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Submitted Papers

History of Flea-Borne Typhus in Los Angeles County, California

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ABSTRACT: Flea-borne typhus is an important vector-borne disease in Los Angeles County, California, with more than 500 human cases reported over the past three decades. The disease is endemic in this and nearby Orange County. It is primarily associated with suburban communities which have a triad of commensal wildlife, domestic animals, and *Ctenocephalides felis* (cat fleas) as the main vector. Feral and companion cats and opossums are the primary hosts of *C. felis*, which transmit the pathogens *Rickettsia typhi* and *R. felis*, the causative agents of flea-borne typhus. The incidence of flea-borne typhus has increased gradually over the past two decades. Although its epidemiology is not well-defined, major policy changes in the recent past have addressed animal welfare with little or no regard to public health and may have exacerbated the endemic status of the disease. In this article, we review the history of flea-borne typhus in human, vertebrate, and vector host species; and the major policy changes concerning animal welfare and public health that have possibly impacted disease prevalence and transmission.

INTRODUCTION

Flea-borne typhus is an acute febrile illness caused by *Rickettsia typhi* and *R. felis*. Most human cases occur in tropical and subtropical regions of the world (Azad *et al*., 1997). In recent decades West Nile virus emerged as a major public health concern throughout the United States; we feel that flea-borne typhus should similarly be considered. For decades the incidence of flea-borne typhus steadily declined (Halverson 1942, Beck and Van Allen 1947), but recently it has regained notoriety as an important infectious disease (Sorvillo *et al*., 1993, Civen and Ngo, 2008, Eremeeva *et al.*, 2012). Similar observations of flea-borne typhus have been documented in other parts of the world including Africa, Southeast Asia, and the Mediterranean (Dumler *et al.,* 1987, Azad, 1990, Gikas, *et al.,* 2002, Jiang *et al*., 2013).

Historical records in Los Angeles County from the early 1900s initially showed high numbers of typhus cases (Anderson, 1915) that declined quickly and remained low over the succeeding decades. In these early years epidemic (louse-borne) and endemic (flea-borne) typhus were diagnostically indistinguishable and combined. Once antibiotics against the former were discovered, the overall numbers plummeted (Beck and Van Allen 1947). It was not until the late 1930s that diagnostic tools were developed to distinguish epidemic from endemic typhus (Halverson, 1942, Beck and Van Allen, 1947, Adams *et al*., 1970). Epidemic typhus is caused by *Rickettsia prowazekii* and transmitted by *Pediculus humanus* (body lice), whereas endemic typhus is caused by *Rickettsia typhi* and *R. felis* and transmitted by *Xenopyslla cheopis* (Oriental rat fleas) and *Ctenocephalides felis* (cat fleas), respectively. *Rickettsia typhi* belongs to the typhi group (TG) and *R. felis* to the spotted fever group (SFG) (Schwan *et al.,* 1985). Endemic typhus is also referred to as murine typhus and is identified throughout this article as flea-borne typhus.

For the next six decades few human cases of flea-borne typhus were documented each year in the United States, except in the states of California, Hawai'i and Texas (Reporter *et al.,* 1996, Eremeeva *et al.,* 2008) where it is considered endemic. The decline of human cases nationwide was so evident over the decades that in 1994 the Centers for Disease Control and Prevention (CDC) delisted flea-borne typhus as a reportable disease (CDPH 2014).

As the CDC was downgrading flea-borne typhus as a reportable disease, its incidence was on the upswing in California's Los Angeles and Orange counties. This trend was also evident in Maui County, Hawai'i, and Cameron, Hidalgo, Nueces and Travis counties in south Texas (Taylor *et al.,* 1986, Dumler *et al.,* 1991, Reporter *et al.,* 1996, Boostrom *et al.,* 2002, Eremeeva *et al.,* 2008). The resurgence of flea-borne typhus in Los Angeles County began in 1996, steadily increased and currently surpasses its previous annual counts. This upsurge is documented in suburban and urban areas of these three states where rodent and human populations co-exist, especially in areas with large concentrations of opossums, cats, and their fleas (Adams *et al.,* 1970, Williams *et al.,* 1992, Sorvillo *et al.,* 1993). This trend should worry public health agencies; however, due to its low case fatality rate and mild symptomatology, the upsurge has not been placed in proper public health context. In this article, we argue that flea-borne typhus is a major disease in Los Angeles County and poses a public health threat especially to its indigent residents living in neighborhoods where conditions conducive to transmission exist. In addition, we identify major risk factors, including well intentioned statutory changes that may be responsible for the resurgence of the disease in southern California.

The life cycle of flea-borne typhus

There are two known life cycles of flea-borne typhus; one for each causative agent and their associated vertebrate and vector hosts. Murine typhus, caused by *R. typhi,* first was described in the 1940s (Dyer, 1944). This pathogen is maintained in the classic urban life cycle which is a rat - oriental rat flea - rat transmission cycle among the urban commensal rats *Rattus norvegicus* and *R. rattus* and their flea *X. cheopis* (Azad *et al.,* 1990). This cycle, though considered classic may also involve other animals and fleas, and has been responsible for many outbreaks in major cities throughout the US (Azad *et al*., 1997).

In the early 1990s another cycle of flea-borne typhus was discovered, especially in areas where *R. typh*i was less prevalent in rodent and flea populations, but present in cats and opossums (Sorvillo *et al*., 1993, Gerhold and Jessup, 2012). In 1990 Adams et al. (1990) described *R. felis.* Soon thereafter it was associated with *C. felis* and domestic and peri-domestic animals (Williams *et al.,* 1992, Azad *et al.,* 1997) and may be maintained in cat fleas alone through vertical transmission (Wedincamp and Foil, 2002). *Rickettsia felis* is serologically indistinguishable from *R. typhi,* but it is distinct when tested by molecular techniques (Henry *et al*., 2007, Schriefer *et al*., 1994). Two recently identified *Rickettsia* (*R. asemboensis* and *R. senegalensis*) may be involved in flea-borne typhus but their role is undefined (Jiang *et al*., 2013, Billeter *et al*., 2016, Maina *et al*., 2016). *Rickettsia felis* and perhaps these other recently described *Rickettsia* occurs in a suburban cycle, maintained in opossums by *C. felis*, with its frequency highest where there is a high population of feral cats (Civen and Ngo, 2008, Perez-Osorio *et al*., 2008). Opossums were introduced into California in 1910 spread throughout the state's urban and suburban areas (Grinell, 1915) and together with an over-abundance of feral cats are sustaining recent typhus outbreaks. Co-infections of cats and *C. felis* with *R. typhi* and *R. felis* (Noden *et al*., 1998, Karpathy *et al.,* 2009, Eremeeva *et al.,* 2008, 2012, Nogueras *et al.,* 2013) and other vertebrate hosts (Abramovicz *et al*., 2011) is rarely reported. Urban and suburban cycles may exist sympatrically; however, the urban cycle seems to have been supplanted by the suburban cycle, as evidenced by the new wave of flea-borne typhus cases.

Diagnosis and treatment

Until recently, most human diagnosis of flea-borne typhus was limited to detecting *R. typhi,* the causative agent for murine typhus. Diagnosis is based on clinical recognition and serology which compares acute- and convalescent-phase sera, and is most helpful retrospectively. Serologic tests can identify the causative agent only to the level of genus. Clinical symptoms include fever, headache, rash, arthralgia, enlarged liver/spleen, cough, and diarrhea (Civen and Ngo, 2008). The US Food and Drug Administration (FDA)-approved serological tests for clinical diagnosis of *R. typhi* are the direct - and the indirect - immunofluorescence assays (IFA). These tests cross react with *R. felis* in the SFG which is serologically indistinguishable from *R. typhi.* Patients presenting with the disease often are in the acute phase and once antibiotics are administered the opportunity for definitive diagnosis is lost.

The polymerase chain reaction (PCR) has been used to diagnose SFG and TG *Rickettsia* in humans and animal (including fleas) tissues. Currently there are no FDA-approved PCR tests for clinical diagnosis. However, PCR-based diagnosis has been used to test peripheral blood in humans, animals, and/or fleas during surveillance studies (Henry *et al.,* 1997, Nogueras *et al.,* 2013).

Flea-borne typhus is treatable with antibiotics. The drug of choice is the tetracycline, especially doxycycline (Civen and Ngo, 2008).

History and ecology of flea-borne typhus

The first reported case of typhus in Los Angeles County was a railroad worker in 1916. The case was mistakenly labeled flea-borne typhus, but records make it highly likely that it was louse-borne typhus. A cluster of human cases involving railroad workers was identified along the railway from Mexicali, Mexico through Laredo to Los Angeles and other Midwestern US states (Halverson, 1942, Beck and Van Allen, 1947, Woodward, 1973, Reif and Macaluso, 2009). There was strong evidence that 32 human cases which occurred in Los Angeles County between 1919 and 1924 were transmitted by fleas. According to Beck and Van Allen (1947), during the following five years (1923 – 1928), 23 human cases were recorded from Los Angeles, San Diego, and San Bernardino counties. In the next 10 years fewer cases of flea-borne typhus were recorded, only to increase again in the 1940s. This may have been because the diagnostic tests became more definitive (see Figure 1). There were 362 flea-borne typhus cases in California from 1940 to 1946, with 70% of these occurring in Los Angeles County. The case fatality rate was 4% during this period (Beck and Van Allen, 1947, Woodward, 1973).

There were fewer reported cases of flea-borne typhus between 1946 and 1995 in California and particularly in Los Angeles County (see Figure 1, Reporter *et al.,* 1996). Human cases started increasing again in the 1990s, and by 1996 there were more than 10 cases annually (CDPH, 1994, 2014). These cases often were diagnosed after several failed attempts by clinicians to stabilize patients without a prescription of antibiotics. Although human cases in the mid-1990s were few and dispersed, by 2010 there were more than 30 cases occurring annually in Los Angeles County. Similar trends were observed in Orange County and statewide (Cummings *et al.,* 2014, CDPH, 2014). Some flea-borne typhus cases appeared in local clusters, for example in 2005 a cluster of six human cases were recorded in Pasadena, and in 2009 a cluster of several cases were recorded in the City of Long Beach (LACDPH, 2013, CDPH, 2014). In 2015, five flea-borne typhus cases were identified at a single location in the City of Pomona (Crocker *et al.,* 2016, Nelson *et al*., 2016).

The impact of plague control on flea-borne typhus

The decline of flea-borne typhus cases from the mid-1920s in Los Angeles County anecdotally has been associated with the plague outbreak of 1924-25 in the city of Los Angeles, and the intensive rat control campaign that followed (see Figure 1, Dickie, 1926, Viseltar, 1974). The campaign focused on poisoning *Rattus norvegicus* and *Rattus rattus* which also removed the vector, *X. cheopis* (Murray, 1964). This control inadvertently also disrupted the transmission of murine typhus by destroying its host and

vector (flea-borne typhus) (Dickie, 1926, Beck and Van Allen, 1947, Nelson *et al.,* 1986). Rat and flea control became the main strategy for plague control throughout the country from the 1940s onwards. More so, after the US Public Health Communicable Disease Control (currently the Centers for Disease Control and Prevention) was established in 1946 and adopted this strategy for plague control nationwide (CDC, 1982).

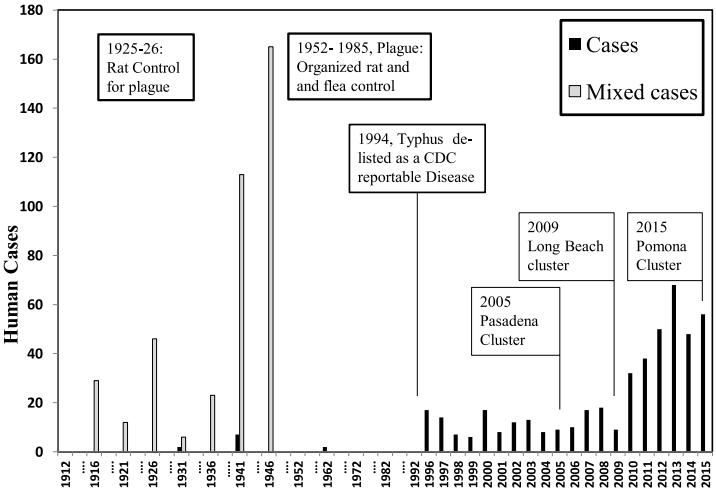

Figure 1. Mixed cases of epidemic and endemic typhus, time line of major events in the diagnosis, treatment, and control of flea-borne typhus in Los Angeles County, California, from 1912 to 2015.

The national rat control program involved surveillance and control by reducing food sources and harborage, trapping and poisoning of rats in urban areas, controlling fleas, and advising homeowners on rodent exclusion. The program funded by the CDC was implemented in mid 1950s and covered major cities and counties nationwide. It was terminated in the mid- 1980s. During the program years the number of recorded human cases of plague declined and rarely was plague detected in commensal rats. There was a gradual shift in the type of vertebrate hosts with which plague was associated, changing from rats to ground squirrels and chipmunks primarily, and secondarily to wild carnivores including coyotes, bobcats, and bears (Rutledge *et al.,* 1979, Schwan *et al.,* 1985). On occasion, domestic cats were infected creating a direct source of human infections (Werner *et al.,* 1984).

The unexpected consequence of the massive CDC-funded rat control program was the dramatic impact on the incidence of flea-borne typhus. The reduction or near elimination of flea-borne typhus in some years during the program period in Los Angeles County and California disrupted the urban ecology of the disease. Because the hosts and vector of urban flea-borne typhus are the same as those for plague and, this may explain why current typhus cases are associated not with *R. typhi* but with *R. felis* (Abramovicz *et al*., 2012, Eremeeva *et al.,* 2012, Cummings *et al.*, 2014) and/ or another yet to be identified *Rickettsia* (Eremeeva *et al.*, 2012).

Just as the urban life cycle for plague in Los Angeles County was being disrupted through organized rat control campaigns, the sylvatic cycle for plague involving ground squirrels, chipmunks, and *Oropsylla montana* (ground squirrel flea) was gaining prominence (Nelson *et al.,* 1986). Similar shift was evident in the dynamics of flea-borne typhus. The urban cycle declined and suburban cycle of flea-borne typhus emerged, involving *R. felis*, opossums, *C. felis*, and colonies of feral or community cats (Sorvillo *et al.,* 1993, Gerhold and Jessup, 2012).

Policy changes and the resurgence of flea-borne typhus

Ironically the CDC removed murine typhus (flea-borne typhus) from the list of reportable diseases in 1994 and thereafter cases of typhus started increasing. Coincidence or not, the trend accelerated over the following decades with human cases surpassing previous annual records (see Figure 1, Reporter *et al.,* 1996, LACDPH, 2013). The downgrade of flea-borne typhus by the CDC occurred a decade after organized rat and flea control programs were terminated. In southern California, the rat and flea control was handled by County Departments of Environmental Health during program years. Current surveillance and control of rodent-borne diseases in Los Angeles, Riverside, San Bernardino and San Diego counties reflects more than 50 years of this rat and flea program history. Over several decades rat and flea control was successful in Los Angeles County and contributed to banishing epizootics of plague from urban and suburban communities to the rural and mountain areas. This ecological phenomenon of shifting disease cycles and associated host species was also observed in other parts of the country. According to Sorvillo *et al.* (1993) the shift in the life cycle of flea-borne typhus was not evident until after the termination of CDC-funded rat and flea control program. The dramatic rise of flea-borne typhus in humans seems to have been fueled by this shift and by additional changes in local and state policies which increased the population of feral and community cats.

Animal control services in Los Angeles County are divided among the various city and county jurisdictions. The county's Animal Control program provides services to the majority of its residents who live in unincorporated areas; however, each city must provide its own services and often contracts for such services with "not for profit" humane societies (see Figure 2). These animal control agencies, private or public, are charged with complying with various city and county policies, codes, and ordinances. Compliance with different regulations within the same county by the same or different animal control agencies created a burden and some have been overwhelmed, ultimately causing several cities to contract animal control services. Unfortunately, insufficient services and increased need led to overcrowding of shelters and eventually many unwanted animals being euthanized. To reduce overcrowding and "euthanasia" at shelters, in 1998 the state passed the Hayden Act that amended the statutes on companion animals (SB1785, 1998). Under the previous law unwanted cats impounded by public shelters could be euthanized after 72 hours. Hayden Act expanded this minimum impound time from 72 hours to six days, and required impounded animals to be released to non-profit animal rescue or adoption organizations in certain circumstances. The law clarified and expanded parts of the statutes that defined feral animals and required that both private and public shelters provide suitable food and shelter for

animals under their care. The state legislature passed the Hayden Act but never foresaw the consequences. In the aftermath no one has been willing or able to reverse even the worst parts of the law (Longcore, 2009).

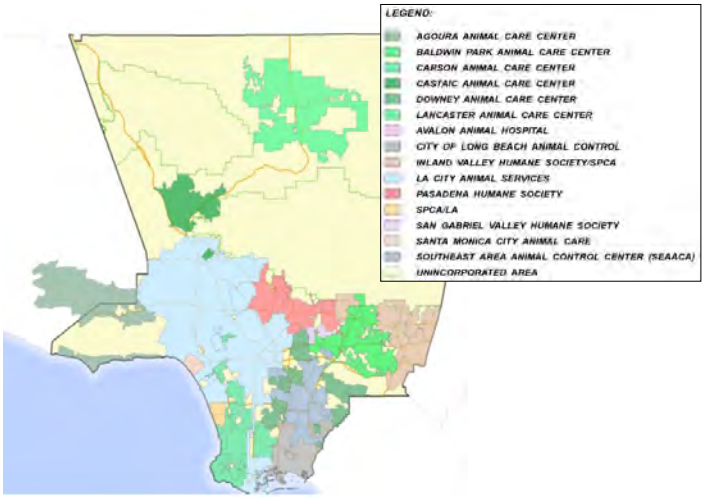

Figure 2. Agencies providing animal control and care services in Los Angeles County – updated January 2013 (published by permission from County of Los Angeles, Department of Animal Care and Control).

The Hayden Act required local governments to expand shelter capacity for animals, and reduce the number of unclaimed animals impounded. Furthermore, local jurisdictions including those in Los Angeles County passed codes and ordinances with several strategies to reduce euthanasia among impounded animals. In addition, these jurisdictions expanded release of animals to rescue and adoption organizations. Some of the codes and ordinances adopted after the passage of Hayden Act had "no kill goals" even after the 6 days of animals being in the pound. Some of the "no kill policies" were sterilization of feral animals that included "trap-neuter-return" (also known as TNR), rehabilitation, and relocation of all outdoor and feral animals (Gerhold and Jessup, 2012, Cummings *et al.,* 2014). The burden imposed on shelters and animal control agencies without equivalent compensation shrunk shelter capacity. Also, penalties on individuals and organizations were increased, but animal holding capacities for shelters were reduced undermining the role of animal control during disease outbreaks. Consequently, this shifted delivery of animal control services from public to private or "not for profit" agencies. On the other hand, penalties against individuals or organizations caught feeding wildlife or feral animals were reduced or eliminated allowing increased feeding of feral cats and encouraged shelters to not impound unclaimed animals. Feral animals left uncared for by owners in the community could legally be fed by anyone in the community without penalties, and food intended for such animals could be consumed by wildlife with individuals responsible not being subjected to the "do not feed wildlife" consequences under the fish and game law (Longcore, 2009, Gerhold and Jessup, 2012)

The Hayden law did not increase funding for shelter capacity. Shelters became increasingly reluctant to impound cats, and many feral animals are released to rescue and adoption organizations. Although saved from eminent euthanasia, "feral" cats were banished to a tenuous life in the wild without food or veterinary care. The high feral cat population increased the interaction of these cats with opossums, skunks, and raccoons, exchanging fleas and exacerbating the likelihood of bringing infected fleas closer to residents, especially with the infection maintained vertically in cat fleas (Wedincamp and Foil, 2002). This is reflected in the increasing number of flea-borne typhus cases countywide over the recent decades. The Hayden law shifted the mission of animal control agencies from being public health to one of animal welfare, and more animal welfare focused "not-for-profit" humane organizations are providing contracted services in southern California. This has steadily eroded public health interests among animal control agencies throughout the region.

In 2015, an outbreak of five cases of flea-borne typhus were clustered at a locale in the City of Pomona and while public health officials scrambled to contain the outbreak, the contracted animal control agency in the city declined to participate (see elsewhere Crocker *et al.,* 2016, Nelson *et al.,* 2016). This was contrary to actions required to mitigate disease outbreaks and to protect the public health. The contractor claimed potential liability for exposing its workers who handled infected feral cats and their fleas at the location. Thus, the contractor failed its duty as an animal control agency in an outbreak of disease by not improving sanitation, reducing hosts (opossums and feral cats), and controlling fleas.

The Districts' flea-borne typhus surveillance and control program

In response to the increasing human cases of flea-borne typhus in the San Gabriel Valley, the District established a surveillance and control program geared to work alongside the Los Angeles County Department of Public Health (LACDPH) disease investigators. In 2014, the District realigned its staff, in addition to performing other duties, to provide enhanced surveillance and control of flea-borne typhus. This address increased service requests to include residents with potential flea complaints. Service requests associated with the presence of feral cats and opossum were given greater attention. The program conducted follow up investigation to suspect, probable and confirmed typhus cases referred from the LACDPH. All activities were geared towards reducing the flea burden in the communities. The program advocates for the reduction of all conditions that increases the risk of this disease in neighborhoods. The program is not intended to replace or supplant activities such as outreach and public education performed by the LACDPH but rather enhance the program. The District provides surveillance and control for flea-borne typhus, including abatement to mitigate disease outbreaks.

The District's surveillance and control program is focused on investigations to determine the potential public health risk and advise property owners and cities on activities that can lower such risk. Some specific activities involved in disease

investigations include property inspections for opossums, feral cats, and rats. These animals are known to sustain populations of cat fleas involved in typhus transmission. Other activities include inspecting neighborhoods to determine conditions suitable for vertebrate infestations such as harborage, food, and water sources; inspections for the presence of flea infestations; trapping of opossums and rats, and combing them for fleas; and testing for typhus in fleas and vertebrate hosts.

CONCLUSION

Flea-borne typhus is caused by *R. typhi* and *R. felis.* In Los Angeles County, this disease has been endemic and continues to increase. Previously, *R. typhi* was maintained in the urban cycle involving commensal rats and rat flea; however, in recent years *R. felis* has increasingly been found and it is maintained by cat fleas, opossums, and feral cats. The ubiquitous distribution of cat fleas among different types of vertebrate hosts including opossums, skunks, and raccoons bring typhus into feral cats, increasing the opportunity for these cats to interact with and infect the indoor and companion cats. The evidence makes this scenario the most probable epidemiological nexus fueling the increase of flea-borne typhus cases in this and the neighboring Orange County for the past several decades.

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Epizootic Plague Activity and Control Measures in Yosemite National Park, 2015[1]

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In August of 2015, two human plague cases associated with exposure in Yosemite National Park were identified. In response, the California Department of Public Health (CDPH), in collaboration with the National Park Service, the Centers for Disease Control and Prevention and other agencies, launched an environmental investigation. Risk assessments were conducted at eighteen locations in the park and surrounding national forests and plague activity was identified in six areas. Based on surveillance results, the participating agencies jointly made the decision to temporarily close and conduct flea control at locations with the highest risk of human exposure. DeltaDust® Insecticide (0.05%) was applied into rodent burrows in five campground or day use areas in Yosemite. Post-treatment assessments found substantial decreases in flea abundance on rodents and/or in their burrows. As a result of flea control operations and coordinated public education efforts, the human plague exposure risk was decreased in Yosemite National Park.

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An Unusual Encounter with Biting Mites (family: Pyemotidae) at a Wildlife Rescue Center

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ABSTRACT: In October, 2015, a non-profit wildlife rescue and rehabilitation center in Northern California contacted the Marin/Sonoma Mosquito and Vector Control District to report an outbreak of pruritic rashes affecting animal care volunteers and staff. This report describes the investigation of this outbreak by district and public health staff to identify the etiology of the rash and the source of the infestation. Straw itch mites (*Pyemotes* sp.) were identified as the likely cause of the outbreak, which affected approximately 20 individuals.

INTRODUCTION

Microscopic, soft-bodied mites of the family Pyemotidae have sometimes been considered beneficial insects for their role as generalist parasites on stored product pests such as moths and beetles. However, their value as biological control agents is diminished by the pain and suffering that the bites of some species can inflict on humans who come into contact with infested products (Coleman et al. 2015, Moser 1975).

Pyemotid mites inject a toxin in their bites that paralyzes arthropods and can cause a blistering rash in humans (Booth 1952, Moser 1975). The resulting pruritic dermatitis has been referred to straw, grain, or hay itch, depending on the type of material involved. These outbreaks of dermatitis have occurred primarily amongst agricultural workers who handle the material infested with the various insect hosts of *Pyemotes* mites (Booth and Jones 1952, Walker and Landis 1994). However, these mites also have attacked people in less predictable situations, including in a store inside a shopping mall where decorative wheat was sold (Betz et al. 1982) and in a house with seasonally-infested furniture (Fine and Scott 1965).

This report recounts the investigation of an itch mite biting incident in a wildlife rescue and rehabilitation facility in Northern California.

MATERIALS AND METHODS

In mid-October, 2015, the Marin/Sonoma Mosquito and Vector Control District ("the District") received a report that 18-19 people working for or volunteering at a local wildlife rescue and rehabilitation center had been experiencing a severe dermatitis of uncertain origin. Onset of symptoms was around October 12, and the timing had coincided with the death of a pair of young mountain lions kept on-site. An abundance of fleas had since been observed in the vicinity of the mountain lion enclosure, and the wildlife facility director requested assistance from the District in assessing if the fleas presented a health risk and whether the dermatitis was caused by the fleas. On-site inspections were conducted by the District on October 21, 23 and 27, 2015.

Wearing Tyvek® suits and rubber boots, the District's scientific programs manager and the wildlife facility director surveyed the mountain lion enclosure for fleas using a flannel flag. Fleas were observed and collected from the enclosure and brought back to the District laboratory.

The wildlife center director also indicated that a number of affected staff had recently worked in a wolf-dog hybrid enclosure located much closer to the main building, which housed the hospital, administrative offices, and a converted garage being used as the animal food preparation room ("the garage"). As the wolf-dog hybrids were held in quarantine while being treated for fleas, this enclosure was swept for ticks, fleas and mites using a flannel flag, and for chiggers using plexiglass-acrylic squares (per Loomis 1956). No biting arthropods were found in this enclosure.

Sticky traps were placed by wildlife facility staff in and around several occupied animal pens, left out for a week, and then retrieved for examination by District staff.

Several samples of materials found around the facility were collected and taken back to the District laboratory for inspection. These samples included: mulch that had arrived on-site around the inception date of the biting incidents, straw stored under tarps outdoors, dust and detritus left in an empty straw storage shed, various types of seeds, nuts and dried animal chow stored in the garage, and spider webs collected from the garage floor. Additionally, swabs were taken from a variety of surfaces in several other rooms, including animal examination rooms in the main building. Each swab was obtained by wetting a paper towel with 70% isopropyl alcohol, wiping a surface with the paper towel, then sealing the towel into an individually labeled zip-top plastic bag.

While standing at the central L-shaped food preparation table in the garage taking samples of animal food the author felt an intense pruritic sensation on her torso. Swabs were taken from the skin onto an alcohol-soaked paper towel using the method described above and preserved for further analysis. Although a Tyvek® suit was not worn at the time of this collection, the author was wearing clothing sprayed earlier with a pyrethrin-based repellent and exposed skin areas (face, neck and arms) had been treated with DEET.

The District rodent specialist conducted a thorough inspection of the main building. The objectives of this inspection were to assess whether a rodent infestation existed that could be producing rat mites, as well as to identify conditions present that might facilitate rodent presence.

Wildlife center staff and contractors on site were interviewed. Staff were asked about whether or not they had experienced bites, and the location and nature of their work activities.

All specimens collected on site were brought to the District laboratory. In order to isolate microscopic arthropods from samples, animal feed and other dry samples were soaked in 70% isopropyl alcohol, and paper towel swabs were flushed using a squirt bottle of alcohol. The resultant alcohol used for the wash was then examined under a dissecting scope. Sticky traps and spider webs were examined directly under the dissecting scope. After candidate biting arthropods were isolated and tentatively identified at the District laboratory, samples were submitted to the California Department of Public Health (CDPH) for confirmation of identifications.

Shortly after the initial inspection was conducted on October 21, the author was contacted by the infectious disease doctor coordinating worker's compensation cases for the wildlife facility. Observations of symptomatic trends and the disease capacity of potential biting arthropods were discussed. This avenue of communication was maintained throughout the investigation.

RESULTS

The swabs taken from the author's abdomen (Fig. 1) contained several non-gravid, female biting mites belonging to the family Pyemotidae (*Pyemotes* sp.), commonly referred to as straw itch mites (Fig. 2). Identification to genus was confirmed by Denise Bonilla via the CPDH. District entomologist Eric Engh further identified the mites as belonging to the *ventricosus* group, based on morphological features using published keys and descriptions (Cross et al. 1981, Walter et al. 2009). It has been suggested that straw itch is only caused by *Pyemotes tritici* (Moser 1975), but there have since been cases of *Pyemotes herfsi* causing a similar dermatitis (Broce et al. 2006, Zaborski 2008). Definitive identification to species was not feasible using available references.

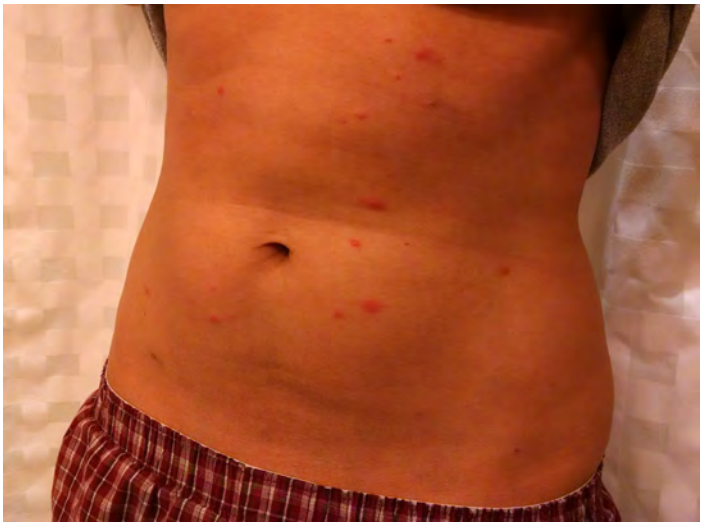

Figure 1. Photo of the author's bites, obtained while collecting samples at the wildlife rescue center.

Figure 2. A *Pyemotes* mite swabbed from the author's torso.

Pyemotes mites were collected in large numbers (> 100) from swabs taken from the countertop in a central L-shaped table in the garage (Fig. 3), the spider webs on the floor beneath this table, and in small numbers (< 10) from swabs of various other surfaces in the same room. No itch mites were collected from the mulch, the straw or straw storage areas, or any of the animal feeds, with the exception of a single *Pyemotes* found in a container of sunflower seeds. However, as these seeds had been stored, uncovered, directly beneath the animal food preparation table, it is likely that this mite simply fell from the heavily infested countertop into the seeds. Other mites found in these samples appeared to be predatory mites, grain or mold mites, and were unlikely to have played a significant role in the severe biting incidents experienced by wildlife center personnel.

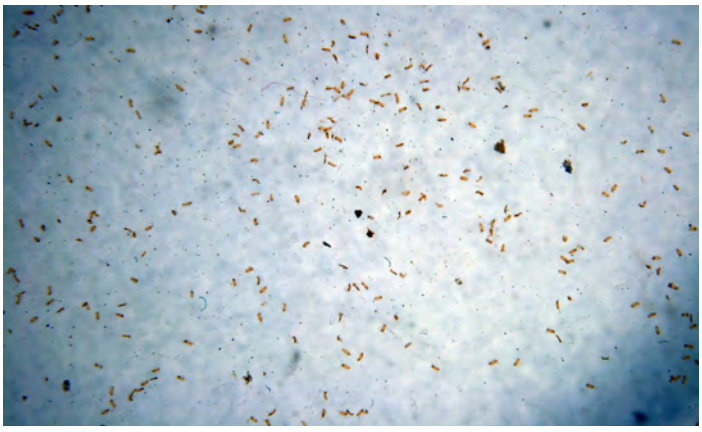

Figure 3. *Pyemotes* mites from a countertop swab in the wildlife center garage/animal feed preparation area. Image has been enhanced for visibility.

Interviews with facility staff revealed that all personnel that had experienced severe bites had spent significant time working in the animal food preparation area in the garage. Several of these staff members, unprompted, displayed their assemblage of bites to the author. Many appeared to have bites numbering in the hundreds, and nearly all of these were located on the front, back, and sides of the torso (Fig. 4). Many bites displayed an area of hyperpigmentation flaring out from each bite site, commonly known as the "comet sign" (Corazza et al. 2014). Several affected employees reported that they had experienced fever and sleeplessness, and some had been prescribed prednisone, which they said was effective in alleviating itching. However, they still reported obtaining new bites every day they were on site. Other employees who worked in the main building in administrative roles or who had done laundry in the garage but did not work with the animal feeds on the central table, had not received bites. Landscape workers and gardeners who worked exclusively outside also did not report any unusual bites.

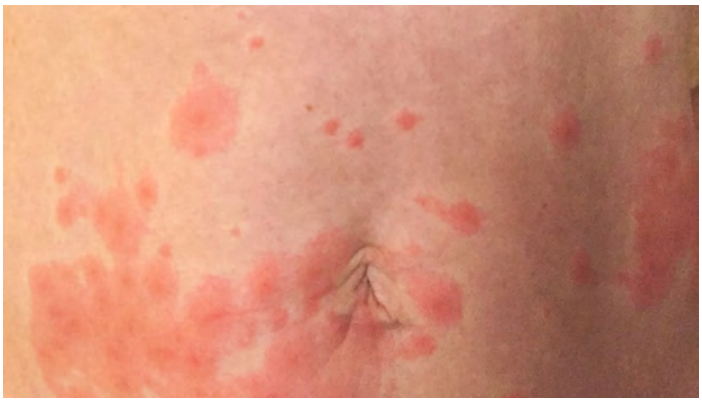

Figure 4. Photo of skin lesions of straw itch mite dermatitis on abdomen of wildlife center staff member.

Cat fleas (*Ctenocephalides felis*) were identified from the mountain lion enclosure collections. However, the flea infestation was limited to the immediate area in and around the lion enclosure; fleas were especially abundant in the straw bedding inside the area where the lions slept. The lion enclosure was located at the far edge of the facility, several minutes' walk from the area around main building, and few staff members had worked in this area. Additionally, the concentration of bites almost exclusively on the torso and back did not match the typical biting habits of fleas.

The District rodent specialist found a few rodent droppings, rub marks, and other signs suggesting a previous rat infestation, but no obvious evidence of current rodent activity. Wildlife facility staff confirmed they had dealt with a serious rat issue in the past. Recommendations for pruning, sanitation, and structural repairs were given to the wildlife facility maintenance manager to prevent a recurrence of rat issues.

Discussions with the coordinating physician confirmed that the appearance, abundance, and location of bites were similar in all of the facility's worker's compensation cases. Also of note was that no patient reported any secondary biting exposures (i.e. their spouses, partners, and children had not been affected). All arthropod findings from on-site investigations were shared with the coordinating physician. In early November, it was reported back to the author that biopsy results from wildlife facility patients were consistent with arthropod bites.

DISCUSSION

As straw itch mites can parasitize a variety of insects, a concerted effort was made to identify infested materials. Although some animal feeds did contain stored product insects, including Anobiid beetles in partially shelled walnuts and in a container of "turkey/pheasant" food and more beetles and psocids in the stored straw, none of these insects were observed hosting *Pyemotes*. Many of the animal foodstuffs used by the wildlife center had been donated by the public, which meant they had arrived in various levels of freshness or decay, and prior storage conditions were largely unknown. Based on the large concentration of mites on the countertop, we speculate that a *Pyemotes*-infested animal feed or bedding material had been processed on the table, which led to the dispersal of itch mites around that area (perhaps repeatedly). As wildlife center staff and volunteers leaned over the counter to measure and prepare other feeds, they were exposed to the biting mites remaining on the countertop, a method inadvertently replicated by the investigator, with similar results.

Cases in the literature cite environmental sources of entomological itch mite hosts aside from stored hay or grains, including wood (wood-boring beetles), wooden furniture and wood flooring (Dermestid beetles), Pin oak trees (oak leaf gall midges), and periodical cicada hatches (Broce et al. 2006, Corazza et al. 2014, Del Giudice et al. 2008, Zaborski 2008). Although the garage floor was cement, and there were no pin oak trees or cicadas present, several stacks of cut wood were kept on site at the wildlife center, and some wood was stored in bins in the garage. Although samples of the wood were not examined in the laboratory, there did not seem to be any direct connection to the location of the stored wood and where the *Pyemotes* were abundant. Additionally, the wood had been present in the garage for at least several weeks before the biting began, without incident. Nonetheless, without closer examination it could not be ruled out as a potential source. The L-shaped food preparation table containing the greatest density of *Pyemotes* mites was constructed of treated, pressed wood, and could potentially have housed furniture beetles, although none were observed during the time spent working in close proximity to the table and countertop.

Straw itch is characterized by often hundreds of tiny red welts, each with a central vesicle, with the bites usually densely concentrated on the trunk of the body (Fine and Scott 1965). The mites are microscopic and patients usually never see what bit them. Additionally, in several published accounts, the entomologists investigating the cases often experienced these bites themselves, a characteristic dubbed "the sign of the infected investigators" (Bellido-Blasco et al. 2009). Despite the lack of identified host insect or infested plant material, *Pyemotes* were collected both from the body of the bitten author and in the working environment common to all affected parties. Circumstances of the current

investigation closely match straw itch case descriptions from the literature.

Working from the hypothesis that *Pyemotes* mites had been the cause of the outbreak, but unable to identify the host insect or source material, the District recommended general sanitation and control measures for the wildlife center, including:

- Thoroughly wet and wipe down surfaces in the garage with Lysol, alcohol, or another cleaning disinfectant. Tyvek® suits, gloves, and dust masks are recommended for cleaning.
- Maintain an ongoing cleaning protocol to regularly wipe down work surfaces with disinfectant.
- Remove wood from garage that is not actively being used.
- Avoid clutter and unnecessary storage in work areas.
- Visually screen donated animal feed; discard any in poor condition or infested with insects.
- Upon acceptance of donated animal feed, freeze the feed for at least 48-h before use.
- Store all animal feed in sealed containers.
- When purchasing new equipment, try to select models made of easily cleanable materials.
- Enact rodent exclusion and environmental management recommendations.
- Work with a licensed, private pest control operator to resolve flea issues in the lion enclosure. This should involve physical removal of the infested animal bedding material. Until the flea population has been reduced, limit staff access to the affected area.

