of three methods. For the first two approaches, the allocation was done randomly using the sample function in R version 2.9.2 (R Development Core Team 2009) with weights (i.e., probabilities of grid cells receiving each trap) directly proportional to either the raw or log-transformed [$\log_e$(population+1)] human population within the cells. For the third method, we ranked all grid cells within each agency in descending order by human population. One trap was allocated to

each cell beginning with the most populated and continuing until the target number of traps had been reached. For example, if 25 traps had been allocated using this method, they would have been placed in the 25 most populated grid cells.

Following a decision on the method to be used, maps of trap allocations were distributed to collaborating mosquito control agencies for a range of total sample sizes, from 30 - 60 mosquito traps and 8 - 12 flocks per agency. Individuals at each agency reviewed the maps and made a final decision on the total numbers of sites to be operated within each agency.

RESULTS AND DISCUSSION

The first two approaches of randomly allocating traps according to each cell's proportion of the raw or log-transformed human population suffered from two drawbacks: 1) Heavy sampling of urban areas resulted with very few traps assigned to grid cells in more rural areas and 2), The small number of traps assigned to rural areas were frequently placed in areas of minimal surveillance value (e.g., remote deserts known to have few mosquitoes) due to the random nature of the allocation. Log transformation reduced the skewness of the population distribution but had the same limitations. Such a sampling design would achieve our goal of population-based sampling, but coverage would be inadequate for detection of enzootic virus amplification that could begin outside or near the edge of densely populated areas.

The third method in which grid cells were ranked by population and traps were assigned to the most populated grid cells to the limit of an agency's resources achieved a much broader coverage of the agency's constituency while still maintaining the population basis (Fig. 2). As a result, this method was chosen for use in the study, and after the initial allocations, maps were sent to each collaborating mosquito control agency for decisions on trap and flock placement. In a few cases, grid cells were removed from the sampling plan if they were inaccessible with existing agency resources or otherwise known to be unsuitable for mosquitoes or arboviruses. Surveillance will be conducted at the selected sites beginning with the 2010 surveillance season, and future work will aim to quantify the relative value of these and other arboviral surveillance indicators in time and space as predictors of human cases of disease associated with West Nile Virus.

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This work is part of a larger collaborative project, and we thank the other agencies and individuals involved for their generous contributions of time and resources, including the Sacramento-Yolo MVCD (Dave Brown), Kern MVCD (Rob Quiring), Coachella Valley MVCD (Branka Lothrop), Center for Vectorborne Diseases, UC Davis (Brian Carroll), Arboviral Diseases Branch, CDC (Roger Nasci, Marc Fischer, Nicole Lindsey), Vector-Borne Disease Section, CDPH (Vicki Kramer, Anne Kjemtrup, Kerry Padgett, Tina Feiszli), and the Viral and Rickettsial Diseases Laboratory, CDPH (Carol Glaser, Cynthia Jean). Funding for this project was provided by the Arboviral Diseases Branch, Centers for Disease Control and Prevention.

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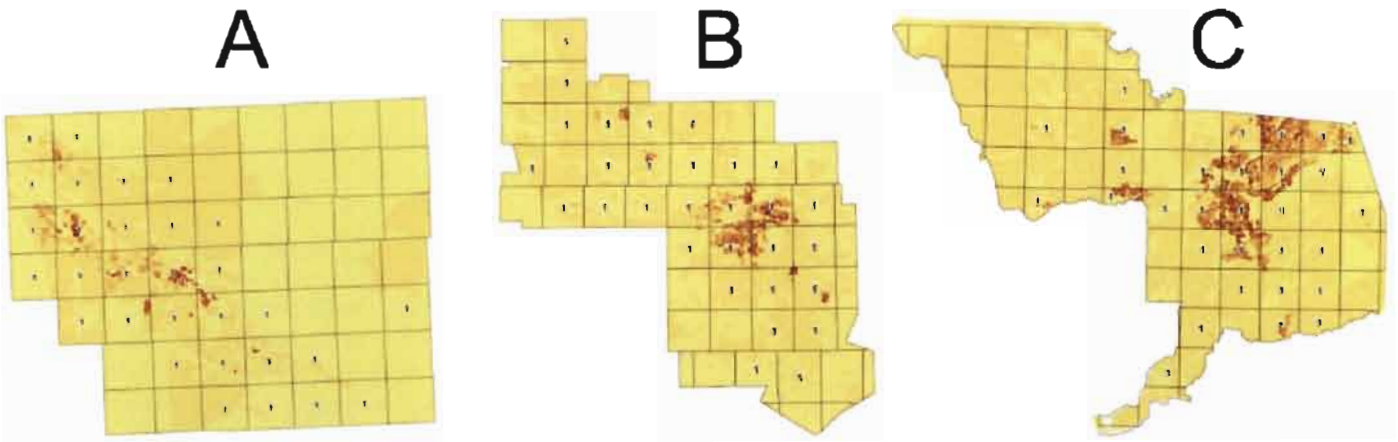

Figure 2. Allocation of mosquito traps to the 30 most populated grid cells within the Coachella Valley MVCD (A), Kern MVCD (B), and Sacramento-Yolo MVCD (C). Grid cells where traps are to be placed are indicated by a "1", and the underlying human population density is indicated by the light to dark gradient from rural to urban areas.

Survey of *Culex* host selection patterns in California

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INTRODUCTION AND METHODS

West Nile Virus (WNV) is an enzootic arbovirus maintained in avian hosts and transmitted primarily by a variety of *Culex* mosquito species. Many bird species can be infected with WNV, but not all species are equally competent as amplifying hosts. For example, corvids such as crows and magpies develop high viremias and easily infect mosquitoes, whereas galliforms such as chickens and quail generally do not produce sufficient viremia for *Culex* infection (Komar et al. 2003, Reisen et al. 2005). Because avian hosts vary in their competency for WNV, host selection by *Culex* mosquitoes may influence the transmission dynamics of WNV in a given area. In addition, *Culex* species responsible for avian transmission also serve as bridge vectors to humans and horses, so characterizing *Culex* bloodfeeding patterns may also be valuable in understanding the timing and efficiency of epidemic transmission.

Several molecular techniques have been developed in recent years to identify bloodmeal hosts (reviewed in Kent 2009). Many of these techniques are limited in that they do not identify the host to species, identify only a few host species, or as with DNA sequencing, are quite expensive. We have developed a high-throughput assay based on the Luminex® platform (Dunbar 2006) that can identify 15 of the most frequently fed-upon birds and mammals in our study areas. This technique uses uniquely labeled fluorescent beads attached to species-specific oligonucleotides that bind to the mitochondrial gene, cytochrome c oxidase I (*COI*). This Luminex® technique can be used in conjunction with DNA sequencing of *COI* (Cooper et al. 2007, Kent et al. 2009) to identify nearly all hosts from mosquito bloodmeals at less cost than sequencing alone.

RESULTS AND DISCUSSION

Over 400 blood-fed *Cx. tarsalis* were collected throughout the year from a farmstead just north of Davis, CA. This farmstead served as a heronry during the summer months, with over 10,000 total black-crowned night-herons (*Nycticorax nycticorax*), snowy egrets (*Egretta thula*), great egrets (*Ardea alba*) and cattle egrets (*Bubulcus ibis*) nesting from June through August. When the herons and egrets were present, bloodmeals from these birds accounted for over 85% of the total collected. As these birds fledged and began to leave the area in September, *Cx. tarsalis* began to feed more frequently on other birds, including mourning doves (*Zenaida macroura*), yellow-billed magpies (*Pica nuttalli*) and house sparrows (*Passer domesticus*). Blood meals taken from mammals [primarily cattle (*Bos taurus*), horses (*Equus caballus*), goats (*Capra hircus*) and dogs (*Canis lupus familiaris*)] also increased. Additionally, two human (*Homo sapiens*) bloodmeals were identified during the summer months. In the winter months, when the herons were absent, *Cx. tarsalis* fed primarily on yellow-billed magpies (45%) and house sparrows (26%), with about 10% of mosquitoes feeding each on American robins (*Turdus migratorius*) and red-tailed hawks (*Buteo jamaicensis*).

Various bloodfeeding patterns emerged from the preliminary *Cx. pipiens* complex data collected throughout California. In Coachella Valley; in total, 86 bloodmeals were identified. The predominant host was chicken (*Gallus gallus*), perhaps due to a lack of hosts other than sentinel chickens near the trap sites. Mourning doves, house sparrows and dogs were also common hosts in this area. Seventy bloodmeals were identified from the Greater Los Angeles area, and house sparrow and house finch (*Carpodacus mexicanus*) accounted for nearly 75% of the total. Chicken and mourning dove blood meals were also common, and one human bloodmeal was identified. *Cx. pipiens* complex were collected primarily from a country club in Kern County. Of the 51 identified bloodmeals, European starlings (*Sturnus vulgaris*) were the most common, representing about 40% of the total. Bloodmeals from chicken, house sparrow, mourning dove and western scrub jay (*Aphelocoma californica*) were also common. At a rural site in Sutter County, 46 bloodmeals were identified with American crow (*Corvus brachyrhynchos*) and American robin accounting for over 50% of the feeds.

Based on these preliminary data, bloodfeeding patterns of *Culex* mosquitoes varied greatly over time and space. At the heronry site north of Davis, CA, *Cx. tarsalis* fed on WNV-competent hosts throughout the year with black crowned night herons serving as the primary host in the summer months and yellow-billed magpies and house sparrows being the most prevalent bloodmeal source in the winter. The *Cx. pipiens* complex fed on a large variety of hosts throughout the state. Thirty-one avian species and five mammalian species were identified from 253 individual bloodmeals. There was marked variation in feeding patterns in different areas of the state with a different species serving as the primary host in Coachella Valley, Greater Los Angeles, Kern County and Sutter County. Further investigation is on-going to increase sample sizes and to determine if the variation in bloodfeeding patterns may account for differences in WNV transmission in these areas.

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Avian WNV Seroprevalence on Stone Lakes NWR, Sacramento Co., California

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ABSTRACT: The arrival of West Nile Virus in California provided us with an opportunity to observe the process of its invasion, spread and establishment in naive free-living bird populations. We were able to collected data on changes in avian serology, host abundance, recapture rate and age ratio before and after the WNV enzootic. We estimated annual survivorship based on relative abundance of new captures per net hour of effort, annual recruitment as the number of hatch-year birds captured, recapture as the relative number of captures from prior years and annual changes in seroprevalence as evidence of the proportion of resistant members of the population. Concurrently, we trapped the associated mosquitoes and tested these in pools to determine the prevalence of WNV in *Culex* mosquitoes. Our goals with this investigation were to detect local infection in individual hosts, to assess the impact of WNV on the host population and to infer the effect of changes in host susceptibility on viral transmission. The focus of this investigation was on the 6,200 acre Stone Lakes National Wildlife Refuge (Refuge) located 12 miles south of Sacramento in Elk Grove, California. The overall sample period for birds and mosquitoes on the Refuge extended from 2000 through 2009 with a sample size of greater than 20,000 birds (~2,000/ year).

The first detection of WNV in Northern California occurred on the Refuge in June of 2004, first in migratory birds and then in a few *Culex* mosquito pools (Armijos et al. 2005). In the following year, 2005, we observed an avian enzootic, noting a substantial decline in capture-abundance of numerous bird species, the greatest diversity in seroprevalence and the highest number of positive *Culex* pools recorded during the study interval (Wright et al. 2006). In 2006, we noted a widespread peak in avian seroprevalence (7% of all species sampled), reflecting the previous years enzootic, coupled with a rebound in capture-abundance consisting largely of young birds. From 2007 through 2008, we observed a comparatively reduced, oscillating up-and-down *Culex* infection and avian seroprevalence with some site dependence, species seroprevalence-dominance (specifically the house finch) and a catch-abundance recovery of most bird species except jays (Wright et al. 2009). The loss of susceptible hosts during the enzootic and the concurrent increase in the proportion of seropositive and resistant hosts established local herd immunity that reduced the intensity of local viral transmission (Wright et al. 2007). Seroprevalence appears to be additive in several species but particularly in the house finch [52% of total seropositive birds sampled on the Refuge (Table 1)]. In the recent years on the Refuge we have observed a reduced WNV infection pressure, a continued reduction in jay predation pressure and a healthy rebound in catch-abundance. These results suggest that, although there seems to be clear variation among species, there was initially a considerable decline in susceptible passerine abundance followed within a year by an overall increase in recruitment and survivorship and a decrease in seroprevalence suggesting a post-enzootic recovery.

Ten most common passerine species based on Refuge biologists' census	Ten most common species based on our mist net captures	Percent of all species captured	Percent of total antibody detected in all species
House Finch	House Finch	23%	52%
Red-winged Blackbird	Song Sparrow	22%	3%
Brewer's Blackbird	Spotted Towhee	8%	1%
Western Meadowlark	Cliff Swallow	8%	2%
Spotted Towhee	Wrentit	6%	>1%
Cliff Swallow	Black-headed Grosbeak	4%	3%
European Starling	Black phoebe	4%	1%
Marsh Wren	Brown-headed Cowbird	4%	3%
Western Scrub-jay	American Goldfinch	4%	1%
Song Sparrow	Brewer's Blackbird	1.5%	>1%

Table 1. Seroprevalence of the ten most commonly captured passerines on SLNWR.

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Towards an Unsupervised Classification Scheme for Detecting Clean and Neglected Swimming Pools from Satellite Data

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ABSTRACT: Neglected swimming pools provide suitable breeding habit for mosquitoes, can contain thousands of mosquito larvae and present both a significant nuisance and public health risk due to their inherent proximity to urban and suburban populations. The rapid increase and sustained rate of foreclosures in California associated with the recent recession presents a challenge for vector control districts seeking to identify, treat and monitor neglected pools. Commercial high resolution satellite imagery offers some promise for mapping potential neglected pools and for mapping pools for which routine maintenance has been reestablished. We present progress on unsupervised classification techniques for mapping both neglected pools and clean pools using high resolution commercial satellite data and discuss the potential uses and limitations of this data source in support of vector control efforts. An unsupervised classification scheme that utilizes image segmentation, band thresholds and a change detection approach was implemented for a sample 5km x 5km region in the Coachella Valley, CA. Comparison with field data collected by vector control personal was used to assess the accuracy of the estimates. The results suggest that the current system may provide some utility for early detection or cost effective and time efficient annual monitoring, but additional work is required to address spectral and spatial limitations of current commercial satellite sensors for this purpose.

INTRODUCTION

The continuing economic recession and accompanying foreclosure crisis in California has led to neglect of swimming pools and water features in residential neighborhoods throughout California. Neglected swimming pools provide suitable breeding habitat for mosquito species, including both *Culex quinquefasciatus* and *Culex tarsalis*, and can contain thousands of mosquito larvae per pool. In neighborhoods with high foreclosure rates, these pools present a significant potential public health risk, and the 2007 outbreak of 140 human cases of West Nile Virus (WNV) (family Flaviviridae, genus *Flavivirus*) corresponded with a 300% increase in the number of mortgage payment delinquency notices issued (Reisen et al., 2008). The rapid increase and sustained rate of foreclosures present a challenge for vector control districts seeking to identify, treat and monitor neglected pools. Airborne imagery and supervised and unsupervised classification and mapping techniques have been successfully applied to map urban features including swimming pools (Thomas et al., 2003), and manual mapping techniques have demonstrated their utility for support of vector control operations in recent years. Commercial high resolution satellite imagery offers some promise for mapping potential neglected pools and for mapping pools for which routine maintenance has been reestablished. These data also have the potential for use in support of vector control operations, particularly if unsupervised classification algorithms can be developed to accelerate data processing and speed delivery of output maps. To evaluate the potential utility of commercial satellite data for mapping of swimming pools and their condition, we analyzed high resolution satellite imagery using multiple classification approaches for a 5km x 5km study region in the Coachella Valley, CA. We present progress on unsupervised classification techniques for mapping both neglected pools and clean pools using high resolution commercial satellite data and discuss the potential uses and limitations of this data source in support of vector control efforts.

DATA AND METHODS

We analyzed three satellite images collected by the BGIS 2000 sensor onboard the DigitalGlobe Quickbird satellite. The BGIS instrument is a multispectral instrument, with data collected in six channels providing spectral coverage for wavelengths of 450-900nm (panchromatic), 450 - 520nm (blue), 520 - 600nm (green), 630 - 690nm (red) and 760 - 900nm (near-infrared). In addition to Quickbird, multiple other commercial satellites provide data of comparable spectral and spatial resolution, including the Ikonos and GeoEye satellites (Table 1).

Data subsets for a 5km x 5km region over the Coachella Valley were obtained from Quickbird satellite images collected on the dates of Oct 11, 2004, Jan 17, 2006 and May 28, 2008.

Satellite	Panchromatic	Blue	Green	Red	Near-IR
Quickbird	450 - 900 nm, 0.6 m	450 - 520 nm, 2.4 m	520 - 600 nm, 2.4m	630 - 690nm, 2.4 m	760 - 900 nm , 2.4 m
Ikonos	526 - 929 nm, 0.8 m	445-516 nm, 3.2m	506 - 595 nm, 3.2 m	632 - 698nm, 3.2 m	757 - 893nm, 3.2 m
GeoEye	450 - 800 nm, 0.4 m	450 - 510 nm, 1.65 m	510 - 580 nm, 1.65 m	655 - 690nm, 1.65 m	780 - 920nm, 1.65 m

Table 1. Spectral characteristics and spatial resolution of three commonly used sources of high-resolution commercial satellite imagery.

The study area was defined by a bounding box with the upper left coordinate 33° 44' 15", -116° 15' 46", and the lower right coordinate 33° 41' 26", -116° 12' 24". The imagery was radiometrically normalized by DigitalGlobe, and the 2006 image was orthorectified by DigitalGlobe. The 2004 and 2008 images were orthrectified against the 2006 image using the ITT VIS ENVI software package to an accuracy of 0.5 m.

Initial analysis revealed that using data from the BGIS sensor, well-maintained or "blue" pools with light colored bottom surfaces were clearly distinguishable from other features in the image, but neglected or "green" pools were spectrally indistinguishable from other common features of suburban and urban environments such as irrigation canals and grass covered medians along roadways (Figure 1). To overcome this obstacle, a change detection approach was employed to identify potentially neglected pools in 2006 and 2008 using a map of locations of blue pools to assist in separating neglected pools from other features with similar spectral signatures. Following orthorectification, images were first pan-sharpened using the principal components (PC) spectral sharpening algorithm in ENVI to create data files with a spatial resolution of 0.5 m for further analysis. Multiple band combinations and indices were developed and tested to map blue pools. Of all combinations evaluated, the ratio of the blue to green band provided the greatest power for discriminating the blue, well maintained swimming pools from other objects within an image.

In addition to use of band thresholds, image segmentation techniques were also used to produce the baseline maps of blue pools based on the scale, color, smoothness and compactness of features in 2004 images. Image segmentation was performed using eCognition (Definiens Inc., Alexandria, VA) and also employed a filter based on the ratios of blue and green bands, which was applied to the segmented image to isolate blue pools from other features. While more intensive to computate and difficult to fully automate, this approach produced reasonably accurate maps of blue pools with minimal user supervision (Figure 2).

Following production of the 2004 blue pool mask, green pools were mapped in the 2006 and 2008 imagery using a combination of band ratio thresholds. The output of this calculation was filtered using the blue pool mask to produce the final map of candidates in 2006. A second blue pool mask was calculated for the 2006 image to account for pools not constructed or visible in the 2004 image, and the combined masks derived from the 2004 and 2006 images were used to filter results from the 2008 images (Figure 3).

Data used in validation and testing were provided by the Coachella Valley Mosquito and Vector Control District (CVMVCD) and included the latitude, longitude, type and condition of pools visually inspected by CVMVCD personal and determined to be neglected and in need of treatment. This dataset was reprojected using ArcGIS 9.2 and used to assess the accuracy of the satellite-based mapping method.

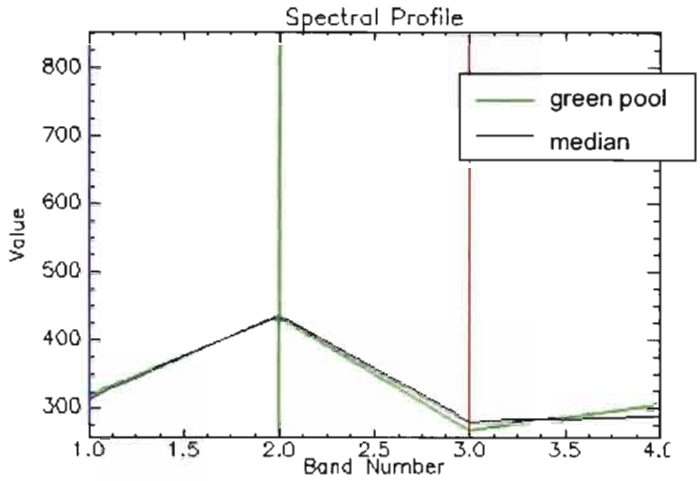

Figure 1. Comparison of the spectral profile of a typical green pool (green line) against the spectral profile of a pixel within a turf-covered street median.

Figure 2. Polygons delineating potential swimming pool locations derived through use of an image segmentation approach.

Figure 3. Output of the unsupervised classification scheme, showing candidate neglected pools mapped by the scheme in green, overlaid upon the 2008 image.

RESULTS

To assess the accuracy of the unsupervised classification scheme, we conducted manual classification based on visual inspection of twenty 200m x 200m subsets of the image to characterize errors. In addition, we also conducted a comparison of the map using unsupervised techniques against 40 known neglected pools provided by CVMVCD to characterize errors of omission and errors of commission. Comparison of results from the unsupervised classification scheme against visual inspection of the imagery and data collected on the ground by VCD personnel yielded initial estimates of errors of omission of 65-71% and errors of commission of 22% for the study area. Visual inspection of the twenty 200m x 200m subsets identified 77 total candidate green pools, of which 55 (71%) were detected by the unsupervised classifications scheme. In addition, of the 71 total candidate pools detected by the algorithm, 16 appeared to be likely false positives based on visual inspection of the image (22% of candidates).

Comparison of the map against the location of 40 known neglected pools provided by CVMVCD indicated that relative to the data collected by field personnel, the unsupervised approach based on the 2008 satellite imagery correctly identified 26 neglected pools (65%) but failed to detect 14 known pools (35%). Of the 14 known pools not detected, six pools were shaded in the 2004 and 2006 imagery, four pools were not present prior to 2008 or were too small to be detected, two pools were empty in 2004 and 2006 and two pools appeared blue and did not appear to be neglected based on visual inspection of the 2008 satellite image.

DISCUSSION

Comparison of the spectral profiles of pools determined to be neglected during on-site inspection by CVMVCD technicians against other features in the image showed that many neglected pools cannot be distinguished spectrally from features common in urban and suburban environments (e.g., including turf-covered roadway median strips, irrigation canals, shaded lawns and asphalt). Thus, it is unlikely that unsupervised techniques will be successful in mapping neglected pools using imagery collected during a single time period.

The relatively distinct spectral characteristics of blue pools facilitated accurate mapping of pool locations, particularly if an image segmentation approach is applied which allows use of information on the spectral dimensions and spatial characteristics (size and shape) to separate blue pools from other features in the image. Use of a change detection approach allowed mapping of potential candidate neglected pools based on shifts in the spectral characteristics of blue pools identified in imagery from prior years. This stage of the mapping can be accomplished using computationally efficient algorithms based on band thresholds and multi-band indexes.

The classification accuracy achieved in this initial study is promising but indicates that additional work is needed to refine the algorithms to improve the mapping accuracies. The maximum achievable accuracy for this method will be limited, however, by factors associated with the spectral and spatial characteristics of current commercial satellite instruments, use of dark materials to line the bottom surface of some well maintained pools, factors associated with normal use of swimming pools (e.g., use of pool covers, construction of shade structures) and limitations inherent in the approach (e.g., interference of sun angle and shading, and inability to detect neglected pools built after the date that baseline satellite imagery was collected).

Despite these limitations, the results suggest that the approach has potential value for supplementing aerial mapping of neglected pools. The relatively low cost of collection and analysis and the ability to process the data rapidly using unsupervised techniques make the use of this approach suitable for early detection or routine monitoring of known neglected pools and foreclosed properties. Very high accuracy mapping of neglected pools will continue to require other approaches such as manual classification of very high resolution airborne imagery and ground-based investigation by field technicians.

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Introduction to the Symposium on Invading and Emerging Arboviruses

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The invasion of the USA and California by West Nile Virus [WNV] provided a wake-up call for public health agencies and demonstrated the inability of the US Public Health system to halt the invasion of mosquito-borne zoonoses (Holloway 2000). Our faltering economy and subsidence of the WNV epidemic have led to reduced spending for surveillance of arboviruses at the national level resulting in the demise of many state programs, a situation that can only facilitate the invasion of the next problem. Many of the factors enhancing this probability were suggested in previous reviews on the pending emergence and dispersal of communicable and vector-borne pathogens (Institute of Medicine 1992). WNV certainly will not be the last vector-borne pathogen to invade California, and this symposium focused on some of the recently emerging arboviruses and their possible introduction to California (Table 1).

Table 1. Listing of the speakers and talks presented in the Symposium on Invading and Emerging Arboviruses.

- Reisen, WK: What will be the next West Nile virus? Receptivity of California and the United States for Invasive Arboviruses
- Clark, D: Detecting invasive viruses: some thoughts for surveillance
- Chen, Ching-I: Chikungunya virus: a macaque model
- Worwa, G: Invasive viruses: the European blue tongue experience
- Mullens, B: Blue tongue virus and Culicoides in California: the next problem for MVCAC?

In this symposium, I first described the ecology and recent movement of selected arboviruses with a high probability of invading California and the USA, including the anthroponoses, chikungunya and dengue viruses, and the zoonoses, Rift Valley fever, Japanese encephalitis and Ross River viruses. David Clark reviewed CVEC's previous attempts to find and identify new viruses within mosquito pools and provided ideas on how to enhance detection and diagnostics. Ching-I Chen described the on-going chikungunya virus epidemic in Africa and Asia and her recent research on experimental infection of Rhesus macaques as a primate model for future pathogenesis and vaccine studies. Gabriella Worwa described the impact of the recent blue tongue virus epizootic in Europe and its financial impact on the livestock and dairy industries. Bradley Mullens provided an update on the blue tongue virus situation in the USA and California and warned of the possible involvement of the MVCAC in *Culicoides* control should an exotic strain invade California.

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What will be the next West Nile Virus? The Receptivity of California for Invasive Arboviruses

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ABSTRACT. The invasion of California by West Nile virus provided a 'wake-up' call for public health agencies and demonstrated the inability of current programs to interrupt the spread and amplification of an invasive zoonosis. The current paper describes the potential for three anthroponoses (dengue, chikungunya and yellow fever) and three zoonoses (Rift Valley fever, Japanese encephalitis and Ross River arthralgia) to invade California. The importance of expanded surveillance for the early detection of invading arboviruses in discussed.

INRODUCTION

Arthropod-borne viruses (arboviruses) are important causes of human and animal disease worldwide. Practically all have their origins as tropical zoonoses where they are maintained at low to moderate enzootic levels, typically with minimal recognized or reported disease (Weaver and Reisen 2010). Changes in landscape, importation of livestock, intensive urbanization in the tropics and invasions of northern latitudes by virulent genotypes have been associated with extensive epidemics as naive host populations are exposed to these pathogens. Extensive human urbanization within tropical ecosystems and the adaptation of several pathogens to these urban environments have given rise to important anthroponoses such as dengue (DENV) and chikungunya (CHIKV) viruses which are transmitted from human to mosquito to human without the need for animal hosts. In these simplified transmission cycles, *Aedes aegypti* and *Ae. albopictus*, which breed in and around homes and feed repeatedly on humans indoors, have become the primary, if not the only, vectors. Other viruses of tropical origin such as Rift Valley fever virus (RVFV) and Japanese encephalitis virus (JEV) have retained their enzootic cycles but recently have shown the potential to expand their global distributions. Other zoonoses such as Yellow fever virus (YFV) and Ross River virus (RRV) are still maintained within enzootic cycles but have the potential to urbanize and be transmitted as arthroponoses.

The decade long invasion of the New World and associated epidemic by West Nile virus (WNV) has been one of the epic sagas of modern arbovirology and has demonstrated the inability of public health agencies to interrupt the advance of an aggressive, mosquitoborne zoonosis (Holloway 2000). Although discovered in Uganda in 1937, it wasn't until the 1950s that this virus was recognized as the cause of large scale febrile epidemics among children in the Nile Delta (Taylor et al. 1956) and the Mediterranean area (Hayes 2001). After repeated incursions and small scale outbreaks of equine and human disease in Europe and Russia (Kramer et al. 2008), Lineage I of WNV carrying a proline mutation enabling infection and high viremia and mortality in American crows (Brault et al. 2007) invaded the New York City area in 1999. Within five short years WNV spread north into Canada and west through the USA to California. By the end of the decade, upwards of a total 1.5 million Americans had been infected, with > 25,000 laboratory confirmed clinical human cases and > 1,000 deaths. In addition, there were > 25,000 equine cases with a 40% fatality rate (Ostlund et al. 2001) and hundreds of thousands of dead birds (Komar 2003). Trend analyses of US bird populations have shown significant declines in multiple corvid and some other species (LaDeau et al. 2007, Wheeler et al. 2008). Thus, WNV has seriously impacted human, veterinary and wildlife health and has had an immeasurable economic cost in morbidity and mortality.

An expanding human population that alters landscapes and climate and progressively concentrates into large sprawling urban areas, coupled with enhanced rapid commercial and tourist travel to the tropics, has set the stage for mosquitoborne arboviruses to extend their current distributions and make incursions into temperate latitudes. Although the current WNV epidemic remains ongoing, it may be useful to examine the potential for other aggressive arboviruses to invade the USA and especially California. The current focus of surveillance testing on high throughput but very specific antigen or RT-PCR assays may prevent the detection of these exotic viruses until they have become established and caused serious human or animal disease. The current paper reviews the potential for several anthroponoses and zoonoses to invade and become established within California.

INVADING ANTHROPONOSES

Three widespread emerging arboviruses, DENV, CHIKV and YFV, have an urbanized transmission cycle that involves *Aedes* mosquitoes in the subgenus *Stegomyia*, including *Ae. aegypti* and *Ae. albopictus,* and humans as the primary host. These viruses have been repeatedly detected in travelers entering or becoming ill within California, but cases resulting from local transmission have not been reported. Although both vector species are well established in the eastern United States, neither species is currently found in California. *Aedes aegypti* has been intercepted at port areas in California and currently has become established in Tuscon, Arizona (Merrill et al. 2005). *Aedes albopictus* was introduced into Los Angeles with 'lucky bamboo' importations

from China, but was eradicated by local mosquito and vector control agencies (Linthicum et al. 2003, Madon et al. 2002). Although eradication here may have been facilitated by the arid conditions of southern California (Washburn and Hartmann 1992), *Ae. albopictus* currently is well established in arid areas of India such as Rajasthan (Angel and Joshi 2009).

Nothing is known of the vector competence or potential for California mosquitoes to transmit any of these three viruses. Although in the subgenus *Ochlerotatus*, *Aedes sierrensis* is one of the few *Aedes* that lives adjacent to and frequently blood feeds on humans; however, their vector competence for these viruses has not been investigated, and their vernal dominated phenology may be somewhat off set with ideal hot temperature conditions required by the tropical viruses. In West Africa, CHIKV (Togaviridae: *Alphavirus*) persists in an enzootic cycle involving several *Aedes* mosquitoes (Chevillon et al. 2008), so therefore it may be useful to investigate the role of California ecological equivalents for their vector competence to evaluate the risk of importation. Currently, the risk of virus importation in travelers would seem high, but the potential for endemic transmission low, because the primary vector species are not found in California, and most local *Aedes* feed frequently on large mammals (Reisen and Reeves 1990).

INVADING ZOONOSES

As illustrated by WNV, zoonoses typically have complex transmission cycles involving a variety of vectors and vertebrate hosts. Humans and domestic animals become infected when transmission in the enzootic cycle amplifies to levels where spill over occurs. Rapid movement by zoonoses is somewhat more restricted than anthroponoses, but still may be facilitated by inadvertent transport of infected vectors, infected vertebrates as potential pets, domestic animals or horses transported to show or sales or by infected migratory hosts. Three viruses, RVFV, JEV and RRV, have expanded their distributions in recent years and may pose a risk for introduction.

RVFV (Bunyaviridae: *Phlebovirus*) is endemic to the Rift Valley of East Africa where it has caused widespread outbreaks in domestic cattle and humans (Meegan and Bailey 1989). The virus persists within vertically infected eggs of several floodwater *Aedes* spp. until rainfall events cause local flooding of ground pools and stimulates hatching (Linthicum et al. 1987). Ground pools producing infectious *Aedes* mosquitoes also produce new grass and thereby attract wildlife and cattle herds which typically calf at this time, ensuring the intersection of immunologically naïve hosts, host-seeking mosquitoes and RVFV within a localized and permissive environment. Outbreaks originating in East Africa have been spread by domestic animal trade throughout Africa, including Egypt and more recently Saudi Arabia. Recently, Turell et al. (unpublished) evaluated several mosquito species found in California for their vector competence to RVFV. *Culex tarsalis* was by far the most competent host, followed by *Cx. erythrothorax, Cx. erraticus* and *Aedes dorsalis*. Cattle (both beef and dairy) and possibly deer presumably could serve as susceptible vertebrate hosts in California.

JEV arose in tropical Southeast Asia and has since invaded Japan, Korea, China, Taiwan and India causing large annual epidemics (Burke and Leake 1988, Erlanger et al. 2009). The virus is maintained enzootically by transmission among *Culex* mosquitoes (mostly *Culex tritaeniorhynchus*) and ardeid birds, including the black-crowned night heron. Domestic pigs provide amplification near human habitation. Childhood vaccination and changes in porcine husbandry now have all but eliminated infection in Japan, Korea and parts of China, but widespread epidemics persist in India and Nepal. In California, seven species mosquitoes in three genera (including *Cx. tarsalis, Cx. pipiens* and *Cx. quinquefasciatus*) were found to be competent vectors (Reeves and Hammon 1946), and several avian species were found to be competent vertebrate hosts (Hammon et al. 1951). In addition, California has a large population of feral swine as well as extensive rookeries of black-crowned night herons. Collectively, this would seem to put California at some risk for the establishment of JEV should it be imported and inadvertently released (Nett et al. 2009).

In Australia, Ross River virus is maintained in a cycle involving several macropods and *Aedes* mosquitoes (Russell 2002). Like CHIKV, RRV causes a severe epidemic arthralgia. The primary vectors, *Aedes vigilax* and *Aedes camptorhynchus*, are floodwater species similar to *Aedes taeniorhynchus* and *Aedes dorsalis/melanimon* which are found in California. Vertical transmission to drought resistant eggs has been reported (Kay 1982) and may be the key reason outbreaks have been tied to high rainfall anomalies (Harley and Weinstein 1996, Woodruff et al. 2002). During outbreak transmission, humans may produce sufficient viremia to infect susceptible *Aedes,* thereby raising the risk of virus importation through infected viremic travelers.

CONCLUDING THOUGHTS

The potential for the importation of unknown or emerging viruses into California would seem high. An expanding human population with increasing travel would seem key to pathogen dispersal, and therefore transportation hubs may be the most likely place for introductions. Successful invasion requires the presence of competent vectors and/or susceptible hosts within a suitable landscape and climate or season. If any of these factors is missing, establishment would seem unlikely. Anthroponoses would seem most likely for introduction because they are prevalent in tropical urban centers frequented by tourists and because human viremias are sufficient to infect mosquito vectors. Detection and therefore containment would seem likely if most infections in humans are apparent. At present, the primary vectors are not found within California, but it is currently unknown if California mosquitoes are capable of transmitting these viruses. Zoonoses will be more difficult to detect until virus amplification reaches levels that include human or domestic animal infection. Containment therefore may be difficult because by the time human infection is detected, the virus has already amplified in animal hosts.

Expanding the current statewide surveillance program to test

for an extended array of viruses may be one way to enhance the detection of arbovirus importations. Current surveillance efforts focus on virus isolation using a variety of different cell culture techniques. These efforts previously detected the presence of California encephalitis and Jamestown canyon viruses within *Aedes* and *Culiseta* that had tested negative for WNV, western equine encephalomyelitis and St. Louis encephalitis viruses (Fang et al. 2009). Subsequent efforts using new molecular tools discovered high infection rates of *Culex* with several new Flaviviruses (Armijos et al. 2009). Although both methods have produced positive results, there is a strong need to improve detection systems to capture with relatively high throughput emerging viruses. During the coming year, our research will focus on new Luminex assays to expand the search for the next emerging arbovirus problem.

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The Application of Metagenomics to Arbovirus Surveillance

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In the past two decades there has been a perceptible shift in the dogma of arbovirus surveillance. Formerly, many regional networks monitored the prevalence and distribution of a small number of pathogens endemic to an area or those pathogens that possessed a history of epidemic potential. Serological assays often failed to identify emergent pathogens because many related viruses exhibit limited antigenic cross-reactivity. Additionally, the broad application of virus isolation in suckling mice or vertebrate cell lines as an initial step in the identification of arboviruses presupposes that the circulating viruses are capable of inducing cytopathic effects in the selected culture system.

The rapid emergence of pathogens such as West Nile Virus in the United States and chikungunya virus across the Indian Ocean have highlighted the unpredictable nature of virus evolution and the inherent dangers of exclusively focusing on the 'devil you know.' Indeed, prior to the mid 1990's, West Nile Virus was considered to be a relatively minor pathogen of humans that was responsible for sporadic outbreaks of a mild disease, principally in Africa and occasionally in the Middle East and central Europe. However, the acquisition of limited numbers of genetic changes permitted increased replication in amplifying avian hosts (Brault et al. 2007), improved vector competence (Moudy et al. 2007) and increased virulence (Beasley et al. 2002, Brault et al. 2007). Similarly, the recent epidemic strain of chikungunya virus acquired a single amino acid substitution that dramatically improved competence of this virus in *Aedes albopictus* (Tsetsarkin et al., 2007). This mutation and the establishment of *Aedes albopictus* in Italy contributed to the first reported local transmission of this virus in Europe (Bonilauri et al. 2008).

Concordantly, there has been an expansion in the use of PCR methodologies that employ virus group-specific primer sets to identify arboviruses directly from homogenized vertebrate or vector samples. This approach has resulted in the identification of a vast array of novel arboviruses (Ha et al. 2005, Hoshino et al. 2007, Aranda et al. 2009, Crabtree et al. 2009, Charrel et al. 2009, Zhioua et al. 2010, Quan et al. 2010). However, the use of virus group-specific primers precludes identification of novel viruses that lack sufficient sequence identity with known arboviruses for which sequence data are available.

The burgeoning field of metagenomics has been employed to identify the viral repertoire in various biological samples including seawater (Rosario et al. 2009), fecal samples (Li et al. 2010) and respiratory aspirates (Willner et al. 2009). By employing random amplification of viral genetic material in such samples, investigators seek to obtain an unbiased representation of the virome of a given microenvironment. Next generation sequencing platforms such as 454 sequencing and Solexa that provide massively parallel short sequence reads have been widely adopted for virus discovery. However, these techniques are too expensive to employ on a routine basis in a diagnostic capacity and require specialized equipment and training.

A relatively inexpensive alternative to this approach is the use of sequence-independent amplification of virus particle-associated genetic material (Stang et al. 2005). Contaminating RNA and DNA present in biological samples are eliminated by treatment with nucleases. The viral genetic material that is encased in the virion is protected from this treatment, resulting in enrichment of viral RNA/DNA. The viral genome is then subjected to sequence-independent amplification using random primers, and the resulting amplicons are then either cloned into plasmids for sequencing or sequenced directly through the introduction of sequence tags at the 5' end of random primers. This methodology is inexpensive and utilizes equipment and reagents commonly available in molecular virology laboratories. Recently, this technique enabled the identification of Eyach virus in *Culex tarsalis* mosquitoes collected in California; the first time that this virus has been isolated in the US (Victoria et al. 2008). It has also been employed to identify dengue virus and Culex flavivirus in *Aedes* spp. mosquitoes.

PCR amplification of particle-protected virus genomes is emerging as an indispensible tool for the identification of viruses that cannot be characterized with sequence-dependent methodologies. The main limitation of this technique is its relatively low sensitivity when compared to virus-specific approaches. However, this technique may find broad use in concert with existing techniques to characterize unidentified isolates and to survey mosquitoes for novel arboviruses.

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Chikungunya Virus Infection in a Nonhuman Primate Model

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INTRODUCTION

The name "Chikungunya" means "that which bends up" in the Makonde language and describes the extreme joint pain (arthrigia) in patients that can last for weeks, months or even years. Similar to western equine encephalomyelitis and Ross River virus, Chikungunya virus (CHIKV) belongs to the genus *Alphavirus* in the Togaviridae family. This virus is an enveloped virus with a positive-sense RNA genome. CHIKV was first isolated from the blood of a febrile patient in Tanzania in 1952, but some researchers believe that enzootic and endemic Chikungunya fever (CHIKF) transmission has been ongoing in Africa, and perhaps Asia, for more than 200 years based on medical historical documents (Weaver and Reisen 2010). CHIKF endemic areas overlap with the distribution of Dengue fever virus, making the exact etiology of infection difficult to determine clinically and complicating the historical documentation of CHIKF emergence as a human pathogen. It is certain that over the past fifty years, CHIKV has expanded its geographic range in eastern Africa, central and southeastern Asia and Europe, with an increasing frequency of urban outbreaks. Examples include an Indian Ocean outbreak in 2004~2007, where more than 2 million CHIKF cases were reported. In the Saint-Denis Reunion outbreak of September 2006, it was estimated that more than 266,000 residents were infected (one third of the population) with 45,000 cases per week during the peak of the epidemic. In addition, there were 248 death certificates issued with CHIKV infection the possible cause of death (Powers and Logue 2007). Most of the fatal cases were patients older than 61 years. Researchers have estimated there were at least 6.5 million CHIKF cases in India during the recent epidemic, but many cases went unreported or misdiagnosed (Townson and Nathan 2008).

Chikungunya disease can range from mild to severe, with symptoms including fever, headache, fatigue, nausea, vomiting, muscle and joint pain, skin problems (e.g., rash, ulcer, hyperpigmentation, keratolysis, bulla, purpura, eczema), conjunctivitis, neurologic complications and even death. In the recent 2004 - 2010 outbreaks, previously unreported neonatal transmission and neurological syndromes were associated with CHIKV infection for the first time (Sudeep and Parashar 2008). These neurological disorders, including meningoencephalitis, seizures and Guillain-Barré syndrome, also have been observed more frequently in newborns and young children. In addition, anecdotal evidence of preterm abortion has been associated with CHIKV infection.

Although the presence of Chikungunya fever has been well documented in Africa and Asia, the vectorial capacity of possible CHIKV vectors is still largely unknown. Historically, *Aedes aegypti* was the primary urban CHIKV vector. However, in recent CHIKF outbreaks (2004 – 2010), *Aedes albopictus* has played an important role in human transmission in various countries world-wide, including Saint-Denis Reunion, India and Italy. Laboratory studies indicate that several *Aedes* and one *Anopheles* species can be experimentally infected with Chikungunya virus (*Ae. aegypti, Ae. albopictus, Ae. furifer, Ae. taylori, Ae. luteocephalus, Ae. africanus, Ae. neoafricanus, Ae. vittatus* and *Anopheles stephensi*) (Chevillon et al. 2008). *Aedes albopictus* has greatly expanded its geographic range in recent years to include much of Mediterranean Europe and the southeastern United States, increasing the risk of CHIKV introduction into these areas. The following elements are specifically of concern:

(1) **The introduction of new mosquito vectors of CHIKF into California.** In California, we have a variety of mosquito species that feed on humans. The introductions of *Ae. albopictus* into California, as well as the widespread distribution of *Ae. aegypti* in neighboring Arizona, raises significant concerns about the possibility of these species becoming established and thereby increase the risk of CHIKV outbreaks. Moreover, the vector competence of endemic California mosquitoes for CHIKV is currently unknown.

(2) **A high-density human population that is immunologically naïve for CHIKV.** The previous lack of CHIKV in California suggests that the resident human population is potentially highly susceptible to infection by this virus.

(3) **Multiple viremic travelers provide a mode of introduction in to the United States.** In 2006, for example, the CHIKV outbreak strain was isolated in Orange County, CA from a viremic traveler. Because California is a popular tourist destination and a major entry point into the United States, the state provides an ideal route for introducing CHIKV through travelers. Moreover, California is also a major importer of agriculture products, providing a route for the introduction of infected mosquito vectors. For example, the importation of lucky bamboo resulted in the introduction of *Ae. albopictus*, the principle vector of recent CHIKF outbreaks, into the Los Angeles area.

(4) **Environmental factors**. The warm weather in California and suburban areas with extensive peridomestic larval habitats would help facilitate establishment of vector populations and CHIKV.

METHODS, RESULTS AND DISCUSSION

In light of the magnitude of recent epidemics, the previously unreported clinical signs and the apparent pre-partum neonatal transmission, a non-human primate model was needed to study CHIKF disease progression and pathogenicity. Specifically, an appropriate model would help to identify prognostic markers of disease and to study the novel disease phenotype of the epidemic (human-mosquito-human transmission) versus enzootic (non-human primate-mosquito-primate transmission) CHIKV strains. As primates possess physiological characteristics more reflective of humans, we developed a pregnant Rhesus macaque model. Complete details of the experimental infection study have been accepted for publication (Chen et al. 2010) and are described briefly below.

