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Editor: Jan O. Washburn, Ph.D.

Reviewer:
Jan O. Washburn, Ph.D.

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

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

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Symposium: West Nile Virus - Collaborative Research Between MVCAC and UC Davis

Surveillance

Operations

University of California - California Department of Public Health - Mosquito and Vector Control Association of California: Partners in Arbovirus Surveillance

William K. Reisen

Center for Vectorborne Diseases (CVEC), Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California Davis, CA 95616, wkreisen@ucdavis.edu

The University of California (UC), the California Department of Public Health (CDPH) and the Mosquito and Vector Control Association of California (MVCAC) have collaborated in the surveillance and control of arboviruses since their discovery in the 1930s. This partnership has been compared to a 3-legged stool, a unique but simple piece of furniture that is very strong and stable, providing that each of the three legs remains intact and firmly attached, and adhere to the overall mission of protecting the health and well-being of the citizens of California that fiscally support these programs.

UC contributes through training, research and service. At UC Davis, the Center for Vectorborne Diseases (CVEC) has developed a strong graduate training program that can lead to a PhD degree with a designated emphasis in Vectorborne Diseases from a variety of graduate groups including Entomology, Epidemiology and Comparative Pathology. Support for two Post Doctoral Scholars and four PhD students are available through an on-going T32 training grant in Vector Biology from the National Institutes of Health (NIH). Research relating to arboviruses has involved a close partnership with MVCAC member agencies and CDPH, and has focused recently on West Nile virus (WNV) ecology, epidemiology and pathogenesis. CVEC service has included operational research and development on data management, decision support systems, predictive models and improved diagnostics. Some of the products in use include the CalSurv Surveillance Gateway, high throughput RT-PCR diagnostics, sentinel chicken bleeding and testing methods, and new assays for testing wild bird blood for arbovirus antibodies.

CDPH coordinates programs at the state level and provides oversight, quality control, certification and training, and outbreak response planning. Decision support and response recommendations have been summarized in the California Mosquito-Borne Virus Surveillance and Response Plan (Kramer 2009), prepared with input from all three 'legs of the stool'. The WNV response team at the CDPH is led by the Vector-borne Disease section with support from the Veterinary Public Health section, Infectious Diseases Branch, Viral and Rickettsial Disease Laboratory and the Communicable Disease Emergency Response Branch.

The MVCAC member districts constitute the heart of the program and do surveillance, control, public education and emergency response by implementing surveillance and control activities recommended in the Plan. Their overall mission of integrated vector management provides the first line of defense against vectorborne pathogens and includes problem detection, effective mitigation and evaluation using scientific methods.

History of collaborative problem solving. The history of the three-legged partnership addressing California mosquito problems dates back to the early 1900s when Dr. HJ Quayle of UC Berkeley collaborated with several newly formed mosquito control districts to control salt marsh *Aedes* near San Francisco. This work summarized in a pamphlet entitled "Mosquito Control" was published in Bulletin No. 178 by the University of California and demonstrated the positive impact of locally-supported mosquito control on property values and the well-being of the residents of California. Shortly afterward, Drs. WB Herms and SB Freeborn at UC Berkeley with HF Gray and several newly formed mosquito control districts collaborated to address the then extensive malaria problem in the Central Valley (Gray 1912, Gray and Fontaine 1957). Malaria was essentially eliminated as a major health problem by the early 1930s (Gray 1956), although there have been subsequent minor outbreaks (Brunetti et al. 1954, Maldonado et al. 1990) and thousands of imported cases during WWII and the Korean and Viet Nam conflicts.

During the late 1920s, extensive summer epidemics of encephalitis in equines almost crippled California agriculture.

Dr. KF Meyer with the Hooper Foundation at the University of California identified the causative agent as a new virus, western equine encephalomyelitis virus (WEEV), which he isolated from the brain of a dead horse (Meyer et al. 1931). Shortly afterward a vaccine was developed and used to protect equines, thereby eliminating these devastating summer equine epizootics. In the late 1930s, WEEV was discovered as one of the viruses causing summer polioencephalomyelitis disease frequently seen in children in the San Joaquin Valley (Howitt 1938). Almost concurrently a second virus was discovered in St Louis, Missouri as the cause of similar summer neurological disease during an large outbreak with more than 1,000 cases (Muckenfuss et al. 1934). Both viruses subsequently were found to be a common cause of disease in humans in Kern County (Howitt 1939). Development of a neutralizing antibody assay enabled serological surveys that demonstrated extensive inapparent human infection in the Central Valley (Howitt 1942). The complex epidemiology of these viruses was unraveled by Drs WM Hammon and WC Reeves during their investigation of an encephalitis outbreak in the Yakima Valley of Washington where they discovered that *Culex* mosquitoes were the vectors and wild birds the reservoir hosts (Hammon et al. 1941a, Hammon et al. 1941b, Hammon et al. 1945, Hammon and Reeves 1943a, Hammon and Reeves 1943b). These discoveries and subsequent studies in Kern County at the Bakersfield Field Station (Reeves and Hammon 1962) changed the focus of mosquito control in California from nuisance *Aedes* and the no longer important malaria vector, *Anopheles*, to *Culex* and especially *Cx. tarsalis*. Working in close partnership with the Kern Mosquito Abatement District, WC Reeves developed many of the surveillance and research tools used today, including the use of chickens as sentinels to monitor transmission, bottled CO_2 gas and then dry ice to collect host-seeking mosquitoes (Reeves 1953), methods to incriminate vectors using laboratory transmission experiments (Hammon and Reeves 1943b), fluorescent dust to mark mosquitoes for release-recapture experiments (Reeves et al. 1948), serological methods to identify mosquito blood-meal hosts (Reeves and Hammon 1944) and the importance of monitoring environmental conditions, mosquito abundance and infection to anticipate outbreaks (Reeves and Hammon 1962).

During the summer of 1952, heavy winter rains and extensive snow pack in the Sierra Nevada led to extensive flooding of the Bakersfield area by the Kern River, resulting in above normal early season abundance of *Cx. tarsalis* mosquitoes and the enzootic amplification of WEEV (Reeves and Hammon 1962). In July, this worsening situation was compounded by a 7.3 magnitude earthquake centered near Tehachapi that caused extensive damage in Bakersfield, forcing many people to sleep outdoors, thereby increasing their exposure to mosquitoes. By the end of the year, 348 laboratory-confirmed human cases of WEEV were documented (Longshore et al. 1956), many of which were children who suffered long term sequellae (Kokernot et al. 1953, Longshore et al. 1960). An additional 48 cases of SLEV occurred, mostly among older individuals. This outbreak and sequent research established the need for an early warning

Table 1. Timeline for key events in the history of the California encephalitis surveillance program.

1943	California Mosquito and Vector Control Association (CMVCA) adopts the NJLT as standard sampling device
1945	DDT use expanded to agriculture and public health
1945	California Department of Health Services (CDHS) tests human and horse samples for WEEV and SLEV
1947	Bureau of Vector Control formed
1952	**WEEV epidemic in San Joaquin Valley**
1953	**California Encephalitis Surveillance Program initiated**
1962	Reeves and Hammon' monograph and Silent Sprint published
1969	CDHS offers testing of mosquito pools for WEEV and SLEV using suckling mice
1969	CDHS/CDPH begins annual reporting of surveillance results at annual CMVCA/MVCAC conference
1972	**UC Mosquito Research Program created with funds from California Senate**
1972	Abundance – Adult Mosquito Occurrence Report issued statewide
1962	CDC and EVS traps used for collecting mosquitoes for virus testing
1988	Gravid female traps used to collect urban *Cx. pipiens* complex
1987	Transition of testing from suckling mice to *in situ* EIA shortening turn-around-time
1995	UC Berkeley Arbovirus Research Unit transferred to UC Davis to become the Center for Vectorborne Diseases
1998	UC Davis begins testing mosquito pools using in situ EIA
1999	WNV detected in New York City
2000	Mosquito pool testing switched to RT-PCR and dead bird program initiated
2001	California Mosquito-Borne Virus Surveillance and Response Plan published
2002	West Nile virus website launched
2003	**West Nile virus invades California**
2006	Surveillance Gateway launched with 'same week' service for testing mosquito pools and dead birds available
2008	Mosquito Research Program terminated
2008	Sentinel chicken testing transferred from Viral and Rickettsial Diseases Branch to the Vector-Borne Disease Section
2011	NPDES permitting required

system to track mosquito abundance and arbovirus amplification to initiate intervention in a timely and effective manner.

Key events in the history of arbovirus surveillance. For a statewide surveillance program to be effective, uniform sampling and testing methodology must be adopted by participating agencies and the resulting data shared in a timely manner to serve as a useful decision support tool for effective vector control. Surveillance data are useful not only for detecting and determining the geographical extent of arising problems, but also for evaluating the effectiveness of intervention. The importance of using consistent methods among agencies was recognized early on when the CMVCA adopted the NJ light trap (Mulhern 1942) as the statewide metric for mosquito abundance.

Table 1 summarizes some of the milestones in the history of surveillance for mosquitoes and arboviruses within California critical to formulating the current program. Tracking the mosquitoborne encephalitis viruses at the state level began in 1945 when CDHS started testing human and horse samples for evidence of WEEV and SLEV infection. After the 1952 outbreak, the California Encephalitis Surveillance Program was initiated in four representative areas of the state (Longshore 1960). In 1969 CDHS launched a statewide testing service for mosquito pools using a suckling mouse system, which was the first step in monitoring enzootic virus transmission for decision support. Results of enzootic and epidemic monitoring by the Viral and Rickettsial Diseases Laboratory were first reported in 1972 (Emmons et al. 1973) at the Annual Conference of the CMVCA/ MVCAC and then at every meeting thereafter, documenting the history of arbovirus activity in California. Turn-around-time for testing was shortened dramatically when the use of suckling mice for virus isolation was replaced by the *in situ* enzyme linked immunoassay (EIA) developed at UC Berkeley (Graham et al. 1986); however, this limited surveillance to those viruses detected by California group, *Flavivirus* and *Alphavirus* antisera. In 1998, due to budget cuts and staffing changes at the CDHS, testing of mosquito pools was transferred from the CDHS to CVEC at UC Davis and for the first time a fee was charged to cover testing expenses. During this period an effort was made to enhance data management to reduce redundant entry for laboratory testing, data reporting and visualization (Eldridge et al. 1998).

In 1999, WNV was detected in New York City (Lanciotti et al. 1999). The ensuing epidemic has been the largest mosquitoborne encephalitis epidemic ever documented in North America and California and the largest WNV outbreak documented globally (Kramer et al. 2008), with more than 30,000 laboratory confirmed cases and probably more than 1.8 million Americans infected. As California watched the rapid march of WNV across the United States, preparation was made to enhance surveillance. In 2000, testing for virus infections in mosquitoes was switched from the *in situ* EIA to a new molecular approach using a real-time multiplex RT-PCR that would detect WEEV, SLEV and WNV in a single assay (Chiles et al. 2004). A dead bird reporting and laboratory testing program was launched in 2002 (McCaughey et al. 2003). WNV invaded the SE desert biome of California in July 2003 and rapidly dispersed into the Los Angeles Basin and to San Diego (Reisen et al. 2004). By the fall of 2004, WNV was detected in every county in California (Hom et al. 2005), and a large outbreak was documented in Los Angeles (Kwan et al. 2010). In 2006, UC Davis launched the CalSurv Surveillance Gateway to manage the large expanding quantity of surveillance data and to accelerate reporting, analysis and visualization of these data (Eldridge 2005, Park et al. 2008). In combination with rapid molecular diagnostics, accelerated reporting improved laboratory turn-around-time from weeks to days (Dannen et al. 2007; Kahl et al. 2005), thereby enabling an almost real-time monitoring of virus activity for mosquito control decision report. Similar improvements for sentinel chicken sera reporting times followed after the Vectorborne Disease Section, CDPH, acquired the sera testing service and new laboratory procedures were adopted (Patiris et al. 2008). Widespread epidemic transmission in 2005 resulted in aerial applications over Sacramento, the first time emergency intervention was used over large urban areas of California to interrupt arbovirus transmission (Carney et al. 2008).

In summary, the tripartite collaboration of academia, public health and operations has produced a world-class surveillance program that has benefited the health and well being of the residents of California through improved and directed mosquito control. The collaboration has benefited UC by enabling operational research programs and providing collaboration and data for epidemiological and modeling studies; CDPH by facilitating management, visualization and program over sight; and the MVCAC membership by providing a science-based decision support system useful in defining risk, targeting intervention and educating the public.

Mosquito research program. An important 'glue' holding the three-legged stool together was the University-wide Mosquito Research Program (MRP) that was founded in 1972-3 with funds allocated by California Senate Bill 310 (Eldridge 2004). The MRP mission was to coordinate University mosquito research in California with the MVCAC and CDPH, with oversight and guidance provided by several committees consisting of individuals from UC, MVCAC and CDPH, each of the three legs of the stool. An important function of the MRP was the awarding of small grants to UC investigators who used these funds to support students and generate preliminary data that were frequently used to obtain larger extramural grants from federal agencies such as NIH. Committee oversight ensured that these funds were directed at California problems. In 2008 the MRP was terminated and its funding support assimilated into the Division of Agriculture and Natural Resources (DANR), first supposedly to enhance administrative efficiency and then to be absorbed within other DANR programs. Since this assimilation, there have been no state funds available for mosquito research, and concurrently there has been a precipitous decrease in interest in California problems by the UC research community, thereby weakening the UC leg of the stool. This deteriorating interest may be exemplified by the 2011 Annual Conference where only a few faculty from CVEC

and one from UC Riverside participated, and only a single PhD student competed for the WC Reeves New Investigator Award. The importance of this loss of UC participation was emphasized in a presentation to the Trustees Business Session (Eldridge 2011) in hopes of raising awareness and developing solutions. During the current period of budgetary crisis at the local, state and federal level, there are limited or no UC resources to address local mosquito operational problems. Therefore, replacing the MRP small granting program with the newly formed Mosquito Research Foundation may be essential to once again attract UC faculty and extend mosquito research to include issues important for the MVCAC.

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Host Antibodies Protect Mosquito Vectors from West Nile Virus Infection

Sarah S. Wheeler and William K. Reisen

Center for Vectorborne Diseases, School of Veterinary Medicine, University of California, One Shields Ave, Davis, CA 95616, sswheeler@ucdavis.edu

ABSTRACT: Our recent data indicate that birds persistently infected with West Nile Virus (WNV) can shed virus despite the presence of neutralizing antibody. The goal of the current work was to test the hypothesis that host neutralizing antibodies protect mosquito vectors from WNV infection. *Culex tarsalis* Coquillett and *Culex stigmatosoma* Dyar were divided into two groups: a control group fed a bloodmeal containing chicken blood, stock WNV and chicken sera that was negative for WNV antibodies and a treatment group fed a bloodmeal containing chicken blood, stock WNV [approximately 7.5 $\log_{10}$ plaque forming units (PFU) of WNV/mL] and Western scrub-jay sera containing WNV antibodies. The viral particles in the *Cx. tarsalis* treatment group bloodmeal were not completely bound by antibody, and the infectious viral titer was estimated to be 2.3 $\log_{10}$ PFU/mL. The *Cx. tarsalis* control group (n = 81) was 90% positive for WNV RNA and 86% positive for infectious virus, whereas the treatment group (n = 81) was 4.9% positive for WNV RNA and 1.2% positive for infectious virus. *Culex stigmatosoma* mosquitoes were fed a bloodmeal containing approximately 7.1 $\log_{10}$ PFU/mL, and here the treatment group bloodmeal was completely bound by antibody. Control group (n = 76) *Cx. stigmatosoma* were 62% positive for infectious WNV and 72% positive for WNV RNA, whereas mosquitoes in the treatment group were negative for both WNV RNA and infectious virus. These data show that host antibodies were protective to mosquito vectors and were not dissociated during the digestion process.

INTRODUCTION

Arboviruses such as West Nile Virus (Flaviviridae: *Flavivirus)* are maintained in a horizontal transmission cycle involving *Culex* vectors and avian hosts (Turell et al. 2002; Komar et al. 2003; Reisen et al. 2005). The mechanism(s) enabling WNV to overwinter where temperate winters drive mosquitoes into inactivity is (are) still not completely understood, but currently there have been examples in the laboratory and field of both vertical transmission in mosquitoes (Reisen et al. 2006; Anderson et al. 2008) and persistent infections in experimentally infected birds (Reisen et al. 2006; Nemeth et al. 2009).

It is interesting that WNV persistent infections in the avian host occur concurrently with high WNV-specific antibody titers (reviewed in Kuno 2001; Nemeth et al. 2009). Both Nemeth et al. (2009) and our laboratory have found evidence of WNV shedding from persistently infected birds. Nemeth et al. (2009) reported infectious virus and RNA in the oral cavity of house sparrows (*Passer domesticus*) 30 and 44 days post infection, respectively. The current authors recently detected WNV RNA in the blood of persistently infected birds as long as seven weeks post infection, despite the presence of high titers of neutralizing antibody.

In the current study, we addressed the hypothesis that within mosquito bloodmeals host antibodies remain bound to WNV and thereby protect mosquito vectors from infection. This finding is important because if host antibodies did not protect mosquito vectors from infection, then WNV shedding seen in some persistently infected birds could play a role in the overwintering of WNV. However, if antibodies were protective to mosquitoes then it would mean that persistently infected birds were dead-end WNV hosts so long as they maintained an antibody response. This finding is especially important for future work which will be done to determine whether persistent WNV infections recrudesce to the point where mosquitoes can be infected and thus reinitiate a WNV transmission cycle.

MATERIALS AND METHODS

Mosquitoes. Two species of *Culex* mosquito were used for this experiment: *Culex tarsalis* Coquillett because it is an important WNV vector throughout California (Reisen et al. 2008) and *Culex stigmatosoma* Dyar because when comparatively evaluated for vector competence this species was found to be especially susceptible to WNV infection (Reisen et al. 2005). Both *Cx. tarsalis* and *Cx. stigmatosoma* used in our studies were from laboratory colonies; the *Cx. tarsalis* colony originated from the Yolo Bypass, Yolo County, CA, and the *Cx. stigmatosoma* colony was established from Chino, San Bernardino County, CA. Both colonies were reared at the Arbovirus Field Station in Bakersfield, CA [22°C, photoperiod of 16:8 (L:D), 3-4 egg rafts per pan] and were shipped overnight to the Center for Vectorborne Diseases (CVEC) in Davis, CA, three to five days post-emergence. At CVEC mosquitoes were housed in a 28°C incubator with 75% humidity and a 16:8 (L:D) photoperiod. Sugar and water were withheld for 24 (*Cx. tarsalis*) or 48 (*Cx. stigmatisoma*) hours, after which a treatment group was offered a bloodmeal containing chicken blood, stock WNV and avian sera containing WNV antibodies. A control group was offered a bloodmeal containing chicken blood, stock WNV and avian sera that was negative for WNV antibodies.

Culex tarsalis females were fed using a Hemotek membrane feeding system (*Discovery Workshops*, Accrington, Lancashire, UK); they were fed for two hours, and the bloodmeal was refreshed after one hour of feeding. *Culex stigmatasoma* were allowed to feed for 1.5 hours using a pledget and hanging drops (*Cx. stigmatasoma* refused to feed from the Hemotek). After the feeding period, mosquitoes were anesthetized with carbon dioxide, and bloodfed females were transferred to clean cartons; five freshly-fed females per group were frozen immediately

at -80°C. The remaining bloodfed mosquitoes were placed 50 per 0.67 liter (1 pint) paper carton, provided with a 10% sucrose solution for 10 days post-bloodmeal and then assessed for WNV infection.

Bloodmeal. The bloodmeals for both the treatment and control groups contained 2.0 mL of whole heparinized chicken blood, 1.0 mL of 8.8 $\log_{10}$ plaque forming units (PFU)/mL stock WNV [CA04; isolated from a yellow-billed magpie (*Pica nuttalli*) found dead in Sacramento, CA, and passaged three times on Vero cells] and 1.0 mL (*Cx. tarsalis*) or 1.25 mL (*Cx. stigmatosoma*) sera either positive (treatment) or negative (control) for WNV antibodies. The chicken blood and sera were collected from WNV antibody negative chickens housed in mosquito-proof enclosures. Because the *Cx. stigmatosoma* were fed with hanging drops, both the control and treatment bloodmeals were sweetened with 2.5% sucrose. The WNV antibody positive sera was collected from a group of six western scrub-jays (*Aphelocoma californica*) which had been wild-infected with WNV, then experimentally challenged with the CA04 isolate of WNV; this re-challenge lead to robust antibody responses in the western scrub-jays. Blood was collected weekly beginning two weeks after experimental WNV-challenge and ending six weeks post-infection. Sera was pooled, heat-inactivated for 30 minutes at 56°C and the antibodies titered. The WNV specific neutralizing antibody endpoint titer, determined by a 90% plaque reduction neutralization test (Beaty et al. 1995), of the pooled western scrub-jay sera was 1:1280. Because of the quantity of sera required and the fact that WNV antibody-negative western scrub-jay sera were unavailable, WNV antibody-negative chicken sera was used in the control group bloodmeal. To prepare the bloodmeals, stock WNV and sera were mixed and incubated at 4°C for 24 hours to allow antibody-virus binding. Immediately before bloodfeeding, the sera and virus mixture was added to whole chicken blood and vortexed to mix. Aliquots of these bloodmeals were clarified by centrifugation and the sera collected and frozen to -80°C until testing.

Diagnostics. Immediately after bloodfeeding, five freshly-fed mosquitoes per species per group were placed individually into cryovials and frozen to -80°C. At ten days post-feeding, the remaining bloodfed females were anesthetized with triethylamine. Using sterile technique, the legs were separated from the body and placed into one cryovial and the body into another, ultimately the legs were not tested. Each tube contained two 8 mm glass ball bearings, and all samples were held at -80 °C until processing. Each mosquito body was homogenized in 1.0 mL of virus diluent (Dulbecco's modified eagle medium [Gibco Invitrogen, Carlsbad, CA, USA], containing 5% penicillin and streptomycin and 20% fetal bovine serum) using a mixer mill (MM300, Retsch, Haan, Germany) at a frequency of 24 cycles/second for four minutes. Total RNA was extracted from the mosquito homogenate using a MagMAX™ 96 following manufacturer protocols (Applied Biosystems, Carlsbad, CA, USA). All samples were then screened for WNV RNA by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), using previously published primers specific for the envelope region of the viral genome (Lanciotti et al. 2000) and a 7900 TaqMan platform (Applied Biosystems, Carlsbad, CA, USA).

The mosquito homogenate was also tested for infectious virus using Vero cell plaque assay (Kramer et al. 2002). Briefly, Vero cells were grown to confluence in 6-well plates, media was removed and 100µL of mosquito homogenate supernatant was pipetted onto the monolayer and allowed to absorb for 1 hour at 37°C. A double-overlay system was used where the first overlay was applied directly after the absorption period and contained 1% agarose, nutrient media and sodium bicarbonate. The second overlay was applied 24 hours after the absorption and was similar to the first overlay except that additionally it contained 3% neutral red. The plates were read 36 hours after the absorption period. The limit of detection for this assay was $\geq$1.0 $\log_{10}$ PFU/mL, the titer if one or more plaques were counted in a well containing undiluted sample.