A follow-up facility inspection was conducted on February 4, 2016. All moldy or insect-infested materials had been removed from the garage, and a new sanitation regimen enacted. In addition, several appliances and items of furniture, including the central L-shaped table, had been replaced by stainless steel items. A tree potentially providing rodent access to the building had been pruned and other entry points sealed. No mites were detected, and no further biting incidents have been reported.

This investigation of an arthropod biting outbreak benefited from a multi-disciplinary approach. Communication with affected individuals, consultation with the medical community, on-site observations and laboratory examination of samples were all key components leading to a satisfactory resolution. Many of the wildlife center staff had been initially convinced that fleas were the cause of their bites. Others harbored vague suspicions about mulch delivered around the same time or of other innocuous conditions such as fruit fly larvae present in the kitchen. Speaking with facility staff both focused our search and mitigated their fears. Similarly, the treating physicians, when presented with what might have been flea bites, had valid concerns about the potential for flea-borne diseases. The information they provided on the bite patterns helped narrow the list of candidate arthropods. The subsequent assignment of causality to *Pyemotes* mites, which do not vector human disease, in turn helped inform medical treatment decisions made by the physicians. The quick response and references offered by CDPH biologists were critical in facilitating a timely and appropriate response to this local outbreak.

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Nymphal *Ixodes pacificus* Tick Exposure Risk While Recreating on Trails in Henry Coe State Park

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ABSTRACT: Located in Santa Clara County, Henry Coe State Park is the second largest California State Park and the largest in Northern California. Tick collection on Forest Trail is unique in northern coastal California because of the dense numbers of ticks that are found on vegetation along the trail. It is common on this trail to pick up over 100 ticks on a 1 meter square flag after dragging only 3 meters. The overall objective of this study was to identify hiker activities and previously unknown tick niches along trails that could possibly put people at risk of nymphal tick exposure at park settings. Specific goals were to: 1.) determine the abundance of nymphal ticks collected from rocks and fallen trees along Forest Trail, 2.) determine the likelihood of someone picking up ticks by touching rocks and logs along trails with their hands, and 3.) determine species and prevalence of Borrelia in I. pacificus nymphs that are acquired by touching trail rocks and logs. Current research suggests that leaf litter plots with downed wood in hardwood forest areas pose a higher risk for nymph exposure, however our study demonstrates there may be equal risk for nymph exposure for those staying on the trail.

INTRODUCTION

Located in Santa Clara County, Henry Coe State Park is the second largest California State Park and the largest in Northern California. It has more than 250 miles of trails with 87,000 acres of open space. Henry Coe SP attracts hikers, backpackers, mountain bikers, equestrians, campers, wildflower enthusiasts, star gazers and fishermen (Henry Coe/Pine Ridge Association Website: http://coepark.net/pineridgeassociation/about-coe-park). At least three common human-biting tick species, the Western black legged tick *Ixodes pacificus* Cooley and Kohls, the Pacific coast tick *Dermacentor occidentalis* Marx, and American Dog tick *Dermacentor variabilis* (Say) are present (D. Bonilla pers. obs.) and are capable of transmitting a range of zoonotic diseases to visitors. Although the risk of acquiring an adult tick bite by walking in other coastal California parks has been classified as low (Lane 1996) certain other behaviors may put visitors at risk of acquiring either adult or nymphal *Ixodes pacificus*. This tick is the primary vector in California of Lyme disease bacteria (*Borrelia burgdorferi*) to humans (Burgdorfer et al. 1985, Lane and Lavoie 1988).

Some studies have shown that exposure to wood in leaf litter areas can increase the chance of acquiring nymphal *I. pacificus* in at least one region of California (Lane et al. 1992, Lane et al. 2004, Lane et al. 2007). This project aims to demonstrate possible risk of acquiring ticks from logs, downed trees, and rocks without leaving the maintained trail.

Tick collection on Forest Trail is unique in northern coastal California. This trail was first targeted because of the dense numbers of ticks that were found on the trail side. Three species of ticks (*I. pacificus, D. occidentalis, and D. variabilis*) were flagged from trailside vegetation. It is common on this trail to pick up over 100 ticks on a 1 meter square flag after dragging only 3 meters. Ticks climb up grasses on top of each other and cluster in interspecies groups, sometimes 10 to a seed head. It is also common to pick up nymphal *Ixodes pacificus* from rocks and logs that border and lie along the trail edge. For example, upon flagging a 1.5 m^2 trailside rock, 10 *I. pacificus* nymphs were found. Similar numbers for trailside logs were found. These are not areas that are traditionally thought of as areas where nymphal ticks are found. Furthermore, when one of us placed a hand on a trailside log, five *Ixodes* nymphs were found on the palm. With 3/7 (43%; MIP= 5.2%) nymph pools from this trail testing positive for *B. burgdorferi* sensu lato (B.b.s.l.) (Table 1), it seemed prudent to further investigate how trail activities could put people at risk of tick bites and potentially tick-borne disease.

Sex/Stage	Collection Date	Number of ticks	Number of pools	Number of positive pools	Minimum Infection Prevalence
Females	3/22/2007	123	13	5	
Males	3/22/2007	157	16	4	
Females	4/10/2008	70	1	2	
Males	4/10/2008	30	3	0	
Total Adults		380	33	11	2.9%
Nymphs	3/31/2007	34	5	1	
Nymphs	6/01/2007	13	1	1	
Nymphs	4/10/2008	11	1	1	
Total Nymphs		58	7	3	5.2%

Table 1. *Ixodes pacificus* testing for *Borrelia burgdorferi* sensu lato from Forest Trail, Henry Coe State Park 2007-2008.

The overall objective of this study was to identify hiker activities and previously unknown tick niches along trails that could possibly put people at risk of nymphal tick exposure at park settings in Northern California. Specific questions we want to answer are :1) In high abundance areas, is it just as easy to pick up nymphs (and potential tick-borne disease) without leaving the trail?; 2.) Is there a significant difference in the number of ticks on rocks, downed wood, tree trunks, and leaf litter?; 3) Are we more likely to pick up *Borrelia* infected ticks on the trail or in the leaf litter?

METHODS

Forest Trail has close proximity to the main visitor center and high numbers of ticks questing on the vegetation bordering the trail. These factors elevate the risk of a visitor unknowingly obtaining a tick on themselves. From 2007-2008, adult and nymphal *I. pacificus* were collected from Forest Trail and the areas directly surrounding it to obtain a baseline infection prevalence of *Borrelia burgdorferi* sensu lato. Flags used for tick collection were standard $1m^2$ white cotton-flannel segments attached to dowel rods. These were dragged over vegetation, leaf litter, and trail substrates, flipped, and the ticks removed. Ticks were placed in a vial and identified at the CDPH Richmond lab. In 2007 and 2008 adult and nymphal *I. pacificus* were tested by nested PCR at the US Army, Public Health Command Region-West, Washington for *Borrelia* spp. For a description of these methods, please see Padgett et al. 2014 (Padgett et al. 2014).

Starting on March 30th, 2, a mile stretch of Forest Trail was surveyed for nymphal *Ixodes* ticks approximately twice monthly (over 8 dates) to August 23rd, 2011 when two sampling sessions found no *Ixodes* nymphs. On March 30th, rocks, logs, downed trees, tree trunks, and benches within 1 meter of the trail edge were flagged with colored tape and numbered for future collection dates. Forty tree trunks, 27 logs and 11 rocks were labeled. Colored tape was removed at the end of the sampling period. Flagging was done as previously described however a modified flagging method was used for trees. In short, a $1m^2$ white cotton flannel cloth was wrapped around the trailside portion of the tree base and then the cloth was rubbed lightly by hand onto the tree surface.

Start times varied between 9:20 a.m. and 10:05 and end times varied between 11:15 a.m. and 2:00 p.m. Temperatures ranged from 49-85° C degrees at the start of sampling sessions and 55-98° C degrees at the sessions' end. Each date, the trail was hiked stopping at each numbered site. The biologist randomly placed her hand upon the substrate five times for 5 seconds each looking for nymphs on the hand between each placement. Any nymphs found were placed in a microcentrifuge tube and labeled with the site number and date. The substrate was then flagged with a cotton flannel flag or by the modified "tree rubbing" method. Tree trunks were sampled 1 meter high from their base. Ticks found were identified, counted, and were placed in tubes labeled with the collection site. A control plot within a mixed oak woodland was identified at the end of forest trail. This plot was divided into five linear side-by-side transects approximately 1 meter in width and 20 m in length. Transects were sampled by dragging the tick flag over the leaf litter and checking for ticks every 3 meters. An exception to this occurred on 3/30/2011 when ticks were sampled and identified but not taken for testing and leaf litter transects were not sampled.

Ticks collected were taken back to CDPH and tested by direct fluorescent antibody test (DFA) and polymerase chain reaction (PCR) for *Borrelia* spp. Ticks were tested individually first by direct fluorescent antibody testing to detect *Borrelia* spp. Positive ticks were then tested by PCR for B.b.s.l. Details of this procedure are described in Padgett et al. 2014.

RESULTS

In 2007 and 2008, 33 pools containing 380 *I. pacificus* adults total were tested from Forest Trail. Of these 33 pools, 11 (MIP=2.9%) were positive for *B. burgdorferi sensu lato*. Nymphs from along and beside these trails collected over three sampling dates were also tested. Of 7 pools containing a total 58 *I. pacificus* nymphs tested, three (MIP=5.2%) were positive for *B.b.s.l*. Table 1.

Trail

Overall, 193 *I. pacificus* nymphs were collected from the trail from 4/12/11-8/3/2011 by either hand placements (n=16) or substrate sampling (n=177). The most nymphs from the trail (n=110) came from wood (log) sampling. Wood sampling yielded an average of 0.58 ticks per wood product sampled over the sampling dates. Tree sampling produced 76 nymphs (avg=0.27 ticks/sample) and rocks produced 7 nymphs (avg=0.9 ticks/sample) (Table 2). The overall infection prevalence (for both sampling methods) during this time from the trail for *B.b*.s.l. was 3.4%.

Leaf litter Transects

Thirty *I. pacificus* nymphs were collected from the 5 leaf litter transects from 4/12/2011-8/3/2011. Of these 7/30 (23%) tested positive for *B.b*.s.l. Table 2. The flag sampling of Forest Trail and the adjacent leaf litter control plot from April-August 2011 yielded a total of 207 nymphal *Ixodes pacificus* (Table 2). Due to the differences in sampling number between substrates, we used a Kruskal Wallis non-parametric test to rank nymphs numbers between substrates. There was no significant difference in the number of nymphs from rocks, tree bases, fallen wood, or transect samples ($p<0.05$) (Figure 1).

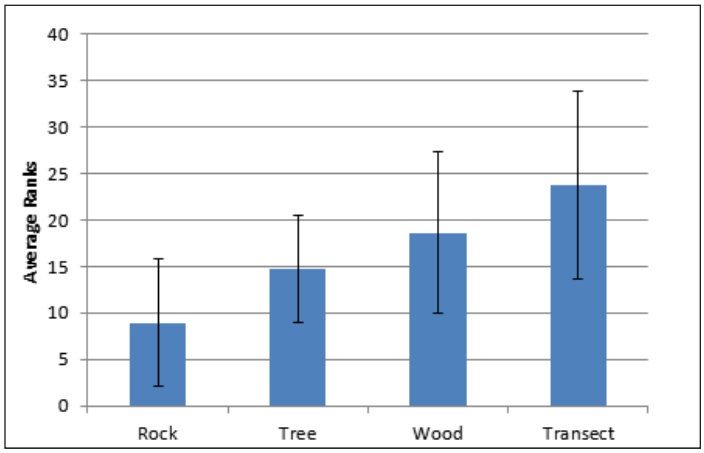

Figure 1. Comparison of substrate ranks using a Kruskal Wallis non-parametric test shows no significance in samples from transects and substrates

Hand placements

Sixteen nymphs were acquired during 2,730 hand placements (5 per substrate number along trail) (0.6%). One of these nymphs came from a rock, 9 from a tree, and 6 from wood products, 1(100%), 3(33.3%), and 1(16.7%) of these tested positive for *B.b.s.l.* respectively (Table 2).

Sex/Stage	Collection Date	Number of ticks	Number of pools	Number of positive pools	Minimum Infection Prevalence
Females	3/22/2007	123	13	5	
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Table 2. *Ixodes pacificus* nymphs sampled by flagging and hand placement tested from Forest Trail 4/12-8/3/2011We also found that the odds a nymphal tick from transect being positive for *Borrelia* is 31.5 times the odds positive tick being found on the wood (p value= 0.0001). The odds of nymphal tick from a transect being positive for *Borrelia* was 4.794 times the odds of a tick being positive for *Borrelia* found on a tree (p value= 0.01885). Lastly, the odds of a nymphal tick on a transect being positive for *Borrelia* (1.523) was not significant (p=0.597) when compared to the rock substrate.

DISCUSSION

Most of the nymphal sampling of *Ixodes pacificus* in California has been off the trail in pockets of leaf litter heavy oak woodland and oak grassland (Clover and Lane 1995, Talleklint-Eisen and Lane 1999, Li et al. 2000, Lane et al. 2004, Lane et al. 2007). Adults are the predominate stage along trails and just off trail in leaf litter, nymphs and larvae are more likely to be found (Clover and Lane 1995, Li et al. 2000). However, similar to our Forest Trail study site, the sides of trails of 4 state parks in Wisconsin were flagged for *Ixodes scapularis*. In this study they found a high number of nymphs (n=933) and larvae (n=2,702) rather than adults (n=68) on the trail side (Paskewitz et al. 2001). A study done in the San Francisco Bay Area was able to sample 501 adult *I. pacificus* over a year period from four trails, but no immature *I. pacificus*; even though 3 other species of immature hard ticks were found (Lane 1996). This may be indicative of the type of habitats the trails transversed. These trails went through open woodland and grassland with little leaf litter in comparison with Forest trail which has thick woodland and ample leaf litter areas. In a study of regional park trails in Northern California, trails wider than 2.5m were associated with higher adult tick count (Li et al. 2000). In contrast, Forest trail is very narrow with its widest spots being just over a meter.

In a subsequent study of another San Francisco Bay Area park, picnic areas and items within the areas: picnic tables, benches, rock walls, tree trunks, logs, and picnic blankets were sampled. *I. pacficus* nymphs were collected from leaf litter most often (37.9%) but when all the wood products were combined, numbers were very similar. They reported a nymphal infection prevalence with *Borrelia* spirochetes of 2.9%. Like our study, this study shows activities that people would often do in recreational settings in the San Francisco Bay area and reports a similar nymphal infection rate with *Borrelia* (Padgett and Bonilla 2011).

In a black oak woodland in Mendocino County, CA sitting on logs was found to be a high risk activity for acquiring *I. pacificus* nymphs (Lane et al. 2004). Furthermore, 87% of logs examined in a Maryland study had *Ixodes scapularis* nymphs (Carroll and Kramer 2001). There is an association of *I. pacificus* nymphs with wood and wood products in Northern California oak woodlands. Notably fallen logs and branches, the base of trees, and picnic tables have been identified (Slowik and Lane 2001, Lane et al. 2004, Padgett and Bonilla 2011).

Ixodes pacificus adult females are the species and stage of tick most often found attached to humans. These are larger chestnut colored and black ticks are found 4 times more often than the dark brown/black poppy seed sized (1.0-1.2 mm) nymphs. However, when nymphs are compared to adults their small size (less likely to be detected), rapid feeding duration, and high infection rate make them generally more important in the transmission of *Borrelia* spirochetes (Clover and Lane 1995). Moreover, nymphal *I. pacificus* in the San Francisco Bay Area are active mostly from March through August when many people are enjoying outdoor activities (Clover and Lane 1995). We found in our study over all years and sampling methods that the *B.b.s.l.* infection rates of *I.*

pacificus nymphs was 5.2% MIP for 2007-2008 and individual infection rate in 2011 was 5.8%. Adult *I. pacificus* had a MIP of 2.9% for 2007-2008. This information is reflective of the fact that *Borrelia burgdorferi* infection rates of *Ixodes pacificus* nymphs (5-15%) in California are generally higher than the infection rates of the adult stage (1-3%) (Burgdorfer et al. 1985, Lane and Lavoie 1988, Clover and Lane 1995, Talleklint-Eisen and Lane 1999, Li et al. 2000).

There was definite difference in the *B.b*.s.l. infection prevalence from the trail (3.1%) and the leaf litter transects (23%). This may be partly a result of low sample size from transects (n=30) but it may also have to do with host preference and availability in the two areas. Grey squirrels (*Sciurus griseus*) were often seen in our leaf litter plots and seem to fuel the *B.b*.s.l. infection rate in oak woodlands in Northern California (Lane et al. 2005, Eisen et al. 2009, Leonhard et al. 2010, Salkeld and Lane 2010). Furthermore, tree trunks act as refugia for nymphal *I. pacficus* but also serve as areas were Western fence lizards (*Sceloperus occidentalis*) reside (Slowik and Lane 2001). When nymphs feed on this lizard something in the lizard blood kills the *Borrelia* in the tick midgut and decreases the B.b.s.l. infection rate in nymphs for the area (Lane and Quistad 1998).

Our 20 m leaf litter control transects had a mean density of 0.89 ticks per transect compared to another Bay area study that found 2.22 nymphs per transect at one park and 1.52 at another park (Li et al. 2000). During our experimental time frame, it was observed by one of us that the nymph numbers were down considerably from earlier sampling sessions (2005-2006). During several dates in March-August 2014 and 2016, this same leaf litter plot was observed to be noticeably drier and more vegetation had grown through the litter. It is possible that this area of previously high risk is transitioning to a lower risk site.

In 2011, we saw an overall drop in trail infection prevalence in nymphs (3.4%) when compared to the 2007-2008 MIP (5.2%) (Table 1, 2). This is probably just a combination of yearly ecological fluctuation and a low sample size during 2007-2008 (n=58).

Overall, there were no significant differences in the number of nymphs collected from trail substrates (rock, tree, wood) and leaf litter transects by flagging. However more nymphs from leaf litter transect areas were infected with *Borrelia* and *Borrelia burdorferi* sensu lato than from trail substrates. It is also concerning that while only 1/177 nymph was *B.b*.s.l. positive (0.6%) from the trail from flagging, 5/16 (31%) nymphs were positive via the hand placement method (Table 2). This may just be a result of sample size, but does warrant further investigation.

To our knowledge, this first published account of acquiring nymphal *I. pacificus* while staying on the trail. People who recreate in wild areas should be mindful that ticks can be found both on and off the trail and should check themselves for ticks several days after being in tick habitat. Showering after hiking and using repellent when hiking are other ways to avoid tick bites. Any attached ticks should be removed as soon as possible.

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William C. Reeves New Investigator Award

The William C. Reeves New Investigator Award is given annually by the Mosquito and Vector control Association of California in honor of the long and productive scientific career of Dr. William C. Reeves.

The award is presented to the outstanding research paper delivered by a new investigator based on the quality of the study, the manuscript, and the presentation at the MVCAC Annual Conference.

This year, there were three applicants for the Reeves Award. First place was awarded to Mary Beth Danforth for her paper "The impacts of cycling temperature on West Nile virus transmission in California's Central Valley." Second place was awarded to Carrie De Jesus for her paper "Timed observations of precopulatory interactions between *Aedes aegypti* and *Aedes albopictus*." The manuscripts or summaries for all Reeves Award candidates appear on the pages following.

Year	Award Winner	Title of Paper
1988	Vicki L. Kramer	A comparison of mosquito population density, developmental rate and ovipositional preference in wild versus white rice fields in the Central Valley
1989	Truls Jensen	Survivorship and gonotrophic cycle length in *Aedes melanimon* in the Sacramento Valley of California
1990	Gary N. Fritz	Polytenes, isozymes and hybrids: deciphering genetic variability in *Anopheles freeborni*
1991	David R. Mercer	Tannic acid concentration mediates *Aedes sierrensis* development and parasitism by *Lambornella clarki*
1992	Darold P. Batzer	Recommendations for managing wetlands to concurrently achieve waterfowl habitat enhancement and mosquito control
1993	Jeffery W. Beehler	The effect of organic enrichment and flooding duration on the oviposition behavior of *Culex* mosquitoes
1994	Merry-Holliday-Hanson	Size-related cost of swarming in *Anopheles freeborni*
1995	Margaret C. Wirth	Multiple mechanisms cause organophosphate resistance in *Culex pipiens* from Cyprus
1996	No award	
1997	John Gimnig	Genetic and morphological characterization of the *Aedes* (*Ochlerotatus*) *dorsalis* group
1998	Yvonne Ann Offill	A Comparison of mosquito control by two larvivorous fishes, the stickleback (*Gasterosteus aculeatus*) and the mosquitofish (*Gambusia affinis)*
1999	Parker D. Workman	Adult spatial emergence patterns and larval behavior of the "Tule Mosquito," *Culex erythrothorax*
2000	Jason L. Rasgon	Geographic distribution of *Wolbachia* in California *Culex pipiens* complex: infection frequencies in natural populations
2001	Christopher Barker	Geospatial and statistical modeling of mosquito distribution in an emerging focus of La Crosse virus
2002	No award	
2003	Laura Goddard	Extrinsic incubation period of West Nile virus in four California *Culex* (Diptera: Culicidae) species
2004	No award	
2005	Troy Waite	Improved methods for identifying elevated enzyme activities in pyrethroid-resistant mosquitoes
2006	Lisa J. Reimer	Distribution of resistance genes in mosquitoes: a case study of *Anopheles gambiae* on Bioko Island
2007	Carrie Nielson	Impact of climate variation and adult mosquito control on the West Nile virus epidemic in Davis, California during 2006
2008	John Marshall	The impact of dissociation on transposon-mediated disease control strategies
2009	Win Surachetpong	MAPK signaling regulation of mosquito innate immunity and the potential for malaria parasite transmission control
2010	Tara C. Thiemann	Evaluating trap bias in bloodmeal identification studies
2011	Sarah S. Wheeler	Host antibodies protect mosquito vectors from West Nile virus infection
2012	Brittany Nelms	Overwintering biology of *Culex* mosquitoes in the Sacramento Valley, California
2013	Kimberly Nelson	The effect of red imported fire ant (*Solenopsis invicta* Buren) control on neighborhoods in Orange County, California
2014	Thomas M. Gilbreath, III	Land Use Change and the Microbial Ecology of *Anopheles gambiae*
2015	Jessica M. Healy	Comparison of the efficiency and cost of West Nile virus surveillance methods in California
2016	Mary Beth Danforth	The impacts of cycling temperature on West Nile virus transmission in California's Central Valley

The Impact of Cycling Temperature on the Transmission of West Nile Virus in California's Central Valley

Mary E. Danforth[1,3], William K. Reisen[2], Christopher M. Barker[2]

INTRODUCTION

As with other arthropod-borne pathogens, transmission of West Nile virus (WNV) is strongly impacted by temperature (Hartley et al. 2012). For example, the extrinsic incubation period (EIP), or the time from a mosquito's initial viremic bloodmeal to when it becomes capable of transmission, is shortened in the presence of warm mean temperatures (Dohm et al. 2002, Reisen et al. 2006, Kilpatrick et al. 2008, Danforth et al. 2015). In addition to the impacts of mean temperature, daily temperature fluctuations typical of those that occur in nature have been shown to alter the transmission of other mosquito-borne pathogens as expected from mean-temperature only studies (Paaijmans et al. 2009, Lambrechts et al. 2011, Carrington et al. 2009). In particular, large daily temperature ranges (DTR) can shorten EIPs at cooler mean temperatures and lengthen them at warmer mean temperatures (Paaijmans et al. 2009, Lambrechts et al. 2011, Carrington et al. 2009). Mosquito behavior also has the potential to influence the temperature to which they are exposed. For example, *Culex tarsalis*, one of the primary vectors of WNV in California, is a night biting mosquito and spends the daytime in underground refugia, protected from the warmest temperatures of the day (Meyer et al. 1990). However, current estimates for the temperature-based risk of WNV transmission used by California mosquito control and public health agencies are based solely on constant temperatures and do not take into consideration mosquito behavior (Reisen et al. 2006). As a result, we hypothesize that daily cycling temperatures in California will shorten the EIP at cooler mean temperatures and lengthen them at warmer mean temperatures. In addition, mosquito behavior will lengthen the EIP when compared to ambient conditions, due to exposure to cooler mean temperatures.

MATERIALS AND METHODS

We used KERN11 stock virus from a *Cx. tarsalis* pool collected in Kern County in 2011 (KERN2000-2011, Gen Bank accession KR348980) and *Cx. tarsalis* from the KNWR colony, which was established from mosquitoes collected at the Kern National Wildlife Refuge (35.7458°N, 118.6179°W). Mosquitoes were infected by feeding on virus-spiked blood via Hemotek feeders. After the bloodmeal, they were incubated under conditions designed to mimic cycling daily temperature patterns recorded during March, May, and July 15 in Bakersfield (NCDC 2015), with a fourth based on temperatures accommodating mosquito behavior during July (Meyer et al. 1990). At predetermined time points, mosquito expectorant was collected via the capillary tube method (Aitken 1977). Mosquito bodies and expectorants were analyzed by quantitative reverse-transcriptase PCR. Data were analyzed via multivariate logistic regression, with the outcome as each mosquito's transmission status, and explanatory variables of time, mean temperature, and daily temperature range. Data from a prior constant temperature experiment were included for comparison to the current cycling temperature results.

RESULTS

When results from mosquitoes under March, May, and July cycling conditions were compared to those from constant temperature estimates based on mean monthly temperature, there was no significant difference in the time to transmission. However, when transmission by mosquitoes incubated under conditions mimicking those of their behavior in July was compared to transmission by *Cx. tarsalis* exposed to ambient conditions from the same month, time to transmission was significantly delayed by three days.

DISCUSSION

Under the conditions found in Bakersfield and large parts of the rest of California's Central Valley, the typical daily cycling temperatures of that region did not impact the rate of extrinsic incubation of WNV by *Cx. tarsalis* as compared to estimates from the same constant mean temperatures. This lack of impact could be due to smaller DTRS than were found to impact dengue (Lambrechts et al. 2011); however, they were within the range of impactful DTRS for malaria transmission (Paaijmans et al. 2009). As a result, estimates for the EIP in California and other areas with similar DTRs can be based on mean temperature alone. However, we determined that mosquito behavior did significantly impact the amount of time required to complete the EIP when compared to estimates based on ambient conditions alone. In addition, we were able to develop a model that more accurately captured the curvilinear relationship between transmission, time, and temperature as observed in nature. Together, those results have the potential to improve prediction of the EIP by mosquito control and public health agencies.

1 A complete version of the manuscript has been published in the Journal of Medical Entomology (Danforth et al. 2016).

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Timed observations of precopulatory interactions between *Aedes aegypti* and *Aedes albopictus*

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ABSTRACT: *Aedes aegypti* and *Aedes albopictus* are both competent vectors of dengue and chikungunya viruses and share similar morphological, ecological, and behavioral characteristics. *Aedes albopictus* has competitively displaced populations of *Ae. aegypti* due, in part, to satyrization. We investigated if *Ae. aegypti* and *Ae. albopictus* differed in their precopulatory interactions, and whether male body size was a factor in mating success. Large and small males of each species were generated by varying larval density, and females were derived from separate rearings at an intermediate density. Five four day old males were placed into conspecific or heterospecific mating cups with 5 four day old females. The number of mating attempts and venter to venter coupling were recorded for periods of 5, 10, 15, 30 or 45 minutes. We found that the number of mating attempts and successful matings significantly differed over time and mating treatment. In conspecific mating groups, there were more attempts made than in heterospecific mating groups. There was a significant interaction between mating treatment and time; however, male size did not significantly affect the number or mating attempts or venter to venter couplings. A few incidences of polyandry were seen in the mating cups. Further investigations of precopulatory behavior of both species are needed to understand why heterospecific matings occur.

INTRODUCTION

Aedes aegypti and *Aedes albopictus* are the two most important vectors of chikungunya, Zika, and dengue viruses. Both species share similar behavioral characteristics: diurnal activity, container breeding, mating near a host, and the formation of small mating swarms. However, differences and similarities in mating behaviors between these two species has yet to be thoroughly investigated. Precopulatory behaviors can play a crucial role in reproductive success of *Ae. aegypti,* but these behaviors have yet to be described for *Ae. albopictus*. In the case of *Ae. aegypti*, mating pairs match the harmonics of their wing beat frequency before copulation (Cator et al. 2009). These matching harmonic traits are passed down to the offspring, and the offspring of harmonic parents are reproductively more successful (Cator and Harrington 2011). An aggregation pheromone for *Ae. aegypti* has also been identified that attracts females to a swarm for mating (Cabrera and Jaffe 2007, Fawaz et al. 2014).

Precopulatory behaviors do not seem to prevent heterospecific mating events. Multiple investigations have found heterospecific mating between A*e. albopictus* males and *Aedes polynesiensis*, *Aedes guamensis, Aedes cretinus* and *Ae . aegypti* females under laboratory and field conditions (Tripet et al. 2011, Bargielowski et al. 2013, Nasci et al. 1989, Leahy and Craig 1967, Gubler 1970, Rozeboom and Bridges 1972, Giatropoulos et al. 2015). Species that incorrectly mated with *Ae. albopictus* have experienced declines in their populations, likely due to satyrization (Gubler 1970, Rozeboom and Bridges 1972, Bargielowski et al. 2013). Matings between *Ae. aegypti* females with *Ae. albopictus* males have been the most well studied case of satyrization (Leahy and Craig 1967, Tripet et al. 2011, Bargielowski et al. 2013). Differences in mating behavior between these species are important to further understand the phenomenon of displacement of *Ae. aegypti* by *Ae. albopictus* (Hobbs et al. 1991, O'Meara, 1993, Bargielowski et al. 2013). The precopulatory behaviors that occur before heterospecific mating have yet to be studied.

For this investigation the number of mating attempts and matings were observed over time for *Ae. aegypti* and *Ae. albopictus* mating swarms in conspecific and heterospecific mating conditions to observe potential precopulatory differences. In addition, we hypothesized that male size is an important factor in mating behavior, and predicted that larger males are more robust and would be more likely to mate faster than small males.

MATERIALS AND METHODS

Eggs of *Ae. aegypti* were previously collected in West Palm Beach, (F_6-F_8) Florida and *Ae. albopictus* (F_3 or F_5) in Raleigh, North Carolina to establish colonies. *Aedes aegypti* and *Ae. albopictus* eggs were hatched in trays (Rubbermaid Egg Tray (5.69x22.81x32.99cm), Rubbermaid, Huntersville, NC, USA) filled with 1L of tap water in a 27°C incubator for 24 hours (Thermo Scientific Precision Incubator 818, ThermoScientific, Marietta, Ohio, USA). Species were hatched separately in their own trays.

To generate large and small males, we varied larval density. Large male trays contained 100 larvae and small male trays contained 250 larvae. Females were reared in separate trays with 150 larvae. Each tray was given 3 pellets of coy fish food (Wardy Pond, Pellet, Secaucus, NJ, USA). Trays were monitored daily for the appearance of pupae. When pupae appeared they were removed immediately and then separated by sex. Pupae were placed into 16oz cups with tap water. Cups with pupae were monitored daily for the emergence of adults. Adults were placed into a climate controlled rearing room at 27°C with relative humidity at 80% with a 14:10 h L:D light cycle and provided with 20% sucrose solution. Adults were then placed into new 16oz cups based on date of emergence and sex. Adults in cups were examined before mating trials and those with both sexes were discarded to prevent the use of previously mated females.

Four-day old adults were placed into mating cups (32oz, 0.95 L) with 5 females and 5 males to mimic mating swarms for

intraspecific and interspecific mating. Males placed in each cup were from 1 specific tray. Cups were observed for continuous periods of 5, 10, 15, 30 or 45 minutes which were repeated five times each. Each cup for each time period observed had a different group of males and females. The observer was close to the mating cups to record behavior and to provide host stimulation. The number of mating attempts (any grasp or directed flight at a female), and time after co-habitation at which successful matings (venter to venter for at least1sec, uninterrupted) (Helinski and Harrington 2012a, Oliva et al. 2013) occurred were recorded. In order for a male to successfully inseminate a female, they must be in the venter to venter position (Clements 1999). Uninterrupted matings were counted, since incomplete sperm transfer could account for multiple matings in a single femaleand incomplete matings can appear as successful mating until insemination can be examined (Helinski et al. 2012). In *Ae. aegypti* females it takes 6 sec of copulation for successful insemination, and this time is similar for *Ae. albopictus* (Spielman 1964, Oliva et al. 2013). The time was reduced for successful matings to 1 sec, because preliminary studies noted heterospecific matings were brief, but were able to transfer sperm.

After each timed observation females were removed from mating cups to be dissected to confirm insemination. Females were anesthetized with CO_2. The spermathecae was then removed with insect pins under a dissection scope. After the spermathecae was removed, it was picked up with a paintbrush tip and moved onto a glass slide and placed in a drop of phosphate buffered saline solution. The spermathecae was then examined under a compound microscope at 100X to check for the presence of sperm.

Male wing length was measured by the distance from the alula to the wing tip excluding any fringe scales. Wing length measurements were taken with a dissection scope and measured with a mounted camera (Olympus SXZ-LLT, Olympus Cell Sens Standard 1.7.1, MA, USA).

A t-test was used to compare male wing lengths of all small and large males (SAS 9.4, SAS Institute Cary, NC, USA). To compare the number of mating attempts, the number of matings, and the number of inseminated females to the mating treatment and male body size over time, a generalized linear mixed model (GLIMMIX) was used. Distribution was checked for goodness of fit by examining the ratio of the chi-square to degrees of freedom, with values close to one suggesting a good fit. For the number of attempts, the negative binomial error distribution fit the data well (chi-square/df = conspecific 1.06). First, second and third order effects were examined, with non-significant third and second order effects removed and the model rerun. However, for the number of matings, while both the negative binomial and Poisson distribution fit well, models with second and third order terms did not converge. In this case we used a normal approximation of the data, which had a good fit (chi-square/df = 1.93) and allowed examination of interactive effects. Again, first, second and third order effects were examined, with non-significant third and second order effects removed and the model rerun.

RESULTS

Male wing lengths significantly differed for small and large reared males in all mating treatment groups ($p > 0.01$). The average wing length for *Ae. aegypti* small and large males for the conspecific group was 1.84 mm and 2.12 mm, respectively. For the heterospecific treatment group *Ae. aegypti* small males wing length averaged 1.82 mm and for large males 2.06 mm. The average wing length for *Ae. albopicuts* males was 1.82 mm for the small group and 1.95 mm for large in the conspecific treatment group. For the heterospecific treatment group *Ae. albopictus* small males wing length averaged 1.78 mm and for large males 1.95 mm.

The number of mating attempts did significantly differ among mating treatments (Table 1). The *Ae. aegypti* female with *Ae. albopictus* male cross significantly differed from all other mating treatments (Fig 1)

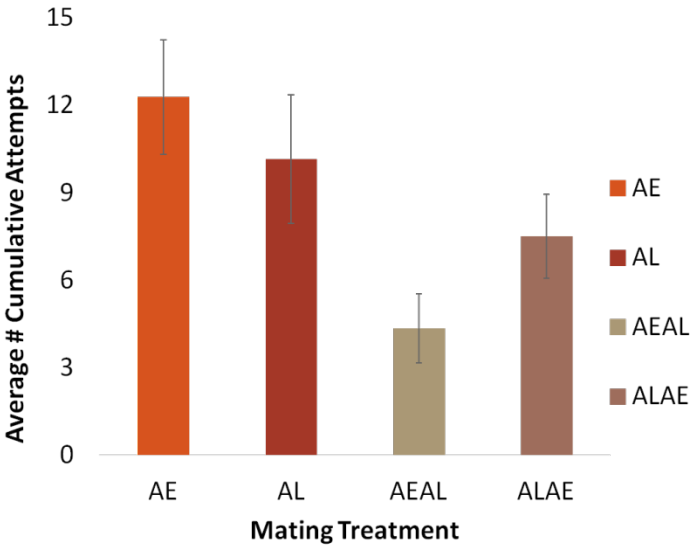

Figure 1: Mean number of mating attempts for each mating treatment. *Ae. aegypti* (AE), *Ae. albopictus* (AL), AEAL and ALAE (Female x Male crosses). Error bars = SE±

Conspecific mating treatments did not significantly differ from each other (Fig 1). Size was not a significant factor in the number of attempts overall (Table 1). The cumulative number of attempts observed increased over the 45-minute observation period in all mating treatments and was significantly different (Fig. 2) (Table 1).

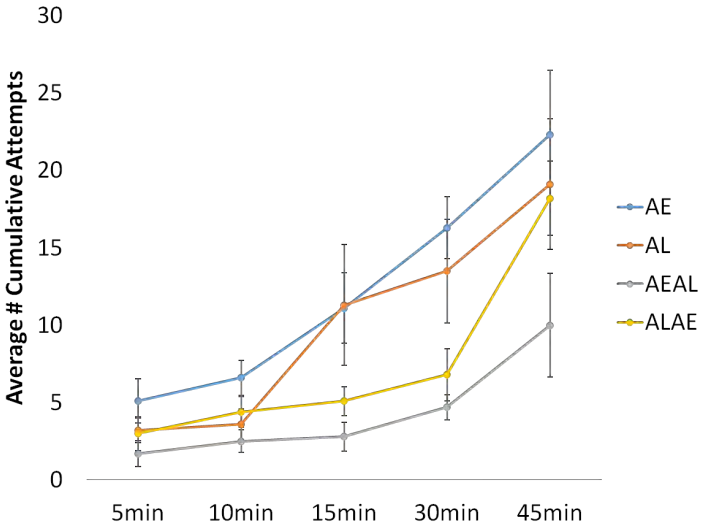

Figure 2: The average cumulative number of mating attempts over time. AE = *Ae. aegypti*, AL= *Ae. albopictus*, AEAL and ALAE (Female x Male crosses). Error bars = SE±

EFFECT	Num DF	Den DF	F Value	Pr > F
MT	3	183	18.43	<.0001
SIZE	1	183	0.44	0.5062
MT*SIZE	3	183	1.52	0.2110
TIME	4	183	36.57	<.0001

Table 1. PROC GLIMMIX table for the number of mating attempts, using a generalized linear mixed model with a negative binomial distribution. Num DF = Numerator degrees of freedom, Den DF = Denomiator degrees of freedom, MT = Mating treatment

The number of matings did significantly differ among mating treatments (Table 2).