To mimic natural transmission by mosquito bite, pregnant rhesus macaques in third trimester were inoculated subcutaneously with biological relevant doses of either a recent 2006 epidemic CHIKV strain or an enzootic strain isolated in 1983. All macaques developed viremias that persisted for 4-5 days with peak viral magnitudes of 5-6 $\log_{10}$ plaque forming units [PFU]/mL observed at 2-3 days post-inoculation (dpi). All macaques showed fever, signs of joint swelling and leucopenia. The macaque infected with the CHIKV-enzootic strain that exhibited the highest viremia titer also showed more severe muscle/joint swelling, an increase in joint temperature and signs of skin rash. These symptoms are comparable to human CHIKF in which muscle lesions are only found in more severely diseased individuals. Viral RNA detection in all maternal lymphoid tissues, some joint-associated skeletal muscle and connective tissues and spinal cord at 21 dpi provided evidence of CHIKV persistence after viremia subsidence in our experimental macques. These findings identify tissues where viral persistence may contribute to the protracted arthralgic syndrome observed in humans and some of the pathophysiological differences observed between CHIKV strains; they also could explain the disease severity of the recent epidemic genotype. However, the absence of viral RNA in fetal tissues and the lack of germinal center development in fetal lymph nodes indicated that trans-placental transmission of CHIKV did not occur in the current experiment. Our results suggest that neonatal transmission likely occurs as a result of active viremia during parturition.

In our study, all Rhesus macaques survived infection and produced viremias sufficient to infect blood feeding *Aedes* mosquitoes. Rhesus macaques are widely distributed through south and southeastern Asia. Based on our and previous findings (Paul and Singh 1968, Sempala and Kirya 1973), Rhesus macaques may serve as excellent reservoir hosts for CHIKV in Asia because of asymptotic infection, elevated viremia and an urban/periurban distribution overlapping with *Ae. aegypti* and *Ae. albopictus*, especially in India. *Aedes aegypti* previously was the primary urban vector of epidemic CHIKV, while the recent 2004~2007 outbreaks were transmitted primarily by *Ae. albopictus*. *Aedes albopictus* has been circumglobally dispersed by commercial trade in used tires (Reiter 1998, Reiter and Sprenger 1987) and can be infected with a lower dose of CHIKV than *Ae. aegypti* (de Lamballerie et al. 2008). As a result, the recent epidemic strain may not require elevated host viremias for infection. Therefore, the current massive outbreaks caused by the CHIKV-epidemic strain, in part, may have been facilitated by the previous expanded circumglobal dispersal of *Ae. albopictus*.

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Invasive viruses: The European bluetongue experience

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ABSTRACT: Although Bluetongue virus (BTV) is distributed worldwide, its epidemiology has profoundly changed in recent years. Our summary points out parallels, but also some of the fundamental differences, between the recent BTV situation in Europe compared to North America.

INTRODUCTION

Bluetongue virus (BTV) is a vector-transmitted orbivirus and member of the family Reoviridae. The 24 different recognized serotypes of BTV are the causative agents of bluetongue disease (BT) in ruminants and are distributed worldwide. The presence of BTV is strictly limited to areas where competent *Culicoides* spp. are abundant and serve as the principal vector for BTV (Mellor 1990). In tropical areas the disease is endemically present throughout the year, whereas in temperate regions BTV outbreaks occur during the warm months of summer and fall which are favorable for both virus and vector (Gibbs and Greiner 1994). BTV re-emerges in most areas each season after over-wintering; however, the mechanism of persistence in temperate regions remains poorly understood (Saegerman et al. 2008). Infected female midges of *Culicoides* spp. transmit BTV by feeding blood on susceptible ruminant hosts (Mellor 1990). Sheep and certain species of deer are most susceptible to clinical disease expression, whereas in cattle infections are typically unapparent, although producing high levels of viremia (Gibbs and Greiner 1994). The pathogenesis of BT is characterized by disorders of the vascular system such as leakage (i.e., edema, hemorrhages) and thrombi formation (MacLachlan et al. 2008). Fulminant cases of BT may occur due to fatal lung edema. Because of its impact on animal health and due to trade restrictions that become effective in BTV-affected areas, BT is regarded to be an emerging disease of major veterinary importance.

DISCUSSION

Epidemiology. BT was first described in Southern Africa, but is now present circumglobally between approximately latitudes 34°S and 53°N in Africa, the Middle East, Australia, Asia, the Americas and Europe (Mellor and Wittmann 2002). In North America BTV occurrence was first reported in the 1950s with the introduction of serotypes -2, -10, -11, -13 and -17. Recently, eight new serotypes (-1, -3, -5, -6, -14, -19, -22, -24) and epizootic hemorrhagic disease virus (EHDV) have been identified in the United States (MacLachlan 2010). Europe was mostly free from BTV until 1998, but thereafter incursions of serotypes -1, -2, -4, -9 and -16 into the Mediterranean Basin and Balkan region were recorded (Purse et al. 2005). During the subsequent eight years, circulation of these serotypes was restricted to the southern parts of Europe. The virus appeared in northern Europe for the first time during 2006 when outbreaks of BTV serotype 8 (BTV-8) were reported in Belgium, the Netherlands, Luxembourg, France and Germany (Saegerman et al. 2008). This is remarkable because BTV-8 was a previously unknown serotype to Europe, and secondly because BTV in general has never appeared that far north. In subsequent years BTV-8 not only re-emerged in the regions of its primary emergence, but also rapidly spread into neighboring countries such as the Czech Republic, Hungary, Sweden, Switzerland, Italy and United Kingdom (Saegerman et al. 2008).

Vectors. The principal vector of BTV in North America is *Culicoides (C.) sonorensis,* whereas in South America it is *Culicoides insignis* (Tabachnick 2004). However, little comprehensive surveillance is currently carried out in order to investigate putative new vectors for BTV in these regions. The emergence of BTV in southern Europe was attributed to *Culicoides imicola* which expanded its distribution further northward, most probably due to global warming (Purse et al. 2005). Its absence in northern Europe falsely raised hopes to remain free from BTV. However, the recent BTV-8 epidemic revealed that *Culicoides* vectors other than *C. imicola* were involved in BTV transmission in northern parts of Europe, including *C. obsoletus*, *C. pulicaris*, *C. dewulfi*, *C. chiopterus* and *C. scoticus* (Meiswinkel et al. 2008). Midges from the *Culicoides obsoletus* complex are also present in North America, but have not been associated with transmission.

Phenotype. During the recent BTV-8 epidemic affecting northern Europe, numerous reports appeared regarding the unique phenotypic behavior of the invading BTV-8 strain. These observations concerned mainly intrauterine infections and unusually pronounced clinical disease in cattle. Although BTV infected cattle rarely developed distinct clinical signs of BT, the BTV-8 outbreak in northern Europe demonstrated increased susceptibility of cattle to that particular strain (Dal Pozzo et al. 2009). Transplacental infections in cattle and sheep resulted in PCR-positive animals and/or congenital abnormalities (Darpel et al. 2009, Worwa et al. 2009). This feature usually was attributed

only to cell-culture adapted and attenuated live virus vaccine strains. Although BTV-8 field viruses seem to show this unique characteristic, it has not yet been proven that transplacental transmission of the virus would be beneficial for its over-wintering persistence, and its epidemiological significance still needs further exploration. The transmission of the virus to calves by feeding colostrum containing BTV represents another potential source of BTV infection that also was described for northern European BTV-8 (Backx et al. 2009). However, a recent study showed that colostral BTV transmission also occurs in California dairy herds (Mayo et al. in press).

Outlook. To date, BTV-8 has been the cause of a major European outbreak affecting animal health and trade causing enormous economical losses in livestock and industry. Affected countries have responded with widespread, on-going vaccination campaigns using monovalent inactivated vaccines. However, incursions of new serotypes such as BTV-15 and further spread of BTV-1 northwards could represent new threats to Europe that will not be resolved by the current vaccination program. Furthermore, discovery of new viruses such as Toggenburg orbivirus, a 25th serotype of BTV, demonstrate the presence of additional, but unknown, orbiviruses in Europe (Hofmann et al. 2008).

The recent incursions of new BTV serotypes and EHDV into North America may be indicative of changes in ecosystems and perhaps competence of the different vector species. These findings stress the need for comprehensive surveillance to increase the preparedness for BTV and to avoid the catastrophic outbreak scenario experienced in Europe.

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Bluetongue Virus and *Culicoides* in California: the Next Problem for MVCAC?

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Scientists and operational personnel from the districts that comprise the California Mosquito and Vector Control Association (CMVCA) have a lot of valuable experience with surveillance and control of arbovirus vectors. It is quite possible that an exotic or virulent strain of one of the *Culicoides*-transmitted ruminant arboviruses, bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV), could break out in the western USA. If that were to happen, it is likely that CMVCA members would at least be expected to know about the issues, and perhaps could assist directly with monitoring, vector suppression and public education. These viruses, in addition to causing ruminant disease, may result in animal movement restrictions that can be expensive or crippling to international trade (MacLachlan and Osburn 2006).

A cline in cattle exposure to BTV exists, with the highest exposure levels (sometimes exceeding 50% prevalence of BTV antibodies in cattle at slaughter) in the southwestern USA. Antibody prevalence declines in the eastern and particularly the northern US states, with almost no transmission between northern Washington and New England. This geographic pattern corresponds with the distribution of *Culicoides sonorensis* which is regarded as the primary vector of BTV (Tabachnick 1996). Literature prior to 2000 refers to this as *C. variipennis* (or *C. variipennis sonorensis*). The three recognized members of what was called the *C. variipennis* complex are mammal-feeders in the subgenus *Monoculicoides*, and their larvae inhabit surface mud in fine sediment beds at the edges of ponds or slowly-moving water (Holbrook et al. 2000). In California, *Culicoides occidentalis* is found in saline and alkaline habitats such as the shoreline of the Salton Sea and Borax Lake, while *C. sonorensis* is common in habitats polluted by animal excrement, such as dairy wastewater ponds (Holbrook and Tabachnick 1995).

Until the last few years, only 5 of the 25 serotypes of BTV were known from the USA; serotypes 10, 11, 13, and 17 are most common, whereas serotype 2 is sporadically reported from the deep southeastern USA. Of the 8 serotypes of EHDV, serotypes 1 and 2 have been found in the USA in the past. However, recently a large number of new serotypes have been discovered from ruminant blood samples in the southeastern and southcentral USA including BTV serotypes 1, 3, 5, 6, 14, 19 and 22 and EHDV-6 (Johnson et al. 2007, Allison et al. 2010). Repeated recovery of live viruses is evidence that they are actually being transmitted there. The vector(s) responsible are unknown, although some sites are outside the range where *C. sonorensis* is common. This suggests that *Culicoides* spp. other than, or in addition to, *C. sonorensis* are involved in the BTV transmission cycle.

There are compelling epidemiological reasons to consider *C. sonorensis* the primary BTV (and perhaps also EHDV) vector to domestic ruminants in the USA (Tabachnick 1996). However, there are several studies in the Southeast and California where other *Culicoides* already have been suspected of transmitting the typical USA virus serotypes in natural settings; this is particularly so where *C. sonorensis* is rare or appears to lack appropriate vectorial capacity characteristics (e.g. Mullens and Dada 1992, Smith and Stallknecht 1996). Further, EHDV is common in deer in parts of the USA such as the northcentral states, where *C. sonorensis* is rare or absent. It is likely that BTV and EHDV transmission by species other than *C. sonorensis* has occurred routinely in parts of the USA, particularly to wild ruminants.

BTV historically has been absent from western and central Europe except for transitory and limited outbreaks in the southernmost regions, mostly near the Mediterranean. Unprecedented and persistent transmission has occurred in southern Europe since 1999, and a major outbreak of BTV-8 began in northern Europe in 2006 and persists to this day (Carpenter et al. 2009). The BTV-8 outbreak, extending as far north as southern Scandinavia, was surprising and has created a great deal of scientific and governmental interest. While the most famous Old World vector, *C. imicola*, was at least known from southern Europe (although frequently uncommon relative to other *Culicoides* spp. in the region), it is completely unknown from the zone of the more recent northern European outbreak (Meiswinkle et al. 2008). While it already was apparent that species other than *imicola* were probably involved in BTV transmission, even in southern Europe, this "smoking gun" (i.e., lack of *C. imicola* in the northern European outbreak zone) led European entomologists and veterinary health authorities to intensify investigations of other *Culicoides* spp.

Americans should pay close attention to what has happened with bluetongue in Europe. In the early stages of the southern European outbreak, it was assumed a range expansion of *C. imicola* contributed substantially to the increased transmission. In fact, little organized surveying or long-term monitoring of *Culicoides* spp. had been done, making it difficult either to support the idea of *C. imicola* expansion or to judge objectively the likelihood of involvement by other potential vector species. After some time, it became apparent that members of the *Culicoides* subgenus *Avaritia*, such as *C. obsoletus*, were involved and were likely primary vectors (Carpenter et al. 2009). Females of this group are notoriously difficult to identify, but European scientists quickly moved to resolve identification issues using molecular techniques combined with traditional morphology (see Meiswinkle et al. 2008).

North America has some of these same issues on a geographic scale similar to that of Europe. We now know quite a bit about *C.*

sonorensis biology, distribution and also its vector competence, because it is one of the very few species of *Culicoides* colonized. However, our knowledge of the more than 150 other species of *Culicoides* in North America is poor. For example, the USA already has what is currently being called *C. obsoletus* and another suspected European BTV vector in the subgenus *Avaritia*, *C. chiopterus* (Borkent and Grogan 2009). It would not be surprising if what we are calling *C. obsoletus* or *C. chiopterus* here prove to be different from their nominal European counterparts, but we just don't know yet. Species of *Culicoides (Avaritia)* are abundant mammal-feeders in North America and a logical group to investigate with regard to potential BTV or EHDV transmission, and that is only one example. *Culicoides* in subgenera other than *Avaritia* certainly also transmit BTV in various global locations, and survey and epidemiological work would need to approach the concept of potential virus vectors region-by-region. Further, we need to think outside of the traditional focus on agricultural ruminants and include wild ruminants, their habitats and the *Culicoides* spp. associated with them in our attempts to understand fully the epidemiology of these arboviruses.

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Surveillance for Mosquito-borne Encephalitis Virus Activity in California, 2009

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INTRODUCTION

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

In 2009, the surveillance program included the following elements:

(1) Diagnostic testing of specimens from human patients exhibiting symptoms of encephalitis, aseptic meningitis, acute flaccid paralysis or with unexplained febrile illness of more than seven days.
(2) Diagnostic testing of specimens from horses exhibiting clinical signs of viral neurologic disease compatible with western equine encephalomyelitis virus (WEEV), WNV and other arboviruses as appropriate.
(3) Monitoring abundance and testing of mosquitoes for the presence of St. Louis encephalitis virus (SLEV), WEEV, WNV and other arboviruses as appropriate.
(4) Serological monitoring of sentinel chickens for SLEV, WEEV and WNV antibodies.
(5) Surveillance and diagnostic testing of dead birds, especially crows and other birds in the family Corvidae, and tree squirrels for infection with WNV.
(6) Weekly reporting in the CDPH Arbovirus Surveillance Bulletin of arbovirus testing results in California and arbovirus activity throughout the United States.
(7) Bi-weekly posting of WNV information, including test results, reports, maps and public education materials on the California WNV website: www.westnile.ca.gov.
(8) Mapping dead bird reports using the WNV Dynamic Continuous-Area Space-Time (DYCAST) model to identify areas of peak WNV activity.
(9) Data management and reporting through the web-based California Surveillance Gateway, including auto-generated weekly outbreak risk estimates for participating agencies.

Only West Nile Virus was detected in 2009; a summary of WNV infections by county is in Table 1.

Table 1. Infections with West Nile virus in California, 2009

County	Humans[a]	Horses	Dead Birds	Mosquito Pools	Sentinel Chickens	Dead Squirrels
Alameda	0	0	10	1	0	0
Alpine	0	0	0	0	0	0
Amador	0	0	1	0	0	0
Butte	2	0	13	5	35	0
Calaveras	0	0	0	0	0	0
Colusa	0	0	2	0	0	0
Contra Costa	5	1	45	17	13	2
Del Norte	0	0	0	0	0	0
El Dorado	1	0	9	0	0	0
Fresno	13	1	62	132	17	1
Glenn	0	0	6	0	3	0
Humboldt	0	0	0	0	0	0
Imperial	0	0	0	3	42	0
Inyo	0	0	0	0	0	0
Kern	21	0	28	138	103	1
Kings	3	0	9	105	0	0
Lake	0	0	2	2	1	0
Lassen	0	0	0	0	0	0
Los Angeles	25	1	82	99	41	0
Madera	1	0	11	3	7	0
Marin	0	0	0	0	0	0
Mariposa	0	0	0	0	0	0
Mendocino	0	0	0	0	0	0
Merced	4	3	9	11	14	0
Modoc	0	0	1	0	0	0
Mono	0	0	0	1	0	0
Monterey	1	0	4	0	0	0
Napa	0	0	0	0	3	0
Nevada	0	0	0	0	0	0
Orange	4	0	10	16	0	0
Placer	0	0	4	40	11	0
Plumas	0	0	0	0	0	0
Riverside	5	1	2	27	37	0
Sacramento	0	2	28	36	2	1
San Benito	0	0	0	0	0	0
San Bernardino	2	0	18	54	14	2
San Diego	4	0	39	5	5	0
San Francisco	0	0	1	0	0	0
San Joaquin	12	3	24	83	10	0
San Luis Obispo	0	0	1	0	0	0
San Mateo	0	0	1	0	0	0
Santa Barbara	0	0	1	0	0	0
Santa Clara	0	0	14	14	0	2
Santa Cruz	0	0	1	0	0	0
Shasta	0	1	9	2	0	0
Sierra	0	0	0	0	0	0
Siskiyou	0	0	0	0	0	0
Solano	0	1	3	2	13	0
Sonoma	0	0	0	0	0	0
Stanislaus	14	2	28	94	25	0
Sutter	0	0	0	25	11	0
Tehama	0	1	0	0	2	1
Trinity	0	0	0	0	0	0
Tulare	9	1	25	131	28	0
Tuolumne	0	0	2	0	0	0
Ventura	0	0	3	0	0	0
Yolo	2	0	7	16	4	0
Yuba	1	0	0	1	0	0
State Totals	**129**	**18**	**515**	**1,063**	**441**	**10**

[a]Includes asymptomatic infections

HUMAN DISEASE SURVEILLANCE

Serological diagnosis of human infection with WNV and other arboviruses was performed at the CDPH Viral and Rickettsial Disease Laboratory (VRDL) and 26 local county public health laboratories. Local laboratories tested for WNV using an IgM or IgG immunofluorescent assay (IFA) and/or an IgM enzyme immunoassay (EIA). Specimens with inconclusive results were forwarded to the VRDL for confirmation or further testing with a plaque reduction neutralization test (PRNT). Additional WNV infections were identified through testing for viral RNA performed at blood donation centers.

The first reported WNV case in 2009 was a 70 year old male resident of Los Angeles County who developed symptoms compatible with West Nile neuroinvasive disease (WNND) on May 12. In total, 112 clinical WNV cases were identified among residents of 19 counties in California (Fig. 1), a 75% decrease from the 445 reported cases in 2008 (Table 2). Case incidence was highest (2.6 cases per 100,000 persons) in Stanislaus County (Fig. 1). An additional 17 asymptomatic infections were identified among blood donors. Of the 112 cases, 45 (40%) were classified clinically as West Nile fever, and 67 (60%) were neuroinvasive disease (i.e., encephalitis, meningitis or acute flaccid paralysis). The median age for all cases for which data were available was 54 years (range: 7 - 88 years) and 67 (60%) of the cases were male. The median ages for West Nile fever and neuroinvasive cases were 51 years (range: 10 - 88) and 57 years (range: 7 - 87 years), respectively. The median age of the 4 WNV-associated fatalities was 69 years (range: 50 - 84 years).

RESULTS

Equine Surveillance. Serum or brain tissue specimens from horses displaying neurological signs were tested for arboviruses at the California Animal Health and Food Safety (CAHFS) laboratory. West Nile virus infection was detected in 18 horses from 12 counties (Table 1); none had been vaccinated against WNV. Eight (44%) of the horses died or were euthanized as a result of their infection.

Mosquito Surveillance. Forty-six agencies in 34 counties collected a total of 745,244 mosquitoes (22,137 pools) which were tested by a real-time polymerase chain reaction test (RT-PCR) for SLEV, WEEV and WNV viral RNA (Table 3) at CVEC and one local mosquito and vector control agency. An additional

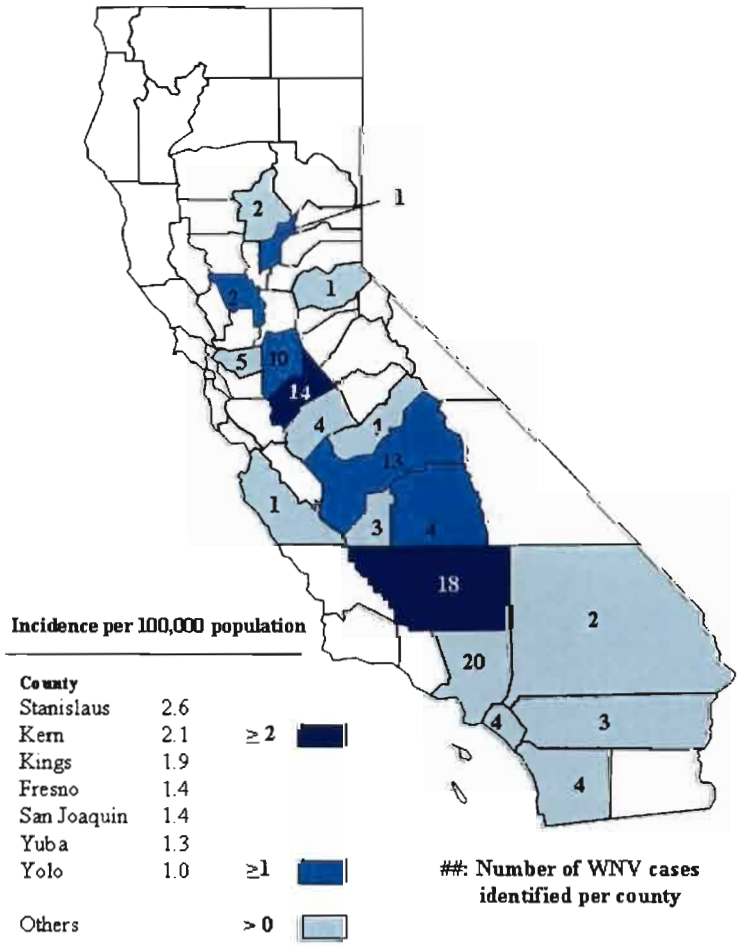


Figure 1. Human cases of West Nile virus infection, California 2009.

Table 2. Reported West Nile virus human cases by county of residence, California, 2003-2009

County	2003	2004	2005	2006	2007	2008	2009	Incidence per 100,000 person-years
Alameda	0	0	1	1	0	1	0	0.0
Alpine	0	0	0	0	0	0	0	0.0
Amador	0	0	3	0	0	0	0	1.1
Butte	0	7	24	31	16	6	2	5.4
Calaveras	0	0	2	0	0	1	0	0.9
Colusa	0	0	2	4	2	1	0	5.5
Contra Costa	0	0	11	8	3	4	5	0.4
Del Norte	0	0	0	0	0	0	0	0.0
El Dorado	0	0	1	2	0	1	1	0.4
Fresno	0	11	59	11	17	3	13	1.7
Glenn	0	3	13	12	7	1	0	16.9
Humboldt	0	0	1	0	0	0	0	0.1
Imperial	1	1	1	1	3	0	0	0.5
Inyo	0	0	0	0	0	0	0	0.0
Kern	0	59	67	49	140	2	18	5.6
Kings	0	0	32	1	7	2	3	4.0
Lake	0	1	0	2	0	0	0	0.6
Lassen	0	1	0	0	0	0	0	0.4
Los Angeles	1	306	40	13	36	156	20	0.8
Madera	0	0	18	0	2	0	1	1.9
Marin	0	0	0	1	0	0	0	0.1
Mariposa	0	0	0	0	0	0	0	0.0
Mendocino	0	0	0	0	2	0	0	0.3
Merced	0	1	25	4	4	1	4	2.1
Modoc	0	0	0	2	0	0	0	2.7
Mono	0	0	0	1	0	0	0	1.0
Monterey	0	0	0	0	0	0	1	0.0
Napa	0	0	0	1	1	0	0	0.2
Nevada	0	0	4	1	0	0	0	0.7
Orange	0	62	17	6	9	71	4	0.8
Placer	0	1	35	8	4	6	0	2.3
Plumas	0	0	1	0	0	0	0	0.7
Riverside	1	109	103	4	17	62	3	2.0
Sacramento	0	3	163	15	25	13	0	2.2
San Benito	0	0	0	0	0	0	0	0.0
San Bernardino	0	187	33	3	4	36	2	1.8
San Diego	0	2	1	1	15	35	4	0.3
San Francisco	0	0	2	0	0	0	0	0.0
San Joaquin	0	2	34	8	10	12	10	1.5
San Luis Obispo	0	1	0	1	0	0	0	0.1
San Mateo	0	0	1	0	0	0	0	0.0
Santa Barbara	0	0	2	0	0	1	0	0.1
Santa Clara	0	1	5	5	4	1	0	0.1
Santa Cruz	0	0	0	0	0	0	0	0.0
Shasta	0	5	1	4	9	1	0	1.5
Sierra	0	0	0	0	0	0	0	0.0
Siskiyou	0	0	0	0	0	0	0	0.0
Solano	0	0	5	8	1	1	0	0.5
Sonoma	0	0	1	0	1	0	0	0.1
Stanislaus	0	0	84	11	21	17	14	3.8
Sutter	0	0	9	12	3	0	0	3.4
Tehama	0	10	4	6	4	4	0	6.2
Trinity	0	0	0	0	0	0	0	0.0
Tulare	0	3	56	6	10	5	4	2.6
Tuolumne	0	0	1	0	0	0	0	0.2
Ventura	0	2	1	3	1	0	0	0.1
Yolo	0	1	11	27	2	1	2	3.1
Yuba	0	0	6	5	0	0	1	2.2
Total WNV disease	**3**	**779**	**880**	**278**	**380**	**445**	**112**	**1.1**

Table 3. Mosquitoes and sentinel chickens tested for St. Louis encephalitis[a], western equine encephalomyelitis[a], and West Nile viruses, California 2009.

County	Agency	No. mosquitoes tested[b]	No. mosquito pools tested	WNV + pools	No. flocks	No. chickens	No. sera tested[c]	WNV + sera
Alameda	Alameda Co. MAD	6,485	186	1	2	13	195	0
Alameda	Alameda Co. VCSD	159	3	0	0			
Alpine		0			0			
Amador		0			0			
Butte	Butte Co. MVCD	2,412	63	5	7	77	1,157	35
Calaveras	Saddle Creek CSD	0			1	10	140	0
Colusa	Colusa MAD	0			1	10	130	0
Contra Costa	Contra Costa MVCD	16,868	472	4	5	50	672	13
Del Norte		0			0			
El Dorado		0			0			
Fresno	Consolidated MAD	20,693	577	115	0			
Fresno	Fresno MVCD	1,975	66	9	2	20	245	12
Fresno	Fresno Westside MAD	10,008	340	7	2	22	286	5
Glenn	Glenn Co. MVCD	2,060	42	0	1	11	132	3
Humboldt		0			0			
Imperial	Coachella Valley MVCD	4,812	100	3	2	20	223	25
Imperial	Imperial Valley VCD	0			4	20	197	17
Inyo	Owens Valley MAP	3,798	81	0	0			
Kern	Delano MAD	0			2	20	239	14
Kern	Kern MVCD	19,726	671	111	9	123	1,221	85
Kern	South Fork MAD	0			1	10	70	0
Kern	UCD Arbourse Field Station	3,517	231	22	0			
Kern	Westside MVCD	2,933	68	5	3	30	384	4
Kings	Consolidated MAD	237	7	1	0			
Kings	Kings MAD	16,674	474	104	0			
Kings	Tulare MAD	20	1	0	0			
Lake	Lake Co. VCD	11,880	259	2	2	12	167	1
Lassen	CA Dept. Public Health	233	6	0	0			
Los Angeles	Antelope Valley MVCD	1,013	29	5	8	48	657	27
Los Angeles	Greater LA Co. VCD	134,190	3,340	91	7	70	1,157	8
Los Angeles	Long Beach VCP	6,290	184	0	3	30	481	0
Los Angeles	Los Angeles Co. West VCD	17,142	504	3	19	119	794	5
Los Angeles	Pasadena Env. Health	191	6	0	0			
Los Angeles	San Gabriel Valley MVCD	35	1	0	11	44	36	1
Madera	Madera Co. MVCD	1,482	35	3	2	21	260	7
Marin	Marin-Sonoma MVCD	0			2	20	297	0
Mariposa		0			0			
Mendocino		0			0			
Merced	Merced Co. MAD	9,601	263	9	8	48	615	14
Merced	Turlock MAD	8,513	211	2	0			
Modoc		0			0			
Mono	Mammoth Lakes MAD	91	4	1	0			
Monterey	North Salinas Valley MAD	0			3	32	391	0
Napa	Napa Co. MAD	1,273	37	0	3	31	356	3
Nevada	Nevada Co. Agric. Dept.	0			2	20	320	0
Orange	Orange Co. VCD	0			0			
Placer	Placer Co. MVCD	36,950	1,111	40	8	48	635	11
Plumas		0			0			
Riverside	Coachella Valley MVCD	72,043	1,883	14	9	135	1,361	21
Riverside	Northwest MVCD	10,675	292	1	6	60	912	11
Riverside	Riverside Co. EH	22,734	581	12	5	60	875	5
Sacramento	Sacramento-Yolo MVCD	80,303	2,271	36	3	34	510	2
San Benito	San Benito Co. Agric Dept.	0			1	10	140	0
San Bernardino	San Bernardino Co. VCP	6,803	375	1	10	132	1,700	9
San Bernardino	West Valley MVCD	1,487	66	0	8	18	48	5
San Diego	San Diego Co. EH	3,394	113	5	4	39	575	5
San Francisco	Presidio Trust	133	7	0	0			
San Joaquin	San Joaquin Co. MVCD	21,119	597	48	1	10	110	10
San Luis Obispo		0			0			
San Mateo	San Mateo Co. MVCD	0			1	10	130	0
Santa Barbara	Santa Barbara Co. VCD	11,826	271	0	5	50	790	0
Santa Clara	Santa Clara Co. VCD	13,366	976	1	5	50	666	0
Santa Cruz	Santa Cruz Co. MVCD	6,508	140	0	2	20	310	0
Shasta	Burney Basin MAD	0			2	20	140	0
Shasta	Shasta MVCD	13,604	298	2	5	55	742	0
Sierra		0			0			
Siskiyou		0			0			
Solano	Solano Co. MAD	1,257	34	2	3	35	313	13
Sonoma	Marin-Sonoma MVCD	0			4	40	578	8
Stanislaus	East Side MAD	175	4	1	2	16	188	9
Stanislaus	Turlock MAD	50,256	1,379	81	4	44	524	16
Sutter	Sutter-Yuba MVCD	12,827	320	25	5	50	640	11
Tehama	Tehama Co. MVCD	0			3	30	300	2
Trinity		0			0			
Tulare	Delano MAD	0			1	10	111	8
Tulare	Delta VCD	39,302	974	126	3	30	372	19
Tulare	Tulare MAD	2,201	91	5	2	19	235	1
Tulare	Kings MAD	575	15	0	0			
Tuolumne		0			0			
Ventura	City of Moorpark VC	0			1	8	128	0
Ventura	Ventura Co. EH	2,248	58	0	4	40	668	0
Yolo	Sacramento-Yolo MVCD	30,969	1,306	16	3	32	510	4
Yuba	Sutter-Yuba MVCD	181	7	1	2	20	280	0
Total		**745,244**	**22,137**	**926**	**224**	**2,056**	**25,313**	**441**

[a]No mosquito pools or sentinel chickens were positive for SLEV or WEEV in 2009

[b]Tested by University of California at Davis Center for Vectorborne Diseases or local mosquito/vector control agency. Does not include mosquitoes tested by local agencies for WNV only

[c]Tested by California Department of Public Health Vector-Borne Disease Laboratory or local mosquito/vector control agencies

Figure 2. West Nile virus in Culex spp., California, 2009.

MIR=number of positive pools/number of mosquitoes tested X 1,000

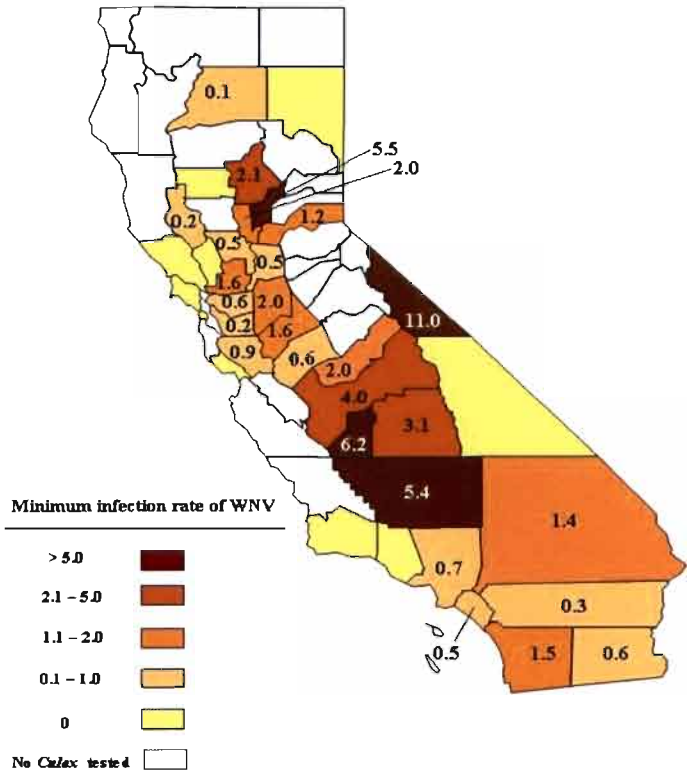
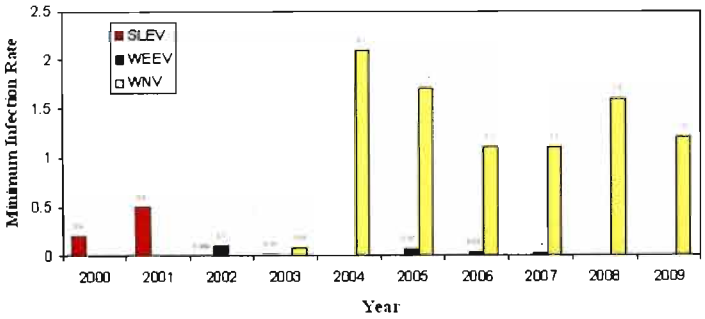


Figure 3. Minimum Infection Rate of St. Louis encephalitis virus, western equine encephalomyelitis virus, and West Nile virus in mosquito pools, 2000-2009.

MIR = number of positive pools/number of mosquitoes tested X 1,000



124,213 mosquitoes (5,456 pools) were tested for only WNV by 10 local agencies using either RT-PCR or a commercial rapid assay-RAMP® (Rapid Analyte Measurement Platform, Response Biomedical Corp).

West Nile Virus was detected in 1,063 mosquito pools from 27 counties (Table 1, Fig. 2); 994 were positive by RT-PCR, and 69 were positive by RAMP only. West Nile Virus was identified from six *Culex* species (*Cx. erythrothorax*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. restuans*, *Cx. stigmatosoma* and *Cx. tarsalis)* and three species from other genera (*Aedes vexans, Anopheles freeborni, Culiseta inornata*) (Table 4). The first detection of WNV in mosquitoes was from *Cx. pipiens* pool collected in Contra Costa County on March 26. The last detection of WNV in mosquitoes was from a *Cx. quinquefasciatus* pool collected in Los Angeles County on November 11. SLEV and WEEV were not detected in mosquito pools in 2009 (Fig. 3).

Chicken serosurveillance. In 2009, 50 local mosquito and vector control agencies in 38 counties maintained 224 sentinel chicken flocks (Table 3, Fig. 4). Blood samples were collected from chickens every other week and screened for antibodies to

Table 4. Mosquitoes tested for West Nile virus, California, 2009.

Culex species	Pools	No. mosquitoes	WNV +	MIR[a]
Cx. boharti	2	9	0	0
Cx erythrothorax	1,685	67,535	15	0 22
Cx pipiens	5,672	153,402	171	1.11
Cx quinquefasciatus	8,475	286,345	483	1.69
Cx restuans	16	589	1	1.70
Cx stigmatosoma	461	7,302	11	1.51
Cx tarsalis	9,898	316,203	377	1.19
Cx thriambus	2	6	0	0.00
All *Culex*	**26,211**	**831,391**	**1,058**	**1.27**

Anopheles species	Pools	No. mosquitoes	WNV +	MIR
An franciscanus	11	136	0	0.00
An freeborni	72	1,541	1	0.65
An hermsi	65	1,329	0	0.00
All Anopheles	**148**	**3,006**	**1**	**0.33**

Aedes species	Pools	No. mosquitoes	WNV +	MIR
Ae dorsalis	29	990	0	0.00
Ae melanimom	202	6,470	0	0.00
Ae nigromaculis	7	291	0	0.00
Ae sierrensis	6	109	0	0.00
Ae squamiger	5	142	0	0.00
Ae taeniorhynchus	33	1,623	0	0.00
Ae vexans	100	3,971	2	0.50
Ae washinoi	19	744	0	0.00
All *Aedes*	**401**	**14,340**	**2**	**0.14**

Other species	Pools	No. mosquitoes	WNV +	MIR
Culiseta incidens	708	18,075	0	0.00
Culiseta inornata	83	1,163	2	1.72
Culiseta particeps	16	376	0	0.00
Coquilletidia peturbans	3	66	0	0.00
Unknown	23	1,040	0	0.00
All other	**833**	**20,720**	**2**	**0.10**

[a] Minimum Infection Rate (MIR) = (No. pools positive/No. mosquitoes tested) X 1000

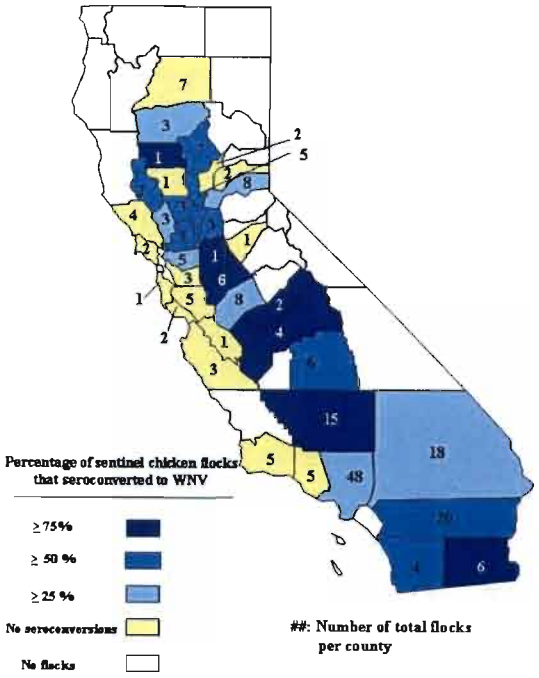
SLEV, WNV, and WEEV by EIA at the CDPH Vector-Borne Disease Section Laboratory (VBDS) and 2 local mosquito and vector control agencies. Positive samples were confirmed at the VBDS laboratory by IFA and western blot, or by PRNT as needed.

A total of 25,313 chicken blood samples were tested for antibodies to SLEV, WEEV and WNV; from these samples, 441 seroconversions to WNV were detected among 94 flocks in 23 counties (Table 3, Fig. 4). In 2009, the first and last WNV seroconversions were detected in Los Angeles County on January 21 and December 17, respectively. No SLEV or WEEV seroconversions were detected in 2009 (Fig. 5).

Dead Bird and Tree Squirrel Surveillance. The WNV dead bird surveillance program is a collaborative program among CDPH, CVEC and over 130 local agencies that was established in 2000 and supported by a CDC Extended Laboratory Capacity grant. The program relies upon the public to report dead birds and tree squirrels to a toll-free hotline (877-WNV-BIRD) or through the WNV website (www.westnile.ca.gov). In 2009, the WNV hotline and website received 15,472 dead bird reports from the public in 57 counties. Of the 2,805 carcasses deemed suitable for testing, WNV was detected in 515 (18%) carcasses from 36 counties: 421 by RT-PCR at CVEC and two local agencies, 67 by RAMP at eight local agencies, 26 by VecTest at five local agencies, and 1 by immunohistochemistry at CAHFS (Tables 1 & 5, Fig. 6). In 2009, the first WNV positive dead bird was reported in San Diego County on January 13, and the last WNV positive dead bird was reported in Los Angeles County on November 18.

In 2009, 307 dead tree squirrels were reported through the WNV Hotline; 103 carcasses were tested, and WNV RNA was detected by RT-PCR in 10 (10%) carcasses from seven counties (Table 1). These included 4 fox squirrels (*Sciurus niger*), 3 eastern gray squirrels (*S. carolinensis*) and 3 western gray squirrels (*S. griseus*).

Figure 4. West Nile virus detection by sentinel chickens, California, 2009.



SUMMARY

Although there was an overall decline in the number of human cases reported in California compared to previous years (Table 2), significant enzootic activity was still detected in mosquitoes, dead birds and sentinel chickens throughout several counties. The minimum infection rate in *Culex* mosquitoes exceeded epidemic levels in four counties (Fig. 2) and statewide was comparable to rates observed during 2006 and 2007 (Fig. 3). Supporting results were also observed for sentinel chicken seroconversions (Figs. 4 & 5) and dead bird analyses (Figs. 6 & 7). Collectively, enzootic data documented virus activity during every season of the year, including the winter period. SLEV was not documented for the sixth consecutive year since the arrival of WNV, perhaps indicating continued competitive displacement.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the cooperation and assistance of the local mosquito and vector control agencies in the collection and submission of samples for testing and their financial support to the testing laboratories; the local public health laboratories which tested samples; the many physicians and veterinarians who submitted specimens from clinical cases; and the valuable contributions of the staffs of MVCAC, CVEC (especially Maureen Dannen, Sandra Garcia and Xiao-Hua Lu), CAHFS (especially Jacquelyn Parker), CDFA Animal Health Branch and the Center for Advanced Research of Spatial Information. From CDPH, we thank VRDL (especially Carol Glaser and Robert Chiles), the Veterinary Public Health Section (especially Ben Sun and Claudia Erickson) and VBDS (especially Anne Kjemtrup, Renjie Hu, Mark Novak, Tim Howard, Laura Zamora, Robert Payne, Ervic Aquino, Jaynia Anderson, Long Her and the WNV Hotline staff). Surveillance funding was augmented by generous support from the Centers for Disease Control and Prevention.

Figure 5. Percentage of sentinel chickens seroconversions to St. Louis encephalitis virus, western equine encephalomyelitis virus, and West Nile virus.

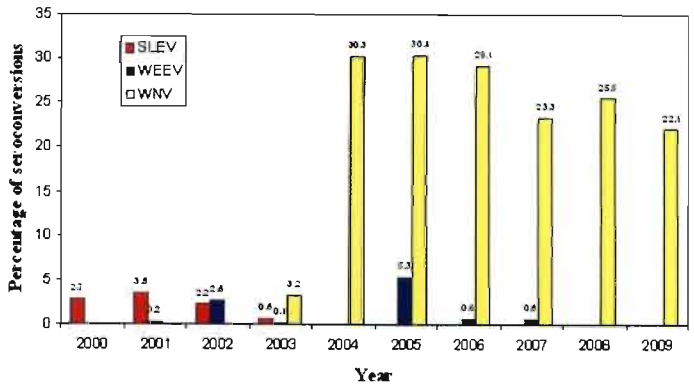


Figure 6. Prevalence of West Nile virus infection in dead birds by county, California, 2009.

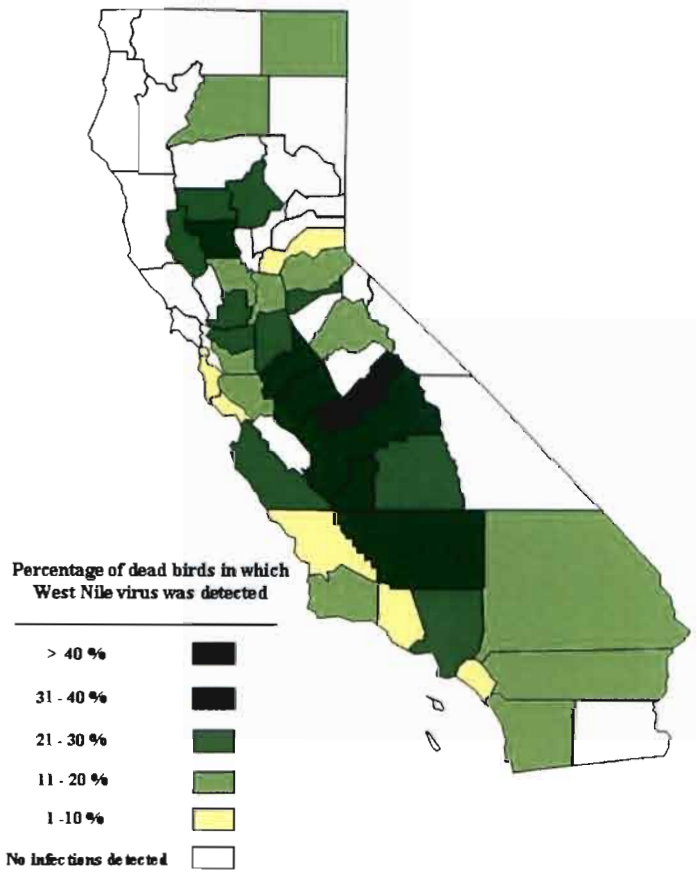


Figure 7. Prevalence of West Nile virus infection in dead birds, California, 2000-2009.