RESULTS

Culex tarsalis. Because the *Cx. tarsalis* mosquitoes did not feed avidly after one hour, they were presented with a second bloodmeal identical to the first to allow a second hour of feeding time. Replacement of the blood meal was necessary due to separation of the sera from the red blood cells. Plaque assays to assess viral titer were conducted on both the first and second bloodmeals of both the control and treatment groups and on the five freshly fed females collected from the control and treatment groups. Approximately half of the mosquitoes fed during the first hour of feeding, and the second half fed during the second hour of feeding. The WNV infectious viral titer of the bloodmeal for the first hour of feeding was 7.6 $\log_{10}$ PFU/mL, and the titer for the second hour was 7.4 $\log_{10}$ PFU/mL. The infectious viral titer for the first bloodmeal of the treatment group was <1.0 $\log_{10}$ PFU/mL, the limit of virus detection for this plaque assay. The plaque assay results of the second hour bloodmeal showed that not all the virus was bound by antibody. There were no plaques when the bloodmeal was plaque assayed undiluted, but two plaques were detected when the bloodmeal was diluted 10-fold; therefore, the second bloodmeal was estimated to contain 2.3 $\log_{10}$ PFU/mL of infectious or unbound WNV.

The freshly fed mosquitoes reflected the above analysis of the control and treatment bloodmeals. All of the freshly fed females in the control group (n = 5) were plaque assay positive for infectious virus, and the mean body titer of these mosquitoes was 4.9 $\log_{10}$ PFU/mL (SD = 0.4). The plaque assays of the freshly fed mosquitoes from the treatment group (n = 5) reflected the breakthrough infectious virus from the second bloodmeal. While four of the five treatment group females had no detectable virus, one showed two breakthrough plaques and had an estimated body titer of 1.3 $\log_{10}$ PFU/mL.

To ensure that the decrease in infectious WNV was attributable to antibody inactivation and not a lack of virus, RNA was extracted from the freshly fed mosquitoes and qRT-PCR used to determine RNA in the bloodmeals. The mean cycle thresholds (Ct) for freshly fed mosquitoes in the control group was 22.27 (SD

=1.04) and not significantly different (t = 0.046, df = 8, P = 0.96) from 22.25 (SD = 0.44) for freshly fed females from the treatment group. This indicated that both treatment and control groups received equal amounts of virus and that the difference between the plaque assay results could be attributed to WNV neutralizing antibodies being present in the treatment group bloodmeal.

After ten days, individual *Cx. tarsalis* mosquitoes from both treatment and control groups were tested by plaque assay for infectious virus and by qRT-PCR for WNV RNA. Overall, the control group (n = 81) was 90% positive for WNV RNA and 86% positive for infectious virus. Conversely, the treatment group (n = 81) was 4.9% (4 females) positive for WNV RNA, and 1.2% (1 female) positive for infectious WNV.

Culex stigmatosoma. The *Cx. stigmatosoma* refused to feed when offered the Hemotek membrane feeder, so hanging drops and pledgets sweetened with 2.5% sucrose were used to present the bloodmeals. The WNV infectious viral titer determined by plaque assay of the control group bloodmeal was 7.1 $\log_{10}$ PFU/mL. Infectious WNV was not detected in the treatment group bloodmeal.

As above, five freshly fed females from each group were collected directly after feeding and were analyzed for infectious virus and WNV RNA. All of the freshly fed females in the control group were positive for infectious WNV; the mean infectious virus body titer was 4.0 $\log_{10}$PFU/mL (SD = 0.2). Infectious WNV was not detected in the five freshly fed mosquitoes of the treatment group. When RNA was extracted and the presence of WNV RNA was quantified by qRT-PCR, there was no significant difference between the Ct scores of the freshly fed control 23.89 (SD = 0.52) and treatment group 23.12 (SD = 0.52) mosquitoes (t = 1.01, df = 8, P = 0.34). As above these finding illustrate that both control and treatment groups were fed equal amounts of WNV; however, the WNV in the *Cx. stigmatosoma* treatment group bloodmeal was completely bound by antibody.

At ten days post-feeding, the *Cx. stigmatosoma* treatment and control group mosquitoes were evaluated for infectious WNV and WNV RNA. In the control group (n = 76), 62% of the females were positive for infectious virus and 72% for WNV RNA. At ten days post-feeding, there was no evidence of infectious virus or WNV RNA in the treatment group (n = 72).

DISCUSSION

These results support our hypothesis that the presence of avian neutralizing antibody bound to WNV within an infectious bloodmeal would protect female mosquitoes from subsequent infection. Susceptible strains of *Cx. tarsalis* and *Cx. stigmatosoma* females were fed high infectious titers of WNV either with or without the presence of WNV neutralizing antibody. The presence of high WNV titers in both groups was demonstrated by the presence of comparable amounts of RNA by qRT-PCR. Even when mosquitoes are exposed to bloodmeals containing high viral titers, it was found that if enough antibody was present to bind all virus, avian antibodies protected mosquito vectors from WNV infection.

There were four individuals in the *Cx. tarsalis* treatment group that were positive for WNV RNA, and of these one was also positive for infectious WNV. These positives were attributed to the fact that the stock virus in the *Cx. tarsalis* treatment bloodmeal was not completely bound by antibody. However, that even a small percentage of females could become infected by a bloodmeal with an infectious viral titer of approximately 2.3 $\log_{10}$ PFU/mL is an interesting finding. Previously Reisen et al. (2005, 2008) evaluated the dose response of *Cx. tarsalis* and *Cx. stigmatosoma;* when fed a bloodmeal containing 3 - 4 $\log_{10}$ PFU/mL, ≤10% of both species were infected with WNV. However, when fed a bloodmeal containing 6 -7 $\log_{10}$ PFU/mL, >70% of *Cx. tarsalis* and *Cx. stigmatosoma* were infected.

The finding that host antibodies protect mosquitoes from WNV infection is especially important with regard to interpreting persistent WNV infections in avian hosts. House sparrows maintain robust neutralizing antibody titers for up to two years post WNV infection (Nemeth et al. 2009), and these high titers may be maintained by shedding of WNV in persistently infected birds. House sparrow sera have been found to contain WNV RNA up to seven weeks post WNV infection, despite the presence of high neutralizing antibody titers (Wheeler unpublished data). Our study showed that, because the house sparrows maintain neutralizing antibody titers viral, shedding would not be infectious to mosquitoes. However, perturbations to the host immune system that compromised the humoral immune response may lead to recrudescent infections that would be infections to mosquitoes. In addition, the response to intermittent shedding may not be immediate if antibody decay has progressed to undetectable levels, as shown by an ephemeral viremia in three of six house finches previously infected with SLEV and then challenged with low titered virus (Reisen et al. 2001). This result was not confirmed in a subsequent experiment (Reisen et al. 2003).

The interplay between host and vector is a complicated balance. Although we now know that virus bound to avian antibodies is not disassociated during blood digestion and is not infectious to bloodfeeding mosquitoes. Future work will be needed to confirm the finding that *Cx. tarsalis* can be repeatedly infected with low titered infectious bloodmeals containing 2.0 - 3.0 $\log_{10}$PFU/mL. If vector mosquitoes can reliabley be infected with such low viral titers, even at low rates, this may have important implications for the role that persistent WNV infections play in the overwintering of WNV.

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Symposium: Collaborative Research Between the Mosquito and Vector Control Association of California and the University of California at Davis: Introduction

William K. Reisen

Center for Vectorborne Diseases (CVEC), Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California Davis, CA 95616, wkreisen@ucdavis.edu

West Nile virus (WNV) invaded California during the summer of 2003 (Reisen et al. 2004), amplified to epidemic levels in Los Angeles in 2004 (Kwan et al. 2010) and rapidly dispersed to every county in the state by the fall of 2004 (Hom et al. 2005). Our research reported at previous meetings tracked epidemic amplification and the endemic establishment of WNV, identified the mosquito and avian hosts, explored different modes of transmission, described advances in laboratory diagnostics and data management to enhance decision support, and evaluated the efficacy of aerial and ground adulticide applications. The current symposium describes research on the mechanisms of WNV persistence, new surveillance methods to sample mosquitoes, evaluate virus genetics, and use climate to predict risk. Most of the research by CVEC is the result of collaboration with MVCAC partners and has been or will soon be published elsewhere. It is described briefly in the current Proceedings.

SYMPOSIUM CONTENT

- Introduction - William Reisen, Research Entomologist and Professor
- Temporal Changes in Persistent West Nile Virus Infections in House Sparrows. Sarah Wheeler, Ph.D. candidate, Graduate Group in Comparative Pathology
- Climate and the Risk for West Nile Virus Transmission. Christopher Barker, Ph.D., Assistant Research Epidemiologist
- Fitness of West Nile Virus Strains in House Finches. Gabriella Worwa, DVM, Ph.D., Postdoctoral Researcher
- Investigation of Sugar Bait For Mosquito-borne Virus Surveillance. Hugh Lothrop, Specialist Entomologist
- Host Selection Patterns of *Culex tarsalis* and the *Culex pipiens* complex in California. Tara Thiemann, Ph.D. candidate, Graduate Group in Entomology
- Detection of Virus CPE and Neutralizing Antibody Using Novel Impedance Technology. Ying Fang, Laboratory Manager
- Role of Migratory Birds in West Nile Dispersal in California. William Reisen, Ph.D., Research Entomologist and Professor

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Temporal Changes in the Persistence of West Nile Virus Infection in House Sparrows (*Passer domesticus*)

Sarah S. Wheeler[1], Meighan P. Vineyard[1], Leslie Woods[2] and William K. Reisen[1]

[1]*Center for Vectorborne Diseases, School of Veterinary Medicine, University of California, One Shields Ave, Davis, CA 95616, sswheeler@ucdavis.edu;* [2] *California Animal Health and Food Safety Laboratory, University of California, Davis, CA 95616*

West Nile Virus (WNV) is now endemic throughout North America and has been detected in California every year since its invasion. The mechanisms enabling WNV to persist overwinter when temperate winters drive mosquitoes into inactivity and halt the transmission cycle are not understood. Understanding these overwintering mechanisms is important for: targeting early season control efforts, improving surveillance and disease forecasting and ultimately disease prevention. We hypothesize that persistent WNV infections in avian hosts may be an overwintering mechanism for WNV. To test this hypothesis, wild-caught house sparrows (*Passer domesticus*) were experimentally infected with WNV and examined for persistent infection for up to 18 weeks post-infection.

Birds were held in groups of eight to ten individuals (aside from the three week group where n =13) 3, 5, 7, 9, 12, 15 or 18 weeks post-infection. Blood was drawn every two weeks, and sera were tested for both WNV neutralizing antibodies and WNV RNA. At the end of each holding period, spleen, kidney, brain and skin were tested for both infectious virus and viral RNA. Infectious virus was detected using a modified Vero cell cocultivation technique described by others (Tesh et al. 2005; Appler et al. 2010), and WNV RNA was detected using qRT-PCR using primers and probe specific for envelop (Lanciotti et al. 2000) and NS1 (Shi et al. 2001) region of the viral genome. The neutralizing antibody titers were quantified by plaque reduction neutralization assay (PRNT) (Beaty et al. 1995).

All infected birds produced neutralizing antibody titers with titers peaking between five and nine weeks post-infection. WNV RNA was detected in the sera of some birds as late as seven weeks post-infection. However, because WNV RNA was detected in the presence of WNV neutralizing antibody, it was unlikely that any infectious virus present would be detected by plaque assay or infectious to mosquitoes (Wheeler and Reisen Submitted).

WNV RNA was detected in tissues at necropsy at every time point except for the 15 week group when persistence was not detected. RNA positivity decreased with time post-infection. For instance, at three weeks 100% (n = 13) of the birds were positive for WNV RNA, at nine weeks 50% (n = 10) were positive, and at 18 weeks 12.5% (n = 8) were RNA positive. The spleen was the organ most often found RNA positive, followed by the kidney, skin and then brain. Infectious virus was isolated by cocultivation from the spleen of three birds at 3 weeks, one bird at 5 weeks, two birds at 7 weeks and one bird at 12 weeks post infection. Infectious virus was only isolated from the kidneys of two birds in the 3 week group; all skin and brain tissues were negative.

Our study confirmed that infectious WNV can persist in avian hosts for up to 12 weeks. Detection of WNV RNA up to 18 weeks post-infection confirmed that the virus may persist long enough to serve as an overwintering mechanism in California. The isolation of infectious virus as long as 12 weeks post-infection confirmed that WNV RNA can be attributed to intact virus and that the RNA detected is not a non-infectious relic remaining from the original inoculation. Whether these persistent infections recrudesce to restart the transmission cycle is unknown, but it is a topic for planned future research.

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Climate and the Risk for West Nile Virus Transmission*

Christopher M. Barker[1,2], David M. Hartley[2,3], Tianchan Niu[3], Arnaud Le Menac'h[4], and William K. Reisen[1,2]

[1]Center for Vectorborne Diseases, University of California, Davis, 530-752-0151, cmbarker@ucdavis.edu; [2]Fogarty International Center, National Institutes of Health, Bethesda, MD; [3]ISIS Center and Departments of Microbiology and Immunology and Radiology, Georgetown University Medical Center, Washington, DC; [4] Health Protection Agency, London, UK

Mosquito-borne pathogens such as West Nile virus (WNV) are particularly susceptible to the influence of temperature because of the time spent in the mosquito vector, where temperatures fluctuate with those of the environment. As a result, temperature plays an important role in determining the likelihood of WNV transmission, as demonstrated in several studies in the laboratory (Dohm et al. 2002, Reisen et al. 2006, Kilpatrick et al. 2008) and field (Nielsen et al. 2008, Soverow et al. 2009, Ruiz et al. 2010). The expectation of increased WNV transmission at warmer temperatures is an important component of the California Mosquito-Borne Virus Surveillance and Response Plan (California Department of Public Health et al. 2011).

To better understand the mechanisms by which temperature affects WNV transmission, we developed an epidemiological model that incorporates variation in host competence and the possibility for vertical transmission in mosquitoes or horizontal transmission between birds, in addition to the typical transmission cycle between mosquitoes and birds. From this model, we derived two measures of transmission risk. The first utilizes published regressions of the WNV extrinsic incubation period (Reisen et al. 2006) and the length of the *Culex tarsalis* gonotrophic cycle (Reisen et al. 1992) on temperature to calculate T, a temperature-dependent ratio of EIP/GP that equals the number of bites that a mosquito would need to take following infection with WNV before it could transmit WNV to a host. A lower number of bites corresponds to a higher probability that a mosquito would survive long enough to transmit WNV. The second measure of WNV risk was the basic reproductive ratio, R_0, that estimates the theoretical number of infections of mosquitoes or birds that would arise from a single infectious individual in a susceptible population. $R_0 > 1$ implies that WNV would be expected to amplify, with each infectious host or vector infecting an average of more than 1 additional individual.

We compared these risk metrics with observed data on WNV seroconversions in sentinel chickens as a spatial indicator of transmission during the period since WNV arrived in California, 2003-2009. Both T and R_0 agreed well with the spatial and temporal dynamics of WNV transmission. Over the entire study period, the average seroconversion rate in areas with $R_0 > 1$ (5.7%) was greater than three times the rate in areas with lower R_0 values (1.7%), and most transmission (59% of all seroconversions) occurred when transmission was expected within 2 or 3 mosquito bloodmeals. Transmission within a single gonotrophic period would not be expected at California's temperatures unless location of a bloodmeal host or oviposition site was markedly delayed. Completion of this mathematical model is an important step toward understanding arboviral dispersal and persistence in California. Mechanistic risk metrics such those presented here help to explain why temperature affects WNV transmission, and they have the added advantage of providing an interpretable quantity that could be used to guide control decisions or communicate outbreak potential to policy-makers or the public.

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Fitness of West Nile Virus Strains in House Finches

Gabriella Worwa[1], Sarah S. Wheeler[1], Payal D. Maharaj[2], Aaron C. Brault[2] and William K. Reisen[1]

[1] *Center for Vectorborne Diseases (CVEC), Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA, gworwa@ucdavis.edu;* [2] *Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, CO*

ABSTRACT: The invasion of North America has required West Nile Virus (WNV) to adapt to different geographical areas. Initial strains of WNV found in the eastern parts of the country were grouped into the NY99 genotype that has been subsequently displaced by a new dominant genotype, WN02, which has not been characterized using natural host systems. In addition, it remains unclear if WNV has further evolved since its introduction in 2003 to accommodate the differing hosts and vectors found in the varying landscape of California. The fitness of WNV also may play a role in overwintering and therefore contribute to the persistence of WNV. Our study targets phenotypic change in current WNV isolates by comparing the fitness of a NY99 strain and different post-invasion California isolates to a genetically labeled founding reference strain from 2003. Comparisons were made using an *in vivo* competition fitness assays in birds and mosquitoes.

INTRODUCTION

The aim of this research project is to examine the persistence and adaptation of newly emerging WNV genotypes in California based on viral fitness. Fitness refers to the replication competence of a virus and is measured *in vivo* comparatively using the viremia response of infected hosts. Overwintering may serve as a genetic bottleneck for WNV and therefore lead to selection for mutations in the viral genome that positively impact fitness. Current WNV strains therefore may contain cumulative genetic changes arising over the past eight years, but it is not clear whether WNV has become attenuated or retained high virulence. Furthermore, we would like to investigate whether outbreaks are related to changes in fitness. Our approach is to assess viral fitness by experimental co-infection of birds and mosquitoes using an *in vivo* competition fitness assay. This study focused on the elaboration of the methodology and fitness comparison between NY99 and WN02 representative isolates as a basis for future characterization of representative WNV isolates from California.

METHODS AND MATERIALS

The *in vivo* competition fitness assay measures replicative capacity between two viruses in a 1:1 ratio mixture inoculated into the same host. This sensitive system allows the distinction of slight fitness differences between two competing viruses with exclusion of host-to-host differences.

For fitness comparisons we selected a previously generated NY99 infectious clone-derived virus representing the displaced NY99 genotype, an infectious clone derived virus from the 2003 COAV997 isolate from a *Culex tarsalis* mosquito pool collected in the Coachella Valley (COAV997ic) and a field isolate from a dead magpie found in 2004 in Sacramento (WN04).

To distinguish diagnostically between these genetically similar viruses, we previously marked an infectious clone from COAV997 by changing five nucleotides by site-directed mutagenesis in the E gene region of the genome (COAV997mut). This virus serves as a marked Californian reference strain representing the founding virus and will be used for *in vivo* competition fitness experiments.

We have selected the house finch (HOFI; *Carpodacus mexicanus*) for our study because this bird species is frequently infected with WNV in nature. Wild HOFIs were collected from traps in Kern County and transported to UC Davis. After a two week cage adaptation period in biosafety level 3 Horsfall-Bauer units, pre-medication with antibiotics and confirmation of sero-negativity for WNV by plaque neutralization assay, the HOFIs were infected with a 1:1 mixture of COAV997mut and COAV997ic (neutrality test), NY99ic and WN04. Control groups with singly infected birds were included for each isolate and one mock-infected negative control group (total 8 groups, n = 6 birds per group). Rectal body temperatures and blood samples were taken daily for seven days and again at 14 days post infection when birds were euthanized. Samples were analyzed by plaque assay titrations, and the amount of RNA copies for each single virus in mixed competition samples determined with a novel specific real-time RT-PCR approach currently being optimized.

PRELIMINARY RESULTS

In contrast to other studies with American crows, the infected HOFIs developed no fever throughout the course of viremia. Lethargy was commonly seen in infected birds, but no clinical signs related to the central-nervous system were observed. Birds at agonal stages of infection developed severe hypothermia and presented a 'puffed up' plumage appearance. All HOFIs, except for the mock-infected birds, became viremic as confirmed by plaque assay titration. Fitness neutrality of COAV997ic and COAV997mut was confirmed by detecting similar viremia titers, meaning that the five nucleotide replacements in COAV997mut did not cause any detectable change in fitness. Differences in the length and magnitude of the viremias and mortality were observed for the three different WNV strains. The NY99ic and WN04 elicited longer and slightly higher viremias in the infected HOFIs compared to COAV997ic and COAV997mut. NY99ic viremia was accompanied by greater mortality compared to the other strains.

DISCUSSION AND OUTLOOK

Our preliminary results indicate that there are fitness differences among the tested WNV strains. Fitness competitions in *Culex tarsalis* mosquitoes with the same experimental design and WNV strains are currently ongoing and will provide further evidence on how these strains alter vector competence in the vector. Avian and mosquito samples then will be analyzed by real-time RT-PCR assay to gain information on the comparative replication rates of the competing viruses. This pilot study is providing proof of principle that our fitness assay is working as intended and will be used for extended fitness studies among WNV isolates from four established California study sites (Coachella Valley, Greater Los Angeles area, Kern County and Sacramento County) representing different biomes with varying hosts, vectors and overwintering conditions. Isolates made from mosquito pools collected in 2008 from the beginning, middle and end of the transmission season from these four sites will be analyzed by comparing their fitness to COAV997mut. Additional isolates from outbreaks with neuro-invasive manifestation in humans in Los Angeles in 2004, Kern in 2007 and Sacramento in 2005 will be included to investigate whether there is a causative association with elevated viral fitness. Full-length sequencing of the genotypes associated with altered fitness will provide information on the cumulative genetic change arisen from over five years of WNV circulation in California.

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Bloodfeeding Patterns of *Culex tarsalis* and the *Culex pipiens* Complex in California

Tara C. Thiemann[1], Debra A. Lemenager[2], Susanne Kluh[3], Brian D. Carroll[1], Hugh Lothrop[1] and William K. Reisen[1]

[1]Center for Vectorborne Diseases, School of Veterinary Medicine, University of California, Davis, CA 95616, tcthiemann@ucdavis.edu; [2]Sutter-Yuba Mosquito and Vector Control District, Yuba City, CA 95992; [3]Greater Los Angeles County Vector Control District, Santa Fe Springs, CA 90670

INTRODUCTION AND METHODS

West Nile virus (WNV) is a mosquito-borne flavivirus now endemic to most of the United States, including California. The virus is primarily maintained in an enzootic cycle involving mosquito-bird-mosquito transmission, but it can be tangentially transmitted to disease-susceptible mammals such as humans and horses (Komar 2003). *Culex* vectors of WNV have been shown to feed on a variety of avian and mammalian host species with varying WNV competence (Molaei et al. 2006, Savage et al. 2007, Kent et al. 2009), so determining the bloodfeeding patterns of these mosquitoes may be crucial for understanding differences in WNV transmission dynamics throughout California.

To explore bloodfeeding patterns, *Culex tarsalis* and members of the *Culex pipiens* complex were collected over a 3-year period from large walk-in red boxes (Meyer 1987), gravid traps (Cummings 1992) and CO_2 traps (Newhouse et al. 1966) set at multiple trapping sites in 5 locations throughout California: Coachella Valley, Los Angeles, Kern County (near Bakersfield), Yolo County (in and around Davis), and Sutter and Yuba Counties. Bloodfed females were stored individually at -80°C prior to processing. The abdomens of the mosquitoes were removed, and DNA was extracted from the bloodmeals. A portion of the mitochondrial gene cytochrome c oxidase I (*COI*) was amplified from the bloodmeal DNA by nested PCR and was identified to host species either by a microsphere-based assay developed for common hosts in California or by sequencing and submission to the 'Identify Specimen' feature of the Barcode of Life Data Systems (www.boldsystems.org).

RESULTS AND DISCUSSION

Nearly 100 avian, mammalian and reptilian host species were identified from over 1400 *Culex* bloodmeals collected throughout California. Adults of both *Cx. tarsalis* and the *Cx. pipiens* complex fed most frequently on avian hosts, though *Cx. tarsalis* fed on a higher percentage of mammals than did those of the *Cx. pipiens* complex. Only one reptilian species, the western fence lizard, was identified.