EFFECT	Num DF	Den DF	F Value	Pr > F
MT	3	174	22.52	<.0001
SIZE	1	174	0.98	0.3248
TIME	4	174	11.63	0.0289
MT*-TIME	12	174	2.69	<.0001

Table 2. PROC GLIMMIX table for the number of matings, using a generalized linear mixed model with a normal approximation. Num DF = Numerator degrees of freedom, Den DF = Denomiator degrees of freedom,MT= Mating treatment.

Conspecific treatments had more matings than heterospecific treatments and did significantly differ (Fig. 3). The number of matings that occurred increased over time (Fig. 4). There was no effect due to size (Table 2). There was a significant interaction between size and mating treatment, but subsequent corrected pairwise comparisons were not significant (all $p > 0.05$, Bonferroni correction (Table 2). A significant interaction was seen between mating treatment and time (Table 2). Over time the number of matings increased in the conspecific mating treatments, while few matings occurred in heterospecific treatments (Fig 4). Insemination rates followed almost perfectly to observations of "successful matings" confirming our assumptions (Table 3).

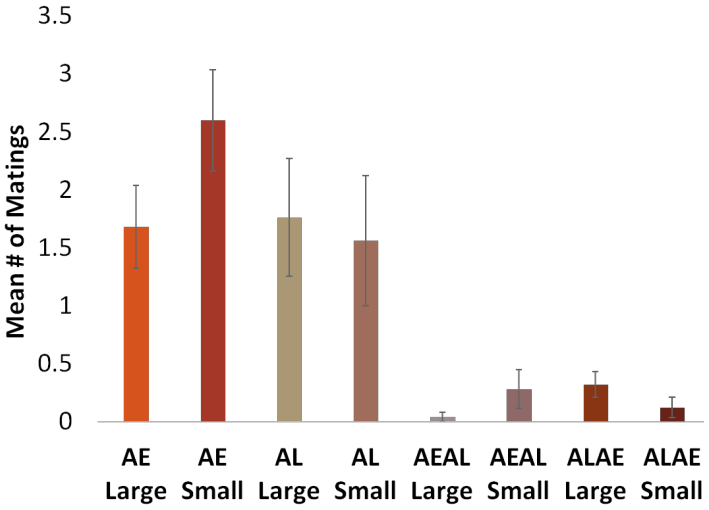

Figure 3: Mean number of matings for each mating treatment. *Ae. aegypti* (AE), *Ae. albopictus* (AL), AEAL and ALAE (Female x Male crosses). Error bars = SE±

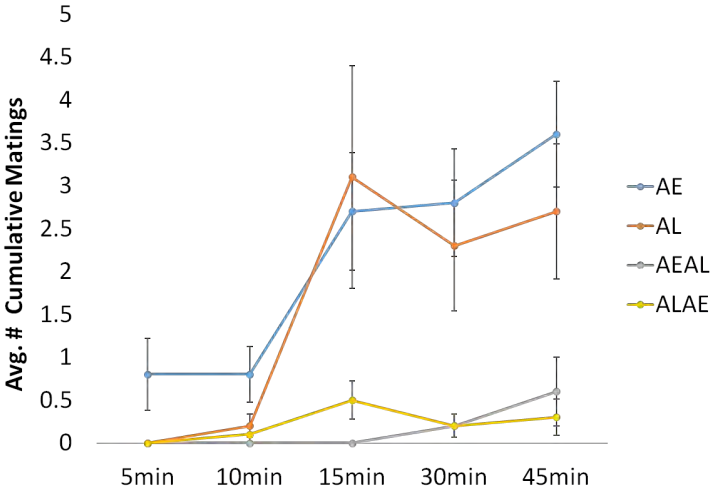

Figure 4: Average number of cumulative matings over time for each mating treatment. *Ae. aegypti* (AE), *Ae. albopictus* (AL), AEAL and ALAE (Female x Male crosses). Error bars = SE±

Some cups had females that mated more than once. In the *Ae. aegypti* and *Ae. albopictus* conspecific treatments, 20% of the mating cups had more than 5 matings recorded, ranging from 6-10 observed copulations, meaning that at least one female mated more than once. Multiple matings of females were seen with both small and large size males. We did not see more than 5 matings in heterospecific cups; however, it plausible that a single female may have mated more than once.

Treatment	Size	Time	Mean proportion inseminated	S.E.M.
AE ♂ x AL ♀	L	5	0	0.000
	L	10	0	0.000
	L	15	0	0.000
	L	30	0	0.000
	L	45	0.04	0.040
	S	5	0	0.000
	S	10	0	0.000
	S	15	0	0.000
	S	30	0.08	0.049
	S	45	0.16	0.117
Ae. aegypti	L	5	0.08	0.080
	L	10	0.16	0.117
	L	15	0.28	0.080
	L	30	0.48	0.150
	L	45	0.64	0.194
	S	5	0.24	0.147
	S	10	0.16	0.075
	S	15	0.68	0.136
	S	30	0.6	0.190
	S	45	0.72	0.120
AL ♂ x AE ♀	L	5	0	0.000
	L	10	0	0.000
	L	15	0.04	0.040
	L	30	0.04	0.040
	L	45	0.08	0.080
	S	5	0	0.000
	S	10	0	0.000
	S	15	0.04	0.040
	S	30	0	0.000
	S	45	0.04	0.040
Ae. albopictus	L	5	0	0.000
	L	10	0.04	0.040
	L	15	0.4	0.210
	L	30	0.64	0.183
	L	45	0.44	0.147
	S	5	0	0.000
	S	10	0.04	0.040
	S	15	0.4	0.245
	S	30	0.24	0.194
	S	45	0.52	0.224

Table 3. Mean proportion of *Aedes aegypti* (AE) or *Ae. albopictus* (AL) females inseminated during mating attempts.

DISCUSSION

We examined the timing of mating interactions in conspecific and heterospecific mating for *Ae. aegypti* and *Ae. albopictus*. We found that the number of attempts significantly differed over time and mating treatment. The number of attempts increased with time. In conspecific mating groups, there were more mating attempts made than in heterospecific mating groups, agreeing with other studies (Bargielowski and Lounibos 2014, Tripet et al. 2011, Leahy and Craig 1967). The same trends were seen for the number of matings, where there were more matings between conspecifics than heterospecifics. There was a significant interaction between mating treatment and time as well.

In our study we attempted to create ideal conditions to observe both conspecific and heterospecific mating. To create ideal conditions, we implemented multiple tactics to mimic natural mating settings. Mating in both *Ae. aegypti* and *Ae. albopictus* typically occur near a host or in small swarms. Many studies on mosquito mating biology have been conducted without host stimulation (Bargielowski and Lounibos 2014, Polnawat and Harrington 2009, Leahy and Craig 1967). However, during our observations the observer remained close to the cup mating arena providing host stimulation. We attempted to reproduce a small male swarm by placing 5 males in each cup and then provided 5 females for mating. Swarms were used because in field settings mating swarms increased copulation frequency (Cabrera and Jaffe 2007). Cups had a limited amount of space compared to field conditions, so the number of males in the swarms were reduced. Density of mosquitoes within mating cages can impact the number of matings that occur and can lead to artificially high insemination rates (Ponlawat and Harrington 2009). Having the higher density of mosquitoes was ideal for heterospecific matings, because they do not occur readily in the field or in laboratory settings. However, the reduced swarm size may have not had enough males for ample swarm activity. In field studies swarms of male *Ae. aegypti* had on average 23 individuals and 3- 40 individuals for *Ae*. *albopictus*, putting our experimental numbers at the low end of that range (Cabrera and Jaffe 2007, Gubler and Bhattacharya 1972). However, the size of mating swarms is variable and may be dependent upon the local population density. We provided sufficient conditions to the mating swarms to allow mating to occur for both conspecific and heterospecific mating. *Aedes aegypti* conspecific matings had the most number of attempts and matings observed. *Aedes albopictus* did not mate as often as *Ae. aegypti* but other factors such as mating density and time to mate may have impacted our results. We observed few matings in the heterospecific mating treatment.

Mosquito swarms were allowed 5 to 45 minutes to mate within the cups. In *Ae. albopictus* swarms in field conditions the highest frequency of matings occurred between 5 to 10 minutes (Gubler and Bhattacharya 1972). In studies previously conducted with heterospecific mating, small swarms were given 15 minutes to mate (Bargielowski and Lounibos 2014). Bargielowski and Lounibos (2014) found *Ae. aegypti* females were inseminated by *Ae. albopictus* males 32-61% of time after 15 minutes of exposure

in strains not resistant to satyrization. Based upon these previous studies and our conspecific mating treatment, the mosquitoes in our study were provided sufficient an amount of time for mating in both mating treatments.

Aedes aegypti females have been shown to more likely mate with *Ae. albopictus* than the reverse cross (Bargielowski et al. 2013, Leahy and Craig 1967, Nasci et al. 1989, De Jesus, 2015). In our study we found that the *Ae aegypti* male with female *Ae. albopictus* cross had more mating attempts and matings, the reverse of what we predicted. Factors including space within in the cups and time might have interfered. Virgin male and female mosquitoes were placed in mating treatment cups 4 days after emergence. Peak mating occurs 3-5 days after emergence (Leahy and Craig 1967). By using 4 days old male and females we expected mating activity in our mating treatments. *Aedes aegypti* and *Ae. albopictus* males take approximately 24 hours for male terminila to rotate and once this is complete males can mate (Roth 1948, Oliva et al. 2012). Since males were exposed to females well after this time period, males should have been capable of mating. In other studies, mosquito pairs were stimulated to mate by tilting a tube back a forth (Oliva et al. 2013). My cups were not shaken or disturbed in any form during observations which could have influenced the number of matings observed across all treatments.

In our study we wanted to observe the interactions leading up to heterospecific mating between *Ae. aegypti* and *Ae. albopictus*. A previous study found that allopatric and sympatric populations of *Ae. aegypti* responded to the presence of *Ae. albopictus* differently (Bargielowshi and Lounibos 2014). Sympatric strains of *Ae. aegypti* were less likely to mate with *Ae. albopictus* than allopatric strains (Bargielowski et al. 2013). The strain of *Ae. aegypti* used in our study was from West Palm Beach Florida and therefore likely to have had a history of exposure to *Ae. albopictus* (Reiskind and Lounibos 2013). Another study found that over 1-3 generations of *Ae. aegypti* exposed to *Ae. albopictus* became resistant to satyrization and *Ae. aegypti* that became resistant to satyrization took longer to mate with conspecifics (Bargielowski and Lounibos 2014). However, this artificially selected avoidance of heterospecific mating was lost over 5 generations in the absence of exposure to heterospecifics (Bargielowski and Lounibos, unpublished data). Because our *Ae. aegypti* population had been kept away from *Ae. albopictus* for at least six generations, we expected that they would respond similarly as allopatric populations in the heterospecific mating treatment. However, low amounts of mating and insemination in the heterospecific mating cups were observed in our study. The factors previously discussed may have played a factor in the low levels of mating observed,

A few incidences of polyandry were seen in our mating cups. In the conspecific treatments 20% of the cups had cases of polyandry which is similar to previous investigations. In a study with *Ae. aegypti* in semi-field conditions, 14% of had mated more than once (Helinski et al. 2012c). For *Ae. albopictus*, approximately 26% of females collected from the field had progeny from more than one male (Boyer et al. 2013). In spite of this empirical data, both species are typically considered monandrous (Clements 1999). Seminal protein fluids (Spfs) alter female behavior after copulation preventing females from mating again for multiple gonotrophic cycles (Helinski et al. 2012a). In heterospecific matings Spfs of *Ae. albopictus* males cause the satyrization of *Ae. aegypti* females, by rendering them refractory to further matings and may occur even in the absence of sperm deposition (Oliva et al. 2013). Using cues for mating in precopulatory behavior is crucial for *Ae. aegypti* females. How *Ae. aegypti* females confuse precopulatory signals or why *Ae. albopictus* males do not recognize correct females is still unknown.

Precopulatory barriers between species should be further examined. Visual, auditory and pheromones should be compared between these two species. Flight tone harmonics and pheromones have proven to play a crucial role in *Ae. aegypti* and could also be important *Ae. albopictus* mating and cross mating between the two species. Understanding the differences between these species is important to the development of new vector control techniques to help prevent disease transmission.

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Borrelia Spirochete Loads in *Ixodes pacificus* Ticks of San Mateo County, California

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ABSTRACT: The Lyme disease agent *Borrelia burgdorferi* and relapsing fever group species *Borrelia miyamotoi* are tick-borne spirochetes known to occur in the San Francisco Bay area. Both agents share the same tick vector, the western blacklegged tick, *Ixodes pacificus.* To study the prevalence of both species in host-seeking *Ixodes pacificus* ticks, we collected 5,082 ticks from 15 recreational areas in San Mateo County. By using a multiplex, quantitative polymerase chain reaction we were able to estimate the prevalence and abundance of spirochetes for both *B. burgdorferi* sensu lato and *B. miyamotoi* in western black-legged ticks. Overall estimated prevalence was twice as high for *B. miyamotoi* (1%) as for *B. burgdorferi* (0.5%). The estimated spirochete counts were over 10 times higher for *B. miyamotoi* compared to *B. burgdorferi*, but there were no significant differences in spirochete load when adult female, male, and nymphal ticks were compared. Regression analysis revealed a positive correlation between average spirochete load per tick and the prevalence of *B. burgdorferi* in San Mateo County parks.

INTRODUCTION

The western blacklegged tick, *Ixodes pacificus*, is widely distributed in California, and is the primary vector of the human pathogen *Borrelia burgdorferi*, the causative agent of Lyme disease in California and the Far-West (Burgdorfer et al. 1985, Clover and Lane 1995). The recent discovery of *Borrelia miyamotoi*, a member of the relapsing fever group of *Borrelia*, has prompted studies to understand its ecology and pathogenesis. Unfortunately, due to limitations of cultivating *B. miyamotoi* outside of an animal host, progress has been slow, and far less is known about this agent compared to its Lyme disease-causing counterpart (Scoles et al 2001). Human disease cases caused by *B. miyamotoi*, characterized by fever with possible meningoencephalitis, have been prevalent in the northeastern United States (Krause et al. 2015). Studies have confirmed the presence of *B. miyamotoi* in California (Mun et al 2006, Padgett and Bonilla 2011, Salkeld et al. 2014, Federova et al. 2014), and it may only be a matter of time before human infections in the far-western United States are recognized.

Quantifying *Borrelia* spirochetes has been used for a number of applications in ecological and clinical studies in order to understand the spatial and temporal distribution of *Borrelia* and the factors that may affect it. Studies have used this approach to describe how spirochetes react to blood-feeding and molting (Piesman et al. 1990, Silva and Fikrig 1995), and to determine spirochete counts in the blood of patients with erythema migrans and acute disease (Liveris et al. 2012). The spirochete load of infected ticks has been shown to differ widely according to the *Borrelia* species and the geographic location of the collected tick. Barbour et al. (2009) and Wilhelmsson et al. (2013) found higher *B. miyamotoi* densities in captured ticks compared to loads of other *Borrelia* species, and the former described a bimodal distribution of *B. miyamotoi* spirochete loads among nymphs. Although they often share vectors and reservoir hosts where they are sympatric (Fukunaga et al. 1995, Barbour et al. 2009, Cosson et al. 2014), the ecology of *B. burgdorferi* and *B. miyamotoi* are distinguished by the ability of *B. miyamotoi,* but not *B. burgdorferi,* to be transovarially transmitted (Rollend et al. 2013). There is still little known about how this transovarial transmission may affect the maintenance of *B. miyamotoi* in an environment and whether there is a possibility of competition with *B. burgdorferi* in tick vectors.

Our study aimed to learn more about the ecology of *Borrelia* spp. by quantifying the spirochetes in infected *I. pacificus* ticks found in San Mateo County, California. As far as we know, this was the first study to examine *B. miyamotoi* spirochete loads in the *I. pacificus* tick. Through comparison of spirochete densities we sought to pinpoint any possible differences between *B. miyamotoi* and *B. burgdorferi* which may influence their ecology. In addition, overall trends of spirochete loads of *Borrelia* within the adult tick gender and geographic location were examined to see if a relationship exists between spirochete load and infection prevalence.

MATERIALS AND METHODS

Study sites and tick collection. All sites were in located San Mateo County with the majority representing recreational areas (e.g. California State Parks, Midpeninsula Open Space Preserves, city parks) (Figure 1). Ticks were collected by the San Mateo County Mosquito and Vector Control District (SMCMVCD) staff, focusing on the winter months because during summers SMCMVCD activities were primarily devoted to mosquito-borne disease surveillance. Collections for this study were completed over the course of two years between January 2014 and March 2015. In San Mateo County, and most of north-western California, questing adult *I. pacificus* populations begin to appear in late October and may be found into mid-June (Clover and Lane 1995, Salkeld et al. 2014). While nymphs may start to appear as early as January, SMCMVCD staff usually does not encounter them until March. Host-seeking ticks were collected by flagging vegetation on the sides of trails using a 1.0 m² white cotton cloth attached to a pole. The cloth was inspected for ticks every 20 m, and ticks found on the flag were transferred to vials using forceps. At the

end of a collection trip, ticks were sorted by species and sex if adult and by developmental stage, counted, and pooled in groups of 5 per vial in 5mL sterile tubes (SPEX Sample Prep, Metuchen, NJ) for DNA extraction. Pooling was performed to increase tick sample size to detect positive samples and keep costs low. These collections were also used for the district's annual prevalence assessment in which pooling is the normal practice.

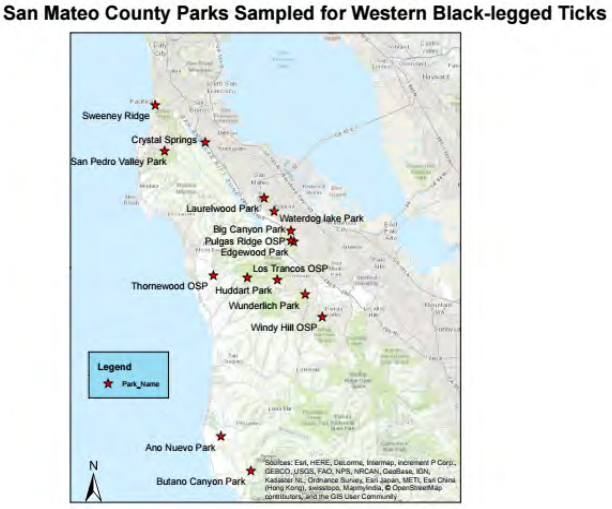

Fig 1. Recreational areas in San Mateo County sampled for western black-legged ticks

DNA extraction and quantitative PCR. Two ceramic beads and 800uL lysis/binding buffer (MagMAX 96-Viral Isolation Kit, Life Technologies, Carlsbad CA) were added to each 5mL tube. The ticks were mechanically crushed efficiently by agitating with a bead mill for 5 minutes (Mixermill 800D from SPEX Sample Prep, Metuchen, NJ). DNA was extracted from tick pools using MagMAX 96-Viral Isolation Kit following manufacturer's protocols and stored at -20°C until further analysis. DNA from all sample pools was subjected to quantitative multiplex real-time PCR (qPCR) following Barbour et al. (2009), with two probes that individually hybridized to regions of the 16S rDNA that differed between *B.burgdorferi* and relapsing fever *Borrelia* species, including *B. miyamotoi*. Forward and reverse primers were 5′GCTGTAAACGATGCACACTTGGT and 5′GGCGGCACACTTAACACGTTAG. The corresponding TaqMan® probes were 5'-6FAM-TTCGGTACTA ACTTTTAGTTAA and 5'- VIC-CGGTACTAACCTTTCGATTA with 3' ends modified with a minor groove binding protein (Applied Biosystems, Foster City, CA). The reaction was performed in 25-µL volume in MicroAmp Fast Optical 96-Well Reaction Plates (Life Technologies, Carlsbad CA) at a final concentration of 900 nmol/L for each primer and 200 nmol/L for each probe. The PCR conditions were 50°C for 2 minutes and 95°C for 20 seconds, followed by 40 cycles of 95°C for 3 seconds and 60°C for 30 seconds on a 7500fast Real-Time PCR machine (Applied Biosystems, Foster City, CA). To monitor for contamination, negative controls, containing only water, were included with all DNA extraction and PCR procedures. DNA standards were prepared according to Barbour et al (2009) using cloned fragments of the targets for qPCR. Tick pools positive for either *Borrelia* were re-tested in triplicate on a simultaneous run with DNA standards for quantification.

Estimation of infection prevalence and spirochete load. In order to determine how many ticks were positive for *Borrelia,* we used a minimum infection prevalence (MIP) calculation: (number of positive pools / total specimens tested) x 100. The MIP assumes that a positive pool contains only one infected tick, an assumption that is considered valid when infection prevalence is low and sample size is high (Gu et al. 2003).

For comparison across the different pools, we estimated normalized spirochete counts per µg of extracted DNA. Raw qPCR results of genome copies/5µl DNA per pool were converted to genome copies/µg DNA by dividing by the dsDNA concentration of each sample using a Qubit 3.0 fluorometer (Thermo Fisher Scientific, Waltham, MA). This was then divided by 10 to achieve spirochetes/µg DNA, because there are 10 genome copies per spirochete cell. Samples with a DNA concentration measuring more than 2 standard deviations below the mean for each sex and developmental stage were discarded.

Statistical tests. Spirochete counts generally followed log-normal distributions and, accordingly, were log-transformed first for calculations of geometric mean and 95% CIs and for performance of *t* tests. Linear regression was used to for analysis of relationship between prevalence of *Borrelia* infection and spirochete load.

RESULTS AND DISCUSSION

Ticks Collected. A total of 5,082 *I. pacificus* ticks, including 2,502 (49.2%) adult males, 2,312 (45.5%) adult females, and 268 (5.3%) nymphs were collected from 15 parks during the sampling period (Table 1). The high proportion (94.7%) of adults was likely due to the flagging during winter months and the focus on adult habitats, such as shrubs and grasses, preferred by questing adults. Sex distribution was fairly even within all of the parks flagged with an average of 169 adult male and154 adult female ticks collected per park.

***Borrelia* infection prevalence varied by park and species.** Overall, the MIP of *B. burgdorferi* and *B. miyamotoi* combined in collected ticks was 1.5% (Table 1). The prevalence of infection with *Borrelia* was similar in male and female ticks at 1.3% and 1.5%, respectively. Prevalence in nymphs was noticeably higher at 2.2%, but sample size was much lower (n=268). Over twice as many pools of ticks were positive for *B. miyamotoi* (50) than for *B. burgdorferi* (24). This is in contrast to *B. burgdorferi* and *B. miyamotoi* in the northeastern and north-central United States and in Europe, and Asia where the ratio of *B. burgdorferi* infections to *B.miyamotoi* infections of *Ixodes* spp. ticks ranges from 5:1 to 10:1 (Wilhelmsson et al 2001, Mun et al. 2006, Barbour et al. 2009, Takano et al. 2014, Federova et al. 2014).

Park Name	Tick Number					B. burgdorferi s.l.		B. miyamotoi	
	Male	Female	Nymph	Total	Pools	Pools +	MIP	Pools +	MIP
Thornewood Open Space Preserve	302	226		528	106	3	0.57%	10	1.89%
Los Trancos Open Space Preserve	162	112	157	431	87	5	1.16%	6	1.39%
Pulgas Ridge Open Space Preserve	275	282		557	112	0	0.00%	9	1.62%
Huddart Park	130	112	1	243	49	2	0.82%	3	1.23%
Laurelwood Park	210	223		433	87	2	0.46%	4	0.92%
Año Nuevo State Park	171	194	1	366	74	1	0.27%	2	0.55%
Wunderlich County Park	370	330	3	703	141	4	0.57%	7	1.00%
Sweeny Ridge	64	89		153	31	1	0.65%	1	0.65%
Edgewood Park	105	69		174	35	0	0.00%	2	1.15%
Big Canyon Park	105	83		188	38	0	0.00%	2	1.06%
San Pedro Valley County Park	86	87		173	35	0	0.00%	1	0.58%
Crystal Springs Regional Trail	149	139	70	358	72	2	0.56%	1	0.28%
Windy Hill Open Space Preserve	225	255		480	96	3	0.63%	2	0.42%
Waterdog Lake Park	128	101	36	265	53	1	0.38%	0	0.00%
Butano State Park	20	10		30	6	0	0.00%	0	0.00%
Total	2502	2312	268	5082	1022	24	0.47%	50	0.98%

Table 1. Prevalence of Borrelia in San Mateo County ticks

B. burgdorferi was detected in 10 sites and *B. miyamotoi* was detected in 13 sites, out of a total of 15 sampled. Park infection prevalence where *B. burgdoferi* was present ranged from 0.3% to 1.2%, whereas the prevalence of *B. miyamotoi* ranged from 0.3% to 1.9%. Butano State Park was the only park with no infections, but the sample size of 30 was low.

Higher spirochete loads in *I. pacificus* for *B. miyamotoi* compared to *B. burgdorferi* When spirochete loads were compared between *I. pacificus* ticks infected with *B. burgdorferi* and *B. miyamotoi*, *B. miyamotoi* loads were significantly higher ($p < 0.001$) (Figure 2A). The spirochete load in *B. burgdorferi* positive pools ranged from 195 to 108,315 and *B. miyamotoi* loads ranged from 15,833 to 1,734,920 spirochetes per µg DNA. Means of *B. burgdorferi* and *B. miyamotoi* spirochete loads were 1,819 (933-3544) and 19,640 (14,162-27,237), respectively. These results resembled those for *I. ricinus* in Sweden, where *B. miyamotoi* spirochete levels were found to be approximately 100 times higher than *B. burgdorferi* (Wilhelmsson et al. 2013), as well as for *I. scapularis* in northeastern and north-central United States, where mean *B. miyamotoi* spirochete numbers in infected ticks were about double of those *B. burgdorferi* (Barbour et al. 2009). Having higher numbers of spirochetes in questing ticks may give *B. miyamotoi* an edge through increasing its likelihood of transmission to a host (Piesman et al. 2001). Unlike the Barbour et al. (2009) study which only included nymphal *I. scapularis* ticks and observed a bimodal distribution of spirochete loads per tick, there was only a single peak of *B. miyamotoi* loads in adult *I. pacificus* at these northern California sites. The bimodal distribution was thought to be caused by half the nymphs who were infected through vertical transmission and half by those that were infected through horizontal transmission as larvae (Barbour et al. 2009). Because our study was done almost entirely with adult ticks, there is a much higher probability that all or most of the positive ticks tested, had fed on an infected competent vertebrate reservoir for *B. miyamotoi* at least once, presumably lowering the relative proportion that might have been infected only by transovarial transmission.

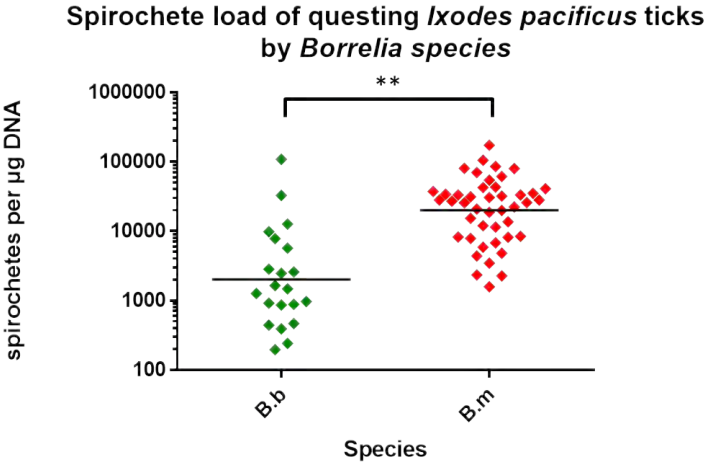

Fig 2. The spirochete load in *I. pacificus* is significantly higher in pools containing ticks infected with *B. miyamotoi*. (A) Spirochete load dot plot comparison of ticks infected with either *B. burgdorferi* (n=22) or *B. miyamotoi* (n=45). Each diamond represents spirochete load data from one pool of ticks and horizontal lines are mean values. Significant differences were found between species. **= P value < 0.001.

Spirochete loads by sex and by developmental stage. When all spirochete load data were separated by sex and stage, mean spirochete numbers were similar across all fields (Figure 3). Mean of spirochete loads for adult female, adult male, and nymph ticks were 6,923 (3,593-13,337), 9,923 (5,760-17,095) and 5,404 (1,000-29,195) spirochetes per µg DNA, respectively. Though previous studies that compared spirochete loads of adult males and females could not be found, Wilhelmsson et al. (2001) did compare adults to nymphs and also found no significant difference in spirochete numbers between these stages. One possible reason for the equal numbers across sex is that these ticks were likely infected as nymphs or transovarially as larvae, when their body size was similar. Though a 5-10 fold drop in spirochetes has been shown to occur with *B. burgdorferi* after molting (Piesman et al. 1990), whether the tick nymph molts into either sex must not be a factor in transstadial survivorship of spirochetes. Many propose that adult male ticks are incompetent vectors because their window of feeding is too short to allow spirochete transmission. Data from the current study shows spirochetes levels in both adult sexes are equal, suggesting spirochete concentration should not be a reason for adult male tick vector incompetence. Since other relapsing fever spirochetes have been shown to need far less feeding time for pathogen transmission than Lyme spirochetes (Schwan and Piesman 2002, Barbour 2005), possibly due to frequent localization in the tick salivary glands (Takano et al. 2012), there is a chance that a short feeding time alone may not be enough to make male *Ixodes* ticks incompetent vectors for *B. miyamotoi*.

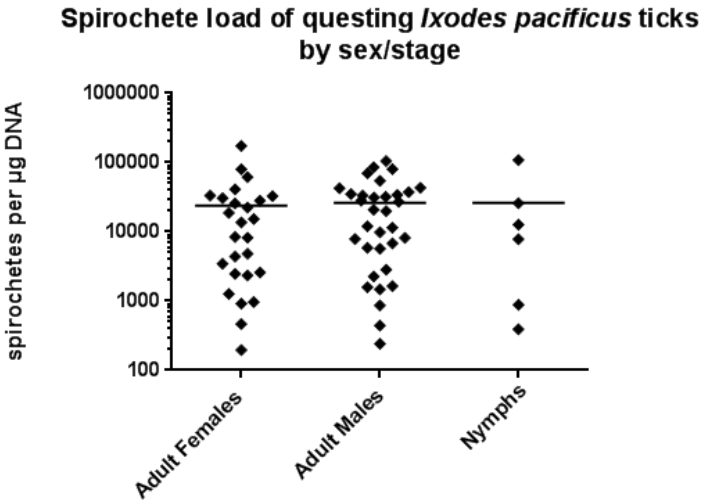

Figure 3. **There is no difference in spirochete load between adult sex or developmental stage.** Comparison of spirochete load between sex and stage found no statistically significant differences. Both *B. burgdorferi s.l.* and *B. miyamotoi* data are included in this figure. Each diamond represents spirochete load data from one pool of ticks and horizontal lines are mean values. Females (n=26), Males (n=33), Nymphs (n=6)

Spirochete load may influence area infection prevalence for *Borrelia burgdorferi*

To test whether there is any association between spirochete load and tick infection prevalence, we plotted the average spirochete load from each park versus calculated infection prevalences for each *Borrelia* species (Figure 4). It is apparent by linear regression plot that a positive relationship exists between *B. burgdorferi* spirochete load and the average MIP of ticks infected by *B.b.* (Figure 4A, R^2= 0.52). On the contrary, at higher overall spirochete loads in the ticks, there was no such discernable relationship for *B. miyamotoi* (Figure 4B, R^2= 0.06). These findings suggest that there are notable differences in the ecology of these two bacteria, and which can occur within the confines of a single county. Though it is not a strong relationship, learning of such a connection hints that the ecological success of *B. burgdorferi* may rely more heavily on maintaining high numbers in their tick vector, while *B. miyamotoi* does not. *B. miyamotoi* has shown an ability to maintain high levels of spirochetes in their reservoir host blood (Barbour et al. 2009), and the current study has shown, in the tick vector as well. Both of these strengths along with transovarial transmission may be a reason to monitor *B. miyamotoi* prevalence in California.

In conclusion, the present surveillance demonstrated that both *B. burgdorferi* and *B. miyamotoi* are widely distributed throughout San Mateo County with low prevalence (0-1.89%) in *I. pacificus*. This county may be one of the few known places where prevalence of Lyme disease borrelia is lower than that of the relapsing fever spirochete *B. miyamotoi.* Our subsequent spirochete load analysis showed that species of spirochete does have an influence on the spirochete load of ticks infected with *Borrelia,* but sex and stage do not. Lastly, regression analysis was able to show a positive relationship between high spirochete load and prevalence of *B. burgdorferi* in San Mateo County parks.

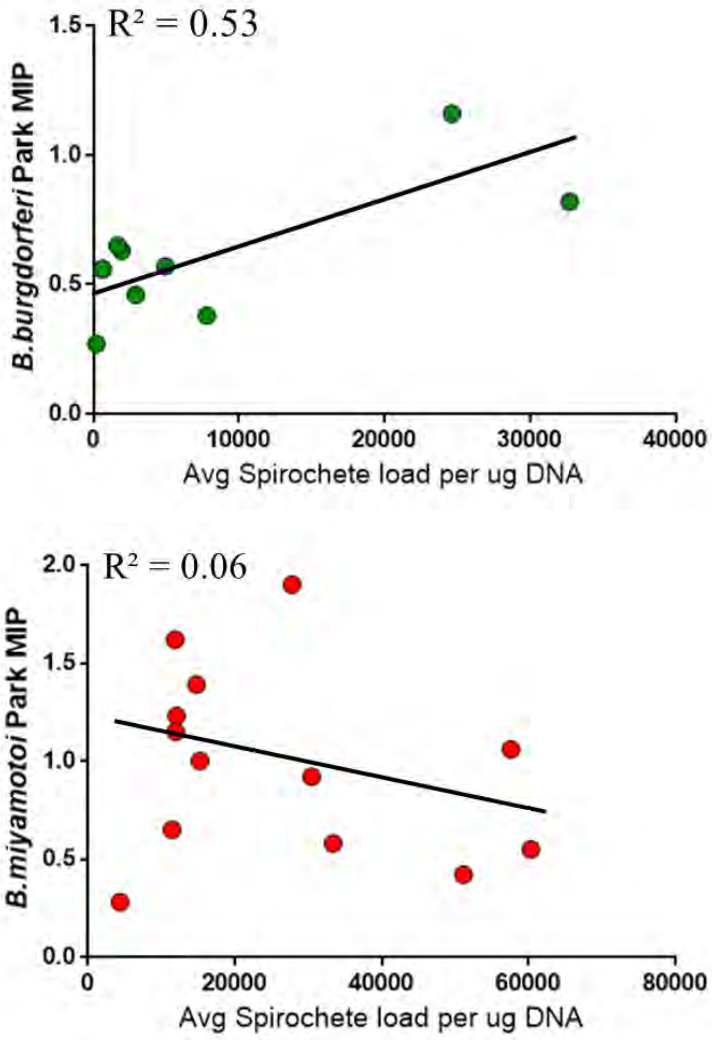

Fig 4. Spirochete load may influence area infection prevalence for *Borrelia burgdorferi s.l.* Linear regression plot of the relationship between the average spicrochete load of ticks in a park and the calculated MIP of *Borrelia* for *B. burgdorferi* (A) and *B. miyamotoi* (B).

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Advocacy and Engagement of Local Elected Officials

Luz Maria Rodriguez

Sacramento-Yolo Mosquito & Vector Control District

A key element in the Sacramento-Yolo Mosquito and Vector Control District's education and outreach program is the government affairs component. Engaging and having the support of elected officials is critical. Therefore each year our District dedicates a significant amount of time reaching out to them as a means of ensuring they are aware of our District, West Nile virus activity in their area, and the District's mosquito control activities that may be taking place within their jurisdiction.

This presentation will highlight the importance of having and implementing a government affairs program. It will provide an overview of our District's strategies which include setting up annual presentations at city council meeting, coordinating 'meet n' greets' with new council or board of supervisor members, obtaining county or city proclamations in support of Mosquito Awareness Week, working closely with key city/county agencies, participating in city sponsored events, and utilizing city/county resources to disseminate our mosquito prevention messages. This presentation will also offer recommendations for Districts who are looking to set up or expand their outreach efforts to elected officials.

Getting Started on Social Media

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It takes only a small investment of time and effort for small public agencies to begin to reap the rewards of an official social media presence, but many agencies face barriers such as confusion about the role of social media, lack of staff time and expertise, and uncertainty about how to begin. Fortunately, social media success is only a few simple steps away. This presentation will provide basic guidance for agencies getting started on social media, from planning and strategy through evaluating the results, along with helpful tips and resources that will minimize time and effort and maximize success.

MVCAC-Community Engagement and Advocacy

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Our initial question when we considered developing a social media program was: Is social media worth a substantial investment in time and money? When we discussed building a presence on Social Media, we had many more questions than answers. Here we describe our attempt to create a favorable perception about vector control, the unpredictable nature of social media, and the flexibility which exists because there are currently are no rules to stifle creativity.

Our effort to attract and keep a community of followers began with the challenge of getting an average person to follow a page dedicated to a very narrow topic (vector control) of public health. Next, we tried to develop messages that would keep our community engaged. Third, we hoped the messages we developed were perceived as "cutting edge" and made public health interesting.

As we proceeded it was important that we controlled the message; this was achieved by using original content that was linked to the District's web site so we became the source of information. We also had to consider how to use the considerable amount of data that we collected, including the valuable component of social media that tracks virtually every entry which is made.

Social media is a dynamic entity, and it is important to evolve with it. We felt that the estimated initial investment of 1,000 hours of labor, $3,500 in services, and $600 in software subscriptions was justified by our ability to reach nearly 700,000 people.

Community Outreach and Education: Chikungunya and Dengue

Lauren Salmo

California Department of Public Health Vector-borne Disease Section

Chikungunya and dengue are emerging infectious diseases that have been detected in travelers returning to California. Community outreach and education is a critical part of mosquito-borne disease prevention. The purpose of this presentation is to highlight key educational materials created by the California Department of Public Health and the Centers for Disease Control and Prevention, and to discuss best practices for material dissemination and community education.