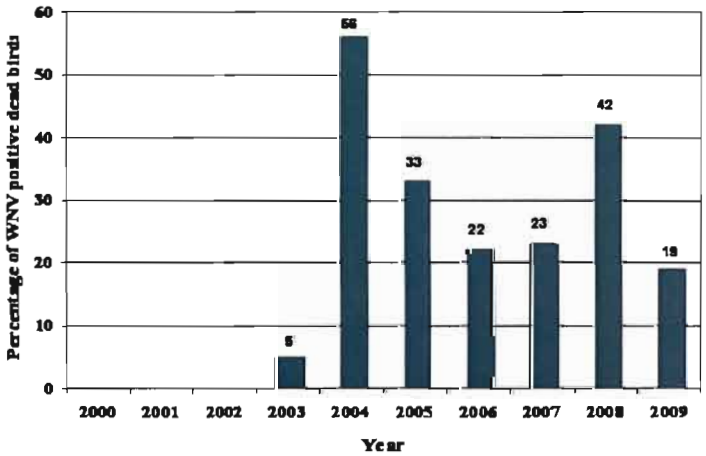


Table 5. Dead birds reported, tested[a], and positive for West Nile virus, California 2009.

County	Reported	Tested	Positive	Percent Positive
Alameda	402	62	10	16.13
Alpine	1	0	0	0.00
Amador	40	4	1	25.00
Butte	371	61	13	21.31
Calaveras	44	3	0	0.00
Colusa	30	6	2	33.33
Contra Costa	1207	198	45	22.73
Del Norte	1	0	0	0.00
El Dorado	283	52	9	17.31
Fresno	977	173	62	35.84
Glenn	55	20	6	30.00
Humboldt	32	8	0	0.00
Imperial	30	0	0	0.00
Inyo	22	6	0	0.00
Kern	606	87	28	32.18
Kings	152	28	9	32.14
Lake	39	8	2	25.00
Lassen	7	1	0	0.00
Los Angeles	1952	386	82	21.24
Madera	108	21	11	52.38
Marin	135	3	0	0.00
Mariposa	28	3	0	0.00
Mendocino	49	6	0	0.00
Merced	293	26	9	34.62
Modoc	13	8	1	12.50
Mono	7	5	0	0.00
Monterey	147	19	4	21.05
Napa	81	5	0	0.00
Nevada	96	17	0	0.00
Orange	836	351	10	2.85
Placer	392	50	4	8.00
Plumas	25	1	0	0.00
Riverside	344	18	2	11.11
Sacramento	1423	214	28	13.08
San Benito	26	5	0	0.00
San Bernardino	522	111	18	16.22
San Diego	453	193	39	20.21
San Francisco	83	12	1	8.33
San Joaquin	576	115	24	20.87
San Luis Obispo	131	19	1	5.26
San Mateo	178	37	1	2.70
Santa Barbara	63	8	1	12.50
Santa Clara	618	86	14	16.28
Santa Cruz	154	23	1	4.35
Shasta	238	48	9	18.75
Sierra	0	0	0	0.00
Siskiyou	6	1	0	0.00
Solano	262	10	3	30.00
Sonoma	220	3	0	0.00
Stanislaus	594	84	28	33.33
Sutter	53	4	0	0.00
Tehama	51	14	0	0.00
Trinity	10	0	0	0.00
Tulare	382	85	25	29.41
Tuolumne	33	11	2	18.18
Ventura	265	41	3	7.32
Yolo	309	44	7	15.91
Yuba	17	1	0	0.00
Totals	**15,472**	**2,805**	**515**	**18.36**

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

West Nile Virus in San Joaquin County, California 2004-2009

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ABSTRACT: After the first appearance of West Nile Virus (WNV) in southern California in 2003, WNV moved north and arrived in San Joaquin County in 2004 where it caused 3 human and 19 equine cases. In 2005, WNV activity intensified and resulted in 36 human and 19 equine cases. Human and equine cases subsequently decreased to relatively low levels. From 2004 to 2009, a total of 9,484 mosquito pools were tested for WNV, and 535 pools were positive. The positive mosquito pools consisted of two dominant mosquito species, *Culex tarsalis* (N = 206) and *Culex pipiens* (N = 329). In addition, human and equine cases coincided with the June to October time frame when positive mosquito pools were frequently detected. There was also close geographic proximity between human cases and positive mosquito pools. These findings indicate that *Cx. tarsalis* and *Cx. pipiens* are the principal vectors in San Joaquin County. *Culex tarsalis* generally reached peak abundance four weeks ahead of *Cx. pipiens* populations, but positive pools for both species appeared at approximately the same time. This finding suggests that *Cx. tarsalis* may play a more important role in early season virus amplification. Over the six year period, 1,013 dead birds were tested for WNV, and 280 were positive with the majority being American crow, western scrub jay and yellow-billed magpie. Positive dead birds have been our earliest indicator of WNV activity, and sentinel chicken flocks were typically the last indicator of WNV presence. On average, positive dead birds appeared 21.5 and 54.4 days earlier than positive mosquito pools and sentinel chickens, respectively.

INTRODUCTION

West Nile virus (WNV) arrived in California in 2003. It was first detected during July from a *Culex tarsalis* pool collected in Imperial County located in the southern end of California (Reisen et al. 2004). That year WNV moved north into Los Angeles and San Bernardino Counties. With the expectation that WNV would continue to move north and ultimately arrive in San Joaquin County, the San Joaquin County Mosquito and Vector Control District (District) responded proactively by implementing a comprehensive surveillance program that included mosquito, dead bird, sentinel chicken, human and equine surveillance components. WNV was first detected in San Joaquin County at the end of June 2004 in a dead barn owl, *Tyto alba*, collected on the southern border with Stanislaus County. WNV virus continued to be detected in dead birds resulting in 57 confirmed carcasses in 2004. The first WNV-positive mosquito pool was detected in the middle of August 2004 in *Cx. pipiens* and in another positive pool in *Cx. tarsalis* in late August. Beginning in the middle of September, equine and human infections were reported with a total of 3 human and 19 equine confirmed cases. In 2005, WNV transmission intensified and caused increased positive mosquito pools, human and equine cases. After 2005, WNV activity decreased but continued to cause human and equine cases (Fig.1).

San Joaquin County is located in the northern most part of the San Joaquin valley, south of Sacramento and to the east of San Francisco. The county covers over 1,426 square miles, consisting mostly of rural agricultural lands as well as highly populated urban areas such as the city of Stockton. The county is home to 685,990 residents, many of whom enjoy recreation along the county's Delta waters and several rivers. Unfortunately, the abundance of waterways in the county, coupled with many agricultural sources in rural areas and neglected swimming pools in foreclosured homes in urban areas, offers prime habitat for the two most common WNV carrying mosquitoes in the county, *Cx. tarsalis* and *Cx. pipiens*. This situation makes the mosquito surveillance and control for WNV particularly challenging. The District maintains a county wide network of permanent trapping sites to collect mosquitoes for WNV testing. The six year surveillance data allow the District to pinpoint the WNV activity foci in the county where control efforts should be focused to reduce mosquito populations likely infected with WNV.

WNV transmission dynamics in different geographic regions around the globe generally follow the same pattern in North America, but the ecological components of the transmission cycle may vary due to the substantial variation of local ecological systems (Hayes et al. 2005). The two important variables dictating WNV transmission dynamics are local mosquito and avian species. This paper examines six years of surveillance data in order to characterize the dynamics and ecological components of WNV transmission in our county. Results are discussed in relation to the utilities of each surveillance component from an operational perspective.

MATERIALS AND METHODS

Mosquito Surveillance. Mosquitoes were collected weekly from a county wide network of permanent trapping sites using CO_2-baited Encephalitis Vector Surveillance (EVS) traps (Rohe and Fall 1979) and hay infusion-baited gravid box traps (Cummings 1992). Additional traps were added in locations at high-risk for virus activity, identified by such factors as high mosquito populations, high virus activity in the area in previous years, prevalence of positive dead birds and/or high numbers of residential service requests. Trap locations were representative of different types of environments, including rural sites such as rice

fields, wetlands, dairies and pastures as well as urban sites such as private residences and parks. With the onset of WNV in 2004, the District began a routine trapping schedule that included 43 EVS and 15 gravid traps. In order to survey virus activity better within the county, in 2009 the number of routine traps was increased to 63 EVS and 8 gravid traps. Mosquitoes were pooled groups of 12 - 50 individuals for each species, and pools were tested either by Rapid Analyte Measurement Platform (RAMP®) at the District or by reverse transcription-polymerase chain reaction (RT-PCR) at the Center for Vectorborne Diseases (CVEC), University of California, Davis.

Avian Surveillance. Dead birds reported by the public were collected throughout the season. Addresses and GPS coordinates were recorded for each dead bird so that the data could be mapped later. Each dead bird that died within 24 hours of collection and was still in good condition was tested. Corvids including the American crow and common raven were tested in house by RAMP® or Vec-Test®. Other eligible bird species were sent to California Animal Health and Food Safety (CAHFS) for RT-PCR testing.

Human and Equine Surveillance. When official horse or human cases of WNV infection were reported in the county, the District took immediate action. Neighborhoods surrounding the cases were inspected for mosquito breeding sources, and these sources were treated. EVS traps were also placed in the surrounding areas, and the mosquitoes caught were tested for WNV as described above.

Sentinel Chicken Flocks. Sentinel chicken flocks used by the District from 2004 to 2009 consisted of 10 chickens per flock, and flocks were maintained open air cages allowing easy access by mosquitoes. The chickens were bled on a biweekly schedule established by the California Department of Public Health (CDPH) starting in the spring and ending in late fall. At its peak, the District sentinel chicken program consisted of six flocks located around the periphery of the county. From 2004 to 2008, chicken sera were tested for presence of WNV in-house using Enzyme-linked immunosorbent assay (ELISA). In 2009, the number of chicken flocks in the county was reduced to one, and chickens were bled onto filter paper which was shipped to CDPH for enzymatic immunoassay (EIA) testing.

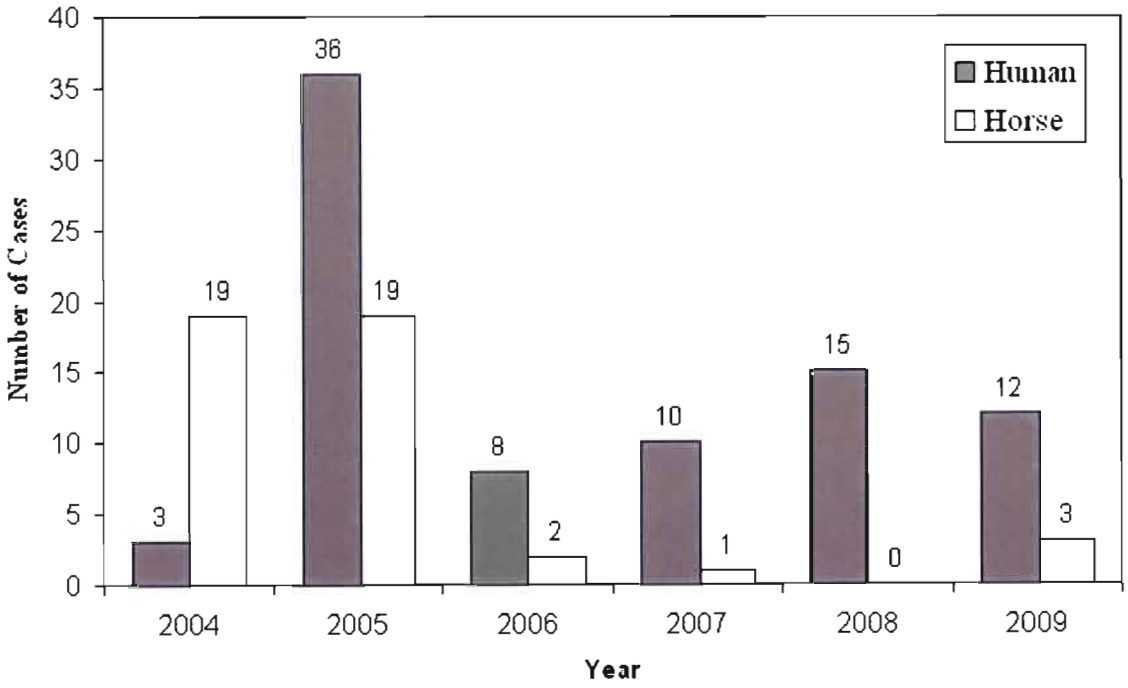

Figure 1. Human and equine WNV cases in San Joaquin County, CA 2004 – 2009.

RESULTS AND DISCUSSION

Mosquito Surveillance. *Culex tarsalis* and *Cx. pipiens* were the most abundant species recovered in EVS and gravid traps. Of the 9,484 mosquito pools tested over the six year period, 535 pools were positive (5.6%, Table 1).

All positive mosquito pools were either *Cx. tarsalis* or *Cx. pipiens*. Furthermore, almost all the human cases occurred within the June to October time frame when positive mosquito pools were frequently detected (Fig. 2). There was also close geographic proximity between human cases and positive pools (Fig. 3). These results suggest that *Cx. tarsalis* and *Cx. pipiens* are the major vectors for WNV in San Joaquin County.

It is important to note that *Cx. tarsalis* abundance peaked on average four weeks earlier than *Cx. pipiens* abundance (Fig. 4). However, both *Cx. tarsalis* and *Cx. pipiens* positive pools were detected about four weeks after the first peak in the *Cx. tarsalis* populations (Fig. 4). This suggests that *Cx. tarsalis* may play a more important role in early season virus amplification. *Culex tarsalis* prefer clean water, both in rural and urban settings, and *Cx. pipiens* prefer habitats with high organic matters. In the same habitat, *Cx. tarsalis* usually breed first when the water is clean, and *Cx. pipiens* follow when the water becomes fouled. This is a very common pattern we see in the unmaintained swimming pools. In rural areas, many agricultural lands are flooded in late spring or early summer and produce large numbers of *Cx. tarsalis* and later smaller populations of *Cx. pipiens*. The largest percentage of positive mosquito pools was detected in rural areas; WNV 'hot spots' were located along the southern border of the county along the Stanislaus River and in the Delta area in the west of the county (Fig. 3). The Delta area is subject to marine air intrusion, and the wind may carry WNV positive *Culex* mosquitoes to the downwind urban areas (Swartzell et al., unpublished data). Thus, early mosquito control efforts that target *Cx. tarsalis* might help reduce or delay WNV activity in the fall.

From 2004 to 2007, several different genera of mosquitoes were tested for WNV by RAMP® and/or RT-PCR, but positive pools were only discovered in *Culex* spp. Consequently, WNV testing was focused exclusively on *Culex* mosquito pools in 2008 and 2009. *Aedes vexans* and *Ae. melanimon* were the most abundant *Aedes* mosquitoes in the summer. Although they have not tested WNV positive in our county, *Aedes* spp. have tested positive in other regions (Andreadis et al., 2004, Feiszli et al. 2007). Because *Ae. vexans* and *Ae. melanimon* are aggressive mammal and human feeders, they could serve as bridge vectors, and control effort might have to be considered according to their population abundance.

Table 1. WNV detection in mosquito pools collected from EVS and gravid traps, California 2004-2009

No. of Traps		2004		2005		2006		2007		2008		2009	
No. of EVS Traps		43		35		53		54		74		63	
No. of Gravid Traps		15		8		9		9		10		8	
Species		Tested	WNV+	Tested	WNV+	Tested	WNV+	Tested	WNV+	Tested	WNV+	Tested	WNV+
Aedes	*dorsalis*					2							
	melanimon	25		225		213		8					
	nigromaculis			1									
	sierrensis					3							
	vexans	22		306		136							
	washinoi					3							
Anopheles	*freeborni*			5						1			
Culex	*erythrothorax*	2		1		1				14		71	
	pipiens	272	1	339	18	619	22	1,201	103	1,215	136	569	46
	stigmatosoma					3							
	tarsalis	209	1	492	20	1,043	27	829	52	1,006	72	581	37
Culiseta	*incidens*	1		39		1							
	inornata			26									
Total		531	2	1,434	38	2,024	49	2,038	155	2,236	208	1,221	83
***Culex* Positive Pool Ratio**			0.4%		4.6%		2.9%		7.6%		9.3%		6.8%
Average MIR			0.112		2.366		2.918		2.17		3.126		2.149

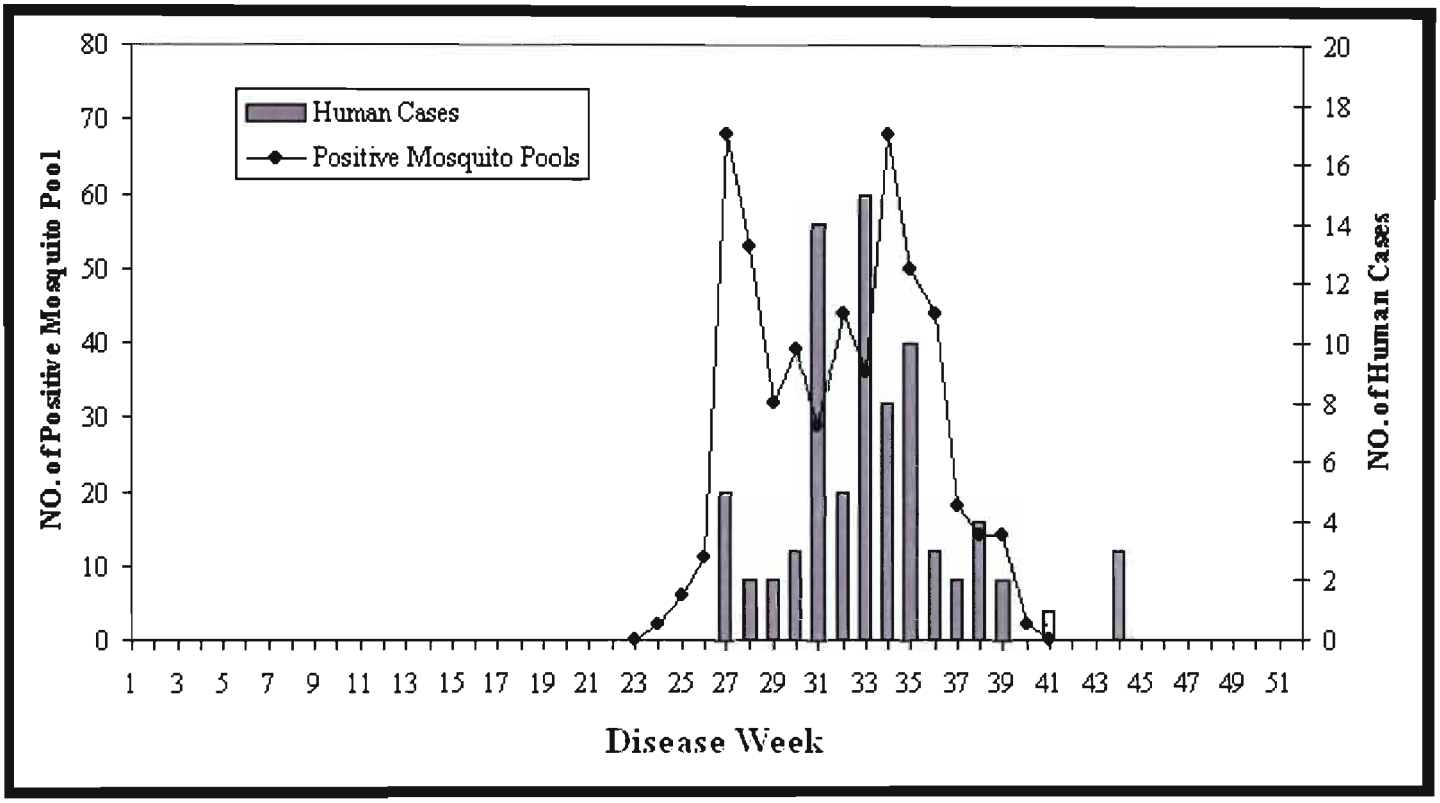

Figure 2. Weekly detection of WNV in mosquito pools and reported human cases in San Joaquin County, CA 2004 – 2009.

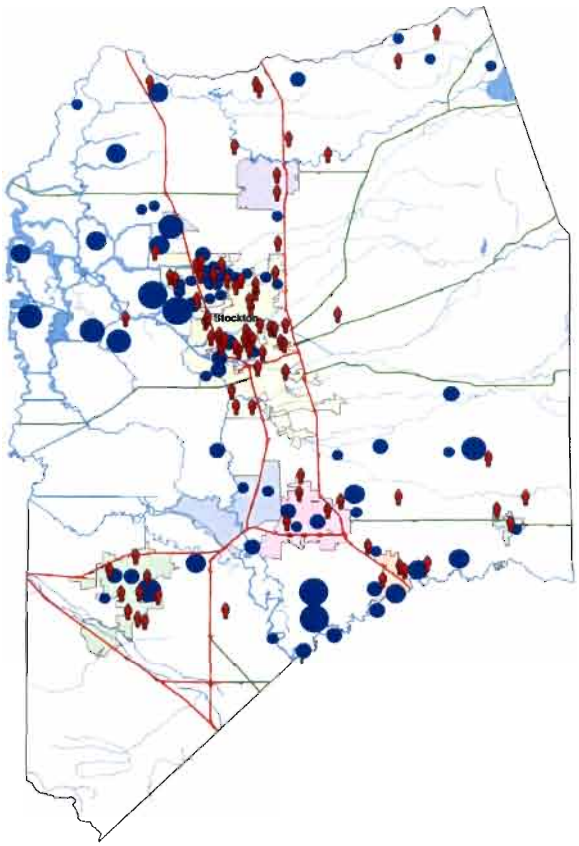

Figure 3. Geographic distribution of positive mosquito pools and human cases in San Joaquin County, CA 2004 – 2009.

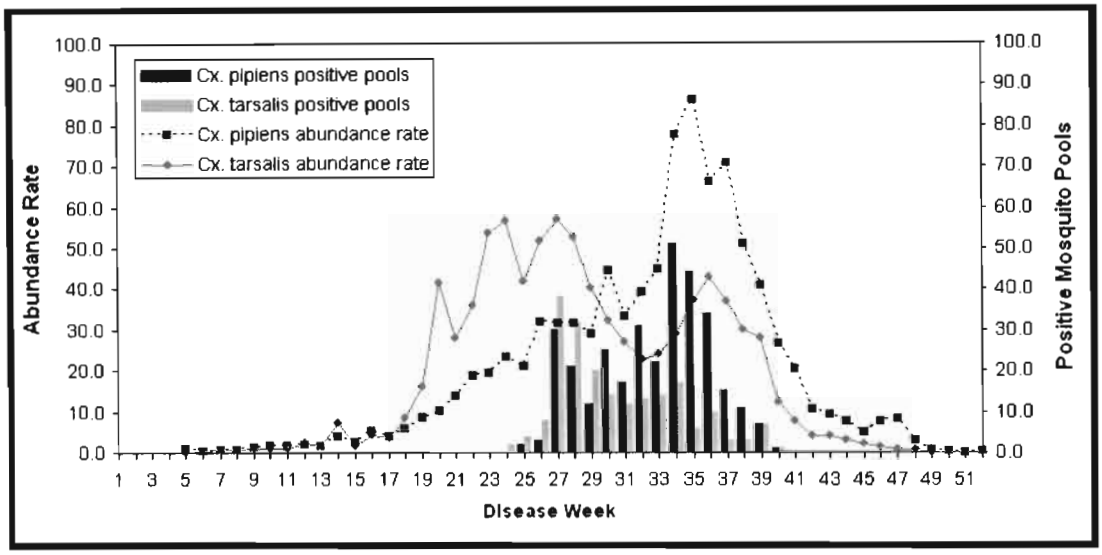

Figure 4. Average weekly abundance rate and number of positive *Cx. tarsalis* and *Cx. pipiens* pools in San Joaquin County, CA 2004 – 2009.

Avian Surveillance. From 2004 to 2009, a total of 12,123 dead birds were reported in San Joaquin County to the state WNV dead bird hotline. Of those birds, 1,013 were tested, and 280 were positive for WNV (Table 2). American Crow (*Corvus brachyrhyncos*), Western Scrub Jay (*Aphelocoma californica*), and Yellow Billed Magpie (*Pica nuttalli*) were the three most commonly reported and also the three most common WNV positive species (Table 3). Combined, these three species comprised 84.6% of the total WNV positive birds collected. The other less frequently tested positive birds include barn owls, house finches (*Carpodacus mexicanus*) and house sparrows (*Passer domesticus*). The species composition of positive birds might have been influenced by public report. Large birds such as American crows, Western scrub jays and yellow billed magpies, are more easily spotted and therefore may be reported by citizens more frequently than smaller birds. The American crow accounted for 43.7% of the total positive dead birds, but it is important to point out that the American crow may not be the major enzootic reservoir. Several studies of blood meal analyses have shown that American crows were seldom fed on by *Cx. pipiens* and *Cx. tarsalis*; moreover, American crows may acquire WNV infection through carnivorous feeding behavior (Molaei et al., 2007, Cummings, et al. 2007).

Dead birds collected in the county have typically been the earliest indicator of WNV activity. On average, virus was been detected in dead birds 21.5 days before the first positive mosquito pool (Table 4). However, dead bird reports may have become less prevalent over time due to evolving herd immunity (LaDeau et al. 2007), decreasing public vigilance and zip code management for reporting. In California, after a positive dead bird was been reported in a particular zip code, the District had the option of 'closing' zip code; after 'closing' dead birds were only reported and not collected for testing. This practice has helped the District to conserve resources during hectic mosquito seasons and may have contributed significantly to the decreased numbers of dead birds collected and tested in 2009. These procedures make it difficult to evaluate the dynamics of avian population in response to WNV.

Sentinel Chickens. Over the course of this study from 2004 - 2009, a total of 17 sentinel flocks were monitored in the field; of these flocks, 15 seroconverted. The two flocks that did not seroconvert (2004) were located where no positive mosquito pools were detected. Similar to other regions in California and other states (Charles, 2008, Cherry et al. 2000), sentinel chickens were not a reliable indicator of WNV activity in San Joaquin County. On average, seroconversion took place 54.4, 23.1 and 10.3 days after the first detection of WNV in dead birds, mosquito pools and humans, respectively (Table 4). Apparently, the utility of sentinel chickens for WNV surveillance is limited.

Human and Equine Surveillance. San Joaquin County had 84 human and 44 equine cases of WNV from 2004 to 2009, with a peak in both human and equine cases in 2005 (Fig. 1). The epidemiological patterns of human infections largely followed the typical WNV transmission patterns reported elsewhere (i. e., low enzootic transmission at WNV introduction, high epidemic transmission at second year and low level thereafter [Hayes et al. 2005]). The reduction in WNV infection in humans and horses after 2005 may be attributed to the WNV intrinsic transmission cycle and/or mosquito control activities. Since the detection of

Table 2. Dead birds reported, tested and positive for WNV in San Joaquin, California 2004-2009

	2004	2005	2006	2007	2008	2009	Total
Reported	1,169	5,300	2,006	1,579	1,508	561	12,123
Tested	188	111	150	117	311	136	1,013
Positive	57	32	50	50	68	23	280

Table 3. Cumulative species composition of dead birds reported, tested and positive for WNV in San Joaquin, California 2004 - 2009.

Total Reported		Total Tested		Total Positive	
Species	Percent	Species	Percent	Species	Percent
American Crow	23.2%	American Crow	26.9%	American Crow	43.7%
Western Scrub Jay	23.0%	Western Scrub Jay	22.0%	Western Scrub Jay	28.3%
Others or Unknown	20.5%	Others or Unknown	20.1%	Yellow-billed Magpie	12.6%
Sparrow	7.9%	Yellow-billed Magpie	7.1%	Others or Unknown	5.5%
Yellow-billed Magpie	6.5%	House Sparrow	4.1%	Barn Owl	2.4%
Rock Dove	3.8%	American Robin	4.1%	House Finch	2.4%
Brewer's Blackbird	3.4%	House Finch	3.2%	House Sparrow	2.4%
American Robin	2.0%	Brewer's Blackbird	2.7%	Chicken	1.2%
Steller's Jay	1.8%	Barn Owl	2.6%	American Robin	0.8%
House Finch	1.6%	Mourning Dove	2.1%	American Kestrel	0.8%
Mourning Dove	1.4%	European Starling	2.1%		
Northern Mockingbird	1.0%	Northern Mockingbird	1.6%		
		Great Horned Owl	1.4%		

Table 4. Dates of first detection of WNV in dead birds, mosquitoes, sentinel chickens, humans and horses in San Joaquin, California 2004 - 2009

Year	Dead Bird	Mosquito	Human	Equine	Chicken
2009	5/18	6/22	7/23	7/30	8/10
2008	5/28	6/09	7/15	N/A	9/08
2007	6/23	6/13	7/01	8/03	7/16
2006	6/23	7/06	7/07	7/18	7/24
2005	6/07	7/05	7/25	7/23	7/15
2004	6/30	8/23	9/27	9/14	8/16
Ave. lag (day)	0.0	21.5	44.7	51.7	54.4

WNV in San Joaquin County in 2004, the District waged an intensive public education and large-scale adulticiding in 2005 and 2006. In addition, horse owners were contacted individually and encouraged to vaccinate their horses. These efforts may have helped achieve the reduction of human and equine cases observed in 2006. The percentage of positive pools in *Culex* mosquitoes followed the same pattern as human and equine cases, rising in 2005 and declining in 2006. The percentage of positive pools rose to even higher levels in 2007, 2008 and 2009, suggesting repeated WNV infections in *Culex* mosquitoes. This could be due to improved testing sensitivity and trapping methods. Traps in later years tended to be better positioned to those WNV activity foci.

Almost all human and horse cases occurred from June to October, the same time period when positive mosquito pools were routinely detected (Fig. 2). The majority of the human cases occurred in the urban areas of Stockton, Tracy, Manteca and Ripon (Fig. 3, colored areas), resulting in a strong correlation between human cases and population density. Typically, positive mosquito pools were detected within a one to three mile radius of human cases. There was a substantial number of positive pools detected in rural areas without accompanying human cases. However, several people confirmed to be infected with WNV reported being bitten by mosquitoes when they spent time in popular recreation areas along waterways.

Although there are additional factors that need to be evaluated in order to understand the WNV transmission cycle in our county, the surveillance system used by the District did provide sufficient information for prompt response to WNV detection, resulting in mosquito treatment and control. The multiyear data allowed the District to identify the target vector species and the foci of WNV activity, enabling the District to direct its resources in the most effective and efficient manner possible to combat WNV in San Joaquin County.

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High Altitude West Nile Virus and Mosquito Surveillance in Mammoth Lakes, CA

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ABSTRACT: During 2008 and 2009, the Mammoth Lakes Mosquito Abatement District (MLMAD) and the California Department of Public Health, Vector-Borne Disease Section collaborated in a joint effort to enhance mosquito and West Nile Virus (WNV) surveillance in and near the MLMAD. Notable outcomes include a WNV-positive pool of *Culex tarsalis* collected on August 21, 2009, implementation of a local mosquito-borne disease response plan, the determination that Mosquito Magnet traps were less efficient than EVS traps in the District and the first known collection of *Aedes sierrensis* in Mono County.

KEY WORDS *Aedes sierrensis*, *Culex tarsalis*, EVS trap, Mono County, Mosquito Magnet, West Nile Virus

INTRODUCTION

The Mammoth Lakes Mosquito Abatement District (MLMAD) was formed in 1969 in Old Mammoth Lakes, encompassing approximately 138 hectares (340 ac). The District employs an integrated pest management (IPM) approach including larval inspections and control, adult surveillance and limited ULV adult mosquito spraying as needed. Because mosquitoes emerging from nearby larval development (breeding) sites outside District boundaries have been a problem in the past, the District routinely provides larval inspection and control beyond District boundaries. MLMAD also had a contractual agreement to provide adult mosquito surveillance in the Hilton Creek area (a rural subdivision near Mammoth Lakes) during 2009 as part of the Hilton Creek Community Services District (HCCSD) effort to evaluate the need for a local control program.

MLMAD began using Mosquito Magnet (MM) traps for surveillance in 2005 because there was no local source of dry ice. However, the District has been dissatisfied with the reliability of the MM traps despite switching propane distributors and tanks, and repeated factory repairs. The recent availability of dry ice from a local supermarket made it feasible for the California Department of Public Health working with the MLMAD to conduct a small scale comparison of MM and dry ice baited EVS traps in the District.

The MLMAD is among the highest elevation mosquito control districts in the United States with an office located approximately 2408 meters (7900 ft) above sea level, resulting in relatively cold temperatures and a short mosquito season. Average daily high and low temperatures in Mammoth Lakes during June through August are approximately 24° C (75° F) and 7° C (45° F) respectively, with an average daily temperature of 15.4° C (60° F) (Accuweather web site, 2009). The frost-free period is approximately 80 days (at the Mammoth Lakes Ranger Station). Because of the cold temperatures and short mosquito season, there is uncertainty about the potential for local West Nile Virus (WNV) replication and transmission in and near Mammoth Lakes. In a study published in 2006, the authors determined that 14.6° C was the minimum ambient temperature at which WNV would replicate in mosquitoes and therefore could be vectored between hosts (Reisen et al. 2006). Average monthly temperatures in Mammoth Lakes relative to the minimum activity temperature for WNV are shown in Figure 1.

West Nile Virus positive dead birds had been found in Mammoth Lakes or the surrounding area from 2004 - 2006, but not in 2007 (CDPH). The finding of two WNV positive dead birds in 2008 (CDPH) prompted the MLMAD, the HCCSD and the local public health officer to develop a WNV response plan.

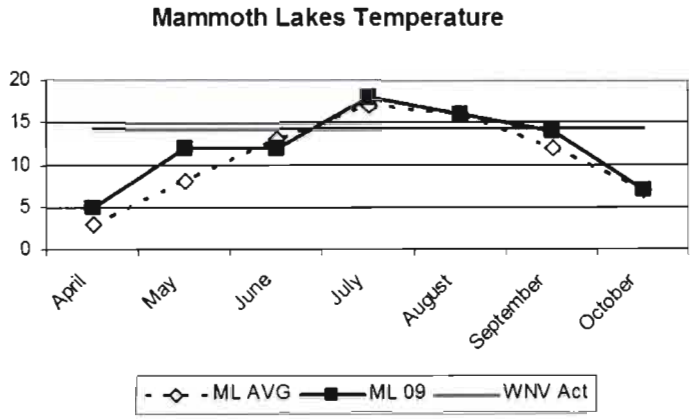

Figure 1. Temperature at Mammoth Lakes, CA. WNV Act is the minimum ambient temperature (14.6° C) at which WNV replicates in mosquitoes (after Reisen 2006).

DISCUSSION

Local WNV Transmission. On August 20 - 21, 2009, six encephalitis virus surveillance (EVS) mosquito traps were set overnight in Mammoth Lakes and the HCCSD surveillance area. Four pools of *Culex tarsalis*, collected in the HCCSD, were submitted to the University of California at Davis, Center for Vectorborne Disease (CVEC) laboratory for WNV testing. Pool size ranged from 17 to 38 mosquitoes with a total of 91 adult females being submitted for testing. One pool of 18 mosquitoes collected at the South Landing trap site (elevation 2134 m [7000 ft] – approximately 15 miles from THE District office tested positive for the virus. The Public Health officer and MLMAD were notified on August 24, 2009.

On August 26, 2009, the MLMAD Manager, Mono County Public Health Officer and representatives from the HCCSD met to discuss adult mosquito control in the Hilton Creek area. Although the local mosquito season was near its end, it was determined that spraying was warranted to reduce the immediate threat of WNV, and the agency representatives present agreed to implement the plan previously established. On August 28, the MLMAD Board of Directors approved adult mosquito ultra-low volume (ULV) spraying outside District boundaries to protect the residents of the Hilton Creek subdivision. Press releases were issued, and adult mosquito spraying was conducted on September 2. The ULV adulticide application encompassed approximately 300 acres (122 ha) within the HCCSD, including the area around the South Landing trap site. The product used was Scourge 4+12 ULV (4% resmethrin, 12% piperonyl butoxide) at a rate of 1 ounce per acre total volume. Post-spray EVS trapping in the HCCSD on September 4 demonstrated a decrease in adult *Cx. tarsalis* mosquitoes in the treatment area, from an average of 30 adult females/trap pre-spray on August 21, 2009 to 4 adult females/trap post-spray on September 4, 2009 in the control area. Comparison traps were not set outside the spray area. The MLMAD has mapped larval development sites in the HCCSD and is working toward plans to implement a limited larval control program in the area targeting *Cx. tarsalis*.

Adult Mosquito Trap Comparison. Total abundance and species composition of trapped mosquitoes were evaluated in a small scale trap comparison between dry ice baited EVS traps and MM traps utilizing propane only. In the evaluation, one trap of each type was set at established trap sites. Mosquito Magnet traps were set at their usual locations and EVS traps were set in visually similar locations approximately 48 m (150 ft) from the Mosquito Magnet trap. On July 25, 2008 and August 21, 2009, both types of traps were set at 5 locations each night. Traps were set in the afternoon and collected the following morning. During the comparison 3 Mosquito Magnet traps stopped functioning each night and could not be used in the evaluation. Comparative trap locations included a dense willow riparian habitat, meadow/ pasture habitat and a wooded residential area. There was one potentially important difference among the trap locations: Mosquito Magnet traps were only placed where they could be chained and locked whereas EVS traps were placed in trees. Based on typical air movement direction, an effort was made to avoid placing either trap upwind of the other. Only captured female mosquitoes were identified (Table 1).

New county record mosquito species. On July 25, 2008 two adult female *Aedes sierrensis* Ludlow were captured in a dry ice baited EVS trap in Mono County, CA. The trap site (Mountain Shadow in Hilton Creek) is located at latitude 37.559915, longitude -118.742120; elevation 2168 m (7113 ft) above sea level. The specimen identifications were verified by Dr. Tom

Table 1: Mosquito Trap Collection Comparison.

Location	Date	Spp.	MM	EVS	EVS % increase
Rowan	7/25/2008	*Culex tarsalis*	1	13	
		Culiseta spp.	0	2	
		Aedes spp.	1	0	
Total			**2**	**15**	**750**
	8/21/2009	*Culex tarsalis*	5	42	
		Culiseta spp.	0	1	
		Aedes spp.	0	4	
Total			**5**	**47**	**940**
Mendoza	7/25/2008	*Culex tarsalis*	3	16	
		Aedes spp.	0	1	
Total			**3**	**17**	**567**
Mt. Shadows	8/21/2009	*Culex tarsalis*	5	17	
Total			**5**	**17**	**340**

Zavortink at the Bohart Museum, University of California, Davis, CA. On August 21, 2009 a dry ice baited EVS trap at the trap site (Mendoza in Hilton Creek) captured two additional *Ae. sierrensis* at latitude 37.567201 and longitude -118.738935; elevation 2102 m (6895 ft) above sea level. The Mendoza trap site is approximately 800 m (0.5 mi) from the Mountain Shadow location. A small sustaining population of this species may exist in the Hilton Creek area, but its full distribution was not determined. These appear to be the first records of *Ae. sierrensis* from Mono County, CA.

CONCLUSION

When a WNV positive pool of *Cx. tarsalis* was collected by MLMAD staff, the local response plan was implemented quickly and smoothly. The cooperative efforts among the MLMAD employees, the MLMAD Board of Directors, the HCCSD and the Mono County Public Health Officer can serve as an example for large and small districts on the value of developing and implementing a mosquito control program to protect public health.

In this small comparison, the EVS traps were more efficient in terms of both diversity of species collected and the number of mosquitoes collected. As a result of the trapping, MLMAD has incorporated the use of EVS traps along with MM traps into their IPM program.

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Evaluation of West Nile Virus Activity in Orange County, California, during 2009

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ABSTRACT: The Orange County Vector Control District (OCVCD) continued its arbovirus surveillance program in 2009 by collecting and pooling mosquitoes, testing avian blood samples drawn from free-ranging wild birds and sentinel chickens and testing dead birds collected from various animal control agencies and the public. Evidence of West Nile Virus (WNV) infection was detected in mosquito pools (21 of 1,567), wild bird seroconversions (66 of 1,087) and dead birds (11 of 367). No sentinel chickens in a flock of ten birds tested positive for WNV antibodies. Four non-fatal human cases of WNV infection were reported in the county during 2009. *Culex quinquefasciatus* Say was the most abundantly trapped mosquito, accounting for the majority of submitted pools (1,152 of 1,567) and positive pools (20 of 21). House finches (*Carpodacus mexicanus* Say) and house sparrows (*Passer domesticus* L.) comprised all of the WNV-seroconversions in free-ranging wild birds (62 of 62), while American crows made up most of the positive dead birds (7 of 11). The Maximum Likelihood Estimate (MLE) in *Cx. quinquefasciatus* from May to October (seasonal) was significantly less in 2009 than 2008 (0.86 vs. 18.07, respectively). Additionally, both the percent of WNV-positive dead birds (3.0% vs. 66.0% respectively) and the number of reported human cases of WNV infection (3 vs.71) were lower in 2009 than 2008. The results of an immature mosquito study of neglected swimming pools revealed that *Cx. quinquefasciatus* was the predominate species with approximately 50% (79 of 161) of sampled pools testing positive for this species of mosquito; *Cx. tarsalis* larvae were also found frequently (46% or 74 of 161), followed by *Cx. stigmatosoma* (20% or 34 of 161) and *Cs. incidens* (17% or 27/161) in larval collections from these sources.

INTRODUCTION

The Orange County Vector Control District (District) encompasses approximately 789 square miles (all of Orange County), and approximately 3.1 million residents reside within its borders (US Census Bureau 2010). Most of the District consists of urban/suburban habitats with a variety of residential mosquito-breeding sources: improperly maintained swimming pools and ponds, debris-choked drainage channels and other man-made habitats. Interspersed within this development are several natural, mosquito-producing fresh and salt-water wetlands. Four important vectors of West Nile Virus (WNV) (*Culex tarsalis* Coquillett, *Culex quinquefasciatus* Say, *Culex stigmatosoma* Dyar, and *Culex erythrothorax* Theobald [Goddard et al. 2002, Reisen et al. 2005]) are collected routinely in the county (Gruwell et al. 1988). The District employed an integrated arboviral disease surveillance system throughout the year, comprised of avian serosurveillance (sentinel chickens and wild birds), testing dead birds and mosquitoes and monitoring veterinarian and physician reports for WNV infections in animals and humans.

METHODS AND RESULTS

Mosquito Surveillance. Mosquitoes were collected weekly from a total of 75 - 80 traps throughout the District, combining CDC/CO_2 - style, host-seeking EVS traps (Rohe and Fall 1979) and Reiter/Cummings gravid female, ovipositional traps (Cummings 1992). Blood-fed mosquitoes were collected in CO_2-baited traps, gravid traps and aspirated at known mosquito resting sites and at locations of service requests.

Culex quinquefasciatus made up the largest component of the specimens collected (27,632 of 40,287) (Table 1). Of 1,567 mosquito pools submitted for arbovirus testing by in-house real-time singleplex reverse transcriptase-polymerase chain reaction

	Total Mosquitoes collected		Positive Mosquito Pools	
	2008	**2009**	**2008**	**2009**
Cx. quinquefasciatus	28,898	27,632	324	20
Cx. tarsalis	8,709	3,684	20	0
Cx. erythrothorax	12,563	8,907	3	1
Cx. stigmatosoma	1,969	58	46	0
Other species	158	6	0	0

Table 1: Comparison of mosquito collection data and minimum infection rates (MLEs) by species for peak activity months, May – October, 2008 – 2009.

(RT-PCR), 21 tested WNV-positive. *Culex quinquefasciatus* comprised the majority of these (20 of 21); Maximum Likelihood Estimates (MLEs) for the months of May – Oct of 2008 and 2009 are shown in Figure 1.

Swimming Pool larval Assessment. District personnel collected larval samples from neglected swimming pools as part of a year-long study to assess the species composition of mosquitoes breeding in these sources. Samples were preserved in small alcohol-filled vials and identified to species (Meyer 2003) by District staff. The species composition from 161 different swimming pools found positive for mosquito breeding are listed in Table 2 and Figure 2.

Sentinel Chickens. The District maintained one sentinel chicken flock of ten chickens near a *Cx. quinquefasciatus* – producing site on Orange County Vector Control District grounds in Garden Grove. Blood samples from the chickens were tested biweekly for SLE, WEE and WNV antibodies by the California

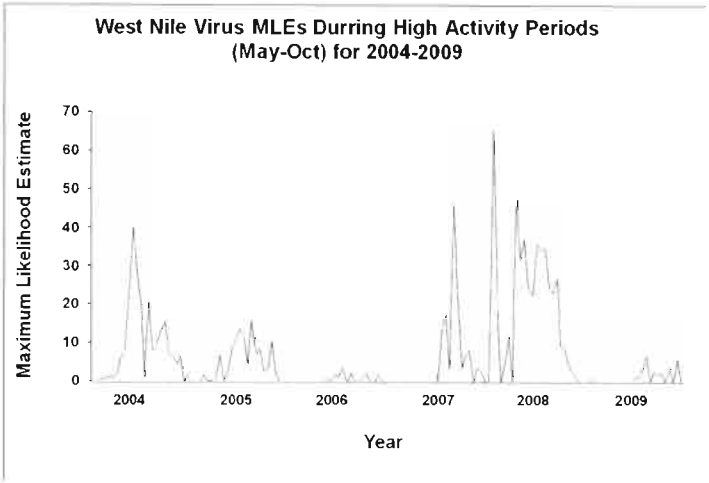

Figure 1. Peak seasonal MLE rates from 2004 to 2009. Each peak represents a week during the respective year.

Neglected Swimming pools	*Cx. quinqufasciatus*	*Cx. tarsalis*	*Cx. stigmatosoma*	*Cs. incidens*	
April	33	17	14	6	9
May	51	24	18	14	10
June	39	15	20	3	8
July	15	7	7	5	0
August	9	5	5	2	0
September	7	5	7	2	0
October	7	6	3	2	0
Total	**161**	**79 (49.1%)**	**74 (46.0%)**	**34 (21.1%)**	**27(16.8%)**

Table 2. Number of larvae-positive swimming pools by species in Orange County during 2009. Results do not add to 100% because some sites were positive for multiple mosquito species.