Despite variation in climate and landscape, several dominant host species emerged throughout the state. House sparrows and house finches were frequent hosts of both mosquito species and may be important in maintaining enzootic WNV, particularly in highly urbanized areas such as Los Angeles. The western scrub-jay, a highly competent corvid species, was frequently utilized by both *Culex* species when available. The western scrub-jay was a particularly frequent host in the Bakersfield area and may serve as an amplifying host, contributing to continued epidemic transmission in this area. The mourning dove was also a frequent host throughout California, and when available, domestic chickens were frequently fed upon. With their low WNV competence, doves and chickens may serve to dampen transmission in some areas.

Differences in mosquito bloodfeeding around the state were largely associated with host availability. Coachella Valley had the highest host species richness, with over 50 host species identified. Several of these species, including the greater roadrunner, great-tailed grackle, and white-throated woodrat, were identified only in this area. House finches and house sparrows accounted for less than 25% of the bloodmeals in the Coachella Valley as compared to over 60% in the less host diverse Greater Los Angeles area. Several other host species were utilized differentially across multiple areas. For example, American robins were fed upon frequently in northern locations, and yellow-billed magpies were fed upon only within its limited range in the northern Central Valley. Despite widespread distribution around much of the state, the American crow was identified as a host from *Culex* bloodmeals at only one study site. The American crow did, however, account for over 20% of the bloodmeals at this site, so the disparity between this and other sites may relate to sampling bias inherent with the crow's focal staging and roosting behavior.

This study offers a broad overview of the bloodfeeding patterns of *Cx. tarsalis* and the *Cx. pipiens* complex throughout California. WNV-competent hosts were fed upon by both *Culex* species in all areas, but variations in bloodfeeding were apparent between mosquito species and among geographic areas. On-going work will take a more detailed look at seasonal feeding patterns, host preferences and the effect of host selection on WNV transmission in California.

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Detection of Virus Cytopathogenesis and Neutralizating Antibody Using Novel Impedance Technology

Ying Fang[2], Xiao Xu[3] and William K. Reisen[2]

[2] Center for Vectorborne Diseases, School of Veterinary Medicine, University of California, Old Davis Rd, Davis, CA 95616, ylfang@ucdavis.edu; [3] ACEA Biosciences, Inc, 6779 Mesa Ridge Road, Suite 100, San Diego CA 92121

Summary of a paper recently accepted in the Journal of Virological Methods (Fang et al. 2011).

INTRODUCTION

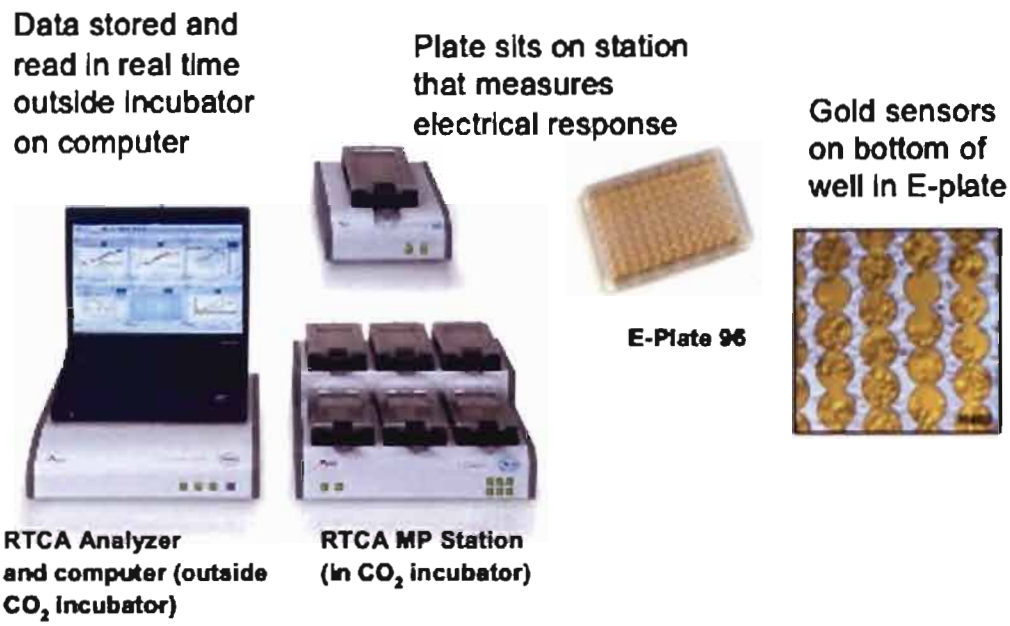
Describing viral growth patterns on different cell types requires the removal of aliquots from replicate cell culture flasks at multiple time points, followed by a series of plaque assays on Vero cell monolayers to estimate viral titer. In addition to the extensive labor effort, time and cost, data may be compromised for some viruses by using mammalian cells for assessment. Plaque reduction neutralization tests (PRNT) are the 'gold standard' for measuring antibody titer. Mixtures of antibody and virus are grown on Vero cells and the titer is the endpoint of the antibody concentration where >10 or 20% of the virus is no longer neutralized. Both plaque and PRNT assays take considerable time, because you must wait for viral growth and then count plaque forming units (PFU).

Recently a real-time cell analysis (RTCA) system (formerly the RT-CES system from ACEA Biosciences, Inc., San Diego, CA) was developed for monitoring cell growth using electronic impedance technology (Solly et al. 2004). A cell index (CI) comparing impedance in control and treatment wells (Xing et al. 2005) allows the RTCA system to track in real time changes to the cell layers cultured on gold microelectrodes integrated into the bottom of 96 well microelectronic cell culture plates or E-plates (Solly et al. 2004). Our research adapted the RTCA system to monitor West Nile (WNV) and St. Louis encephalitis (SLEV) viral growth patterns and to measure the inhibition of viral growth by neutralizing antibody.

MATERIALS AND METHODS

The RTCA system basically consists of three parts: one or more 96 well E-plates with gold sensors, a docking station for the E-plates that measures electricity flow through the sensors, and a reader attached to a computer to determine monitoring frequency, calculate the cell index, and display CI (and thus virus growth) curves (Fig. 1). In our research, plates and docking station were placed inside a standard incubator set at 37°C and 5% CO_2 and were connected to the reader outside the incubator with a standard computer electrical cable.

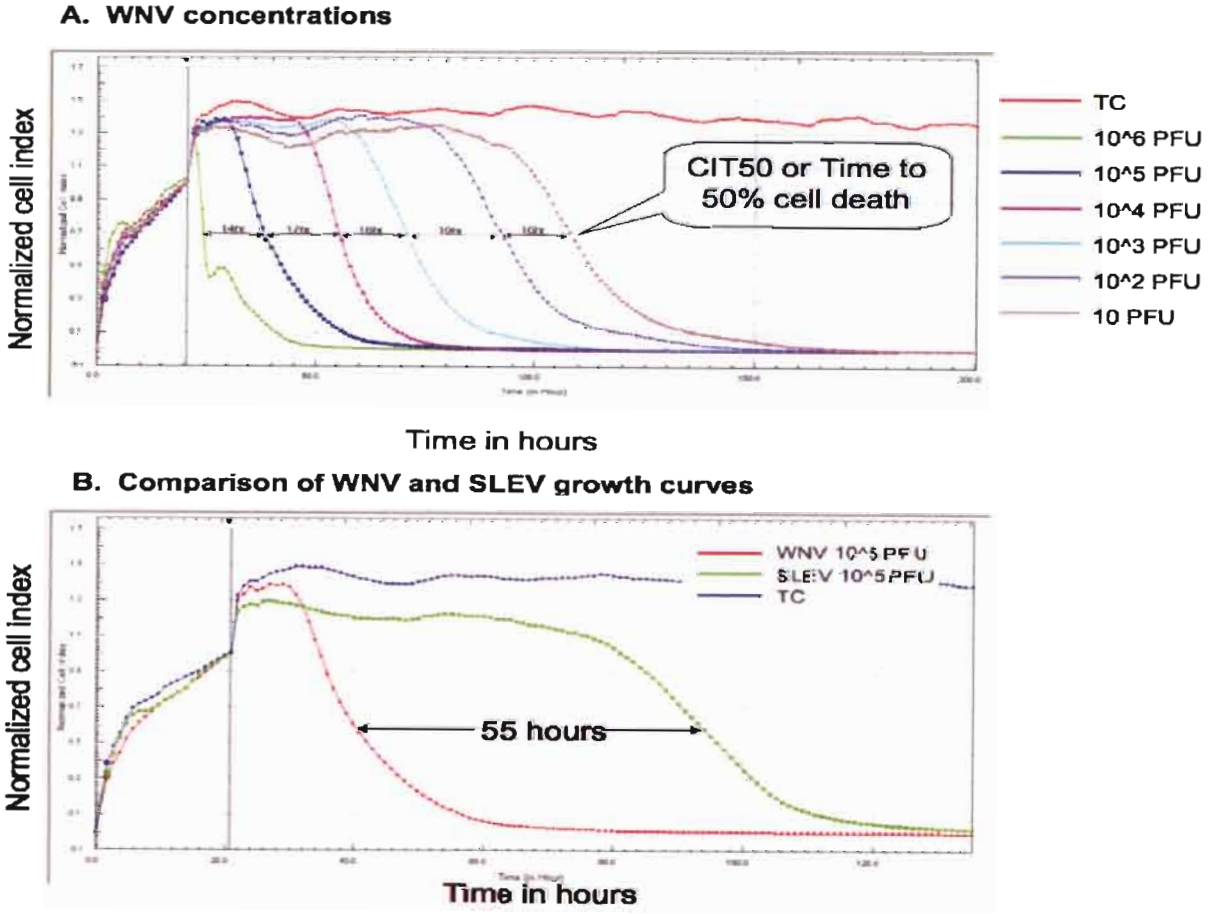
Figure 1. RTCA system showing gold sensors in e-Plate and plate sensor docking station.



Viral growth curves. Initially 50 µL of viral solution of varying titers mixed with 50 µL of a Vero cell culture suspension containing ca. 50,000 Vero cells were loaded onto the E-plates and monitoring initiated. Our experiments used the NY99 strain of WNV and the Kern217-89 strain of SLEV. Fig. 2A shows the concurrent increase in impedance for all groups as the Vero cells attach to the sensors and grow, until ca. 22 h when initial cytolysis occurred resulting in the immediate loss of impedance for the replicate 10^6 PFU titer wells where all the cells either lysed or released from the sensors. The time until median cytolysis, or the cell index time 50 (CIT_{50}), was virus titer dependent and progressively increased as a linear function of decreasing virus titer ($y = -17.11x + 124.5$, $R^2 = 0.99$, where $x = \log_{10}$ titer, $y = CIT_{50}$). The RTCA system also differentiated between different viruses with different growth rates. Fig. 2B shows a difference of almost 55 h in CIT_{50} between cultures of WNV and SLEV initiated at the same 10^5 PFU titer.

Antibody measurement. 50,000 Vero cells in growth media were added to the E-plate and incubated for 24 h. House Finch serum of known titer (1:2560) was serially 2-fold diluted in DMEM, and 100 µl of the serially diluted anti-sera mixed with 100 µl of a solution containing $10^{5.3}$ PFU of WNV. After 30 min incubation in standard 96 well plates at 37°C, 100 µl of the antibody/virus mixtures were added to wells of the E-plate after the original growth media was removed. The E-plate then was loaded onto the docking station within the CO_2 incubator and the cell index monitored for 6 days (Fig. 3A). The time until CIT_{50} increased as a linear function of increasing antibody titer between titers of 1:20 and 1:160; titers ≥1:320 neutralized virus for the duration of the experiment precluding an estimate of the CIT_{50}. The titers used in Fig. 3A were exceptionally elevated

Figure 2. Real time monitoring of WNV and SLEV growth patterns on Vero cells using the RTCA system. A) Variation of cell index as a function of WNV concentration. B) Comparison of WNV and SLEV growth curves at the same virus titer and temperature.



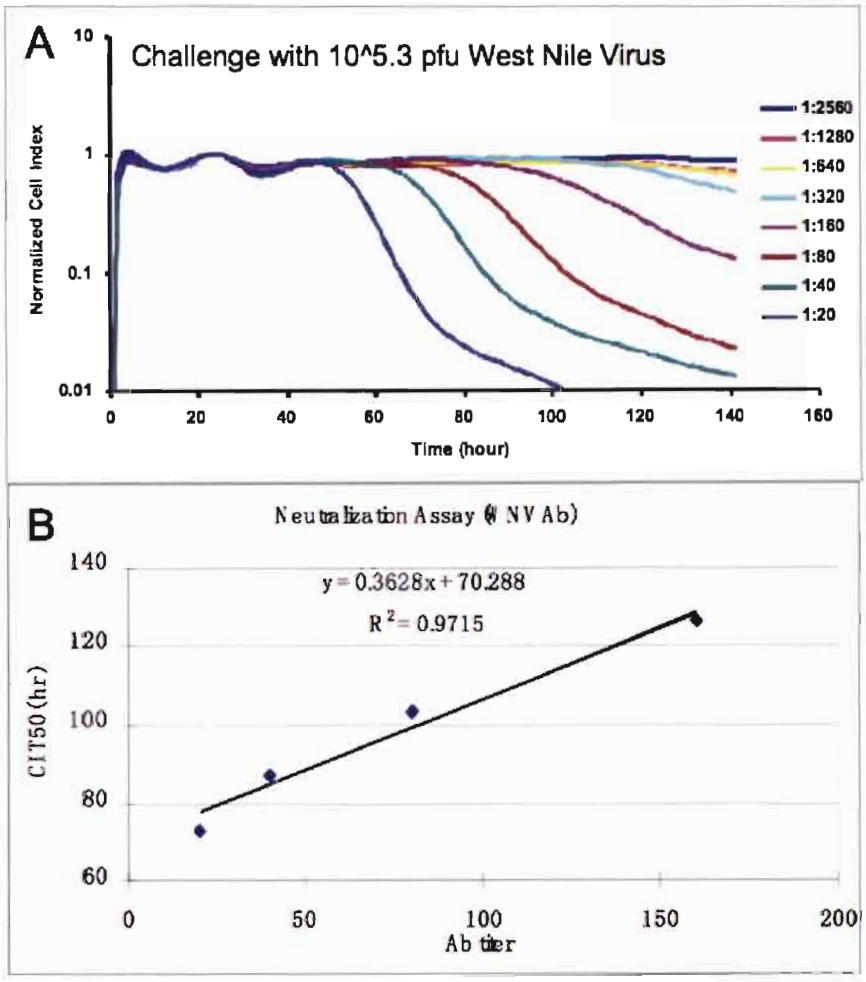
and were created only after SLEV infected birds were challenged with WNV (Fang and Reisen 2006). Most free ranging birds have titers <1:320 after a single infection.

Because CIT_{50} values were antibody titer dependent, we felt it was possible to estimate $PRNT_{80}$ titer by the RTCA system using a single well of a 96 well E-plate with a known concentration of virus mixed with a field serum sample of unknown titer. Titers were estimated from a standard antibody dilution vs. CIT_{50} curve as shown in Fig. 3B. We evaluated sera from 40 field samples that previously had been screened by EIA and confirmed by standard $PRNT_{80}$. Results were essentially congruent. Overall, 35 of 36 samples estimated to be positive by the RTCA system agreed within a dilution of the standard $PRNT_{80}$ estimate. Four of 4 samples were negative by both RTCA and $PRNT_{80}$, but positive by EIA, and 1 sample positive by EIA and the RTCA system was negative (<1:20) by $PRNT_{80}$.

SUMMARY

The RTCA system has been extremely useful for our research, because it greatly reduced labor and reagents, and produced fine time-scale and real-time monitoring of viral growth curves. These benefits far out-weighed the increased cost of the E-plates with their gold sensors. We have used the RTCA system to compare the virulence of different strains of western equine encephalomyelitis virus on mammal, avian and insect cell cultures (Zhang et al. 2010) as well as the growth patterns of different genetic constructs of WNV (unpublished). Future use may include the confirmation of EIA positive avian sera as part of our wild bird herd immunity monitoring program, because SLEV has been essentially eliminated from California precluding confusion due to cross reactivity.

Figure 3. Delay in the time until CIT50 due to increasing concentration of PRNT antibody titer.



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Role of Migratory Birds in the Maintenance and Persistence of Arboviruses in California

William K. Reisen, Sarah S. Wheeler, Sandra Garcia, Ying Fang and the Arbovirus Research Team

Center for Vectorborne Diseases, Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Old Davis Rd, Davis, CA 95616, wkreisen@ucdavis.edu

A complete version of this paper was published recently (Reisen et al. 2010)

INTRODUCTION

The endemicity of arthropodborne viruses such as West Nile virus (WNV) at temperate latitudes depends largely on its ability to over winter successfully. Possible mechanisms for the persistence of WNV in California have been reviewed (Reisen et al. 2006) and include local persistence by: a) Continued transmission by non-diapausing female mosquitoes such as *Cx. quinquefasciatus* at warmer southern latitudes; b) Vertical passage of virus to diapausing female F1 progeny mosquitoes such as *Culex tarsalis* who then carry the virus over winter to the following spring when they transmit to avian hosts, thereby renewing the cycle; c) Gonotrophic dissociation where infection is acquired through blood meals are taken by prediapausing or diapausing females such as *Cx. pipiens* without egg development; d) Chronic or persistent infections in birds acquired during acute infection that recrudesce/relapse during the following spring; and e) Bird to bird transmission by fecal contamination at communal roosts and/or cannibalism and predation. Alternatively viruses may become regionally extinct and require repeated reintroduction by: a) 'Rolling' epizootics'; b) Inadvertent movement by tourism or commerce; and c) Migratory birds. Although multiple studies (Dusek et al. 2009, Malkinson and Banet 2002) have shown the North to South movement of WNV by migrating southbound birds after transmission seasons at northern latitudes, few have demonstrated the vernal introduction of virus by northbound birds. In fact, although Nearctic encephalitis viruses can be detected in the Neotropics (Shope et al. 1966, Turell et al. 2005), they rarely seem to cause human disease and don't amplify to epizootic levels. However, our studies along the Pacific Flyway showed the repeated replacement of St. Louis encephalitis virus (SLEV) in Coachella Valley with new genotypes appearing after periods apparent local extinction (Reisen et al. 2002), perhaps indicating re-introductions.

The current research addressed the overall hypothesis that north-bound migrants introduced arboviruses into California, with four specific aims: a) Compare seroprevalence between migrants collected at the Salton Sea and the San Joaquin Valley of Kern County to determine if birds entering California have a higher prevalence of antibody; b) Determine infection prevalence among migrants collected as part of the dead bird program to determine if they readily die after infection; c) Attempt to detect viremia in migrants entering California at the Salton Sea; and d) Determine the host competence of some common migrant species.

METHODS AND RESULTS

A complete presentation of the methods and data were recently published (Reisen et al. 2010) and are summarized briefly here.

Seroprevalence in living birds. Birds were collected in Coachella Valley from 1996 – 2007 and in Kern County from 1997 – 2008 using mist nests and seed baited traps, banded, bled and released. Sera were screened for antibodies against alphaviruses or flaviviruses using an enzyme immunoassay (Chiles and Reisen 1998) and positives confirmed by a plaque reduction neutralization assay (PRNT) (Table 1). Of 5,600 sera from 43 Neotropical migrant species, only 7 birds (0.1%) were confirmed by PRNT to be positive for flavivirus and only 3 for WEEV. By comparison, of 50,574 sera from temperate resident birds, 1,899 (3.8%) were positive for flaviviruses and 231 (0.5%) for WEEV. These data indicated transient migrants were not frequently infected with these viruses and that there was no difference in seroprevalence between the Coachella and San Joaquin Valleys. (Table 1)

Table 1. Seropositivity for arboviruses of migrating and total birds collected at the Salton Sea in Coachella Valley (COAV) and Kern County near Bakersfield.

			EIA		PRNT			
	Agency	Total	WEEV+	FLAV+	WEEV+	SLEV+	WNV+	FLAV+
Migrants	COAV	3,442	8	8	1	0	3	0
	KERN	2,158	5	13	2	0	2	2
Total wild birds	COAV	26,708	201	663	91	119	248	88
	KERN	29,466	386	1,737	140	1	1,407	196

EIA, enzyme immunoassay; PRNT, plaque reduction neutralization test, FLAV, flavivirus positive.

Infection in dead birds. Birds reported dead by the public were shipped to the California Animal Health and Food Safety laboratory at Davis where they were necropsied and kidney samples tested by the Center for Vectorborne Diseases for WNV RNA by RT-PCR using previously published primers (Lanciotti and Kerst 2001). Birds were not tested for WEEV and SLEV because these viruses rarely killed birds during experimental infection studies (Reisen et al. 2003). From 2003-2007 a total of 1,109 migrants were reported dead and tested, of which 126 (11%) were found WNV RNA positive (Table 2). However, only 3 species (barn swallow, cliff swallow and western tanager) of the 126 RNA positive birds tested positive during spring, whereas 123 sera from 33 species were positive during summer. These data indicated that birds entering California probably became infected in California during the summer transmission season and were rarely involved in vernal transmission (Table 2).

maximum viremia (mean = 8.45, range = 7.6–10.5 $\log_{10}$ plaque forming units [PFU]/mL), longest duration viremia (max = 7 d) and most frequent mortality following infection (5 of 6 birds died by 7 d post infection). Two control birds survived the experimental period. In marked contrast, four yellow warblers and a single common yellowthroat produced significantly lower mean maximum viremias (mean = 6.76, range = 5.6 – 7.5 $\log_{10}$ PFU/mL), a shorter viremia period (3–4 d post infection) and a lower mortality rate (1 of 5 birds died) than the orange-crowned warblers. In agreement, 4 of 17 (23%) orange-crowned warblers and 6 of 29 (21%) yellow warblers found dead by the public were positive for WNV.

DISCUSSION

The general lack of evidence for previous or current arboviral infection in northbound migratory birds entering California from southern latitudes indicated that migrants reported WNV RNA positive by the dead bird program probably were infected locally rather than on their overwintering grounds or during their northbound migration. The passage of these migrants through Coachella Valley after WNV infection was detected in *Cx. tarsalis* may indicate that these birds acquired their infection locally and then dispersed virus northward from southern California into the Central Valley. In agreement, during the summer of 2004, WNV rapidly moved >800 km northward from the Coachella Valley and Los Angeles basin into every county in California.

Although our research has yet to resolve the primary method for WNV overwintering in California, annual autumnal extinction and vernal re-introduction by northbound migrants does not seem to be an efficient and therefore likely mechanism. On-going projects are exploring chronic infection of birds and vertical infection of mosquitoes as local persistence mechanisms.

Table 2. Migratory birds reported dead by the public and tested for WNV RNA during spring and summer, 2003 – 2007.

	Spring		Summer		
Migrant species	WNV-	WNV+	WNV-	WNV+	Total
33 Positive species*	67	3	509	123	702
22 Negative species			46		46
43 Negative species	361				361
Totals	428	3	555	123	1,109

*Species that tested positive for WNV on 1 or more occasions

Virus isolation attempts. If migrants entering California had been recently infected during their northbound journey or acquired WNV infection in southern California, they could be viremic, but their sera negative for antibodies. To test this possibility, blood samples from birds collected from along the Salton Sea during the springs of 2006 and 2007 were expelled into viral diluent and immediately frozen on dry ice to preserve virus. Overall, 1,222 blood samples from 50 species were tested for WNV using a Vero cell plaque assay with negative findings. These samples were also screened for antibodies by EIA of which 6 were positive; of these, on two were confirmed by PRNT.

Host competence. Birds were mist netted in either the Coachella Valley or Kern County, tested to assure that they were serologically negative for WNV, SLEV and WEEV, and held in a mosquito-proofed and air conditioned facility where they were provided water *ad libitum* and fed live meal worms, finely ground trout food (Aqua Max Carnivorous, Purina Mills, St. Louis, MO) and cut citrus. After acclimation, birds were inoculated subcutaneously with 1,000 plaque forming units (PFU) of the NY99 strain of WNV. Birds were bled daily for 6 to 7 d by jugular puncture to monitor viremia response. Additional birds that were inoculated with viral diluent were maintained and bled as controls.