Public Usage of the West Nile Virus Dead Bird Hotline and Website in 2015

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ABSTRACT: The West Nile virus (WNV) dead bird surveillance program (DBSP) is the most public-interactive component of the California Department of Public Health Vector-Borne Disease Section's (CDPH-VBDS) comprehensive Arbovirus Surveillance Program. Californians can report dead birds and obtain health information about WNV via the hotline and website. From mid-April to mid-October, hotline operators speak to callers, record critical information about their dead birds, and address any questions or concerns. Suitable dead birds are collected and tested by the University of California Davis Arbovirus Research and Training (DART) laboratory or by local agencies. WNV positive birds indicate where and when the virus is active in the environment. We conducted a survey of hotline calls and website reports during the 2015 season to better understand caller interaction with the program. The most common public questions in 2015 were similar to those recorded in 2007. Callers heard about the hotline and website to report dead birds through more than twenty sources, with the most common being local wildlife or animal control centers, a vector control agency, or internet search. Online news stories and social media were influential in bringing visitors to the WNV website. The results of this study may be useful in the development of educational materials, staff training, and online activity about the WNV DBSP, geared to deliver the most relevant information to the public and promote reporting.

INTRODUCTION

The West Nile virus (WNV) dead bird surveillance program (DBSP) is the most public-interactive component of the California Department of Public Health Vector-Borne Disease Section's (CDPH-VBDS) comprehensive Arbovirus Surveillance Program. The public can access information about WNV and report dead wild birds by calling the Dead Bird Hotline (1-877-WNV-BIRD), or by visiting the California (CA) WNV website (www.westnile.ca.gov) (both established in 2002; McCaughey et al. 2003). The ability to report dead birds via the website was added in 2013.

Dead bird reports to the DBSP are funneled through a screening process whereby hotline operators determine carcass suitability for WNV testing. With the collaboration of local agencies (mosquito and vector control, mosquito abatement, environmental health agencies, and veterinary public health laboratories), the University of California Davis Arbovirus Research and Training (DART) laboratory, and CDPH, dead birds are collected, sampled, and tested for WNV. Dead birds provide early and ongoing indications of WNV activity to help focus mosquito control efforts (Carney et al. 2005; Anderson et al. 2012).

Public Interaction

In addition to reporting dead birds on the telephone hotline, callers can also listen to recorded health information about mosquito repellents and WNV disease in humans and animals (in English and Spanish). Although available year-round, this feature may be underused. Another feature of the hotline is the Zip Code Locator: this automated function reads out the name and phone number of the caller's nearest vector control agency when he/she enters a zip code on the keypad.

The CA WNV website is an excellent resource for current WNV information. During the WNV season (mid-April through mid-October), the CA WNV website features weekly updated maps and statistics on WNV positive dead birds, mosquitoes, sentinel chickens, and human cases. Permanent resources include copies of media news stories and answers to commonly asked questions. The WNV hotline and website have been identified as public education tools for many years (Her and Husted 2008), as well as a means to promote public participation in the dead bird surveillance program. Each year, hotline operators report commonly-fielded questions and concerns from callers. One report about public usage of the WNV hotline (Her and Husted 2008) discussed the most common questions, concerns, and complaints of callers during 2007. In the years since, the telephone hotline has remained important for dead bird reporting, but internet reporting is nearly as popular today. Social media is also an increasingly important tool for government agencies to interact with and educate the public; many local agencies as well as CDPH utilize social media to broadcast messages and calls to action. One of our objectives was to determine whether or not the public was better informed today about WNV compared to 2007, due to increased internet usage, social media, and seasonal reappearance of the virus (in mosquitoes, dead birds, and humans) in the last thirteen years.

Despite the website's resources and hundreds to thousands of monthly visitors, educating the public about WNV is an ongoing endeavor. For example, media stories about new disease threats can lead to public fatigue or forgetfulness about WNV. The public may also confuse the risks of WNV with other current diseases (i.e. Avian Influenza, Zika). As such, operators not only work with callers to report, collect, or dispose dead birds, but also inform callers about WNV facts and risks.

With these points in mind, our objectives were to 1) summarize and identify trends in public usage of the WNV hotline and website, 2) identify the most common questions and concerns of the public and determine if they had changed since 2007, and 3) identify areas where improvements could be made to the hotline, website, or other parts of the DBSP to best educate Californians about WNV while promoting dead bird reporting.

METHODS

The Survey

In spring 2015, three seasonal hotline operators were hired with a background in a science field and demonstrated abilities to professionally manage calls from the public. Staff worked from mid-April through mid-October to coincide with peak WNV activity and the majority of WNV-related bird mortality. One operator worked three days per week, and two operators worked five days per week. Training consisted of written materials and guided practice.

We created a ten-question survey using the website SurveyMonkey™ (https://www.surveymonkey.com). To generate random intervals, operators first generated a random date (www.randomlists.com/random-date), excluding their days off. They repeated this to generate a second day of the week. Next they generated two, separate, random 30-minute intervals (www.random.org/clock-times). During each appointed interval, operators filled out the survey after each of the next five calls they handled, which included call-backs to internet reporters. There was no limit to the length of time necessary to reach five calls, except if the shift ended (typically there are fewer calls in the late afternoon). To prevent a bias of surveys on days worked by the part-time employee, he completed just one randomized interval per week; the full-time employees completed two intervals per week.

Survey Questions:

1. Name of hotline operator
2. Report identification number
3. How many calls (both operator and caller) were made before successful contact (includes voice messages but does not include initial internet report)?
4. Animal reported (i.e. dead bird, sick bird, squirrel)
5. Bird was identified by: caller, operator by description or photo, or could not be identified and reason.
6. Caller questions/concerns (if any). Categories for questions: adults, children, mosquitoes, dog, cat, horse, chicken, swimming pool, or other.
7. Caller complaints or compliments (if any; open-ended).
8. Based on the caller's questions/concerns, referred them to (i.e. local MVCD, MAD, etc.)
9. How did the caller find out about the hotline? (i.e. television, a website, newspaper)
10. Duration of the call (minutes).

The study was designed to be minimally intrusive on both caller and hotline operator time. Hotline protocol was almost completely unchanged from usual and proceeded as follows: operators filled out an electronic report during each call (using the Bird Information Reporting Database; B.I.R.D.) and recorded critical information such as the location or address where the dead bird was found, decomposition state of the carcass, and species or type of bird. After determining that the bird was suitable for pick-up and testing (i.e. accepted species, dead <24hs, non-trauma death, and few insects), the operator asked the caller to place the bird in a bag using gloves or a shovel, and to leave it on their front porch for their local agency to pick up. The operator also told the caller that if the bird tests positive for WNV, their local agency will call to inform them, but testing takes one to two weeks. The operator thanked the caller for reporting and saving the dead bird. At this time, some callers voiced questions or comments which were addressed appropriately. At the end of the call, hotline operators asked callers "How did you find out about our hotline?" (question #9). This was the only addition to hotline protocol for phone calls within survey intervals. After the call, the operator filled out the survey.

Additional data about the call (i.e. caller's zip code, county, and bird species) were obtained from the B.I.R.D. database. Call reports from the telephone company provided information about caller usage within the hotline phone tree. Website statistics from the WNV website were added to these data.

RESULTS

Report and Call Summary

In total, the WNV DBSP received 10,850 reports via the hotline and website in 2015; the majority of which occurred during the season (mid-April to mid-October). To offer perspective, the program has received over 400,000 dead bird reports from 2000 to 2015, with an average of 26,500 reports per year. In 2015, forty-seven percent of reports received during the season were internet reports, and our busiest hours were 08 – 1100 h. The number of callers who listened to automated information on the hotline during the season was totaled. Out of 6,953 phone calls, 235 (3.4%) used the Zip Code Locator, and 300 (4.3%) listened to health information about WNV. Twenty-six Spanish speakers listened to the health information in Spanish, and six Spanish voicemails were received. Sixteen percent of English-speaking callers (over 1,000) left voicemails, and the remaining callers (about 76%) spoke with a live operator. It should be noted that the hotline receives repeat callers (see Table 3).

Hotline Survey

Surveys occurred from May 20 to October 7. The three operators filled out a total of 380 surveys. Due to varying start times, each of their portions of the total surveys was 47%, 36%, and 17% (with the smallest number from the part-time operator).

Number of calls until contact. The number of calls made until successful contact between the caller and an operator ranged from 1 to 8. In 56% of reports (209), one call was made before successful contact; in 30% (112) two calls were made; in 10% (38) three calls were made; and in 3% (11), four calls were required. In less than one percent of reports, five, six, and eight calls were required until successful contact between caller and operator.

Animals reported. In 95% of the reports in the survey, the citizen was reporting a dead bird. In 4%, he/she was reporting a dead squirrel (tree squirrels were tested for WNV in the DBSP until 2013), and one percent of reports reported a sick bird.

Identification of birds. The dead bird was identified to species or type by the caller in 74% (282) of the reports in the

survey, and was identified by the operator based on the caller's description in 11% (43) of reports (correct identification could not be guaranteed). In 0.8% (3) reports, an operator identified the bird based on a photo sent by the caller, and in 13.7% (52) reports, the bird could not be identified to species (reasons included: difficult based on description alone; the bird had already been disposed; or the caller did not want to go near the bird).

Caller Questions and Concerns. Callers voiced questions or concerns in 18% (68) of the 380 calls in the survey which were categorized (Table 1). Many focused on the risk of WNV transmission to the caller's child, dog, or an adult in the home, due to being near the carcass (Table 1). Callers also inquired about how WNV is spread, how to prevent mosquito bites, and if there is a risk of contracting avian influenza (AI) from their dead bird. These top caller questions (rows 1-5 in Table 1) were similar or identical to callers' top questions in 2007 (Her and Husted 2008). However, we also noted that some questions from callers suggested a familiarity with WNV: "I submitted a bird last year and it tested positive, and that's why I'm concerned about WNV this year" and "I'm worried about WNV because I know someone who had it."

Category	Questions or Concerns	Proportion (%)	# Callers
Adults	Concern that they will contract WNV from the dead bird (DB). Concern about WNV because they know someone who was ill from the disease.	13	9
Mosquitoes, Transmission	How do mosquitoes spread WNV? How do birds get WNV? How do I prevent mosquito bites? What are some natural repellants?	10	7
Dogs	Concern that dog will contract WNV from being near DB	9	6
Children	Concern that child will contract WNV from being near DB	9	6
Avian Influenza	Is there a risk of getting bird flu from a DB?	9	6
Bird feeder	Is my feeder contaminated or a source of contamination?	6	4
Cats	Concern that cat will contract WNV from the DB. OR Concern that cats will steal DB before it is picked up.	6	4
WNV detections	Is WNV in my local area?	4	3
Sick bird	What do I do with a sick wild bird?	3	2
Bird pick-up	When will technician arrive to pick up the DB?	3	2
Birds	What is the test result of my previously submitted DB?	3	2
Previous positive	General concern because he/she submitted a DB in the past which tested positive.	3	2
Swimming pool	Is my pool contaminated after a DB was found in it?	1	1
Horses	Is my horse at risk for contracting WNV?	1	1
Miscellaneous	Fleas, ticks, bird bands, etc.	19	13
Total		100	68

Table 1: Questions and concerns received from the public, May-October 2015 West Nile virus dead bird surveillance program hotline survey.

Caller Complaints or Compliments. For 18% (70) of the 380 survey responses, hotline operators received unprompted caller complaints and compliments. Twenty were neutral comments (e.g., "caller wanted to bury bird") and were disregarded. The 50 remaining responses were categorized (Table 2). In ten (20%) of these 50 responses, callers expressed concern or a squeamishness about bagging the bird for pickup. Sometimes they agreed to bag the bird when the operator explained that doing so (using protection such as gloves) would not put them at risk for contracting WNV. In nine responses (18%), callers were unhappy because it took them considerable time to find our hotline (they were referred to several other numbers until finally being referred to the hotline) (Table 2). Interestingly, this was the number one complaint in 2007: "Your number was too hard to find" (Her and Husted 2008).

Complaints	Proportion (%)	# Callers
Scared or reluctance to bag dead bird (DB)	20	10
It was difficult to find out who to call about my DB	18	9
DB will not be picked up and tested	16	8
Misinformation from other sources led to misunderstanding (i.e., "I thought you picked up all birds.")	14	7
Program is closed on weekends	6	3
Phone Tag	4	2
Complaint that squirrels are not tested	4	2
Complaint that pigeons are not tested	2	1
Website map was difficult read	2	1
Compliments		
The WNV website was very helpful and easy to use	6	3
Thank you for the call back or for the recommendations	8	4
Total	100	50

Table 2: Complaints and compliments received from the public, May-October, 2015 West Nile virus dead bird surveillance program hotline survey.

Referrals. Hotline operators filled out the referral question in 369 surveys. In most of those calls (95%), the operator did not need to refer the caller to another organization or agency. In 17 calls they made referrals. When the caller did not want to dispose of the bird on his/her own, the operator recommended calling a local animal control facility (six callers). A local wildlife rescue location was recommended for live, sick, or injured birds (four callers); the local MVCD or MAD was recommended for mosquito or green pool issues (three callers); California Department of Fish and Wildlife was recommended for four or more dead birds found together (two callers); and the California Department of Food and Agriculture's number was recommended for backyard chicken deaths (one caller). Our website (www.westnile.ca.gov) was referred to one caller, most likely to direct him/her to information or current statistics about WNV in California.

Sources: Callers discovered the WNV dead bird program/ hotline through more than 20 sources (Table 3). The five most common were: WNV website (www.westnile.ca.gov), wildlife care or animal control facility, local mosquito or vector control agency, search engine (the most popular search phrases were "dead bird" and "West Nile virus,") and newspaper. It is unclear how callers originally found the WNV website; perhaps some callers actually found it through a search engine or another source but did not remember (see Website Analytics). Five persons (1%)

spoke to more than one entity before being directed to our hotline (for example, the caller telephoned city hall, which directed her to the police department, which directed her to the hotline) (Table 3).

Source	Proportion (%)	# Callers
Website (www.westnile.ca.gov)	17	66
Wildlife rescue or rehabilitation center	16	59
Local MVCD, VCD or MAD	12	47
Google ("dead bird" and "West Nile virus" most common)	8	31
Newspaper	8	29
Repeat caller	7	25
Television news	5	18
Health department	4	17
Friend, family or acquaintance	4	16
Occupation, workplace, or co-worker	2	9
County or city offices	2	8
Police department	2	8
Radio	2	8
Physical flyer, brochure, newsletter, or posting	2	6
Social website (i.e. Facebook©, Nextdoor©)	2	6
Called 311	1	5
County website	1	5
Spoke to 2+ organizations before finding the hotline	1	5
Unknown	1	5
Birding website (i.e. Audubon)	1	2
California Fish and Wildlife Department	1	2
Phone book	1	2
Agriculture department	<1	1
Total	100	380

Table 3: Sources of discovery of the West Nile virus Dead Bird Surveillance Program's hotline (1-877-WNV-BIRD) and/or website (www.westnile.ca.gov) by the public, May-October 2015.

Duration of calls. Call duration was recorded in 366 surveys. Calls ranged from 1 to 20 minutes in duration (Figure 1). Three minutes was the most common duration (28%), followed by four minutes (22%), and then two minutes (16%) (Figure 1).

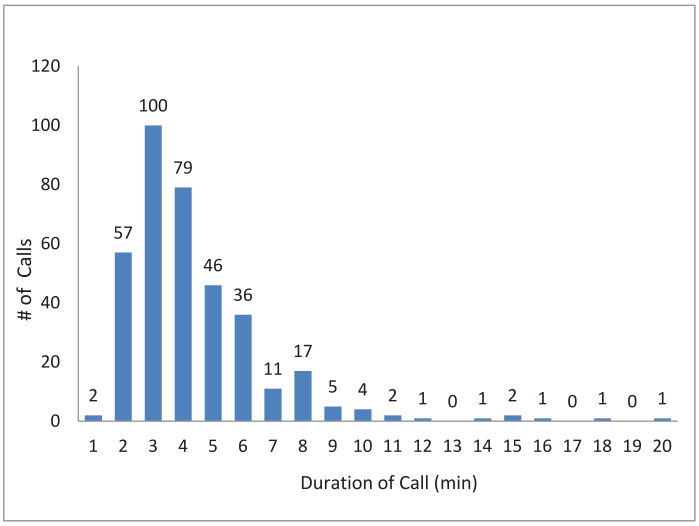

Figure 1: Frequency distribution of the duration (in minutes) of 366 hotline phone calls, May-October 2015, West Nile virus dead bird surveillance program hotline survey.

Website Analytics. Website analytics revealed trends in website visitor behavior. The website received a total of 257,411 visitors in 2015; 65% of those visits occurred from April through October. Of the total visitors in 2015, 56% (144,682) were new (unique) visitors. Unique visitor totals have been steadily climbing each year since 2012. In September, the website received the most visitors (~27,000) compared to any other month; September also had the most unique visitors (~16,000). This coincided with peak WNV activity in September, particularly in human cases (~138 cases for the month), which were widely publicized in media news (Figure 2).

Online news: We evaluated the top external links responsible for sending traffic to the WNV website. While many news stories brought visitors to the site, a few were among the top external links during the WNV season. For example, a press release about the unprecedented WNV activity in 2014 (http://www.cdph.ca.gov/Pages/NR15-027.aspx) may have contributed to a jump in April 2015 visitors (26,138). The April visitor total was the second highest of the year followed by September (Figure 2).

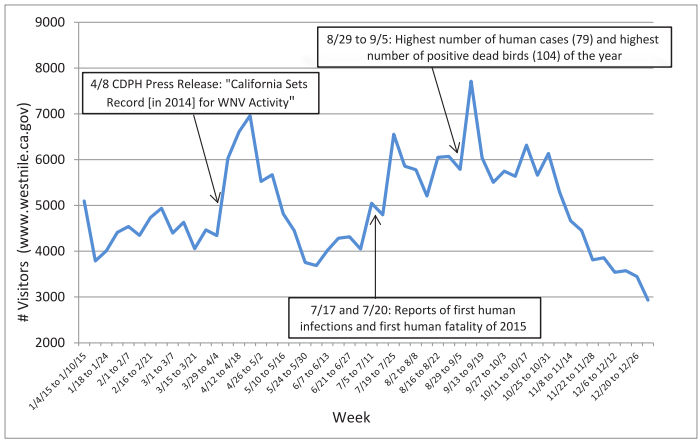

Figure 2: Number of weekly visitors to the California West Nile virus website (www.westnile.ca.gov) in 2015, and three possible causes of increased visitor volume.

In October, the Los Angeles Times article "Aggressive Nonnative Mosquitoes Spread across the State" (http://www.latimes.com/local/california/la-me-disease-mosquitoes-20151025-story.html) discussed the threat of invasive *Aedes* mosquitoes in Southern California. The article also mentioned *Culex* mosquitoes, WNV, and provided a link to the website; 220 people clicked on this link. Finally, an online news story from 2011 described common arthropod bites and disease risks (The Hidden Dangers of Insect Bites http://www.huffingtonpost.com/glenn-d-braunstein-md/the-hidden-dangers-of-bug_b_915050.html). In the article, potential for mosquitoes to spread WNV was mentioned with a link to the WNV website. From May through October 2015, 764 people clicked on this link and visited the WNV website. Online news in general proved to be a valuable internet conduit; online news covering local WNV detections or mosquito control efforts with the WNV website link embedded led visitors to our website during the entire season.

External Links: Several mosquito and vector control agencies' websites were consistently among the top ten external links bringing visitors to the website. Many agencies place a link on their webpage to the WNV website for visitors to check the latest WNV surveillance totals or report dead birds. There were other sources of visitors to the WNV website which seemed to be singly interested in pesticide and repellent information: a chemistry class at an out-of-state college, and a commercial repellent company. CDPH and many local agencies curate Facebook© pages for publicity; the social media platform was the number one external link in July, August, and September. Facebook© was also among the top ten external links during remaining months excepting January and December. The public also found the WNV website through inputting key words (i.e., "West Nile virus" and "dead bird") into search engines. Online searches about mosquito control and classification of pesticides also fueled visitor volume.

DISCUSSION

Report volume during the 2015 season was manageable by three hotline operators working staggered schedules, because 86% of callers were able to speak to an operator in one to two calls. Our total report volume was ~10,000 reports, but there have been years when report volume was much greater (such as 2012 with 20,000+ reports), and hotline activity can be as unpredictable as WNV activity from year to year.

The typical hotline phone call was two to four minutes long, and callers are more likely to phone in the morning. Certain questions from the public appear to be uppermost in the public mind; the most common questions of 2015 were similar to those of 2007. One frequent concern was that a person or animal in the caller's household would contract WNV from the dead bird on their property. This top concern could be addressed in public interaction (website, operators, and social media) to assure people of the very low risk of contracting WNV from a dead bird and the much higher risk of being infected from a mosquito bite.

Also similar to the 2007 study, avian influenza (AI) detections posed a threat to California in 2014-early 2015, and news coverage prompted alarm. We found that the public confounded facts and risks about AI with WNV. This is understandable since dead wild birds can be indicators of both diseases, although highly susceptible species to AI (waterfowl, poultry, and game birds) are not the same species which are highly susceptible to WNV (corvids, other songbirds, and raptors). Since AI is a recurrent threat to wild birds in North America, we could post permanent facts and links regarding AI on the WNV website.

The variety of ways in which people found out about the hotline or website illustrate the importance of both "traditional" forms of advertising (i.e., newspapers, flyers, television), and internet-based advertising and sharing. There are some people who do not have access to the internet or prefer not to use it. While different demographics tend to obtain news from their favorite sources, we theorize that all channels of communication with the public help to spread the word about reporting dead birds (for example, anyone may hear about reporting dead birds listening to the car radio or by word of mouth). Although many people said they searched the internet to find the WNV website, it is unclear what initially brought them to it; this question could have been phrased differently.

Many callers in our survey called a local wildlife care/animal control, city or county office, or even their police department when they found a dead bird. Local agencies conducting WNV dead bird surveillance may want to forge contacts with their neighboring agencies, perhaps touching base each spring since staff turnover may occur.

Interestingly, the online article "The Hidden Dangers of Insect Bites" delivered numerous visitors to our website and continued to make impact years after written. It was a source of many online visitors during warm weather months. Evidently, people were spending time outdoors, encountering insects, and wanted to know more about insect bites. This finding could be useful for media campaigns striving to make the public aware of WNV (or other vectorborne diseases). A well-timed spring article about biting insects and the diseases they can transmit could reach a wide audience.

Three major internet "channels" brought visitors to the WNV webpage: Mosquito and Vector Control Agencies' websites, Facebook©, and search engines. Given these results, agencies conducting WNV surveillance might increase their public's awareness by maintaining a website and a Facebook© page and periodically posting updates and stories throughout the WNV season. This proves to be effective in promoting public participation in the dead bird surveillance program. For local agencies, being active online can also help promote mosquito control activities and a positive public image.

There are study limitations to note. Common questions and concerns from the public were captured, but only as volunteered by callers, and therefore should not be construed as citizens' knowledge base of WNV. It is likely there were unintentional website visitors, because we found a handful of irrelevant sources. In addition, an unknown portion of our hotline callers and website visitors are staff from local agencies and CDPH.

Although our study was not exhaustive, we gained insights about our audience in 2015. Looking ahead, we aim to serve the public's needs by maintaining relevant resources on the WNV website, staying up-to-date in technology (the latest improvement was a mobile-friendly report system), and training hotline staff with the most common public questions in mind. There may be no better public education mechanism than speaking directly to a live person; moreover, the hotline still receives about 53% of reports via phone. Among our findings it was a surprise to discover that although WNV has been in California for over a decade, top public concerns regarding WNV have not changed, and advertisement of the dead bird hotline is still critical to ensure public participation and maximize the effectiveness of dead birds as a WNV surveillance tool.

ACKNOWLEDGEMENTS

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Now it's "Next Gen?" What's Next?

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The San Gabriel Valley MVCD Education Department decided to go beyond the straight forward "mosquito talk" classroom program and anchor our main messages within the context of state life science standards in Kindergarten through Grade 12. This added value to our programs and has resulted in long term positive relationships with teachers and school administrators. By incorporating the latest changes in national and state educational standards, we are able to keep our classroom programs fresh and relevant. We have come to realize online material is only as good as the extent to which we are willing to promote it. The assumption, "if you build it…will they come," holds little credibility in the real world and without vigorous promotion our online materials are doomed to languish in electronic purgatory. Our most recent challenge with invasive Aedes mosquitoes forced us to be innovative and gave rise to a popular citizen science project. Next Generation Science Standards have provided new opportunities to inject relevance into our evolving outreach message of shared responsibility.

Teachers Tools: Educating Students about Mosquito Control In and Out of the Classroom

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ABSTRACT: Educating youth about vectors and vector-borne disease is an integral part of the Coachella Valley Mosquito and Vector Control District's public outreach program which strives to raise awareness about mosquitoes, their habitats, and the diseases they can transmit. In an effort to expand vector education beyond classroom presentations by District staff, we developed an online curriculum for local students in elementary, middle, and high school to learn about mosquito biology, habitat, public health significance, control, and prevention. The curriculum was developed in adherence to California's Next Generation Science Standards with input from local science teachers. The curriculum is divided into four programs geared at grades K-2, 3-5, 6-8, and 9-12 and incorporates videos and interactive activities which can be done online, in the classroom, or at home. By making the educational resources available to students 24 hours a day, seven days a week, we aim to reach more students than in-person presentations and inspire students on a deeper level with ideas for science fairs, school projects, and a desire to create mosquito-free habitats in their community.

If it "Bleeds it leads" or sometimes it is, a Mammal with Wings infected with Rabies (Bats, Rabies, and the Media in Alameda County)

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ABSTRACT: An important program element of the Alameda County Vector Control Services District is the "Rabies Control and Surveillance Program". We monitor the rabies control activities of our local animal services organizations, and investigate potential wildlife rabies situations, in coordination with the Alameda County Public Health Department, their Public Information Officer, and our County Health Officer, as well as the Public Health Laboratory (PHL). Rabies in our bat population is an ongoing threat to public health, and we take this very seriously, and occasionally, so does the news media. Our spectacular media response to some of the rabid bat detections earlier in 2015, can fall in the category of "if it bleeds, it leads", though in our situations no blood was spilt. Often, a press release regarding a rabid bat will barely catch the media's attention, but rabid bats found on school grounds and a local community center were enough to result in over 20 television news interviews, radio and print reports, and may have led to substantially more reporting by the public of suspect rabid bats. The mystique of bats and the deadliness of rabies in the presence of our children, was a real 'attention getter', and some of the details of the 13 rabid bats, involving four different species we detected in Alameda County during 2015 are quite interesting. The current paper presents the details of our 2015 wildlife rabies surveillance and outreach in Alameda County.

INTRODUCTION

The saying "if it bleeds, it leads" is an old adage; the origin is not easy to trace, but I suspect it may go back to the early days of the print media, where sensational headlines sold papers and not much has changed in media aims and goals over the years. The news media is in business to make money, and things like market share, distribution, viewers, or listenership are key value components when you plan to sell advertising to businesses that are trying to sell their products. This is not to say that the ethical media outlet has a complete lack of social interest. They do include less glamorous news of public interest, but if they are able to spice up their 'teasers' with something that has the sensational draw, to keep you tuning in until the next, or even last news segment, then the media has done a fine job.

Public Health outreach needs the media to help disseminate information to the public regarding public health risks. Risks from vectors of disease may not seem that glamorous, and occasionally, may get very little, or marginal attention, as a public health issue. On another side of "if it bleeds, it leads" are issues that some mental health practitioners, like Deborah Serani (Serani, 2011), who feels that the tendency of the media to "milk" disasters, tragedies, and horrible situations for all that they are worth, increases anxiety, and leads to depression in some individuals. She suggests it may be better to turn off the television, and read an occasional newspaper, which because of the sedate presentation, provides less of an emotional impact. Situations like the 9/11/2001 attack on the World Trade Center, where the video was repeatedly broadcasted for weeks (McNaughton-Cassill, 2009) showing people falling from the burning buildings, and "the results indicate that greater levels of exposure and attention were related to increased distress is a memorable example." There are a number of critiques of the over reliance on the 'bleeding and leading' technique of garnering viewers' attention. The newscast editors will say that if they did not run the story, their competitors would, and they would lose viewership. A cynical view may be that this is what the viewers want.

METHODS

With our Rabies Control and Surveillance Program in Alameda County, we monitor the rabies activities of our local animal services organizations, and investigate wildlife rabies situations—coordinating with our County Health Officer, our Public Health Department's Public Information Officer, the Public Health Department, and the Public Health Laboratory. This is a "passive surveillance" program where reported, suspect animals are captured/collected by our staff, or local animal control services, and then are submitted for rabies testing by our PHL. Typically, these animals are picked up by our staff from City/County Animal Services, veterinarians, veterinary hospitals, and occasionally, from other organizations, such as wildlife rescue and rehabilitation centers.

On the occasions where a rabid animal is detected, coordinating with a news-tip, our staff will distribute rabies information to the adjacent neighborhood, businesses, or institutions, such as schools and community centers. Our outreach message is straightforward: avoid contact and do not touch any wild animal, report sick, injured, or animals behaving strangely to your local animal control or vector control, especially bats and skunks, and keep your pets' rabies vaccinations up-to-date.

RESULTS

Case Investigations: On February 2, 2015, we had our first rabid Mexican Free-tailed bat detection submitted from Jefferson Elementary School in San Leandro, CA (Table 1). Subsequent to our media release, we had a couple of interviews—one very positive with an uplifting theme "everyone did what they were supposed to do, thereby protecting the public health from rabies". Overall, good press, with the children and school administration doing the right thing so that the situation reflected well on everyone.

A month later, on March 9, 2015, a second rabid Mexican Free-tailed bat was found at the Irvington Community Center in Fremont. This is a very busy community center with many programs for small children. Some negative press arose when "the media" found out that the bat initially reported on Friday evening after 5 pm, was not picked up by the local animal services, until mid-day Saturday—everyone had gone for the day, so the bat sat in the bushes along the community center entrance walkway. None-the-less, we did receive bountiful media attention with several TV teams following our staff around as they left notices at residences in the adjacent community.

One week later, on March 16, 2015 a third rabid Mexican Free-tailed bat was found at Niles Elementary School, also in Fremont. This provoked a media frenzy, with several TV teams patrolling the neighborhood and school grounds throughout the evening and the following day after our press release. In this situation, a local dog, walking with his guardian, picked up the bat with his mouth and the guardian demanded that the dog drop the bat. The guardian conscientiously and safely, collected the bat and turned it in to the Fremont Animal Control. The bat was positive for rabies, and the dog, though current on his rabies vaccine, had to be re-vaccinated, and then undergo a 30-day rabies quarantine in compliance with state law. This was all good press and by getting the message out through this bay-area-wide media blitz at this early part of the year, may be responsible for the record number bats submitted for testing and rabies-positive bats detected in 2015.

The subsequent rabid bat submissions were all from private property, with no human or pet contact, and received little attention from the media, except the second-to-last rabid bat detection of the year.

On September 16, 2015, the twelfth rabid bat was detected in an unfortunate manner. A young woman, after leaving class at a local community college, found a weakly moving bat in the leaf-litter, at the curb of the road, in front of a local coffee shop. She "rescued" the bat with her bare hands, and a local merchant gave her a box to put the bat in. She then took the bat to work with her, where someone in the bank suggested the bat may have rabies, and she should contact vector control. The bat was positive, and the woman had to undergo post exposure rabies prophylaxis. She survived the ordeal, but it is unfortunate, that even with the media blitz regarding bats and rabies, someone was still inclined to handle a bat. Our vector biologist for Fremont discovered a bat colony living in the roof of the coffee shop and advised on exclusion methods that resulted in the bats moving on.

Incident	Animal	Date Tested Positive	Address	City	Species
1	Bat	2/2/2015	Jefferson Elementary School	San Leandro	Mexican Free-Tail Tadarida brasiliensis
2	Bat	3/9/2015	Irvington Community Center	Fremont	Mexican Free-Tail Tadarida brasiliensis
3	Bat	3/16/2015	Niles Elementary School	Fremont	Mexican Free-Tail Tadarida brasiliensis
4	Bat	3/18/2015	Kilkare Road	Sunol	Mexican Free-Tail Tadarida brasiliensis
5	Bat	3/20/2015	Moncado Court	Pleasanton	Mexican Free-Tail Tadarida brasiliensis
6	Bat	4/9/2015	Park Meadow Drive	Fremont	Mexican Free-Tail Tadarida brasiliensis
7	Bat	4/20/2015	Suffolk Way	Pleasanton	Little Brown Bat Myotis lucifugus
8	Bat	4/30/2015	Cabernet Way	Livermore	Townsend's big-eared bat Plecotus Corynorhinus townsendii
9	Bat	5/6/2015	Bluebird Way	Livermore	Hoary Bat Lasiurus cinereus
10	Bat	5/22/2015	Kilkare Road	Sunol	Little Brown Bat Myotis lucifugus
11	Bat	6/1/2015	Kilkare Road	Sunol	Mexican Free-Tail Tadarida brasiliensis
12	Bat	9/16/2015	Washington Boulevard	Fremont	Mexican Free-Tail Tadarida brasiliensis
13	BAT	9/22/2015	Royalton Court	Pleasanton	Little Brown Bat Myotis lucifugus

Table 1. Bats testing positive for rabies during 2015

Historical perspective: These first three public and child-centric locations were sensational enough to elicit concern from parents, public health officials whose job it is to protect the public health, and certainly our partners in the media who are instrumental in getting the word out, using the creepy glamour of rabid bats to captivate the audience's attention.

Over the past 19 years, we have always had at least one rabid bat each year detected in Alameda County (Figure 1). In 1999, we detected nine rabid bats, but this was combined with five rabid skunks and an opossum, to total 15 rabid animals. Interestingly, we have not detected any rabid skunks since 2005 and rabid opossums since 1999. The North Carolina State Laboratory of Public Health (SLPH), states that only about 5% of bats tested for rabies end up being positive for the virus, although in Alameda County from 1997-2015, 890 bats were tested for rabies, with 100 positive for the virus, resulting in about 11% of all bats tested being positive for rabies virus. In 2015, our public health laboratory tested 77 bats, with 13 (16.9%) being positive for rabies (Figure 2). Klug from the University of Calgary (Klug et.al 2011), suggests from their studies and testing of migratory bats, that only about 1.0% of all bats have rabies.

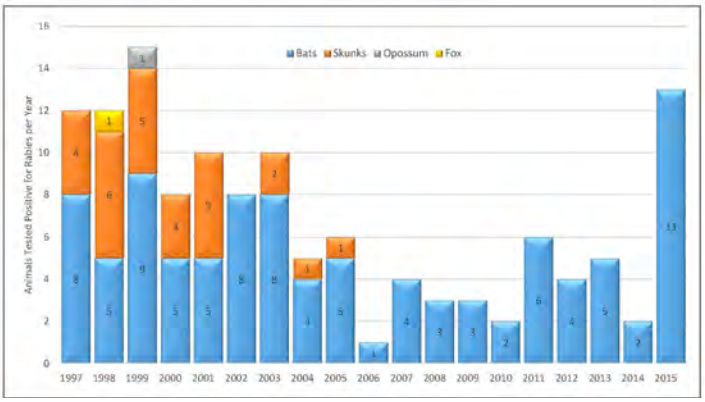

Figure 1. Rabid animals detected in Alameda County, 1997-2015

DISCUSSION

Rabies surveillance in Alameda County is an ongoing passive surveillance program, mandated by California State law, and designed to protect the public health. In Alameda County from, 1997-2015 we detected 129 rabid animals, of which 100 were bats, 27 skunks, 1 opossum, and 1 fox. Although in 2015 we detected the highest number of rabid bats within this timeframe, we have had years with high numbers of rabid bats, as in 1999 when nine rabid bats were detected.

What is not clear about this passive surveillance program is if residents actually take the time to report dead or strangely behaving wild animals. Scrutiny of data from previous years may lead us to believe that higher public awareness results in more suspect animals reported for testing. During 2015, 77 bats were submitted for testing, resulting in 13 rabies positive bats, which is indeed the highest number of bats submitted for rabies testing since 1997. This circumstantial evidence leads one to suspect the "if it bleeds it leads" categorization of rabid bats detected on school and community center campuses, is well applied. It may also be that during 2015, there actually were more bats dying throughout the County of Alameda, which resulted in more bats being noticed and subsequently reported.

Our conclusion is that the media can be a powerful ally in increasing public awareness regarding potential public health threats. This symbiotic relationship rests upon the marketability of the information that we provide and the circumstances which embody the situation. People cherish their children and want to protect them from harm, and the perceptive media recognizes this and utilizes this concern to bolster their ratings, also increasing public awareness and protecting public health.

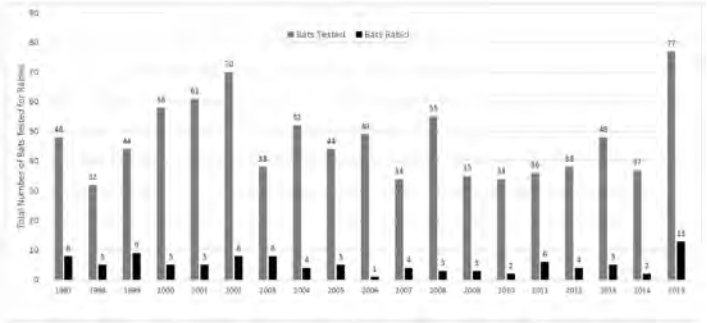

Figure 2. Bats tested and positive for rabies, 1997-2015

ACKNOWLEDGEMENTS

All of our staff at Alameda County Vector Control are involved in the various aspects of the rabies control program, and deserve a sincere acknowledgement for all the hours invested. The Alameda County Public Health Laboratory is instrumental for their timely testing, and good data collection. The Alameda County Public Health Department, their Public Health Nurses that investigate the human contacts with rabid animals, their Public Information Officer, and our Health Officer are key players in this program. The 13 Animal Control Organizations throughout the County are essential, and provide professional and thoughtful assistance year round.