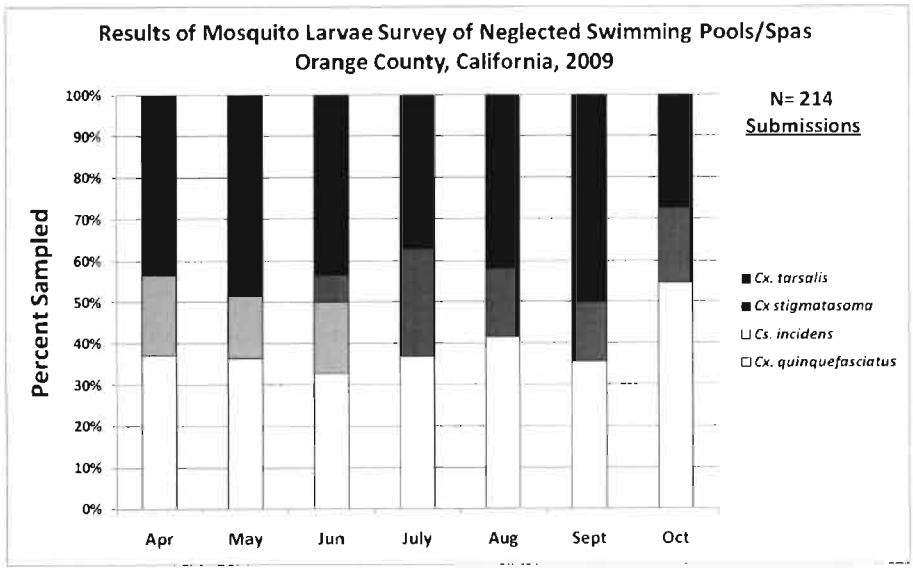

Figure 2. Relative proportions of larval samples from neglected swimming pools (N = 161) in 2009.

Department of Health Services' Viral and Rickettsial Diseases Laboratory (CDHS/VRDL) by enzymatic immunoassay (EIA) (Reisen et al.1994) from April - November and blocking ELISA at the District (Hall 1995, Jozan et al. 2003). None of the sentinel chickens tested positive for exposure to any arbovirus.

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

Birds were sampled at each site on alternate weeks (3 sites/ week). Newly captured birds were banded, aged, sexed (if possible), bled and released. Blood samples (0.2 ml) were taken from the jugular vein with a 1.0 ml syringe and a 28-gauge needle, dispensed into a 1.8 ml field diluent solution kept cool and processed at the District's laboratory by EIA for SLE and WEE antibodies (Gruwell et al. 1988) and blocking ELISA for evidence of WNV infection (Jozan et al 2004).

Of the 865 house finches sampled in 2009, 53 birds (6.1%) seroconverted for WNV antibodies; 11 (7.1%) of the 154 house sparrow samples and 2 mourning doves (5.7%) out of 35 tested showed evidence of WNV-seroconversions during the year (Table 3). New seroconversions to WNV antibodies in house finches and house sparrows were detected in every month of 2009, except December (Table 3).

Dead Bird Surveillance. Dead birds were collected from the public via dead bird phone calls and through cooperation with various animal control agencies. A total of 638 phone calls were received, 553 birds were collected, but only 367 were suitable for testing. Eleven of these birds were found PCR-positive. Rates of WNV-positive dead birds declined from 66% (692 of 1,583) in 2008 to 3% (11 of 367) in 2009. The species composition of positive dead birds included American crows, (64% or 7 of 11), house finches (18% or 2 of 11), a mourning dove (9%, or 1 of 11) and a Cooper's hawk (9% or 1/11). (Table 4)

DISCUSSION

Data from all three arboviral surveillance systems demonstrated a significant decline in detectable WNV activity in Orange County during 2009 compared to the previous year. Similar declines in the WNV MLEs in the mosquito population and levels of WNV-positive dead birds were observed in adjoining southern California counties in 2009 (Riverside, San Bernandino, Los Angeles, and San Diego) (http://westnile.ca.gov). The WNV MLEs for *Cx. quinquefasciatus*, Orange County's primary WNV vector (Schwedes et al. 2005, Weir et al. 2006) peaked at 1.58 (0.70 – 3.12, upper and lower limits) in September, 2009. This value is much lower than the previous year's high of 30.83 (25.65 - 37.02, upper and lower limits) in August, 2008.

	Total Blood Samples		No. WNV Positive		Percent Positive	
Species	**2008**	**2009**	**2008**	**2009**	**2008**	**2009**
House Finch	854	865	86	53	10 %	6.1 %
House Sparrow	221	154	11	11	5.0 %	7.1 %
Other	40	35	2	2	5.0 %	6.0 %

Table 3: Wild bird data and WNV-seroconversion rates by species, 2008 - 2009.

Year	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Totals	Percent
2008	0/19	0/12	0/16	1/18	40/86	78/153	237/278	216/246	88/127	20/46	12/37	0/9	**692/1583**	**44%**
2009	0/25	1/19	1/42	0/57	2/92	1/94	1/62	1/64	2/47	2/19	0/17	0/15	**11/367**	**3%**

Table 4. Numbers of WNV-positive dead birds/total tested per month, 2008– 2009.

Analysis of the mosquito larval collection data showed a relative difference among larvae-positive neglected swimming pools, with *Cx. quinquefasciatus* as the most common species recovered, at approximately 50% (79 of 161) of these sites. Larvae of *Cx. tarsalis* (46%, or 74 of 161), *Cx. stigmatosoma* (20%, or 34 of 161), and *Cs. incidens* (17%, or 27 of 161) were also recovered from many of these sites. These data suggest that neglected residential swimming pools in urban areas can increase mosquito breeding sources for multiple WNV vectors (Table 4, Figure 2).

WNV-positive dead birds were found in every month except December during 2009, but overall monthly numbers and rates declined substantially for the year compared to 2008 (Table 3). The decline in dead bird reports from the public, through which the District collects many of its dead bird samples, may partially be caused by a lack of public interest. The perceived threat of this disease has waned from media attention, despite the District's efforts to involve the residents of the county in WNV surveillance. Hence, some of the reduction in numbers may reflect causes other than biological factors.

The detection of WNV antibody-positive wild birds had foreshadowed the discovery of WNV in mosquitoes and dead birds in 2004, ultimately indicating the emergence of multiple WNV transmission foci throughout Orange County (Schwedes et al. 2005). Reduced seroconversion levels in wild birds, however, may have indicated antibody persistence in adult passerine birds as the result of infection to WNV from the previous year (Schell et al. 2006). Unlike 2008, WNV-seroconversion rates in free-ranging wild birds did not rise during the summer/fall months of 2009.

From June through September, four human cases (no deaths) were reported in 2009, a dramatic decline from the 71 cases (3 deaths) reported in 2008. Prior to the first known human infection, avian serosurveillance in free-ranging house sparrows and house finches and testing of dead birds had demonstrated reduced, but continuous WNV transmission throughout 2009 in the county, suggesting a persistent, low-level risk to the public. Overall, fewer human cases may have been the result of a combination of wild bird immunity, lower mosquito infection rates, better mosquito control and public awareness of disease prevention.

CONCLUSION

Year-round arbovirus surveillance in 2009 continued to demonstrate that WNV is endemic in the suburban *Cx. quinquefasciatus*-peridomestic small bird cycle of Orange County and remains a threat to the public. Although detectable WNV activity declined throughout the county during 2009 compared to the two previous years, seasonal MLE (< 1.0) and avian antibody seroprevalence levels (~ 6.0%) remained relatively high at several foci. Future WNV abundance is likely to undergo yearly oscillations, decreasing and increasing with changes in avian immunity and shifts in mosquito numbers and species composition. The relative roles of vertical transmission, vector competency and host preferences in the mosquito populations, coupled with the roles of chronic infections and the attenuation of WNV amplification via herd immunity in avian reservoirs, remain to be determined for predicting the occurrence of WNV epizootics in Orange County.

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Comparison of RT-PCR West Nile Virus Test Results for Paired Samples of Kidney and Bilateral Intraocular Cocktail from Dead Birds Submitted to Orange County Vector Control District in 2009

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Orange County Vector Control District personnel have been performing in house necropsies and testing of dead birds for West Nile Virus (WNV) infection since 2004. Testing transitioned from imunnohistochemistry to real time Polymerase Chain Reaction (PCR) techniques in 2007. In an attempt to simplify bird processing, we initiated sampling of eye tissue in 2009 using the Rapid Bilateral Intraocular Cocktail (BIC) sampling methodology developed by Lim et al. (2009) in San Diego. With BIC sampling, the entire eye is extracted and probed for WNV RNA for smaller passerine birds; for larger birds, the retinal tissue is disrupted using the tip of a needle, and the contents aspirated into a syringe. All survey necropsies were performed in a bio-safety hood, notes on pathology and the proximal cause of death were recorded, and kidney samples removed for later WNV RNA testing. After eye and kidney tissue maceration, viral RNA was extracted using Ambion's *MagMAX magnetic particle processor and real time PCR performed on an ABI 7300* Real-Time PCR System. A positive WNV test result was considered to have a CT value ≤ 30. Results of the BIC and kidney assay methodologies were compared. A total of 272 birds were tested from January 2009 to October 21, 2009. Our findings showed that West Nile Virus activity for 2009 was exceptionally low with only ten West Nile Virus (WNV) positive dead birds identified following testing with both methods. Of the ten total WNV positive birds detected, nine were positive by both BIC and kidney tissue, and one bird was positive by kidney tissue but negative by BIC (Table 1). Our results were similar to those of the prior study; one specimen tested positive solely by kidney sample, and the remaining nine yielded positive results via both techniques. As in Lim et al. (2009), the majority of WNV positive birds showed a lower CT value for the kidney tissue. While the BIC technique is faster and somewhat more sensitive than the kidney necropsy, there was little difference in our perceived risk of WNV exposure to District personnel while processing specimens. We found no compelling reason to switch to BIC sampling for our in-house dead bird WNV testing program, and our District will continue to compare the two methodologies during 2010.

Table 1. Comparison of BIC and rtPCR of kidney sample, 2009.

All Species	BIC+/ Tissue +	BIC+/ Tissue -	BIC -/ Tissue +	BIC -/ Tissue -	Total
American Crow	6	0	1	83	90
Cooper's Hawk	1	0	0	7	8
House Finch	1	0	0	24	25
Mourning Dove	0	0	1	45	46
Total	**8**	**0**	**2**	**159**	**169**

Table 2. Comparison of Ct values for paired eye and kidney samples in 2009.

Species	Eye	Kidney
American Crow	28.58	18.97
American Crow	24.29	16.24
Cooper's Hawk	27.42	25.10
American Crow	21.07	17.10
American Crow	28.13	22.54
House Finch	17.52	19.66
American Crow	25.59	23.91
American Crow	29.91	18.19
Mourning Dove	39.80	29.72
American Crow	19.28	23.23

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Using the Collection Bottle Rotator Trap for Mosquito Abundance and Temporal Activity in Fresno County

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ABSTRACT: The Consolidated Mosquito Abatement District (District) employed a modified Collection Bottle Rotator (CBR) Model 1512 trap (John W. Hock Company) at specific surveillance sites in 2009 to examine abundance and temporal activity of mosquito species. The CBR trap was used: (1) in conjunction with a study to evaluate the efficacy of ground ultra low volume (ULV) adulticide applications against *Culex pipiens sensu lato*, and (2) as an additional sampling mechanism in areas of abundant and diverse mosquito species. Trap collections indicated significant temporal variation in mosquito activity. The CBR was also used in combination with other carbon dioxide-baited traps to collect mosquitoes that were subsequently tested for West Nile Virus (WNV). This function was enabled by constructing sampling containers for the CBR that facilitated the capture of live specimens so that subsamples could be pooled for testing. Pools of adult mosquitoes were submitted to the University of California, Davis Center for Vectorborne Diseases for testing.

INTRODUCTION

The Consolidated Mosquito Abatement District (District) covers about 1,058 square miles—approximately one-sixth of the total area of Fresno County and a small portion of Kings County. The combined area serviced by the District will be referred to as "the County". The District conducts surveillance of adult mosquitoes using a variety of mechanisms to measure abundance of vector populations, identify species variety, and detect the presence of mosquito-borne disease. Abundance and disease data are used to facilitate decisions about adult mosquito suppression and to provide public information for the District's residents.

The District employs traps to sample the adult mosquito population. These include New Jersey light traps at fixed sites as well as carbon dioxide-baited (CO_2) and oviposition (gravid) traps at both fixed sites and temporary locations. When adult mosquito populations reach high levels or when vector-borne disease is detected in sampled specimens, the District conducts suppression in designated areas using truck-mounted aerosol generators (foggers) to apply ultra low volume (ULV) insecticides for most situations. Although nuisance mosquitoes have been an annual concern in Fresno County throughout the history of organized mosquito control, mosquito-borne disease prevalence was relatively low during the last three decades of the 20th century. Following the introduction of West Nile Virus (WNV) into the County in 2004, the disease has been detected in local mosquito populations every year thereafter.

The District has acted to combat the occurrence of WNV with timely adult mosquito suppression in areas where the principal vectors of the disease are prolific. Studies suggest that ULV insecticides for adult mosquito control should be applied during flight times of host-seeking females in order to maximize efficacy (Anderson et al. 2007, Reisen et al. 1997). Because this requires knowledge of the temporal activity of the target species, the employment of a time-segregated trap was incorporated into a larger study designed to evaluate the efficacy of the District's adulticiding program in 2009 (Holeman unpublished). The time-segregated trap was also used to study temporal abundance of various species in several distinct locations.

MATERIALS AND METHODS

Equipment. The time-segregated trap used in the surveillance studies was a modified Collection Bottle Rotator, Model 1512 (John W. Hock Company). The instrument, on loan from the Orange County Vector Control District, was equipped with a programmable timer, a suction fan operated with a 6-volt motor and eight plastic open-ended killing jars (half-jars) that fit into corresponding positions on the trap's rotating platen. The trap was supported by insertion of its central steel rod into a pipe fastened to a wooden base. Two screws were tightened to the rod so that the platen would rotate properly. When set up, the top of the cylinder surrounding the fan (where mosquitoes enter) was 49 inches above the ground.

Collection containers were constructed for live capture of specimens. The containers were made from clear plastic jars, each equipped with glued screens on the bottom and sides to allow for ventilation. A hole was cut into each plastic jar lid so that a cloth sleeve could be glued over the outer edge, allowing the assembly to be drawn over the outside of the half-jar and held in place with a rubber band. An inverted cone of soft, fine-mesh screen was glued inside each half-jar to prevent captive specimens from escaping.

Power to the suction fan and gear motor was supplied with a 6-volt and 12-volt rechargeable battery, respectively. This arrangement precluded the synchronization of the trap rotator with the fan operation but enabled the fan to spin at a slower rate than it would if powered by the 12-volt battery. Each battery was secured inside a plastic coffee can with lid to provide protection from the environment.

CO_2 was used as a lure in the form of dry ice kept inside an insulated plastic bucket with four holes to allow escape of gas. The bucket was hung on a metal stake of angle iron and positioned several inches over the suction fan.

Study Areas. The CBR was used at two distinct sites in conjunction with the ULV efficacy study. One site, California State University (CSU) Fresno, was located within farmland on the university campus and 275 meters from the closest urban residents. The other site, Riverdale, was on a farm 1.7 km SE of the nearest residential community. Three additional study sites included two

in the vicinity of Laton—Upton and Gonsalves, a farmstead and a commercial dairy, respectively. Each of these sites was surrounded by agriculture, but both were within 650 m of residential neighborhoods. China Creek Park, a mixed riparian habitat operated by the County in a rural environment directly SW of Centerville, was the final study site. This site lies 3.5 km NE of the City of Sanger.

CSU Fresno. The CBR was operated on five nights in an orchard at 36.819489° N, 119.730150° W. Surrounding the site was a mixed agricultural habitat consisting of stone fruit, citrus and sweet corn. The trap was programmed to rotate each of the eight positions at 90-minute periods for a total time of 12 hours. The platen rotated at the commencement of each period. To ensure that the first position would be underneath the fan during the first period (2000-2130 hours), the trap was always set up earlier with the fan on and over the eighth position. This procedure was followed in all setups of the CBR during 2009. Weather data was collected from the California Irrigation Management Information System (CIMIS) at CSU, Fresno.

Riverdale. The CBR was operated on two nights in a stone fruit orchard at 36.411917° N, 119.833972° W. Silage corn was in abundance N, W, and S of the site as well as a commercial dairy 700 m SE. The trap was programmed to rotate at 90-min periods (1930-0730) on the first night and at 60-min periods through the first seven rotations (1900-0200) on the second night.

Laton. The CBR was operated once at Upton (36.425667° N, 119.695583° W) and once at Gonsalves (36.439194° N, 119.672583° W) three nights later. The trap was programmed for 90-min periods (2000-0800) at both sites.

China Creek Park. The CBR was operated on four nights situated adjacent to a wetland pond at 36.720933° N, 119.505208° W. Four additional CO_2-baited traps were simultaneously set up in the vicinity, spread out over an area of approximately forty acres. For the two August trap nights, the first period was set to begin at hour 2000. To adjust for shorter days in September, the commencement of the first period was adjusted to hour 1930 and hour 1900, respectively, for the final two trap nights. Subsamples of CBR collections were pooled with specimens from the additional traps and submitted for arbovirus testing.

RESULTS

CSU Fresno. Mosquito species collected (%) included *Culex pipiens sensu lato* (93.5), *Culex tarsalis* (6.4) and *Culiseta incidens* (0.1). The number of female *Cx. pipiens* collected for each of the five nights along with the five-night average abundance plotted against the corresponding averages for temperature, humidity, and wind velocity is shown in Figure 1.

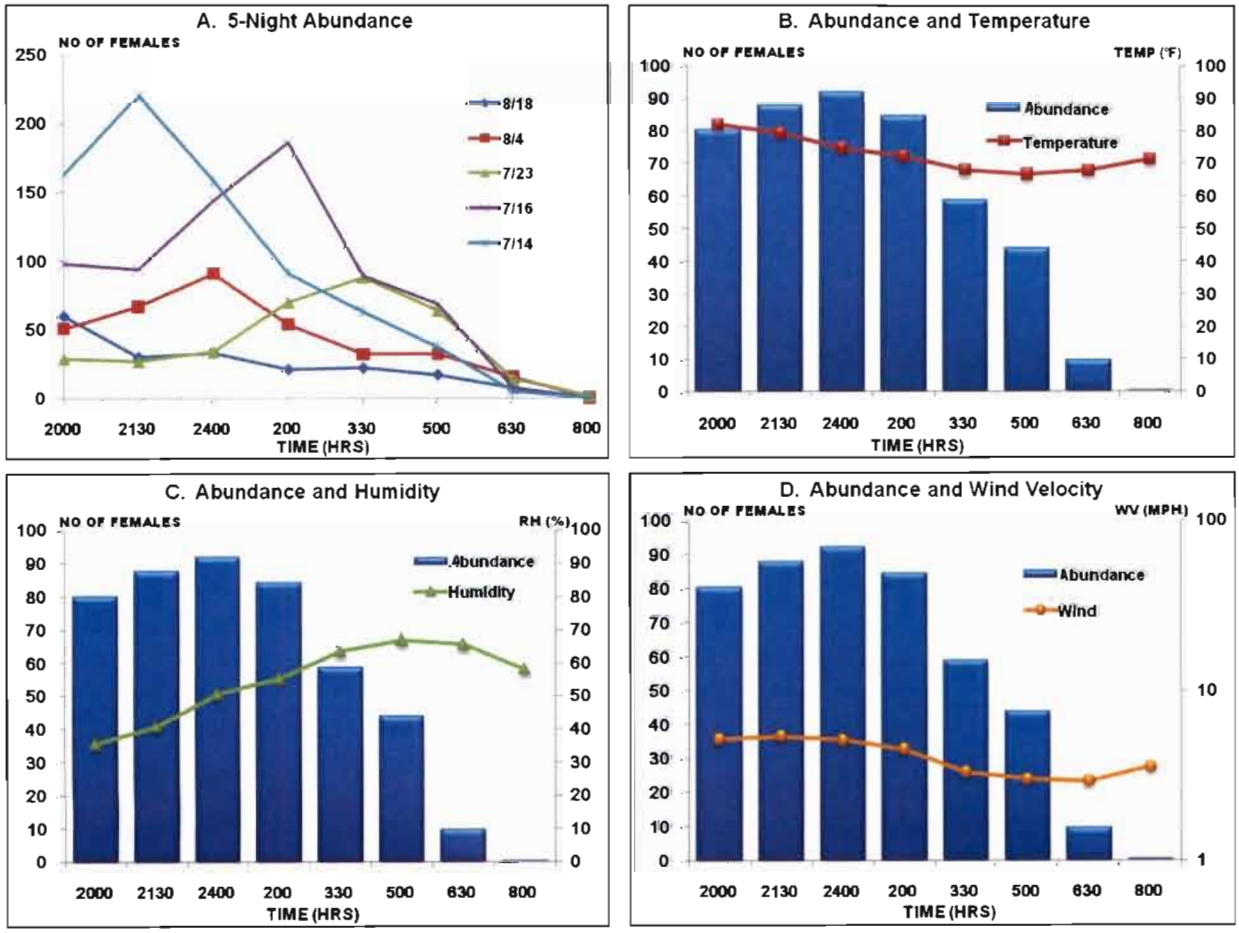

Figure 1. Temporal activity of *Culex pipiens sensu lato* in females per 90-minute period at CSU, Fresno, 2009.

Riverdale. Mosquito species collected (%) included *Cx. pipiens sensu latu* (90.6) and *Cx. tarsalis* (9.4). The first trap night (Aug 28) indicated that the highest activity was during the first period (1930-2100) for both species. There was a consistent temporal decline in *Cx. pipiens* abundance thereafter, with specimens captured during all but the last period (0630-0800). Using shorter sampling periods for the second trap night (Sep 16), the highest activity was during the second period (2000-2100) which ended at the same time as the first period for Aug 28. Temporal activity of *Cx. pipiens* at the Riverdale site is shown in Figure 2.

Laton. Mosquito species collected (%) from both sites combined included *Cx. pipiens sl* (96.5) and *Cx. tarsalis* (3.5). Activity of *Cx. pipiens* appeared to be significant throughout the night at the Upton site, with the highest collection in the third period (2300-2430). The Gonsalves site had fewer adults, with the most specimens collected in the fifth period (0200-0330).

China Creek Park. Mosquito species collected for the four nights are combined in Table 1. Specimens submitted for arbovirus testing included *Cx. erythrothorax* (Aug 20, Sep 29), *Cx. stigmatosoma* (Aug 6), and *Cx. tarsalis* (Aug 6). Temporal activity of the *Culex* species collected is illustrated in Figure 3. *Culex* activity on August 6 tended to be highest during period 0200-0330 hours in contrast to August 20 when it was highest during 2000-2130. All *Culex* appeared to be highly active during 0130-0300 on September 4, following an earlier peak of *Cx. erythrothorax* at 2100-2230. This trend was somewhat similar on September 29, albeit with a smaller sample. Collections of *Aedes*, *Anopheles* and *Culiseta* spp. for the first three trap nights were relatively few, and the temporal patterns observed were highly variable. Only on Sep 29 were high numbers of *Aedes vexans* collected, and the activity decreased precipitously after the first period (1900-2030).

DISCUSSION AND CONCLUSIONS

Efficacy Study. Using the CBR enabled the District to learn more about the temporal activity of *Culex pipiens*, the most abundant species inhabiting the study site communities and a principal vector of WNV. This information was incorporated into the determination of optimum timing of ULV treatments at CSU Fresno and Riverdale. Host-seeking activity of *Cx. pipiens* at CSU Fresno as measured by captive females varied considerably at different time periods, averaging the highest level during the period between 2130 and 2300 hours. Temperature, relative humidity and wind velocity varied slightly during the trap nights. Activity of *Cx. pipiens* at Riverdale exhibited a consistent pattern during two trap nights: peak activity occurred between the hours of 1930 and 2100, followed by a steady decline throughout the evening. Because the first trap night at Riverdale commenced ten

Table 1. Total specimens collected by species in the modified CBR trap at China Creek Park, Fresno County, 2009.

Species	4-Night Totals	Highest 1-Night
Aedes vexans	173	147
Culex stigmatosoma	108	65
Culex erythrothorax	78	34
Anopheles punctipennis	78	27
Culex pipiens s.l.	76	27
Culex tarsalis	70	29
Culex restuans	24	15
Culiseta particeps	20	7
Anopheles freeborni	10	5
Culex thriambus	2	2
Culiseta inornata	1	1

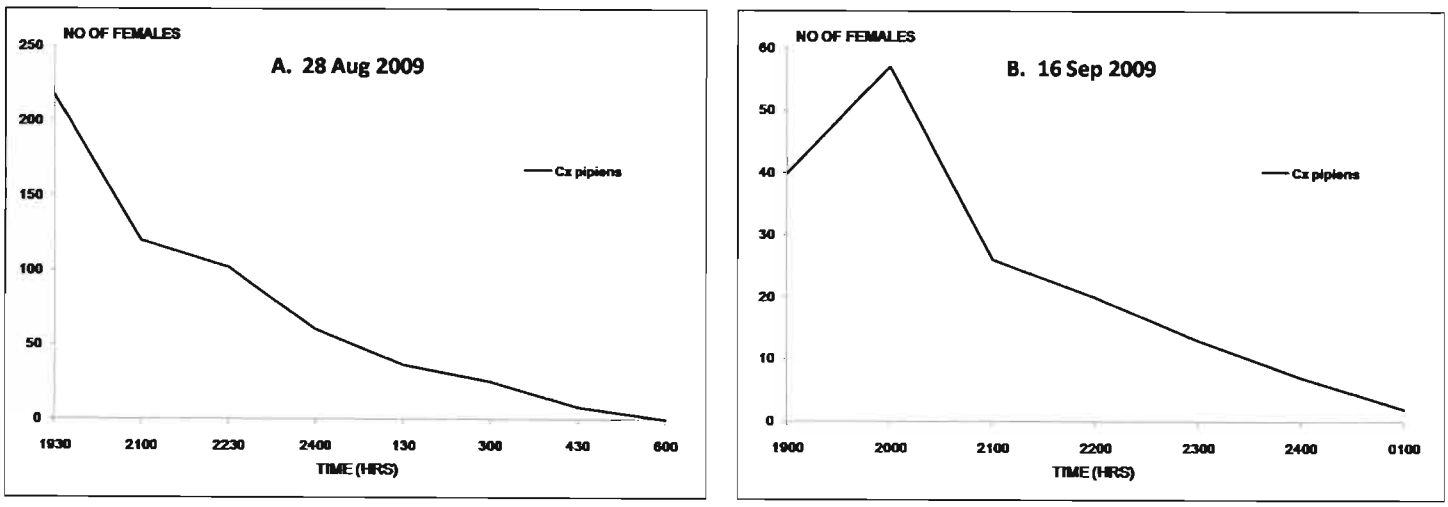

Figure 2. Temporal activity of *Culex pipiens sensu lato* in females at Riverdale, Fresno County, 2009.

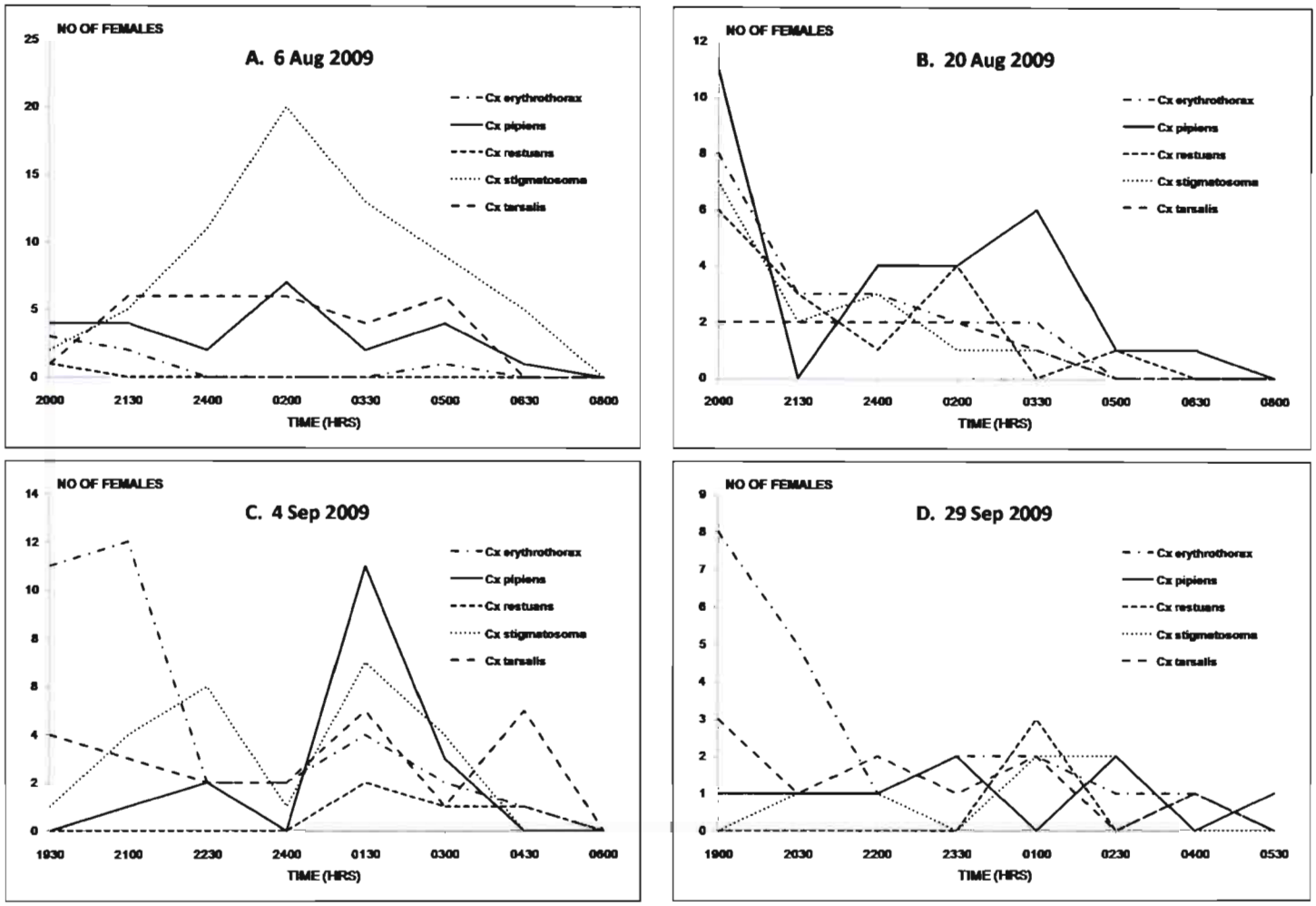

Figure 3. Temporal patterns in the host-seeking activity of *Culex* species in females per 90-minute period at China Creek Park, Fresno County, 2009.

days following the final night at CSU Fresno, perhaps different temperatures and shorter days influenced the temporal activity of the Riverdale mosquitoes. Employment of the CBR in the efficacy study was valuable to the District in conjunction with its *Cx. pipiens* adult suppression program and is likely to be used in future endeavors.

Abundance and Diversity. In four trap nights, the CBR collected eleven species of mosquitoes at China Creek Park. *Aedes vexans* represented the largest sample with nearly all specimens captured on one night. Among the six species of *Culex* mosquitoes captured, *Cx. stigmatosoma* had the highest numbers. *Culex erythrothorax*, *Cx. pipiens*, *Cx. tarsalis* and *Anopheles punctipennis* were trapped in nearly equal numbers. The subsamples of *Cx. erythrothorax*, *Cx. stigmatosoma* and *Cx. tarsalis* that were pooled and submitted for arbovirus testing had negative results. Temporal activity varied significantly among the species with disparate peak levels occurring on different nights. Although nightly samples were generally small, there appeared to be some similarity in the temporal patterns of *Culex* species. Among this group, the Aug 20 collection indicated that the highest host-seeking activity occurred in the early evening (2000-2130), whereas the Sep 4 collection suggested an active period from 2400 to 0300. Weather conditions, photoperiod and species' life cycle timing are all likely contributors to the observed variance in temporal activity. In a habitat containing such a diversity of mosquito species, the CBR may be helpful in learning more about the behavior of the adults that compete for available blood meals.

ACKNOWLEDGEMENTS

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West Nile Virus Dead Bird Surveillance Program, 2010 Survey Results

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INTRODUCTION

Since 2000, the California Department of Public Health's (CDPH) West Nile Virus (WNV) Dead Bird Surveillance Program (DBSP) has been an important component of statewide WNV surveillance (McCaughey et al. 2003). This report highlights the utility of this program as a surveillance tool as well as summarizes results from a recent survey to participating mosquito and vector control districts in California.

Overview of Dead Bird Surveillance Program. CDPH's West Nile Virus Dead Bird Surveillance Program has been operating for ten years. In 2000 the program was initiated with grant funds from the U.S. Centers for Disease Control and Prevention with the goals of facilitating early detection of WNV, monitoring ongoing transmission of the virus and enhancing public education throughout the state of California. The program was expanded in 2002 to include the Dead Bird Reporting Hotline (1-877-WNV-BIRD) and California's WNV website (www.westnile.ca.gov) to increase public reports of dead birds (McCaughey et al. 2003).

The DBSP is dependent upon cooperation and participation from California residents and functions in collaboration with local vector control agencies, mosquito abatement districts, the University of California Davis laboratories- Center for Vectorborne Diseases (CVEC) and the California Animal Health and Food Safety Lab (CAHFS). Passive surveillance is effective for WNV as crows and other corvids most susceptible to WNV-caused mortality are common in urban and suburban areas (Eidson 2001). The DBSP expands surveillance by covering areas that may have been overlooked or unable to be reached with active surveillance practices such as mosquito pool testing. Because dead bird reporting is highly dependent upon public outreach and education, the more educated and informed the public is, the more likely dead birds will be reported. For example, it is common to find an increase in dead bird reporting regionally after a local news story (CDPH, personnel observation).

Together the dead bird hotline and the California WNV website are able to offer the public and regional agencies up-to-date information about WNV as well as facilitate submission of dead birds and tree squirrels for WNV testing. The toll-free dead bird hotline operates seven days a week from 8:00am-5:00pm with at least one operator on staff. Hotline operators have college undergraduate or graduate degrees in biology, public health or a related field. After hours callers can receive readily available recorded information on WNV and are able to report a dead bird or squirrel via voicemail prompts. The WNV website is also a resource for residents to report dead birds online and access reports and information on WNV activity throughout the state at any time.

COMPARATIVE RESULTS

There have been 342,269 birds reported to the DBSP since its initiation in 2000; 11.3% of these were tested for WNV (Table 1). Of the 38,798 dead birds tested over the past 10 years, 31.7% were positive for WNV (n = 12,300). Between 2004 and 2008 there were over 30,000 dead birds reported each year; 2005 was the highest reporting year with 109,375 dead birds reported. In 2009, the number of bird reports was significantly lower than the previous year, decreasing from 33,568 reports in 2008 to 15,472 reports in 2009 (~54% decrease). In addition, the number of birds that tested positive decreased by 79.9% between 2008 and 2009. This decrease in WNV activity was also noted in other surveillance elements; the number of positive human cases and mosquito pools, declined by 74.8% and 46.9% respectively. The prevalence of positive dead birds also varied from year to year (Fig. 1), and fluctuations ranging from 19% to 56% were seen between 2004 and 2009. Highest levels of prevalence were seen in the years 2004 and 2008, with 56% and 42% positive, respectively.

Year	Reported	Tested	Positive
2000	40	20	0
2001	68	18	0
2002	3,666	653	0
2003	8,650	1,765	96
2004	93,057	5,728	3,232
2005	109,375	9,263	3,046
2006	46,345	6,508	1,446
2007	32,028	5,942	1,396
2008	33,568	6,098	2,569
2009	15,472	2,803	515
Total	**342,269**	**38,798**	**12,300**

Table 1. Dead birds reported, tested and positive, 2000-2009.

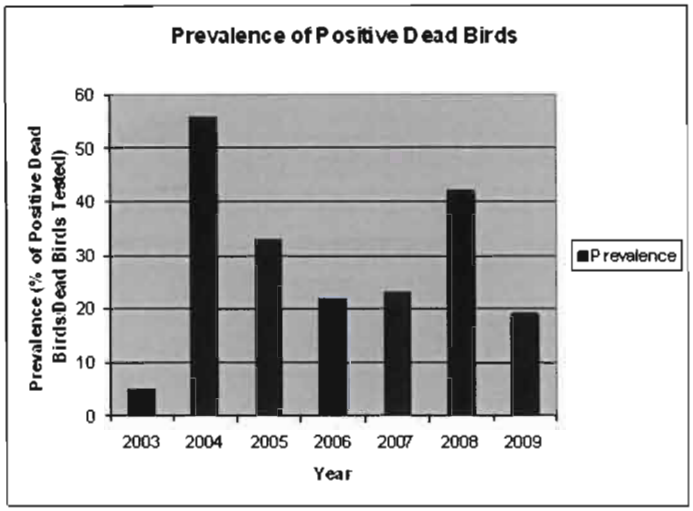

Figure 1. Prevalence of positive WNV dead birds, 2003-2009.

Along with dead birds, dead tree squirrels are also collected for WNV testing in California (Padgett et al. 2007). As tree squirrels have relatively small ranges compared to birds, they can provide highly localized evidence of WNV transmission. Similar to dead bird reports, dead tree squirrel reports have been gradually declining since 2007 and declined greatly between 2008 and 2009. The highest level of reporting occurred in 2007 when 669 tree squirrels were reported (Table 2). In the years 2004 - 2006 the prevalence of WNV in tree squirrels was comparable to the prevalence in dead birds, but from 2007 to 2009 the prevalence of dead WNV infected tree squirrels began to decrease, falling below the level of prevalence in dead birds.

Year	Reported	Tested	Positive	Prevalence
2004	194	77	49	63%
2005	493	155	48	31%
2006	375	142	32	23%
2007	669	218	26	12%
2008	647	217	32	15%
2009	307	103	10	10%
Total	**2685**	**912**	**197**	

Table 2. Dead tree squirrels reported, tested, positive and prevalence, 2004-2009.

First Detection-Comparative WNV Surveillance Elements. One of the goals of the DBSP is to facilitate early detection of WNV. This early detection in dead birds provides an early warning of the risk for transmission to humans (Eidson et al., 2001) and aids in prioritizing areas for mosquito control intervention (Watson et al. 2004). Dead birds have reliably been the first surveillance element to detect WNV in most California counties, showing evidence of viral activity prior to sentinel chickens, mosquito pools, tree squirrels, horses and humans. Since the first positive dead bird was identified in 2003, dead birds have continuously had a high rate of first detection, greater than 60% (Table 3). The rate is calculated based on the number of counties with dead birds as the first positive WNV element relative to the total number of counties with WNV activity. In 2009, 29 of the 42 counties with WNV activity were first detected by positive dead birds. Although a significantly lower number of dead birds tested positive in 2009, the first detection rate was 69%, and dead birds were detected an average of 49 days before any other surveillance element.

Year	Counties w/ Dead Bird as First Positive Element	Total Positive Counties	First Detection Rate
2009	29	42	69%
2008	38	48	79%
2007	42	51	82%
2006	41	53	77%
2005	39	48	81%
2004	48	57	84%

Table 3. First detection rate of dead birds, 2005-2009.

2010 WNV Surveillance Survey Design. In January 2010, CDPH conducted a survey to assess current and projected WNV surveillance requirements of mosquito and vector control agencies throughout California. The survey was designed on a website platform and distributed to 65 agencies throughout California in an email containing the link to the survey and instructions encouraging all agencies to participate. There were a total of fourteen questions; the first four recorded the name of the person completing the survey, their agency, telephone number and email address. The remaining ten questions covered different elements and aspects of California's WNV surveillance program. Questions pertained to the DBSP, mosquito pool testing, chicken seroconversions, human infections, adult mosquito occurrence reports, monthly educators teleconference calls and taskforce

meetings. Seven of these questions specifically pertained to the DBSP, as discussed below.

Mosquito and Vector Control Districts Survey Results. The results of the survey are based on 36 completed surveys received from mosquito and vector control agencies from 30 counties (response rate of 55.3% of 65 agencies surveyed). Results of survey questions (presented in italics below) that pertain specifically to the DBSP are as follows.

Question 1) In 2010, does your agency intend to collect dead birds to test for WNV? The majority of agencies intended to collect dead birds for testing (34 of 36 [94%]); two agencies (6%) did not plan to collect dead birds.

(2) In 2010, does your agency plan to test dead birds in-house? 17 of 36 (47%) agencies plan to test birds in-house and 19 (53%) agencies do not plan on testing in-house.

(3) If your agency does test in-house, what type of test will you use? (more than one choice allowed). Of the 17 agencies that do plan on testing birds in-house, nine agencies plan to use VecTest, six agencies plan to use RAMP, and three agencies plan to use RT-PCR.

(4) If your agency does test in-house what sample type will be used for testing? (more than one choice allowed). Of the 17 agencies that do plan on testing birds in-house, 15 agencies plan to use oral swabs, one agency plans to use the kidney for testing, one agency plans to use ocular tissue, and one agency plans to use cloacal swabs.

(5) In 2010, does your agency intend to collect dead squirrels to test for WNV? Of the 36 agencies that responded, 15 (42%) intend to collect dead squirrels for testing, 15 (42%) do not intend to collect dead squirrels and 6 (16%) are undecided.

(6) Did your agency advertise, or refer callers to the CDPH WNV hotline or website (www.wnv.ca.gov) for reporting dead birds? Of the 36 agencies that responded, 34 (94%) responded that they would advertise or refer callers to the CDPH hotline or website, and 2 responded that they would not (6%).

(7) Please assign a number to each item below according to the value each has to your agency (1 = not important, 5 = very important): Dead bird reports, Positive Dead Birds, DYCAST, Wednesday weekly bird report and the WNV website. The score presented represents an average score of all 36 respondents. Dead bird reports received an average rank of 4.0; Positive dead birds received an average rank of 4.6; DYCAST received an average rank of 3.1; Wednesday weekly bird report received an average rank of 4.0; and the WNV website received an average rank of 4.4.

DISCUSSION

With the survey results, CDPH was able to assess the surveillance activities of the mosquito and vector control agencies and their intended participation for the upcoming 2010 testing season. From the survey, we determined that the majority of agencies will continue to collect dead birds for testing, and nearly half of these agencies will test birds in-house using VecTest, RAMP or RT-PCR. For agencies that test birds in-house, the most common sample type used will be an oral swab, with few agencies using kidney sample, ocular tissue and cloacal swabs. The remaining agencies will submit dead birds to be tested to the CVEC laboratory at UC Davis. While bird collection and testing will remain high, only 37.5% of agencies intend to collect dead tree squirrels in 2010. All aspects of the DBSP were assigned positive rankings of importance, receiving evaluations above 3.0 and the majority receiving rankings of 4.0 or above.

The DBSP is a valuable and integral component of the WNV surveillance system in California. Positive dead birds continue to be one of the earliest indicators of WNV activity in California counties. While a primary role of the WNV hotline and website is still the collection of dead bird reports, both are increasingly important in providing information to the public and local agencies. For example, operators can respond directly to concerns and questions from the public regarding WNV. Similarly, the public and local agencies can access reports and bulletins on the WNV website. Ongoing education and outreach to the public encouraging them to report dead birds is essential to maintaining the value of the DBSP as an important WNV surveillance tool.

ACKNOWLEDGEMENTS

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Analysis of Mosquito Abundance and Arbovirus Surveillance in Northwestern Riverside County 2009

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ABSTRACT: Since it's creation in 1959, the Northwest Mosquito and Vector Control District (NWMVCD) has been conducting surveillance of mosquitoes, mosquito-borne arboviruses and other disease vectors in the northwestern part of Riverside County, California. Mosquito populations were surveyed and sampled with Dever-Northwest traps (modified CDC/EVS carbon dioxide-baited traps herein referred to as DN traps), gravid traps and resting boxes. Arboviral surveillance was conducted for Saint Louis encephalitis (SLE), West Nile Virus (WNV) and Western Equine encephalomyelitis (WEE) by sampling and testing mosquitoes and screening of sentinel chicken flocks, dead birds and trapped wild birds. Using DN traps, a total of 97,817 mosquitoes were collected. As in previous years, the most common genus in the District was *Culex* (96%), and *Cx. erythrothorax* was by far the most abundant species collected. A total of 244 mosquito pools of host-seeking females were collected by the DN traps, and all tested negative for SLE, WNV and WEE viruses. One of 50 pools of *Cx. quinquefasciatus* females collected in the gravid traps was positive for WNV, and 11 of 60 sentinel chickens in four separate flocks seroconverted for WNV. Of the 155 wild birds tested, one pigeon that was bled on 18 June 2009 was found seroconverted for WNV; this was the first indicator of WNV activity within our district in 2009. None of the 14 dead birds tested in-house with the Immunohistochemistry (IHC) technique were found positive for any arbovirus. Despite lowered WNV activity in 2009, mosquito populations increased within the District boundaries; in contrast, we observed a drastic reduction in the number of dead bird-related service requests received from District residents. Possibly, WNV is evolving in terms of it's host specificity and is infecting new hosts from other bird families such as house finches, sparrows, cowbirds and starlings; these birds are significantly smaller and their carcasses perhaps not easily detected by the public. When the WNV related results from years 2003-07 were compared graphically, a cyclic pattern in the activity of WNV appeared; activity increased to a peak and subsequently declined every three years. If this pattern continues in the future, then there will likely be a slight increase in WNV activity in 2010 and a large peak of activity in 2011. The cyclic pattern could be due to the evolution of this virus in term of it's infectivity/pathogenicity, possible movement of this virus into new hosts or may be related to cyclic pattern of factors which trigger it's activity.