Orange-crowned warblers were the most susceptible of the three species tested for WNV, producing the highest mean

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Detection of Spotted Fever Group Rickettsia and *Borrelia burgdorferi* in San Diego County Rabbit Ticks

Nikos Gurfield[1], DVM, Dipl ACVP, Saran Grewal[1], MVSc., Deborah Doggett[1], Karen Ferran[2], Ph.D., and Rebecca LaFreniere[1]

[1]San Diego County Dept. of Environmental Health, Vector Control, 5570 Overland Avenue, Suite 102, San Diego, CA 92123, nikos.gurfield@sdcounty.ca.gov;[2] Early Warning Infectious Disease Surveillance Program, California Office of Binational Border Health, 5353 Mission Center Road Ste. 215,San Diego, CA 92108

INTRODUCTION

The spotted fever group of (SFG) rickettsia belongs to the Rickettsiaceae, a family composed of a mixed group of bacteria that live and divide within cells. The most pathogenic member of this group, *Rickettsia rickettsii,* causes the disease Rocky Mountain Spotted Fever (RMSF, Ricketts 1906). An outbreak of RMSF in Mexicali, Mexico in 2009 claimed 8 lives and sickened over 155 people (CDPH 2009). Because the epidemic originated from brown dog ticks (*Rhipicephalus sanguineus*), the San Diego County Vector Control Program, in collaboration with the Early Warning Infectious Disease Surveillance program and Project Wildlife, performed a regional study to assess the presence of spotted fever group rickettsia and other potential pathogens in different tick species along the southern border region (Eremeeva 2011). *Borrelia burgdorferi*, the causative agent of Lyme disease, is transmitted by *Ixodes scapularis* (deer tick), *Ixodes pacificus* (western black-legged tick) and *Amblyomma americanum* (lone-star tick) (Burgdorfer 1982, 1991); of these, only *Ixodes pacificus* is found in San Diego. This study resulted in tick pools of *Haemaphysalis leporispalustris* testing positive for spotted fever group rickettsia and other pools testing positive for *B. burgdorferi*. To our knowledge this is the first report of spotted fever group rickettsia and *B. burgdorferi* from rabbit ticks in Southern California.

METHODS

Ticks were removed from wild rabbits rescued and undergoing rehabilitation by Project Wildlife staff. Ticks removed from the same animal were pooled together, placed in 2.0 ml screw top tubes and refrigerated without a preservative until delivered to the San Diego County Vector Disease and Diagnostic Laboratory. Upon receipt, vector ecologists sorted the ticks by species, sex and life stage. Ticks were washed, cut in half to expose gut material and lysed. Real time PCR was performed using Applied Biosystems 7500 Fast with SYBR Green PCR Core Reagents to amplify a 100-150 bp segment of the rOmpA gene of *R. rickettsii* (GenBank accession number M31227.1, Eremeeva 2003). Primer sequences specific for the *B. burgdorferi* sensu stricto *ospA* gene were used (Ullmann 2005) to detect *B. burgdorferi*.

RESULTS AND DISCUSSION

Thirty-three pools of *H. leporispalustris* ticks were obtained from adult Western brush rabbits (*Sylvilagus bachmani*). Using real-time polymerase chain reaction testing techniques eight pools tested positive for SFG rickettsia (24.2%) and two pools (6.1%) tested positive for *B. burgdorferi*. Genetic sequencing of the rOmpA gene amplicons was attempted; however, sufficient sequence data was not obtained due to low copy number. Interestingly in one case, ticks removed from the ear of a rabbit as well as sections of the ear itself, tested positive for SFG rickettsia.

This is the first report of SFG rickettsia and *Borrelia burgdorferi* occurring in rabbit ticks in San Diego. The most common rickettsia found in rabbit ticks is a low-virulence strain of *Rickettsia rickettsii* (serotype Hlp [Hlp]) (Parker 1951). Although Hlp has never been associated with human disease, it has been shown to be lethal to human endothelial cells *in vitro* (Eremeeva 2001). Hlp has never been documented in southern California (Eremeeva, pers. comm.). A prior study of SFG rickettsia in southern California identified 1 out of 365 *Dermacentor occidentalis* ticks positive for *Rickettsia rickettsii* and 28 out of 375 *D. occidentalis* and *D. variabilis* ticks positive for the closely related 364D rickettsia (Wikswo 2008). A study of brown dog ticks (*Rhipicephalus sanguineus)* collected from Riverside County revealed one male tick containing *Rickettsia ricketsii* DNA (Wikswo 2007). Unlike *D. occidentalis*, *D. variabilis* and *R. sanguineus*, *H. leporispalustris* rarely bites humans, preferring to feed on rabbits and birds instead. Although the risk of people becoming infected from the bite of a rabbit tick is likely low, in Canada two suspect cases of Lyme disease in people bitten by *H. leporispalustris* have been reported (Banerjee 1995).

Borrelia burgdorferi is typically thought to be transmitted by *Ixodes* ticks. On the west coast, *Ixodes pacificus* is the primary vector. Although *B. burgdorferi* has been isolated from the kidneys of the eastern cottontail rabbit (*Sylvilagus floridanus*), rabbit ticks collected from infected rabbits did not yield positive cultures (Anderson 1989). This is in contrast to the SFG rickettsia detected in a rabbit and the ticks removed from it in this report. The Western brush rabbit and its ticks may represent a previously unrecognized reservoir for rickettsial and spirochetal pathogens in San Diego. Human clinical cases with symptoms of spotted fever-like disease or Lyme disease warrant investigation as to rabbit and rabbit tick contact.

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Spring Season Survey of Blackflies (Diptera: Simuliidae) in Santa Clara County

Noor S. Tietze and Nayer S. Zahiri

Santa Clara County Vector Control District, 1580 Berger Drive, San Jose CA 95112, 408-918-4770, noor.tietze@deh.sccgov.org, nayer.zahiri@deh.sccgov.org

ABSTRACT: Complaints for small biting flies along the headwaters of Coyote Creek in Morgan Hill, California, prompted the Santa Clara County Vector Control District to research and survey the species composition of blackflies (Diptera: Simuliidae) in the region. Sampling methods were developed to optimize collection of pupal stage simuliids from the riffled zones of creeks and streams. The survey yielded nine blackfly species: *Simulium vittatum, S. argus, S. piperi, S. aureum, S. vandalicum, S. saxosum, S. canadense, S. virgatum* and *Greniera abditoides*. *Simulium vittatum* and *S. argus* are known human pests that are widespread in distribution, while the other species blood-feed on birds or mammals including horses, sheep and cattle. During 2010 and 2011, immature blackflies were readily recovered from major creeks in populated regions of Santa Clara County. Further investigation into the distribution and composition of blackflies is warranted for this group of blood-feeding and potentially disease-transmitting flies.

INTRODUCTION

Santa Clara County has a network of over 800 miles of creeks and other waterways flowing through a variety of habitats ranging from manmade channels to natural riparian corridors. The county is largely composed of sprawling suburban neighborhoods that are frequently proximal or adjacent to these waterways that have seasonal or continuous flow during the course of the year. The creeks are managed by the Santa Clara Valley Water District and provide important aesthetic and recreational benefits to local communities

These same waterways have been the focus of vector control. Since its creation in 1989, the Santa Clara County Vector Control District has inventoried and monitored the known mosquito sources within creeks and other waterways to check for mosquito development and manage their development. The immature stages of the woodland malaria mosquito, *Anopheles punctipennis* have adapted to develop along flowing creeks, whereas *Culex pipiens* and *Culiseta incidens* develop in stagnant puddles within creek beds.

On certain occasions, however, residents living near creeks have complained about bites or irritation from small flies that do not resemble mosquitoes. Species of potentially biting or nuisance flies in the area include the local "no-see-ums" or valley black gnat (*Leptoconops torrens*), the canyon fly (*Fannia benjimini*) and simuliid blackflies. Preliminary investigations into these complaints recovered larval blackflies developing in creeks adjacent to the complainant's property, as well as recovery of adult blackflies in carbon dioxide-baited traps (EVS and Fay traps) placed there.

Due to the lack of scientific information on blackfly biology and ecology in our county, we conducted a survey to describe blackfly species composition and distribution. The goal of the survey was to identify what simuliid species occur in the county, but also whether anthropophilic species were present and how widely they were distributed.

MATERIALS AND METHODS

The survey for blackflies in Santa Clara County targeted pupal stage flies to facilitate identifications of species that are remarkably easier than that of other stages. Development of a pupal stage blackfly sampling device included attempts using a 20 inch piece of braided contractor chord attached to a six inch section of reinforcement bar. The device was placed into the riffled streambed of Coyote Creek in Morgan Hill, California during 2009. Preliminary tests using the latter device yielded larval and pupal stage specimens, however further tests using yellow and black caution tape (4 inches wide by 30 inches long) similarly attached to rebar, proved to be easier to recover from the streambed, as well as aided in locating and picking of the pupae off the device and provided a larger surface area for blackfly recruitment (Fig. 1).

Figure 1. Immature blackfly sampling device composed of 6 inch reinforcement bar and 20 inch length of yellow caution tape.

With the assistance of five District Technician III's, blackfly pupal sampling devices were deployed in major creeks countywide during April - May of 2010 and 2011. To preclude unintended "take", sampling activities were delayed for creeks hosting anadromous steelhead populations until May of each year, while upper reaches above reservoirs were sampled earlier.

During weekly sampling visits to each creek site, pupal stage blackflies were removed from the sampling device using fine-tipped forceps and placed into vials for transport to the laboratory. Later in the laboratory, specimens were pickled in 70% ethyl alcohol and using a dissecting microscope, identified the species present and number collected. Pupal stage blackflies were identified by number and configuration of respiratory filaments or gills, as well as by cocoon shape and other features using the taxonomic key in (Adler et al. 2004). During 2010, voucher specimens were submitted to Prof. Adler at Clemson University for assistance in species identification.

Blackfly collection data were stored in a Microsoft Access database that included geographic coordinates, number of pupae for each species type, date, collector and comments. The blackfly species distributions were plotted using ESRI ArcGIS 10 (Redlands, CA).

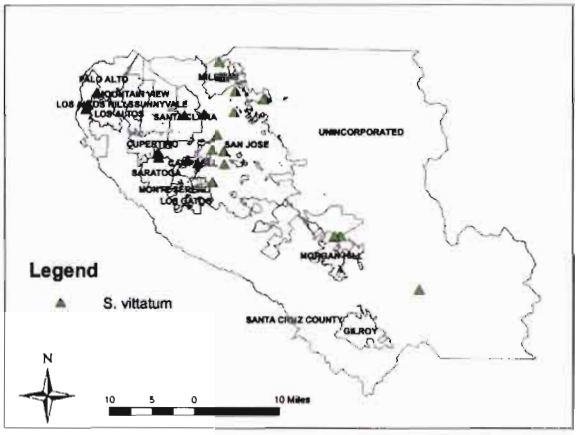
RESULTS AND DISCUSSION

After sampling in 2010 and 2011 a total of 83 samples were taken from 23 separate creeks resulting in a total of 574 pupal stage blackflies collected and identified (Table 1). In 2010, pupal blackfly voucher specimens were sent to Peter Adler for taxonomic verification. He identified or verified the identity of the 9 species mentioned in this paper. Only 14 of the samples were negative for blackflies or alternately, 83% of the samples taken contained one or more blackflies. Species richness for blackflies ranged from zero to seven species per creek, averaging 2.46 overall (Table 1).

Table 1. Blackfly species recovered by creek sampled in Santa Clara County in 2010 and 2011.

Creek Name	Blackfly Species								
	S. piperi	*S. vittatum*	*S. vandalicum*	*S. donovani*	*S. canadense*	*S. argus*	*G. abditoides*	*S. saxosum*	*S. virgatum*
Adobe	0	0	6	0	0	0	0	0	0
Almaden Golf Crs	0	0	0	0	0	0	0	0	0
Arastadero	31	6	2	4	0	0	0	0	0
Arroyo	0	26	0	0	0	0	0	0	2
Berryesssa	4	2	0	3	0	3	0	0	0
Calabazas	0	4	0	0	0	0	0	0	0
Calera	3	2	0	9	0	0	0	0	0
Coyote	9	25	3	0	0	0	0	0	0
Guadalupe	7	48	14	6	0	2	2	1	0
Hunting Hollow	6	1	0	0	0	0	0	0	0
Llagas	23	0	17	4	0	0	0	0	0
Los Alamitos	6	0	11	0	0	0	1	0	0
Los Gatos	0	4	1	0	0	0	0	0	0
Matadero	7	2	0	0	0	0	0	1	0
Penitencia	60	20	3	9	44	2	0	0	3
Randel	3	0	0	2	0	0	0	0	0
Rodeo	0	7	0	0	0	0	0	0	0
Ross	1	10	0	2	0	9	0	0	0
San Tomas	0	4	44	6	0	4	0	0	0
Saratoga	2	0	16	0	0	0	0	0	0
Silver	0	0	0	0	0	0	0	0	0
Spring	0	0	0	0	0	0	0	0	0
Upper Coyote	0	0	0	0	0	0	0	0	0

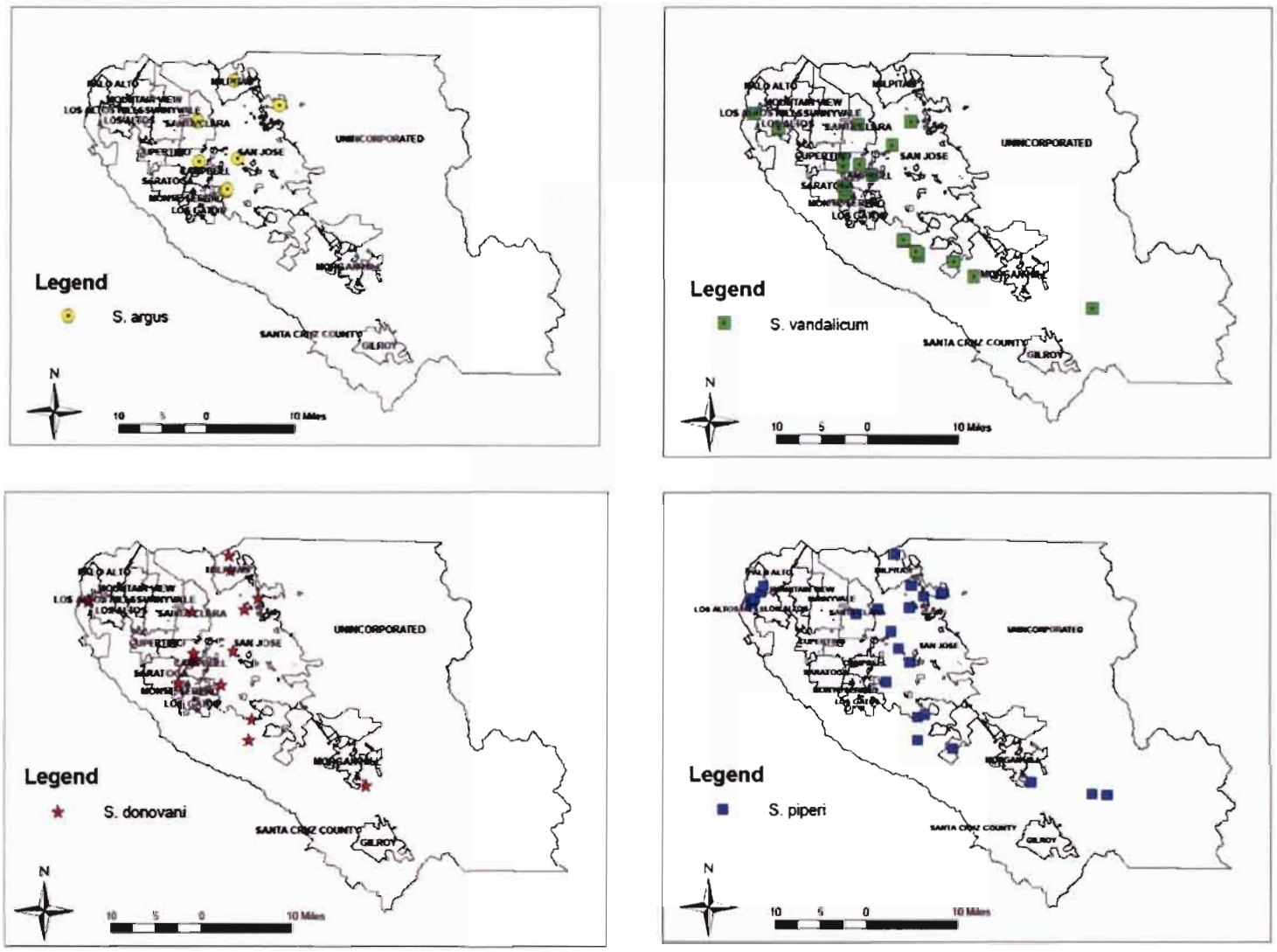
Figure 2. Geographic distribution of the blackfly, *Simulium vittatum* in Santa Clara County based on collections made in April and May of 2010 and 2011.



The geographic distribution of the anthropophilic or human pest species, *Simulium vittatum* was widespread occurring in large, medium and lower flow creeks county-wide (Fig. 2). About 25% of the locations sampled yielded specimens of *S. vittatum*. *Simulium vittatum* was found to be sympatric to *S. piperi* , *S. donovani, S. argus and S. vandalicum* on 5 or more occasions (Table 1).

In addition to *S. vittatum, S. argus, S. donovani, S. piperi* and *S. vandalicum* were widespread in geographic distribution during the April-May sampling periods (Fig. 3). *Simulium argus* was recovered in the northern half of Santa Clara County, while the others were found in samples from as far south as Morgan Hill and Pacheco Pass, east of Gilroy.

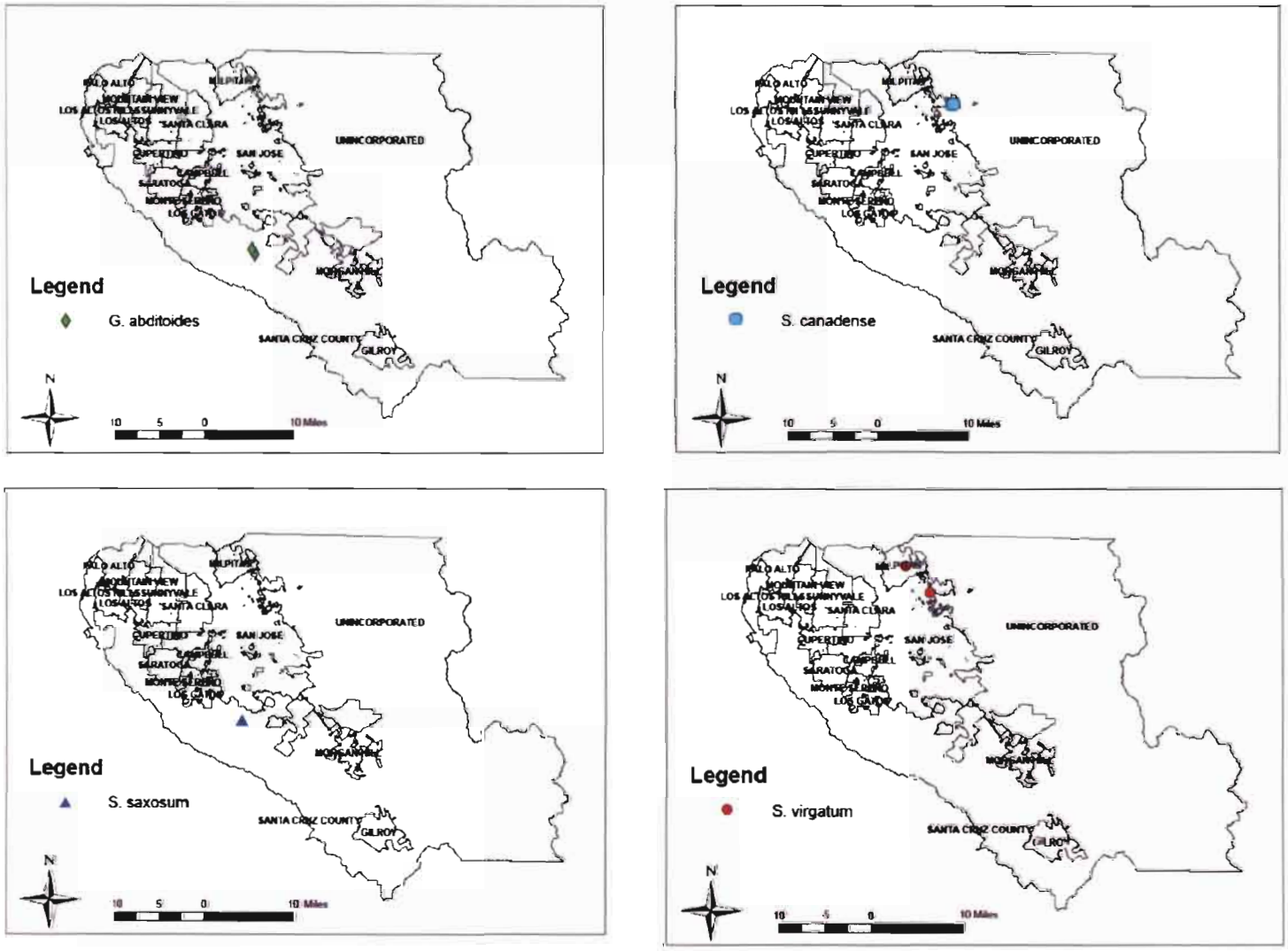
Figure 3. Geographic distribution of blackflies, *Simulium argus*, *S. vandalicum*, *S. donovani* and *S. piperi* in Santa Clara County based on collections made in April and May of 2010 and 2011.



Greniera abditoides, S. canadense, S. saxosum and *S. virgatum* were detected less frequently than the other blackfly species, typically at one or two sampling locations (Fig. 4). *S. canadense* was found to be locally abundant at one sampling site at the northeast end of the valley. *Greniera abditoides, S. saxosum* and *S. virgatum* were less abundant with totals of 3, 1 and 5 specimens collected, respectively. The lower number of sites for these species may be an artifact of our limited sampling duration that targeted mainly early-season species.

This survey found *S. vittatum* to be the most reputed anthropophilic species present in the county. That species is known to be transcontinental in distribution and biting pests of horses and cattle (Adler et al. 2004). Swarms of these blackflies can cause a severe nuisance to people although actual bites are rare. *Simulium piperi* was reported to bite humans in California (Coleman 1951), as well as horses, cattle and sheep as summarized in Adler et al. (2004). What is known of the host preferences for the other blackfly species include: *Simulium virgatum* and *S. argus* are known to feed on horses and cattle. *Simulium donovani* and *G. abditoides* are presumed ornithophilic; *S. vandalicum,* whereas the hosts of *S. saxosum* and *S. canadense* are unknown, but presumed to be mammals (Adler et al. 2004). In another study conducted in northern California by Anderson and Yee (1995), *S. argus, S. vittatum , and S. virgatum* were collected from ears of a horse model baited with carbon dioxide and a real horse. In the latter study, *S. griseum* and *S. trivittatum* were also collected from the model horse, but their preference was the model body underside as opposed to the ears.

Figure 4. Geographic distribution of *Greniera abditoides*, *S. canadense*, *S. saxosum* and *S. virgatum* in Santa Clara County based on collections made in April and May of 2010 and 2011.