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The Effects of Wildland Fire on Vector Control

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ABSTRACT: The 2015 fire season proved to be a disaster for Lake County and the Lake County Vector Control District. Starting in late July, we experienced three large fires that dramatically changed the landscape and the people living within our boundaries. Although wildland fires are not uncommon in California, 2015 was a particularly severe fire season due to the dry conditions caused by a four-year drought. The purpose of this paper is to identify and discuss the effects of wildland fires on the environment and the Lake County Vector Control District, identify the unique Vector Control problems created by the fires and provide possible solutions to the problems. This paper also will discuss the District's loss of revenue as a direct result of the fires, how this will affect the District overall, and how we expect the workload to be affected and adapted to accommodate these struggles as seen by me, the actual technician in the field. Changes in landscape have altered the environment in ways that make the mosquitoes and other vectors much more difficult to control, which in turn could potentially lead to the rise and spread of disease. Our goal is lessen the impact of this natural disaster on the residents who survived the fires and for the tourists who continue to enjoy Lake County.

INTRODUCTION

Lake County is a rural community situated in the Mayacmas Mountain Ranges between the coast and the Sacramento Valley. There is a population of roughly 65,000 people living in a total area of 1,329 square miles, 73 of which are water. Currently the Lake County VCD has the county divided into four zones for four vector control technicians. My zone, the area that will be the main focus of this discussion, lies in the southernmost portion of the county that encompasses the majority of the 2015 fires and associated damage.

THE ROCKY FIRE

On July 29 the Rocky Fire started on the southeastern tip of Clear Lake in the town of Lower Lake.

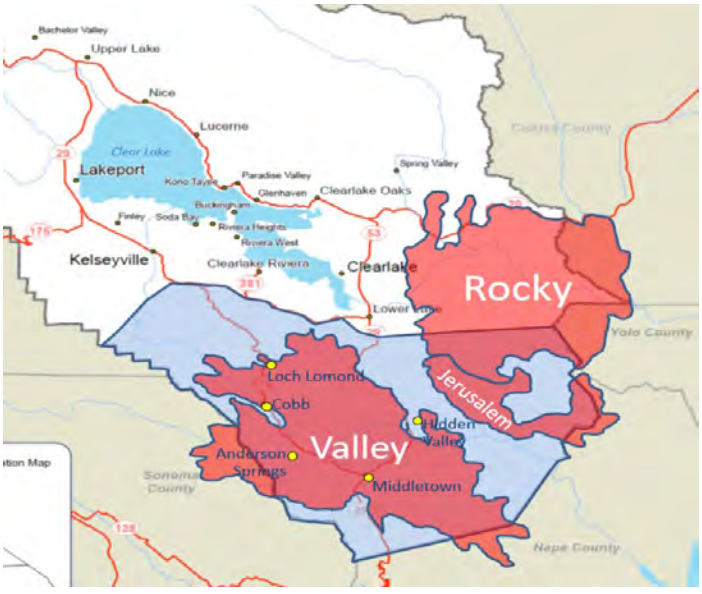

Figure 1. The southern portion of Lake County with my zone highlighted in blue, and the three major fire areas highlighted in red.

It burned south and east through thick brush and dense chaparral into the counties of Yolo and Colusa, eventually closing Highways 20 and 16, which are the only ingresses into the county from the east (Figure 1). During this time, more than 13,000 people were urged to leave their homes and seek shelter in temporary evacuation centers throughout the county, and nearly 9,000 firefighters arrived to help fight the fires. With so many people displaced and camped outdoors during our peak West Nile virus (WNV) season, we thought there would be an elevated risk of WNV transmission to humans. Fortunately, we experienced no increase in WNV associated with the fires at that time.The Rocky Fire burned 69,438 acres (108 square miles) and destroyed 43 residences, 53 outbuildings, and 8 structures. There were no human fatalities. Although the Rocky Fire was huge and terrifying, it turned out to be just the tip of the iceberg for us.

THE JERUSALEM FIRE

On August 9, four days before the Rocky Fire had even been declared contained, we experienced our second large fire of the summer, the Jerusalem Fire. The Jerusalem Fire also started in the southeastern part of the county and burned south and east, this time burning into the County of Napa (Figure 1). It burned 25,118 acres (39 square miles), and was declared contained on August 24. Six residences and 21 outbuildings were lost. Again there were no human fatalities.

THE VALLEY FIRE

On September 12 at 1:30pm on a Saturday afternoon, we experienced our third and largest fire of the summer, the Valley Fire. This was the third most destructive wildland fire in California history in terms of property damage. The Valley Fire burned in the south-southwestern flanks of the county and spread south and west into the counties of Napa and Sonoma (Figure 1). Because of the severe impacts of several years of drought, the decimation of trees left in the wake of the bark beetle, and the 40-60 mph winds that blew that day, the Valley Fire exploded into an inferno. In 18 short hours, the Valley Fire burned 40,000 acres at an astounding speed of 37 acres per minute! This fire

prompted the rapid evacuation of 20,000 people that weekend, including me and my family. Thankfully, I had a home to return to, but that wasn't the case for thousands of people. Returning to my zone was heartbreaking: entire towns, neighborhoods, and communities were burned to the ground (Figure 2).

Figure 2. Neighborhood in Middletown destroyed by the Valley Fire.

Interestingly, the flood-irrigated pastures (Figure 3) in this area became a refuge for people who stayed to defend their homes or were trapped by the fire and smoke. The people who live around and work on these pastures sat out in these fields all night on September 12 and watched their houses burn down around them. These pastures saved their lives! Because of that, I'll probably never be able to convince these people to water less again.

Figure 3. Flood irrigated pastures near Hidden Valley Lake, the only green left for thousands of acres in any direction.

In total, the Valley Fire burned 76,076 acres (119 square miles) and destroyed an estimated 1,280 homes, 27 multi-family structures, 66 commercial properties, and 585 other structures. Four human lives were claimed by the Valley Fire as well as those of countless animals and livestock. In less than seven weeks, these three fires combined burned approximately 267 square miles, more than a fifth of the total area of Lake County (Table 1).

The Three Large Fires of Lake County

	Date	Acres Burned (square miles)	Homes Lost
Rocky Fire	July 29-Aug 13	69,438 ac (108 sq mi)	43 residences 53 outbuildings 8 structures
Jerusalem Fire	Aug 9-Aug 24	25,118 ac (39 sq mi)	6 residences 21 outbuildings
Valley Fire	Sept 12-Oct 6	76,076 ac (119 sq mi)	1,280 residences 27 multi-family 66 commercial 585 structures
Total	57 Days of Fire	170,623 ac (267 sq mi)	1,329 residences 27 multi-family 66 commercial 667 structures, etc.

Table 1. A summary of the damages associated with each fire.

THE SOURCES

One of the first amazing things we noticed when we were allowed back into the area were changes to the creeks. Creeks that had been reduced to a trickle or had stopped flowing altogether because of the drought, started abundantly flowing again due to the destruction of vegetation within their watersheds. This was the end of a long, dry summer and we were seeing substantial flow. Because this was coupled with debris that was sloughing off from burned homes nearby, we found some new mosquito sources forming. A hot tub discovered in the creek contained *Culiseta incidens, Culex tarsalis, Culex thriambus* and *Culex apicalis* in all life stages (1st instar through pupae) just weeks after the fire.In fact, further inspections revealed that every property contained a technician's worst nightmare: ornamental ponds, water features, bath tubs, swimming pools, septic tanks, flower pots, bird baths, wheelbarrows, and melted boats all containing water and larvae (Figure 4). We were amazed that within just weeks after the fires and continuing into October and November, mosquitoes were found in almost every source we located. I promptly treated these sources with Agnique MMF, a Vecto- product, or Altosid. All of these cryptic sources would have been maintained when the people were living there, but now these residents were gone.

Figure 4. Examples of sources on properties destroyed by the fires. (A) Vector Biologist, Bonnie Ryan, sampling a water feature on a burned property. (B) Cryptic sources (e.g. ponds, bird baths, flower pots, etc.) prove to be a challenge. (C) An ornamental water feature on a burned property. (D) Vector Control Technician, Sandi Courcier, sampling a bathtub on a burned property.

Although there has now been some major cleanup in the area and many of these small sources have been removed, we will continue to have problems with the sources that could not be removed such as in-ground swimming pools, large fountains, horse troughs, and septic systems. Even people's basements and foundations have been found holding water (Figure 5). Mosquitofish have been placed in many of these large sources, but for all the sources we didn't find, we plan to have a large fish giveaway in the fire-affected areas in the Spring, and will advertise that in the local paper.

Figure 5. A basement holding water is all that remains of this house.

Another problem the District is facing is lack of addresses. When the houses burned down, so did the addresses, making the locating of properties and reporting pesticide use extremely challenging. This has encouraged the District to change its online Service Request forms for fire victims. Previously, things like 'Address' and 'House Color' were required fields. Obviously, these descriptions no longer apply to these areas. We are working to add features to our online Service Request forms to help locate properties, such as the ability to upload a picture or drop a pin on a map where you want to report a mosquito problem.

Figure 6. A neighborhood in Cobb immediately after the Valley Fire.

Many of the most heavily damaged neighborhoods were revisited in January 2016 and were found to be completely cleaned up. Figure 6 is an example of a neighborhood directly after the fire, and that same neighborhood post-cleanup (Figure 7).

As you can see in Figure 7 of the post-cleanup area, orange mesh construction fences have been erected around huge holes in the ground. These holes are where septic tanks are located or where large trees once stood, and they're now full of water. If the septic tanks didn't melt or burn in the fire, they were damaged or cracked when the large equipment came in to remove all the trees and home debris. These holes are safety hazards for us and others working in the burned areas as well as potential mosquito sources; at least the fences let us know where to focus our efforts.

Figure 7. The same neighborhood in Cobb after cleanup was complete. Orange construction fences surround damaged septic tanks and other holes in the ground.

LOSS IN REVENUE

Not only did the fires create an entirely new set of mosquito problems for us, they also took a toll on the District financially. Currently, the District receives its revenue from a combination of property taxes and a voter-approved Proposition 218 Benefit Assessment. The properties destroyed by the fires were reassessed and will pay less in property taxes and assessments until the structures are rebuilt. Unfortunately, many people are talking about not returning and rebuilding or are replacing their homes with mobile homes, which are assessed at a lower rate. The Valley Fire Long-Term Recovery Task Force conducted a survey and reported that only 55% of those who lost their homes were planning on rebuilding.

Initial estimates predicted that the County and local agencies will lose more than $2.2 million annually in property taxes and assessments, including a $66,750 annual loss for the Lake County Vector Control District itself. The actual loss will likely be much greater than these initial estimates.

CONCLUSION

Although at this point the future looks challenging and I have no idea what to expect for the upcoming mosquito season in terms of workload, one thing remains certain: nothing is permanent except change itself. This is now an area of constant change. It is important to understand that our recovery is in the beginning stages of a very long process, and we hope to be able to meet those challenges as they arrive.

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I gratefully acknowledge Jamesina Scott, PhD for her assistance in helping me learn and navigate PowerPoint. A very special thanks to Lake County Vector Control District (LCVCD) staff who helped me locate and treat sources, take photographs, and edit this paper. I would also like to thank the LCVCD Board of Trustees for their support.

2015 Drought Related Mosquito Control Challenges and Observations in Lower Sacramento Valley

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Some of the unique observations and challenges the Sac-Yolo MVCD faced as a direct result of California's ongoing drought ranged from reduced urban runoff to flooded organic fallow agricultural fields and waterfowl habitat concerns. The district combated catch basin breeding during winter months due to warmer and dryer weather, isolated stagnated puddles in creeks, streams, and urban drainages as well as utilized combinations of treatment application methods and products in flooded organic fallow rice fields and wetland habitats. Drought related water management practices may continue to impact mosquito control into the future as a result of local and statewide policy and program changes. Potential areas of concern include groundwater recharge, storm water commodities, and habitat accommodations that may require specialized surveillance and application methods such as the potential use of drones or other imagery options. Stormwater regulations will continue to dominate the municipal design and management of stormwater systems which will have to be closely monitored for mosquito production as they age.

Impact of Aerial Applications of Adulticides on Mosquito Vector Populations and West Nile Virus Activity in San Joaquin County, 2015

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ABSTRACT: In the summer of 2015 high vector abundance coupled with increased West Nile Virus (WNV) activity necessitated aerial adulticide applications in the Delta and Stanislaus River habitats of San Joaquin County. Aerial applications of the organophosphate Naled resulted in a significant reduction in adult populations of both Culex pipiens and Culex tarsalis mosquitoes, and substantially decreased WNV activity measures such as minimum infection rate (MIR) and Vector Index (VI) for those species. This paper compares the reduction in vector populations and WNV presence in treated locations with untreated locations to estimate the difference that aerial applications made in WNV activity during 2015 in San Joaquin County.

KEY WORDS

Aerial Applications, Adulticide, West Nile virus, Vector population

The 2015 mosquito season presented many challenges for mosquito control in San Joaquin County. Due to the ongoing drought conditions, limited water sources caused an ecological shift in mosquito production. Regions of the county such as the Delta and the southern riparian habitat that historically were greater sources of Aedes mosquitoes, instead produced primarily Culex mosquitoes. In a typical year, many of the mosquitoes produced by these habitats would be flood water mosquitoes such as Aedes vexans. However, during the drought year of 2015, water levels did not reach sufficient depth to inundate and trigger Aedes eggs to hatch. Instead, the water dried up and left small stagnant pools, ideal for Culex mosquito production. The result was an abnormally high Culex population for the county in 2015, further increasing the risk of WNV transmission, especially in the southern riparian and Delta regions of San Joaquin County.

The Delta is the epicenter of WNV enzootic transmission in San Joaquin County. The close proximity of mosquito breeding sources puts Stockton at an especially high risk for nuisance mosquitoes and exposure to mosquito borne diseases such as WNV. Its habitat presents challenges to effective treatment. A long system of levee roads and waterways combined with thick brush and limited access points to mosquito harborage areas makes treatment by ground difficult. Aerial adulticide applications are the only effective way to respond quickly to high populations of WNV vector mosquitoes.

The southern riparian region along the Stanislaus River is near two small cities, Manteca and Ripon, causing them to be vulnerable to nuisance mosquitoes and exposure to WNV. Much of this area also is difficult to reach because of poor levee roads and thick brush making it almost impossible to effectively reach flying mosquitoes with a mosquito adulticide applied from a truck-mounted fogger.

The abundance of Culex pipiens and Cx. tarsalis mosquitoes, both vectors of WNV, required San Joaquin County Mosquito and Vector Control District (SJCMVCD) to treat these two habitats by the aerial application of adulticides several times during 2015, including four large scale (>10,000 acres) applications. Aerial applications for adult mosquito control are an effective method to control mosquito vector populations and reduce WNV transmission risk (Carney 2005). In addition, past aerial applications have been more successful in controlling mosquitoes in these environments (Smith 2015), which is why they were chosen for treatment in 2015.

MATERIALS AND METHODS

Each of the four aerial applications analyzed in this paper were conducted by Vector Disease Control International (VDCI). Each of the applications was flown with a Cessna 402 equipped with Micronair AU 5000 nozzles applying Naled (as Trumpet EC at ~0.75 oz/acre) to between ~11,000 - ~15,000 acres. These large scale aerial applications were made twice to each habitat in the period between late July and early September (Table 1).

Treatment #	Date	Habitat	Acres	Material	Rate (oz/acre)	Time	Wind (mph)
1	7/28/2015	Delta	15,200	Trumpet EC	0.77	8:40 PM	15
2	8/16/2015	Delta	15,158	Trumpet EC	0.76	8:30 PM	4-15
3	8/20/2015	Ag/Riparian	11,947	Trumpet EC	0.75	8:20 PM	10-15
4	9/3/2015	Ag/Riparian	10,923	Trumpet EC	0.75	8:07 PM	10-17

Table 1. Data parameters for each aerial treatment analyzed.

Several parameters were analyzed to determine the effectiveness of these treatments, including: 1) The average number of Culex vectors caught per trap night, used to calculate the mathematical and Mulla control reduction of vector abundance (Mulla 1971), 2) The number of mosquito samples tested positive for WNV, 3) the Minimum Infection Rate (MIR) calculated by Maximum Likelihood Estimate (MLE), and 4) the Vector Index (VI). MLE and VI statistics were calculated using the statistical package PooledInfRate v. 4.0 (Biggerstaff 2004). The data used for this analysis was taken from weekly dry-ice baited Encephalitis Virus Surveillance (EVS) Mosquito Traps and in-house testing of mosquito samples for WNV presence using a quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR). EVS traps were set in the treated habitats once weekly and run over night.

The population numbers and qRT-PCR data were collected three times for each treatment. The first occurred 2-3 days prior to aerial treatment. The second collection was made the following week after aerial treatment, 1 week later than the first, and the third collection was made one week after the second. For example, a typical cycle of trapping, testing, and treating the mosquitoes in the two habitats consisted of setting traps on Monday with collection and testing on Tuesday, aerial treatment on Thursday or Friday, and follow up trapping and testing done the following two weeks in the same manner.

Multiple EVS trap locations were selected in both habitats to represent the mosquito populations of each (Figure 1). In the Delta habitat 8 EVS trap sites were selected and in the Ag/Riparian Habitat 4 EVS trap sites were selected as treated. Outside of either treated habitat, 10 EVS traps adjacent to the treated blocks were selected as a non-treated control to compare treated areas to those that were untreated.

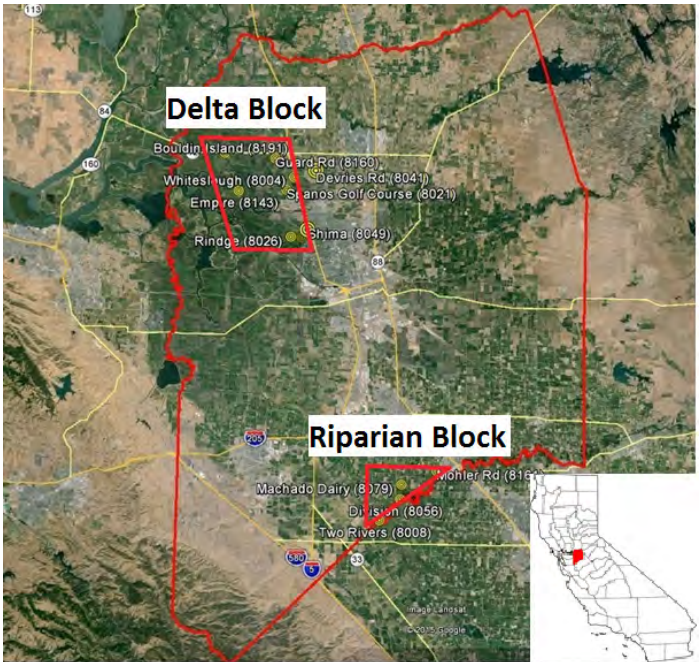

Figure 1. Map of San Joaquin County displaying spray blocks and EVS trap locations.

RESULTS AND DISCUSSION

The first treatment conducted on July 28, 2015 in the Delta Block resulted in a reduction in both Culex species numbers and WNV prevalence (Table 2). Pre-treatment trap counts averaged 116 Culex/trap night, with post-treatment counts averaging only 30 Culex/trap night for an overall reduction in Culex population of 75% or 88.8% Mulla control reduction (Mulla 1971). Additionally, the number of WNV positive pools, MLE, and VI decreased 73%, 37% and 70%, respectively. As a comparison, the Culex populations in the Control area at the same time period experienced an increase in Culex mosquito abundance (120%) and an increase in VI (264%), although the MLE was slightly reduced by 11%.

Treatment #1		Delta Block				Stockton Control Traps		
7/28/2015		Pre-Trt	Post-Trt	Change		Pre-Trt	Post-Trt	Change
Avg *Culex*/Trap		116 ± 30	30 ± 28	-75%		8 ± 15	18 ± 9	120%
WNV+ Pools		11	3	-73%		1	1	---
MLE		33.08	20.83	-37%		5.82	5.16	-11%
VI		1.723	0.519	-70%		0.017	0.062	264%

Avg=Average; WNV+ =WNV Positive; MEL = Maximum likelihood estimate of minimum infection rate (MIR), VI= Vector Index; Trt=Treatment.

Table 2. Data from the first aerial treatment of the Delta Block.

The second treatment conducted on August 16, 2015 in the Delta Block had similar results as the initial treatment (Table 3). Culex mosquito abundance was reduced by 66% for 69.8% Mulla control reduction. WNV levels also decreased, with the number of WNV positive mosquito pools, MLE, and VI reduced by 83%, 75%, and 88%, respectively. In contrast, there was an increase in all the parameters for the control area.

Treatment #2		Delta Block				Stockton Control Traps		
8/16/2015		Pre-Trt	Post-Trt	Change		Pre-Trt	Post-Trt	Change
Avg *Culex*/Trap		68 ± 20	23 ± 5	-66%		11 ± 6	13 ± 7	18%
WNV+ Pools		6	1	-83%		0	3	+
MLE		34	7	-75%		0	87	+
VI		1.001	0.125	-88%		0	0.554	+

Avg=Average; WNV+ =WNV Positive; MEL = Maximum likelihood estimate of minimum infection rate (MIR), VI= Vector Index; Trt=Treatment.

Table 3. Data from the second aerial treatment of the Delta Block

The third treatment conducted on August 20, 2015 involved the Stanislaus River Block along the southern border of San Joaquin County. Results of post-treatment indicated a decline in Culex abundance coupled with a steep decline in WNV activity based upon mosquito pool testing data (Table 4). The average number of Culex mosquitoes per trap night was reduced by 58% from pre-treatment to post-treatment counts or a 78.1% Mulla control reduction. All of the WNV data parameters were reduced to 0, with no WNV positive mosquito pools found after the treatment was completed. In comparison, average mosquito population numbers increased by 85% during the same time

period in the untreated control area. WNV parameters also decreased in the untreated area, but not to the degree that was seen in the treated area.

Treatment #3		Stanislaus River Block				Stockton Control Traps		
8/20/2015		Pre-Trt	Post-Trt	Change		Pre-Trt	Post-Trt	Change
Avg *Culex*/Trap		143 ± 28	60 ± 13	-58%		13 ± 7	24 ± 17	85%
WNV+ Pools		11	0	-100%		3	1	-67%
MLE		62	0	-100%		87	4.51	-95%
VI		4.95	0	-100%		0.554	0.098	-82%

Avg=Average; WNV+ =WNV Positive; MEL = Maximum likelihood estimate of minimum infection rate (MIR), VI= Vector Index; Trt=Treatment.

Table 4. Data from the first aerial treatment of the Stanislaus River Block.

The fourth treatment was conducted on September 3, 2015 in the Stanislaus River Block. In this instance, mosquito counts decreased only 17% from pre-treatment to post-treatment (Table 5), or 15.6% Mulla control reduction. The data parameters regarding WNV presence all decreased to 0, with no WNV activity detected post-treatment. Mosquito counts in the untreated Stockton control group also declined during the same time period (27%). However, similarly to the August 20, 2015 treatment period, WNV activity decreased (34%) but did not completely disappear as in the Stanislaus River Block population that was treated.

Treatment #4		Stanislaus River Block				Stockton Control Traps		
9/3/2015		Pre-Trt	Post-Trt	Change		Pre-Trt	Post-Trt	Change
Avg *Culex*/Trap		79 ± 11	66 ± 26	-17%		11 ± 5	8 ± 3	-27%
WNV+ Pools		8	0	-100%		3	2	-34%
MLE		71	0	-100%		41.65	55.6	33%
VI		2.628	0	-100%		0.375	0.312	-17%

Avg=Average; WNV+ =WNV Positive; MEL = Maximum likelihood estimate of minimum infection rate (MIR), VI= Vector Index; Trt=Treatment.

Table 5. Data from the second treatment of the Stanislaus River Block.

Overall, each of the four aerial treatments during the 2015 season resulted in a great reduction in both mosquito abundance and WNV infection. Post-treatment Culex mosquito populations were reduced 56% on average across the four treatments compared to their pre-treatment levels. These findings indicated that the aerial applications had a strong effect on reducing mosquito abundance and especially infected females in the Delta and Stanislaus River areas. Additionally, WNV was greatly reduced by each of the treatments: on average WNV positive mosquito pools, MLE, and VI were reduced 89%, 86%, and 96% respectively. Even in cases where the mosquito population was not markedly reduced, such as treatment #4, the presence of mosquitoes carrying WNV was reduced. Each of these applications effectively disrupted the WNV transmission cycle of WNV and greatly reduced the risk of WNV infection for local residents.

ACKNOWLEDGEMENTS

The authors would like to thank Mary Iverson for her efforts in collecting mosquito populations from the two habitats discussed. The diligent work of the SJCMVCD staff to reduce and eliminate nuisance and vector mosquitoes in these habitats should also be noted. The SJCMVCD Board of Directors should also be thanked for their support and interest in this study.

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Aerial Adulticide Spray Block Design

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The Sacramento-Yolo Mosquito and Vector Control District frequently uses aerial applicators to perform adulticide treatments during the mosquito season in rural/agricultural and urban areas when West Nile virus activity warrants it. This talk will cover how the District determines the timing of treatments as well as the block size, shape and location using the surveillance information we have available and the mosquito population involved.

Field and cage enclosure studies investigating interactions between invasive aquatic weeds and mosquitoes

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A field-scale survey, three outdoor cage enclosure experiments, and a laboratory experiment were conducted to elucidate the impact of the invasive aquatic weeds *Eichhornia crassipes* (floating water hyacinth), *Ludwigia hexapetala* (emergent water yellow-primrose), and *Egeria densa* (submersed Brazilian waterweed) on the population, behavioral, and physiological ecology of *Culex pipiens*. This mosquito is a primary vector for West Nile Virus (WNV), and both +WNV mosquito incidence and human cases have increased over the past decade in agricultural and urban regions of the Sacramento-San Joaquin Delta, a period during which weed abundance has also increased. In a two-year field survey, *C. pipiens* larval abundance was higher among *E. crassipes* mats in closed water bodies than in open, flowing sloughs, in which mosquito abundance was negligible. In an outdoor cage experiment, in mesocosms containing larval *C. pipiens* and mosquitofish *(Gambusia affinis)*, mosquito survival was significantly higher by 1 to 18 among high densities of the three plant species than open water. In intermediate plant densities, mosquito survival was 7 percent higher among water hyacinth than both Brazilian waterweed and water primrose. In low plant densities, mosquito survival among water hyacinth was 5 and 6 percent higher than Brazilian waterweed and open water, respectively. Thus, in some cases water hyacinth provided more protection for *C. pipiens* against mosquitofish than Brazilian waterweed and water primrose. In separate mesocosms in the absence of predators, *C. pipiens* larval development time was completed more rapidly (by 0.5 days) in the presence of intermediate densities of water hyacinth than with other densities of this weed, or in the presence of the other two weeds. In an outdoor caged adult mosquito choice experiment, females laid 34 percent more eggs in mesocosms containing intermediate densities of water hyacinth than in mesocosms containing intermediate densities of water primrose, and 99 to 100 percent more eggs in mesocosms subject to all other treatments. Laboratory choice tests in cages and an olfactometer experiment revealed that *Culex pipiens* was more attracted to open water that had contained water hyacinth for 48 hrs, than to water pre-treated with other invasive aquatic plant species. These results indicate that water hyacinth is conducive for *C. pipiens* mosquito reproduction and larval survival. Effective management of invasive water hyacinth in Delta waterways may thus reduce *C. pipiens* populations and reduce human health risk.

The Host-seeking Behavior of *Culicoides occidentalis* at Borax Lake, California

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ABSTRACT: The largest known population of *Culicoides occidentalis* Wirth and Jones (Diptera: Ceratopogonidae) in the world is along the shores of Borax Lake in the city of Clearlake. This lake is within a terminal basin; water collects to form a lake, but there is no outlet to drain water away. As a result, the water in Borax Lake is alkaline and boron-rich and provides excellent larval habitat for the hematophagous gnat *C. occidentalis*. A single CO2-baited trap can collect more than 45,000 females per night. Female *C. occidentalis* are not known to vector disease, but are morphologically indistinguishable from *Culicoides sonorensis*. The latter is a vector of Blue Tongue virus in the United States; a zoonosis that can cause disease in wild and domestic ruminants.

Past efforts to mitigate the larval populations of *C. occidentalis* through larvicide application, biological control, and physical destruction yielded variable results. Experimental larvicidal treatments began in 1975 and concluded in the early 1990's. Long-term adult abundance of *C. occidentalis* has been tracked with a New Jersey Light Trap near the lake beginning in 1985.

The current study describes the potential biting nuisance of *C. occidentalis* to nearby residents. Host-seeking periodicity was monitored with a CO2-baited collection bottle rotator trap operated during three eight-day periods in 2010. We also documented the seasonality of host-seeking adults from spring 2013 to spring 2015 using two CO2-baited traps that were set every other week.

Watching ants: How insect behavior impacts protocol

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ABSTRACT: Know your pest is the first rule for any control treatment. The Coachella Valley Mosquito and Vector Control District re-examined the diurnal foraging activity of red imported fire ants, *Solenopsis invicta*, from May until November 2015. The goal was to determine the most effective time to conduct surveillance and to make treatments to control these ants. Thirty-two mounds were surveyed every two weeks during the time of day when technicians were working (May 1 – September 30: 6:00 am to 11:00 am; October 1 – November 30: 8:00 am – 1:00 pm). A hot dog slice was placed 1 m (3 ft.) from the mound. After 60 minutes, the number of ants was estimated, the hot dog slice was removed, and a new hot dog slice was placed 90° from the previous in a cardinal direction (for instance, if the first slice was north of the mound, the next slice was east of the mound). Temperature and relative humidity were measured, and temperature was found to be a good predictor of ant activity. The District is using this study to revise its Standard Operating Procedures to make effective and efficient treatments.

INTRODUCTION

A component of the Coachella Valley Mosquito and Vector Control District's (the District) Integrated Vector Management program is to protect residents from the red imported fire ant, *S. invicta*. The ants are aggressive and inflict a painful sting to animals and humans. Daily irrigation from residential properties and golf courses provide moist and cool conditions for the ants allowing them to survive in the Coachella Valley. When the District's red imported fire ant control program began in 2005, a study was done to determine the foraging activity of *S. invicta*. Monitoring when the ants forage is necessary, so that control efforts are successful at managing the ants. The products that the District uses are baits with corn grit bases. When the baits get wet, the corn becomes mushy and unattractive to the fire ants. Given the high temperatures in the Coachella Valley, many residents and businesses water their grass multiple times per day to keep the grass from burning; to prevent the product applications from being wasted, the District requires that the water being kept off throughout the day of application. Knowing when the ants are attracted to and picking up bait allows the District to request that residents and businesses stop irrigation for long enough for the treatments to be effective.

The District has also adopted a procedure where technicians estimate the level of infestation using food lures to determine the type of treatment made when a property is smaller than 3 acres. Hot dog baits are placed in locations where ant activity is suspected to occur and are checked after approximately one hour. If 30% of the hot dog baits have red imported fire ants, a broadcast treatment is made. If fewer than 30% of the hot dog baits have fire ants, a mound treatment is made. This division was made to ensure that these small properties were not being over-treated with large amounts of chemicals. This study examined whether the timing of this baiting would impact the treatment decision.

To determine if the ants have adapted to the local environment, the foraging hours of *S. invicta* were re-examined. Food lures were used to determine the optimal foraging hours of *S. invicta* and if abiotic factors, such as, air temperature, relative humidity, sun or shade, impact the ants foraging behavior.

MATERIALS AND METHODS

A one-acre site located at the Vintage Country Club, Indian Wells, California, was selected based on a history of *S. invicta* activity. Thirty-two mounds located at the base of palm trees were chosen at the site. From May through November 2015, the site was sampled every two weeks. The sampling method consisted of using 1/8" hot dog slices as bait anchored with a marking flag to the ground. The anchored bait was placed one meter (3 ft.) from the base of each of the thirty-two trees at the beginning of each hour, so that there was a single bait per tree (Figure 1). The baits were evenly distributed to have eight located on the north, east, south, and west side of the trees (Figure 2). Ants were allowed one hour to forage, and the number of ants found at the bait at the end of the hour was counted. An estimate of the number of ants on the bait was recorded based on a 0 – 10 score (Perezchica-Harvey and Henke 2016) and if the bait was in the sun or shade. The bait was replaced, and the location of it was switched 90˚ around the tree clockwise at the beginning of each hour. Air temperature, relative humidity, wind direction, and wind speed was measured with an ambient weather WM-4® comprehensive wind meter before each set of baits was placed.

Figure 1. Left: Placement of the hot dog slice as bait on the south side of a mound. Right: Fire ants actively foraging on a hot dog slice.

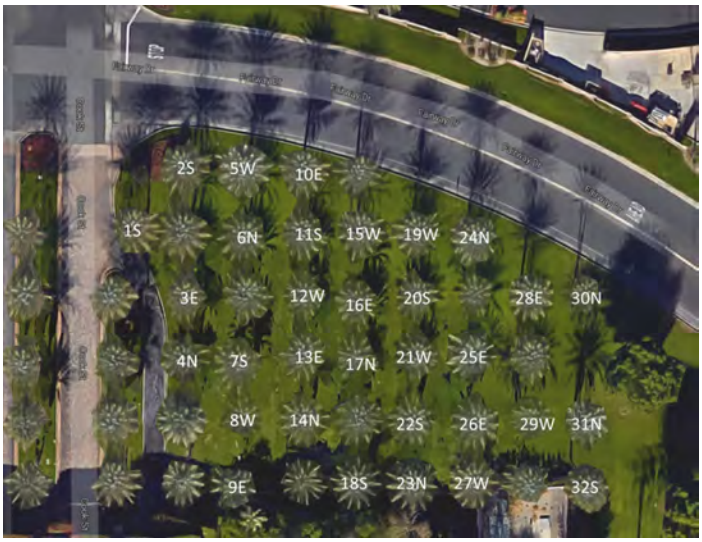

Figure 2. A one acre site at The Vintage Country Club, Indian Wells, California, was selected. Hot dog baits were randomly distributed to have eight on the north, east, south, and west location of each tree (as indicated by the number and the letter for the bait's starting position). The bait was replaced every hour and switched 90° around the tree. Map was created using Google Maps (Google Earth 2016).

Baiting was done at times when technicians were working. From May through September, baits were set from 0600 – 1100 and from October and November, the baits were set from 0800 – 1300.

RESULTS

Ant activity during the third sampling period was considered to be representative of earlier foraging. Comparing the third hour with the sixth hour, there tended to be more ants foraging at the third hour compared to the sixth hour, especially during the summer (Figure 3), but this is most apparent in September. In May, October, and November, ants were active throughout the day, with a trend towards lower activity later in the day in June.

The level of infestation was calculated for each sampling by taking the number of hot dog slices where fire ants were detected and dividing this by the total number of slices placed (32). Sampling later in the day during the summer resulted in a substantially different infestation rate (Table 1). False negatives were observed when sampling was done later in the day in July, August, and September.

Relative humidity was recorded and ranged from 14% to 70%. As air temperature increased during the day, relative humidity decreased. The number of ants on the baits exposed to sun or shade was counted. Although there were slightly more ants on the baits in the shade during the summer and more in the sun during November, there was no difference if the baits were exposed to the sun or shade for all months (Table 2). In May and October, there were about the same number of ants on the baits exposed to both sun and shade for each hour. During the summer, there were slightly more ants on the hot dog baits that were in the shade and in November, there were more ants on the baits that were in the sun.

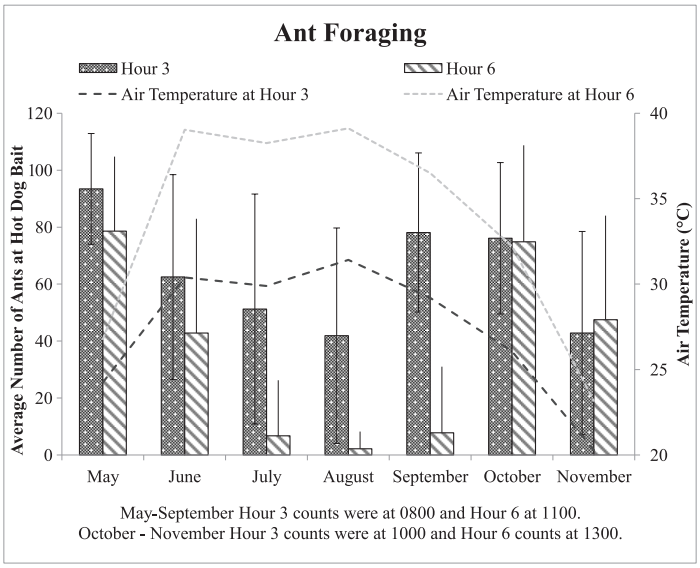

Figure 3. From May through November, there were more ants foraging at the third hour, especially during September. Bars represent standard deviations.

Hour	May	Early Jun	Late Jun	Early Jul	Late Jul	Early Aug	Late Aug	Early Sept	Mid Sept	Late Sept	Early Oct	Late Oct	Early Nov	Late Nov
1	84	84	34	91	88	81	84	84	100	100	100	100	78	56
2	94	88	91	88	94	94	72	84	100	100	100	100	88	47
3	100	97	97	88	94	94	78	94	100	100	100	100	100	84
4	100	100	97	78	100	97	88	78	66	94	100	100	97	84
5	100	88	84	50	56	53	44	34	41	59	94	100	91	94
6	97	94	63	**13**	34	**25**	**9**	**9**	**22**	**25**	91	100	91	94

Table 1. The level of infestation (percent) for each sampling from May through November was calculated by dividing the total number of positive locations by the total number of locations sampled (32 trees). The percent infestation table shows where false negatives occurred (shown in bold and highlighted in dark grey).

		Hour 1	Hour 2	Hour 3	Hour 4	Hour 5	Hour 6	Average
May	sun	6.00	8.72	9.19	9.19	8.83	7.82	8.29
	shade	6.07	8.60	9.23	8.77	9.36	9.00	8.51
June	sun	6.79	6.08	6.20	6.53	3.70	2.00	5.22
	shade	3.47	5.80	6.34	6.08	5.00	6.20	5.48
July	sun	4.86	4.34	4.95	3.19	1.33	0.31	3.16
	shade	6.50	6.36	5.21	5.04	3.80	1.65	4.76
August	sun	5.41	5.55	5.74	3.25	0.79	0.15	3.48
	shade	6.31	5.06	4.50	4.63	2.90	1.60	4.17
September	sun	9.25	8.48	7.25	3.26	1.75	0.81	5.13
	shade	8.11	8.56	8.07	4.88	4.14	0.76	5.75
October	sun	6.71	9.00	8.22	5.77	7.78	6.97	7.41
	shade	7.00	8.86	7.00	7.08	8.23	7.84	7.67
November	sun	4.05	7.41	5.56	6.15	7.00	5.88	6.01
	shade	3.18	3.37	5.28	3.75	5.27	4.32	4.20

Table 2. An estimate of the number of ants on the hot dog baits exposed to sun and shade was recorded for each hour. The estimates were based on a 0 – 10 score (Table 1).