INTRODUCTION

The NWMVCD encompasses over 240 square miles of area and provides service to over 500,000 residents. The service area includes the cities of Norco, Corona, Lake Elsinore, part of Riverside and several outlying communities. WNV invaded California in 2003 and was first detected in Riverside County in 2003 (Wisniewska-Rosales et al. 2004). Since then it has been regularly detected every year in our county. To monitor arbovirus activity, District personnel surveyed and sampled mosquito populations with DN traps, gravid traps and resting boxes. Arboviral surveillance was conducted for SLE, WNV and WEE by sampling and testing mosquitoes, screening seroconversion of sentinel chicken flocks and analyzing dead and trapped birds for antibodies.

MATERIALS AND METHODS

Mosquito Surveillance

Dever-Northwest Trap. Dever-Northwest (DN) traps (Fig. 1) differ from standard Encephalitis Virus Surveillance (EVS) traps by their larger flight path openings for mosquitoes, as well as their usage of rechargeable batteries (Williams et al. 2009). Traps were deployed at 25 fixed trap sites once a week from March to November. All mosquitoes captured by these traps

Figure 1. Dever-Northwest (DN) Trap.

were anesthetized with triethylamine (TEA) and sorted by species and sex. A total of 244 pools (12 - 50 female mosquitoes/pool) were submitted on dry ice to the Center for Vector-borne Diseases (CVEC) at University of California, Davis for arbovirus screening using the singleplex RT-PCR Taqman assay and positives were confirmed with a second primer set.

Gravid Traps. Gravid traps were placed and operated overnight at nurseries, horse ranches and other urban environments between June and September, 2009. Collected mosquitoes were anesthetized with TEA and sorted by species and sex. Fifty pools consisting of 12 - 50 females were submitted on dry ice to CVEC for arbovirus screening with RT-PCR.

Resting Boxes. Three resting box traps (H x D x W: 2m x 2m x 1m) were deployed (two in Norco and one in Mira Loma cities) to collect blood-fed mosquitoes. Blood-fed mosquitoes from these boxes were collected once a week using a mechanical aspirator. A total of 826 blood-fed females were collected between July and December 2009; these will be analyzed in the future for the sources of their blood meals. Six pools of 12 - 50 gravid mosquitoes were collected from these boxes and submitted to CVEC for arbovirus screening.

Avian Surveillance

Sentinel Chicken Flocks. Six sentinel flocks consisting of ten white leghorn hens each were maintained at strategic locations within the District boundary. Blood samples were collected every two weeks, placed on filter strips, air dried and submitted to CVEC for detecting seroconversion using indirect enzyme immunoassay (EIA).

Wild Birds. Two modified Australian crow traps were deployed by the District, one each in Corona and Norco. Birds were trapped and sampled at approximately two to three week intervals throughout the year. Specimens were also collected from traps deployed by the Orange County Water District (OCWD) for brown-headed cowbird control. Additionally, several residents volunteered to have their pet birds screened for seroconversion against arboviruses. Blood samples from all birds were submitted to Orange County Vector Control District Laboratory for seroconversion against SLE and WEE by serum hemiglutination inhibition (Gruwell et al. 2000) and WNV by a blocking ELISA test (Jozan et al. 2003).

Dead Bird Surveillance. Between April and November, 14 wild dead birds were either reported directly to the District or via California Department of Public Health (CDPH) dead bird hotline (1-877-968-2473); these cadavers were collected and transported on blue ice to the District laboratory where they were tested in-house with the IHC technique for arboviruses.

RESULTS

Mosquito Surveillance

Dever-Northwest Traps. Using DN traps, a total of 97,817 mosquitoes were collected in 2009 (Table 1). As in previous years, *Culex* spp. were the most commonly collected mosquitoes in the District, although species of *Aedes*, *Anopheles* and *Culiseta* were also present. *Culex* spp. comprised 96% of all mosquitoes that were collected in DN traps, compared to 98% in 2008 (Sandhu

Table 1: Total number of mosquitoes caught by Dever-Northwest (DN) traps in year 2009.

Month	# TRAPS	*Culex*					*Anopheles*		*Culiseta*		*Aedes*	
		stig	*tars*	*thriam*	*erythro*	*quing*	*hermsi*	*incidens*	*inornata*	*particeps*	*washinoi*	*vexans*
		Total	Total	Total	Total	Total	Total	Total	Total	Total	Total	Total
January	*	*	*	*	*	*	*	*	*	*	*	*
February	*	*	*	*	*	*	*	*	*	*	*	*
March	12	54	149	0	246	2	0	1	7	1	0	1
April	72	59	911	0	6063	35	40	118	89	31	12	0
May	74	419	1937	2	6643	183	148	81	67	74	1	2
June	120	314	1939	99	20227	416	1095	234	73	86	1	0
July	97	178	1351	30	7747	455	732	49	6	69	0	0
August	120	220	1239	100	8265	470	485	7	13	26	0	0
September	97	288	11435	53	3256	555	183	4	9	44	0	0
October	99	397	3729	35	7080	515	138	4	22	17	0	0
November	75	177	283	13	5462	749	17	8	29	13	0	0
December	*	*	*	*	*	*	*	*	*	*	*	*
Totals		2106	22973	332	64989	3380	2838	506	315	361	14	3

* No samples were collected during this time.

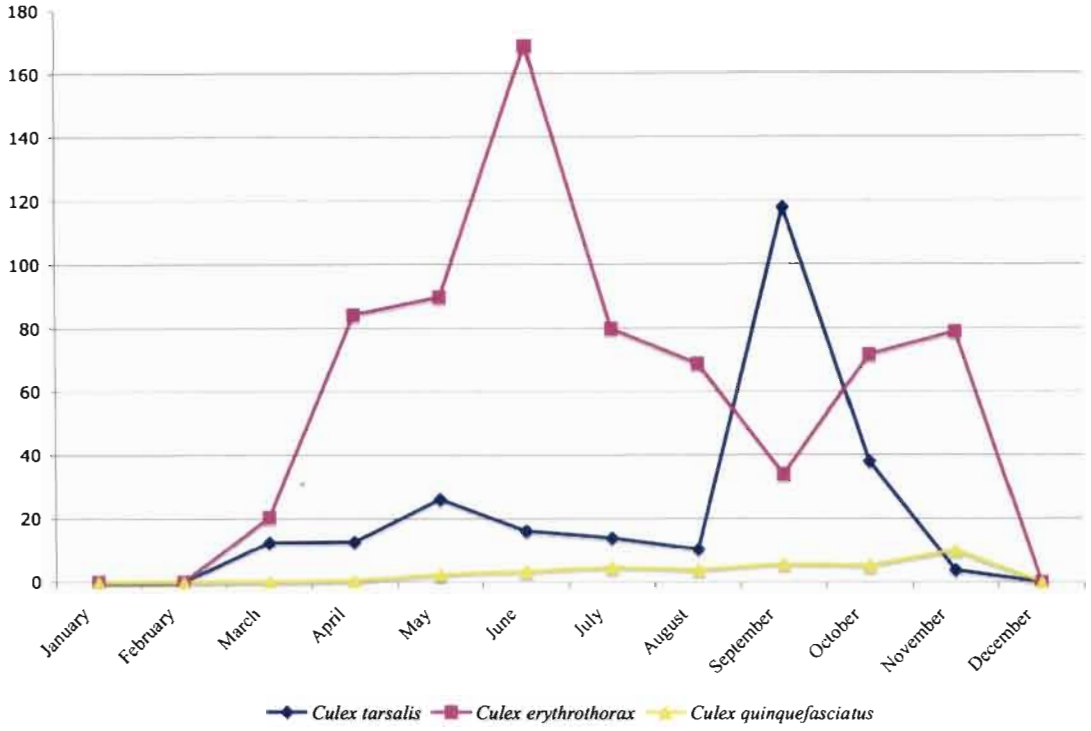

Figure 2: Mean numbers of mosquitoes/ Dever-Northwest trap during 2009.

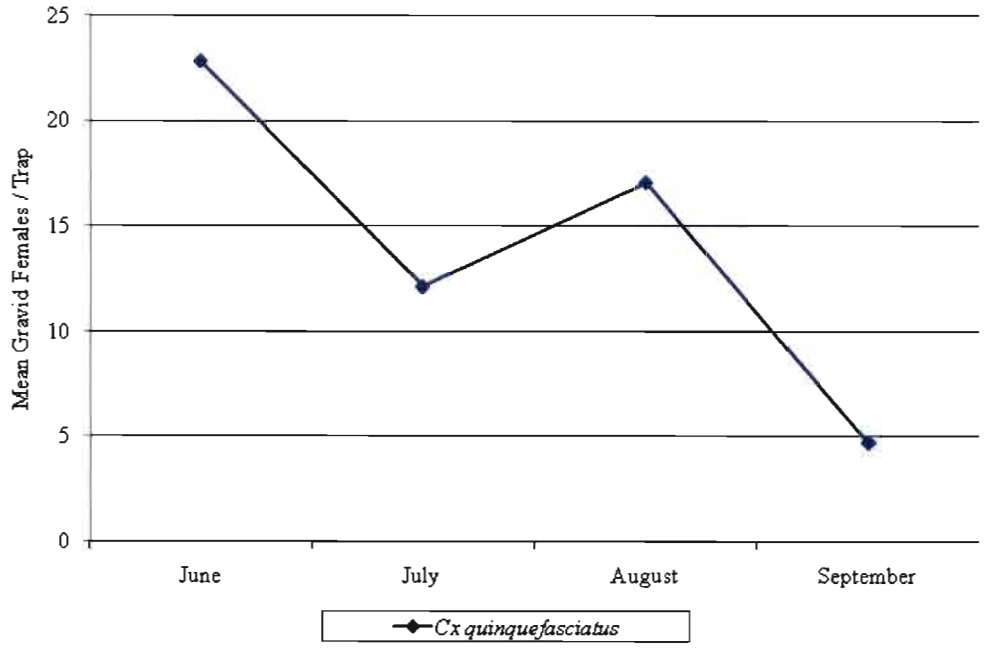

Figure 3: Average Numbers of *Cx quinquefasciatus* captured in gravid traps during 2009.

et al. 2009). Mean DN trap counts for the most common species captured in different months during year 2009 are shown in Figure 2. *Culex erythrothorax* was by far the most abundant species, followed by *Culex tarsalis*. *Culex erythrothorax* numbers peaked in June, while *Culex tarsalis* populations peaked in September. The CVEC tested 244 mosquito pools from our DN traps, but none were found positive for SLE, WNV or WEE.

Gravid and Resting Box Traps. A total of 42 gravid traps were deployed in the 2009 trap season, capturing a total of 649 mosquitoes. *Culex quinquefasciatus* was by the most common species (92% of the total), and 50 pools of this species were sent to CVEC for arbovirus testing. Average numbers of *Cx. quinquefasciatus* per trap are shown in Figure 3. In addition to *Cx quinquefasciatus*, gravid trap catches included *Cx. stigmatasoma* (3%), *Cx. tarsalis* (1%), *Cx. thriambus* (1%), *Cx. erythrothorax* (3%) and a single *Anopheles hermsi*. Of the 50 submitted gravid mosquito pools, only one pool of *Cx quinquefasciatus* collected on September 2, 2009 in Norco tested positive for WNV. Although resting box traps were primarily sampled for a different blood-meal related study, 6 pools of 158 gravid mosquitoes from these boxes were pooled and tested at CVEC; none were positive for SLE, WNV or WEE.

Avian Surveillance

Sentinel Chicken Flocks. First evidence of seroconversion for WNV in a sentinel chicken was detected in a blood sample collected on July 15 from the sentinel flock deployed at Corona Municipal Airport. In total, 11 sentinel chickens belonging to 4 different flocks (3 from Temescal, 5 from Rancho Jurupa, 2 from Corona airport and 1 from Mockingbird Canyon) seroconverted for WNV.

Wild Bird and Dead Bird Collections. Table 2 gives the species breakdown of 155 wild birds that were bled for arbovirus surveillance during 2009. A majority of these birds were either brown-headed cowbirds (*Molothrus ater*) (37%), house finches (*Carpodacus mexicanus*) (24%) or chickens (*Gallus gallus*) (19%). One pigeon owned by a local resident in Mira Loma, which was bled on 18 June 2009, seroconverted for WNV and was the first indicator of WNV activity within our District in 2009. Additionally, there were two more WNV positive wild birds (both house finches) collected from the Australian crow trap located in Corona. Fourteen dead birds were collected, necropsied and tested in-house with IHC. None was found positive for any arbovirus.

DISCUSSION

As compared to 2008, there was a marked decrease in WNV activity during 2009. Despite this lowered WNV activity, mosquito populations increased within the District boundaries. Other than *Cx. tarsalis*, all mosquito species increased in numbers in 2009 compared to 2008. *Culex erythrothorax* was by far the most abundant species followed by *Cx. tarsalis* and *Cx. quinquefasciatus*. Populations of *Cx. erythrothorax* peaked in June with a mean of 169 female mosquitoes/trap, *Cx. tarsalis* peaked in October with 118 mosquitoes/trap, and *Cx. quinquefasciatus* peaked in November with 10 mosquitoes/trap. Only eleven sentinel chickens seroconverted for WNV in 2009, compared to

Table 2: Species and number of wild birds bled during 2009.

Species	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	**TOTAL**	**WNV +**
Brown-headed cowbird	16	29	0	0	0	2	1	9	0	0	0	0	**57**	**0**
House finch	0	0	0	0	0	14	2	0	0	0	12	9	**37**	**2**
House sparrow	0	0	0	0	0	3	3	0	0	0	0	0	**6**	**0**
Pigeon	0	0	0	0	0	5	0	0	0	0	0	0	**5**	**1**
Plymouth rock	0	0	0	0	0	4	0	0	0	0	0	0	**4**	**0**
Rooster	0	0	0	0	0	10	10	10	0	0	0	0	**30**	**0**
Song sparrow	0	0	0	0	0	5	0	8	0	0	0	0	**13**	**0**
Turkey	0	0	0	0	0	2	0	0	0	0	0	0	**2**	**0**
Wild fowl	0	0	0	0	0	1	0	0	0	0	0	0	**1**	**0**
Total	**16**	**29**	**0**	**0**	**0**	**46**	**16**	**27**	**0**	**0**	**12**	**9**	**155**	**3**

- In multiple recaptures, birds captured multiple times are counted once.
- For brown-headed cowbirds recaptures refer to multiple bleedings of sentinel birds in cages.

Table 3: Number and composition of collected mosquito pools during 2009.

Month	*Cx. tarsalis*	*Cx. quinque.*	*Cx. stig.*	*Cx. erythroth.*	*Cx. thri.*	*Cs. Inor.*	*C.s partic.*	*Cs. incid.*	*An. hermsi*	Total
Jan.	*	*	*	*	*	*	*	*	*	*
Feb.	*	*	*	*	*	*	*	*	*	*
Mar.	*	*	*	*	*	*	*	*	*	*
Apr.	3 (86)	0	0	5(250)	0	1(10)	0	0	0	9(346)
May	15(485)	5(204)	2(20)	8(367)	0	0	0	1(14)	2(34)	33(1124)
Jun.	26(932)	22(860)	1(28)	16(747)	0	0	1(16)	4(91)	14(451)	84(3125)
Jul.	20(601)	19(564)	0	2(100)	0	0	3(41)	0	5(177)	49(1483)
Aug.	14(388)	22(639)	0	3(150)	0	0	0	0	3(64)	42(1241)
Sep.	29(1387)	7(265)**	0	2(87)	0	0	0	0	0	38(1739)
Oct.	13(582)	10(404)	3(68)	8(393)	0	0	0	0	0	34(1447)
Nov.	1(21)	2(65)	0	8(400)	0	0	0	0	0	11(486)
Dec.	*	*	*	*	*	*	*	*	*	*
Total	**121 (4482)**	**87 (3001)**	**6 (116)**	**52 (2494)**	**0**	**1 (10)**	**4 (57)**	**5 (105)**	**24 (726)**	**300 (10991)**

*No traps were deployed during this time period.
**One pool tested positive for WNV.

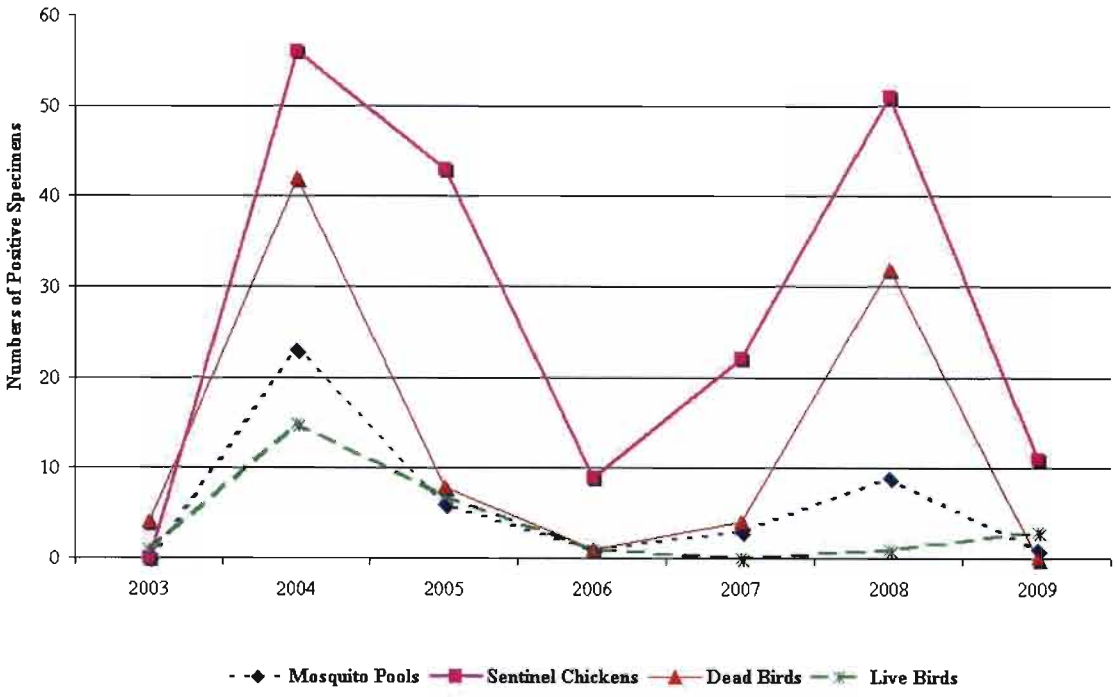

Figure 4: Analysis of West Nile virus activity during the years 2003-2009.

51 in 2008. As depicted in Table 3, in 2009, 300 mosquito pools (10,991 mosquitoes) were screened, and only one pool comprising of *Cx quinquefasciatus* tested positive for WNV; in contrast 250 mosquito pools (10,400 mosquitoes) were screened in 2008, and 15 pools tested positive for WNV. Similarly, 32 dead birds tested positive for WNV in 2008 (Sandhu et al. 2009) while none were positive in 2009.

During 2009, wild bird serology served as the first indicator of WNV activity within our District. In the previous year, dead bird screening served as the first indictor for WNV activity (Sandhu et al, 2009), but none were found positive this year. Further, there were drastically fewer dead bird-related service requests received from the District residents in 2009. During the first peak of WNV activity in 2004, all virus-infected dead birds species were in the family Corvidae (Wisniewska-Rosales et al. 2005); in 2008, WNV positive dead birds included corvids, finches, sparrows, starlings, cowbirds and a scarlet macaw (Sandhu et al. 2009). It is possible that WNV virus is evolving, changing host specificity and now infecting hosts from other families of birds. Many of these birds are smaller (e.g., house finches, sparrows, cowbirds, starlings) which may be less easily detected and reported by the public. Additionally, American crows with weak immune systems might have been eliminated from the population, or their offspring may have developed immunity to this flavivirus. Perhaps this hypothesis needs to be investigated in the future.

Since the appearance of WNV in Riverside County in 2003 (Wisniewska-Rosales et al. 2004), this arbovirus has maintained a constant presence within the District, although it's incidence has been reduced in some years. Over the past seven years, our trapping locations and methods have remained constant, but our results have varied. When the results were graphically plotted, a cyclic pattern of WNV activity appeared; viral activity increased, peaked and decreased every three years (Figure 4). If this pattern holds true, then there will likely be a slight increase in WNV activity in 2010 and a large peak of activity in 2011. This pattern could be due to the evolution of this virus in term of its infectivity/ pathogenicity, possible movement of this virus into new hosts or may be related with cyclic patterns that trigger its activity.

ACKNOWLEDGMENTS

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Efficacy of VectoMax CG for Mosquito Control in Wild Rice in Lake County, California

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ABSTRACT: In 2009, Valent BioSciences collaborated with LCVCD to evaluate VectoMax CG for mosquito control in cultivated wild rice. VectoMax CG was applied to approximately half of the wild rice acreage and provided effective initial control of both *Anopheles freeborni* and *Culex tarsalis* and continuous control of *Cx. tarsalis* larvae for 21 days. Adult mosquito counts were also reduced compared to previous years. In addition to providing improved control of the primary vector of WNV, the LCVCD anticipates that incorporating VectoMax CG into its wild rice mosquito control program will reduce the total annual treatment cost by 10% to 20% due to the reduced number of aerial applications.

INTRODUCTION

Cultivated rice fields produce extraordinary numbers of mosquitoes, and larval control is even more difficult in cultivated wild rice because its taller, denser canopy limits the amount of larvicide reaching the water. For more than 20 years, the Lake County Vector Control District (LCVCD) has achieved moderate levels of control in about 900 acres of cultivated wild rice through aerial applications of VectoBac G (8 lbs/acre) at 8- to 12-day intervals during the growing season at an annual cost of approximately $100,000 per year in aircraft and materials. A key drawback to this approach is that VectoBac G provides only 2-3 days of larval control, and application intervals of 8- to 12-days provide discontinuous larval control; however, more frequent applications are beyond the LCVCD's financial means. In 2009, Valent BioSciences collaborated with LCVCD to evaluate VectoMax CG for mosquito control in cultivated wild rice. VectoMax CG is a new larvicide formulation that combines *Bacillus thuringiensis* subsp. *israelensis* Strain AM65-52 and *Bacillus sphaericus* Strain 2362 in a carefully selected ratio using BioFuse™ Technology to assure optimal toxin ratio delivery in every microparticle. Mosquito larvae are exposed to the entire suite of *Bti* and *Bsph* toxins which results in a quick kill, and in many habitats also provides extended residual control. This study compared the effectiveness of VectoMax CG to the District's usual VectoBac G treatments in cultivated wild rice.

MATERIALS AND METHODS

Two separate treatment blocks were established 1.4 miles apart (Figure 1). On July 13, 2009 VectoMax CG was applied at a rate of 8 lbs/acre to Tule Lake, a 369-acre area northwest of the community of Upper Lake. VectoBac G was applied on July 14 to the Reclamation District acreage, a 410-acre area located between Clear Lake and the community of Upper Lake, and additional VectoBac G applications were made on July 23 and August 4. All applications were made using an Air Tractor 401B flown by Harding Flying Services. The aircraft applied 8lbs/acre in a 55-foot swath when flying at 115 mph. Five 200-foot sampling transects perpendicular to the direction of flight were established in each treatment area. Five 5-gallon buckets were placed at even intervals along transects in the VectoMax CG treated block prior to application in order to verify treatment of the area and actual application rate.

Figure 1. The two study sites were 1.4 miles apart. Tule Lake (369 acres), the northernmost site, was treated with VectoMax CG on July 13. The Reclamation District (410 acres) was treated with VectoBac G on July 14, July 23 and August 4, 2010.

All plots were sampled by dipping immediately prior to treatment. Post-treatment samples were collected at 48-hours post-treatment, and on days 7, 14, 21, 28 and 35 post-treatment. Twenty dips were taken from each transect, and all immature mosquitoes were returned to the LCVCD laboratory for identification and enumeration. Each immature mosquito was classified as early instar (1st and 2nd instars), late instar (3rd and 4th instars) or pupa.

Two carbon dioxide-baited CDC traps (John W. Hock Company, Gainesville, FL) were set weekly in each block (four traps per week total). Trap collections were returned to the LCVCD laboratory for identification and enumeration. Subsamples of female mosquitoes were submitted to the Center for Vectorborne Diseases (CVEC) at the University of California, Davis for arbovirus testing.

RESULTS

Three immature mosquito species were recovered from the sampling transects during the trial: *Culex tarsalis, Anopheles freeborni* and *An. franciscanus*. Too few immature *An. franciscanus* were collected for significant statistical analysis, and historical surveillance data show that this species is of limited importance relative to the other two species in Lake County; therefore, it was not included in the data analysis. Similarly, although ten mosquito species (*Aedes increpitus, Ae. sierrensis, Ae. vexans, An. franciscanus, An. freeborni, An. punctipennis, Coquillettidia perturbans, Cx. erythrothorax, Cx. tarsalis,* and *Cs. inornata*) were collected in the four CO_2-baited CDC traps, only *Cx. tarsalis* and *An. freeborni* are considered in this data analysis because the other species are either absent from the wild rice ponds, or, as in the case of *An. franciscanus*, did not occur in significant numbers in the wild rice ponds and would not have been affected by these larvicide applications.

The wild rice fields were drained in preparation for harvest beginning on day 24, which was earlier than expected. Consequently, the final two larval samples taken on days 28 and 35 were from fields that were mostly drained and in some cases post-harvest, which significantly altered the habitat; thus samples collected on these dates are not directly comparable to those samples taken earlier. After the study was completed on day 35, VectoBac G applications were made on day 36 to both the Tule Lake and Reclamation District fields, and on day 46 to the Reclamation District field to control the larvae remaining in the wet portions of the harvested wild rice fields.

Both VectoMax CG and VectoBac G controlled *Cx. tarsalis* and *An. freeborni* for 2 days following treatments (Figures 2 and 3). Recruitment of early instar larvae of *Anopheles freeborni*, but not *Cx. tarsalis*, was seen on all post-treatment sampling dates within the VectoMax CG-treated fields. Recruitment of all species of early instar larvae was observed in the VectoBac G-treated fields up to day 21. In the VectoMax CG-treated fields, no *Cx. tarsalis* larvae were detected for 21 days following the application, but both early and late instar *An. freeborni* exceeded treatment thresholds beginning on day 7. Late instar *An. freeborni* numbers increased to 0.1 on day 7, and declined on the subsequent two sample dates. While we did observe some control of the *An. freeborni* during the trial, the percent reduction could not be calculated since the VectoBac G treatments in the control field eliminated their populations for most sample dates. For this reason, there was a significant difference observed in the *An. freeborni* population between the VectoMax CG and the VectoBac G treated fields when using the two-tailed Student's t-test ($P = 0.005$, $df = 6$, $t = 2.44$).

Repeated applications of VectoBac G maintained control of *An. freeborni* in the Reclamation District during the study period, however, the *Cx. tarsalis* population was not suppressed and rebounded after each application of VectoBac G. On day 21 the

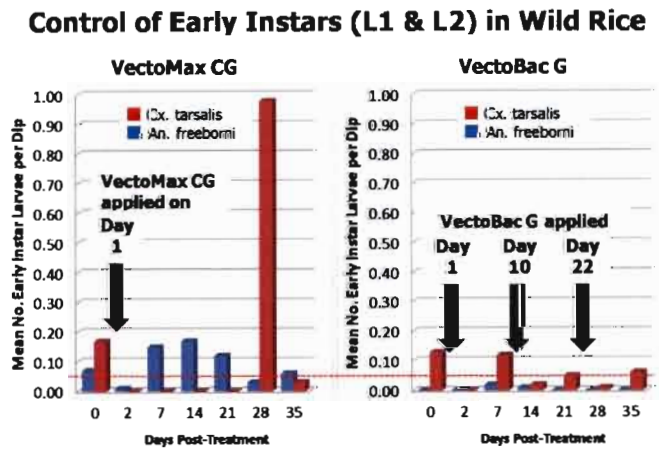

Figure 2. Early instar larval results in VectoMax CG treated wild rice fields (shown on left) and VectoBac G treated wild rice fields (right).

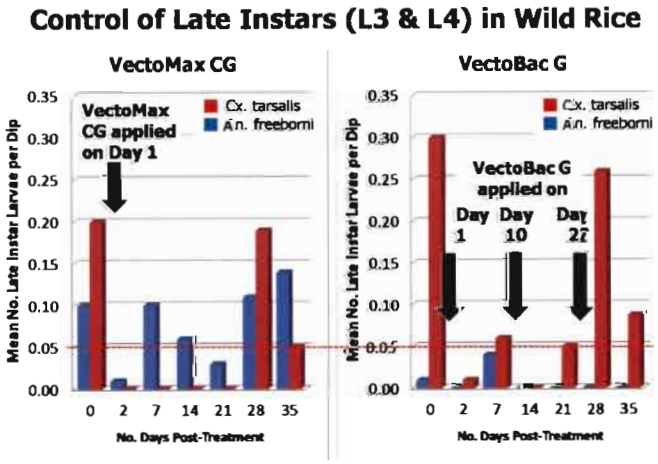

Figure 3. Late instar larval results in VectoMax CG treated wild rice fields (shown on left) and VectoBac G treated wild rice fields (right).

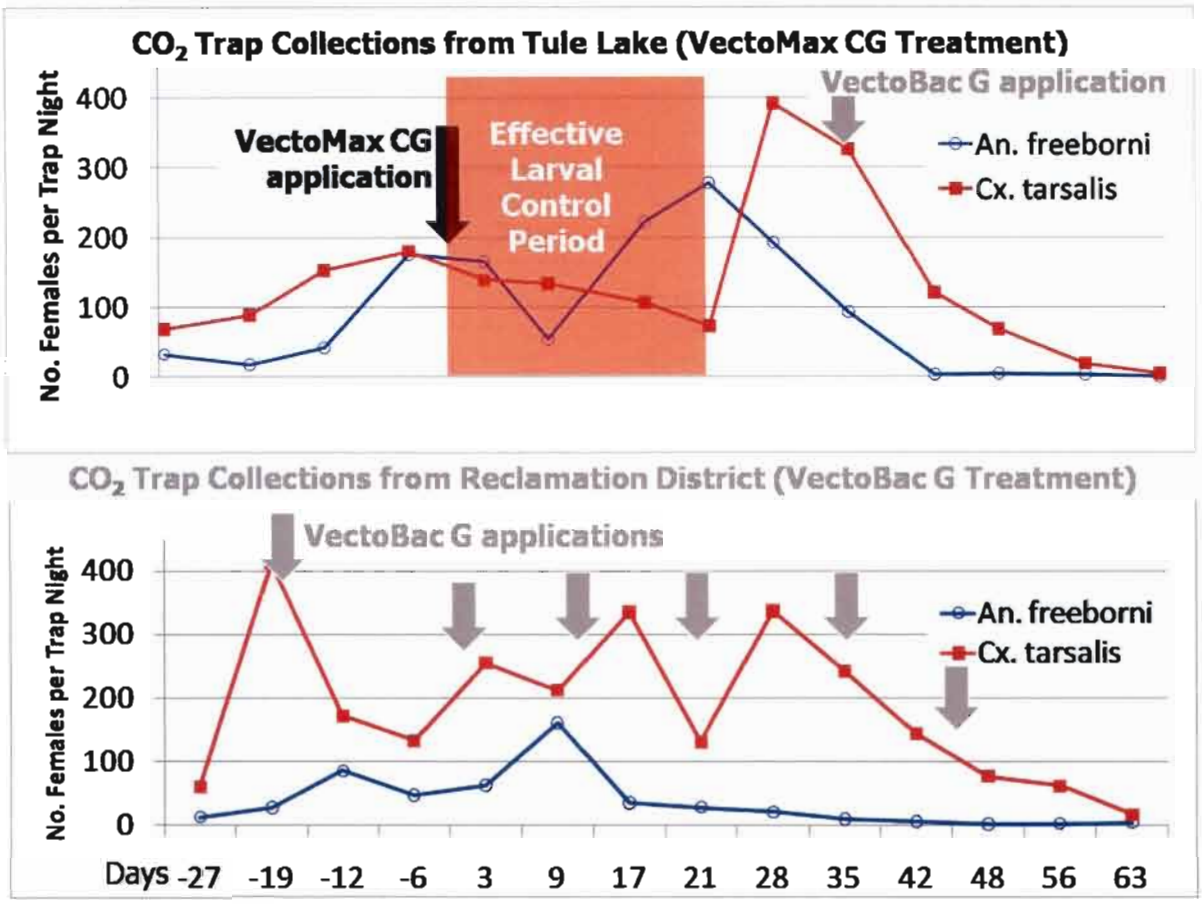

Figure 4. Carbon dioxide-baited trap collections of *Culex tarsalis* and *Anopheles freeborni* from VectoMax CG-treated wild rice fields (upper graph) and VectoBac G-treated wild rice fields (lower graph).

Cx. tarsalis population did not decrease after an application of VectoBac G. Applying a two-tailed Student's t-test to the data showed that VectoMax CG significantly suppressed the late instar *Culex* population ($P = 0.01$, $df = 6$, $t = 2.44$) for 28 days without the need for a re-application.

Throughout the 35 days of the trial there was an initial decrease in adult *Anopheles* populations that rebounded after about one week (Figure 4). The reduction in adult *Cx. tarsalis* lasted four weeks after the VectoMax CG treatment. Two pools of *Culex tarsalis* collected from the wild rice fields were positive for West Nile virus.

DISCUSSION

The LCVCD's treatment threshold for larval mosquitoes in wild rice is 0.05 larvae per dip. VectoMax CG provided initial control of *Anopheles freeborni* larvae and continuous control of *Culex tarsalis* larvae for 21 days. Control may have extended beyond 21 days, but an earlier-than-anticipated draining and harvest of the rice fields significantly altered the larval habitat and limited meaningful comparison of samples from the first four samples dates to those collected after day 21. Interestingly, although early instar *An. freeborni* populations remained at 2-3 times the treatment threshold, late instars rebounded strongly by day 7 but declined thereafter. By day 21, larval populations were below the treatment threshold, which suggests that VectoMax CG may have provided some control of *An. freeborni*, but that they required more time to ingest a lethal dose. This observation may warrant further investigation under more controlled conditions.

Adult *Cx. tarsalis* counts were also reduced in the VectoMax CG-treated fields compared to the VectoBac G-treated fields during the period of effective larval control. The sharp increase in the adult *Cx. tarsalis* population on day 28 can be attributed to the harvesting of the rice fields, as a similar rapid increase occurs each year when the wild rice is harvested. The draining of the wild rice ponds and subsequent cutting of the rice eliminate an important resting site for adult mosquitoes, and large increases in the number of adult mosquitoes collected in traps and resting box mirror the increase in complaints from nearby residents.

The presence of large populations of both *Cx. tarsalis* and *An. freeborni* necessitates an effective control program. *Culex tarsalis* is the primary local vector of West Nile Virus, and *An.*

freeborni is an aggressive human-biting mosquito that will fly several miles from its larval source, generating many complaints from residents. As a publicly funded agency, the LCVCD has a responsibility to use its funding as effectively as possible. In this study, VectoMax CG provided continuous control of *Cx. tarsalis* for 21 days and controlled *An. freeborni* for two days after application. Two features of VectoMax CG were attractive to the LCVCD—the possibility of continuous control and a reduction in application costs. Although VectoMax CG is more expensive than VectoBac G, fewer applications are required. For the LCVCD, if one application of VectoMax CG can replace three applications of VectoBac G, the total application cost is reduced by 10% (material and flight cost combined), plus it provides continuous control of *Cx. tarsalis*. VectoMax CG provided superior control of *Cx. tarsalis* as compared to VectoBac G, but VectoBac G provided better control of *An. freeborni*. We plan to use a combination of products in future seasons to maximize control of both mosquito species and to reduce total control costs.

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From the Bottle to the Bowl, Determining VectoMax® CG Application Strategies in Sonoma County, California

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ABSTRACT: The diversity of Sonoma County landscapes offers many challenges in the application of biorational mosquito larvicides, particularly in winery ponds and wastewater treatment facilities. During the 2009 mosquito season, VectoMax ® CG was used in multiple water retention sources utilizing a variety of field equipment at an application rate of 10 - 20 lbs/acre to control *Anopheles* and *Culex* mosquito species. This study evaluates seven separate mosquito sources where VectoMax® CG was used: four winery ponds, two wastewater treatment ponds and one recreational lake. In most sources, we noted control lasting up to 21 days; however, beginning in late summer/early fall, a reduction in the length of control was noted at certain winery ponds. Following a laboratory trial to determine if the efficacy of the product was responsible, it was later determined that the fluctuation in water levels due to active grape crush was the real cause of the decrease in duration of control. This study outlines the efforts to determine the effectiveness of VectoMax ® CG for these sources, with emphasis on one winery pond.

INTRODUCTION

Marin/Sonoma Mosquito and Vector Control District (MVCD) began evaluating the efficacy of VectoMax® CG in various sources to determine where the product could effectively be used, which species were effectively controlled, what application rates would provide satisfactory results and how long the control lasted. District personnel were impressed by the quick knockdown provided by the product as well as the length of control observed in the field. It was determined that a retrospective evaluation of the sources treated with VectoMax® CG could provide the District with valuable information for the 2010 mosquito season.

Seven sources were selected for this retrospective evaluation, each with its own unique problems. For instance, Spring Lake Recreational Area typically produces *Anopheles* spp.; however, this year as an algal bloom began to decompose, the lake produced a large population of *Culex* spp. An application rate of 10 lbs/ac was used along the perimeter of the lake to control both the *Anopheles* and *Culex* mosquitoes. Some of the winery ponds chosen for this evaluation were too large to treat from land so boats were used to deliver the product into the thick vegetation in the middle of the ponds (i.e., Jackson Estate), whereas other winery ponds could be treated by walking the perimeter (i.e., Mazzocco Winery). An application rate of 10 lbs/ac was most frequently used at the winery ponds, with the exception of Martin Ray Winery, a highly organic and consistently problematic breeding source for *Culex* spp., which was treated at a rate of 18 - 20 lbs/ac. In addition, two wastewater treatment plants with different levels of vegetation in their ponds were evaluated for this study. Both treatment sites contained varying types of aquatic vegetation in addition to developing summer algal blooms. One of the treatment plants routinely produced *Cx. pipiens, Cx. tarsalis* and *Cx. stigmatosoma*, while the other treatment plant produced both *Culex* spp. and *Anopheles* spp. We tested application rates of 8 - 10 lbs/ac for these two sites.

As the 2009 season progressed, VectoMax® CG began to emerge as a valuable control tool against both *Anopheles* and *Culex* species found in many sources containing dense vegetation and high organic content. However, an optimal application rate for the winery ponds had yet to be determined, so an intensive examination of one of the winery ponds (Mazzocco Winery) is reviewed in this paper.

MATERIALS AND METHODS

Study Site. Seven treatment locations were evaluated retrospectively; however, for the purpose of this paper, emphasis is going to be placed on one of the winery ponds. Mazzocco Winery in Healdsburg, CA was chosen as the trial site on September 30, 2009. This winery has two ponds measuring 0.25 and 0.125 acres in size. The larger pond was skirted with cattails measuring over 10 ft in height, whereas the smaller pond was clear of any large vegetation. The larger pond typically produces *Culex* larvae, and the smaller pond produces *Anopheles*.

Larval Sampling. At the Mazzocco Winery location, four transects measuring 30 m (100 ft) each were selected and staked with PVC poles. Transect selection was based on high late instar densities, and at each sampling date, a standard one pint dipper was used to take 20 dips/transect. Pre-treatment sampling was recorded immediately prior to larvicide application. Post-treatment densities were recorded at two and seven days post-treatment and every seven days thereafter for the duration of the study. The District recorded early instar (L1/L2), late instar (L3/L4) and pupal densities at each sampling date. Live pupae were returned to the laboratory and held for emergence. At all other study site locations compared in this study, a standard one pint dipper was used to take a minimum of 10 dips per location. This is consistent with the District's standard operation procedure for monitoring treatment efficacy of bacterial larvicide products.

Characterization and Transect Setup. Prior to the trial, a Solo backpack sprayer was characterized to ensure an application rate of 20 lbs/ac and a swath width of 30 ft. Five PVC poles were

placed along the bank of the two ponds, each of them spaced 20 ft apart from the adjacent pole. Five gallon buckets were placed on each of the PVC poles to verify the application rate. As stated above, each transect measured 100 ft in length with three transects in the large pond and one transect in the small pond.

VectoMax Application. On the morning of 30 September 2009, after a high density of late instar *Anopheles* spp. and *Culex* spp. were sampled, VectoMax® CG was applied at a rate of 20 lbs/ac to the two ponds. Wind speeds of 0 - 3 MPH were recorded from the NW during the application. An average of 52 granules was counted in each bucket, confirming the desired application rate of 20 lbs/ac. After the granules were counted and recorded from each of the five gallon buckets, the buckets were emptied into their respective ponds.

RESULTS AND DISCUSSION

While each of the seven treatment sites selected for this retrospective study offered unique challenges, one site, Mazzocco Winery, stood out because the level of control provided was less than anticipated. The District observed control lasting 28 days at sites such as Spring Lake Recreational Area. At this site, an airboat equipped with a solo sprayer was used to apply VectorMax® CG at a rate of 10 lbs/ac to the perimeter of the lake. This site typically produces *Anopheles* spp. however, this year the District saw an unexpected spike in the *Culex* spp. Only one application of VectoMax® CG was required to reduce the populations to acceptable levels. Large scale application of this product was never warranted again at this site. The District is prepared to apply the product again in 2010 should a similar algal decomposition occur.

The District also evaluated two wastewater treatment plants, each with different levels of vegetation. Application rates of 10 lbs/ac were used at these sites, and control lasted from 15-21 days depending on the level of vegetation (Fig. 1). Treatment ponds with less vegetation showed longer control, whereas ponds with greater vegetation showed reduced length of control. The reduction in the length of control could be due to the presence of mosquito refuges.

The four winery ponds chosen for this evaluation varied greatly based on the presence or absence of vegetation. For instance, Jackson Estates had thick vegetation in the middle of the pond making it difficult to treat for mosquitoes. Treatment at this winery was done by boat so an even coverage with the product was possible in the heavy vegetation in the middle of the pond. Other ponds were lined with vegetation along the perimeter, such as Mazzocco Winery, and could be treated with a backpack sprayer. Control at these four wineries ranged from 15.0 - 23.5 days depending on the timing of the application. Ponds treated prior to grape crush showed extended control, while those ponds treated during crush had reduced length of control. Mazzocco Winery was treated with VectoMax® CG at a rate of 20 lbs/ac and it was anticipated that control of at least 21-24 days would be seen; however, control was lost by day 14, and a second application had to be made. A detailed evaluation of this treatment was warranted and the following data are related to this study site.

Prior to treatment, the density of late instar larvae along the four transects exceeded the District threshold of 0.1 larvae per dip. Within these transects, late instar larval densities ranged from 0.06 - 1.49 larvae/dip depending on the proximity to the vegetation and the species. A total of five species were collected at the two ponds. Transects nearest to the cattails had more late instar larvae present at the time of the pre-treatment dip counts and a higher density of pupae. The transect located in pond 2 had the greatest density of *Anopheles* larvae, and *Cx. stigmatosoma*, *Cx. thriambus* and *Cx. tarsalis* were the most abundant species collected at these two winery ponds. During the application of the VectoMax® CG, five gallon buckets were placed adjacent to the transect poles to collect granules and verify the application rate. Granules were counted, recorded, and then emptied into the water.

Application of VectoMax® CG achieved >92% control of late instar larvae and pupae and >97% control of early instar larvae at 48 hours post treatment. Within seven days, 100% reduction in L3/L4 larvae was achieved (Fig. 2). A steady recruitment of early instar larvae was identified throughout the study (Fig. 3). While we

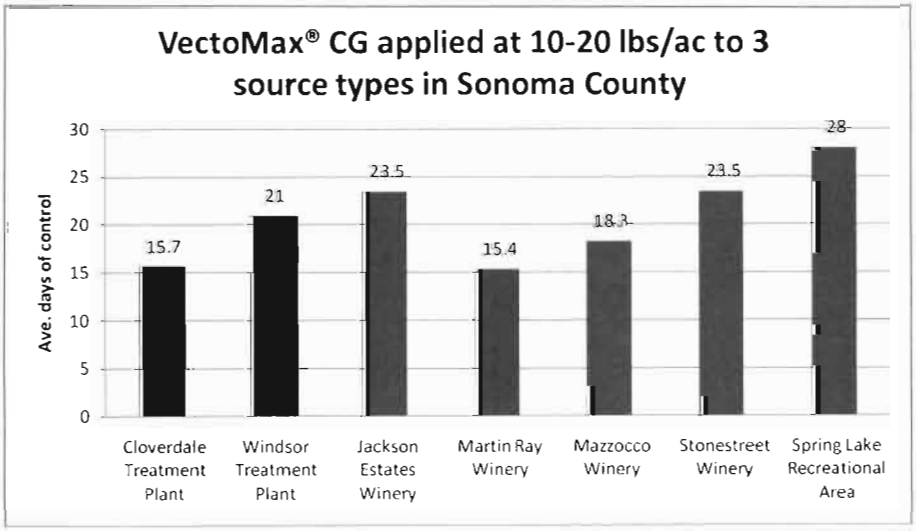

Figure 1. Efficacy of VectoMax® CG at 3 source types in Sonoma County.