CONCLUSIONS

In summary, this survey yielded an assemblage of blackflies developing in urban, suburban and rural creeks in Santa Clara County. While two species recovered in this survey are known to cause "severe nuisance" or biting to humans, other mammalophilic species may also be a pest to man. Further research is warranted into elucidating local blackfly host preferences, distance traveled when host seeking; and seasonal abundance in creeks throughout the year. Operational control measures against blackflies may only be approved once this potential pest's impacts on the community (i.e., humans and domestic animals) has been properly established.

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Surveillance for Mosquito-borne Encephalitis Virus Activity in California, 2010

Tina Feiszli[1], Kerry Padgett[1], Bborie Park[2], Bruce Eldridge[2], Ying Fang[2], William K. Reisen[2], Cynthia Jean[1], Erin Parker[1], James Glover[1], and Vicki Kramer[1].

[1] *California Department Public Health, 1616 Capitol Ave. Sacramento, CA 95814;* [2] *Center for Vectorborne Diseases, University of California, Davis, CA, 95616; tina.feiszli@cdph.ca.gov*

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 on, and contributed to, the West Nile Virus (WNV) component of the arbovirus surveillance program.

In 2010, the surveillance program elements included the following:

(1) Diagnostic testing of specimens from human patients exhibiting symptoms of encephalitis, aseptic meningitis, acute flaccid paralysis or with unexplained febrile illness of more than seven days.

(2) Diagnostic testing of specimens from horses exhibiting clinical signs of viral neurologic disease compatible with western equine encephalomyelitis virus (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 WNV diagnostic testing of dead birds and tree squirrels.

(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.

Only West Nile virus was detected in 2010; a summary of WNV infections by county is in Table 1.

Table 1. Infections with West Nile virus in California, 2010

County	Humans[a]	Horses	Dead Birds	Mosquito Pools	Sentinel Chickens	Dead Squirrels
Alameda	1	0	1	0	0	0
Alpine	0	0	0	0	0	0
Amador	0	1	0	0	0	0
Butte	1	1	6	7	7	1
Calaveras	1	0	0	0	0	0
Colusa	0	0	1	0	4	0
Contra Costa	5	0	8	4	4	0
Del Norte	0	0	0	0	0	0
El Dorado	1	0	2	0	0	0
Fresno	28	4	22	130	7	2
Glenn	2	0	3	0	0	0
Humboldt	0	0	0	0	0	0
Imperial	0	0	0	10	15	0
Inyo	0	0	0	0	0	0
Kern	15	1	13	277	86	0
Kings	2	0	0	65	0	0
Lake	0	0	0	3	0	0
Lassen	0	1	0	0	0	0
Los Angeles	4	0	41	57	2	1
Madera	10	5	13	9	9	0
Marin	0	0	0	0	0	0
Mariposa	0	0	0	0	0	0
Mendocino	0	0	0	0	0	0
Merced	2	0	14	9	8	0
Modoc	0	0	0	0	0	0
Mono	0	0	1	0	0	0
Monterey	0	0	0	0	0	0
Napa	0	0	0	0	0	0
Nevada	0	0	0	0	0	0
Orange	1	0	17	19	0	2
Placer	4	0	9	36	4	0
Plumas	0	0	0	0	0	0
Riverside	0	0	1	71	44	0
Sacramento	13	2	115	205	6	4
San Benito	0	0	0	0	0	0
San Bernardino	5	0	10	41	11	2
San Diego	0	0	2	1	0	0
San Francisco	1	0	0	0	0	0
San Joaquin	7	1	26	57	1	0
San Luis Obispo	0	0	0	0	0	0
San Mateo	0	0	0	0	0	6
Santa Barbara	0	0	0	0	0	0
Santa Clara	0	0	32	10	0	6
Santa Cruz	0	0	4	0	0	0
Shasta	0	0	4	0	0	0
Sierra	0	0	0	0	0	0
Siskiyou	0	0	0	0	0	0
Solano	0	1	1	1	2	0
Sonoma	0	0	0	0	0	0
Stanislaus	12	1	34	86	21	0
Sutter	0	0	1	26	13	0
Tehama	0	0	0	0	1	0
Trinity	0	0	0	0	0	0
Tulare	15	1	21	168	26	0
Tuolumne	0	0	0	0	0	0
Ventura	0	0	0	0	0	0
Yolo	0	0	14	11	5	0
Yuba	1	0	0	2	5	0
State Totals	**131**	**19**	**416**	**1,305**	**281**	**24**

[a]Includes asymptomatic infections

Table 2. Reported West Nile virus human cases by county of residence, California, 2003-2010

County	2003	2004	2005	2006	2007	2008	2009	2010	Incidence per 100,000 person-years
Alameda	0	0	1	1	0	1	0	1	0.03
Alpine	0	0	0	0	0	0	0	0	0.00
Amador	0	0	3	0	0	0	0	0	0.93
Butte	0	7	24	31	16	6	2	1	4.73
Calaveras	0	0	2	0	0	1	0	0	0.79
Colusa	0	0	2	4	2	1	0	0	4.73
Contra Costa	0	0	11	8	3	4	5	4	0.41
Del Norte	0	0	0	0	0	0	0	0	0.00
El Dorado	0	0	1	2	0	1	1	0	0.33
Fresno	0	11	59	11	17	3	13	23	1.74
Glenn	0	3	13	12	7	1	0	2	15.38
Humboldt	0	0	1	0	0	0	0	0	0.09
Imperial	1	1	1	1	3	0	0	0	0.46
Inyo	0	0	0	0	0	0	0	0	0.00
Kern	0	59	67	49	140	2	18	15	5.02
Kings	0	0	32	1	7	2	3	1	3.49
Lake	0	1	0	2	0	0	0	0	0.56
Lassen	0	1	0	0	0	0	0	0	0.33
Los Angeles	1	306	40	13	36	156	20	4	0.68
Madera	0	0	18	0	2	0	1	7	2.16
Marin	0	0	0	1	0	0	0	0	0.05
Mariposa	0	0	0	0	0	0	0	0	0.00
Mendocino	0	0	0	0	2	0	0	0	0.27
Merced	0	1	25	4	4	1	4	1	1.83
Modoc	0	0	0	2	0	0	0	0	2.31
Mono	0	0	0	1	0	0	0	0	0.84
Monterey	0	0	0	0	0	0	1	0	0.03
Napa	0	0	0	1	1	0	0	0	0.18
Nevada	0	0	4	1	0	0	0	0	0.61
Orange	0	62	17	6	9	71	4	1	0.66
Placer	0	1	35	8	4	6	0	3	2.05
Plumas	0	0	1	0	0	0	0	0	0.57
Riverside	1	109	103	4	17	62	3	0	1.67
Sacramento	0	3	163	15	25	13	0	12	1.99
San Benito	0	0	0	0	0	0	0	0	0.00
San Bernardino	0	187	33	3	4	36	2	5	1.55
San Diego	0	2	1	1	15	35	4	0	0.23
San Francisco	0	0	2	0	0	0	0	1	0.05
San Joaquin	0	2	34	8	10	12	10	6	1.38
San Luis Obispo	0	1	0	1	0	0	0	0	0.09
San Mateo	0	0	1	0	0	0	0	0	0.02
Santa Barbara	0	0	2	0	0	1	0	0	0.09
Santa Clara	0	1	5	5	4	1	0	0	0.11
Santa Cruz	0	0	0	0	0	0	0	0	0.00
Shasta	0	5	1	4	9	1	0	0	1.30
Sierra	0	0	0	0	0	0	0	0	0.00
Siskiyou	0	0	0	0	0	0	0	0	0.00
Solano	0	0	5	8	1	1	0	0	0.43
Sonoma	0	0	1	0	1	0	0	0	0.05
Stanislaus	0	0	84	11	21	17	14	12	3.55
Sutter	0	0	9	12	3	0	0	0	2.93
Tehama	0	10	4	6	4	4	0	0	5.34
Trinity	0	0	0	0	0	0	0	0	0.00
Tulare	0	3	56	6	10	5	4	12	2.57
Tuolumne	0	0	1	0	0	0	0	0	0.21
Ventura	0	2	1	3	1	0	0	0	0.10
Yolo	0	1	11	27	2	1	2	0	2.67
Yuba	0	0	6	5	0	0	1	0	1.87
Total WNV disease	**3**	779	880	278	380	445	112	111	**0.95**
Asymptomatic Infections[a]	0	51	55	14	29	53	17	20	
Total WNV infections	**3**	**830**	**935**	**292**	**409**	**498**	**129**	**131**	**1.03**

[a] WNV infections detected through blood bank screening; no associated illness reported

HUMAN DISEASE SURVEILLANCE

Serological diagnosis of human infection with WNV and other arboviruses was performed at the CDPH Viral and Rickettsial Disease Laboratory (VRDL), county public health laboratories and commercial diagnostic laboratories. Local laboratories tested for WNV using an IgM or IgG immunofluorescent assay (IFA) and/or an IgM enzyme immunoassay (EIA). Locally-tested specimens with inconclusive results were forwarded to 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 case of WNV in 2010 occurred in a 32-year-old female resident of Tulare County who developed symptoms compatible with West Nile neuroinvasive disease (WNND) on June 15. In total, 111 clinical WNV cases and 20 asymptomatic WNV infections were identified among residents of 21 counties in California (Table 1). Case incidence was highest (6.5 cases per 100,000 persons) in Glenn County (Fig. 1). Of the 111 clinical cases, 38 (34%) were classified clinically as West Nile fever and 73 (66%) were neuroinvasive disease (i.e., encephalitis, meningitis, or acute flaccid paralysis) (Fig. 2). The median age for all cases for which data were available was 56 years (range: 10 - 89 years) and 63 (57%) of the cases were male. The median ages for West Nile fever and neuroinvasive cases were 55 years (range: 23 - 89) and 57 years (range: 10 - 89 years), respectively. The median age of the six WNV-associated fatalities was 66 years (range: 49 - 75 years).

Figure 1. Human cases of West Nile virus infection by county, California 2010.

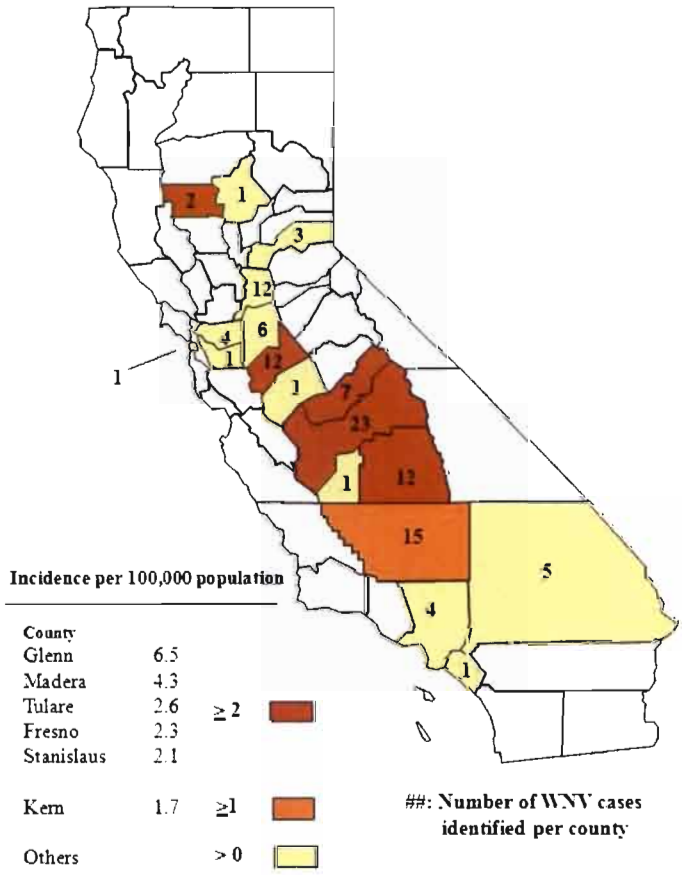


Figure 2. Human cases of West Nile virus infection by clinical syndrome, California 2004-2010. Data do not include cases with unknown clinical syndromes.

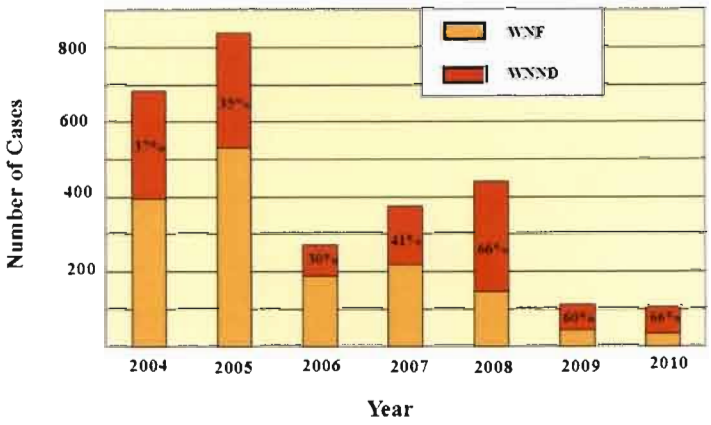


Table 3. Mosquitoes and sentinel chickens tested for St. Louis encephalitis[a], western equine encephalomyelitisa, and West Nile viruses, California 2010.

County	Agency	No. mosquitoes tested[b]	No. mosquito pools tested	WNV + pools	No. flocks	No. chickens	No. WNV positive flocks	WNV + sera
Alameda	Alameda Co. MAD	3,958	118	0	2	14	0	0
Alpine		0			0			
Amador		0			0			
Butte	Butte Co. MVCD	5,771	121	7	7	84	3	7
Calaveras	Saddle Creek CSD	0			1	10	0	0
Colusa	Colusa MAD	0			1	10	1	4
Contra Costa	Contra Costa MVCD	13,107	370	1	5	55	2	4
Del Norte		0			0			
El Dorado		0			0			
Fresno	Consolidated MAD	24,455	670	117	0			
Fresno	Fresno MVCD	1,058	39	3	2	14	2	4
Fresno	Fresno Westside MAD	10,859	250	10	2	12	1	3
Glenn	Glenn Co. MVCD	1,314	27	0	1	11	0	0
Humboldt		0			0			
Imperial	Coachella Valley MVCD	3,633	74	10	1	26	1	15
Inyo	Owens Valley MAP	1,772	40	0	0			
Kern	Delano MAD	0			2	20	2	14
Kern	Kern MVCD	58,528	1,470	263	10	122	9	70
Kern	UCD Arbovirus Field Station	1,979	102	4	0			
Kern	Westside MVCD	2,421	53	10	3	30	1	2
Kings	Consolidated MAD	385	11	2	0			
Kings	Kings MAD	27,628	773	63	0			
Lake	Lake Co. VCD	13,556	340	3	2	12	0	0
Lassen		0			0			
Los Angeles	Antelope Valley MVCD	877	25	0	8	48	1	1
Los Angeles	Greater LA Co. VCD	132,671	3,515	56	7	70	0	0
Los Angeles	Long Beach VCP	7,918	198	0	3	30	0	0
Los Angeles	Los Angeles Co. West VCD	12,549	399	0	19	114	0	0
Los Angeles	San Gabriel Valley MVCD	0			11	66	1	1
Madera	Madera Co. MVCD	2,655	87	9	2	22	2	9
Marin	Marin-Sonoma MVCD	434	10	0	1	6	0	0
Mariposa		0			0			
Mendocino		0			0			
Merced	Merced Co. MAD	3,636	105	8	8	48	4	8
Merced	Turlock MAD	5,783	147	1	0			
Modoc		0			0			
Mono	Mammoth Lakes MAD	0			0			
Monterey	North Salinas Valley MAD	0			2	20	0	0
Napa	Napa Co. MAD	1,334	45	0	3	31	0	0
Nevada	Nevada Co. Agric. Dept.	0			2	20	0	0
Orange	Orange Co. VCD	0			1	10	0	0
Placer	Placer Co. MVCD	36,104	1,496	36	7	42	2	4
Plumas		0			0			
Riverside	Coachella Valley MVCD	133,297	3,386	69	11	174	6	40
Riverside	Northwest MVCD	9,189	250	2	6	60	2	4
Riverside	Riverside Co. EH	28,830	677	0	5	60	0	0
Riverside	West Valley MVCD	50	1	0	0			
Sacramento	Sacramento-Yolo MVCD	63,627	4,779	205	9	71	4	6
San Benito	San Benito Co. Agric Dept.	0			1	10	0	0
San Bernardino	San Bernardino Co. VCP	18,192	789	21	10	100	4	11
San Bernardino	West Valley MVCD	6,447	296	5	8	16	0	0
San Diego	San Diego Co. EH	4,640	152	1	2	20	0	0
San Francisco		0			0			
San Joaquin	San Joaquin Co. MVCD	685	18	1	1	10	1	1
San Luis Obispo		0			0			
San Mateo	San Mateo Co. MVCD	58	2	0	1	10	0	0
Santa Barbara	Santa Barbara Co. MVMD	14,707	338	0	5	49	0	0
Santa Clara	Santa Clara Co. VCD	1,376	209	0	6	42	0	0
Santa Cruz	Santa Cruz Co. MVCD	543	12	0	2	20	0	0
Shasta	Burney Basin MAD	0			2	12	0	0
Shasta	Shasta MVCD	9,075	324	0	5	44	0	0
Sierra		0			0			
Siskiyou		0			0			
Solano	Solano Co. MAD	1,352	30	1	3	36	1	2
Sonoma	Marin-Sonoma MVCD	2,619	68	0	4	24	0	0
Stanislaus	East Side MAD	2,849	85	1	2	16	1	5
Stanislaus	Turlock MAD	44,288	1,156	80	3	36	3	16
Sutter	Sutter-Yuba MVCD	13,606	342	26	5	50	2	13
Tehama	Tehama Co. MVCD	0			3	30	1	1
Trinity		0			0			
Tulare	Delano MAD	0			1	10	1	7
Tulare	Delta VCD	39,836	983	154	3	30	3	19
Tulare	Kings MAD	93	6	0	0			
Tulare	Tulare MAD	4270	111	14	0			
Tuolumne		0			0			
Ventura	City of Moorpark VC	0			1	10	0	0
Ventura	Ventura Co. EH	973	23	0	3	30	0	0
Yolo	Sacramento-Yolo MVCD	24,146	1,265	11	4	29	1	5
Yuba	Sutter-Yuba MVCD	2,381	69	2	2	20	1	5
Total		**801,714**	**25,856**	**1,196**	**221**	**1,966**	**63**	**281**

[a]No mosquito pools or sentinel chickens were positive for SLEV or WEEV in 2010.
[b]Tested by University of California at Davis Center for Vector-borne Diseases or local mosquito/vector control agency. Only includes pools tested for all 3 viruses.
[c]Tested by California Department of Public Health Vector-Borne Disease Laboratory or local mosquito/vector control agencies.

EQUINE SURVEILLANCE

Serum or brain tissue specimens from horses with neurological symptoms were tested for arboviruses at the California Animal Health and Food Safety (CAHFS) laboratory. West Nile virus infection was detected in 19 horses from 11 counties (Table 1); none had been vaccinated against WNV. Five (26%) of the horses died or were euthanized as a result of their infection.

MOSQUITO SURVEILLANCE

A total of 801,714 mosquitoes (25,856 pools) collected in 34 counties were tested either at CVEC or at one of two local agencies by a real-time [TaqMan] reverse transcriptase-polymerase chain reaction [qRT-PCR] for SLEV, WEEV and WNV viral RNA (Table 3). Nine local agencies also tested an additional 119,256 mosquitoes (5,574 pools) for WNV or WNV/SLEV using either RT-PCR or a commercial rapid assay-RAMP® (Rapid Analyte Measurement Platform, Response Biomedical Corp).

West Nile Virus was detected in 1,305 mosquito pools from 24 counties; 1,286 were positive by RT-PCR, and 19 were positive by RAMP only (Table 1). Statewide, the minimum infection rate (MIR - defined as 1,000 times the number of infected mosquito pools divided by the number of mosquitoes tested) of WNV in mosquitoes was 1.2; the MIR was highest (4.4) in Kern County (Fig. 3). Since 2003, the MIR of WNV in CA has ranged between the low of 0.08 and the high of 2.1 (Fig. 4). West Nile virus was identified from seven *Culex* species (*Cx. erythrothorax*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. restuans*, *Cx. stigmatosoma*, *Cx. tarsalis* and *Cx. thriambus)* and three other species (*Aedes vexans, Anopheles freeborni, Culiseta incidens*) (Table 4). In 2010 the first detection of WNV in mosquitoes was from a *Cx. tarsalis* pool collected in San Bernardino County on May 4, and the last detection was from a *Cx. tarsalis* pool collected in Riverside County on November 23.

Table 4. Mosquitoes tested for West Nile virus, California, 2010.

Culex species	Pools	No. mosquitoes	WNV +	MIR[a]
Cx erraticus	4	189	0	0.00
Cx erythrothorax	1,823	66,614	5	0.08
Cx pipiens	6,467	107,747	243	2.26
Cx quinquefasciatus	9,440	332,390	555	1.67
Cx restuans	8	227	3	13.22
Cx stigmatosoma	826	10,571	9	0.85
Cx tarsalis	11,350	356,671	467	1.31
Cx thriambus	48	125	1	8.00
unknown	3	46	0	0.00
All *Culex*	**29,969**	**874,580**	**1,283**	**1.47**

Anopheles species	Pools	No. mosquitoes	WNV +	MIR
An franciscanus	13	346	0	0.00
An freeborni	99	3,030	1	0.33
An hermsi	73	1,596	0	0.00
All *Anopheles*	**185**	**4,972**	**1**	**0.20**

Aedes species	Pools	No. mosquitoes	WNV +	MIR
Ae dorsalis	6	133	0	0.00
Ae fitchii	1	50	0	0.00
Ae melanimom	257	10,254	0	0.00
Ae nigromaculis	1	29	0	0.00
Ae sierrensis	11	121	0	0.00
Ae squamiger	4	74	0	0.00
Ae vexans	24	745	1	1.34
Ae washinoi	33	1,386	0	0.00
All *Aedes*	**337**	**12,792**	**1**	**0.08**

Other species	Pools	No. mosquitoes	WNV +	MIR
Culiseta incidens	577	13,721	2	0.15
Culiseta inornata	63	817	0	0.00
Culiseta particeps	22	549	0	0.00
Coquilletidia peturbans	12	480	0	0.00
Psorophora columbiae	31	1,359	0	0.00
Unknown	234	11,700	18	1.54
All other	**939**	**28,626**	**20**	**0.70**

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

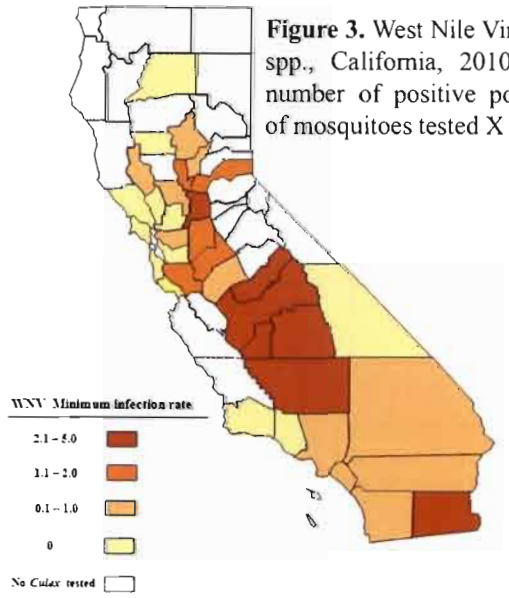


Figure 3. West Nile Virus in *Culex* spp., California, 2010. MIR = number of positive pools/number of mosquitoes tested X 1,000.