DISCUSSION

Control products used by the District consist of bait formulations that red imported fire ants carry back to the nest. Applying the control product when the ants actively forage provides the best control. Because ants are sensitive to changes in temperature, air temperature is a good predictor of when the ants will more likely go to the bait. When air temperatures are between 16° and 38° C (60° and 100° F), technicians use food lures to determine the level of fire ant infestation. When temperatures remain cool, such as in May and October, ants were actively foraging throughout the day, and baiting early or late in the day should yield similar results. During the summer months, more ants were actively foraging earlier in the morning when the air temperatures were below 38° C (100˚ F), and baiting at this time would yield better results. In early November, ants were active throughout the day, and as the temperatures got colder, there were more ants foraging later in the day. When air temperatures are below 16° C (60° F), baiting later in the day can yield better results when the air temperatures are warmer. Although humidity may impact the foraging behavior of ants, air temperature remains a reliable inidcator of ant activity.

The positive percentage result determines whether a technician should make a broadcast or mound treatment. If 30% or more of the total number of baits have fire ants, the technician makes a broadcast treatment; less than 30%, a mound treatment is made. During the summer, false negatives were observed during late periods when temperatures were hot (Table 1). Hot dog baits that were set at 11:00 a.m. during the summer would have resulted in a mound treatment, but if sampling was done earlier the same day, a broadcast treatment would have been made. Baiting should take place earlier in the day during the summer months, because baiting later in the day can result in an ineffective treatment application.

In May and October, the number of ants on the baits that were in the sun and shade were similar. From June through September, when air temperatures were above 38°C (100°F), there were slightly more ants on the baits exposed to the shade later in the day. In November, there were more ants on the baits exposed to the sun; however, no preference was seen regarding the placement of baits in sun versus shade overall (Table 2).

CONCLUSION

Air temperature was found to be a good predictor of *S. invicta* foraging activity. In the Coachella Valley, District technicians monitor air temperature when conducting surveillance for the red imported fire ant. To prevent false negatives, baiting with hot dog slices for ants should take place early in the day if the air temperature will exceed 100°F and later in the day when temperatures are below 60°F. Treatments can be made later in the day during the summer, provided that the control product is not exposed to water. This information was used to review and update the District's Red Imported Fire Ant Standard Operating Procedures.

ACKNOWLEDGEMENTS

The authors thank Bobbye Dieckmann, Field Supervisor, and Rod Chamberlain, Lead Supervisor, for their assistance setting up the field study area. Greg White, Vector Ecologist, provided useful discussions on the study.

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Endosymbiont interference of Spotted Fever Group Rickettsia infection in the Pacific coast tick, *Dermacentor occidentalis*

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ABSTRACT: Previous studies have demonstrated that non-infectious endosymbiotic bacteria can interfere with *Rickettsia* co-infections in ticks. We hypothesized that interference also exists between other bacteria and Spotted Fever Group *Rickettsia* (SFGR) within *D. occidentalis* ticks. Using PCR amplification and sequencing of the *romp*A gene and intergenic region, we identified a cohort of SFGR-infected and non-infected *D. occidentalis* ticks collected from San Diego County. We then amplified a partial segment of the 16S rRNA gene and used next-generation sequencing to elucidate the microbiomes of the ticks. The SFGR *R. philipii* str. 364D and *R. rhipicephali* were detected in 2.3% and 8.2% of the ticks, respectively. Interestingly, there was an inverse relationship between infection with *Francisella*-like endosymbionts (FLE) and *Rickettsia* that is consistent with the hypothesis of interference. Excluding the *Rickettsia* and FLE endosymbionts, there was a small, but significant, difference in microbial community diversity and a pattern of geographic isolation by distance between collection locales. Our findings suggest that FLEs and to a lesser extent, other bacteria, interfere with and negatively affect the ability of *D. occidentalis* to be infected with certain SFGR.

A deterministic model to quantify risk and guide mitigation strategies to reduce bluetongue virus transmission in California dairy cattle

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INTRODUCTION

Bluetongue virus (BTV) is an economically important cause of disease in ruminants, and in North America, the virus is transmitted primarily by the biting midge, *Culicoides sonorensis*. Like other arboviruses, transmission of BTV is highly sensitive to variation in temperature (Mullens et al. 2004), and the northward expansion of BTV into many parts of Europe has been blamed, in part, on climate change (Purse et al. 2005). In this study, we modeled the effects of temperature on bluetongue transmission dynamics and estimated seasonal variation in basic reproductive rates for BTV throughout California.

METHODS

Data used for estimation of host, vector, and environmental states and parameters were obtained from literature and surveillance conducted on four dairy farms, which were intensively monitored during 2009-2010 (Mayo et al. 2012a, Mayo et al. 2012b). Host and vector competence parameters were assumed to be constant over time, and the model was evaluated for a typical herd of 1,000 dairy cows. Year-round temperatures for all of California were used for estimation of temperature-dependent parameters.

A compartmental, ordinary differential equation (ODE) model of BTV transmission was constructed (Figure 1). Populations of cattle and *Culicoides* midges consisted of susceptible (S), incubating (E), and infectious (I) animals. Infected cattle recovered from infection with immunity (R), and adult *Culicoides* were assumed to emerge from uninfected eggs (P). A defining feature of this model in comparison with other vector-borne disease models is the incorporation of temperature-dependent parameters for *Culicoides* biting rates and the extrinsic incubation period (EIP) of BTV, and vector dynamics defined by *Culicoides* trapping data. The basic reproduction number (R_0) for BTV was computed from the system of ODEs (van den Driessche and Watmough 2002).

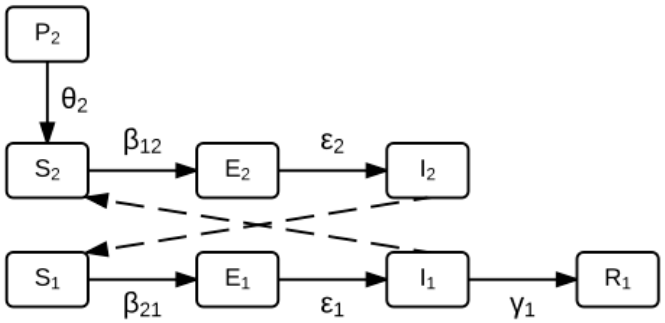

Figure 1. Flow diagram of the bluetongue virus transmission model where host (cattle) compartments are denoted by the subscript 1 and vector (mosquito) compartments are denoted by the subscript 2. Each compartment is denoted by letter as susceptible (S), exposed (E), infected (I), or recovered (R). Parameters include the emergence rate of adult mosquitoes (θ), transmission rates between hosts and vectors (β), BTV incubation rates (ε), and the recovery rate of cattle (γ).

RESULTS AND DISCUSSION

The highest R_0 values (1.7-2.3) were in southern California, southeastern deserts, and the Central Valley, which have hotter summers and cooler winters than the coastal regions of California. Remaining portions of the state had a heterogeneous distribution of R_0 values ranging from 0.03-1.72.

Seasonal patterns of the proportion of BTV-infected dairy cattle were evaluated. Predicted outcomes generated by the model were compared to prevalence values based on surveillance data in 2010. The seasonal increase of BTV infection in dairy cattle was closely associated with increases in the mean number of female *C. sonorensis*, which did not peak until late August, but began to increase during the middle of May.

The time lag from the spring onset of predicted BTV transmission to dairy cattle to the seasonal peak of expected transmission in summer suggested that optimal control measures for reducing the cycle of BTV infection may be most effective several months prior to the peak of infection in dairy cattle. The spatial and seasonal results indicate that simple and cost-effective strategies to reduce vector abundance (i.e. disrupting larval habitat) might be the most effective mitigation strategy to decrease BTV transmission among dairy cattle within an endemic region such as California.

ACKNOWLEDGEMENTS

We gratefully acknowledge producers, staff, and herd managers of the farms from which the samples were obtained. These studies were supported by funds provided by the Agriculture and Food Research Initiative competitive grant 2012-67015-19527 from the U.S. Department of Agriculture–National Institute of Food and Agriculture, and the Center for Food Animal Health at the University of California-Davis, the U.S. Department of Agriculture under the Animal Health Act, 1977, Public Law 95-113.

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Using Data-Informed Decision Making to Improve Efficacy

Joel Buettner, General Manager

Placer Mosquito and Vector Control District

Use of data to inform business and mosquito control decisions is a much promoted and worthy goal, however, for a many reasons most decisions are made using a combination of data and intuition with statistically significant data trends on one end of the spectrum to "this is how we have always done it" on the other. Historically, limitations on the amount and type of data available has been a major barrier to producing timely information on which to base important decisions. With relatively recent data collection, management and analytics tools, the question has become how do we collect, process, and display information in a timely, useful and insightful way? This presentation will discuss the concepts and processes that allow more insight to be gained from operational data that can help to increase efficiency and efficacy of a mosquito and vector control program.

Data visualization for vector control: Potential applications of Tableau software

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BACKGROUND

Data visualization with Tableau software can enable a vector control agency to more quickly and clearly communicate complicated data to all levels of staff, from management to field technicians. Other commonly used software for creating graphs and presenting data can be limited by the time required to create images. Additionally, data visualizations currently utilized by most vector control agencies represent a fixed point in time, and must be re-created to include new information. Real-time updates "pushed" out to users using Tableau software allow quick, informed decisions to be made independently, eliminating the barriers of having to request information from another party and wait for a response. Examples presented here focus on adult mosquito surveillance, but the software and principles could be applied to other vector control agency data such as larval surveillance, control operations, employee activities, or administrative functions.

METHODS

Tableau software (Tableau Software, 837 North 34th Street, Suite 200, Seattle, WA 98103, tableau.com) is an interactive data visualization product, usually applied to business intelligence. The software provides data visualization such as graphs, tables, charts, infographics, and maps, and can connect to many types of data sources including Microsoft Excel files, CVS files, and databases such as MySQL and SQL Server. Active, real-time data connections mean that when the data changes, the visualization changes as well.

An example of the use of Tableau at a vector control district is the Placer Mosquito and Vector Control District weekly trap report. For this report, data is retrieved from California Surveillance Gateway (gateway.calsurv.org) using an API (application programming interface) as well as from Placer MVCD's internal inspection and treatment database (MapVision) using an SQL query, with weather data (wunderground.com) also retrieved using an API.

RESULTS

Good visualizations are intuitive, can be rapidly interpreted, and provide several types of information at once. For example, in the map visualization shown in Figure 1 each mosquito trap's location is represented by a pie chart on the map. The overall size of each pie chart shows mosquito abundance at that site, while the size and color of each "slice" of the pie chart presents the proportion of each species within that collection. When viewed in Tableau or with the free Tableau reader, a slide bar or animation can represent the change in mosquitoes trapped at each site over time.

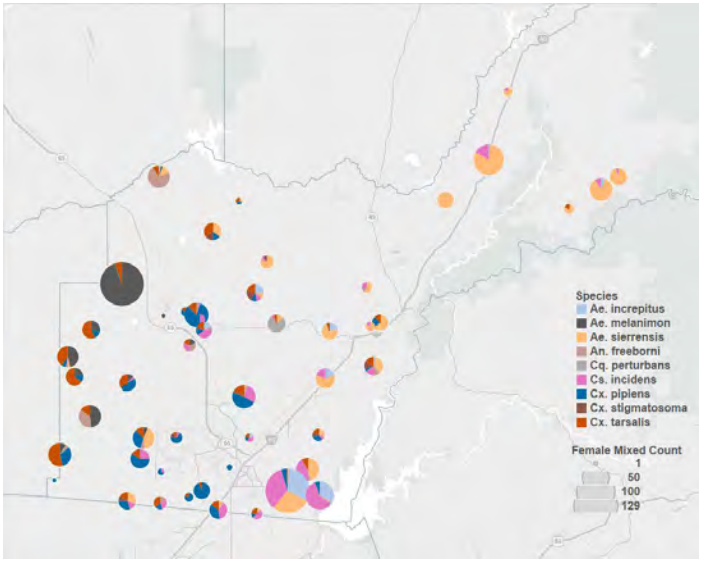

Figure 1. Visualization of mosquito trapping data created with Tableau software. Each pie chart is a trap. Position on map indicates location, size of pie chart indicates total mosquito count, and pie section colors and size indicate the proportion of each species collected in the trap.

If visualizations are viewed with full Tableau software, details about each data point can be viewed by "hovering" with the mouse. Underlying data for any point can be accessed by clicking, and slide bars and filters can be added to make the visualizations even more interactive. End users may also view reports using the free Tableau Reader with more limited functionality, or reports may be exported as PDF or JPG files for static viewing or incorporation into text documents or other reports.

Although there are many advantages to Tableau software for visualizing mosquito and vector control district data, there are some disadvantages as well. The cost of the software may be impractical for smaller agencies. Also, while little training is required to create visualizations with Tableau and to connect the software to Microsoft Excel data, some specialized knowledge is required to establish API, SQL, or other database connections.

CalSurv Gateway tools for managing and visualizing data on pesticide applications and resistance testing (PART)

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ABSTRACT: The rationale for mosquito control relies on the ability of available tools to achieve reductions in mosquito biting and disease risk. For larval treatments or other control actions that are not defined by large, discrete events, it can be difficult to measure control success. The CalSurv Gateway's new modules for recording data on pesticide application and resistance offer new possibilities for combining surveillance and control data for better summaries and evaluations of districts' control actions. This presentation will provide an update on the latest Gateway tools for analyzing pesticide applications and resistance.

CalSurv Gateway: survey results and new tools for invasive Aedes

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ABSTRACT: The rapidly growing importance of invasive Aedes aegypti and Aedes albopictus in California has necessitated modifications to the CalSurv Gateway to accommodate new trap types and data entry needs. This presentation will summarize results from a statewide survey of mosquito control districts' needs and will provide an update on the latest changes to the Gateway, including public and password-protected versions of surveillance maps that provide real-time updates on statewide distributions derived from Gateway data.

Field Evaluation of DeltaGard (deltamethrin) using field-collected *Culex tarsalis* and *Anopheles freeborni*

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ABSTRACT: A field trial evaluating a new ULV mosquito adulticide, DeltaGard, manufactured by Bayer was conducted on August 11, 2015 near Robbins, CA, a rice production area. This work was done in cooperation with the Sutter-Yuba Mosquito and Vector Control District and Colusa Mosquito Abatement District. DeltaGard contained 0.17 lbs/gallon of deltamethrin in a water diluFig. formulation using Bayer's patented FFAST technology that inhibits water evaporation from the droplets. Applications of undiluted DeltaGard Insecticide at 1 g active ingredient(ai)/ha deltamethrin (0.00089 lb. ai/acre) were made over test plots using a truck mounted Clarke ProMist Dura Fogger with Smartflow, variable speed flow control calibrated to deliver a flow rate equivalent to 4.04 oz/minute at 10 mph. The truck was driven at 10 mph. This application was replicated, with the first application at 8:57 pm and the second at 9:46 pm. The temperature at release height at the time of the first application was 68 degrees F, with a temperature inversion of 4.6 degrees F (sensors at 10 and 30 meters. The windspeed at release height was 1.7-3.0 mph. The conditions at the time of the second applicaton were 67 degrees F, with a temperature inversion of 3.5 degrees F. and a windspeed of 2.1-3.2 mph. The RH was 75% for both runs. The test plot consisted of one row of stations placed at 30.5, 61, 91.5, 161, 229 and 381 m (100, 200, 300, 528, 750 and 1,250 ft.) perpendicular to and downwind from the fogger truck path. Each station consisted of two standard sentinel mosquito cages (Townzen and Natvig, 1973) and one droplet impinger (manufactured by Leading Edge Associates) ,with two Teflon slides, placed about 1.5 m high Two mosquito cages containing mixed species were placed at each distance on stakes, one cage containing mosquitoes collected in Sutter County and one containing mosquitoes from Colusa County. Two additional cages were set up-wind of the treatment area to serve as controls. Each mosquito cage contained an average of 15 *Culex tarsalis* and 5 *Anopheles freeborni*. Caged mosquitoes were provided sugar water via soaked cotton wicks throughout the trial. All mosquito cages and slides were collected at 15 min after application. Mosquito cages were put in open plastic bags and placed in ice chests. Upon returning to the laboratory the sugar water was replenished. Knockdown was recorded at 2 hours after application and mortality recorded at 12 hours. There was no mortality in the controls. Knockdown was recorded as mixed populations and declined over distance with 83% knockdown at 30.5 m and 20% at 381 m for Colusa populations and 68% and 10% for Sutter-Yuba populations, respectively (Figure 1). Mortality was greatest for *Anopheles freeborni*, with 100% mortality at 12 h out to 229 m for both populations (Figure 2). At 381 m there was 80% mortality with the Sutter population and 100% with the Colusa population. *Culex tarsalis* mortality at 12 hours for Colusa was 100% out to 91.5 m and 96% out to 381 m, whereas the Sutter population mortality was 85% at 91.5 m and 13% at 381 m (Figure 3). There is a history of pyrethroid resistance with *Cx. tarsalis* where the Sutter population was collected. Teflon slides were analyszed within 12 hours after treatment using Leading Edge DropVison. This analyisis indicated that spray droplets maintained their integrity of 18-20 microns out to 381 m, though droplet density declined beginning at 229 m. DeltaGard is formulated to minimize evaporative loss but it was still surprising to see droplet integrity from a water-based product out to 381 m under these conditions. Field testing indicated that DeltaGard can be an effective ULV adulticide treatment in the Central Valley of California.

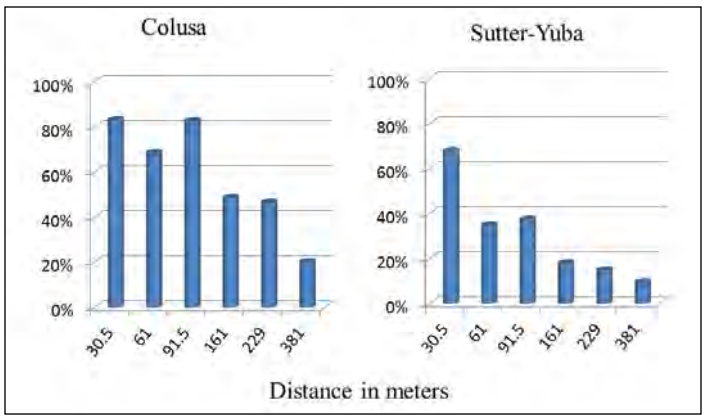

Figure 1. Average % knockdown at 2 hours after application mixed population (*Culex* & *Anopheles*)

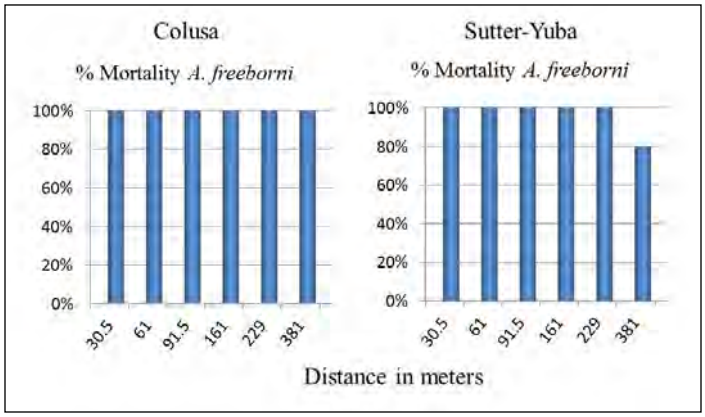

Figure 2. Average % mortality at 12 hours *Anopheles freeborni*

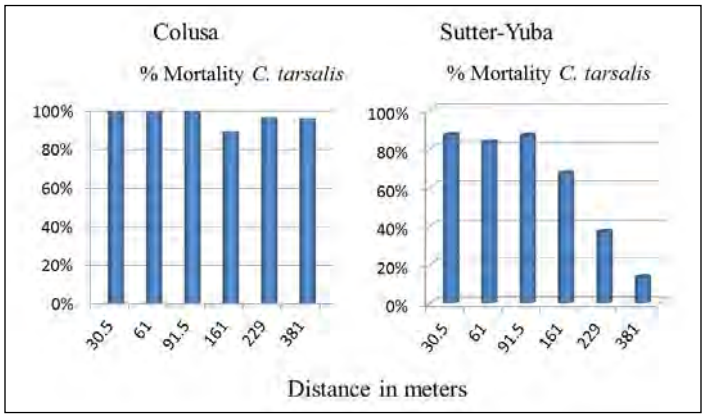

Figure 3. Average % mortality at 12 hours *Culex tarsalis*

Development of Etofenprox-Treated U.S. Military Combat Uniforms

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Historically, the loss of personnel from death and disease has greatly outnumbered the corresponding losses due to combat during military conflict. In 1951, the U.S. military elected to treat combat uniforms with a blend known as M-1960 for the purpose of personal protection from arthropods that transmit disease pathogens. In the late 1970s, alternative uniform fabric treatments were explored and in the 1980s, permethrin was selected as the best insecticide for use on arthropod repellent treatment for uniform fabrics. In 1991, permethrin became the standard treatment of U.S. military combat uniforms.

In 2013, the United States Department of Agriculture-Agricultural Research Service partnered with Mitsui Chemicals Agro, Inc. and Landis International, Inc., to evaluate etofenprox as a new fabric treatment against mosquitoes. There have been numerous documented cases of resistance to permethrin and other pyrethroids. Etofenprox, is a pyrethroid-like compound that has a better toxicological profile than permethrin and does not exhibit cross-resistance in a number of strains of pyrethroid-resistant mosquitoes. For this study, etofenprox was applied to unwashed and 3 levels of washed U.S. Army Fire-Retardant Army Combat Uniforms (FRACU) to evaluate the protective efficacy against the bites of Aedes aegypti and Anopheles albimanus mosquitoes. The three wash levels (0x to 75x) demonstrated greater than 90% bite protection over that of the untreated control fabric. The data will be used for registration of this new clothing treatment and registration is expected sometime in 2016.

Evaluation of Three Granular Bacterial Products

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ABSTRACT: In the rural agricultural area of the Coachella Valley, desert leakage sites from broken well pumps create ponds of standing water. The sites used in this trial were known to be sites that had previously produced mosquito larvae. The efficacy and residual activity of three short term Bti-based microbial products, FourStar® Bti CRG, Aquabac® 200G, and Vectobac® G, were compared at 6 sites which were 1.5 acres or less. The products were applied at a rate of 15 or 20 lbs. per acre. Sites were checked twice each week until larval mosquitoes were found. Water parameters (temperature, pH, dissolved oxygen, conductivity, and change in depth) were measured. No significant difference was seen betweenthe two products of Aquabac 200G which averaged approximately 21 days of control and Vectobac G which averaged approximately 17 days of control. If both products are readily available, using Aquabac 200G is more cost effective.

INTRODUCTION

Although there are few active ingredients available for larval mosquito control, some active ingredients are used by several manufacturers, such that several trade names may refer to the same active ingredient. Determining the best product to purchase can depend on a number of variables such as cost, availability, and effectiveness. The Coachella Valley Mosquito and Vector Control District (the District) compared three products containing the active ingredient *Bacillus thuringiensis israelensis* (Bti). Bti is a stomach poison which must be ingested and breakdown the midgut to result in the death of the mosquito larvae. FourStar Bti® CRG is a multi-brood controlled release granule of Bti strain BMP 144 with solids, spores and insecticidal toxins with a stated label residual action of up to 40 days (FourStar Microbial Products). Its active ingredient is 10% Bti insecticidal toxins. Aquabac® 200G and Vectobac® G are very similar products (2.86% and 2.80% Bti, respectively). Both are comprised of Bti solids, spores, and insecticidal toxins (Aquabac is strain BMP 144, whereas Vectobac is strain AM 65-52). Both provide a residual activity for 7 to 14 days (Becker Microbial Products, Valent Biosciences Corporation).

In the rural agricultural area of the Coachella Valley, broken well pumps under artesian pressure create ponds that allow for mosquito production. These ponds can be temporary (a few weeks) to nearly year round, depending on the flow from the pump. Bti has been selected as the active ingredient to be used in these sites until the permanence of the site can be determined.

MATERIALS AND METHODS

Six sites which were each 1.5 acres or smaller were used for the applications. A second application and monitoring period was completed following after the first application was determined to no longer be efficacious. The second application was with a different product used in each site (e.g., if Aquabac was applied at the site first, then either Vectobac or Fourstar would be used for the second application).

Depending on area of the site, between 3 and 6 floating larval cages (modified BioQuip breeders in Styrofoam floats – Figure 1) were placed in each and secured to the pond substrate with a wooden stake and twine. Each cage was stocked with 20 lab-reared *Culex quinquefasciatus* larvae no older than third-instars. After inspection, the cages were re-stocked with new larvae. Water samples were taken from each area where the floating cages were located and tested for activity with larvae as individual samples. Water samples returned to the lab were used to verify that lab-reared larvae were controlled by treatments. Water parameters (temperature, pH, dissolved oxygen, and conductivity) were measured using the YSI Multi-probe. The change in water depth was also noted.

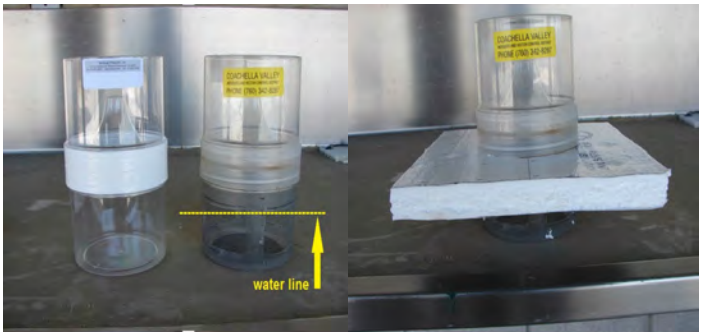

Figure 1. Modified floating larval cages were placed in each site. The bottom of the breeder and windows into the sides were cut and lined with screen to allow water to pass into the chamber without the larvae escaping. Left: Comparison of un-modified cup and modified cup showing water line. Right: Modified cup inserted into Styrofoam float.

RESULTS

Larval mortality was similar in treatment water that was brought back to laboratory and in larvae sequestered in the floating cages at the field sites, so only the results from the larvae sequestered in floating cages were presented. In the first application, there was 100% mortality for the first 8 days' post

treatment (Figure 2). The application of FourStar Bti CRG was effective for approximately 8-10 days following treatment. The applications of Aquabac were effective for 8 days and for 17 days, while the application of Vectobac was also effective for 17 days.

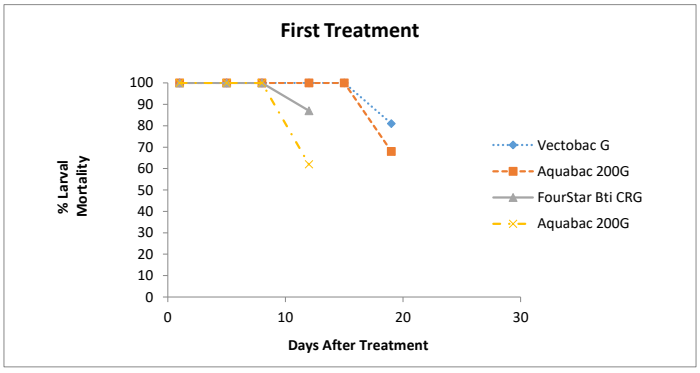

Figure 2. First treatment comparisons. Each line indicates a similar site. Vectobac G and Aquabac 200G were effective between and beyond label residual. FourStar Bti-CRG controlled larvae for 8-10 days.

In the second application, only Aquabac and Vectobac applications were made. One site treated with Vectobac dried out at 10 days after treatment. The other site treated with Vectobac had 100% larval mortality for 28 days after application. The two applications of Aquabac were effective for 10 and 35 days following application (Figure 3).

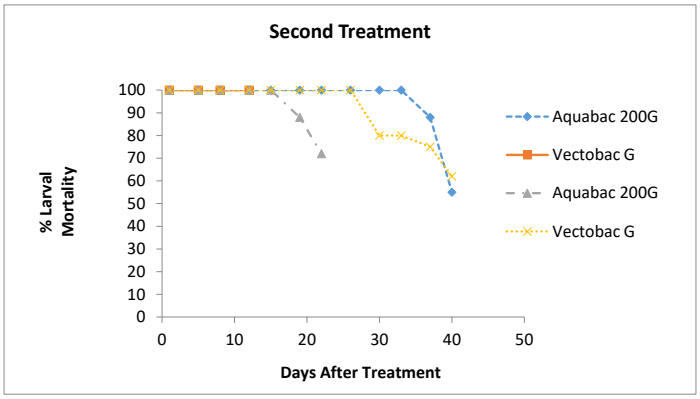

Figure 3. Second treatment comparisons. Each line indicates a similar site. Vectobac G and Aquabac 200G were effective between and beyond label residual. The red line represents a site treated with Vectobac G which remained effective until the site dried out at 10 days.

Conductivity and pH remained constant for all sites throughout the study. In June 2015, water depth and volume began to decrease at a steady rate. Two of the six sites dried up during the first treatment (both sites had no breeding). Dissolved oxygen declined during June and July. Water temperature increased steadily and averaged 85°F at the end of May to 95°F in mid-July.

DISCUSSION

These trials were conducted to evaluate and compare the efficacy of three granular bacterial products, Aquabac 200G, Vectobac G, and FourStar Bti CRG. No difference was seen in two of the products: Aquabac 200G averaged approximately 21 days of control and Vectobac G averaged approximately 17 days of control, based on mortality of larvae deployed in sentinel cages in the field and confirmed with field-treated water brought back to the laboratory. The newest granular product tested, FourStar Bti CRG, did not perform as indicated on the label. Although there was control for up to 9 days, residual effect did not persist beyond 10 days.

Aquabac 200G and Vectobac G are similar in their effectiveness as "quick-kill" products, however, the cost of Vectobac G is 58% more per pound than Aquabac 200G. If both products are readily available, it is recommended that the District favor using Aquabac 200G. Both products will provide at least 2 weeks of 100% control in rural flooding sites.

ACKNOWLEDGEMENTS

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Entomopathogenic Fungi in Mosquito Control

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ABSTRACT: Background. Biological control efficacy of two commercially available formulations of *Beauveria bassiana* and *Metarhizium anisopliae* against laboratory *Culex quinquefasciatus* mosquitoes was determined. Fungi were exposed to conditions in underground storm drain systems (USDS) in the Coachella Valley in two month-long trials during the spring and autumn seasons. Fungi were bioassayed for mosquito infectivity using two methods. In method #1, fungi were applied to filter papers placed in open plastic vials secured in hanging containers within each USDS. After exposure for varying time periods in the field, papers were returned to the laboratory and exposed to colonized *Cx. quinquefasciatus* for 24 hr. In method #2, fungi were sprayed directly on a 1 m^2 USDS concrete wall surface. After varying periods, colonized mosquitoes were transported to the field, exposed *in situ* to the surfaces overnight, and then returned to the laboratory. Filter papers or USDS wall surfaces treated with deionized water served as uninfected controls to monitor natural mortality. Following both methods of exposure, mosquito health was monitored in plastic vials under laboratory conditions for 21 days.

RESULTS

Fungal-linked mortality of mosquitoes differed by fungal species, the length of USDS aging, site location, and method of exposure. Fresh preparations of both fungal species (maximum label-recommended application rates) in laboratory and field exposure methods killed 50% of mosquitoes within 1-2 weeks and greater than 80% of mosquitoes within 3 weeks. Fungal efficacy was markedly reduced after one week of USDS aging in both species; however, *Beauveria bassiana* persisted on USDS walls for up to 4 weeks and on filter paper for up to 11 weeks in the spring trial. *Metarhizium anisopliae* infectivity was minimal after more than 2 weeks of USDS exposure and yet produced greater mosquito mortality in the fall season compared to the spring season. Differences in fungal efficacy among sample seasons could not be explained by differences in environment alone. USDS wall sprays demonstrated higher fungal infection rates at two sites that were deeper (3.7 m) or were flooded continuously (depth of standing water ~ 0.3 m); however, site location did not impact fungal efficacy in aged filter paper exposures.

CONCLUSIONS

Entomopathogenic fungi maintained on different surfaces in the USDS environment effectively killed mosquitoes if specific conditions were satisfied. A delayed onset of mortality and possible sublethal reductions of mosquito fitness point to potential complementary uses of these fungi with other control agents that warrant further investigations targeting underground mosquito sources in abatement programs.

ACKNOWLEDGEMENTS

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Evaluation of Siesta™ Insecticide Fire Ant Bait against red imported fire ants

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ABSTRACT: The Coachella Valley Mosquito and Vector Control District (the District) examined Siesta™ Insecticide Fire Ant Bait (active ingredient: 0.063% metaflumizone) for control of the red imported fire ant, *Solenopsis invicta*. The efficacy and residual activity was compared to the District's currently used Advion® Fire Ant Bait (active ingredient: 0.045% indoxacarb) to determine if it is as effective at controlling the fire ants at parks. Both formulations share a similar mode of action but are not thought to promote cross-resistance. Broadcast treatments were made using a Scotts® Hand-Held Spreader at two parks. Siesta was applied at 1 lb. per acre to one park; at the second park, half of it was treated with Siesta at 1.5 lbs. per acre, while the other half was treated with Advion at 1.5 lbs. per acre. To determine ant activity, ten hot dog slices were deployed in a circle, about 15 ft. from the center of each evaluation plot. After 60 minutes, an estimate of the number of ants on the hot dog slices was recorded as a metric of ant abundance. The temperature, relative humidity, wind direction, and wind speed was measured with an ambient weather WM-4 comprehensive wind meter. Evaluations were made at 0, 1, 2, 4, 9, 12, and 16 weeks after treatment. At the high application rate, both products reduced ant activity immediately following treatments. One week after treatments were made, ant activity was reduced 100% with Advion and 96% with Siesta. The greatest reduction in ant activity was during the first four weeks. At 1 lb. per acre, Siesta did not provide sustained control. Although ants were reduced 39% following treatment for the first four weeks, ant counts were high and mounds were seen throughout the grass plots. Siesta performed nearly as well as Advion by reducing ant populations quickly and consistently for four weeks.

INTRODUCTION

In 2005, the Coachella Valley Mosquito and Vector Control District (the District) began conducting surveillance and control of the red imported fire ant, *Solenopsi invicta.* The ants are aggressive and inflict a painful sting to animals and humans. Daily irrigation to parks and golf courses provides ideal conditions for the ants to survive. The District evaluated Siesta™ Insecticide Fire Ant Bait (active ingredient: metaflumizone; up to 8 week residual) for the control of *S. invicta* at two parks and compared it to the District's currently used Advion® Fire Ant Bait (active ingredient: indoxacarb; up to 16 week residual). Both formulations share a similar mode of action. Metaflumizone is a semicarbazone class of chemicals and indoxacarb is an oxadiazine. Both products are voltage dependent sodium channel blockers, causing paralysis and death. Although the products share a similar mode of action, their mode of action or chemical structure is different enough to not promote cross-resistance (IRAC 2015).

MATERIALS AND METHODS

Pre-treatment evaluations were done at Monticello Park in La Quinta and Dominguez Park in Indio, California. A measuring wheel was used to divide the properties into ten ¼ acre evaluation plots, four at Monticello Park and six at Dominguez Park. There was a minimum of a 25-foot buffer between each plot (Figure 1).

Figure 1. Monticello Park (left) and Dominguez Park (right). Shown are the ten plots and the treatments: Siesta at 1 lb. per acre (plots 1 – 4: purple), Siesta at 1.5 lbs. per acre (plots 5 – 7; orange), and Advion at 1.5 lbs. per acre (plots 8 – 10; yellow).

Ten hot dog slices were placed in a circle, about 15 ft. from the center of the plot. After 60 minutes, an estimate of the number of ants on the hot dog slices was recorded. Ants were estimated in tens, such that a score of 1 was given for every 10 ants (Table 1).

Siesta was applied at 1 lb. per acre (low rate) to Monticello Park; Siesta and Advion both were applied at 1.5 lbs. per acre to appropriately half of Dominguez Park. Broadcast treatments were made using a Scotts® Hand-Held Spreader (Figure 2). To determine ant abundance, post-treatment evaluations were made at 1, 2, 4, 9, 12, and 16 weeks after treatment using the hot dog bait method used for the pre-treatment evaluation. The number of ants on the hot dog slices after 60 minutes at each sampling date was counted, and the air temperature, relative humidity, wind direction, and wind speed was measured with an ambient weather WM-4 comprehensive wind meter®.

Figure 2. Broadcast treatments were made at the parks with a hand-held spreader.

RESULTS

In Advion-treated plots, compared to abundance pre-treatment, ant abundance was reduced 100% seven days after treatment, and few ants subsequently were observed for four weeks. At 1.5 lbs. per acre, Siesta reduced ant activity by 96% by day 7, with similar control as that of Advion during the first four weeks. At 1 lb. per acre, Siesta reduced ant activity by only 39% seven days after treatment. At the low rate, the populations of ants quickly recovered. At the high rate, the portion of Dominguez Park treated with Siesta took three months before ant abundance returned to pretreatment levels, while it took those treated with Advion nearly four months (Figure 3).

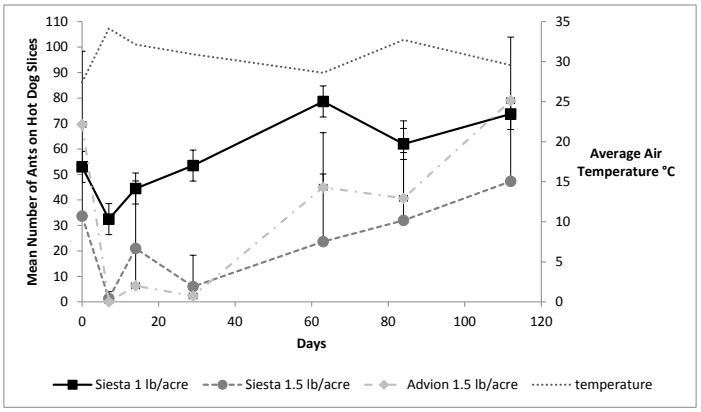

Figure 3. At 1.5 lbs. per acre, Advion and Siesta provided better control than Siesta at 1 lb. per acre. Bars represent standard deviations.