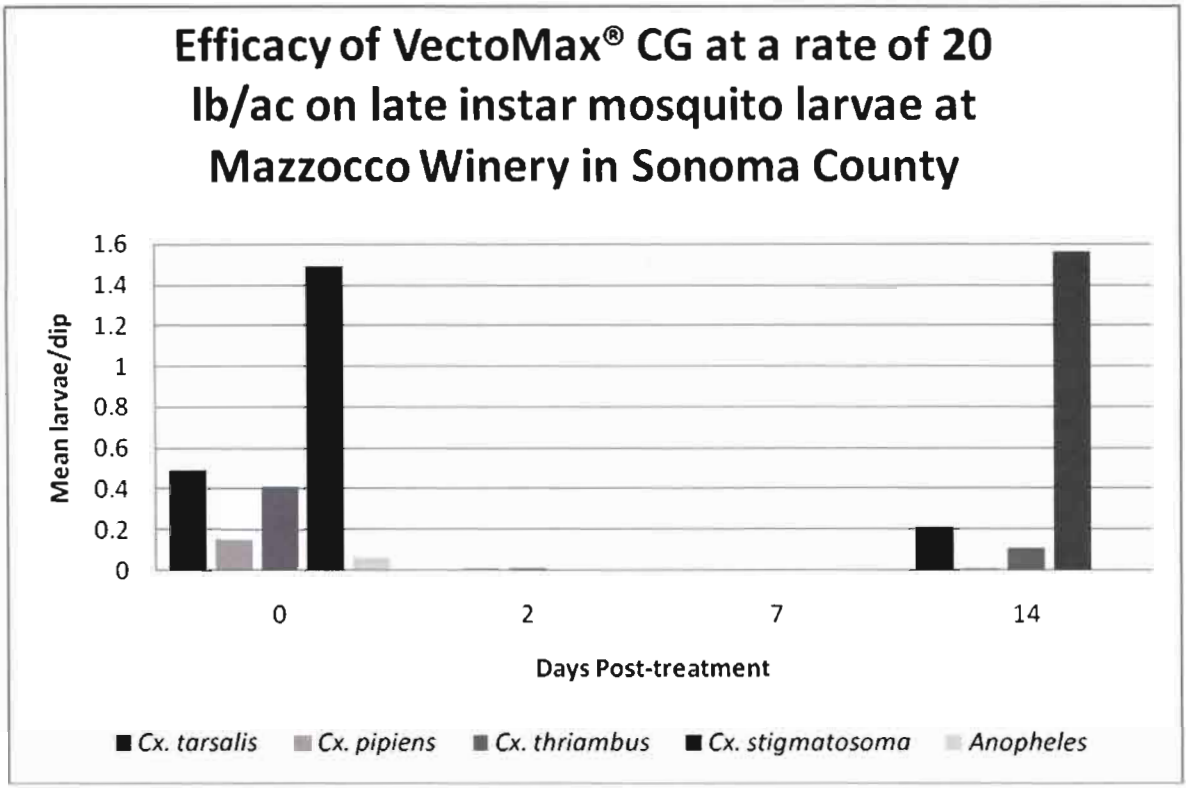

Figure 2. Control of late instar larvae at Mazzocco Winery lasted 14 days when treated with VectoMax® CG at a rate of 20 lbs/ac.

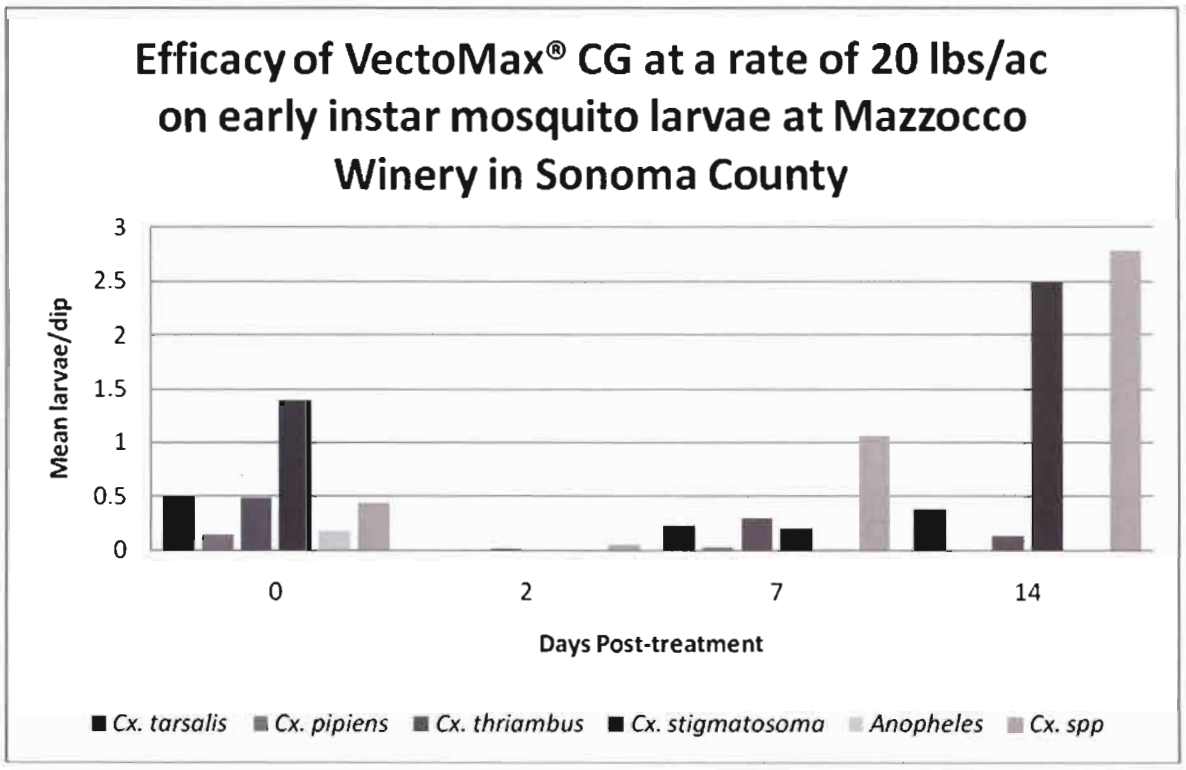

Figure 3. Early instar larvae continued to be recruited to the ponds at Mazzocco Winery.

expected at least 21 days of control based on previous treatments at wineries, late instar counts began to exceed treatment thresholds after 14 days. It was determined that another application was needed to control the population. The lack of extended control raised many questions about the efficacy of the product and what could have caused the marked change in the length of control that was normally observed. A laboratory trial was set up to determine if the product lost its efficacy or if something else caused the decline in efficancy. In the lab trial, two buckets were treated with an application rate of 20 lbs/ac. of VectoMax® CG, and two buckets were used as a control. Ten *Culex pipiens* larvae were placed in each bucket daily and the mortality recorded. All pupae were removed from the buckets daily, placed in emergence cages and adult emergence was recorded. Results showed 100% control for over 26 days in both treatment buckets indicating that something else was responsible for control failure in our field study. Further evaluation of the study site showed that during our field experiment, water levels were fluctuating daily because of the active grape crush at Mazzocco Winery. Because of this, new water was continuously being added to the ponds and excess water was flushed out and used to water the fields. The fluctuation in water depth and the possible removal of product may have influenced the length of control. Therefore, it would be more cost-effective for the District to use a less expensive and shorter lived product in the winery ponds during active crush in 2010.

CONCLUSION

Based on 2009 results, the District can effectively control mosquito populations within 48 hours and can observe up to 21 days control using VectoMax® CG at a rate of 10 lbs/ac. This product provides effective control of larvae in winery ponds throughout much of the mosquito season; however, due to multiple factors during crush, more work is needed to determine the cost-effectiveness and efficacy of VectoMax® CG during this critical time in the wine country.

ACKNOWLEDGEMENTS

We would like to thank Peter DeChant of Valent Biosciences Corporation for his professional consultation on the trial and for supplying the VectoMax® CG material for this project. We would also like to acknowledge Stephanie Whitman and Jim Wanderscheid for their encouragement and general support for the project. We are grateful for the field expertise of Jason Sequeira for his contribution of data and pictures. Finally, we would like to thank the following Marin/Sonoma Mosquito & Vector Control District employees; Mike Wells, Nathen Reed, Sarah Klobas and Kimberly Heilig for their assistance with collections and mosquito identification.

Natular™ Stewardship Initiatives Over the Past Season

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ABSTRACT: Natular™ represents both a successful collaboration between Clarke and DowAgro Sciences (DAS) and the first new larvicide for the control of mosquitoes in decades. The active ingredient, spinosad, was first discovered in 1982 and first registered for use in 1997 under the US Environmental Protection Agency's (EPA) Reduced Risk initiative. US EPA registration of Natular™ for public health use as a mosquito larvicide was achieved beginning in 2007. To date, six formulations of Natular™ have been registered in the US, two single-brood and four multi-brood formulations. Field trials with US cooperators began in 2008 and have continued through the 2009 mosquito season. These stewardship initiatives have provided invaluable documentation of label use patterns and insight into potential operational influences.

INTRODUCTION

The active ingredient in Natular™ is generated through fermentation of a naturally occurring soil actinomycete, *Saccharapolyspora spinosa*, by DAS in Michigan. The organism itself was first discovered in 1982 by Dr. John Mynderse, an Eli Lilly Company chemist, from a soil sample taken near an abandoned rum distillery in the Caribbean. Spinosad is comprised of 2 insecticidal factors (A and D) present in an 85:15% ratio. Insects are exposed to spinosad through both ingestion and contact, and the active ingredients affect the nervous system of the insect at unique sites on the nicotinic acetylcholine and gamma aminobutyric acid (GABA) receptor sites (Saldago and Sparks 2005).

First registered by the US EPA in 1997 under that agency's Reduced Risk Initiative, DAS focused on the incorporation of the active ingredient, spinosad, within the agricultural industry. It is now registered for use on more than 250 crop types in 80 countries. In addition, it is used in the turf and ornamental industry, animal health and pet care industries and stored grain industry among others. In 1999 spinosad was given the President's Green Chemistry Challenge Award, one of only six insecticides that have met the requirements of the this award. Clarke entered into collaboration with DAS in 2004 to bring spinosad into the public health market, specifically for use as a mosquito larvicide. This was achieved through Clarke's Product Development team three years later, with the first of six Natular™ formulations (single brood G and 2EC) granted registration by the US EPA in October of 2007. The multiple brood formulations (XRG, T30, XRT and DT) were registered the following year in June 2008.

All Clarke formulations of Natular™ meet the US EPA's reduced risk criteria. To date, four of six formulations (EC, XRG, T30 and XRT) have successfully been reviewed and have met the standards for use in organic food production sites and are so listed by the Organic Materials Review Institute (OMRI). The DT tablet was developed primarily for international use in potable water sites; as such this formulation is not subject to review by OMRI.

METHODS AND DISCUSSION – STEWARDSHIP INITIATIVES

During the mosquito season of 2008, limited "blind trials" were performed in the US by four cooperators. These cooperators received the final formulations of Natular™; however, all samples and associated paperwork such as the Material Safety Data Sheet (MSDS) identified the formulation by code as a DAS formulation rather than bytrade name. All communication necessary to initiate and complete these blind trials was performed by Dr. David Dame.

Figure 1 below represents work which was carried out by the Merced County Mosquito Abatement District. At the maximum label rate for the two habitats in which the evaluations took place (i.e., pastureland and freshwater wetlands) the single brood corn cob based formulation (0.5% AI) proved efficacious in controlling *Aedes nigromaculis* and *Culex tarsalis*. At the same time in 2008, Clarke's Environmental Sciences department initiated evaluations

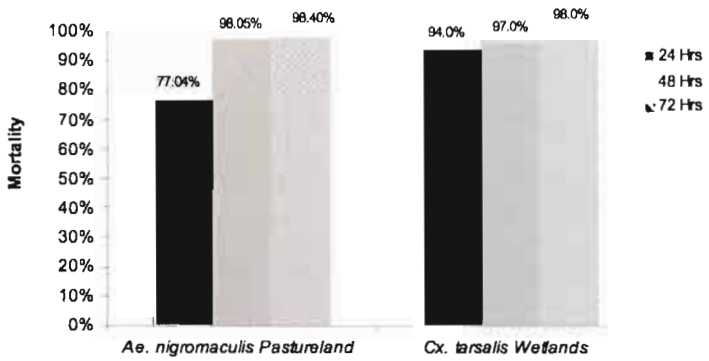

Figure 1. Mortality as a result of the application of Natular™ G at the rate of 9 lbs/acre over a 72 hour period. Application and monitoring were conducted by the Merced County MAD.

in the Chicagoland area of Illinois. Evaluations of three formulations were performed: (1) The XRG sand core granular containing 2.5% active and labeled for applications between 5 and 20 lbs per acre with an expected 30 day duration; (2) The T30 tablet (8.33% AI), also rated for 30 days of efficacy; and (3) The XRT tablet (6.25% AI) that has the potential to provide up to 180 days of control.

Figures 2 and 3 represent the XRG formulation applied in retention sites. In both cases XRG was applied at 10.5 lbs per acre, slightly less than mid-label rate of 12 lbs per acre. The two sites differed in stage of flooding; the site in Figure 2 was already flooded at the time of application and supported populations of *Culex pipiens*, whereas the site in Figure 3 was dry at the time of application and XRG was applied as a pre-hatch application. The results from these evaluations, displayed as per dip numbers (each number the average of two dips) in treated and non-treated (control) sites indicate that labeled use patterns can provide the type and duration of control that a mosquito control district would expect from a larvicide within their Integrated Mosquito Management (IMM) program. *Culex pipiens* populations were controlled in this evaluation for 35 days, the extent of the surveillance and evaluation (Fig. 1).

Similarly, data in Figure 3 indicate that XRG applied as a pre-hatch has the ability to provide control once flooding occurs. In this case, a rain event induced hatch of an *Aedes vexans* brood between weeks one and two. *Aedes vexans* production was subsequently controlled at the treated site and the *Cx. pipiens* populations in the flooded retention site were also mitigated for 35 days, the entire length of the evaluation.

Maximum mortality in treated populations at times may take 72 hours to achieve, a trend that has been noted previously and documented in the literature (Hertlein et al 2010). Often, however, significant control is achieved in the first 24 hours following exposure as shown in Figure 1. In 2008, numerous evaluations were performed by Dr. Grayson Brown's laboratory at the University of Kentucky. Figure 4 is provided as an example of the "ladder of efficacy" over a 72 h post-treatment period for, in this instance, *Anopheles quadrimaculatus*. Labeled application rates of Natular™ G for typical *An. quadrimaculatus* production sites range from 3.5 – 9 lbs per acre. The rates found in Figure 4 represent the minimum and mid-label rates for this formulation and indicate exceptional efficacy versus this species.

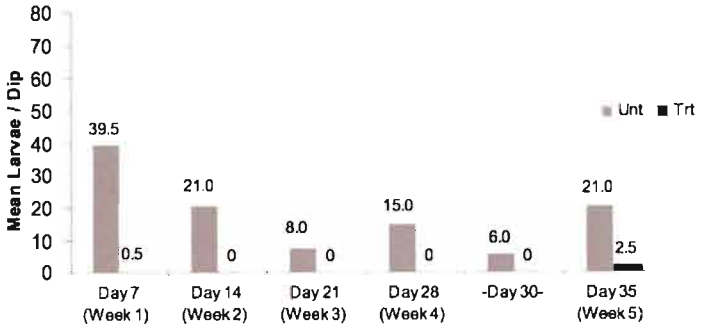

Figure 2. Natular™ XRG applied at the rate of 10 lbs/acre to a retention pond in Illinois. Efficacy for controlling *Cx. pipiens* was evaluated using standard dip counts in treated and control sites for 35 days post treatment.

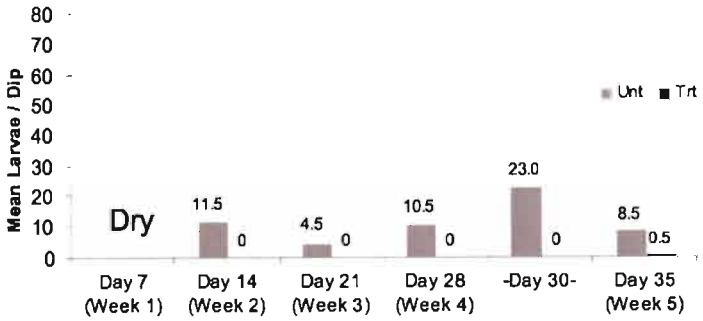

Figure 3. Natular™ XRG applied as a pre-hatch treatment at the rate of 10 lbs/acre to a dry retention pond site in Illinois. Efficacy for controlling *Ae. vexans* and *Cx. pipiens* was evaluated using standard dip counts in treated and control sites for 35 days post flooding.

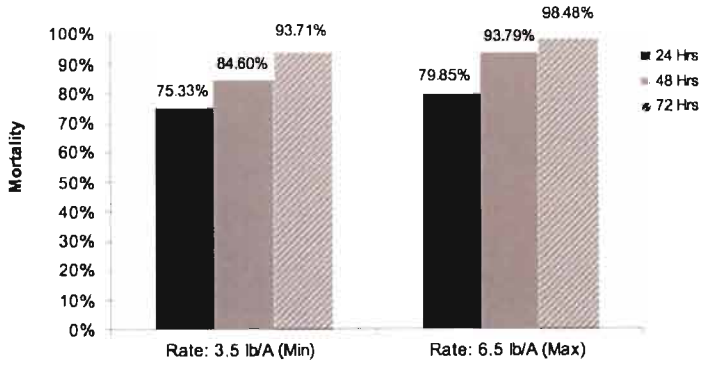

Figure 4. Natular™ G induced mortality over a 72 hour period. Application and monitoring were conducted by personnel at the University of Kentucky.

In 2009, Natular™ efficacy evaluations were performed in 21 states in collaboration with more than 40 operational and university collaborators. These collaborators collected vital information related to product performance and provided keen insight into the influence of a variety of environmental and application factors, as well as species interactions.

The tablet formulations of Natular™, T30 and XRT, are pharmaceutical grade tablets that enhance handling and application by technicians. These are dust free tablets formulated to provide applicators with a clean handling experience. The light, white color as more than several collaborators observed is "easy to see" when submerged in habitats ranging from mangrove swamps to catch basins. Both tablets validated label claims of residual activity when applied to catch basins. In both Illinois and Minnesota, the T30 controlled *Cx. pipiens* and *Cx. restuans* for a 30 day period as shown by data from the Metropolitan Mosquito Control District (MMCD), MN (Fig. 5). MMCD's efficacy evaluation, both blind trials, utilized pupae as a proxy for successful development; a lack of pupae demonstrated positive product efficacy.

Cumulative pupal populations of mosquitoes over the duration of the MMCD catch basin season following treatment with T30 tablets are shown in Figure 5. The same control (untreated) data were used in evaluating both the T30 and XRT treatments. The control curve of cumulative pupation rose steadily over the season, showing that the untreated catch basins continued to serve as *Culex* production sites (Figs 5 and 6). Clearly, in T30 treated catch basins (one per basin), mosquito pupae were absent over the expected 30 day period of residual activity (Fig. 5). Comparable results were achieved in catch basins treated by the MMCD with Natular™ XRT prior to flooding and the onset of larval populations (Fig. 6). Again, the curves associated with treated and untreated basins provide clear evidence supporting labeled expectations of the formulation's ability to provide season-long control.

Results from Natular™ XRT treated catch basins in Illinois are shown in Figure 7. In these trials, XRT tablets were applied to catch basins with larvae already present, and mosquito populations were monitored for a period of 150 days. Also delineated on this figure are three significant rain events. The trend between basins treated with the XRT tablet (one per basin) and those untreated is distinct.

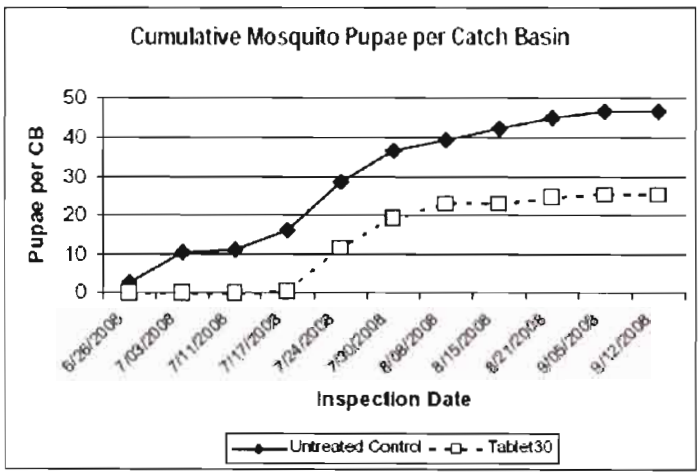

Figure 5. Cumulative pupal populations in Natular™ T30 treated and control catch basins in 2008 in Minnesota. Application and monitoring were conducted the Metropolitan MCD (2008).

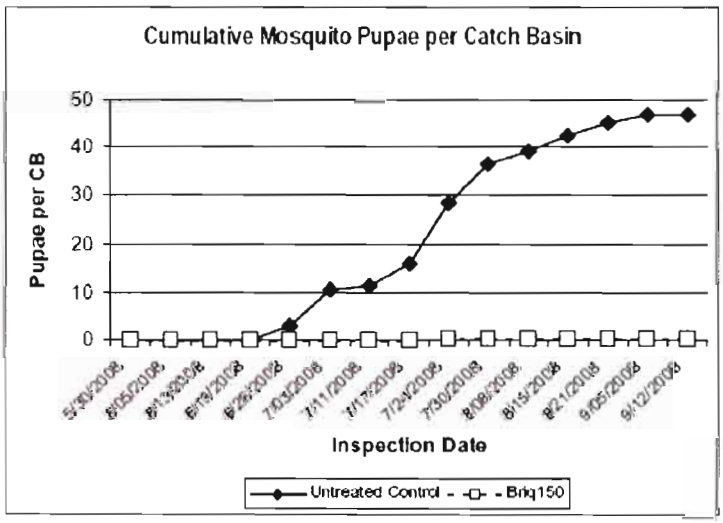

Figure 6. Cumulative pupal populations in Natular™ XRT treated and control catch basins in 2008 in Minnesota. Application and monitoring were conducted the Metropolitan MCD (2008).

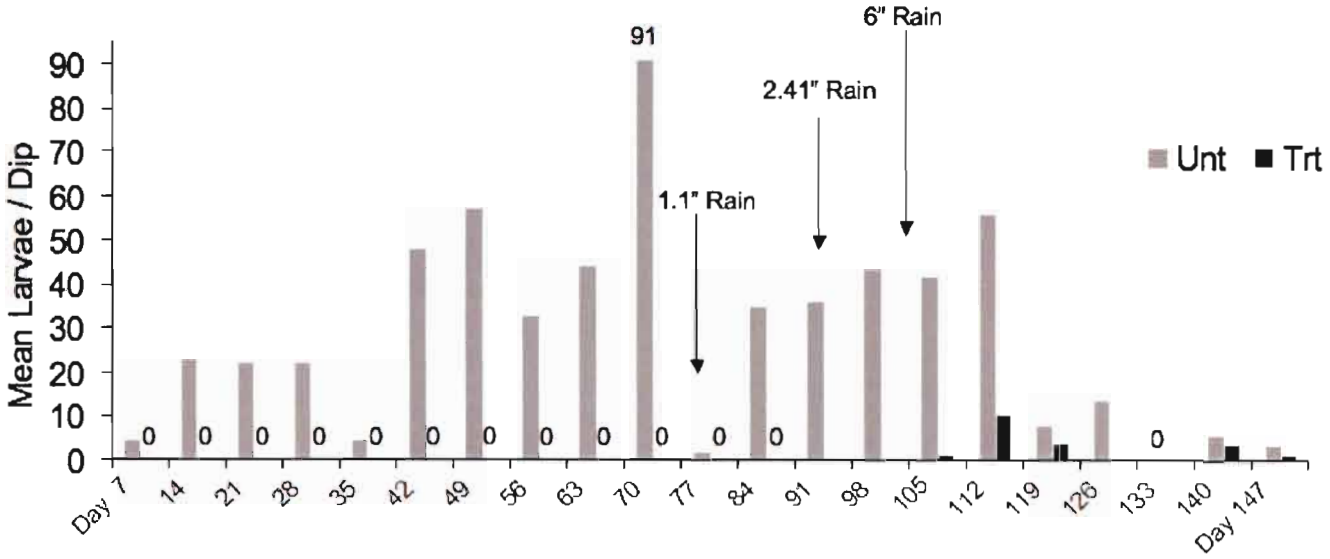

Figure 7. Larval populations in Natular™ XRT treated and control catch basins in Illinois with several major rain events denoted.

The spinosad based Natular™ formulations have been used across a wide range of mosquito production sites during the past two seasons. The 2EC formulation delivered on efficacy expectations in tidal wetlands populated by *Aedes sollicitans* in New Jersey, following application via fixed-wing aircraft, as well as in Waste Water Treatment Plants (WWTP) in Louisiana. The corn cob G formulation has provided control of *Aedes taeniorhynchus* in the Florida Keys as well as control of *Aedes vexans* and *Aedas dorsalis* in Montana, and *Aedes melanimon*, *Ae. nigromaculis* and *Culex tarsalis* in California. Residual formulations such as the XRT tablet have provided districts with season-long control of *Culex spp.* production in Minnesota catch basins and abandoned swimming pools in Saginaw, Michigan. To date, more than 14 mosquito species, including several arbovirus vectors, have been successfully controlled during operational evaluations.

CONCLUSIONS

Spinosad is designated a Group 5 insecticide by the Insecticide Resistance Action Committee (IRAC). IRAC is a global industry organization committed to promoting the development of insecticide resistance management strategies to maintain efficacy, support sustainable agriculture and improve public health. The unique mode of action of spinosad makes the Natular™ formulations perfect candidates for inclusion within a rotational or resistance management program.

Stewardship of our insecticide control options is paramount to our ability to mitigate the public health impacts of mosquito mediated disease. Evaluations of new agents, such as those carried out by industry professionals across the US in 2008 and 2009 with Natular™ are vital to the stewardship of this novel compound. Understanding the role of Natular™ within IMM programs will continue to be defined by the professionals of our industry for years to come.

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Ground ULV & Backpack Application Trials of Etofenprox (Zenivex® E20) in Sonoma County, California

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ABSTRACT: Three ULV field trial applications and one operational field application of Zenivex® E20[1] (etofenprox) were conducted on September 22 – 24, 2009 in Sonoma County, California at an application rate of 0.0035 pounds active ingredient per acre (AI/ac), delivered by a truck mounted ULV Beecomist sprayer and ULV backpack sprayer calibrated to deliver a $DV_{0.5}$ of 22.5 and 19.2 microns, respectively. Weather conditions were monitored at all three trials at a six foot elevation. Four to seven stations with bioassay cages containing 25 mosquitoes per cage, combined with three mm spinning impingers to monitor droplet flux values, were deployed throughout the trial application sites. Additionally, pre- and post-treatment trap counts were monitored and evaluated to determine the effectiveness of the adulticiding efforts in two of the three trials and the actual field application.

Treatment bioassay results with caged *Culex pipiens* and *Culex erythrothorax* mosquitoes showed variable mortality rates (sometimes as high as 88 - 100%) at all three application sites. In the ULV operational field application, pre- and post-treatment trap results demonstrated an average control of the adult population of *Culex erythrothorax* of 93.7 to 99.2 % up to one week after the application of Zenivex®.

INTRODUCTION

In mid-September 2009, the non-ester pyrethroid Etofenprox (Zenivex® E20, a trademark of Wellmark International d/b/a Central Life Sciences) became registered for use in California for adult mosquito control. Marin/Sonoma Mosquito and Vector Control District (MSMVCD) was interested in evaluating this new adulticide product because of its low toxicity to mammals and birds (oral LD_{50}mg/kg >5,000 according to the manufacturer, Wellmark International) and because it was classified by the EPA as a reduced risk insecticide. Additionally, Zenivex® has a unique formulation in that it does not contain piperonyl butoxide (PBO) as a synergist, yet it is still applied at ultra low volumes by ground and aerial equipment for effective adult mosquito control.

Initially, one site located in Healdsburg, CA was selected to perform a field trial with Zenivex® by truck mounted ULV ground fogging on September 22 with a Beecomist® sprayer (Clarke Mosquito Control, Roselle, IL 60172). After reviewing the initial results, it was determined to include another trial, this time utilizing a ULV backpack sprayer the following day, September 23. Both trials were conducted around a one half acre waste water treatment pond privately owned and maintained by Rio Lindo Academy, a nearby dormitory style high school. The main target species was *Culex pipiens*, although *Culex tarsalis* and *Culex stigmatosoma* were also found breeding in this area. A third trial was also conducted at District headquarters in Cotati, CA on September 23; this trial was outlined as a two by three grid, and the target species, *Culex erythrothorax*, was placed in cages within the grid. Droplet analysis for all three field trials were evaluated with DropVision® technology (Leading Edge Associates, LLC, Waynesville, NC, 28785). Wind speed and direction, ambient temperature and relative humidity were recorded at six foot elevation for all three field trials. A truck mounted ULV operational application of Zenivex® was made on the evening of September 24 at a large waste water treatment plant located near Petaluma, CA. The treatment plant was entirely covered in heavy vegetation (bulrush) and was producing large numbers of *Cx. erythrothorax*. Pre- and post-treatment trappings were used to evaluate and monitor the application of Zenivex®. This paper details the efficacy of etofenprox on natural populations of *Cx. pipiens* and *Cx. erythrothorax* by utilizing caged adult mosquitoes and incorporating droplet analysis (i.e. DropVision®) to evaluate the effectiveness of a new synthetic pyrethroid recently made available to mosquito control districts in California.

MATERIALS AND METHODS

Trial Sites and Mosquito Surveillance. Three field trials were conducted for the purposes of this study; two located at Rio Lindo Academy near Healdsburg, CA near the Russian River and one located at the District headquarters in Cotati, CA. The Rio Lindo site had four waste water ponds measuring 0.25 – 0.50 acres which are flooded alternately during the season. During this trial, only two of the ponds (one 0.25 ac. and one 0.5 ac.) contained water and were producing mosquitoes. The larger pond was mostly surrounded by cattails with some areas of vegetation being 10 feet deep into the perimeter of the pond. Larviciding treatments earlier in the season with biorational products produced less than satisfactory results, possibly due to high organic loads in the pond and the difficulty in treating effectively amongst the dense vegetation. Pre- and post-trapping at the Rio Lindo site were conducted with four EVS, two CDC miniature light and three BG Sentinel traps (BioQuip Products, Rancho Dominguez, CA 90220). The EVS and CDC traps were set along the perimeter

Figure 1. BG Sentinel trap at Rio Lindo waste pond.

road which circumvented the pond, and one EVS trap was hung on a cat walk above the pond. The three sentinel traps were placed on floating stations directly in the pond; two were located deep within the cattails (Fig. 1) and one in the open in the middle of the pond. All traps used dry ice placed in buckets above the trap and were set overnight to be collected early the next morning. The traps were monitored once a week for two weeks and then daily one week prior to the trial. Wild caught female *Cx. pipiens* were collected at the site and used in the bioassay cages of both trials at this location.

The grid trial located in Cotati, CA was performed in the early evening of September 23 and utilized wild caught, adult female *Cx. erythrothorax*. This trial was conducted in a relatively flat, open grass field that is owned and maintained by the District. There was no water at this location at the time of application, and trapping for adult mosquitoes was not performed pre- or post-treatment. All mosquitoes for the grid trial were collected from one EVS and two BG Sentinel traps at a large waste water treatment plant near Petaluma, CA. This same waste water treatment site was also the location of the actual field application by truck mounted ULV fogging which was conducted on the evening of September 24. The operational field application site contained a large 14 acre pond which was completely planted with bulrush to facilitate water filtration by the facility. This facility has been monitored regularly as a mosquito breeding habitat with trapping conducted weekly at various sites around the plant. For the purpose of this paper, trap results were reported two days prior to the application of Zenivex®, the next day following the treatment and one week post–treatment to evaluate percent control of the *Cx. erythrothorax* population.

Treatment bioassay, weather data and product application. For the three field trials, 25 female mosquitoes were placed in four inch diameter cardboard bioassay cages with tulle screening provided by Central Life Sciences (Central Life Sciences, Schaumburg, IL 60173). Five foot PVC t-post stations were set up with two bioassay cages per station. A spinning impinger with two 3 mm Teflon slides was attached on top of the t-post between the two cages to capture and evaluate the droplet spectrum. At the Rio Lindo site, seven stations were set up for the truck mounted ULV fogging on September 22, and four stations were used for the ULV backpack fogging performed on September 23. At the District property, six stations were arranged in a 2 X 3 grid trial and set up with three rows of two t-post stations 50 feet apart at 100, 200 and 300 feet downwind from the spray truck. Additionally, four control cages of 25 mosquitoes each were placed within one quarter mile of the treatment areas at each of the three trial locations. Weather was documented prior to, during and 20 minutes following all trial applications. Temperature and relative humidity at Rio Lindo were 59°F and 12% RH for the truck mounted ULV spraying on September 22, and for the ULV

backpack application on September 23 the weather was recorded at 74°F and 46% RH. A critical difference between the two trials at Rio Lindo was the documentation of wind (or lack thereof); virtually no wind was recorded during the truck mounted ULV application, and a seven MPH wind occurred the next day during the ULV backpack spraying. The weather data for the grid trial was 68°F, 46% RH and a seven MPH wind speed. At the Petaluma treatment plant, winds were variable and gusting up to 10 MPH during the operational ULV field application. All three trials and the operational application were performed at the same mid-label application rate for Zenivex® at 0.0035 lbs/acre with a flow rate of 1.8 ounces at 10 MPH. Droplet flux values were determined with the DropVision® software after each of the three field trials, but not for the operational application of Zenivex® at the Petaluma site.

RESULTS AND DISCUSSION

The initial trial performed on September 22 at the Rio Lindo pond generated mixed results in evaluating the efficacy of Zenivex®. Four of the seven t-post stations were staked along the rim of the pond, and all of these revealed an excellent mortality rate of 94 - 100% in the caged mosquitoes. However, three of the seven t-post stations were placed deep in the cattail vegetation, and a mortality rate of 4 - 52% was observed at these stations. While the average droplet size of 19.38 microns recorded at all seven impinger stations was within the desired range of 10 - 30 microns (as defined by the Zenivex® label), the $DV_{0.5}$ (VMD) was significantly different from the four stations set along the rim of the pond as compared to the droplet densities recorded in the three stations down in the vegetation. Droplet densities of less than 3.97 drops/mm^2 squared correlated to low mortality rates of less than 4% in the bioassay cages at these stations. Conversely, cages that were determined to have a mortality rate of 100% showed average droplet densities of 86 - 100 drops/mm^2, and these were all found to be in the stations along the rim. Another primary cause of lack of mortality in the bioassay cages was the lack of wind to assist in moving the material down into the dense vegetation. Therefore, we conducted a second trial the next day, this time with a ULV backpack sprayer which enabled the field technician to walk near the edge of the pond and apply the material into the vegetation. This trial proved only slightly more successful with mortality rates ranging from 8 - 96% and droplet densities from 18 - 102 drops/mm^2, respectively. Fortunately, the trapping results at this location showed an overall reduction in the adult population occurring at the pond (Fig 2).

The grid trial conducted at the District property on September 23 was very successful against the wild caught *Cx. erythrothorax*. Mortality was recorded at 92 - 100% at 100 feet downwind distance from the spray truck, 80 - 92% mortality at 200 feet and 76 - 88% mortality at 300 feet. The lower mortality rates further downwind are indicative of the wind direction (SE) at the time of application and the seven MPH wind speed that assisted movement of the material across the bioassay cages and impingers. Additionally, this site was an open grass field that was devoid of dense vegetation as noted in the previous two trials at Rio Lindo.

The truck mounted ULV operational application on September 24 at the Petaluma waste water treatment plant proved to be the most successful of all the of Zenivex® trials for the District. While the treatment pond was completely covered in heavy vegetation (Fig. 3), there was still sufficient wind to help carry the product

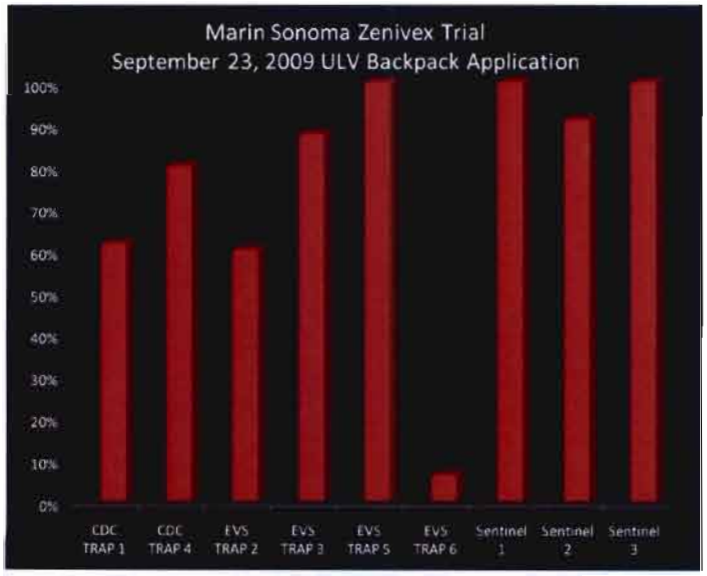

Figure 2. Trapping results showing percent reduction of adult mosquitoes at the Rio Lindo site.

Figure 3. Petaluma waste water treatment facility pond covered with bulrush.

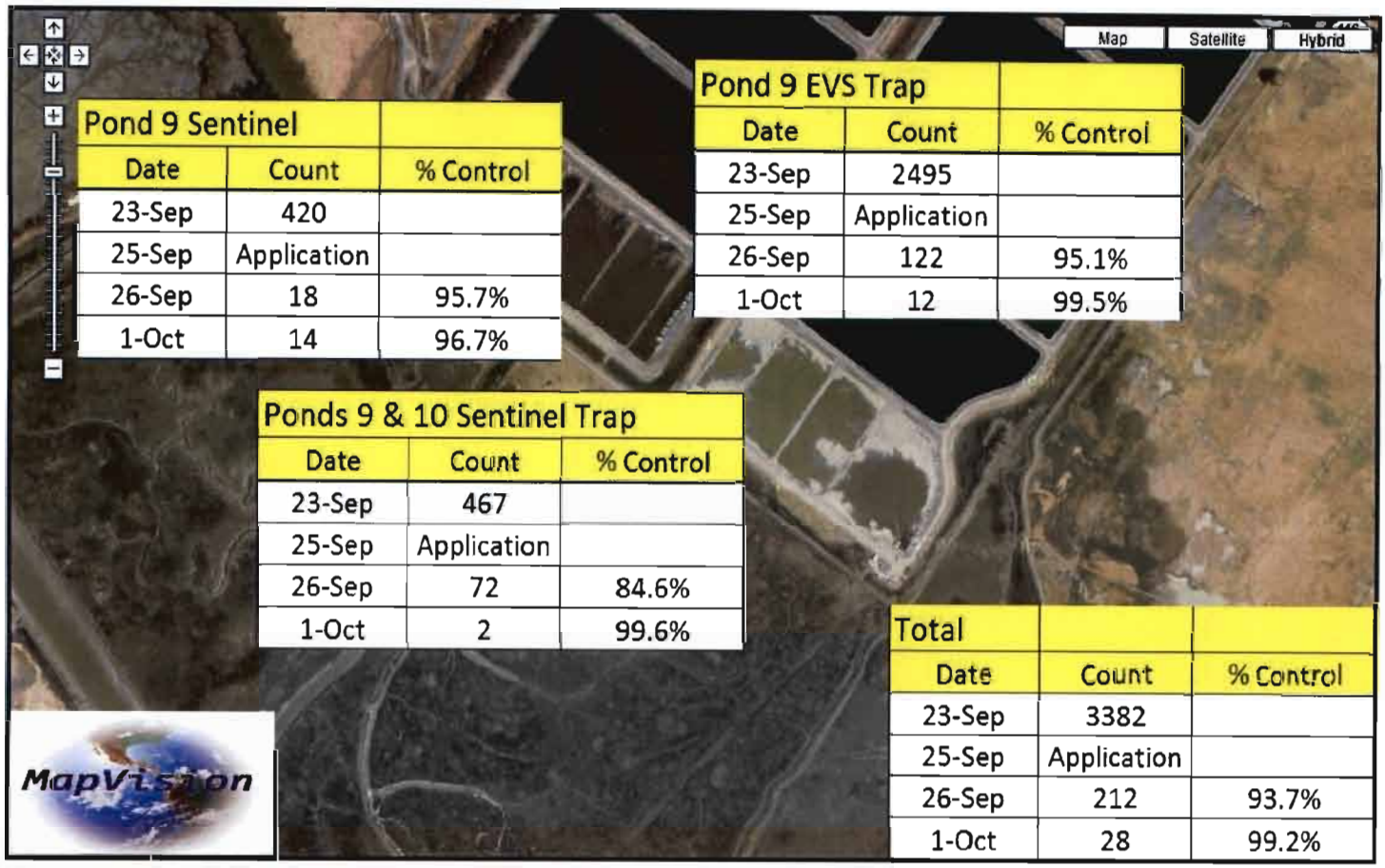

Figure 4. MapVision® image overlay with trapping results at the Petaluma treatment facility *(MapVision® imagery courtesy of Leading Edge Associates, LLC Waynesville, NC 28785).*

into the bulrush and cattails; this provided adequate control of the adult population of *Cx. erythrothorax*, reducing the population from 3,382 to 212 adults per trap night. An overall control of 93.7% was reported on September 26, and control of the adult mosquito population was still evident one week later at 99.2 % control (Fig. 4).

CONCLUSION

The three trials and one field application of Zenivex® produced promising results for this new adulticide product, particularly against *Culex* mosquitoes that are found commonly in Sonoma County. It became apparent that droplet analysis (i.e., DropVision®) correlated very well with the bioassays of caged mosquitoes and was instrumental in defining the mortality rates during the field application process, as well as setting standards for droplet flux values with the adulticiding equipment used by the District. We hope to complete more trials in the future on other mosquito species such as *Aedes sierrensis* utilizing Zenivex® and the DropVision® technology.

ACKNOWLEDGMENTS

We would like to thank Larry Smith and Ted Sleek of Central Life Sciences for the donation of Zenivex®. We are also appreciative of Doug Schmidt, administrator of the Rio Lindo Academy, for allowing us to use their property for the trials. We would like to thank the following employees of the Marin/ Sonoma MVCD: Steve Delucchi, Mark Farmer, Paul Filippi, Kimberly Heilig, Kristen Holt, Sarah Klobas, Nathen Reed and Mike Wells for their assistance in this project. And finally we are grateful to Jim Wanderscheid, District Manager, for his support and guidance.

Host-seeking Preferences of Three Species of *Culex* Mosquitoes in Southern California

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ABSTRACT. Feeding preference (host biting preference) plays a significant role in determining human and animal risk of infection with mosquito-transmitted pathogens like WNV. The intrinsic biting preference of common southern California *Culex* mosquitoes was examined in the field by determining their feeding success on four bird species, American crow (*Corvus brachyrhynchos* Brehm), house sparrow (*Passer domesticus* Linnaeus), house finch (*Carpodacus mexicanus* Müller) and mourning dove (*Zenaida macroura* Linnaeus), presented equally within a net trap to wild host-seeking mosquitoes. Blood meals in engorged mosquitoes captured within the net trap were identified to avian species using a multi-plex PCR assay targeting the *cytochrome b* gene sequence. There were significant differences in host biting preference among the three *Culex* species, with *Cx. tarsalis* Coquillett preferentially biting house sparrows and house finches over mourning doves ($P = 0.001$), *Cx. quinquefasciatus* Say preferentially biting American crows and house finches over mourning doves ($P < 0.01$), and *Cx. erythrothorax* Dyar preferentially biting American crows over mourning doves ($P = 0.0001$). The greater intrinsic biting preference of all three *Culex* species for passerine birds (sparrows, finches and crows) relative to the non-passerine mourning dove may result in higher infection prevalence in wild populations of these mosquito species when passerine bird species are available.

INTRODUCTION

Detailed knowledge of the blood-feeding behavior of mosquito populations in nature is an essential component for evaluating their vectorial capacity and for assessing the role of various vertebrates to serve as reservoir hosts of vector-borne viruses (Molaei et al., personnel comm.). As a component of vectorial capacity, feeding preference (host preference) plays a significant role in determining human and animal risk of infection with mosquito-transmitted pathogens like West Nile Virus (WNV). Since the introduction of WNV into southern California in 2003, few studies have been conducted on vector-host interactions governing the transmission of this disease in the wild. Recent work by Molaei et al. (personnel comm.) demonstrated that the house finch (*Carpodacus mexicanus* Müller), and few other mostly passeriform (perching) birds serve as main hosts for blood-seeking mosquitoes in southern California, suggesting their importance in enzootic cycling of WNV. Since mosquito blood meal studies often represent host contact opportunities more than actual host preference, the purpose of this study was to determine if *Culex* mosquitoes exhibit a feeding preference for common bird species in southern California after the influence of habitat is removed. Further, host preference was compared for *Culex* species from different locations in southern California to assess variation in host preference by site.

MATERIALS AND METHODS

Studies were conducted from 24 April through 8 October 2008 and from 27 May through 26 June 2009 at four locations in southern California: three wetlands (Bolsa Chica Wetland Reserves, UC Irvine Marsh and San Jacinto Wildlife Area) known to local mosquito control agencies for high populations of *Culex* mosquitoes; and one urban site, the Orange County Vector Control District (OCVCD) facility. At each study site, blood fed mosquitoes were captured using a 3 m x 3 m net trap baited with wild birds or on some occasions both wild birds and a single CO_2-baited suction trap (CO_2 trap) without the LED light (Model # 2780, Bioquip, Rancho Dominguez, CA, USA). The net trap consisted of a steel frame with a covering of green fabric on top and mosquito netting on all four sides.

Birds offered to host-seeking mosquitoes were American crow (*Corvus brachyrhynchos* Brehm), house sparrow (*Passer domesticus* L.), house finch and mourning dove (*Zenaida macroura* L.). All four bird species are commonly found in southern California. To reduce bird stress, individual birds were not used on consecutive trap nights.

On each trap night, two birds of each of the four bird species (total of eight birds) were placed into individual wire mesh cages (2.5 cm x 5 cm mesh size) suspended 0.5 m from the ground on rebar support poles. Individual bird cages were evenly distributed around the circumference of a 2 m diameter circle centered within the net trap, with each bird randomly assigned to a cage. On nights when the single CO_2 trap was also placed inside the net trap, the CO_2 trap and the eight bird cages were evenly distributed around the circumference of the 2 m diameter circle centered within the net trap. Trapping was conducted two nights per week, one night with and one without the addition of the CO_2-baited trap. Traps were set up in late afternoon and left overnight. Near dawn the next day, mosquitoes were collected from the interior walls and

roof of the net trap by mechanical aspirator and immediately placed on dry ice. Mosquitoes were transported to the lab on dry ice to be sorted by sex, blood meal status (engorged or unfed) and species (Meyer 2003).