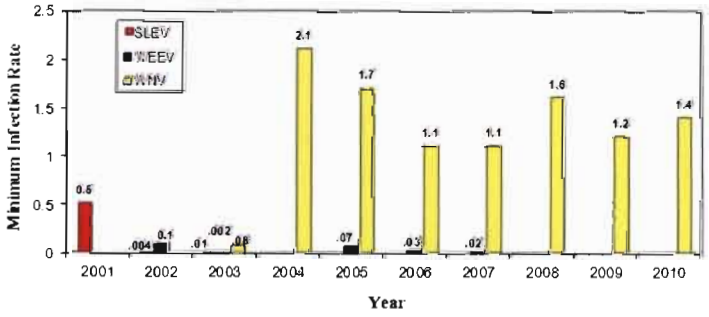


Figure 4. Minimum Infection Rate of St. Louis encephalitis virus, western equine encephalomyelitis virus and West Nile Virus in mosquito pools, 2001-2010. MIR=number of positive pools/ number of mosquitoes tested X 1,000.

CHICKEN SEROSURVEILLANCE

In 2010, 48 local mosquito and vector control agencies in 39 counties maintained 221 sentinel chicken flocks (Table 3). Blood samples were collected from chickens every other week and tested for antibodies to SLEV, WEEV and WNV by EIA at the CDPH Vector-Borne Disease Section Laboratory (VBDS). Positive samples were confirmed at the VBDS laboratory by IFA and western blot or by PRNT.

Out of 25,945 chicken blood samples that were tested, 281 seroconversions to WNV were detected among 63 flocks in 21 counties (Table 3, Fig. 5). Statewide, 14.3% of sentinel chickens seroconverted to WNV. Since 2003 WNV seroconversions in chickens have ranged between the low of 3.2% and the high of 30.4% (Fig. 6). In 2010 the first WNV seroconversions were detected in Imperial and Riverside counties on June 28, and the last seroconversion was detected in Riverside County on November 15.

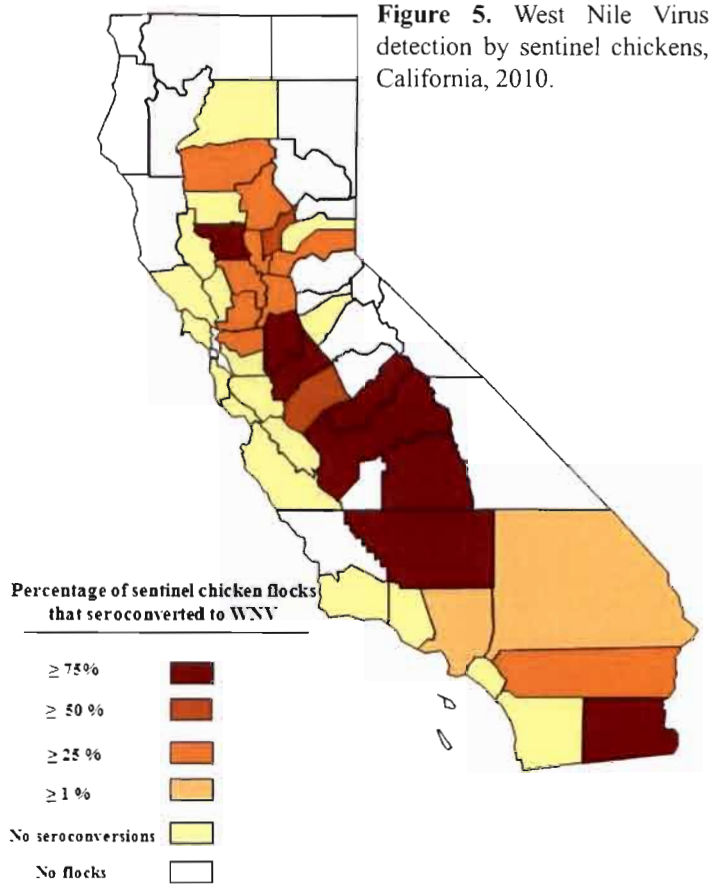
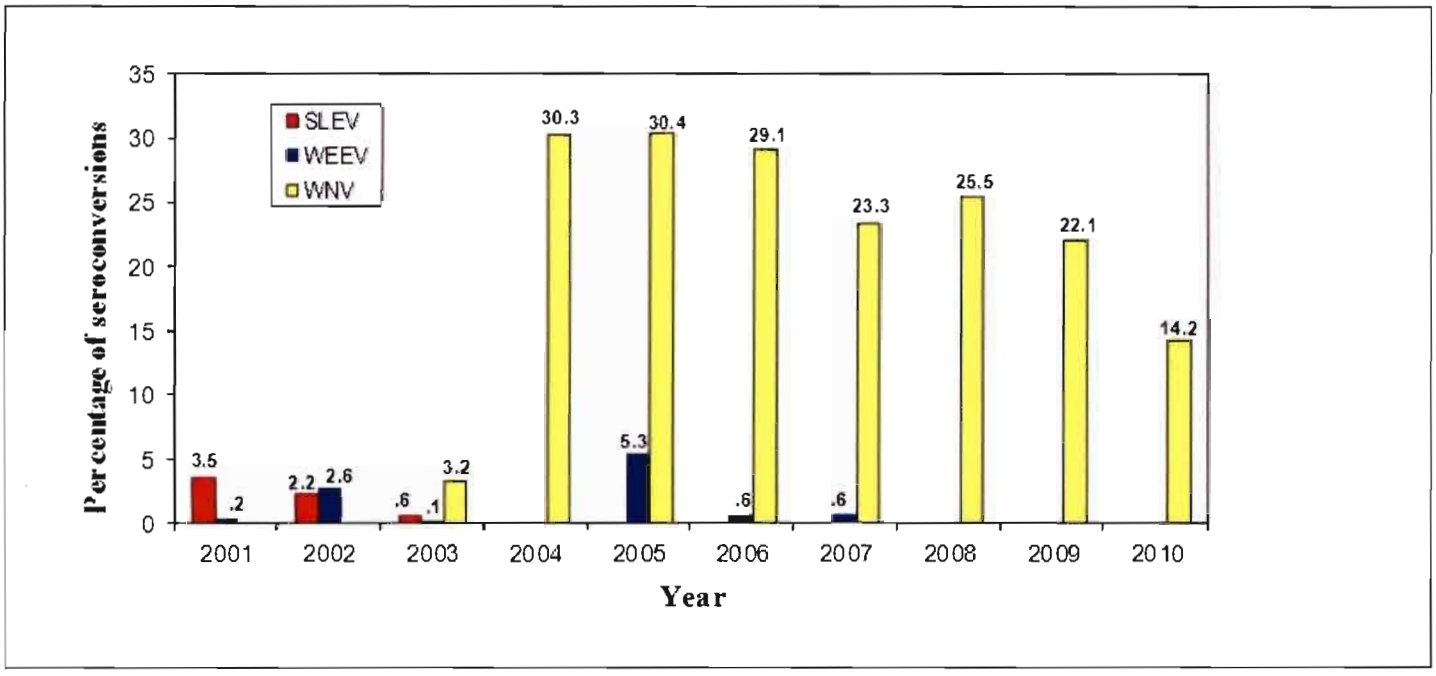


Figure 5. West Nile Virus detection by sentinel chickens, California, 2010.

Figure 6. Percentage of sentinel chicken seroconversions to St. Louis encephalitis virus, western equine encephalomyelitis virus, and West Nile Virus, 2001-2010.



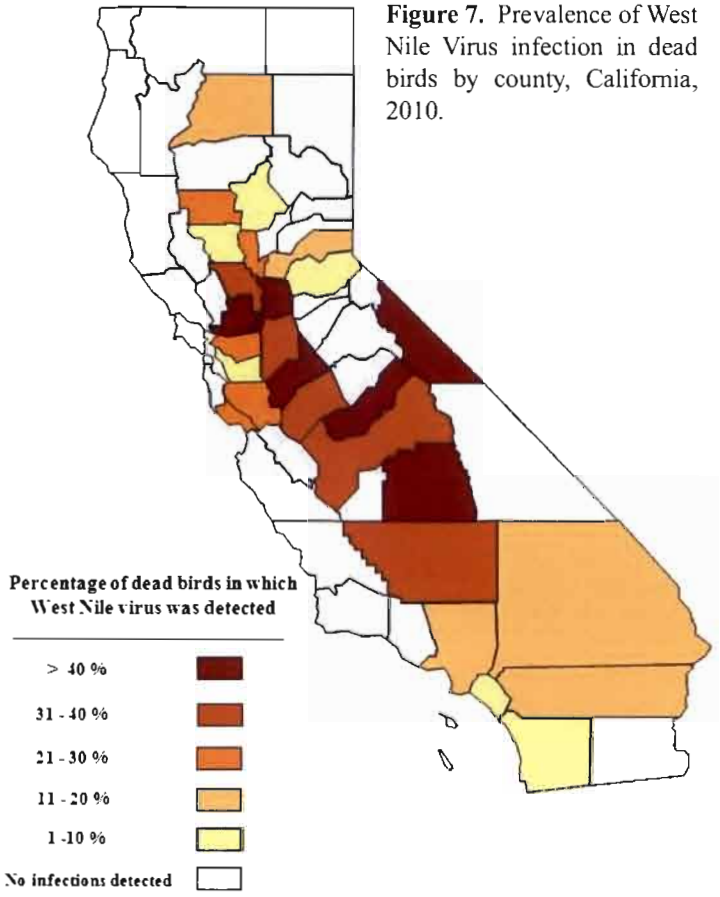
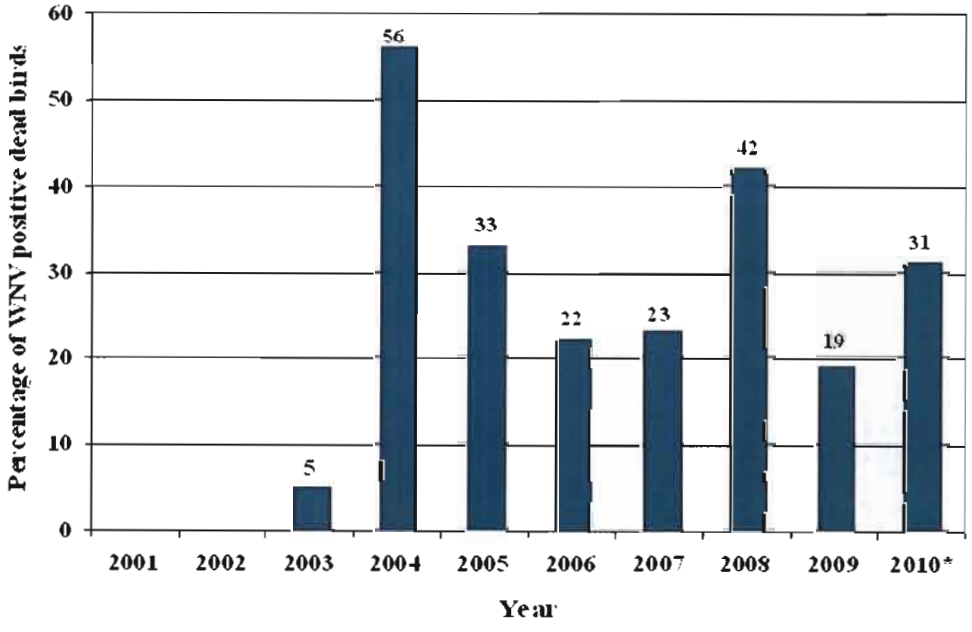


Figure 7. Prevalence of West Nile Virus infection in dead birds by county, California, 2010.

DEAD BIRD AND TREE SQUIRREL SURVEILLANCE

Established in 2000 and supported by a CDC Extended Laboratory Capacity grant, the WNV dead bird surveillance program is a collaborative program between CDPH, CVEC and over 130 local agencies. 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 2010 the WNV hotline and website received 10,462 dead bird reports from the public in 56 counties. Bird carcasses were tested either at CVEC by RT-PCR, or at one of 25 local agencies by RT-PCR, RAMP or VecTest (Medical Analysis Systems, Inc., Camarillo, CA). In 2010 CVEC began differentiating between chronic and acute infections in West Nile virus positive dead birds based on research conducted by CVEC and increased testing capabilities (Reisen et al. 2006; Fang et al. 2010). Of the 1,953 carcasses deemed suitable for testing, WNV was detected in 598 (31%) carcasses from 36 counties; 416 were reported as recent positives (acute infections), and 182 were reported as chronic positives. Of the recent positives, 373 were confirmed positive by RT-PCR, 31 by VecTest and 12 by RAMP (Tables 1 and 5, Fig. 7). Since 2003 the prevalence of WNV positive dead birds has ranged from the low of 5% to the high of 56% (Fig. 8). In 2010 the first WNV recent positive dead bird was an American crow reported from Los Angeles County on February 28, and the last recent positive was an American crow reported from Santa Clara County on December 17.

Figure 8. Prevalence of West Nile Virus infection in dead birds, California, 2001-2010. Data include chronic and recent infections.



In 2010, 415 dead squirrels were reported through the WNV Hotline; 108 squirrel carcasses were tested at CVEC, and WNV RNA was detected by RT-PCR in 24 (22%) carcasses from eight counties (Table 1). These included ten fox squirrels (*Sciurus niger*), eight eastern gray squirrels (*S. carolinensis*), five western gray squirrels (*S. griseus*) and one California ground squirrel (*Otospermophilus beecheyi*); notably, this was the first time WNV has been detected in a ground squirrel from California.

SUMMARY

In 2010, 131 human WNV infections were reported from 21 counties, comparable to the 129 infections reported in 2009. Although the number of reported human infections in 2009-2010 has greatly declined compared to previous years (Table 2), significant enzootic activity continues to be documented; these data together with the increasing proportion of WNND cases among all reported cases (Fig. 2) suggest that at least part of the decline in human infections may be due to underreporting of more mild infections.

In 2010 significant enzootic activity was detected in mosquitoes, dead birds and sentinel chickens throughout several counties, primarily those located within the south San Joaquin (SSJ) region; counties in this region include Fresno, Kern, Kings, Madera and Tulare. More than 50% of the reported human cases were residents of the SSJ region (Fig. 1), and correspondingly, this region reported the highest minimum infection rates in *Culex* mosquitoes (Fig. 3). Counties in this region also documented significant levels of transmission via sentinel chicken seroconversions and dead bird test results (Figs. 5 and 7).

Throughout California, enzootic data continued to document WNV activity during every season of the year, including the winter period. For the 3rd consecutive year, only WNV was detected. WEEV was last detected in California in 2007, and SLEV has not been documented since 2003, perhaps indicating continued competitive displacement with WNV.

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Maximizing Kill: Field Trial of VectoMax® CG on a Duck Club Pond in Contra Costa County

Steve Schutz[1], Eric Ghilarducci[1], Damien Clauson[1], Carlos Sanabria[1] and Stacy Bearden[2]

[1] *Contra Costa Mosquito & Vector Control District, 155 Mason Circle, Concord, CA 94520, sschutz@ccmvcd.net;*
[2] *Lucky 7 Research Services, LLC, P.O. Box 83, Berry Creek, CA 95916*

ABSTRACT: A field trial of VectoMax® CG was conducted on a duck club pond in the San Joaquin Delta area of eastern Contra Costa County during October 2010. The material was applied at the label rate of eight lbs per acre to an area of approximately 13 acres during flood-up, using a seed spreader mounted on a tracked all-terrain vehicle. A nearby 11 acre field was simultaneously treated with Altosid pellets to prevent adult emergence while serving as a control for comparison of larval dip counts. Dip counts were conducted along four-station transects of each field (four dips per station) pre-treatment and on days 2, 5, 7, 14, 21 and 28 post-treatment. Samples were counted and identified by species and stage (early instar larvae, late instar larvae and pupae). The product achieved approximately 95% suppression of *Aedes melanimon* larvae within 48 hours of treatment, compared with the control, based on Mulla's formula. In addition, we observed delayed colonization of the treated field by *Culex tarsalis* (counts remained well below one per dip until day 28, versus eight per dip in the control field at day five), very low pupal counts and good control of *Culex* larvae (80% or greater) for at least 28 days, after which the study was terminated due to the start of the hunting season. This product appears to be a good choice for mixed-brood habitats like duck club ponds because it provided rapid knock-down of single brood *Aedes* as well as long-term suppression of *Culex tarsalis*.

ACKNOWLEDGMENTS

We wish to acknowledge Valent Biosciences for providing material and protocols, David Wexler and Tim Mann for conducting the application, and Greg Howard for coordinating with the property manager to arrange the preparation and use of the fields.

Assessing the Risk of Sewer System Entry

Cheryl P. Sebay and Chindi Peavey

San Mateo County Mosquito and Vector Control District, 1351 Rollins Road, Burlingame, CA 94010, cpeavey@SMCMAD.ORG

ABSTRACT: Atmospheric gases were sampled in 689 sewer manholes to assess the health risk to vector control personnel conducting vector control work in these sites. The concentrations of carbon monoxide, hydrogen sulfide, methane and oxygen were measured in 689 manholes using a portable gas tester. Sample sites were classified as industrial, residential or commercial, and flat, lowland or sloped, high elevation neighborhoods. Carbon monoxide and hydrogen sulfide were not detected in any of the manholes. Methane concentrations at or above 10% of volume were detected in 1% of manholes tested in residential areas and 3% of those in industrial areas. Methane was undetectable in manholes in commercially developed areas. In a low proportion of sites (3 – 5% of those tested), oxygen levels were low enough to be considered hazardous (19.5% of volume or less). Carbon monoxide and hydrogen sulfide were not detected in any of the manholes tested.

INTRODUCTION

During the course of vector management activities, employees of the San Mateo County Mosquito and Vector Control District occasionally find it necessary to open sewer manholes. Manholes are the vertical pipes that connect sewer lines to the street surface; their purpose is to vent sewer gases and facilitate maintenance activities. District field staff open these manholes to search for sources of mosquito larvae or resting adult mosquitoes, and although they do not actually enter the sewers, they may be required to reach inside. In 2008, the District also became responsible for overseeing several municipal baiting programs for domestic rats in sewer manholes. The private pest control operator regularly reaches into these manholes to install poison bait blocks for rat control, and District staff must inspect their installations. Shortly after assuming oversight of this program, the District became aware of changes in regulations governing sewer opening and entry. Occupational Safety and Health Administration regulations at the federal (1910.146.App E) and state (Title 8, Section 5157) levels require that operators test for atmospheric gases prior to entering a sewer system. Entry is defined as actually climbing into the confined space or simply removing the cover and reaching into the opening (referred to as "breaking the plane"). Sewers may contain toxic gases including methane, carbon dioxide, sulfur dioxide and nitrous oxides. Methane is of particular concern because it is explosive. It is lighter than other gases and may concentrate at the top of the pipe, near the manhole opening. Therefore, methane is the gas that workers are of most likely to be exposed to while removing manhole covers and reaching into the opening. In addition, if the action of opening the metal cover of the sewer manhole were to generate a spark, there is risk of an explosion when methane levels are high. Lack of oxygen in sewer systems can also pose a hazard to workers. Oxygen is displaced when other gases are generated in sewers and it is consumed by the decay of organic material. If an operator enters a manhole or places his or her head inside the opening, low oxygen could lead to a loss of consciousness or asphyxiation.

Methane and other gases are formed by the decay of organic material in sewage, and their levels may vary depending on the speed with which material moves through the system. Gases can accumulate upstream of sewer line blockage and may be expected to reach higher concentrations in sections of sewer with little flow. In conversations with local public works departments, the view was expressed that manholes in hilly areas would be less likely to contain toxic gas because the sewage flows more rapidly through them. Some departments felt that high concentrations of these gases were more likely to occur in industrial areas than residential ones because industrial waste might be more likely to contain dangerous chemicals.

The following project was designed to assess the presence for gases at toxic levels. The distribution of manholes with toxic levels of gas was assessed to determine whether the risk of opening them could be predicted by their location.

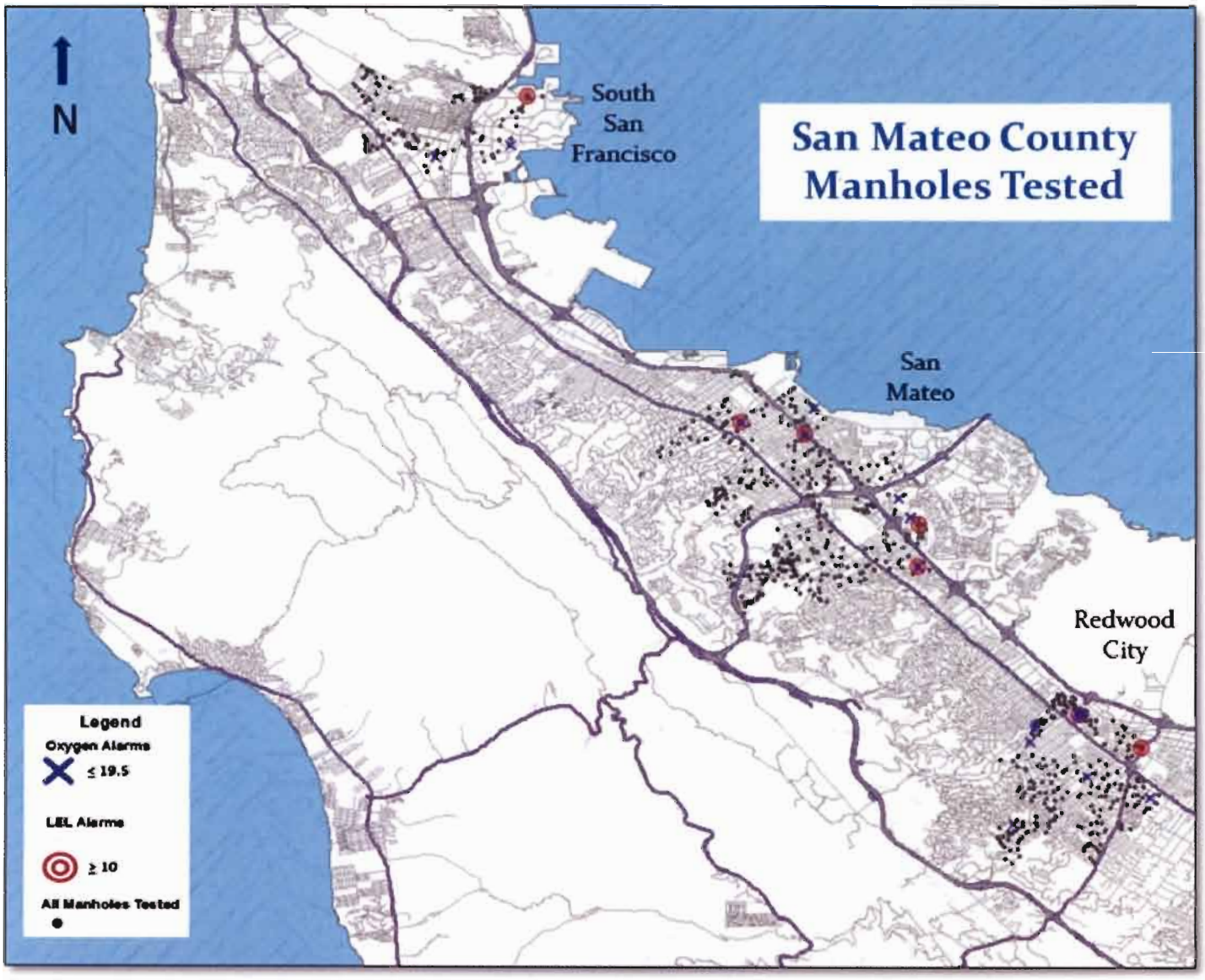
MATERIALS AND METHODS

Testing was conducted between August and October of 2010. Sewer manholes were sampled in three cities representing the north, central and southern parts of the county (Fig.1). All manholes sampled were among those in which the District oversees installation of poison bait blocks for rats, since these are the places in which District staff are most likely to open manhole covers during regular operations. Manholes were categorized as industrial, commercial, or residential. These were further categorized as being in elevated or lowland areas. Atmospheric gases were sampled in 689 manholes overall, representing 30% of the 2,183 sewer manholes in the sewer baiting program in these cities. The majority of manholes were located in residential areas. Thirty-eight manholes were located in industrial areas. This number was limited because San Mateo County does not have a great deal of industry and the manholes tested represented all of the manholes in the baiting program that lie in industrial areas. Similarly, the number of manholes baited for rats in areas of commercial development was limited by the number of baited manholes in commercial areas. The twenty manholes in

commercial areas sampled comprised most of those baited for rats in these areas (Figure 1).