Percent relative humidity ranged from 19 to 51% and wind speeds from 0.1 to 3.5 mph. These environmental factors did not appear to impact the foraging activity of the ants. During the summer, counts were done earlier in the day because of the hot summer temperatures.

DISCUSSION

At 1.5 lbs. per acre, both products reduced ant activity immediately following treatments. One week after treatments were made, ants were reduced 100% with Advion and 96% with Siesta. The application rate on the chemical label (Syngenta 2014) states to retreat with Advion at 12 – 16 week intervals. After four weeks, the number of ants foraging on hot dog slices steadily increased until the end of the study. The application rate on the Siesta label (BASF Corporation 2013) states that product may provide control for up to eight weeks or longer, and to retreat with a broadcast treatment after 4 to 8 weeks if needed. The greatest reduction in ant activity was during the first four weeks. At the low rate, Siesta did not provide sustained control at this application. Although ants were reduced 39% following treatment, ant counts were still high and mounds were seen throughout the grass plots at Monticello Park.

At similar application rates, Siesta performed nearly as well as Advion by reducing ant populations quickly and provided the best control the first four weeks. The difference in cost to treat one acre with Siesta compared to Advion is $6.35 (Table 2). The price for Siesta was based on a purchase of 15 lbs., and a purchase of a larger amount is expected to be lower per pound (Sandra Torry, personal communication, April 14, 2015). For both Advion and Siesta, the pesticide labels state that no more than four applications can be applied to a site within a one year period (BASF Corporation 2013; GreenCast Syngenta 2014).

The District will incorporate Siesta into product rotation for managing *S. invicta* at parks. At the high rate, Siesta could provide control between Advion applications. The use of a second product would mean that additional applications could be made without exceeding the limit of four applications for one product in a year.

ACKNOWLEDGEMENTS

The authors thank Bobbye Dieckmann, Field Supervisor, for assisting with the site selection, and Gregorio Alvarado, Lead Vector Control Technician, and Paul Bustamante, Vector Control Technician I, for making the treatments.

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Within-population variability in bottle bioassay results for mosquito adulticide resistance testing: implications for replication and data interpretation

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Resistance to adult mosquito control products in populations of *Culex quinquefasciatus* and *Cx. tarsalis* mosquitoes is a growing problem in California, where these species are the primary vectors of West Nile virus. The time-response CDC Bottle Bioassay is a commonly used method to detect and track insecticide resistance. To aid in interpreting the data produced by this method, it is important to understand the capabilities and limitations of resistance bioassay methods to detect resistance and differentiate between populations. The current study found a high level of within-population variation in response to etofenprox and naled using the CDC Bottle Bioassay and insecticide-susceptible colony strains of mosquitoes (*Cx. tarsalis* BSF strain, and *Cx. quinquefasciatus* CQ1 strain). At any given time point in the bioassay, percent mortality could vary by as much as 60%. Preliminary results suggest that with the commonly used replication of four bottles of 25 mosquitoes each per population, time-mortality curves showing less than 60 minutes of difference in time to knockdown should be interpreted with caution, because less than a 60-minute difference may reflect within-population variation rather than a true difference in resistance between two test groups. Further information about variability in bottle bioassay response, as well as more specific guidelines for analysis and interpretation of bottle bioassay data, are needed to improve resistance monitoring efforts.

Monitoring resistance in *Culex pipiens* collected from a single site over time

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It is recommended that abatement districts test for insecticide resistance in mosquito populations at least 1-2 times a year to help plan their integrated pest management (IPM) programs. Traditional methods for testing resistance provide snapshots of a sample population in a particular time and place. But isolated testing might not reveal if patterns of resistance vary within a single site during peak population abundance. Our goal is to evaluate the variability of the resistance profile of a population from a single site over time. Our approach is to (1) test mixed-age samples of F1 generations from wild gravid *Culex pipiens* multiple times during the local peak population or season at a single collection site. (2) Evaluate the resistance profile of each sample using enzymatic assays namely: alpha-esterase, beta-esterase, acethylcholinesterase (iAChE), mixed-function oxidases (MFO), glutathione-S-transferase (GST), and protein along with the CDC bottle bioassay. The *Cx. pipiens* collections will also be evaluated with genetic screening for knockdown resistance (kdr). Abatement districts could use these results to decide if testing 1-2 times a year accurately reflects resistance profiles over a season or would help direct control decisions.

Optimization of rearing conditions for *Culex tarsalis* and *Cx. quinquefasciatus* production in mosquito and vector control agencies

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BACKGROUND

Vector control districts often require large numbers of experimental mosquitoes of known genetic background for laboratory studies such as insecticide resistance testing and field evaluations of adulticide efficacy. This need for mosquitoes is often met by maintaining colonies in the laboratory. Species maintained in colony are often those species which are the target of control activities and/or are vectors of important pathogens, or the species which are relevant to specific projects or research. Genetic strains reared may include those of known pesticide resistance or susceptible status, or those representing certain geographic regions of interest.

At one extreme, rearing of mosquitoes may be as simple as leaving a container of water outdoors and allowing mosquitoes to oviposit and develop. At the other extreme would be the rearing of carefully selected genetic strains of species of interest, with temperature, light cycles, water quality, larval and adult food supply, rearing densities, and other conditions carefully regulated.

At a vector control agency, controlled or consistent rearing practices may be desirable for a few reasons. Controlled rearing practices can predictably produce large quantities of mosquitoes, ensuring enough material to maintain a colony and to supply mosquitoes for projects. Controlled rearing practices can also ensure that mosquitoes produced are fit enough to be used for projects and to continue the colony. Ensuring that all mosquitoes produced are similarly fit maintains consistency in terms of size, robustness to handling, susceptibility to pesticides, longevity, and reproduction, and increases the replicability of experiments. Under ideal conditions mosquito size should be consistent and similar to the size of wild mosquitoes.

Development time can be a further indicator of health in mosquito colonies. Very short or very long development times can be indicative of poor rearing conditions (mosquitoes without enough food may develop slowly, but in some cases may simply grow to a smaller size and fewer actually emerge earlier.)

PLACER MVCD EXPERIMENTS

Experiment 1. Effects of tray cleaning. An experiment was conducted to evaluate the effect of cleaning methods for larval rearing trays, as well as initial water conditions, after it was hypothesized that the soap used for cleaning and fresh tap water were sources of mortality. Treatments included 1) washing with 1% Liquinox soap, 2) washing with 100% Liquinox soap, 3) one week old tap water, 4) tap water with Aquasafe dechlorinator added, and 5) fresh tap water. For treatments 1 and 2, week-old tap water was used. For treatments 3, 4, and 5, brand-new trays were thoroughly rinsed before use. Each treatment was replicated three times for each of two species, *Culex quinquefasciatus* (CQ1 colony strain) and *Cx. tarsalis* (BSF colony strain originating in Bakersfield, CA), and percent adult emergence was measured in each treatment. Water treatment did not have a significant effect on adult emergence of *Cx. quinquefasciatus* (ANOVA, $F_{(4,10)}$=1.3, P=0.33), but for *Cx. tarsalis* water treatment did have a significant effect (ANOVA, $F_{(4,10)}$=4.17, P=0.03), with adult emergence significantly lower in tubs with fresh tap water (Tukey's HSD post-hoc test, P<0.05, Figure 1). Additionally, the 100% Liquinox soap treatment showed a lot of variability in adult emergence for both species, which was consistent with the hypothesis that the rinsing process after washing was failing to remove all the soap, and that the soap could be causing mortality in some trays.

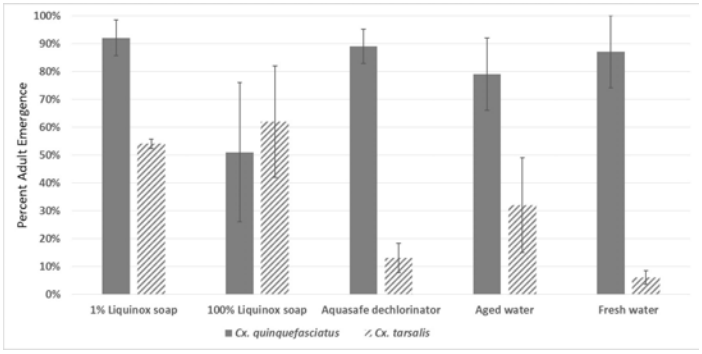

Figure 1. Adult mosquito emergence after rearing in different water treatments and tray cleaning conditions was not significantly different for *Cx. quinquefasciatus* (ANOVA, $F_{(4,10)}$=1.3, P=0.33), but was significantly different for *Cx. tarsalis* (ANOVA, $F_{(4,10)}$=4.17, P=0.03).

Experiment 2. Ions in fresh tap water. A second experiment examined the effects of chlorine and fluoride as possible explanations for the mortality observed in fresh tap water treatments. The three treatments included 1) water that had been reverse-osmosis filtered to remove chlorine, fluoride, and other ions; 2) water that had been dechlorinated with Aquasafe; and 3) fresh tap water. Tap water was from the city of Roseville, CA, where the water has fluoride added. The 2015 water quality reported from Roseville, CA Environmental Utilities showed an average of 0.76 ppm fluoride (range 0.05-1.2 ppm) and an average

of 0.65 ppm chlorine (range 0.03-1.17 ppm). Each treatment was replicated three times, and only *Cx. tarsalis* mosquitoes were used, as these were found in the first experiment to be more sensitive to water conditions than *Cx. quinquefasciatus*. Overall, the effect of water treatment was significant (ANOVA $F_{(2,23)}$=7.23, P=0.003), and pupation rate in the reverse osmosis filtered water was significantly higher than in the dechlorinated or fresh tap water treatments (Tukey's HSD post-hoc test, P<0.05, Figure 2). Although the reverse osmosis process removed more than just chlorine and fluoride, fluoride is one likely source of the observed mortality. Fluoride concentrations as low as 0.5mg F-/L may be harmful to invertebrates (Camargo 2003), but recommended drinking water concentrations to prevent tooth decay are 0.7-1.2 mg/L.

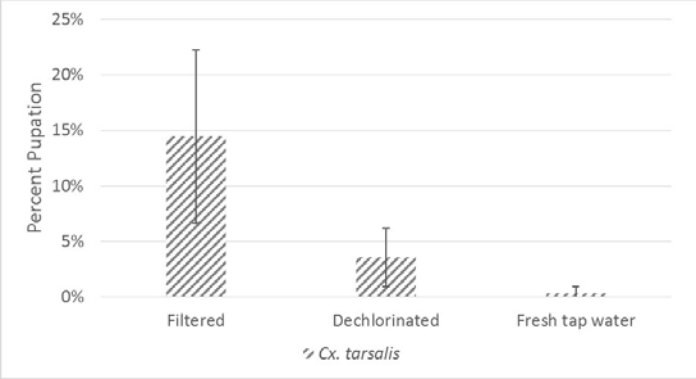

Figure 2. Overall effects of water treatment on number of *Cx. tarsalis* pupae produced was significantly different among water treatments (ANOVA $F_{(2,23)}$=7.23, P=0.003).

Experiment 3. Nutrition. Further tests were directed at identifying ideal rearing densities and food amounts for these two mosquito species. Larvae were reared in styrofoam drinking cups with 250ml of dechlorinated water in each cup and a variable number of larvae and amount of food per cup, with 3 replicates for each combination of larval and food density per cup. Larval densities were 20, 40, 80, or 160 larvae per cup, and food amounts were 2.5, 5, 10, and 20 mg per 250 ml cup. Different combinations of larval densities and food amounts led to food availability of 0.06, 0.13, .25, .5, 1, 2, or 4 mg food per larvae. Percent pupation and hind tibial length of adult females were measured for each treatment. For *Cx. quinquefasciatus*, pupation rates were best above 0.25 mg of food per larva and below densities of 160 larvae per cup (Figure 3). For *Cx. tarsalis*, pupation rates increased with increasing food amount and decreasing larval density up to the 4 mg food/larva and 20 larva/cup treatment (Figure 3), suggesting that to find ideal rearing conditions for *Cx. tarsalis*, the experiment would have to be repeated with even higher feeding rates and even lower larval densities. Data for hind tibial length for both species showed a similar pattern, with hind tibia generally longer in treatments with greater food availability and lower larval density, though with more variability than in results for pupation rates (Figure 4).

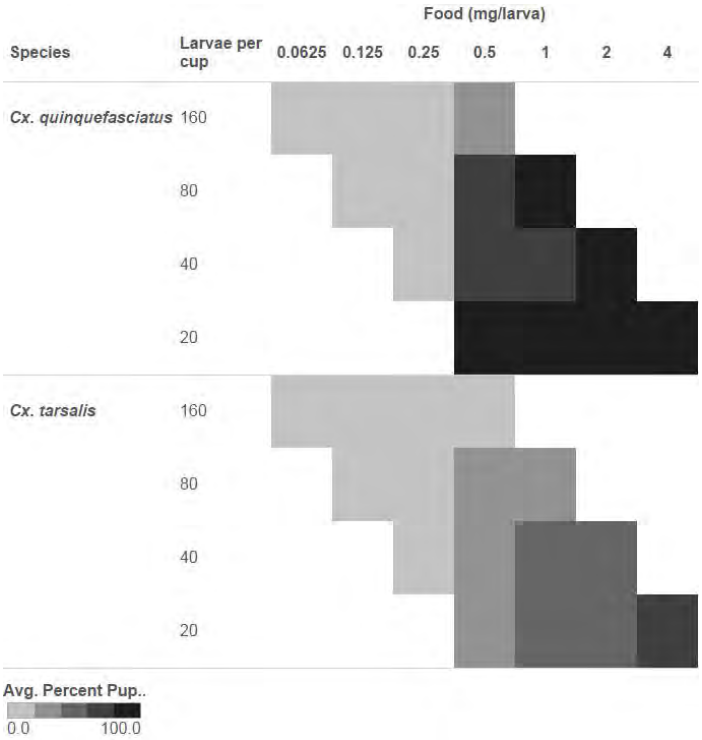

Figure 3. Average percent pupation of *Cx. quinquefasciatus* and *Cx. tarsalis* reared in 250ml of water in Styrofoam cups with varying numbers of larvae and amounts of food per larva. Shading indicates percent pupation.

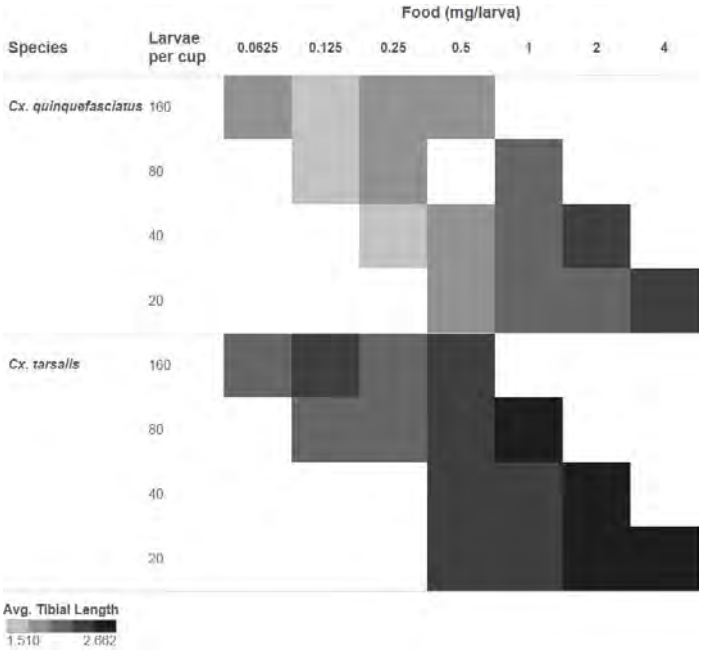

Figure 4. Average tibial length of adult female *Cx. quinquefasciatus* and *Cx. tarsalis* reared in 250ml of water in Styrofoam cups with varying numbers of larvae and supplied with varying amounts of food per larva. Shading indicates mean tibial length in mm, with darker shading indicating longer tibial length.

Experiment 4. Large rearing trays. When extrapolating the results of the cup study for larval density and food amounts to larger trays for colony rearing of larvae, the question arose of what was most important when considering larval density: volume or surface area of water. To answer this question, larvae were reared in three containers with the same volume of water, but with different surface areas. Each container received 8 L of water, 640 larvae, and equal amounts of food. Surface areas were 2,562 cm^2 (large), 1,134 cm^2 (medium), and 433 cm^2 (small). As for the previous experiments, treatments were replicated three times for each of two species, *Cx. quinquefasciatus* and *Cx. tarsalis*. No significant difference was observed in adult emergence in any of the treatments, for either *Cx. quinquefasciatus* (ANOVA $F_{(2,6)}$=0.10, P>0.05) or *Cx. tarsalis* (ANOVA $F_{(2,6)}$=0.58, P>0.05, Figure 5).

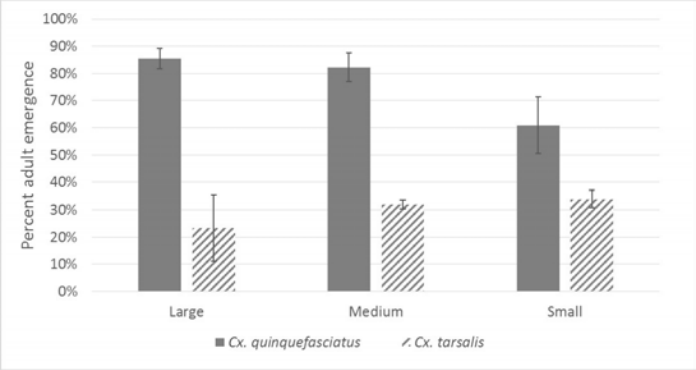

Figure 5. Average percentage of larvae emerging as adults from larval rearing containers (tray sizes) with the same water volume but different surface areas.

CONCLUSIONS

Our studies produced actionable conclusions that helped improve mosquito rearing in the Placer MVCD mosquito colonies. Cleaning procedures, water filtration, feeding and larval density, and smaller rearing trays were adopted to incorporate these study findings, and have led to an improved quantity and quality of mosquitoes.

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A new rapid diagnostic test to identify *Aedes* eggs

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INTRODUCTION

The invasive mosquitoes, *Aedes aegypti* and *Aedes albopictus*, have been detected in many new cities in California in recent years, with a sharp increase in new detections during 2015. This has presented a number of challenges for mosquito control districts. Surveillance is difficult because these mosquitoes fly relatively short distances and utilize cryptic larval habitats that can be hard to detect. Collecting mosquito eggs in oviposition traps is an effective surveillance strategy to complement other more labor-intensive methods, with the complication that many of the eggs collected may be those of *Aedes sierrensis*, a common, native treehole-breeding species of lower concern for human health than the invasive species. Here, we describe initial validation of a new protein-based assay that takes advantage of each species' unique protein profile to provide rapid, cost-effective identification of eggs without the need for hatching and rearing.

METHODS

Matrix-assisted Laser Desorption/Ionization - Time of Flight Mass Spectrometry (MALDI-TOF MS) combines an ionization process with mass spectrometry based on mass and charge of the resulting ions to obtain unique signatures for each sample tested. Initially, we developed a library of known signatures for each species from laboratory colonies of *Ae. aegypti* (Los Angeles and Puerto Rico), *Ae. albopictus* (Los Angeles), *Aedes mediovittatus* (Puerto Rico), and *Aedes sierrensis* (Sonoma County). Validation was performed by blinded testing of egg samples of each species from insectary colonies.

RESULTS AND DISCUSSION

Results from preliminary laboratory testing have shown that the MALDI-TOF MS assay can reliably distinguish between unknown laboratory samples of the species listed above. Nearly all unknown samples have been accurately identified, including a mixed sample of *Ae. aegypti* and *Ae. albopictus* from Los Angeles. Single-species samples of *Aedes aegypti* or *Aedes albopictus*, both of which are in the subgenus *Stegomyia*, occasionally match both species, which is consistent with their close relationship within the subgenus *Stegomyia*. In all samples, invasive *Aedes* species were readily distinguished from California's native and more distantly related *Aedes sierrensis*. Testing of unidentified field samples submitted by mosquito control agencies will continue during the 2016 surveillance season with the goal of establishing MALDI-TOF MS as a useful tool for routine surveillance for invasive *Aedes* in California.

ACKNOWLEDGMENTS

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Larval emergence from floating cages in log decks

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INTRODUCTION

In the Placer Mosquito & Vector Control District, there is a sawmill facility that produces a large number of *Culex pipiens* mosquitoes. The District currently introduces (S)-methoprene larvicide (Alstosid® Liquid Concentrate SR20, Central Life Sciences) into the sprinkler system that distributes water to the holding areas where the stored logs are stacked into "log decks". To test the efficacy of the larvicide treatments, floating emergence containers (ECs) were built and placed in tubs that collect water from the irrigation system. If the mosquito larvae emerge as adults in these cages, they fly up into the holding area, where a rubber seal prevents them from getting wet from the sprinkler system.

Methods. The ECs were created from "mosquito breeders" (item 1425, Bioquip, Rancho Dominguez, CA) by modifying them in several ways. Screen covers were added to cut-outs on the bottom section to allow water, control product, and food particles to enter. Early prototypes used 1mm size mesh, but the smaller larvae were found to escape so the mesh size was changed to 0.2 mm. Additionally, early prototypes had water entering the upper holding chamber and preventing accurate counts of emerged adults. A rubber seal was cut (general purpose 40A rubber, 2mm thick, Grainger, Lake Forest, IL) and added to prevent water from the sprinkler system from entering the bottom of the upper chamber. Similarly, top containers without a screened ventilation area (specimen jar, item 715730, Carolina Biologicals, Burlington, NC) were added to allow the containers to be sprayed but keep the emerged adults dry so that they did not degrade in a wet environment.

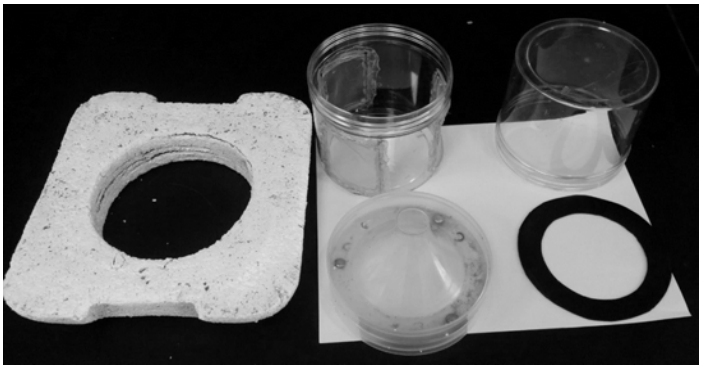

Figure 1: Components of larval emergence container, including Styrofoam float, rubber gasket, and modified top without ventilation. The larval emergence container is designed to measure emergence of adults from larvae developing in a wet, humid environment treated with methoprene larvicide.

RESULTS

During the first tests of the ECs, adults did not emerge from the control groups, even though there was no larvicide product in those locations. Test cages received food material through the organic material in the recycled sprinkler water, but control cages in early tests did not receive any food from treatment water or any supplemental food, beyond what was present in initial gathering of log deck water. A second set of experiments was designed to test the hypothesis that there was not enough food, causing the larvae to starve and not emerge as adults. The second set of experiments examined the best way to supplement food in the cages without under or over feeding to ensure survival of test larvae in the field.

Through this series of experiments adding 2.5 grams of solid alfalfa pellets when the cages were first placed in the field proved to be the best option. The first test used nine tubs (30 x 35 x 14cm) in three treatments with three replications each. All tubs were filled ¾ full with reverse-osmosis filtered water and placed outside in a shaded area. In each of the tubs 2.5g, 5g, or 10g of food was placed along with an EC containing 60 3rd to 4th instar larvae. The tubs were left for 2 weeks and then collected.

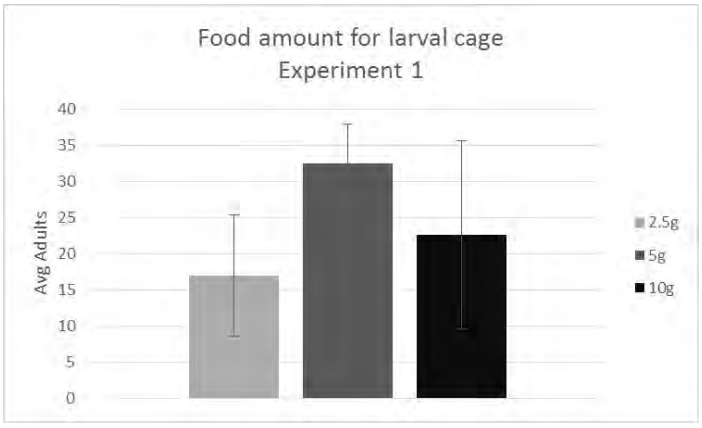

Figure 2: Results of the first trial investigating the amount of food to be added to larval containers to support 60 3rd-4th instar larvae through pupation. This trial did not provide enough information and was later repeated in Figure 3 as a second trial.

This first food experiment had too many unplanned variables such as weather, ants eating some adults, and extra organic matter falling into some tubs but not others. The 10g treatment had far too much food that produced a thick film across the surface of the water. Not enough data could be determined from this test so it was repeated with some of the variables removed.

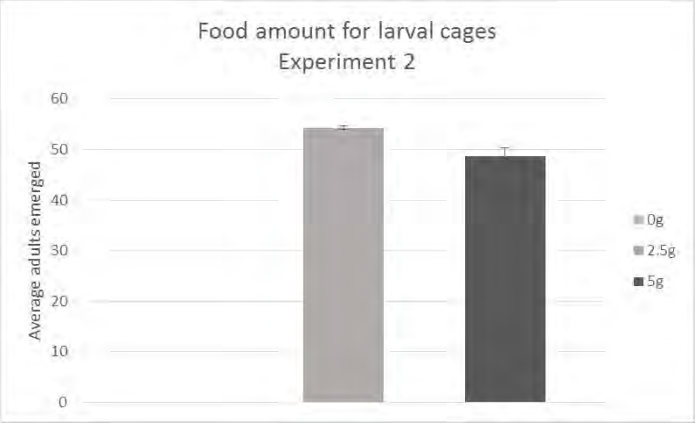

Figure 3: Results of a second trial investigating the amount of food to be added to larval containers to support 60 3rd-4th instar larvae through pupation.

The second food experiment was carried out in the larval rearing room, which is temperature and light controlled and does not have ants or foreign organic matter. This test was set up same, but the treatments were 0g, 2.5g, and 5g of food.

This test produced more consistent data within each treatment. The 0g food treatment produced no adults, showing that food is definitely needed. The 5g treatment did produce a thin film across the surface of the water, but also produced a number of adults. The 2.5g treatment produced the most adults with minimal variation between replicates.

With the information produced by these experiments, the larval cages will be used to measure larvicide efficacy in field trials at the log deck sawmill facility beginning in summer 2016.

Detection, identification, and characterization of mechanisms of insecticide resistance in *Culex tarsalis*

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Insecticides have played a significant role in reducing the transmission of a myriad of vector-borne viruses such as yellow fever, dengue, and West Nile (WNV). However, the success of these insecticides is being threatened worldwide by the increasing number of insect species, more specifically mosquitoes, showing resistance to one or more insecticides. WNV, among other arboviruses, is transmitted by Culex mosquitoes and is considered endemic in San Joaquin county Two major mechanisms of resistance are found in mosquitoes: target site insensitivity and enhanced enzymatic metabolism.

Previous studies on resistance in *Culex tarsalis*, a major vector of WNV, have identified enhanced enzymatic metabolism as a mechanism of resistance. However, to our knowledge, no studies have investigated target site insensitivity in *Culex tarsalis*, and therefore, the molecular tools needed to detect this genetic mutation do not exist for this species. We investigated the presence of resistance in field-caught mosquitoes collected from Sutter-Yuba and Sacramento-Yolo counties and are further interested in mechanisms of resistance found in these mosquitoes.

Culex tarsalis adult females were collected in Sutter-Yuba and Sacramento-Yolo counties and were tested to see if resistance was present. Twenty to twenty-five females were placed into bottles coated with either Naled (an organophosphate) or Sumithrin (a pyrethroid) along with a laboratory-reared colony strain with known susceptibility to insecticides. We found that there was resistance to Sumithrin in field populations. Specimens were separated based on "susceptible," "some resistance," and "resistant" and stored for further enzymatic and genetic testing.

After individually extracting the DNA from these mosquitoes, we will amplify the sodium channel gene and sequence them to determine if there is a mutation at codon 1014. From here, we will use these sequences to develop a primer probe to detect the kdr and ace-1 mutations. Further, we will test each mosquito for elevated levels of enzymatic activity, such as esterases, hydrolases, and mixed-function oxidases. This information will be useful for vector control districts to better monitor resistance in these populations.

Use of DropVision to optimize ULV applications in Coachella Valley

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ABSTRACT: The Coachella Valley Mosquito and Vector Control District (CVMVCD) responds to potential arbovirus transmission risks using a variety of methods in an Integrated Vector Management (IMV) program. One of the methods used to reduce virus transmission is to control adult mosquito populations through ultra-low volume (ULV) spray applications. ULV applications are conducted either by truck mounted or aerial equipment depending on the environment. The CVMVCD has been re-evaluating ULV applications over the past few years to determine if adult control methods can be improved. In order to increase the speed and accuracy of the evaluations the DropVision system was purchased and used to evaluate ULV applications. Using DropVision the Laboratory and Operations staff were able to determine that the current aerial ULV application device was not optimal for the needs of the District. When a new ULV sprayer was purchased, the use of DropVision allowed CVMVCD to quickly troubleshoot the installation to ensure the spray output was optimal for adult mosquito control.

The 2016 Saint Louis encephalitis virus outbreak in Maricopa County, Arizona

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In May of 2015, the CDC confirmed one Maricopa County human case of Saint Louis encephalitis virus (SLEV). There were several suspect cases at this time, but CDC confirmation was delayed until July. Beginning July 1, the Vector Control laboratory began Real-Time PCR mosquito testing for SLEV and identified eight positive pools from the first sample. Currently, 86 positive SLEV pools were detected, with 44 pools testing positive for both SLEV and West Nile virus. Seventeen human cases that were neuroinvasive. Two human cases that were non-neuroinvasive with one death confirmed. In response, CDC sent a team of experts consisting of epidemiologists, geneticists, entomologists and ornithologists to study this outbreak and work closely with our county and state health departments. This presentation will summarize this outbreak, both on the human and vector side of the equation, and review the future implications that will be incorporated within the medical community and vector control programs.

Establishing an *Aedes aegypti* response threshold in Maricopa County, Arizona

James Will & Kirk Smith

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The Maricopa County Environmental Services Department Vector Control Division mosquito surveillance program primarily targets vectors of West Nile virus (WNV) and Saint Louis encephalitis virus (SLEV). We set 756 CDC CO_2 traps weekly of which 12% collect *Aedes aegypti*. Chikungunya and Dengue fever are 'knocking' at our southern border. To prepare for future vector control activities, we need to establish an adult surveillance system for this species. One method is to establish a local threshold for adult *Ae. aegypti* based on trap counts. In this study, we have compared data from the CDC CO_2 traps with those such as the BG-Sentinel, Fay Prince, and the Omni-Directional Fay Prince traps. These traps are bulky, expensive and prone to theft and vandalism. We have sufficient data to correlate results from these traps with our CO_2 traps. The latter are affordable and easily replaced if need be. Results from our field trials are presented.

Simulation of overwintering potential for *Aedes albopictus* in California

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ABSTRACT: The mosquito, *Aedes albopictus*, is among the world's most invasive species. Its spread has been facilitated by rapid global transport of cargo and potentially by climate warming, and it is now established on every continent except Antarctica. This species represents a "triple threat" to human health, being a day-biting pest, a competent vector of globally important dengue, chikungunya, and Zika viruses, and a potential bridge vector of several zoonotic arboviruses. As a result of its importance, the biology of *Ae. albopictus* is also well-studied, but the fine-scale processes by which it becomes established in a given location are poorly understood. Fine-scale spatial models for mosquito dynamics and movement offer a way forward, combining our understanding of *Ae. albopictus* biology with surveillance paradigms and detailed data on the real landscapes where invasions occur. Here, we use hierarchical modeling with remote sensing and surveillance data from Los Angeles to account for heterogeneities in household-level receptivity, then we model the stochastic dynamics of *Ae. albopictus* on this landscape using the suitability surface and a temperature-dependent, dynamical model for reproduction and spread. Using the model, we consider the potential for overwintering success across a range of temperature scenarios representing several cities in California.

Mapping climatic suitability for invasive *Aedes aegypti* and *Aedes albopictus* in the United States: a process-based modeling approach

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ABSTRACT: Rapid changes in the distributions of the mosquitoes, *Aedes aegypti* and *Aedes albopictus,* in the continental United States alter the potential for local transmission of dengue, chikungunya, and Zika viruses. All three viruses have caused major disease outbreaks in the Americas recently, with infected travelers now returning regularly to the U.S. Recent outbreaks of Zika, dengue, and chikungunya have proven that these viruses are capable of invading and being transmitted within the habitat ranges of *Ae. aegypti* and *Ae. albopictus*. The expanding range of these mosquitoes and discovery of new populations within the U.S. raises questions about whether recent spread has been enabled by climate change or other anthropogenic influences. In this analysis, we used daily average temperatures for the United States to model *Ae. aegypti* and *Ae. albopictus* population growth rates using a stage-structured matrix population model to understand current habitat suitability of both species across the U.S. Understanding the range of these mosquitoes should be considered a high priority for public health officials and vector control agencies.

INTRODUCTION

Dengue, chikungunya, and Zika viruses are transmitted from *Aedes aegypti* and *Aedes albopictus* mosquitoes to humans, and are responsible for a massive disease burden globally. The distributional ranges of both *Ae. aegypti* and *Ae. albopictus* are expanding, and with them so are the ranges of the pathogens these mosquitoes transmit (Weaver 2014); e.g., currently over half of the world's population is estimated to be at risk of dengue virus infection (Brady et al. 2012; Bhatt et al. 2013). Understanding the climate-driven spatial and temporal dynamics that limit the ranges of these mosquitoes is critical for establishing effective vector control and minimizing virus transmission in the United States. In this paper, we model and then map *Ae. aegypti* and *Ae. albopictus* daily reproductive rates (daily population growth rates), a combination of individual life stage development and survival rates as functions of temperature, using daily U.S. temperatures over a single year.

METHODS

Climate data. Temperature data sets were obtained from the PRISM Climate Group (http://prism.oregonstate.edu) which has generated gridded estimates of climatic parameters using regression of climate data against elevation, using data obtained from weather stations. In this study, model output was generated from the 2014 daily mean temperatures at 2.5 arc minute grid cell resolution.

Mosquito biological parameters. To model reproductive rates for *Ae. aegypti* and *Ae. albopictus* throughout 2014, we used biological parameters estimated from laboratory experiments using a Thai population of *Ae. aegypti*, whereas parameters for *Ae. albopictus* were obtained from an experimental study using a colony from La Reunion Island (Carrington et al. 2013; Delatte et al. 2009). In order to focus on daily temperature-dependent development, we did not include the diapause mechanism characteristic of temperate *Ae. albopictus*, which we expect to have a greater influence on seasonal population growth rates and persistence than daily rates.

MODELING

To translate life-history parameters into expected reproductive rates and potential species spread across heterogeneous landscapes and climates, we built a deterministic, stage-structured matrix model for both *Ae. aegypti* and *Ae. albopictus* population dynamics (Caswell 2001). This model allowed us to identify regions where we would expect to see the highest potential reproductive rate, and how these rates varied seasonally in the absence of density dependence, predation or other competition factors. This model used median egg, larval, pupal, and adult development and survival rates, as well as female egg-laying rates, as functions of temperature fitted using the Sharpe and DeMichele enzyme kinetics model (Sharpe and DeMichele 1977; Focks et al. 1995). Egg stage duration often depends on stochastic environmental events (in addition to diapause, which we avoided), and so we assumed a period of 10 days from egg laying to egg hatch. Daily larval and pupal survival were fitted to laboratory estimates as functions of temperature, and we assumed daily survival rates of 90% for eggs and adults (Delatte et al. 2009; Carrington et al. 2013).

Mapping current reproductive rates. Reproductive rates produced by the stage-structured matrix model were analyzed in R version 3.3.1 (R Development Core Team) and mapped in QGIS version 2.10.1 (http://qgis.org). The daily temperature values for each 2.5 arc minute pixel from the 2014 mean temperature data

set were fed into the stage-structured matrix model for both *Ae. aegypti* and *Ae. albopictus*.

RESULTS AND DISCUSSION

During the days of 2014 when reproductive rates were above zero, *Ae. aegypti* reached a maximum average of 13% daily population growth, and *Ae. albopictus* reached a maximum average of 9% daily population growth (Figure 1, Figure 2). In the experiments used to parameterize the model, *Ae. aegypti* developed faster and survived at more extreme temperatures. Our reproductive rate model outputs exemplified these trends; modeled reproductive rates for *Ae. aegypti* were faster for all seasonal temperature ranges than those of *Ae. albopictus*. *Ae. aegypti* are known to be able to survive at more extreme hot and cold temperatures relative to *Ae. albopictus* (Brady et al. 2013), so it is expected that in warmer areas, especially areas that reach extremely hot daily temperatures, *Ae. aegypti* populations would have on average faster development rates. Our model indicates that *Ae. aegypti* would develop at a faster rate in northern latitudes; however, we know that *Ae. albopictus* have populations established at more northern latitudes than *Ae. aegypti*. We expect that the *Ae. albopictus* diapause mechanism plays an important role in allowing populations to persist at northern, colder environments; however, Brady et al. (2013) found that *Ae. albopictus* mosquitoes have a slower mortality rate that *Ae. aegypti* in the field, indicating that environmental factors not accounted for in laboratory studies are also contributing to population growth and distribution differences between the two species in nature. The maximum length of the reproductive season (the total number of days that had positive reproductive rates) for *Ae. aegypti* was 313 days and the longest reproductive seasons were characteristic of southern California, western Arizona, southern Texas, and southern Florida. The maximum length of the reproductive season for *Ae. albopictus* was 302 days. These long seasons were found in the same southern states.