The source of the blood meal acquired by each engorged *Culex* mosquito was determined by polymerase chain reaction (PCR) to amplify a fragment of the *cytochrome b* (cyt-b) gene present in all vertebrates, but lacking in invertebrates. Due to the capture of very large numbers of *Cx. erythrothorax* Dyar, a randomly selected subsample of this species captured from each location was analyzed. For each bird species used in this study, PCR primers were developed from a consensus sequence found by aligning all cyt-b sequences for the species that were available from GenBank (http://www.ncbi.nlm.nih.gov/) in December 2007.

RESULTS

Only *Cx. erythrothorax, Cx. tarsalis* Coquillett and *Cx. quinquefasciatus* were collected in large enough numbers for statistical analysis. At the three wetlands locations, a total of 2,855 mosquitoes comprising 7 species were collected, of which *Cx. erythrothorax* was the most common species collected. In addition, 288 engorged *Cx. quinquefasciatus* were collected at the urban OCVCD location in 2009. Blood meals were identified from 450 of 454 *Cx. erythrothorax*, 137 of 138 *Cx. tarsalis,* and 222 of 231 *Cx. quinquefasciatus*, with blood from exactly two avian hosts identified in 1-3 individual mosquitoes of each species. Few non-*Culex* mosquitoes were collected during this study, and only one was engorged (a single *Aedes vexans* [Meigen] which was not subjected to blood meal analysis0.

For all three *Culex* species, there were significant differences in host biting preference among bird species ($F \geq 6.75$; $df \geq 3, 27$; $p \leq 0.002$), with engorgement on mourning doves significantly reduced relative to the most commonly fed upon bird species. Of particular note, none of the 225 *Cx. quinquefasciatus* blood fed on a mourning dove. *Culex erythrothorax* fed most often on American crows while *Cx. tarsalis* fed most often on house sparrows; however, blood feeding rates on bird species other than mourning doves was not significantly different. *Culex quinquefasciatus* fed equally on all bird species other than mourning doves. Host biting preference was not different between collection sites ($p > 0.05$) for *Cx. erythrothorax* and *Cx. tarsalis*, the only species collected at all three wetlands sites in numbers that were sufficient for analysis.

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A Simple *in vitro* Blood Feeding Method for *Culex pipiens*

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ABSTRACT: A low-cost method for maintaining a *Culex pipiens* colony using a simple *in vitro* blood feeding system for adult mosquitoes is described. This procedure was developed using the laboratory mosquito colony at the San Mateo County Mosquito and Vector Control District. Our colony had been maintained previously on live quail blood feeds. Mosquitoes were supplied blood meals using a simple blood delivery system composed of a mixture of chicken and sheep blood and sugar suspended in a cotton ball. The temperature of the blood was maintained at approximately body temperature for several hours using a chemical hand warmer in an insulated plastic cup. The district's mosquito colony has been maintained successfully on this simple blood feeding system for multiple generations. Observations from experiments and suggestions for maximizing *in vitro* blood-feeding success are discussed.

INTRODUCTION

Mosquitoes of the *Culex pipiens* complex are found worldwide and carry many significant human and wildlife disease agents. In addition to vectoring a handful of filarial, bacterial and protozoan diseases, these mosquitoes are among the most important arboviral encephalitis vectors (Vinogradova 2000) and played a major role in the spread of West Nile Virus across the United States. Many local vector control agencies maintain in-house colonies of *Cx. pipiens* complex mosquitoes; these colonies typically rely on live humans or animals as blood sources. However, there are financial, operational and ethical drawbacks to using *in vivo* sources for blood meals. Viable feeding alternatives for a number of mosquito species have been developed using an assortment of extracted blood types, membranes and delivery systems. Unfortunately, many published techniques have not been designed or tested specifically for *Cx. pipiens* (Tseng 2003). Moreover, some techniques may also require specially fabricated or purchased equipment, which can involve considerable staff time and expense (Cosgrove, et al. 1994, Rutledge, et al. 1964).

Since 2003, the San Mateo County Mosquito and Vector Control District has maintained an in-house *Cx. pipiens* colony, primarily using live quail for adult mosquito blood feeding. Beginning in 2008, efforts were launched to develop a simple, low-cost *in vitro* blood feeding method as an alternative to using live animals in the lab as a blood source. As of May 2010, the district is currently using the methods described herein.

MATERIALS AND METHODS

Blood. A variety of different types of fresh animal blood were obtained from Hemostat Laboratories (Dixon, CA) in bottles of 50 or 100 ml. Types of blood acquired (with anticoagulant method indicated) included: chicken blood (Alsever's solution), bovine blood (sodium citrate) and sheep blood (sodium citrate or defibrinated). Upon arrival, blood was aliquoted into 5 ml Eppendorf tubes and frozen at - 20°C. For setting up individual mosquito cages for blood feeding, between 5 and 10 ml of blood was brought to a temperature of approximately 38°C in a water bath. Warmed blood was then mixed with sugar (about ½ of a cube per 10 ml of blood) and placed in the delivery system as described below.

Delivery System. After initial experiments using parafilm and sausage casings as feeding membranes proved both inconvenient to prepare and relatively unattractive to colony mosquitoes, a very simple blood delivery system was assembled using the following materials: Small cloth or paper towel, shallow plastic deli container or bowl, cotton ball, watch glass or other small glass dish and a chemical hand warmer (e.g., "Grabber," Grand Rapids, MI 49509 or "Heat Factory," Vista, CA 92081) (see Figure 1).

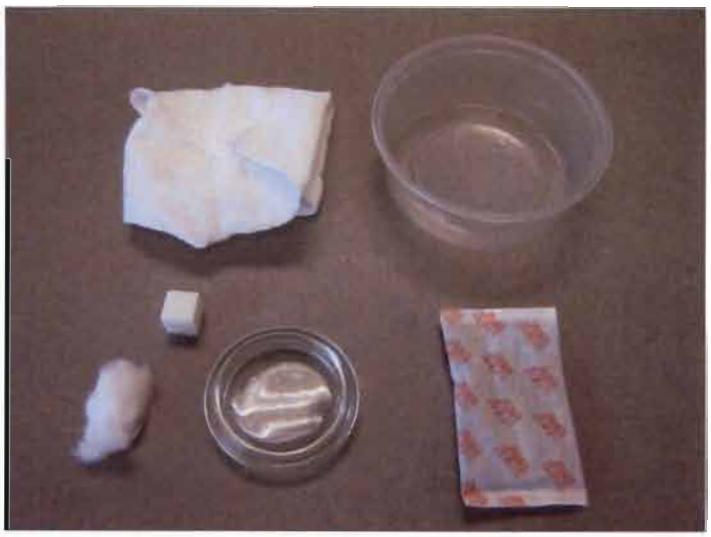

Figure 1. Materials used in the mosquito blood-feeding delivery system.

Procedures. Blood meals were provided to mosquitoes as follows:

1) The chemical hand warmer was activated according to the package instructions, and the watch glass (or small glass dish) was heated in a water bath to ~38° C and dried before use.
2) A cloth or paper towel was rubbed on the skin of the person preparing the blood meal in order to transfer natural attractants (Schreck et al. 1990). The cloth, hand warmer and warmed watch glass were then layered in plastic container to form a simple heat containment system (Fig. 2).
3) A blood/sugar mixture was added to warm watch glass.
4) A single cotton ball was added to the blood mixture. Cotton balls were tapped down into the mixture in order to soak up blood, but some dry surface area was left for mosquitoes to land on. This helped reduce the number of mosquitoes that drowned or became stuck in blood mixture (Fig. 3).
5) The entire blood feeding system was then placed inside an adult mosquito cage. The person preparing the blood meal then exhaled a few breaths of air into the cage to stimulate the mosquitoes to blood feed. The cages were left undisturbed in the dark for at least several hours or overnight to allow the mosquitoes to feed.
6) Mosquitoes fed in this manner subsequently oviposited into available water three to six days after blood feeding.

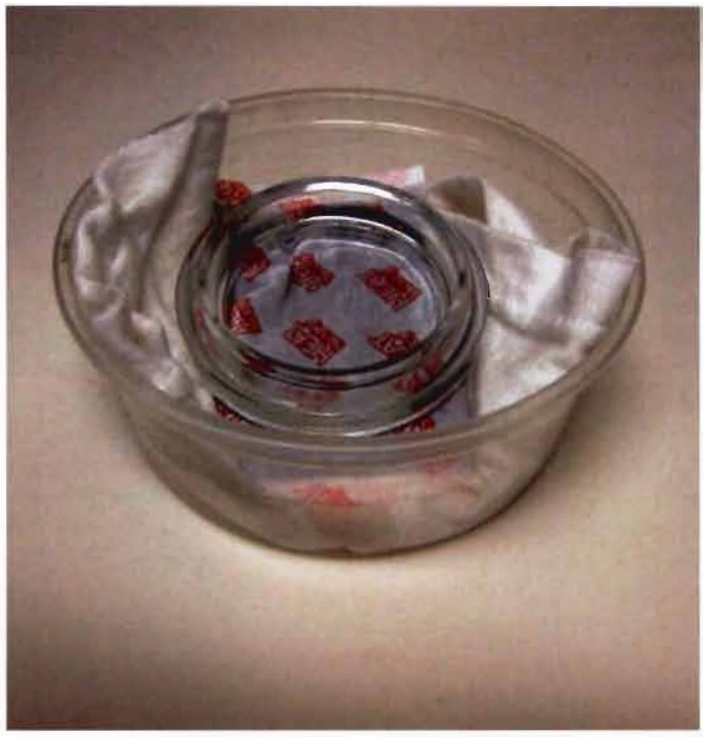

Figure 2. Assembled blood delivery system.

RESULTS AND DISCUSSION

Both lab-reared and wild-caught *Cx. pipiens* fed readily on blood and successfully oviposited using this procedure. Based on repeated experiments with variations of this method, the district has determined that certain practices produced optimal results when applying this *in vitro* feeding technique.

Although some mosquitoes readily fed on any of the blood types tested, chicken blood consistently resulted in a higher proportion of blood-fed mosquitoes than non-avian blood types. However, chicken and other avian blood is generally more expensive to obtain than mammal blood. To mitigate the cost of using chicken blood, a mixture of chicken blood (in Alsever's) and defibrinated sheep blood was also tested and found to be effective. For these tests, we prepared between 5 and 10 ml of 1:2 mixture of chicken:sheep blood (by volume), in order to balance the apparent *Cx. pipiens* preference for avian blood with the lower cost of sheep blood. We also found that blood frozen for longer than six months lost some attractiveness to mosquitoes. Raising the proportion of chicken blood offered in blood mixtures (to 50 - 100% chicken blood), however, partially offset the decline in mosquito interest to the older blood. It should be noted that blood stored frozen at temperatures colder than - 20°C resulted in some blood feeding, but many of the blood fed mosquitoes died before oviposition occurred. This effect may have been due to negative changes in the properties of the blood at ultra-low temperatures (e.g., hemolysis).

Figure 3. *Culex pipiens* feeding on *in vitro* blood using our blood-feeding delivery system.

Although the district experiments did not determine the precise quantity of sugar that was optimal for mosquito feeding, an effective amount of sugar in blood mixture can be ascertained experimentally and may vary based on conditions (e.g., age of blood, type of blood used). Kogan (1990), Clements (1992) and others have noted that an excess of sugar in the blood mixture can alter the destination of the blood meal within the mosquito, resulting in a diminished oviposition. Aside from this, the practical concern of not making the blood mixture too "sticky" (which causes mosquitoes to become entrapped) underscores the need to adding as little sugar as possible while still rendering the blood meal attractive to mosquitoes.

Certain preparatory measures taken with the adult *Cx. pipiens* mosquitoes were found to maximize blood feeding. As Gerber, et al. (1969) has noted, it is ideal to offer the blood feed 3 - 10 days after emergence. Also, removal of the sugar supply from colonies 24 hours before blood is offered has been found to increase feeding percentages for both female and male mosquitoes (Gerberg 1970). Although adult mosquitoes require a moderate level of humidity for survivorship, colony mosquitoes had greater rates of both blood feeding and oviposition when their colony cage was removed from the district's environmental chamber for the duration of blood feeding. The high noise level inside the climate-controlled chamber may have inhibited blood feeding behavior.

Extending the period of blood availability (e.g., leaving the blood meal in cage for several hours to overnight) dramatically improved the proportion of blood-engorged mosquitoes. Additionally, a heat source must be selected that maintains temperature over this time or the blood loses attractiveness. Griffith and Turner (1996) found that their chemical hand warmer heating system maintained a mosquito-feeding formula at 33 - 37°C for up to 6 hours. It also may be necessary to increase the amount of blood added to the cotton to ensure the blood does not dry completely during the offer period.

The district maintains its insectary on a light/dark cycle of at least 14 - 16 hours of light per day, with an hour of transitory dim lighting in the late afternoon to simulate "dusk." This was accomplished by installing a simple timer into the light switch panel. Blood feeds were typically set to coincide with the insectary's "dark hours," beginning with an hour of dimmed light, to allow mosquitoes access to blood during their natural "dawn and dusk" host-seeking times. When exposed to shorter light cycles, a smaller proportion of blood-fed mosquitoes oviposited. This may be related to the behavior of the specific subspecies of *Cx. pipiens* in our mosquito colony that was originally established from a mixture of different wild mosquito populations collected in San Mateo County. The current genetic composition of the colony has not been characterized.

CONCLUSION

The district's *Cx. pipiens* colony has been maintained on a combination of autogenous egg production and the described method of *in vitro* blood feeding for over 24 generations to date (~ 16 months). Egg raft size is roughly equal for both types of egg production with each raft generally containing between 30 and 60 eggs. While this egg raft size is smaller than the published average of 175 eggs per egg raft (Gerberg et al. 1969), the substantial volume of egg rafts produced and the frequency of feeds afforded by this simple method easily sustains laboratory needs for the colony.

ACKNOWLEDGEMENTS

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Vector Concepts Design Standards for the Prevention of Vector-Borne Diseases

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ABSTRACT: This paper outlines new standards for combating vector-borne diseases. Typically most vector control districts reduce mosquitoes by larviciding and adulticiding, focus on source reduction, have a fish program and provide educational materials on mosquito abatement. The program described here adds another component by utilizing planning designs for new development to prevent the spread of disease. With the dramatic shift away from agriculture to urban development, placing conditions on new developments has demonstrated effective prevention. All annexations, parcel maps, subdivisions, special use permits, site plans building plans and final maps are sent to the Vector-Borne Diseases Program for review. This article explains how development projects are reviewed by requiring conditions in the designs of detention/retention basins, channels, catch basins, ponds, wetlands and landscape designs.

DISCUSSION

Vector-borne diseases are a global public health issue that Nevada has been combating with a unique and effective program. In particular, one of our newest threats is West Nile Virus, a vector-borne disease carried by mosquitoes that has spread across the United States since 1999. Originally, pesticides were used to combat mosquitoes following the first use of petroleum hydrocarbons for larviciding. Early practices emphasized the elimination of places where mosquitoes were found to be breeding. This method, referred to as source reduction, is in current use. However the most profound change occurred just after the Second World War when chemical control methods became available that allowed for large area treatments (Schaefer 1993). Currently, pesticides are still used for controlling mosquitoes, to reduce both nuisance biting and exposure to diseases they transmit. Yet, as this article demonstrates, with resistance and limitations due to pesticide use, regulations in the following Vector Borne Diseases Program (VBDP) help combat health risks through community development planning by designing urban infrastructure that reduces the ponding of water.

The VBDP at the Washoe County Health District has evolved from reliance on pesticide applications to prevention based Vector-Borne Diseases Populations Management (VBDPM). Vector-Borne Diseases Populations Management is defined as an environmentally safe-based strategy that provides long-term solutions to pest problems with minimum impact on human population and the environment (Monsen 2000). With VBDPM, insect management becomes proactive as a prevention tool to minimize the impacts of pest nuisances, and more importantly, to reduce the adult threshold of disease transmission. The VBDPM tools used in our program specifically target known insect populations through preemptive treatments as well as biological, cultural and source reduction which call for redesigns in existing drainage facilities. Furthermore, the newest tool used by the Vector-Borne Diseases Program is through the review of new developments and redevelopments proposals in the cities of Reno, Sparks and Washoe County. As planners, participation in the infrastructure design of projects will minimize the impacts of insects to residents who move into these new developments.

New development and redevelopment (3% growth rate with a population increase of 1000,000 from 1997 to 2007) have dramatically occurred in Washoe County (specifically in the Truckee Meadows Community) since 2000 with land converted from agricultural use to urban development. Importantly, it is this conversion where there has been a shift in the type of mosquitoes that affect the public. Mosquitoes in agriculture are primarily nuisances that irritate those who work and participate in outdoor activities. On the other hand, insects in new development and redevelopment areas exploit the public infrastructure that not only creates a nuisance factor, but also creates a health threat from diseases to the urban population. This shift between mosquitoes and people has brought about a new focus on how we can better serve and protect the public against insects that cause nuisances and transmit disease.

With the recognized change away from agriculture to an urban environment, the need to protect the increase in population and tourism became an important focus. As a result in Washoe County, the Vector-Borne Diseases Program of the Washoe County Health District initiated health regulations governing the prevention of vector-borne diseases. The program established regulations for assisting professionals, such as city and county planners and engineers, in that the VBDP would become a planning partner in development. Potential vector conditions and diseases affecting the public were reduced through better designs of urban infrastructure. The regulations represent standards for the prevention of vector-borne diseases in land development and redevelopment. Importantly, these standards establish conditions that are required during the review of development and redevelopment projects.

In the fall of 2002, regulations were drafted with specific language for residential subdivisions, commercial centers, professional offices and industrial sites (all development) to design hydrology features in a better way. This included designing detention/retention basins, catch basins, man made channels, wetlands, landscape detail, lakes and ponds as well as redesigning existing infrastructure to meet design criteria for the VBDP. Another component to the regulations was rodent prevention such as rockery walls and talus treatments to discourage rodent habitat for new development. Proposed changes to the Washoe County

Health District schedule of service charges and permit fees were added to the regulations. These included fees for annexations, parcel maps, master plans, rezones, tentative subdivision maps, site plans, special use permits, building construction plans and final maps.

In April of 2003, a series of three publicly advertised work shops were established to provide public comment on the proposed regulations. Stakeholders who could be impacted by the proposed regulations were contacted throughout the Reno area; these included Washoe County Community Development departments, engineering firms including the building industry, Builders' of Northern Nevada and Associated General Contractors (District Health Procedures and Amending or Adopting Regulations V.3). An impact statement was included for the potential financial impact of the new regulations. When the public meeting process was completed, the Vector-Borne Diseases staff reviewed the issues presented during the meetings prior to considering revisions to the draft regulations. After the final draft of the regulations and business impact statement was completed, the draft was sent to the deputy district attorney representing the Washoe County Health District and the Washoe County District Board of Health for review (V.5). Once approved, the draft regulations were then presented to the Washoe County District Board of Health and adopted unanimously on May 22, 2003. The final version of the draft regulation was sent to the secretary of the State Board of Health for approval. The draft regulations were placed as an agenda item and approved in June of 2003.

As a result of the approval of the regulations, copies of all annexations, parcel maps, changes in land use, subdivisions, special use permits, site plans, abandonment's, amendments, reversions, building construction plans and final maps are sent to the Washoe County Health District for review as to health and safety issues following set standards and regulations (Carnahan 2003). These reviews include designs for grading, landscape, rockery walls, detention/retention basins, catch basins, channels and any type of development or construction that would cause a potential vector-borne disease issue (Cona 2003).

As growth in the past ten years has moved from the valley floor to higher elevations, rockery walls were constructed to stabilize slopes. Design standards minimized the threat ground squirrels have in the possible transmission of plague which is endemic to Washoe County. When rockery walls are included in the grading process of subdivisions, commercial and industrial projects, the Vector-Borne Diseases Program required the voids in the walls were filled by placing smaller rock within six inches of the face for the entire height of the wall. In some instances, rock rip rap was placed on 2:1 slopes for slope stabilization. These talus treatments require the placement of ¾ inch to 1/12 inch D size rock, four inches in depth, prior to or in some instances after, the placement of rip rap rock to discourage void formation for rodent habitat.

The VBDP also required modifications in the construction plans for the irrigation of residential and commercial landscapes. Excess irrigation, combined with the windy conditions endemic to Washoe County, created water runoff (nuisance waters) that flowed from curbs into storm catch basins, detention ponds and ephemeral channels, eventually reaching the Truckee River. These nuisance flows not only created water quality issues such as pollutants, sediment and erosion, they produced ponding water in infrastructure that was exploited by ovipositing adult mosquitoes. To curtail the nuisance water runoff in landscapes, a wind sensor control unit was included as part of the standard for irrigation standards in these settings. To reduce water runoff further, an 18 inch "catchment area" (xeriscape design) from the back face of impervious surfaces was installed which would allow excess irrigation water to percolate on site thereby minimizing runoff of nuisance waters from standing in the storm catch basins.

Staff of the VBDP collaborated with Washoe County community development engineers to develop a new storm catch basin design that would not collect and hold water. The previous standard design for catch basins collected water and sediment, including oils, prior the eventual discharge to river(s). The new design for catch basins requires a series of weep holes (one inch in diameter) on the side and end walls; water passing though these holes is filtered thru Class C backfill rock placed around the catch basin (Lindeman 2007). A marifi 140N fabric or equivalent is placed adjacent to the basin structure prior to the Class C rock to prevent sediment from back flowing into the basin and plugging the weep holes. In addition, an oil absorbent sock is placed in the basin to absorb oils, replacing the ineffective previous design. This modified catch basin design was approved and became the new Washoe County standard in 2007. The importance of this new detail design is that it eliminated standing water. Our GIS data base from mosquito surveys had previously determined that one out of three catch basins, and in some developments two out of three, were breeding mosquitoes in Washoe County (Murray). These mosquitoes were identified as *Culex pipiens* (house mosquitoes), an important vector of West Nile Virus.

Due to the increase in urbanization, the landscape of ephemeral channels in Washoe County has been altered. With the increase in water runoff in natural channels due to the development of impervious surfaces such as parking lots, roadways and rooftops, detention basins have become a standard feature for infrastructure development. The purpose of these detention basins is to decrease the peak flow rates of storm water events, thereby delaying the flow of water to tributaries of the Truckee River. If these basins are not designed properly, they will pond water and create insect nuisances to the urban population. To minimize this, a 4 - 6 inch cobble rock low flow channel (V channel), 1 foot deep and 2 feet wide and connecting the inlet(s) to the outlet pipe, was designed to allow the water to trickle thru the facility during the spring and summer months, while having the capacity of storing water for flood events during the winter.

In some new developments, channels are constructed to receive storm nuisance water runoff because no natural channels exist. Prior to the program design standards, city and county engineers constructed these as flat bottom structures. These flat bottom channels pond water over time, support weed growth

and create larval habitats for *Culex tarsalis*. To prevent this from occurring, a low flow channel (V channel) channels the nuisance water thru the structure while still having the capacity for flood flows.

Ponds are another aesthetic feature for developers in urbanization. If these bodies of water are not constructed and maintained properly, they provide breeding habitats for *Culex pipiens* and midges. Designing ponds with 3:1 slopes, with cobble rock placed 2 foot above and 2 feet below mean water level, curtails weed growth around the perimeter of the pond. Another insect that breeds in ponds are midges that can be troublesome to urban populations. While this insect neither bites nor transmits diseases, it can affect the quality of life because they create a nuisance to people by hovering in entryways and covered patios. To minimize pesticide use to control midges, the Vector Borne Diseases Program requires the installation of fountain aerators to eliminate these insects. The size and number of these units is based on the size and configuration of the pond and determined by aquatic specialists. These units are required to run at a minimum of two hours in the morning and two hours in the afternoon during the months of March thru October. The agitation of the water surface by these aerators discourages adult midges and female *Culex* mosquitoes from laying eggs in this water. Fountain aerators have the additional benefit of improving water quality by moving low oxygen water from the top to the bottom. This minimizes algal blooms, reduces weed growth and eliminates smells from decaying organic vegetation and debris that results from reduced oxygen content in the water.

As development encroaches into areas known for jurisdictional wetlands, mitigation is required by the Army Corp of Engineers with wetland loss. Wetland consultants, state and federal agencies participate in the planning of creating a wetland habitat. Environmental health is often times ignored when it comes to the planning design of wetlands that are usually situated adjacent to residential development. As a result, the VBDP developed a model wetland design composed of a series of meandering low flow channels with native plant species planted on the upland slopes and an earthen path designed for public use. Outfall pipes that convey storm and nuisance waters from nearby developments connect to the low flow channels in the wetlands. A micro pool basin is constructed below the outfall pipes to remove sediment and urban debris from entering the wetland, thereby maintaining the pristine nature of the wetland.

Low Impact Development (LID) design standards have recently been adopted by Washoe County and the cities of Reno and Sparks for improving water quality and reducing storm water runoff. The LID Handbook assists planners, developers, architects and landscape professionals, city and county community development, public works staff and others with the selection and design of features and practices that mimic natural hydrologic functions (page 1-1 Kennedy Jenks Consultants 2007). The Vector-Borne Diseases Program supports the LID concept that reduces run off from impervious surfaces such as roads, parking lots, storm events and excessive irrigation. One such LID design is bioretention systems which consist of depressions with vegetation engineered with porous soils designed to capture, treat and infiltrate urban water runoff (pollutants, oils etc) to the subsurface. Urban runoff is reduced thru infiltration, soil retention, plant uptake and evapotranspiration, thereby minimizing water from ponding in the conventional storm drainage systems. Another such design is LID swales that are lined or unlined open channels that convey and treat runoff from roadways, parking lots, etc. so that it can be utilized in residential, commercial and industrial land uses. Low flow channels and under drain systems are the preferred design for the VBDP because it reduces the potential for extended ponding and mosquito breeding.

Xeriscape buffer strips are the more typical landscape for the semi arid climate of Washoe County; these buffer strips are planted with low to no water use plants and covered with decorative rock allowing for water conservation. These strips are designed at the back face of sidewalks and impervious surfaces to reduce runoff. Less runoff will occur as the xeriscape buffer strip captures and infiltrates the water leaving the lawn area (Kennedy Jenks Consultants, 2007). These LID designs allow water to flow thru unconventional storm drain systems, mimicking the natural hydrologic functions while reducing the habitat of insects that exploit the urban infrastructure and pose a threat to the public health of human populations.

CONCLUSIONS

When the regulations were initiated in the fall of 2002, few realized the positive effects that they would have in the Community Development Departments of Reno, Sparks and Washoe County and the citizens of these communities. The Vector-Borne Diseases Program, through the establishment of regulations, became a planning partner in development and redevelopment projects. This has assisted professionals in designing and modifying infrastructure to improve environmental health and mitigate the impact and severity of diseases from mosquitoes. Additionally, the VBDP recognized the need for becoming involved with planners to minimize the threshold of insects, while utilizing a more permanent approach of eliminating water habitat in the urban environment.

An action plan is necessary if nationwide public health needs are to be adequately addressed (Hazeltine 1993). The action plan our program under took in our community allowed us to step out of our role as an agency reliant on the use of pesticides for insect control, to becoming active in the planning process in community development. Furthermore, involvement in planning allowed us to review development proposals and condition these projects thru design standards for hydrology features that would reduce standing water habitat for insects. Due to this, "non vector friendly" urban infrastructure is designed in new developments and redevelopment projects.

Vector control agencies in the United States rely heavily on pesticides as their major tool to combat mosquito problems. Yet, while this reduces the disease concerns, irritation and annoyance

from these insects, it does not fully address prevention and long lasting cost saving measures. The forward approach of the Vector Borne Diseases Program of the Washoe County Health District focuses on sites thru preemptive treatments in targeting the immature stages which reduces the threshold of adult mosquitoes from emerging. Furthermore, participation in the planning process through community development prevents insects from exploiting the urban infrastructure thru improved design standards that ultimately reduce the dependency on the use of pesticides.

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Can Public Health Agencies Establish Working Relationships With Private Pest Control?

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ABSTRACT: Private pest control companies (PCOs) operating within the district boundaries were surveyed to assess services available to local residents for control of domestic rodents. Information gathered was used to compile a list of vendors and the vertebrate control service(s) each offers along with guidelines on how to choose a PCO.

INTRODUCTION

In April of 2008, responsibility for services related to domestic rodents was transferred from the Health Department of San Mateo County to the San Mateo County Mosquito and Vector Control District (SMCMVCD). The existing program included answering service requests, conducting inspections of residential property and overseeing a baiting program in sewer lines. When the SMCMVCD responds to resident complaints, technicians visit the property and advise residents on how to exclude or control rodents. The control work itself is the responsibility of the property owner. Homeowners are informed about the nature of the work that needs to be done, and then they must either do it themselves or hire a Private Pest Control Operator (PCO). Residents frequently ask for a list of local PCOs during these visits. Staff members are also frequently asked for advice on how to evaluate proposals from PCOs or how to assess the work after it is done. Many residents have expressed uncertainty about how to evaluate the work proposed by PCOs. Some of these problems appear to come about from a lack of understanding on the homeowner's part about rodent control methods in general and their problem specifically. Others resulted from a lack of information provided by PCOs regarding the work proposal or its expected results. In addition, some residents claimed that PCOs had given them false or misleading information about local regulations or the role of the agency in rodent control. For example, one resident said she had been told that the PCO was required to report the presence of rodents on her property to the county, that the county would require them to hire a PCO and that they would be given a citation if they did not hire one. Another resident stated that one company had told her that her child would get sick if she was allowed to play in the yard where rats had been seen.

To address these issues, the SMCMVCD decided to conduct a survey of PCOs working in the district, to learn about the services available to residents from these companies and to compile a list of local companies for distribution to residents. Compiling and distributing a list of private service providers carries potential liability for public agencies, and as a result most do not provide information of this kind. Publicly funded agencies are prohibited from promoting the financial interests of one private company over another and must not have the appearance of guaranteeing their work. An informal poll of other vector control districts belonging to the MVCAC revealed that of the thirty-five districts with programs related to rodents, only one provides residents with a list of PCOs offering rodent control. One other district provides a list of companies offering exclusion work. Both districts are located in the Coastal Region. One district in the Southern California Region is currently considering a list of PCOs offering rodent control. Overall, the general policy among the majority of vector control agencies is to avoid referral lists altogether.

Because the SMCMVCD requires residents to do rodent control work on their property, district staff felt it was important to have an understanding of the services offered by local PCOs and to develop guidelines that residents could use in choosing a company and evaluating its work. Therefore, to learn more about the scope of services offered by PCOs operating in the county and to understand what local residents face in hiring a company, a telephone survey of local PCO's was conducted. Following the survey, all of the responding companies were invited to a forum hosted at district headquarters to share information about rodent issues within the county.

DATA COLLECTION

A list of PCOs serving San Mateo County was compiled from the local phone directory and the internet, both readily accessible sources of information for the general public. Each company was contacted by telephone and asked a standardized list of questions (Table 1). The questions sought information about services offered, monitoring methods, the use of poison bait and whether

Table 1. List of survey questions posed to private pest control operators (PCOs) during phone survey.

- Does your company provide residential rodent control?
- What rodent control services (trap, bait, exclusion) are offered?
- Describe in more detail you company's rodent control method(s).
- What is the initial cost of inspection and rodent control work?
- Is the rodent control work guaranteed?
- If a contract is required, what is the duration?
- How does you company determine the effectiveness of the rodent control work?
- Throughout the control work phase, does your company provide feedback to the resident?

residents were required to sign a long term contract. To determine whether companies would respond differently to a district inquiry than to a member of the public, each company was contacted twice, first with the caller representing themselves as a member of the general public; and then again as a district employee.

SURVEY RESULTS

The telephone directory and internet search revealed 47 companies conducting rodent control or exclusion work in San Mateo County. Of these, six (13%) work only on commercial properties, four (8.5%) could not be reached, one (2%) does not work with rodents, one (2%) does not provide services in San Mateo County, and three (6.5%) were out of business. The scope of services offered by the remaining 32 companies varied widely. For example, four companies use only poison baits, 18 offer only non-poisonous control methods (trapping and/or exclusion), and ten offer both poison baits and non-poisonous control, depending on the homeowner's preference (Table 2). Eighteen of the 32

Table 2. Breakdown of services offered by companies conducting rodent control in San Mateo County.

Service Type	No. of Companies	
Exclusion only	3	
Inspect / Trap	6	
Inspect / Trap/ Exclude	9	
Total offering strictly nonpoisonous control		18
Inspect / Bait	4	
Inspect / Bait / Exclude	3	
Inspect / Bait / Trap	4	
Inspect / Bait / Trap / Exclude	3	
Total using poison baits		14
Total Number of Companies	32	32

companies offer exclusion work, either as an adjunct to trapping or baiting or as a stand-alone service. Most companies (30 of 32) did not require homeowners to sign a long term contract. The number of return visits for a single incident varied from none to a series of visits occurring at intervals from three days to a few weeks. Many companies did not routinely conduct follow-up inspections, relying instead on the homeowner to call back if the rat activity continued. For those companies that did conduct follow-up inspections, the effectiveness of their control work was assessed by the presence of new droppings, consumption of bait or other signs of new rodent activity. Results of these follow-up inspections were not routinely provided to the resident, unless specifically requested. Companies stated that in general, few residents had expressed an interest in receiving such information. Twelve of the 32 companies guaranteed their work. Guarantees varied from 30 days to two years (only on exclusion work).

The cost of rodent control services also varied tremendously. Prices quoted over the telephone for an initial inspection ranged from $0 (free) to $195. Control work costs were between $35 and $425 (Figure 1). Cost differences among companies are difficult

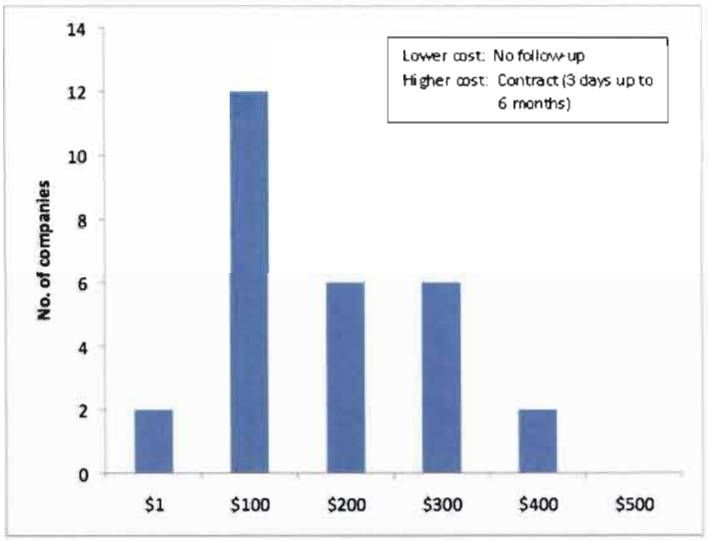

Figure 1. Variation in cost of initial rodent control work as quoted by private pest control companies in San Mateo County.

to evaluate because each bills its services in a different manner. For example, some companies charged a one-time fee for rodent control service that included a single visit to do bait placement, trapping or exclusion with no follow-up visits. If further work was needed after this initial visit, an additional cost was charged. The cost of the work depended on the amount and type of work required. Two companies offered free inspections but required homeowners to sign a contract of four weeks to three months before work could begin.

It became clear during the survey and subsequent follow-up discussions that the price of rodent control also varies because of the individual nature of any rodent infestation. Thus, the scope of work needed on any particular property depends on the characteristics of the property and the rodent infestation occurring there. Therefore, it is almost impossible for a PCO to quote a single price over the phone for control work.

Finally, responses to the second survey, in which the caller identified themselves as a representative of the mosquito and vector control district, did not differ greatly from those to the first survey.

FOLLOW-UP MEETING WITH PEST CONTROL OPERATORS

A second goal of the telephone survey was to gather information on local rodent populations and to obtain a more complete overview of rat populations as a whole. Every company

contacted during the survey was invited to attend a meeting at district headquarters to learn about district programs and exchange information about rodent control and rodent problems within the county. The companies were told that attendance was open to all companies and that those that attended and provided information about their services would be placed on a referral list. At the meeting, each company was asked to describe the scope of services offered and their approach to rodent control. The district presented a description of its own program and the services it offers to residents. This was followed by a discussion of rodent activity in the county in general.

CONCLUSION

In conducting the telephone survey, it became apparent that choosing a private pest control operator is a complex undertaking for most residents. Residents are faced with a dizzying array of choices in the services offered, the method of control used and the way in which work is billed. Some residents stated that they did not clearly understand what they were paying for. Other residents stated that the rodent control work conducted by some companies seemed costly and open-ended. Without a general understanding of rodent biology and the specific issues that need to be addressed on their own property, residents find it difficult to determine whether the work proposed by a PCO suits their needs. The utility of the district's inspection services lies in giving residents a basic understanding of rodent biology, exclusion and control and providing an unbiased assessment of the specific rodent issue on their own property. The district's goal is to give homeowners the ability to ask meaningful questions of service providers, to evaluate the appropriateness of the work proposed by PCOs and to assess the effectiveness of the work after it is completed.

Information gathered at the first annual meeting with PCOs and from the survey was used to construct a list of companies offering rodent control or exclusion work. This list will now be provided to residents on request. It categorizes services offered by each company: physical (exclusion, trapping) or chemical control work (poison baits). The list includes a disclaimer stating that the district does not guarantee the work of any company and does not recommend one company over another. There is a link at the bottom of the page to three websites to assist residents with researching the qualifications of PCOs (Table 3). Through these websites, residents can verify a PCO's licensing, company status and professional affiliations. The format is modeled after that of a list provided by the Santa Cruz County Mosquito and Vector Control to its residents.

Providing a list of potential providers and having a general knowledge of the companies operating in the county will enhance the district's ability to advise residents. In providing a list of potential PCOs to residents, the district does not propose to guarantee the work of these companies or to suggest residents choose one provider over another. Rather, the goal is to educate residents in how to assess work proposals from PCOs and how to judge the results of the work after it is completed. It remains the homeowner's responsibility to:

1) Understand what will be done on their property and why.
2) Obtain a clear written estimate of the work proposed and what it will cost.
3) Verify that they understand the results that can be expected from the work and how its success will be assessed.
4) Verify that the company they hire has a valid license and good reference.,

The district plans to meet annually with PCOs to update the information on the referral list. Invitations will be sent to all companies listed in the phone directory, allowing new companies to be included in the future. These meetings also provide a forum to exchange information about the status of rodent issues in the county. At the conclusion of our first meeting, all the representatives present expressed significant interest in attending presentations on topics such as integrated pest management and biology of vertebrate and invertebrate vectors and vector-borne diseases.

The district strives continuously to improve our IPM programs while responding to the concerns of county residents. Providing a referral list of PCOs and conducting annual coordination meetings with PCOs will enhance the district's programs and expand our knowledge about rodents within the county.

Table 3. Consumer information referral websites.

California Structural Pest Control (www.pestboard.ca.gov)
This state regulatory agency oversees licensing of companies that exterminate mice, rats, pigeons, spiders, ants, roaches and other household pests. The person who negotiates the contract with you and the pesticide applicator must be licensed. A licensee should possess an identification card which shows the expiration date and type of license. This website provides information on current licensing/registration, a company's history of complaints and how to formally file a complaint.

Pest Control Operators of California (www.pcoc.org)
This nonprofit trade association provides a list of member companies. This website has consumer information on finding a company and has many links to regulatory agencies and university publications on pest vertebrates and insects.

Better Business Bureau (www.bbb.org)
This nonprofit organization provides a list of accredited pest control companies that meet certain high standards for their business practices. The BBB also acts as a neutral mediator in dispute resolutions between individuals and businesses or between businesses. This website provides information on a company's history of complaints and how to formally file a complaint.

Control of West Nile Virus within the Kern Mosquito and Vector Control District

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ABSTRACT: Enhanced coordination and rapid response to trap data lessened mosquito abundance in urban / suburban areas and consequently reduced the threat of West Nile Virus transmission to humans. Daily, as opposed to weekly, reporting of trap counts determined when and where nocturnal fogging operations were done in response to areas with increased mosquito abundance. These adulticide operations were performed daily after-hours during July and August, the peak of mosquito activity during the year. The numbers of both gravid and EVS traps were increased to improve sensitivity to detect increased mosquito populations. Aerial surveillance for unmaintained swimming pools in early and mid seasons of 2007 and 2008 showed a marked increase in the number compared to previous years. Subsequent weekend inspection and treatment of pools were implemented. Rapid turnaround time for virus testing by the Center for Vectorborne Diseases enabled a rapid operational response to focus where positive mosquito pools were collected. Rapid response to surveillance indicators reduced vector abundance and infection rates and the number of human cases detected during 2008.

INTRODUCTION

Between 1969 and 2003, only 17 human cases of any type of arboviral disease were reported from Kern County. These were all cases of Saint Louis Encephalitis, mostly clustered during the 1989 outbreak centered in Bakersfield (Reisen et al. 1992, Tueller 1990). Upon the arrival of West Nile Virus (WNV) in Kern County during 2004, there were 60 human WNV cases during the first year. In subsequent years there were 68 and 49 human cases, until in 2007 there were a record 140 human cases. The Kern MVCD management was under extreme pressure to develop intervention solutions to stem the onslaught of WNV cases and protect the public health Nearly every enzootic indicator of arboviral activity (mosquito pools, dead birds and sentinel chickens) showed a relatively large number of WNV positives (Table 1) that were well above the California Department of Public Health's risk assessment model threshold for epidemic conditions (Reisen et al. 2009). The current paper describes how rapid, focused and effective mosquito control reduced mosquito abundance and the numbers of human cases within the Kern MVCD during 2008.

Table 1. Numbers of human cases reported by Kern County Health Department, positive enzootic surveillance indicators including mosquito pools, laboratory tested dead birds and sentinel chicken seroconversions, and the numbers of residential swimming pools treated within the Kern MVCD from 2004 to 2008.

Year	2004	2005	2006	2007	2008
Human cases	60	68	49	140	2
Mosquito pools	213	223	221	206	7
Dead Birds	61	44	24	124	10
Sentinel Chickens	90	111	117	82	18
Swimming pools treated	542	552	398	856	2,257

MATERIALS AND METHODS

Prior to 2007 mosquito surveillance trap results were summarized and reported to management weekly, usually on Fridays. However, in late summer of 2007 and in subsequent years, trap counts and species abundance were reported daily, and those areas that had high adult mosquito abundance were usually treated with adulticides that night. The number of traps deployed weekly was increased from 28 gravid traps and 38 CDC-style dry ice-baited traps at the start of 2007 to 44 gravid traps and 63 CDC traps by the end of 2008, an increase of about 40% for each type of trap.

During routine residential inspections in 2006 and 2007, it became apparent that swimming pools were becoming a major source of both *Culex tarsalis* and *Culex quinquefasciatus*. This situation was exacerbated by abandoned swimming pools caused by high number of foreclosed properties during an economic crisis starting in mid 2006 (Reisen et al. 2008b). To worsen matters, mild temperatures in Bakersfield often allowed mosquito larvae and pupae to survive in swimming pools throughout the winter. In late summer of 2007, aerial surveillance of swimming pools was contracted to an independent aerial company. In the first sets of photos, about 3,500 unmaintained or 'dirty/green' pools were identified by the aerial company. Subsequently, in 2008 aerial surveillance was done in April and August and yielded about 4,000 potentially dirty/green pools. On each occasion the maps were distributed to area forepersons that checked the accuracy of the addresses and then inspected and treated the pools as necessary. Prior to 2007 there was an average of about 550 swimming pools that were treated annually. In 2007 that number increased to 856 swimming pools and in 2008 the treated pools increased to 2,257 (Table 1). The number of personnel devoted to swimming pool inspections and general mosquito control increased from about 40 individuals at the start of 2007 to about 50 by the end of 2008, an increase in the work force of 20%.

A factor contributing to the effectiveness of our quick response was the rapid testing and reporting of positive mosquito pools by the Center for Vectorborne Diseases. These results allowed us to concentrate treatments on areas of WNV positive mosquito activity in a timely manner. Results for mosquito pools that were sent by overnight UPS were often reported to us by the following day by email using the California Surveillance Gateway.