A portable multi-gas detector (model M40, Industrial Scientific, Oakdale, PA) was used to measure the concentration of oxygen, hydrogen sulfide, carbon monoxide and methane in the sewer manholes. Devices of this kind are used by public works personnel to test sewer air before opening and entering manholes. Gases were sampled by inserting a hollow plastic tube from the gas detector into the sewer through a 1 inch diameter hole in the manhole cover. The tubing was inserted to a depth of 2 feet. This sample depth was considered a reasonable measure of the level at which vector control staff might risk exposure to dangerous gases. District staff do not climb down into the sewer access pipe, but simply lean over it to inspect or attach a wire to the upper part of its wall. A hydrophobic filter was installed in the tube to prevent fluid that might be present in the sewer from entering and damaging the testing device.

Figure 1. Site locations of 689 municipal sewer line manholes tested and among those, the specific manholes that went into alarm for low level oxygen and high levels of methane.



Note: LEL is a measure of the low explosive limit of combustible gases. For sewers, methane is the combustible gas that was tested.

RESULTS AND DISCUSSION

Methane. Methane was detected at levels high enough to set off an alarm on the sampling device in 7 of the 689 manholes tested overall (1%) (Table 1). The alarm sounds when the amount of methane present is above of 10% of the total volume of air present; 100% of volume represents the point of explosion (Table 1).

Table 1. The location and total number of sewer manholes that went into alarm for high levels of methane in San Mateo County.

Area	South San Francisco	San Mateo	Redwood City	Overall Total
Residential	0% (0/76)	1% (4/345)	1% (2/210)	1% (6/631)
Industrial	3% (1/34)	0% (0/2)	0% (0/2)	3% (1/38)
Commercial	0% (0/0)	0% (0/0)	0% (0/20)	0% (0/20)

Overall Total Manholes tested: 689
% = Manholes in Alarm/Total Manholes Tested

Six of the sites in which methane was detected at potentially dangerous levels were located in residential areas. Positive manholes represented 15% of sites tested in residential areas. Of the 20 manholes tested in industrial areas, only one contained an elevated level of methane; no methane was detected in any of the 38 manholes tested in commercial areas. Methane was present at hazardous level in both lowland and elevated neighborhoods and was not significantly higher in either (Fig. 1). Based on these results, dangerous levels of methane may occur in any type of development, including residential, and type of development does not indicate whether opening a manhole presents a safety risk.

Oxygen. Low oxygen levels (below 19.5%) were detected in 18 (3%) of the 631 manholes sampled in residential areas and 2 (5%) of the 38 manholes in sampled industrial areas. None of the manholes in commercial areas contained less than 19.5% oxygen (Table 2). Under normal atmospheric conditions, oxygen makes up 20% of the volume of air, levels between 15 and 19% decrease a person's ability to work strenuously. Oxygen concentrations below 15% of total volume cause impairment of coordination, perception and judgment even at rest. As with methane, the presence of dangerously low levels of oxygen cannot be predicted on the basis of development.

Other Gases. The sampling device did not detect carbon monoxide and hydrogen sulfide in any of the 689 total manholes sampled.

CONCLUSION

The overall risk of exposure to toxic levels of methane, hydrogen sulfide, carbon monoxide or oxygen while inspecting sewer manholes is low. However, some manholes did contain levels of these gases at levels considered to be dangerous and federal and state laws require measuring these gases before entering these confined spaces. District policy will now be to test atmospheric gases before removing manhole covers. District staff will use caution if manholes exhibit high levels of methane gas. When methane is detected at dangerous levels the staff will: 1) Reschedule inspection of that sewer manhole for another time to allow time for gas concentration to decline to a safe level, and 2) Consider selecting another manhole in the same vicinity for future bait placement. It is the duty of the District to maintain safe working conditions for the staff. Although the majority of manholes do not contain toxic levels of gas, some do and their status is not predictable based on location.

ACKNOWLEDGEMENTS

Thanks are due to operations and laboratory staff of the San Mateo County Mosquito and Vector Control District for collaborating efforts to complete the field study testing. Additionally, thanks to Tom Kirby from Industrial Scientific for his training of the District Staff and follow-up support on the use of testing equipment.

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The federal regulations under the Office of Safety and Health for entering sewer systems can be found at http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=standards&p_id=9797.

The state regulations under the California Department of Occupational Safety and Health can be found at http://www.dir.ca.gov/title8/5157e.html.

Using Data from a Sewer Baiting Program to Look for Patterns in Norway Rat Populations in San Mateo County

Theresa L. Shelton

San Mateo County Mosquito and Vector Control District, Burlingame, CA 94010, tshelton@smcmad.org

ABSTRACT: Norway rats are common urban pests that are involved with the spread of bacteria and serve as a host for flea vectors. Sewer baiting for Norway rats is done on a continual basis in eight cities or sanitary districts in San Mateo County, with a total of 3,384 baited sewer manholes. The bait is placed on a wire within the sewer manholes and inspected for evidence of rat activity every four months by a private pest control company that has contracts with the cities and sanitary districts. The San Mateo County Mosquito and Vector Control District has been overseeing the program since 2008 and has accumulated more than two years of data on inspection results. In order to better understand the distribution of the Norway rat in the county and potentially make changes to the program to improve efficacy, ArcMap 2010 software was used to analyze the data. Two years of data (six inspection periods) were included for each city or sanitary district. Seasonal patterns, yearly variation and variability among cities were examined, and cluster analysis was used to identify areas with particularly high levels of rat activity.

INTRODUCTION

Baiting programs for the Norway rat, *Rattus norvegicus*, in municipal sewers can be effective at keeping populations at a minimum and lessening the interactions between rats and people (Brooks 1962). Norway rats are undesirable in business and residential communities because of their role in the spread of disease. They serve as hosts for fleas that vector plague and typhus. Additionally, they can transmit viral and bacterial diseases including rabies, rat bite fever, salmonella and leptospirosis (Meehan 1984).

In San Mateo County, eight cities and sanitary districts participate in a sewer baiting program. The participating cities and sanitary districts have contracts with a private pest control company, Dewey Pest Control, to place bait in sewer manholes and periodically inspect for rat activity. Bait blocks were placed in 3,384 sewers throughout San Mateo County (Fig. 1). The bait blocks are inspected by a technician from Dewey every four months. The technician records signs of rat activity, whether the bait needed to be replaced or reinstalled, or if it appears untouched. The work performed by Dewey Pest Control is overseen for the cities and sanitary districts by the San Mateo County Mosquito and Vector Control District.

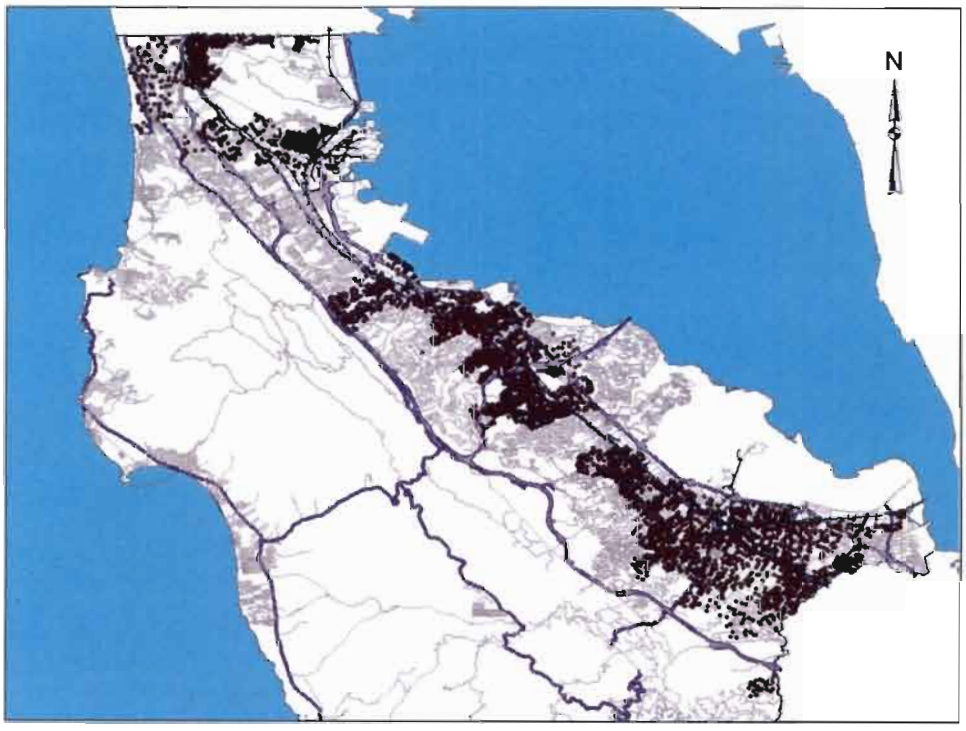

Figure 1. Location of rat baited sewers within San Mateo County.

METHODS

The technician from Dewey Pest Control recorded the status of the bait blocks on a large cardboard map. The map is submitted to the Mosquito and Vector Control District, where the results are entered into a Microsoft Access database. The data are linked to ArcGIS maps, showing the placement of the baited sewers and their associated inspection results throughout San Mateo County.

Two years of inspection results were analyzed to determine patterns or trends in the distribution of Norway rats in San Mateo County and to assess the effectiveness of the sewer baiting program at keeping the population under control. Three questions were addressed by analyzing these data:

1) Does the amount of rat activity vary seasonally?
2) How much variability of rat activity is detected between the two years?
3) Are there areas of the county with particularly high levels of rat activity?

The season variability of rat activity was tested with one-way ANOVA. The variability between years was tested with a paired t-test, with the level of rat activity in 2008-2009 and 2009-2010 as a pair for each city or sanitary district. To search for geographic areas with higher than normal areas of rat activity (i.e., "hotspots"), I performed cluster analysis with ArcGIS 10 software using the Average Nearest Neighbor tool. This tool considers the distance between sewers with rat activity and compares it with the average expected distance assuming the sewers with rat activity are distributed randomly (Allen 2011). In order to have a large enough sample size, I combined results from all the inspection dates for each of the cities or sanitary districts.

RESULTS

Rat activity was low throughout the county, with indications of rat activity in only 3-4% of sewers countywide during any season (Fig. 2). No significant differences in rat activity were detected among any of the seasons, and overall, rat activity appeared to be consistent but minimal year round. Rat activity in sewers was also consistent between the two years of 2008-2009 and 2009-2010. When separated by city and sanitary district, no significant differences were observed. The highest level of rat activity occurred in the Bayshore sanitary district during 2009-2010, with almost 9% of bait in sewers showing signs of rats. Bayshore sanitary district had the highest percentage of active sewers during both years. The city of San Mateo had the lowest percentage of active sewers between the two years, with less than 3% of sewers having signs of rats during both years.

Most areas of the county did not have any hotspots of rat activity. However, there were clusters present in portions of three cities: Redwood City, South San Francisco and Menlo Park. Additionally, parts of Burlingame and San Mateo, although they did not have statistically significant clusters, were less non-randomly distributed than most other areas of the county. More years of data might show definite hotspots in these areas.

CONCLUSIONS

The consistently low level of rat activity throughout the baited regions of the county suggests that the sewer baiting program is effective at keeping Norway rat populations under control. The program, which has been continuous for decades, may explain why San Mateo County appears to have fewer Norway rats than other bay area counties. Other factors that can limit the size of Norway rat populations are age and condition of the sewers and the availability of food around the breaks in the lines. Some rats are territorial and do not venture from the sewer lines into the areas under manholes (Bentley et al. 1958).

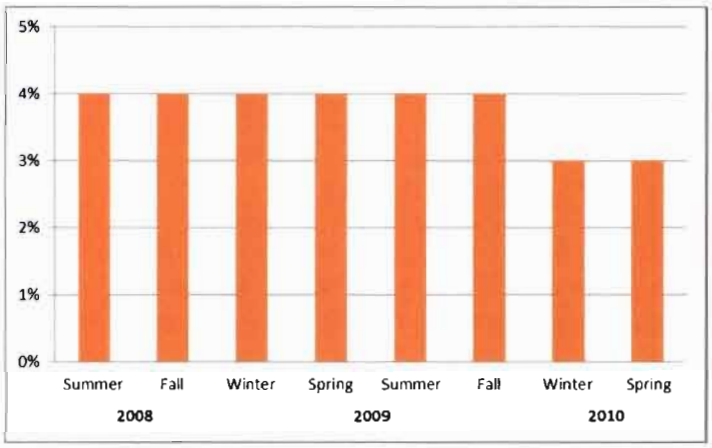

Figure 2. Seasonal variation of rat activity; bars represent percentages of sewers with signs of rat activity. No difference in the amount of rat activity was observed among seasons.

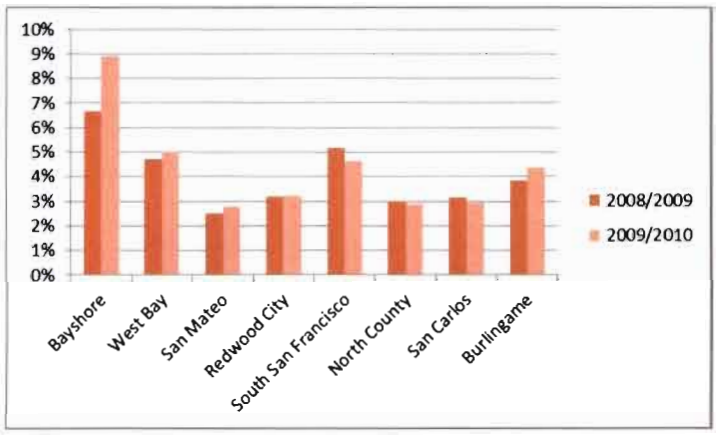

Figure 3. Annual variability in levels of rat activity within each city or sanitary district. Darker bars represent percentage of sewers with signs of rats from summer 2008 to summer of 2009. Lighter bars represent percentage of sewers with signs of rats from summer of 2009 to summer of 2010. Although levels of rat activity varied among cities and sanitary districts, none of them had significantly different levels of activity between years.

The evenness throughout the seasons and between years is probably because of the stability in environmental conditions within the sewers. They remain humid and fairly warm at all times because of the underground shelter. In addition, the coastal climate of San Mateo County does not have the irregularity of temperatures and weather that are present in inland areas.

Although there were few geographic clusters of rat activity, a few were detected using the ArcGIS cluster analysis. The district can attempt to improve the sewer baiting program by shifting bait from areas of little rat activity into these areas of high activity. Continual collection of data will allow the district to observe whether this method is successful in further reducing rat activity or results in new hotspots of rats in areas that previously had low levels. With additional years of data, rat activity hot spots might become more apparent. Thus far, data analysis is limited by small sample sizes from overall low levels of rat activity.

The San Mateo County Mosquito and Vector Control district intends to continue collecting inspection results and annually analyzing it for trends or patterns to better understand the Norway rat population in the county. We are also incorporating a field study with census blocks to observe whether rat activity levels have changed in a section of the county that has stopped participating in the baiting program in September 2009 (nineteen months prior to data analysis). With more information about the distribution of Norway rats the district can better protect county residents from the risk of rodent-borne diseases.

ACKNOWLEDGMENTS

I gratefully acknowledge Dewey Pest Control for their work inspecting the sewer bait, especially technician Mitsu Kimura. I thank the sanitary districts Bayshore Sanitary District, North County Sanitary District and West Bay Sanitary District and the cities of Burlingame, Redwood City, San Carlos, San Mateo, and South San Francisco for their participation in the baiting program. I also thank Chindi Peavey of the San Mateo County Mosquito and Vector Control District for her guidance on this project.

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Assessment of Nuvan ProStrips+® Application in Storm Drain Manhole Chambers

Steven F. Vetrone, Paul O'Connor and Susanne Kluh

Greater Los Angeles County Vector Control District, 12545 Florence Ave. Santa Fe Springs, CA 90670, svetrone@glacvcd.org

ABSTRACT: The underground storm drain systems (USDS) within Los Angeles County serve as ideal larval habitat, and adults of certain mosquito species often utilize storm drain manhole chambers as resting sites. While routine larvicide applications achieve adequate control of larval populations within these systems, resting adults are unaffected. In an attempt to reduce these populations further, adulticide trials were conducted in various USDS throughout the Greater Los Angeles County Vector Control District service area. In particular, this study used a multi-step approach to investigate the efficacy of Nuvan ProStrips+® (AMVAC Chemical Corp.) when applied to the manhole chambers. This included testing for repellency associated with the pesticide, efficacy of label recommended dosage at varying depths and the longevity of the product in actual field conditions. Preliminary results show that Nuvan ProStrips+® were effective at controlling mosquitoes within manhole chambers to limited depths and demonstrated no repellency, suggesting that Nuvan ProStrips+® could be used as a viable control method for resting and overwintering mosquito populations within USDS manhole chambers.

INTRODUCTION

The underground storm drain systems (USDS) within Los Angeles County are a complex matrix of catch basins, drains, manhole chambers and lateral trunk line conduit of varying lengths and diameters. When properly maintained, these systems collect storm water and urban runoff and channel it to the ocean. Improper construction, lack of maintenance and accumulation of debris can all result in the retention of water within the system. USDSs have long been recognized as a major problem for their ability to produce large numbers of mosquitoes. These systems can maintain very stable environmental conditions in terms of temperature and humidity, creating ideal larval habitat for some species of mosquitoes (Dhillon and Mulla 1982, 1983, 1984). In Los Angeles County the vast majority of mosquitoes collected from these systems are *Culex quinquefasciatus* Say (Kluh et al. 2006).

The service area of the Greater Los Angeles County Vector Control District (GLACVCD) sits atop more than 8000 miles of city and county managed USDS. Currently the only strategy used by the USDS Program at GLACVCD is the routine application of larvicides to control mosquito populations. Although these treatments achieve a satisfactory level of larval control, they have no effect on populations of resting and overwintering adult mosquitoes that find refuge within the manhole chambers. Earlier studies have demonstrated that resting and overwintering mosquitoes showed a preference for the vertical surfaces within the USDS, particularly the upper portions of manhole chambers (Dhillon and Mulla 1983, 1984, 1985). Accordingly, the search to find an effective method of control for these mosquitoes has been an ongoing pursuit at GLACVCD in recent years (Kluh et al. 2006). The current study assessed the efficacy of Nuvan ProStrips+® (AMVAC Chemical Corp. Los Angeles, CA) for use as a non-residual adulticide within manhole chambers using a multi-step approach.

MATERIALS AND METHODS

Study sites. *Repellency:* A manhole chamber with a diameter of 3 ft. and a depth of 4 ft. located at 1000 N. Figueroa Ave., Wilmington, CA was chosen due to its history of producing large numbers of *Cx. quinquefasciatus* mosquitoes year-round.

Efficacy and longevity: For the final two phases of the field trials, mosquito production was not considered as site selection criteria because sentinel cages were utilized to determine mortality. The sites were chosen based on sufficient depth of the manhole chambers, safe traffic conditions as well as the proximity to our facility. Two adjacent manholes located on the 16000 block of Foothill Blvd, Sylmar, CA, served as the study site for these trials. Both manhole chambers had a diameter of 3 ft., a depth of 8 ft., and were spaced 0.1 miles apart. One manhole chamber was used as the untreated control.

Manhole chamber insert. In an effort to investigate the efficacy of Nuvan Pro-Strips+® thoroughly, we first needed to determine if the pesticide possessed any repellent properties. Any repellency would negate the possibility of the product being used successfully in the USDS. Toward that end, we fabricated a device that, when deployed within the manhole chamber, would allow mosquitoes to enter and exit the treatment area freely, while also having the ability to collect any mosquitoes that would die as a result of exposure to the pesticide. The device was constructed of 24 inch length of 6 inch diameter polyvinylchloride (PVC) pipe, attached to which were eight 12 gauge steel wire, articulated arms. Fine mesh bridal veil was used to cover the wire creating a collection basket (Fig. 1). The hinged articulated arms prevented the device from becoming entangled with the access ladders during deployment and retrieval. The device was also outfitted with two color-coded lanyards; one lanyard was used to control the position of the arms while the other lanyard supported the entire device when it was suspended inside the manhole chamber at the desired depth.

Pesticide. The pesticide used was an 18.6% dichlorvos impregnated resin strip called Nuvan ProStrips+®. For repellency and efficacy trials, the full strip (65 grams) was cut into 4 gram strips in order to match the label specified application rate with the volume of the manhole chamber (56.6 ft^3) at test sites. The longevity portion of these field trials required the use of the full strip, as the manhole chamber was not a closed system. The full size strip will treat an area of 900- 1200 ft^3 for the duration of 16 weeks as indicated by the label.

Study Design. The purpose of this study was to investigate the efficacy of Nuvan ProStrips+® when used in manhole chambers associated with USDS to control mosquitoes. In order to do this effectively, the study was conducted in three phases. We examined the potential repellency of the pesticide, its efficacy when used for this application and the longevity of the product under actual field conditions.

Repellency trial: A qualitative approach was taken to establish if the Nuvan ProStrips+® exhibited any repellency. Prior to the actual repellency evaluation, tests were conducted to establish whether or not the manhole chamber insert did in fact allow mosquitoes to freely enter the treatment area. First, a CO_2 baited EVS trap was placed in the manhole chamber for 24 hours to estimate the adult population density without the chamber insert in place. This was then repeated with the chamber insert in place (Fig. 2), with the trap suspended between the device and the manhole lid. Each trapping event spanned consecutive 24 hour periods in the same manhole chamber.

Figure 1. Manhole Chamber Insert. The construction of the manhole chamber insert consisted of a 24 inch by 6 inch diameter PVC pipe, to which wire arms and fine mesh bridal veil were attached. From left to right, manhole chamber in closed position (A), in an open position (B) and deployed within a manhole chamber (C).

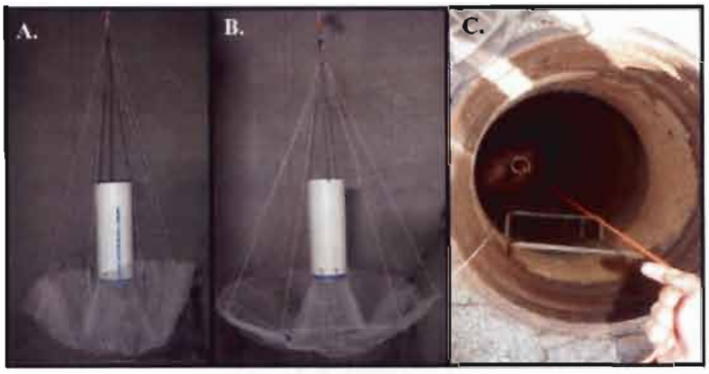
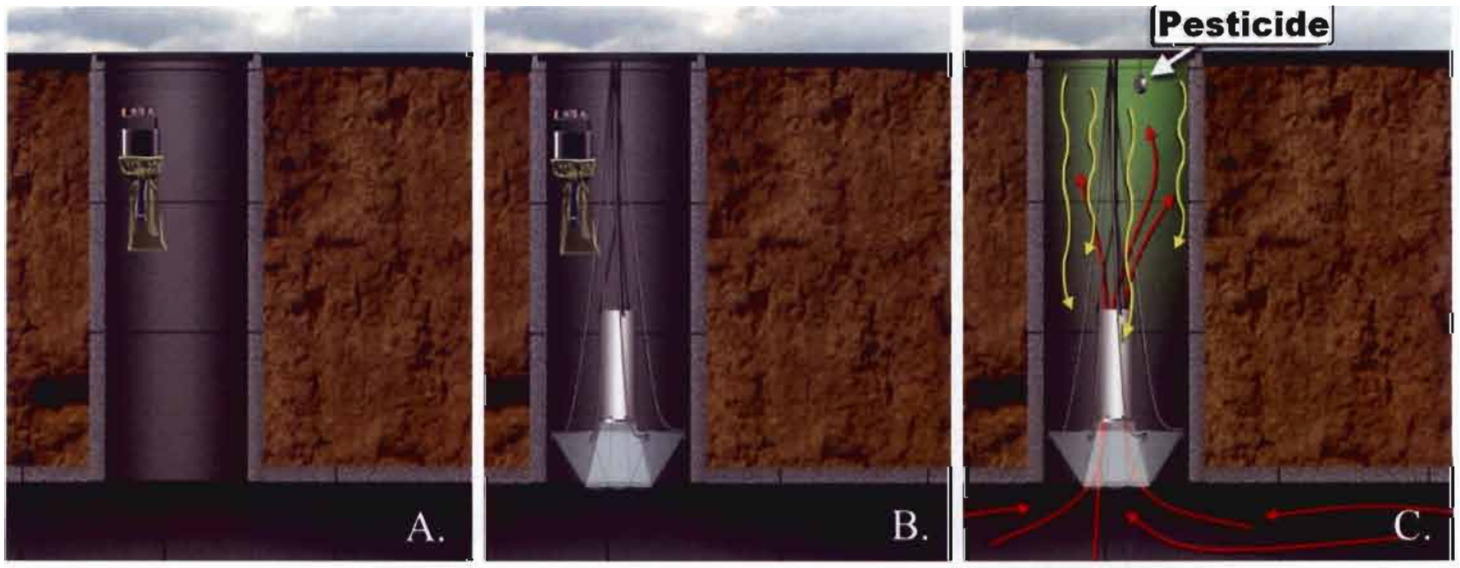


Figure 2. Repellency Trial Study Design. In order to evaluate thoroughly the efficacy of Nuvan ProStrips+® as a possible adulticide for USDS, repellency trials were conducted. From left to right, EVS trapping within manhole chamber (A), EVS with manhole chamber insert (B), and manhole chamber insert with pesticide (C).



A third trial was conducted to assess the ability of the mosquitoes to exit the manhole chamber insert through the 6 in. diameter tube running through the center of the device. The chamber insert was left in place for seven days (without a CO_2 trap) at which time the netting was examined for any dead mosquitoes. Mosquitoes unable to find or utilize the exit tube would have desiccated and died.

The actual repellency evaluation was conducted by suspending the pesticide strip in the manhole chamber with only the manhole chamber insert in place. The content of the netting on manhole chamber insert was inspected for mortality after seven days.

Efficacy trials: Caged sentinel mosquitoes were employed to assess the efficacy of Nuvan ProStrips+®. Four cages were suspended at two foot intervals down to a depth of eight feet. An untreated control was run concurrently in the adjacent manhole chamber 0.1 miles away. Each cage contained 25 laboratory reared *Cx. quinquefasciatus* mosquitoes. The sex ratios for each cage varied throughout the trials. The sentinel cages were exposed at various depths to four grams of the Nuvan ProStrips+® for a 24 hour period. The pesticide was suspended from a bar just below the manhole lid. A new piece of the Nuvan ProStrips+® was used for each replicate trial (n = 6).

Longevity trials: This phase of the field study was conducted in two stages using either 4 gram strips or full-size 65 gram strips. Sentinel cages were exposed to the pesticide for a 24 hour period every seven days.

RESULTS AND DISCUSSION

Repellency Assessment: Manhole chamber insert performance trials demonstrated that the device had little effect on mosquito movement into the manhole chamber as CO_2 trap counts with and without the chamber insert in place yielded similar numbers. The results of the third trial in the same manhole chamber demonstrated the device also allowed mosquitoes to exit the manhole chamber as no dead mosquitoes were found in the netting after seven days where mosquito activity was known to occur. No repellency of mosquitoes to Nuvan ProStrips+® was detected in our trials during the seven day exposure period in the test chamber. A total of 51 *Cx. quinquefasciatus* were collected from the netting of the manhole chamber insert, strongly suggesting the pesticide exhibited no repellency. This assessment is based on the two preceding performance tests conducted with the manhole chamber insert, demonstrating that the mosquitoes were able to exit the treated area if the pesticide had any repellent characteristics. Mosquitoes entered the treated area, either through the hole in the manhole lid or from within the USDS, through the opening in the device and died as a result of exposure.

Efficacy Assessment: The results of the efficacy trials showed higher mortality rates as a result of exposure to the pesticide, when compared to the control, through all depths of the manhole chamber (Fig. 3). However, statistical significance was only obtained for the 2 and 4 ft depths (p= 0.0001 and 0.0002 respectively). This finding indicates that the pesticide did not reach a sufficient concentration at the 6 and 8 ft depths for effective control. A possible explanation could be that the sentinel cages at these depths are closer to the trunk line of the USDS, making them more susceptible to air movement within the system. As a result, the concentration of the pesticide is diluted below effective control levels.

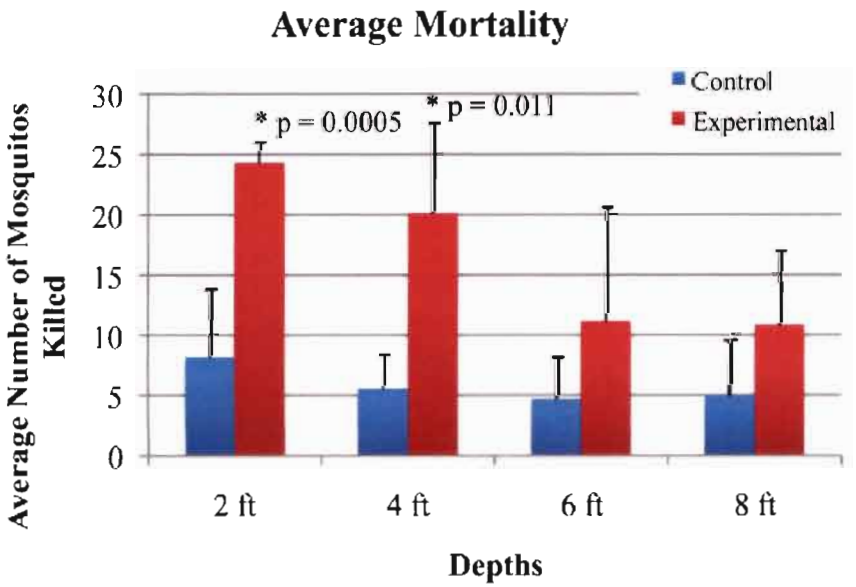
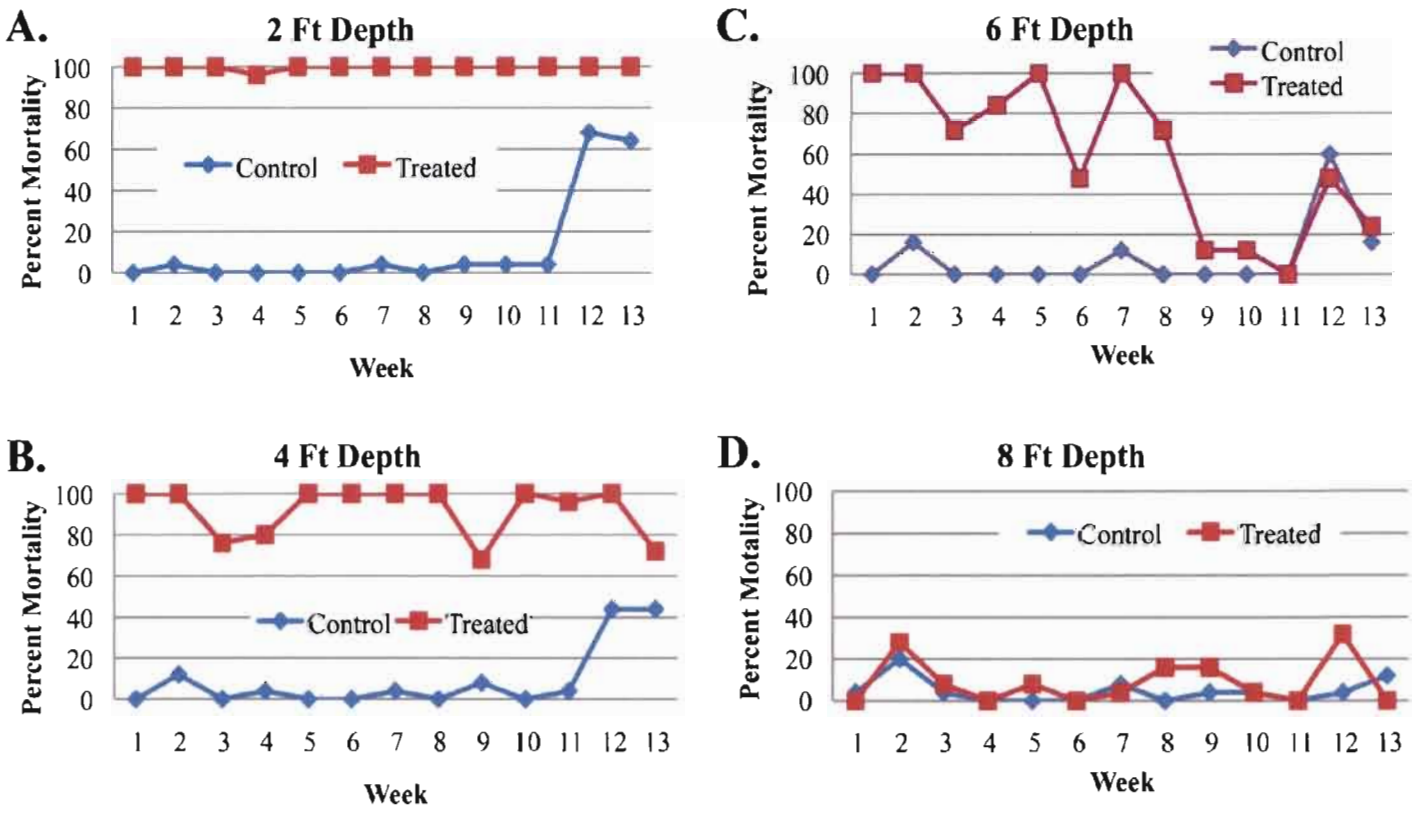


Figure 3. Nuvan ProStrips+® demonstrate significant mortality at 2 and 4 ft. depths within manhole chambers. Sentinel cages at various depths were inserted into the manhole chamber for both control and treated (4 g of pesticide) sites for a period of 24 h and mortality was calculated. The experiment was repeated six times, and each sentinel cage contained a total of 25 *Cx. quinquefasciatus* adults. Error bars represent one standard deviation of the mean.

Longevity Assessment: Longevity trials were initially conducted by placing a 4 gram portion of the Nuvan ProStrips+® into the manhole chamber. After a period of seven days, caged sentinel mosquitoes were exposed for 24 hours. No mortality was detected for either the treated or control sites. This demonstrated that the length of control at this application rate was less than seven days. After further examination of the study sites, it was determined that the manhole chamber was not a closed space and that the dimensions of the connected trunk lines should be taken into account when calculating total volume of the treatment area. The volume of the treatment area was then recalculated to be 3788.8 ft^3, thus allowing for the application of the entire 65 gram Nuvan® strip. At this application rate, control was maintained down to the 6 ft depth for duration of eight weeks. Effective control lasting beyond twelve weeks was only observed to the 2 and 4 ft depths. No control was detected at the 8 ft depth during the course of this trial (Fig. 4). As previously mentioned, a possible reason is that the sentinel cages in the lower portions of the manhole chamber were exposed to more air movement within the USDS; as a result mortality was reduced due to lower pesticide concentrations. Although control was limited to the depth of 6 ft, we do not anticipate this to be problematic as manhole chambers within our district have an average depth of 5.2 ft. It should also be noted that unseasonably high temperatures occurred during weeks twelve and thirteen, resulting in increased mortality due to desiccation within both the treated and control sites.

Figure 4. Nuvan ProStrips+® demonstrate increase mortality over time to adult mosquitoes to a depth of four feet in USDS manhole chambers. Manhole chambers treated with pesticide strips (squares) or untreated controls (diamonds) were monitored for the ability to kill adult mosquitoes over a period of 13 weeks. Depths of 2 ft (A), 4 ft (B), 6 ft (C) and 8 ft (D) from street level were assessed.



CONCLUSIONS

Nuvan ProStrips+® demonstrated positive results in its ability to control adult *Cx. quinquefasciatus* mosquitoes within manhole chambers of underground storm drain systems for a period lasting up to twelve weeks for depths of 4 ft or less and eight weeks for depths up to 6 ft. These findings indicate that the use of Nuvan ProStrips+® can contribute to an overall increased level of mosquito control within USDSs when used in combination with larvicide applications. Future studies will focus on the attempt to assess how late fall and or winter applications of Nuvan ProStrips+® will impact spring populations of mosquitoes within USDS.

ACKNOWLEDGEMENTS

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Rapid Response to Mosquito Abundance and West Nile Virus Positive Elements

Richard Takahashi

Kern Mosquito and Vector Control District, 4705 Allen Road, Bakersfield, CA 93314, Rtakahashi44@gmail.com

ABSTRACT: Increased surveillance and rapid responses to high mosquito counts and West Nile Virus (WNV) positive surveillance elements (i.e., mosquitoes, sentinel chickens, dead bird carcasses and human cases) were initiated in the latter half of 2007 and continued through 2010. These efforts appeared successful in the early control of mosquito populations. Housing foreclosures in Kern County peaked at over 8000 in 2008 and remained above 7000 in both 2009 and 2010. This exasperated the breeding of mosquitoes in neglected, dirty swimming pools and consequently increased the number of pools that had to be treated for mosquito larvae. Although mosquito WNV infection rates in 2009 and 2010 rose significantly from the previous year, human case counts significantly decreased compared to the first four years WNV was detected in Kern County. Because human blood transfusions and organ transplants are carefully screened for WNV in Kern County, mosquito transmission is the most likely way a human will contract this disease. Therefore the early reduction of mosquitoes in each mosquito season and in each cohort will abate subsequent amplifications of the virus in bird reservoirs by significantly reducing the population of infected vectors. We believe these actions consequently influenced the reduction of WNV human cases in 2008, 2009 and 2010 within the Kern Mosquito and Vector Control District. The continued reduction of human cases for three years despite the increasing mosquito infection rates and the relatively small percent of the human population that was already confirmed infected indicated that not all of the decrease in human cases could be due to herd immunity.

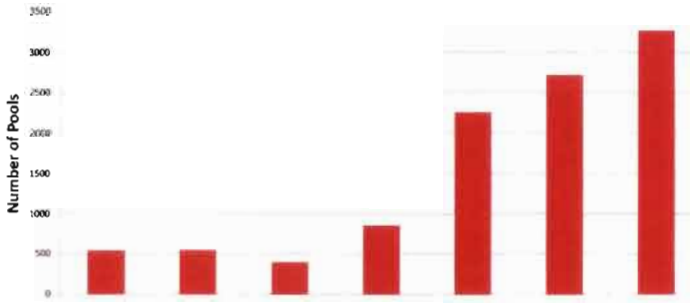
INTRODUCTION

Prior to 2004 West Nile Virus (WNV) was detected in California only in the counties south of the Tehachapi mountain range; subsequently the pathogen made its debut in Kern County in 2004. During the first year after its arrival in Kern County, there were 60 WNV human cases in the county. In the following years of 2005 and 2006, there were 68 and 49 human cases, respectively. In 2007 the WNV human cases in Kern County had risen dramatically to 140, accounting for one third of all cases in California. A Kern County WNV epidemic was evident, and it was clear that changes to the normal mosquito control procedures would have to be made.

In subsequent years, gravid surveillance trapping was increased from about 18 traps in 2006 to 48 in 2008, and EVS CO_2 trapping was increased from 28 in 2006 to 42 in 2008. The frequency of trapping was also increased from every other week to every week. During late 2007 and 2008, rapid deployment of adulticide treatments in areas of high mosquito adult counts and an increase in personnel devoted to lavalciding and swimming pool inspections/treatments contributed to a significant decline in human cases (Takahashi et al. 2010). However, because only one year of favorable data was produced subsequent to the 2007 WNV epidemic, we are unsure if this effect was due to Herd Immunity or because of our increased efforts.

MATERIALS AND METHODS

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



Personnel. From 2008 through 2010, the number of personnel devoted to mosquito control increase to a level 20% higher than the beginning of the 2007 season. Rapid deployment of adulticiding which began in 2007 continued through 2010 in response to higher adult mosquito abundance, presence of WNV in mosquitoes captured in CO_2 CDC or Gravid traps and presence of WNV human cases. In many cases the response to higher mosquito abundance occurred the same night that they were

captured. Inspections of swimming pools continued not only during the mosquito season but also throughout the winters from 2008 through 2010.

Dead birds. KMVCD and other vector control agencies in Kern County participated in the California WNV dead bird program administered statewide by California Department of Health Services, Vector-Borne Disease Section (CDHS-VBDS). Dead birds reported to the CDHS-VBDS dead bird hotline were picked up by various Kern County agencies and submitted to the California Animal Health and Food Safety (CAHFS) laboratories where necropsies were performed. Tissue samples were subsequently submitted to the University of California, Davis Center of Vector-borne Diseases (CVEC) laboratory for WNV testing by reverse transcriptase-polymerase chain reaction (RT-PCR) and/or virus isolation from Vero cell culture.

Sentinel chickens. Surveillance with 16 sentinel chicken flocks was conducted every year, and each flock was bled every two weeks. Blood samples were obtained from the comb or wing vein of the chickens and sent to Viral and Rickettsial Disease Laboratory in Richmond, CA, for testing for IgG antibody by indirect enzyme immunoassay (EIA) and subsequently confirmed by indirect fluorescent antibody assay (IFA) and endpoint plaque neutralization test (PRNT).

Mosquitoes. Mosquitoes were collected by CO_2 baited CDC traps (Sudia and Chamberlain 1962) and by Reiter/Cummings gravid traps (Cummings 1992). After the 2008 mosquito season, surveillance trapping was intensified by deploying traps numbering 42 in 2008 and 70 in 2010. The number of gravid traps remained about the same or fewer because some sites were designated or reassigned to EVS CO_2 traps to detect *Cx. tarsalis* as well as *Cx. quinquefasicatus* (and *Cx. pipiens* complex) after sampling revealed that both species of larvae were breeding year round in urban and suburban swimming pools. Adult mosquito collections were identified to species and processed for virus in accordance with "Procedures and Processing Mosquitoes for Arbovirus Detection – 2004," an annual protocol published by CDHS. Mosquitoes captured with CDC CO_2 traps and gravid traps were stored at -70° F for two to five days before being sent to Center of Vector Borne Diseases (CVEC) for testing by multiplex RT- PCR (Chiles et al. 2004).

RESULTS

Dead birds. The number of dead birds reported (i.e., all calls to the hotline) in Kern County and has substantially reduced since West Nile Virus first appeared in the county. Consequently there was a large reduction in the number of birds submitted for testing to the CDHS-VBDS (Table 1). However it is unclear if this phenomenon is due to bird die-off, herd immunity in the bird population, public apathy towards reporting or a combination of these factors. As a consequence it is difficult to assess reductions in human cases based on dead bird surveillance only.

Table 1. Dead birds reported, tested and positive in Kern County vs. California.

Year Dead Birds	2007 Kern	2007 Calif.	2008 Kern	2008 Calif.	2009 Kern	2009 Calif.	2010 Kern	2010 Calif.
Reported	1739	27611	1572	33594	606	15472	247	10465
Tested	329	6000	193	6124	87	2805	40	1954
Positive for WN	124	1396	10	2568	28	515	13	416
% of those Reported	7 %	5 %	0.6	42 %	4 %	3 %	5 %	4 %
% of those Tested	38 %	23 %	5 %	7.6 %	32 %	18 %	33 %	21 %

Table 2. Number of swimming pools treated in Kern MVCD 2004 to 2010.

2004	2005	2006	2007	2008	2009	2010
542	552	398	856 Aerial Surveillance for S. pools	2257 (+1400)	2718 (+500)	3268 (+500)

Sentinel chickens. High percentages of sentinel chickens were infected with WNV nearly every year since its arrival in Kern County, and the WNV seroconversion of flocks came approximately 2 weeks later than positive detections in mosquitoes and dead birds. Hence, the value of sentinel chickens as a means of rapid response was limited. However, because the chickens were virus free at the start of each season, they did show the presence of new viral infections and the subsequent rapid spread of the virus throughout each flock.

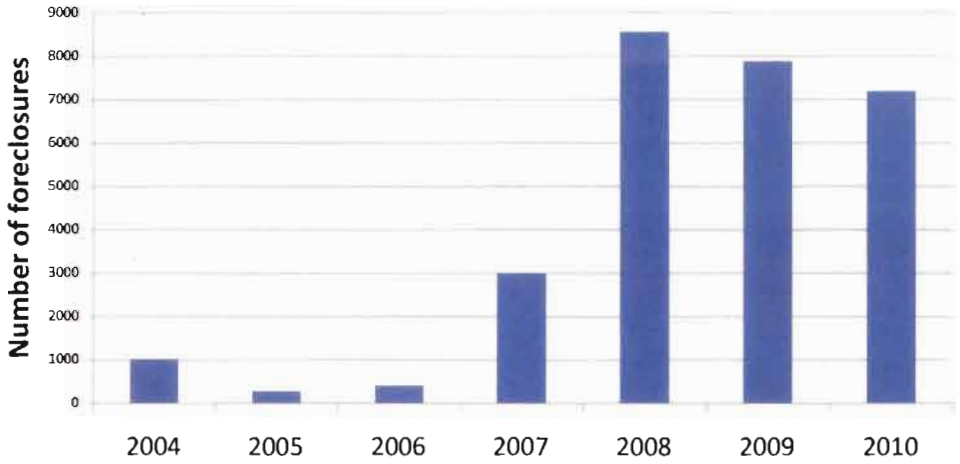
Personnel. Personnel increase allowed the district to increase surveillance for larval sources and, perhaps more productively, for inspection of swimming pools during a period when housing foreclosures were rampant (Tables 1 and 2).

Mosquitoes. Mosquito surveillance using CDC CO_2 and gravid traps has shown that there is still a threat to humans because of the abundance of positive mosquitoes being trapped and the pervasiveness of positive collections throughout the KMVC District (Fig. 2).

DISCUSSION AND CONCLUSIONS

Increased surveillance and the rapid response to high mosquito counts and to West Nile Virus positive surveillance elements (i.e., mosquitoes, sentinel chickens, dead bird carcasses and human cases) was initiated in the latter half of 2007 and continued through 2010. These efforts appeared successful in the early control of mosquito populations. We believe these actions also contributed to the decline of WNV human cases in Kern County, despite the increase in mosquito abundance and mosquito infection rates after 2008.

Figure 2.



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Sacramento, California 95814
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E-mail: abradley@mvcac.org

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