RESULTS AND DISCUSSION

Rapid and focused operational responses to surveillance data markedly reduced West Nile Virus activity during 2008. The number of human cases dropped dramatically from 140 in 2007 to 2 in 2008 (Table 1). *Culex tarsalis* abundance decreased concurrently in 2008, with peak trap counts delayed until September (Fig 1a). The abundance of *Cx. quinquefasciatus* in CDC traps dropped similarly during the heavy mosquito season, July through August (Fig 1b). Most strikingly, the abundance of *Cx. quinquefasciatus* in gravid traps dropped from a peak of

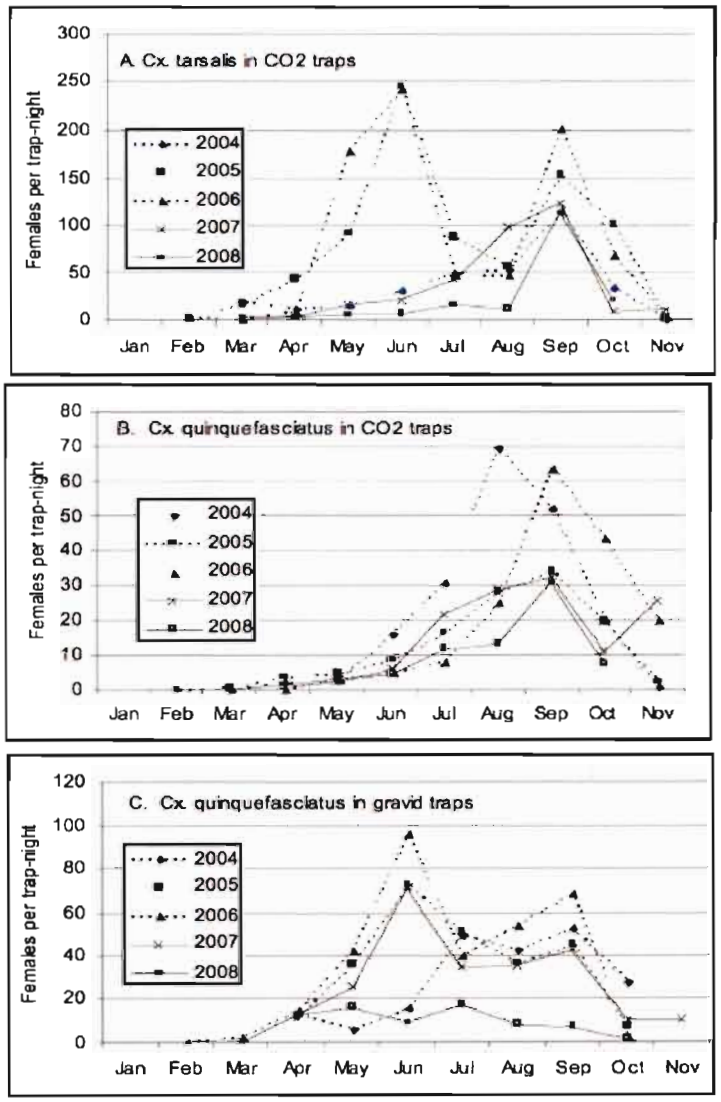

Figure 1. Monthly changes in the abundance per trap-night of: (A) *Cx. tarsalis* in CO_2 traps, (B) *Cx. quinquefasciatus* in CO_2 traps and (C) *Cx. quinquefasciatus* in gravid traps operated within the Kern MVCD during 2004 – 2008. Data from 2007 epidemic years is shown in bold and the 2008 year with extended control is shown with open points for comparison.

over 70 per trap night in Jun 2007 to less than 20 for the entire season (Fig. 1c). In agreement with the decline in human cases and mosquito abundance, the combined WNV infection rate (MLE) for *Cx. quinquefasciatus* and *Cx. tarsalis* remained below epidemic levels during 2008 (Fig 2).

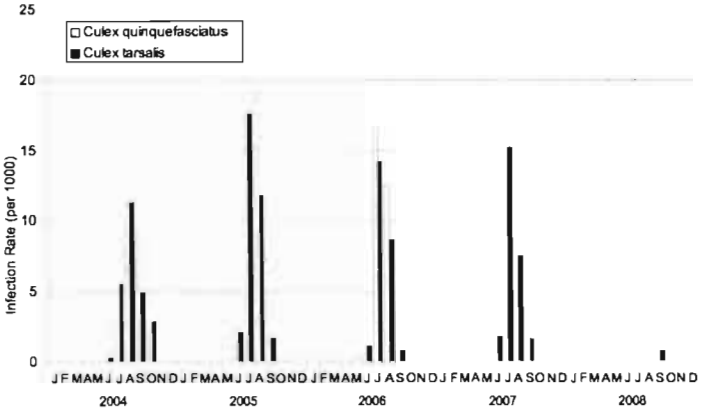

Figure 2. Monthly changes in the WNV infection incidence per 1,000 in *Cx. tarsalis* and *Cx. quinquefasciatus* within the Kern MVCD during 2004 – 2008 calculated using the maximum likelihood method. Infection incidence > 5 per 1,000 is considered epidemic levels.

The enhanced surveillance and rapid response methods used by the Kern MVCD are being continually evaluated and may not always be as successful as shown for 2008. Frequently, WNV amplification following outbreak years such as 2007 is dampened by epidemiological factors such as herd immunity in avian host populations (Kwan et al. 2010, Wilson et al. 2006), variation in winter and spring climatic conditions (Reisen et al. 2008a), availability of surface water and the creation of new or recurring larval sources such as swimming pools. However, prior to the extended control operations initiated during 2008, Kern County annually had among the highest incidence of West Nile illness in California and consistently elevated epidemic risk shown by consistently high mosquito infection rates (Fig. 2). Effective control of swimming pools in 2008 seemed to reduce urban *Cx. quinquefasciatus* populations markedly (as measured by gravid traps [Fig. 1c]), and this seemed key in reducing transmission previously concentrated in the urban/suburban areas of Bakersfield. We feel strongly that enhanced mosquito control decreased early season vector abundance and thereby delayed and limited WNV amplification and spill-over transmission to the human population during 2008.

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We especially thank Y. Fang, M. Dannen, S. Garcia, H. Lu and previous staff of the Center for Vectorborne Diseases for excellent laboratory support. Dead bird reports were provided by the Dead Bird Surveillance Program lead by Stan Husted and staff of the Vectorborne Disease Section, California Department of Public Health. Tissues were collected at necropsy by the California Animal Health of Food Safety Laboratory at U.C. Davis. Reports of human cases were provided by the Kern Count Health Department. Sentinel chicken sera were tested by E. Baylis and staff at the Viral and Rickettsial Diseases Laboratory, California Department of Public Health. This research was funded, in part, by Grant No. RO1 AI55607 from the National Institute of Allergy and Infectious Diseases, NIH; special funds Mosquito Surveillance provided by the Centers for Disease Control and Prevention to the California Department of Public Health; NASA Earth-Sun Science Applied Sciences Program Research Opportunities in Space and Earth Science; Decision Support through Earth-Sun Science Research Results grant; and funds from the Mosquito Research Program allocated annually through the Division of Agriculture and Natural Resources, University of California.

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Are Late Season Mosquitoes More Likely to be Infected with Filarial Nematodes? A Study of Age and Infection in *Aedes sierrensis*

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ABSTRACT: Between March and September of 2009, a total of 1,121 female *Ae. sierrensis* were collected and examined individually for parity. The number of gonotrophic cycles completed by females increased progressively through the season to a maximum of 8 gonotrophic cycles. Size of females decreased seasonally, with smaller females completing more gonotrophic cycles. A total of 689 of the females were also assessed for the presence or absence of filarial nematodes. Fourteen were infective, and the number of nematodes isolated from individual females ranged from 1 to 18. *Aedes sierrensis* females were first collected in April, however all filarial infections occurred between June 1 and August 25. No filarial infections were detected in nulliparous females. The percent of females infected with filarial nematodes increased from 0.7% in 1-parous females to 7.1% in females that had completed four or more gonotrophic cycles. Linear regression analysis showed a significant relationship between reproductive age (i.e., the number of gonotrophic cycles completed) and percent of females infected; however, there was no statistically significant relationship between the size of females and percent infected. Filarial nematodes isolated from *Ae. sierrensis* females could not be positively identified as *Dirofilaria immitis*. The identities of the nematodes have not yet been confirmed, but they are most likely *Setaria yehi*.

INTRODUCTION

In northern California, the western treehole mosquito, *Aedes sierrensis* (Ludlow), is considered to be the primary vector of dog heartworm, *Dirofilaria immitis* (Leidy) (Sacks et al. 2004) and deer body worm, *Setaria yehi* (Rudolfi) (Lee 1971). Female mosquitoes become infected with these parasitic filarial nematodes when they ingest microfilariae in the blood of a definitive host. When ambient temperatures are high enough, the microfilariae undergo two larval molts to third-stage larvae (L_3) and migrate to the head and mouthparts (labium and labellae) of the vector (Fortin et al. 1981). Only female mosquitoes with L_3 stage larvae in the mouthparts are considered to be infective. Relatively few *D. immitis* L_3 stages have been recovered from vector populations in the field (Grieve et al. 1983); however, Scoles et al. (1993) developed a technique for rapidly assessing the infectivity of individual mosquitoes.

Paradoxically, Sacks et al. (2003) demonstrated that the risk of *D. immitis* transmission increased in northern California during the late summer months when *Ae. sierrensis* populations were low and declining. More recently, Mills et al. (2009b) showed that *Ae. sierrensis* females had completed progressively more gonotrophic cycles from April to September, concurrent with a decrease in size during the same interval. Taken together, these studies indicate that smaller, multiparous females present in late-summer play a key role in filarial nematode transmission. During the 2009 season, we investigated the seasonality of filarial nematode infection in *Ae. sierrensis* females collected in Lake and Mendocino Counties and examined the relationships between the age and size of *Ae. sierrensis* females and their potential to vector parasitic filarial nematodes.

MATERIALS & METHODS

Study Areas and Mosqutio Collection. The study was conducted in five oak woodlands known to support populations of *Ae. sierrensis*. Dogs (domestic or coyotes) and deer were also known to be present at each woodland. The sites included an interior-live oak (*Quercus wislizenii* Candolle) and Pacific madrone (*Arbutus menziessii* Pursh) woodland with dense canopy cover near Potter Valley (39°14'05"N, 123°06'24"W, elevation 304 m) in Mendocino County, CA. The Lake County study sites were two black oak (*Q. kelloggii* Newb.) woodlands on Cobb Mountain (38°51'48"N, 122°41'46"W, elevation 870 m and 38°53'32"N, 122°47'14"W, elevation 878 m) with moderately dense canopy cover and two blue oak (*Q. douglasii* Hooker and Arnott) woodlands in Lakeport (39°01'24"N, 122 °55'14"W, elevation 424 m and 39°00'48"N, 122 °55'52"W, elevation 441 m) with open canopy cover.

Host-seeking *Ae. sierrensis* females were collected with CO_2-baited suction traps that were operated for one day per week in each oak woodland from March to October. Females were also collected from the Cobb Mt. and Lakeport sites by vacuuming oak treeholes and large resting boxes (LRB) during the same interval (Mills et al. 2009).

Infectivity and Age Determination. Field-collected *Ae. sierrensis* females were held in the laboratory for ten days to allow larval nematodes to develop to the infective stage. Females were placed in individual cages (8.5 cm h x 8.5 cm diam.), provided with 10% sucrose and held at 21°C, 80% RH and 16 hours light: 8 hours dark. Bloodfed and gravid females were also provided with a small cup of treehole water (30 ml) lined with Teri-wiper® towels as an oviposition substrate. Some bloodfed and gravid females

were held longer than 10 days to allow for oviposition and were maintained for an additional 48 hours to allow the local stretching of the *tunica intima* around the developing ovariole to contract and form a distinct nodular swelling or dilatation (Bertram 1962). Females were anesthetized with CO_2, decapitated and analyzed for the presence of L_3 nematodes using the method of Scoles et al. (1993). Third stage larvae occasionally emerged from the head and thorax immediately after decapitation (Fig. 1). If L_3s

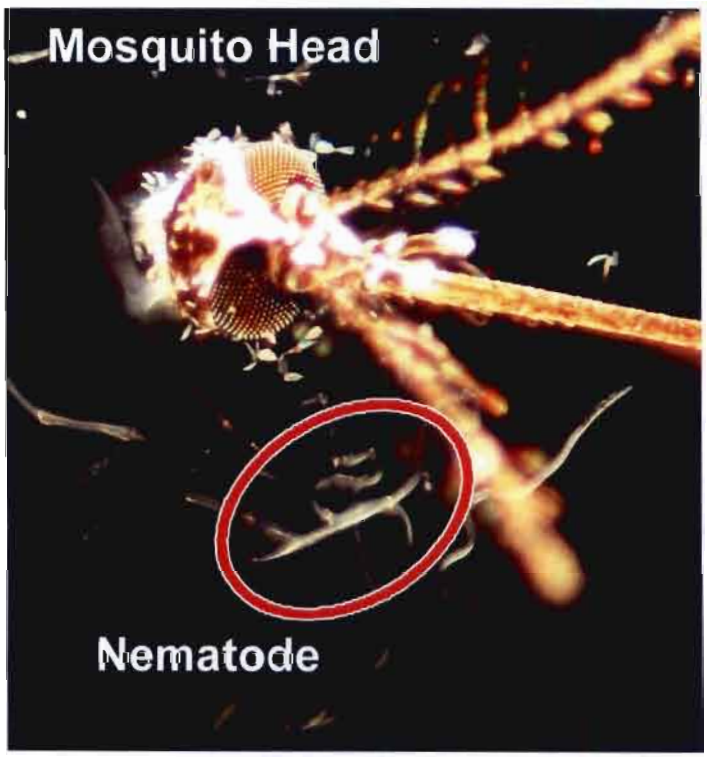

Figure 1. Infective stage filarial nematode larvae that emerged from the head and thorax (not shown) of an *Ae. sierrensis* female (40X).

were not immediately apparent, each mosquito head was placed in 200 µl of phosphate buffered saline on a tissue culture plate and incubated at 37° C for 90 minutes. Each well was examined for L_3s at 40X under a dissecting microscope. All nematodes were saved in 90% ethanol for identification using molecular techniques. Size determinations of female mosquitoes were made by measuring the right wing from the alula to the wingtip. The bodies of decapitated females were placed in individual vials and stored at -70° C for age grading using the tracheole and ovariole examination methods of Detinova (1962) and modified by Mills et al. (2009a).

RESULTS

Nematode Infections. Among 689 *Ae. sierrensis* females assessed for the presence of L_3s, 14 (2.03%) were infective, and a total of 43 filarial nematodes were recovered. A range of 1 to 18 nematodes was recovered from each infected female. Nematodes recovered from females could not be identified as *D. immitis*; initial PCR results on two of the nematodes sampled were inconclusive and retesting is in progress (A. Gerry, personal comm.). It is likely that the remaining nematodes are *S. yehi*, the deer body worm, a species previously isolated from *Ae. sierrensis* in Mendocino County (Lee 1971). Mermithid infections were detected in two *Ae. sierrensis* females; these are likely *Octomyomermis troglodytis*, the only mermithid known to infect *Ae. sierrensis* (Washburn et al. 1986). Two females had protozoan infections that were not identified (Fig. 2).

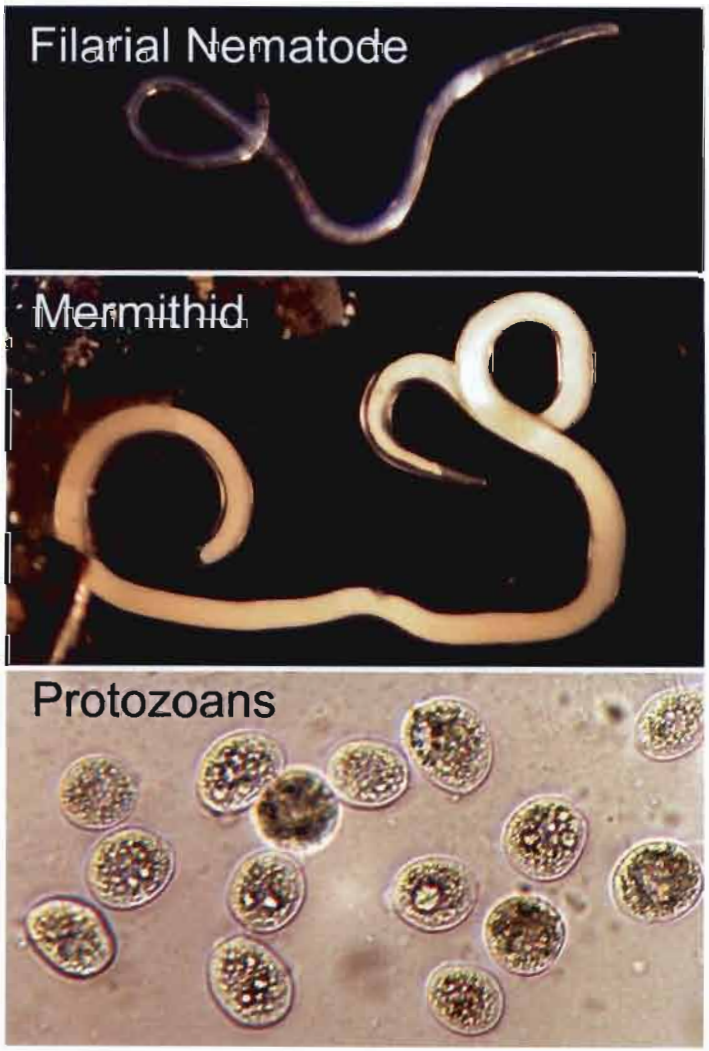

Figure 2. Three different parasites of *Ae. sierrensis* were extracted from females during the 2009 season: filarial nematodes (60X), mermithids (40X), and protozoans (200X).

Relationship between Age, Size & Infectivity. Parity was determined for 1,121 *Ae. sierrensis* females; all mosquitoes were examined using the tracheole method, and 1,039 were also assessed by the ovariole technique. The number of gonotrophic cycles completed by *Ae. sierrensis* females increased as the season progressed with the mean number of cycles completed positively correlated with the month of collection ($r = 0.68$; $P < 0.0001$). Some females completed as many as eight gonotrophic cycles by the end of the season (Fig. 3). Conversely, average wing length, used as a measure of adult size, decreased ($r = -0.95$; $P = 0.0009$) as the season progressed (Fig. 4).

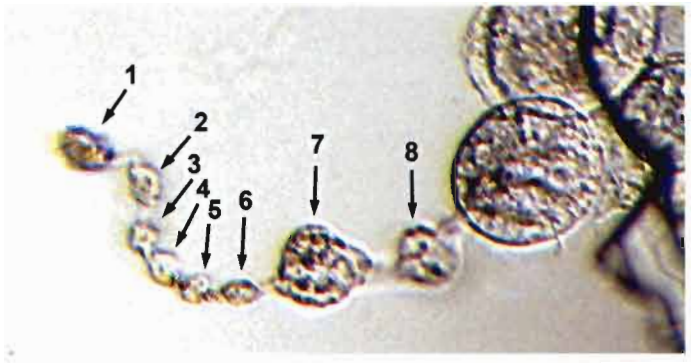

Figure 3. Ovariole from an *Ae. sierrensis* female that completed eight gonotrophic cycles during the 2009 season (100X).

Aedes sierrensis females were first collected in April; however, infective females were only collected in June (n = 5), July (n = 3) and August (n = 3) (Fig. 5). More than 86% (12/14) of the infective females had completed two or more gonotrophic cycles. Figure 6 shows that the percent of females infected with filarial nematodes increased from zero in nulliparous females to 7.1% in females that had completed four or more gonotrophic cycles. The relationship between number of gonotrophic cycles completed and percent infected was significant ($r = 0.94$; $P = 0.015$) (Fig. 6). On the other hand, there was no relationship between the size of females and the percentage that were infected with filarial nematodes ($r = 0.34$, $P > 0.05$) (Fig. 7). Female wing lengths ranged from 1.91 to 4.21 mm; however, most of the filarial infections occurred in the quintiles with wing lengths between 2.54 and 2.85 mm in length.

DISCUSSION

This study evaluated the infectivity rates of *Ae. sierrensis* females throughout the 2009 season to determine what reproductive ages and body sizes, if any, were likely to have acquired a filarial nematode infection. Multiparous females were more likely to harbor filarial nematode infections than those with lower parity. The filarial nematodes collected in this study could not be confirmed as *D. immitis* and re-testing is in progress.

The low numbers of *Ae. sierrensis* collected during the

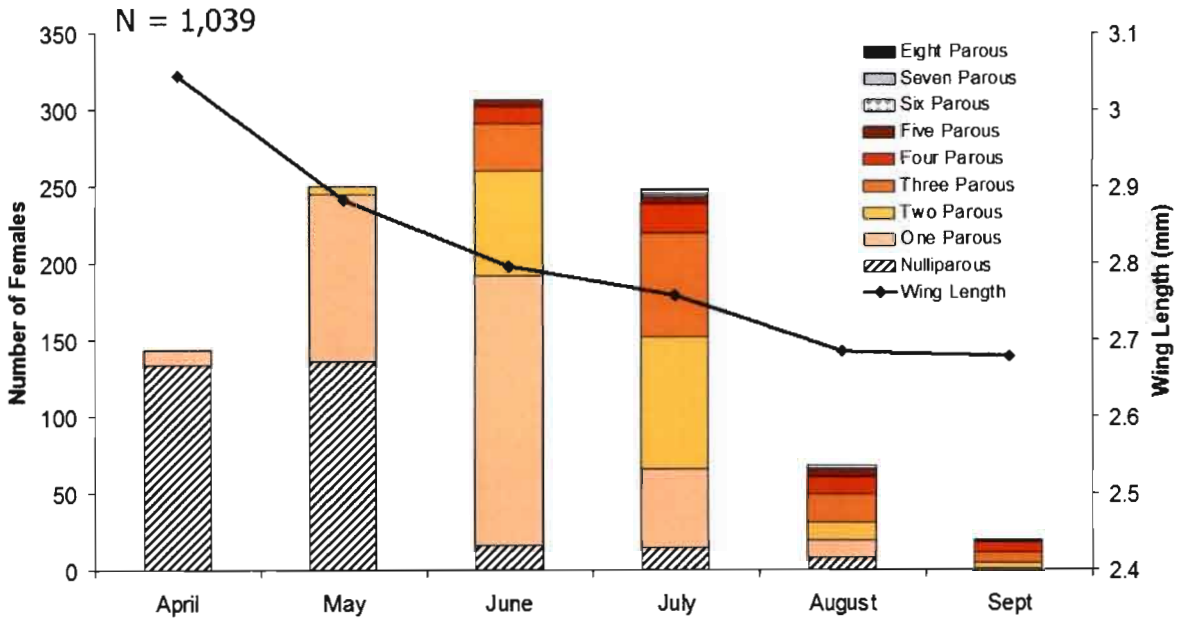

Figure 4. Seasonal parity profile and average wing length (mm) of *Ae. sierrensis* females collected at all study sites and with all collection methods. Wing length was used as a determinant of adult size.

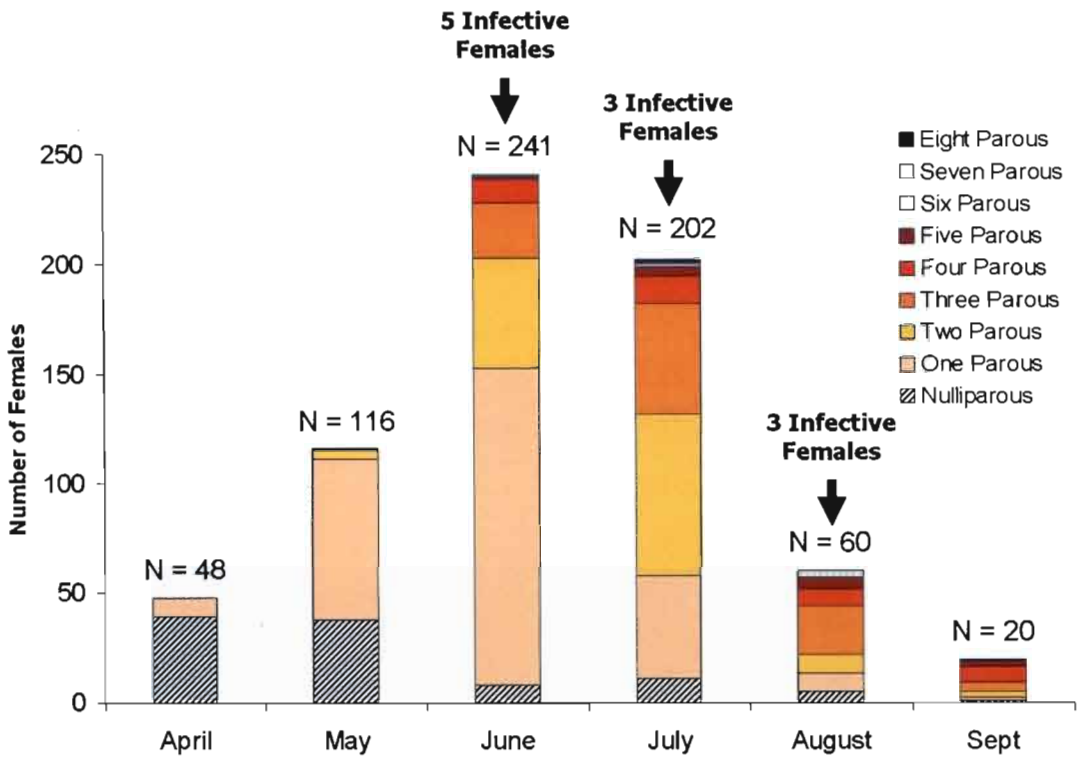

Figure 5. Seasonal parity profile of female *Ae. sierrensis* assessed for filarial nematodes and collected at all study sites. Infective females were only collected in June (n = 5), July (n = 3) and August (n = 3).

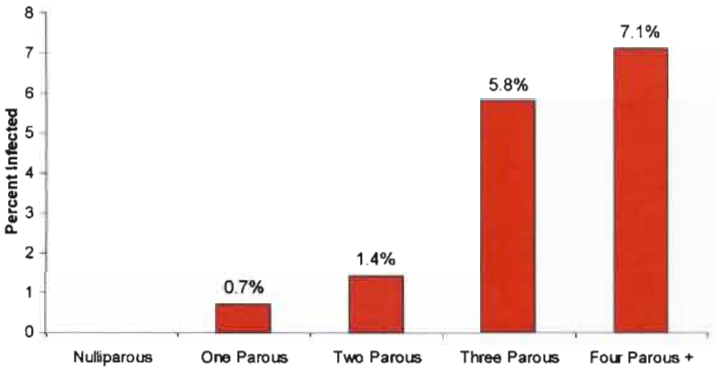

Figure 6. The percent of *Ae. sierrensis* females infected with filarial nematodes is shown for each parous group.

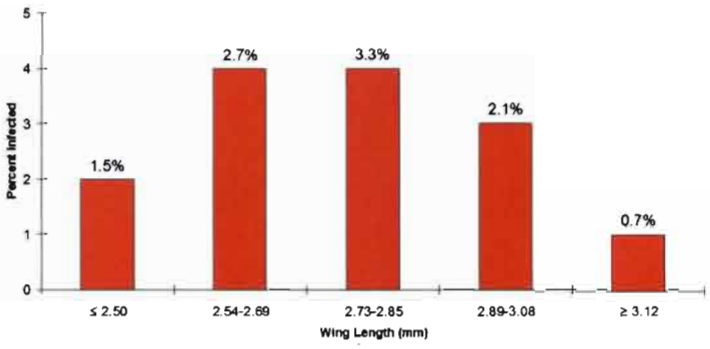

Figure 7. The percent of *Ae. sierrensis* females infected is shown for five size classes based on wing length.

2009 season were most likely due to low rainfall (Woodward, unpub. data). Sacks et al. (2003) also demonstrated that low annual rainfall can lead to a reduction in the incidence (biting rate) and transmission intensity of *D. immitis* in coyotes which are reservoirs for dog heartworm. This suggests that heartworm prevalence in mosquitoes may be affected by weather patterns and particularly rainfall. Future studies conducted over the course of many seasons, in different habitats and under different environmental conditions, should be conducted to characterize better the year-to-year variability in the proportion of the infective population.

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Flea-Borne *Rickettsia* in San Bernardino County, California

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DISCUSSION

San Bernardino County (SBC) is a neighbor to Los Angeles and Orange Counties that are known endemic areas of flea-borne rickettsioses in California. Although no recent human cases have been reported, there is a long history of the diseases in SBC. Human cases of murine typhus were reported in SBC in from the 1920s through the 1940s (one human case in 1924, another in 1943 and two others in 1944). The purpose of this investigation was to determine the prevalence of murine typhus and any other flea-borne rickettsioses. One hundred and three opossums (*Didelphis virginiana)* were trapped at 32 locations of the suburban residential and industrial zones of the south-western part of SBC from March 2007 through April 2008. Of these, 67 *D. virginiana* trapped from March to October 2007 from 27 locations were analyzed further. Each opossum was combed for fleas, and seven opossums were flea-free. A total of 1258 fleas were recovered from 60 opossums, and all were identified as *Ctenocephalides felis*. The number of fleas per animal ranged from 1 to 289 with a median of 14, and an average of 31. All the fleas were shipped frozen to the CDC in Atlanta, GA and were tested for the presence of *Rickettsia typhi* and *R. felis* using a TaqMan assay for citrate synthase gene (*glt*A). Opossums with ≤ 10 fleas were put in group A and those with ≥ 11 fleas were in group B. Twenty-seven opossums with an average of four *C. felis* per animal were placed in group A, and 33 opossums with an average of 35 fleas per animal were placed in group B. Group A fleas were analyzed individually, and group B fleas were tested in pools with two individuals. DNA of *R. felis* was detected in fleas from 45 of 60 opossums (75%) with a prevalence of 26.2%. DNA of *R. typhi* was not detected in any of *C. felis* tested. No evidence for circulation of *R. typhi* in cat fleas removed from opossums was found during this study. The prevalence of *R. felis* is significant, and its role as a cause of human rickettsiosis in SBC needs to be evaluated.

Looking for Ticks in All the Right Places: A Novel Approach for Surveillance of Adult *Ixodes pacificus* Ticks

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ABSTRACT: Knowing the abundance of adult host-seeking ticks is necessary for assessing the risk of exposure to tick bites and subsequent risk of tick-borne disease transmission. Determining abundance is also essential in the appraisal of the efficacy of any control methods applied. We propose a visual examination of vegetation as an alternative to traditional flagging that may offer several advantages. Conditions such as wet vegetation are less likely to adversely affect visual surveillance. In addition, visual methods provide a more comprehensive accounting of ticks present. Non-questing ticks may not be picked up by flagging but would still be visible on the tips, underside and non-trailside facing portions of vegetation. If repeated surveys are intended, visual methods are less likely to disturb, displace or damage ticks. We investigated the use of a visual surveillance method for counting adult ticks on vegetation within a meter of a trail edge. The Null hypothesis investigated in this study was that there would be no significant difference in tick abundance estimates calculated from standard flagging methods compared to visual methods. At each site, two 150 foot transects were established and marked at 10-foot intervals. These transects were along the uphill edge of existing trails. On each survey date each transect was first visually inspected. A visual inspection of each 10-foot section included scanning the vegetation along the transect length and up to one meter in from the trail edge. Subsequently the investigators traded transects and surveyed for ticks using a one-meter square flannel flag. The numbers of ticks observed by visual inspections were compared to those collected by flagging with a paired t-test. Values of $p < 0.05$ were considered statistically significant. Mean (M) and standard deviation (SD) were calculated for both the flagging and visual methods. Estimates of ticks within a 150 foot area by the visual method ($M = 38.5$, $SD = 26.44$) were significantly greater than the number of ticks captured by flagging ($M = 29.56$, $SD= 20.29$, $p= 0.0026$). The time period to flag or visually check each transect was recorded. The total time for visual inspections for the 16 surveys was 496 minutes ($M = 31$) while total flagging time was 317 minutes ($M = 19.81$). The paired times for each observation/flagging event were also evaluated with a paired t-test ($t= 4.5290$, two-tail $p = 0.0004$). This test revealed that the time to inspect for the presence of ticks visually took significantly longer than to flag for their presence.

In summary the visual method took significantly longer to implement than the flagging method. The flagging method is more efficient and cost-effective; however the visual method may better approximate the true number of ticks in a given area. In addition, if a site is too wet to flag, the visual method can be a viable option to having to reschedule the survey. We found that surveying for ticks visually provided a consistent and comparable alternative to standard flagging techniques and should be considered when designing studies to assess tick populations.

Host Ecology of *Ixodes pacificus* at the Quail Ridge Preserve, Napa County, California

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ABSTRACT: A variety of bird species act as hosts and transporters of ticks and function as reservoirs for Lyme borreliosis in eastern North America (Richter et al. 2000, Durden et al. 2001, Ginsberg et al. 2005). The role of birds in western North America with *Ixodes pacificus* has yet to be fully described (Eisen et al. 2004). Widespread investigations in northern California have demonstrated that birds are important hosts for subadult stages of *I. pacificus* and that some species harbor *Borrelia burgdorferi* sensu stricto (Wright et al. 2000, 2006; Eisen et al. 2004). As yet, there has been no demonstration that birds can transmit *B. burgdorferi* spirochetes to feeding *I. pacificus* larvae.

The goals of our investigation on the Quail Ridge preserve were to identify reptile, mammal and bird hosts of the larvae and nymphs of *I. pacificus*; to observe the seasonality of host infestation by larvae and nymphs; to compare the relative infestation of simultaneously sampled lizard, rodent and bird hosts; and to identify potential avian reservoirs and search for spirochetes in blood and larvae from these species.

Questing adults and nymphal *I. pacificus* were collected from canyon trails via flagging. Lizards were noosed or hand caught, aged, sexed and measured, and feeding nymphal and larval *I. pacificus* were numerated and collected. Mammals were captured with Sherman and National live traps then tagged, aged, sexed, measured and bled; all feeding nymphal and larval *I. pacificus* were numerated and collected. Birds were captured with mist nets, banded (provided by the USGS Bird Banding Laboratory), aged, sexed, measured, bled and all feeding nymphal and larval *I. pacificus* were numerated and collected.

The infection at Quail Ridge of adult ticks of *I. pacificus* with *B. burgdorferi* in 2008 was 1.3% of 150 *I. pacificus*, and in 2009 it was 0.8% of 130 *I. pacificus*. Abundance of *I. pacificus* based on flagging was 40 ticks/20 minutes of flagging or approximately 2 ticks/minute sampling. One other species of trail questing tick was encountered, the Pacific Coast tick, *Dermacentor occidentalis*.

Subadult stages of *Ixodes pacificus* were collected from 3 species of reptiles, 3 species of rodents and 12 species of birds. More than half of the collected *I. pacificus* nymphs came from spirochete refractive lizards, leaving less than half from birds and less than 0.1% from rodents. Over 70% of all subadults came from lizards. Birds that were largely infested with larvae came from ground foraging specialists, whereas nymphs infested birds of tree foraging specialists. *Ixodes pacificus* larvae (N = 152) that fed from a variety of birds were tested for the presence of *B. burgdorferi* sensu stricto via RT-PCR. *Borrelia burgdorferi* spirochetes were identified from three larvae of *I. pacificus* that fed on two Dark-eyed Juncos, *Junco hyemalis*. For the first time, we report larvae of *I. pacificus* feeding on birds infected with *Borrelia burgdorferi* sensu stricto. The significance of this finding in the maintenance of the cycle of Lyme borreliosis is still under investigation. Migrant birds at Quail Ridge have infestations of sub-adult *I. pacificus* making them available as transporters of these ticks, and perhaps *B. burgdorferi* spirochetes, to other supportive habitats in the far west.

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Utilizing Real-Time PCR to Detect *Borrelia burgdorferi* Infection in *Ixodes pacificus*

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ABSTRACT: Our goal was to develop a protocol using Real-Time Polymerase Chain Reaction (RT-PCR) to test field-collected *Ixodes pacificus* ticks for *Borrelia burgdorferi*, the Lyme disease bacterium, by conducting a Minimum Infection Rate (MIR) study. An acceptable protocol should yield an MIR comparable to those previously found in northern California. We used the flannel flag method to collect 1,806 adult *Ixodes pacificus* from five northern California state parks over the 2008/2009 winter season. Ticks were sorted by sex and collection location, placed in pools of five, and stored at - 80° C until tested. DNA was extracted on the Applied Biosystems 6100 Nucleic Acid PrepStation using the NucPrep® DNA Chemistry for Tissues Protocol. A positive control of heat-killed *Borrelia burgdorferi* cells was used. RT-PCR was performed on the Applied Biosystems 7500 Real-Time PCR System, using TaqMan® probe-based detection. Pools with Ct values of 40 or below were considered to be positive. Of 357 pools tested, 26 were positive for *Borrelia burgdorferi*. Assuming one positive tick per positive pool, and with a total of 1,806 individual ticks tested, the MIR in this study was 1.44%. The RT-PCR protocol developed in this study is effective in testing *Ixodes pacificus* for infection with *Borrelia burgdorferi* because the MIR in this study is comparable to infection rates found in previous studies conducted in northern California.

INTRODUCTION

Marin/Sonoma Mosquito and Vector Control District (MVCD) encompasses all of Marin and Sonoma Counties, an area of 2,100 square miles with 715,000 residents. The District's topography includes coastal regions, vast wetlands, several mountain ranges, extensive grasslands and abundant areas of oak woodlands.

There are several state parks within Marin and Sonoma Counties that offer the public varied wilderness experiences, including camping, hiking, picnicking and mountain biking. Activities in these rustic locations carry a significant risk for exposure to Ixodes pacificus, also known as the western black-legged tick, the vector of *Borrelia burgdorferi* on the West Coast.

The Marin/Sonoma MVCD conducts testing of ticks for *B. burgdorferi* and sends a yearly report of the results to the California Department of Parks and Recreation. In the past, the District used the Indirect Fluorescent Antibody (IFA) technique for tick testing. In 2006 the District purchased a Real-Time PCR system for testing mosquito pools for West Nile virus. In order to maximize utility of the PCR system, this protocol was designed for testing ticks for *B. burgdorferi*.

MATERIALS AND METHODS

Tick Collection. We collected 1,806 adult *Ixodes pacificus* by the flannel flagging method from five northern California state parks over the 2008/2009 winter season. Ticks were sorted by sex and collection location, placed in pools of five and stored at -80°C until tested.

DNA Extraction. DNA was extracted on the Applied Biosystems 6100 Nucleic Acid PrepStation, using the NucPrep® DNA Chemistry for Tissues Protocol (Applied Biosystems, Foster City, CA). A positive control of heat-killed *Borrelia burgdorferi* cells was used (KPL, Gaithersburg, MD). Cells were rehydrated with 1 mL reagent quality water and diluted to 1:25 using 1X TE buffer.

Real-Time PCR. A 500 base-pair sequence from the flagellin gene of *B. burgdorferi* (Pahl et al. 1999) was submitted to Applied Biosystems Custom Assay Services, where a set of primers and a probe were designed (Table 1).

Table 1. Custom *B. burgdorferi* primers and probe designed by Applied Biosystems Custom Assay Services based on GenBank accession number X15660.

Forward Primer:
5' CAAACCAAGATGAAGCTATTGCTGTA 3'
Reverse Primer:
5' CTTCCTGTTGAACACCCTCTTGAA 3'
MGB Probe:
5' FAM-CAGCCTGAGCAGTTTGA-MGB 3'

The TaqMan® probe was labeled at the 5' end with a fluorescent reporter dye (FAM) and with a non-fluorescent quencher dye at the 3' end (MGB). RT-PCR was performed on the Applied Biosystems 7500 Real-Time PCR System, using TaqMan® probe-based detection (Applied Biosystems). The PCR reactions were performed in 25 µL volumes containing 2X TaqMan® Universal PCR Master Mix (Applied Biosystems), 900 nM forward primer, 900 nM reverse primer, and 250 nM probe. Five micro liters (5µL) of extracted DNA were added and the following cycling conditions applied: one cycle at 95° C for ten minutes, 45 cycles

at 95° C for 15 seconds and 60° C for one minute. Previously extracted *B. burgdorferi* DNA was used as a positive control, and a no-template control was prepared using sterile, nuclease-free water. Pools with Ct values of 40 or below were considered to be positive.

RESULTS

Of 357 pools tested, 26 were positive for *Borrelia burgdorferi*. Assuming one positive tick per positive pool, and with a total of 1,806 individual ticks tested, the MIR in this study was 1.44%.

DISCUSSION

The RT-PCR protocol developed in our study is effective in testing *Ixodes pacificus* for infection with *Borrelia burgdorferi* because the MIR we determined is comparable to infection rates found in previous studies conducted in northern California (Bissett and Hill 1987, Burgdorfer et al. 1985, Holden et al. 2006).

Future goals include using the District's recently purchased MagMax™ Express magnetic particle processor (Applied Biosystems) for DNA extraction in the tick testing protocol, developing a confirmation assay and expanding the testing program to include other tick-borne pathogens.

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The Vertebrate Pest Control Research Advisory Committee – 10 Reasons Why You Should Be Interested

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ABSTRACT: The vertebrate pest research program, administered by the California Department of Food and Agriculture, was established to maintain the state's current vertebrate pest control product registrations and to investigate effective alternative control methods. The Vertebrate Pest Control Research Advisory Committee (VPCRAC) establishes research priorities to promote the objectives of the control program. While the primary focus of VPCRAC is to fund research on agricultural pests, many of VPCRAC's activities and issues are of potential interest to vector control professionals. Examples of these include developing alternative vertebrate control materials and methods, providing vertebrate control resources and information, monitoring regulatory changes, providing continuing education and training opportunities and addressing the potential public health impacts of vertebrate pests.

Each year ground squirrels, rats, birds and other animals cause millions of dollars of damage to agriculture and other sites in California. In response to this adverse impact, the California Legislature established a vertebrate pest control research program. While this program deals mainly with agricultural pests and related issues, the Vertebrate Pest Control Research Advisory Committee (VPCRAC) should be of interest to the vector control community for several reasons.

1. Research Funding. The vertebrate control research program is a pesticide user-based program; it is funded by the purchase of rodenticide products that are registered by the California Department of Food and Agriculture (CDFA). The program was established by legislation passed in 1990 which created Sections 6025-6029 of the state Food and Agricultural (F&A) Code. The Rodent Surcharge Program (AB 2776) funded vertebrate control research by placing a surcharge of 50 cents per pound on vertebrate pest control material sold, distributed or applied by counties. Currently, this surcharge is applied to sales of six CDFA-registered rodenticides. The assessment may be raised to $1 per pound, but has not changed since the inception of the program. Recent annual revenues from the surcharge program have averaged $500,000.

2. Roles and Responsibilities. The specific purposes of the research program include: 1) the investigation of effective and economical alternative materials for the control of vertebrate pests; 2) the solicitation and consideration of research proposals for alternative humane methods of control; 3) the continuation of current vertebrate pest control product registration at the state level until alternative products are developed that prove to be effective and economical; 4) the funding of research for the development of scientific data to fulfill registration requirements; and 5) cooperation with the United States Department of Agriculture in funding research programs to maintain, develop and register vertebrate pest control materials used in this state. Section 6026 of the F&A Code established The Vertebrate Pest Control Research Advisory Committee (VPCRAC) to recommend priorities for conducting and funding various vertebrate pest control research projects. The Committee consists of 11 members, one each from CDFA, California Agricultural Commissioners and Sealers Association, University of California, California State University, California Department of Public Health, the general public and five members of agricultural industry representing affected commodities.

3. An Information Source for Vertebrate Control Research. VPCRAC-funded research projects have examined a wide variety of vertebrate pest issues and control techniques. Examples of the diversity of research topics include anticoagulant field efficacy studies, ground squirrel underground baiting, evaluation and control of wild turkey damage and field tests of a bait delivery device for coyote management. In addition to research on specific control methods, various other funded projects have addressed related needs such as assessing environmental impacts from agricultural anticoagulant uses, determining economic impacts of damage to commodity-producing counties and the development of a vertebrate pest control digital library. Summaries for all funded research projects are available via the VPCRAC website: http://www.vpcrac.org/research/

4. The Vertebrate Control Handbook. Another valuable information resource available at the VPCRAC website is the Vertebrate Control Handbook (http://www.vpcrac.org/about/handbook.php). This on-line guide provides detailed information on vertebrate pest biology, control products and techniques, laws and regulations and other issues related to vertebrate pest control in California.

5. Continuing Education Credits. VPCRAC meets semi-annually (typically April and October) to discuss current vertebrate control issues, review currently funded research projects and consider new research proposals. Continuing education credits are available for CDPR pesticide applicators and may be available, upon request, for CDPH Category D (vertebrates).

6. New Approaches for Certification and Training. Among the innovative projects recently funded by VPCRAC have been several in which vertebrate pest control information and training is provided via computer-based kiosks located in county agricultural commissioner offices or at home and garden retail outlets. Long-term plans include exploring the use of these kiosks or internet-based delivery systems for certification/licensing and continuing education for pesticide applicators. For summaries of these projects, see the Research Section of the VPCRAC website and enter key words: kiosk or computer.

7. Public Health Implications of Agricultural Pests. Recent food-borne disease outbreaks have focused public health concerns on the impact of vertebrate pests in food crops. New food safety standards have proposed mitigation strategies that may increase the need for vertebrate control in and around agricultural fields and may increase pesticide use. In response, VPCRAC has funded research to document the extent of vertebrate pest intrusion into agricultural fields and to evaluate the appropriateness of the rodent control recommendations.

8. Rodenticide Label Changes. VPCRAC has been actively monitoring the status of the USEPA's Final Risk Mitigation Decision (RMD) for Ten Rodenticides, and the RMD's potential impact on agricultural uses, particularly CDFA-registered products. The new requirements have an implementation deadline of April 2011. Significant changes include restricting consumer use of 1st generation anticoagulant products and toxicants to pre-loaded bait stations. Second generation rodenticide products will no longer be marketed to consumers and will only be available for professional use. First generation anticoagulant field uses will be restricted use, meaning applicator certification will be required for most agricultural applications. Second generation products for use above ground, around agricultural buildings, will be limited to bait stations.

9. Future Regulatory Changes. The implementation of USEPA's RMD may not be the final word for rodenticide use regulation changes in California. The California Department of Pesticide Regulation (CDPR) is also reviewing rodenticide use and has the authority to institute additional restrictions in the state. Past and future projects funded by VPCRAC may have bearing on the need for or degree of additional use restrictions. Because agricultural uses of first generation anticoagulants will soon require private applicator certification, VPCRAC is currently advocating for a rodenticide-specific CDPR private applicator exam/certification.

10. Potential Source of Research Funding. The stated purpose of the research program is to control vertebrate pests that pose a significant threat to the welfare of the state's agricultural economy, infrastructure and the public. While VPCRAC typically prioritizes the funding of projects directly related to agricultural damage, research proposals that address effective and economical alternative control materials or alternative humane methods of control are appropriate for consideration. See the VPRCRAC website for additional application information and submission requirements.

Through VPCRAC and the rodenticide surcharge program, California has developed a well-funded and effective vertebrate control research program that has helped to maintain the use of a variety of control materials and develop alternative methods for vertebrate pest control.

Guidelines for Contributors

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Proceedings and Papers, also referred to as *Proceedings*, is the official, professional publication of the Mosquito and Vector Control Association of California. The *Proceedings* is published once each year and includes papers based on presentations, including young investigators award competition, given at the Association's annual conference. Publication of submitted papers is also encouraged. The *Proceedings* publishes articles on the biology, ecology and control of mosquito and other vectors of disease.

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