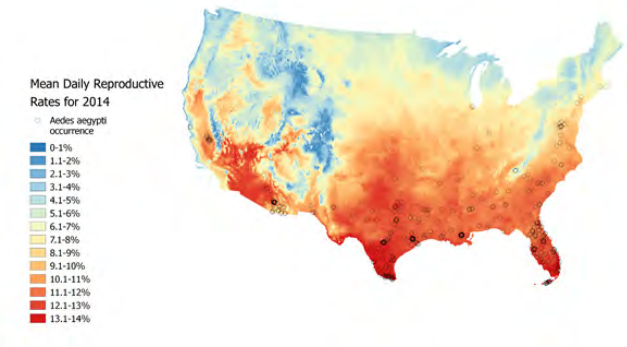

Figure 1. National map of the 2014 estimated daily reproductive rate for *Ae. aegypti* during periods when positive population growth would be expected. Black circles indicate known occurrences of *Ae. aegypti* through 2014.

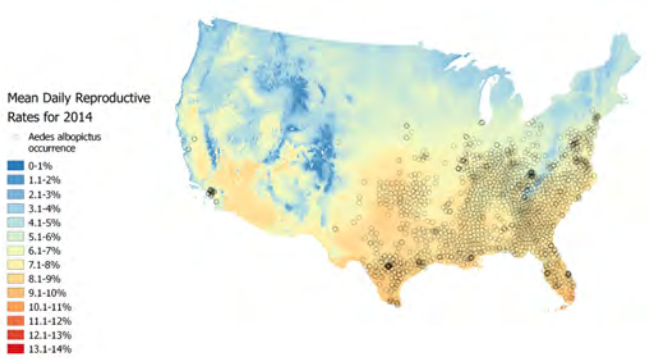

Figure 2. National map of the 2014 estimated daily reproductive rate for *Ae. albopictus* during periods when positive population growth would be expected. Black circles indicate known occurrences of *Ae. albopictus* through 2014.

The modeled reproductive rates were highest for both species in the southern U.S., and southern California, Arizona, Texas and Florida had the highest average rates for both species. For most of the U.S., the winter months were unsuitable due to consistently low temperatures; however, in the southernmost regions it was most likely warm enough to sustain low-to-moderate populations throughout the winter (Monaghan et al. 2016). This agrees with our hypothesis that the southern part of the U.S. would be the most suitable due to those regions' characteristically warm climates. Our findings that *Ae. aegypti* and *Ae. albopictus* would have the fastest development in the southern U.S. agree with previous studies that have examined seasonal abundance and probability of occurrence in the U.S. (Monaghan et al. 2016; Kraemer et al. 2015). Although these studies have predicted greater abundances and probabilities of *Ae. albopictus* populations in the northeastern U.S. relative to *Ae. aegypti*, our study found that development rates are actually slower in these northeastern regions for *Ae. albopictus* than they are for *Ae. aegypti*. Persistence of *Ae. albopictus* populations in these areas and the absence of *Ae. aegypti* populations is attributed to the diapause mechanism *Ae. albopictus* eggs have that *Ae. aegypti* do not, allowing *Ae. albopictus* populations to survive through harsh winters, in addition to other unaccounted for environmental influences affecting mortality rates of the two species (Takumi et al. 2009, Brady et al. 2013).

In conclusion, we have found that much of the southern United States is suitable for both *Ae. aegypti* and *Ae. albopictus* mosquito development. Many regions in the US, where continuous, year-round reproduction of either species is not expected, had periods of time where development of both species could occur, given an introduction event. Outbreaks of yellow fever transmitted by *Ae. aegypti* occurred in the U.S. until the early 1900s, and have occurred as far north as Boston (1691) and New York City (1699) (CDC 2016), which highlights that outbreaks can occur at latitudes where we would not expect *Ae. aegypti* and *Ae. albopictus* populations to survive through the winter if introductions of mosquitoes and arboviruses occur with enough regularity during the warm part of the year. Understanding the range and seasonal dynamics of these mosquitoes should be considered a high

priority for public health officials and vector control agencies for assessing the risks associated with dengue, chikungunya and Zika virus infected travelers returning to the U.S.

ACKNOWLEDGMENTS

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Dispersal of male *Aedes aegypti* in residential Clovis, California

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In October 2015 a mark-release recapture trial of male *Aedes aegypti* was undertaken in a residential neighborhood of Clovis, California. Male mosquitoes originating from a colony from Clovis, were reared and marked with fluorescent dust at the University of Kentucky and shipped to California in tubes. A total of 8,543 dusted males were released from within a park in the center of the study area. For a period of two weeks after release, 16- 24 BG Sentinel traps were used to recapture marked males, by placement at ground level in the front yards of homes at various distances from the release point. Five percent (435) of marked males were recaptured, the majority of which (167) were collected from houses adjacent to the park which were about 50 m from the release point. Marked males were collected at 200m and 250m from the release site at 24 hours and 48 hours, respectively. The maximum distance a marked male was collected from release point was 300m at 10 days after release. Based on personal observations, we contend that *Ae. aegypti* following people walking and jogging in the neighborhood could likely contribute significantly to dispersal.

Efficacy of ULV applications to control Clovis *Aedes aegypti*

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Efficiency of ground-applied pyrethroid and pyrethrum + PBO formulations at killing native Clovis *Aedes aegypti* (Clovis) in open and residential environments were evaluated. Male and female Clovis and susceptible Rockefeller strain *Ae. aegypti* (Rock) were held in sentinel cages placed at specific distances from the spray source. Applications of pyrethrum + PBO, etofenprox and deltamethrin in open settings resulted in 100% mortality in the susceptible Rock strain at all distances from the spray source (up to 91.4m). The mortality rate in Clovis strain was significantly lower than the Rock strain for both etofenprox (Wilcoxon rank sum test P-value = 0.00021) and pyrethrum + PBO (P-value=0.00073) applications. In the open field ULV deltamethrin application, 100% mortality was achieved against both *Ae. aegypti* strains. In the ULV trial conducted in the residential area, there was no significant decline in mortality over distance (linear model P>0.05) from spray source in sentinel caged mosquitoes (Wilcoxon rank-sum test P-value > 0.05). Clovis strain had significantly lower mortality rate than the Rock strain with mortality of 55.6% along the full swath length.at (Wilcoxon rank-sum test P-value = 6.26×10^{-5}). We recommend that formulations with deltamethrin as the active ingredient can be use during emergency situations to control *Ae. aegypti* in residential areas.

The Biting Nuisance of *Aedes albopictus* in the San Gabriel Valley, Los Angeles County, California - How Many Bites are too Much?

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BACKGROUND

The San Gabriel Valley Mosquito and Vector Control District detected *Aedes albopictus* on September 2, 2011 in the city of El Monte (Fujioka 2012). Despite a tremendous effort to eradicate the initial populations of *Ae. albopictus* in 2011 and 2012, the area infested by this mosquito kept expanding. The expansion of infested area where *Ae. albopictus* was detected was initially geometric but over time became exponential (Wekesa *et al*., 2014). In 2012 the area infested by *Ae. albopictus* in the San Gabriel Valley was 2,780 acres compared to 1,787 acres in 2011. In 2013, a total of 5,266 acres were infested followed by 29,059 acres in 2014 (Wekesa *et al.,* 2014). The infestation in 2015 covered a total 65,483 acres in the San Gabriel Valley. This infestation was in the City of El Monte in 2011, and in 2012 and 2013 it spread to parts of Arcadia. In 2014 *Ae. albopictus* had extended to twelve cities and by 2015 a total of seventeen cities were infested. The cities infested were Alhambra, Arcadia, Azusa, Baldwin Park, Bradbury, Covina, Duarte, El Monte, Glendora, Irwindale, La Puente, Monrovia, Monterey Park, Rosemead, San Gabriel, Temple City, and West Covina.

In 2013, a total of 7,891 inspections, including door to door, service requests, and re-inspections, were conducted, with 423 positive for *Ae. albopictus*, of which 236 were new sites (properties). In 2014, the "Albo Crew" conducted 17,770 inspections, with 1,073 inspections positive for *Ae. albopictus,* of which 533 were from new properties. A similar number of inspections were conducted in 2015 where 17,100 inspections were completed, 1,527 were positive, and 927 were from new properties. Despite having almost the same number of inspections in 2015 as in 2014, the number of properties positive for *Ae. albopictus* in 2015 was twice that of 2014. Despite our efforts, the number of inspections did not increase significantly due to the increased requests for service caused by the *Ae. albopictus* biting nuisance. The increased requests for service required pesticide treatments of those properties with high abundance of *Ae. albopictus* (Urena *et al*., 2016).

SURVEILLANCE

To determine if biting nuisance generated by *Ae. albopictus* was similar throughout the infested area, we compared service requests received from cities with longest and shortest presence of this mosquito. Previous reports showed that the largest *Ae. albopictus* population in the San Gabriel Valley occurs between the months of August through October (Brisco *et al*., 2015, Wekesa *et al.,* 2014). This may also be the time when the nuisance factor due to *Ae. albopictus* bites are highest, generating numerous service requests from residents.

The Cities of El Monte and Arcadia have had more than two years of continuous presence of *Ae. albopictus* (Fujioka *et al.,* 2012) and were considered "old" cities. All other cities with *Ae. albopictus* including Alhambra, Azusa, Bradbury, Covina, Duarte, Glendora, Irwindale, La Puente, Monterey Park, Rosemead, San Gabriel, Temple City, and West Covina were considered "new" cities, because *Ae. albopictus* has been present for two years or less. Based on these criteria, a total of 70 service requests were received in the months of "August through October" in 2014 from the old cities compared to only 24 from the new cities. In contrast, for the same months of 2015, there were 166 service request calls from the "old cities" compared to 441 from the "new cities" (Figure 1).

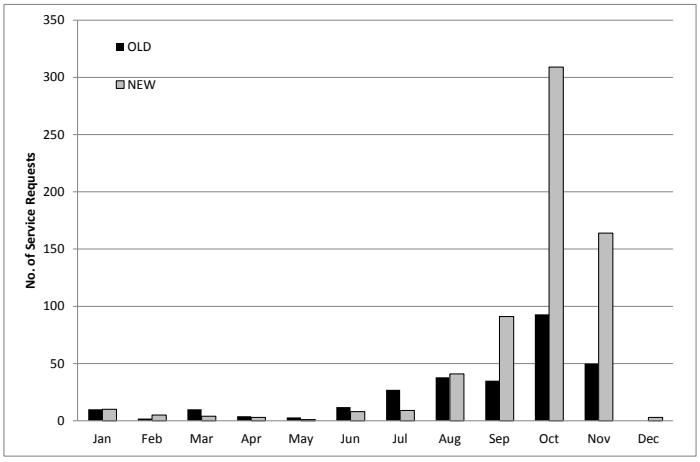


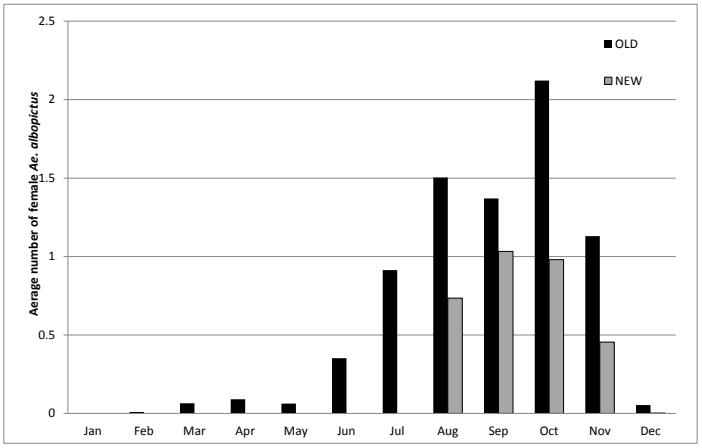
Figure 1. Number of service requests in cities with older infestations (>2 years) compared to cities with newer infestations (< 2 years)

The traps used to collect mosquitoes included BioGents Sentinel (BG Sentinel), CDC- Autocidal Gravid Ovitraps (AGO), and oviposition traps (Brisco *et al*., 2015). The average numbers

of female *Ae. albopictus* collected in the AGO and BG traps were plotted monthly in old and new cities. Additional data was generated, through a model developed by Barker *et al.* (2013) and our *Ae. albopictus* egg oviposition analysis, where a female *Ae. albopictus* was predicted to lay an average of seventeen eggs per oviposition trap (ovitrap) (Ruedas *et al.,* 2017 manuscript) per week. The adult females collected by AGO and BG traps were combined with the estimated females from the egg numbers in the ovitraps.

DISCUSSION

Service requests, in this scenario, are presumed to have been initiated by individuals bothered by bites from *Ae. albopictus*. To determine if service requests regarding *Ae. albopictus* were due to bites of adult mosquitoes in the surrounding neighborhood, counts of mosquitoes from a variety of traps in new and old cities were compared. Despite receiving more service request calls from newer cities compared to older cities in 2015, we found there were greater numbers of adult mosquitoes in our traps (see above and Ruedas *et al.,* 2017 manuscript) in older cities than newer ones (see Figure 2). It seems individuals recently exposed to bites of *Ae. albopictus* were quicker to be bothered and called



in with a service request more frequently than individuals already accustomed to the bites in areas with the longest infestation.

Figure 2. Average number of female *Ae. albopictus* collected in cities with older infestation (N = 3,408) compared to cities with newer (N = 2,075) detected infestations (where N is cumulative collection throughout the study period by all trap types).

An additional explanation for the spike in service requests may have been unrelated to nuisance bites by *Ae. albopictus* or their population levels. The increase may have been associated with access to critical information about *Ae. albopictus*. On October 11, and October 24, 2015, the San Gabriel Valley Tribune and the Los Angeles Times published news articles, respectively. During weeks following each of these publications, there was a significant increase in the number of calls requesting for vector service. In addition, areas that were recently inspected by "Albo Crew" and received informational pamphlets showed the highest increase in service requests. Therefore, the increase of service requests from new cities, although associated with lower adult mosquito counts, also may have been influenced by increased awareness among residents. This awareness was primarily through word of mouth, door-to-door inspections, pamphlet distribution, and articles in the local newspapers. The increased service requests may not be significantly associated with adult mosquito counts, but associated more with the perception of the individuals living the experience and their access to information explaining their circumstances.

ACKNOWLEDGEMENTS

We thank the District's staff, especially the front desk who answered hundreds of service request calls from the public. "The Albo Crew" for 2014 and 2015 were instrumental in responding to service requests, in addition to doing all door-to-door inspections, mosquito trapping, pesticide treatment, and property sanitation. The 2014 "Albo Crew" included Leslie Conner, Bernadine Dizon, Alex Freire, Marco Gaytan, Robert Krutosik, Hendricks Peña, Sonia Oran, Leticia Rios, Javier Romo, and Ignacio Urena. The 2015 Crew included Mark Castillo, Alex Freire, Martha Gonzalez, Leticia Rios, Javier Romo, and Eddie Torres. We also appreciate the support from residents of those cities in the District experiencing the biting pressure associated with *Aedes albopictus*.

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ADAM, Auto-Dissemination Augmented with Males: A novel strategy to control *Aedes aegypti* mosquitoes

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The recent invasion of *Aedes aegypti* L. into several, dispersed areas of California, beginning in 2013, has created significant public health issues for mosquito and vector control agencies. A primary vector for dengue and chikungunya viruses, this mosquito is also closely associated with human habitation for oviposition sites and exhibits a preference for humans as hosts. In invaded neighborhoods, *Ae. aegypti* is a huge biting nuisance and source of resident complaints, yet it is difficult to control utilizing conventional treatment methods. Thus innovative approaches are called for in combating this important vector.

During 2015, the University of California, Davis and the Consolidated Mosquito Abatement District collaborated with MosquitoMate Inc. and the University of Kentucky to evaluate their novel control strategy, auto-dissemination augmented by males (ADAM), against *Ae. aegypti* in a small neighborhood of Clovis, Fresno County, California. ADAM incorporates the mass rearing and release of large numbers of male mosquitoes that have been dusted with the insect developmental inhibitor, pyriproxyfen (ppf). After release, dusted males mate with local females and transfer ppf to the females. PPF contaminated females and released males visit and transfer the insecticide to oviposition and larval development sites where the material disrupts immature development.

We will discuss the elements and issues involved in development of the study; including the selection of study sites, gaining access and acceptance from homeowners, development of procedures and protocols specific to the evaluation of ADAM against *Ae. aegypti* in an arid habitat, as well as dealing with media and providing and disseminating public education materials.

The distribution of *Aedes aegypti* and *albopictus* in Hawaii

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ABSTRACT: The Yellow Fever Mosquito (*Aedes aegypti*) and the Asian Tiger Mosquito (*Aedes albopictus*) are major vectors of arboviruses. They were introduced to the Hawaiian Islands in the 1890s. After a concerted effort to eradicate the human associated *Aedes aegypti* from the islands ending in the 1960s, most of the main Hawaiian Islands remained free of this highly efficient vector. The exception to this pattern was the island of Hawaii, where along the leeward coasts this species still persists. During the fall of 2015, Dengue resurged on the island of Hawaii. To predict areas that are likely to harbor one or both of these *Aedes* species, we synthesized distributional maps from existing vector-control surveys from 2002 and our own subsequent observations. We focus on the comparative distribution of both species on the island of Hawaii. Our goal is to assess the most likely regions suitable for *Aedes aegypti* as well as identify areas that are hot-spots for *Aedes albopictus* to aid vector control efforts. Finally, we discuss the distribution of both of these species in Hawaii in light of the current epidemic of Dengue virus on the island of Hawaii.

INTRODUCTION

Invasive mosquitoes impact wildlife and human health worldwide. Two species in the genus *Aedes* (subgenus *Stegomyia*) in particular, the Yellow Fever Mosquito (*Aedes aegypti*) and the Asian Tiger Mosquito (*Ae. albopictus*), are problematic where they occur, with the former species being the primary vector of Dengue and other arthropod borne viruses such as Chikungunya and now the Zika, while the latter species has spread globally throughout the tropics and subtropics and represents a looming threat where it likely transmits all three of these arboviruses (Lambrechts, Scott, and Gubler, D.J. 2010, Higgs and Vanlandingham 2015, Chouin-Carneiro *et al.* 2016). In the Hawaiian Islands these two invasive species have been interacting longer than most places where they co-occur, at least since the 1890s (Winchester and Kapan 2013). Furthermore as the focus of the Pan American Health Organization's eradication campaign, *Ae. aegypti* was successfully eliminated from four of five main Hawaiian Islands by the end of the 1960s and now only persists in a relict population on the Island of Hawaii (Winchester 2011, Winchester and Kapan 2013). During the fall of 2015 and into Spring 2016 there was a Dengue epidemic with local transmission on the Island of Hawaii with over 260 confirmed cases detected (see http://health.hawaii.gov/docd/dengue-outbreak-2015/). During the outbreak, *Ae. aegypti* was not widely detected (HDOH and CDC *pers. comm.*), but our previous work had found it in areas identified as having active Dengue transmission. Therefore, we present the following report on the contemporary distribution of *Ae. aegypti* and compare this to *Ae. albopictus* in order to better inform epidemiological investigations and vector control efforts on the island of Hawaii. We used presence records for both species along with publicly available data on temperature, rainfall and other similar information to estimate the probability of occurrence using species distribution modeling.

METHODS

Presence records were collated from the Hawaii Department of Health (HDOH) Vector Control Branch records (P. Yang, *pers. comm*) with methods outlined in Winchester (2011). A total of 67 presence records for *Ae. albopictus* were used from the Island of Hawaii, while 26 modern presence records from both DOH 2002 vector control records and J. Winchester's fieldwork were used for *Ae. aegypti*. Geographic information on climate

included several variables such as temperature; rainfall and other "BIOCLIM" (Houlder *et al.* 2001, Beaumont *et al.* 2005, Frazier, *et al.* 2015, Giambelluca, T.W. *et al.* 2014.) variables as input (see Winchester 2011 for details). All areas above >1500 meters were masked, because they are inhospitable to these species. To calculate the potential range of these species, we used Maximum Entropy Species Distribution Modeling (SDM) software (Maxent version 3.3.3e: Phillips *et al.* 2006; Phillips and Dudík 2008) methodology implemented in the DISMO package for the R software environment (Hijmans *et al.* 2011, R Core Team 2016). The predictions of the final model are presented in Figure 1, with the results of the model analysis in Table 1.

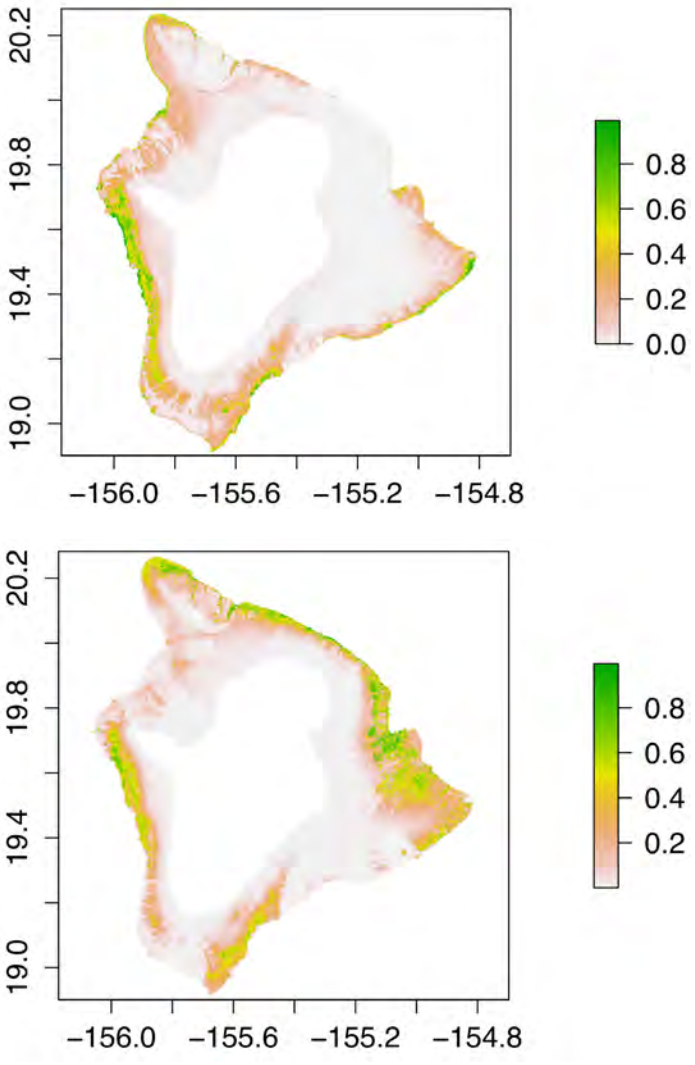


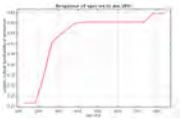
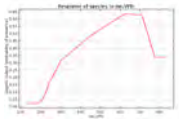
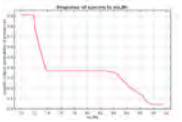
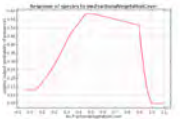
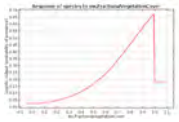
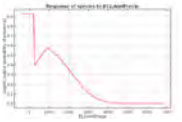
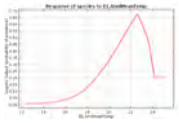
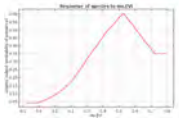
Figure 1. The predicted distributions of *Ae. aegypti* and *Ae. albopictus* are shown from the final model selected. The range of color indicates the predicted probability of presence from low (pink) moderate (yellow) to high-predicted presence (green). The high altitude white area in the middle of the island are masked.

RESULTS

Figure 1 shows the predicted distributions of these two species based on a reduced set of input variables (Table 1). Each model had an area under the receiver operating characteristic curve (AUC) of 0.976 (*Ae. aegypti*) and 0.939 (*Ae. albopictus*), respectively, indicating that the models have relatively high utility (Phillips and Dudík 2008). For the *Ae. aegypti* model the geographic layers that explained the most variance in the combined model included minimum vapor pressure deficit, maximum relative humidity, maximum fractional vegetation cover for the top 66% of the variance (Table 1). Permutation tests measure the impact of dropping a particular layer, and for the *Ae. aegypti* model the layer that had the highest impact on the model was annual precipitation with nearly 70% permutation importance and no other variable had over a 10% impact for this model alone (Table 1). For the *Ae. albopictus* model the minimum vapor pressure deficit and maximum enhanced vegetation index explained nearly 60% of the variance, whereas the variables that had the largest impact when removed were annual mean temperature, maximum enhanced vegetation index, and maximum fractional vegetation cover (Table 1). Maxent output includes the shape of the response curve of each variable taken separately: for widespread *Ae. albopictus* these were peaked responses to most variables, with the maximum probability of occurrence at intermediate to higher values then falling off, including vapor pressure deficit, enhanced vegetation index, annual mean temperature and fractional vegetation cover (see Table 1). In contrast, the *Ae. aegypti* model shows increasing predicted occurrence with increasing minimum vapor pressure deficit, decreases in precipitation and relative humidity, and a peaked distribution at intermediate values of fractional vegetation cover (Table 1).

DISCUSSION

Based on HDOH records and our own observations, *Ae. aegypti* is currently distributed on the leeward side of the Big Island of Hawaii, where drier environments prevail, especially at lower elevations near shorelines. These same data suggest that *Ae. albopictus* is more widespread, basically found throughout the island in areas with vegetation and abundant water, but were found exclusively in the wetter windward side of the island. The SDM predicted distributions for both species overlapped the area of highest Dengue activity from the 2015/16 epidemic (Hawaii Department of Health 2016). The SDM's also suggested some differences between the factors influencing the two species, with *Ae. aegypti* favoring dry areas with lower annual precipitation, lower relative humidity and increasing vapor pressure deficit and only intermediate levels of vegetation cover. Contrastingly, *Ae. albopictus* favored increasing temperature and vegetative indices and decreasing (peaked) vapor pressure deficit as is typical of the wetter vegetated areas where it is generally found.

Species: ->	*Aedes aegypti*			*Aedes albopictus*		
Variable:	Percent contribution	Permutation importance	Relationship+	Percent contribution	Permutation importance	Relationship
Min. vapor pressure deficit	**29.6**	3		**36.9**	0	
Max. relative humidity	**18.7**	3.4		3.7	0.5	
Max. fractional vegetation cover	**17.9**	6.3		7.3	**12**	
Annual precipitation (B12)*	9.8	**69.4**		2.3	3.5	
Annual mean temperature (B1)*	5.5	0		2.2	**36.1**	
Max. net radiation	4	1		2.7	4.4	
Max. enhanced vegetation index	3.9	1.8		**22.7**	**20**	
Mean diurnal range (B2)*	3.9	7.4		4.8	1.3	
Temperature Annual Std. Dev x 100 (B4)*	2.1	2.4		2.5	9.2	
Max. actual evapotranspiration	1.7	0.9		9.9	3.3	
Min. Soil evaporation in W/m^2	1.7	2.4		0.7	2	
Min wet canopy evaporation W/m^2	1	2		0	0	
Precipiation STD deviation (B15)*	0	0		1.2	4.5	
Isothermality (B3)*	0	0		3.1	3.1	

* Bioclim variables are noted by (number). +The relationship between predictor variable (left) and species probability of occurrence is indicated for variables with greater than 10% contribution or permutation importance.

Table 1. Results of Maximum Entropy Species Distribution Models for island of Hawaii with 14 input variables. Variables selected and their impact on the final model fit. Variables are presented in decreasing order of variance explained for *Aedes aegypti* (columns 2 & 3). All variables with a > 10% impact on variance explained or permutation importance are bold-faced. For bold-faced variables, the response curves are shown where the response is the logistic output (probability of presence) versus increasing function of input variables.

In this report, we show that sparse surveillance data provided an initial basis for a coarse prediction of the distribution of two *Aedes* species in the Island of Hawaii that are significant vectors of Dengue virus and emerging arboviruses such as Chikungunya and Zika that threaten Hawaii's shores. Further work is needed to test the predicted distributions and determine their utility for vector control and suppression efforts. To foster further data collection and community involvement, we started the 'Mosquitoes in Hawaii' project in May 2015 which as of July 8th, 2016 has 248 observations of 6 species collected by 28 observers and identified by 14 identifiers (for more information see (http://www.inaturalist.org/projects/mosquitoes-in-hawaii). Further analysis of historic data, results for the remaining Hawaiian Islands and the impact of citizen observations on our understanding of vector ecology in Hawaii will be published elsewhere.

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Use of Abatement to Reduce Intensity of a Flea-Borne Typhus Outbreak in the San Gabriel Valley, Los Angeles County, California

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BACKGROUND

Flea-borne typhus is an acute febrile disease caused by *Rickettsia typhi* and *Rickettsia felis* transmitted to humans by the bite of *Xenopsylla cheopis* (rat fleas) or *Ctenocephalides felis* (cat fleas) found on many vertebrate hosts (Azad *et al*., 1997). More than 200 human cases of flea-borne typhus occur in the United States every year, but the disease is only endemic in California, Hawai'i, and Texas (Civen and Ngo, 2008, CDPH, 2014). In California, most cases occur in Orange and Los Angeles counties. Within Los Angeles County, approximately 40% of the human cases occur in the San Gabriel Valley, which has attracted the attention of county public health officials (Civen and Ngo, 2008, Wekesa *et al.*, 2016a). It is therefore important that the San Gabriel Valley Mosquito and Vector Control District (District) develop strategies and nurture interagency collaboration to limit the occurrence of flea-borne typhus in the District (Wekesa *et al.,* 2016b). An opportunity to do so occurred when a cluster of human cases was identified at an address in the eastern end of the District in 2015.

In May 2015, the Los Angeles County Department of Public Health's (LACDPH) Acute Communicable Disease Control Program reported to the District three human cases of flea-borne typhus among residents of a 95-unit mobile home community (MHC). These cases had onsets between 9 Apr and 5 Jun 2015 and prompted an investigation to evaluate whether a public health risk existed. The initial survey of the MHC found potential risks in the form of excessive free roaming cats, overfilled trash containers, outdoor feeding stations, high flea counts, and excessive animal feces throughout the property. Traps set on the property yielded two opossums; each of which was infested with more than 600 *C. felis*. Two samples each containing five fleas was tested using Real Time Polymerase Chain Reaction (RT-PCR). Although *R. felis* was detected in both samples, *R. typhi* was not. These findings bring into question the species of *Rickettsia* responsible for this outbreak. The public health control measures required to mitigate this outbreak would apply to either species. These observations also indicated that flea-borne typhus was actively being transmitted at the MHC and required immediate action to reduce the risk to public health.

ABATEMENT ACTION

The MHC was abated by the District on 24 Jun 2015. The summary abatement order was issued to the owner of the MHC property and copied to the property manager, but not to the 90 tenants renting spaces at the MHC. The summary abatement notice required the property owner to comply within 45 days with their own MHC rules, the City ordinance, and the State of California Housing and Community Development's regulations regarding pets in MHCs. For example, California Housing and Community Development general requirements under Title 25, Chapter 2, Section 1114 of California Code of Regulations states that "(a) all dogs and other domestic animals, and cats (domestic or feral) shall not be permitted to roam at large (free) in any park; and (b) animal feces shall not be permitted to accumulate on any lot or common area to the extent that they create a nuisance."

The summary abatement specifically required that the property owner remove all feces present on the grounds of the MHC, ensure no outdoor pet food was available throughout the MHC, and enforce the MHC rules which limit the number of pets to one animal per unit and require that the pet be registered with property management. The abatement order also required the property owner to provide bi-weekly flea control and remove feral animals. To comply with the summary abatement, the property manager notified residents to remove outdoor pet foods and to register their pet with the MHC office.

On 24 Aug 2015 a multi-agency community event adjacent to the MHC was hosted by LACDPH and involved staff from the District, the City, the office of California State Senator Connie Levya, and programs within LACDPH, including Community Health Services, Environmental Health Vector Management, Veterinary Public Health, and the Acute Communicable Disease Control Program. The California Department of Housing and Community Development participated in planning the event but

did not attend. Tenants were informed of the outbreak and that an excess of fleas and a large population of free-roaming cats and opossums throughout the MHC were the major contributor. Residents were advised about flea-borne typhus and informed of the community's role in preventing new cases. They were further instructed to immediately remove outdoor feeding stations, and while the property owner managed the fleas outdoors, residents were responsible for controlling fleas inside their units. Tenants were reminded that the MHC allowed them to keep only one dog or cat per property. The LACDPH donated one Seresto® (similar to Frontline®) collar per tenant for their indoor pet. Sera were collected from individuals who were currently symptomatic or were symptomatic for flea-borne typhus in the past three months. Two additional cases of flea-borne typhus at the MHC were identified bringing the total of human cases in this outbreak to five (Croker *et al.,* 2016).

On 3 Sep 2015, the District implemented a multi-agency plan which involved the LACDPH Environmental Health Division's Vector Management Program, the City's, the District, the landlord, and the tenants of the MHC. The property owner was required to provide both flea control for the outdoor space of the MHC and wildlife trapping services. The pest control operators who were hired to provide these services did so under direct instructions from the District's disease surveillance program.

EVALUATION OF ABATEMENT

The summary abatement order issued on 24 Jun 2015 was followed with monitoring of animals and outdoor feeding stations. Feral cats, opossums, and outdoor feeding containers were counted to assess the effectiveness of this order. The bi-weekly monitoring showed declining populations of wildlife and feeding stations in the MHC (as shown in Figure 1), but indicated that additional action was required for this abatement order to be effective. The coordination of multi-agency approach culminated with a community event on 24 Aug 2015 and implementation of the multi-agency action plan thereafter. To assess the impact of the multi-agency action plan and overall effectiveness of the summary abatement order, monitoring of adult flea populations was necessary. Bi-weekly monitoring of fleas was conducted by placing six glue boards of 16 cm x 11 cm in size (PIC Corporation, Linden, NJ) throughout the MHC. In addition, outdoor feeding stations and the counts of feral cats were recorded. The percent change was then calculated by subtracting the current value from each sampling period from the initial value and then dividing that number by the initial value (Current Value – Initial Value/ Initial Value) to analyze the effect of the MHC's actions. Also, a regression analysis was conducted to obtain the R^2 value and the level of significance of these actions on decreasing this public health issue. As shown in Figure 1, the number of outdoor wildlife and feeding stations had significantly declined within the five month monitoring period (n=87, r^2= 0.914, $P \leq 0.05$) throughout the MHC. This was further confirmed by a similar decline in the flea population monitored on glue boards between Sep and Nov 2015 (Figure 2), such that no fleas were observed for the last two consecutive sampling days. These findings indicate that the situation at the MHC continued to improve and that the outbreak was considered mediated by Nov 2015.

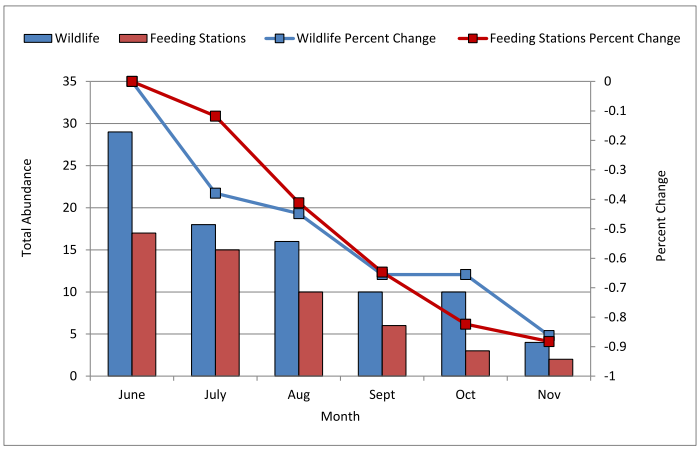

Figure 1. Abundance of outdoor wildlife and outdoor feeding sources from Jun to Nov 2015 at the Mobile Home Community in San Gabriel Valley, California.

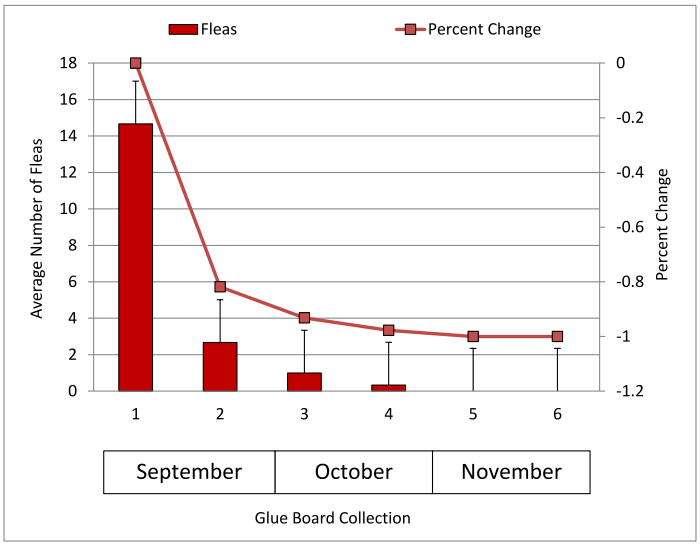

Figure 2. Average number of fleas (plus standard error) collected on glue boards (n=36) from Sep to Nov 2015 at the Mobile Home Community in San Gabriel Valley, California.

SUMMARY

This approach represents the first successful attempt to use the California Health and Safety Code in an abatement process to control flea-borne typhus in southern California. We hope to employ this strategy at other mobile home communities that have excessive feral animals, animal feces and fleas. To ensure that this condition does not recur, the District will continue to monitor this MHC for fleas and the property owner will continue with monthly flea control and wildlife removal until all tenants are considered compliant to MHC rules, city ordinances, and state regulations on feral animals.

ACKNOWLEDGEMENTS

We thank Jeffrey Gunzenhauser, MD MPH, the Interim Health Officer and Medical Director of Los Angeles County, Department of Public Health (LACDPH) for activating a public health response befitting a disease outbreak; special thanks to Laurene Mascola, MD MPH, Benjamin Schwartz, MD, and Van Ngo, MPH of the Acute Communicable Disease Control Program of the LACDPH for approaching this outbreak investigation with dedication. Thanks to Cristin Mondy, RN, MPH, the Area Health Officer for SPA 3 (San Gabriel Valley) for coordinating and ensuring the public health risk was clearly identified and mitigated. We thank Brenda Lopez, Gracelin Shin, Kelsey Onaga, Maria Dalusong, Terri Williams, and Yvette Boston of Los Angeles County Environmental Health Vector Management Program for the outbreak response and community outreach. Additional thanks go to Mark Gluba of the City of Pomona for the assistance and cooperation. Sincere thanks go to Senator Connie Leyva's office, especially to District Director Manuel Saucedo and District Representative Benny Ayala for their involvement and making sure all local and state agencies responsible for mobile homes were engaged, especially for getting Sonia Semlow of California Department of Housing and Community Development to enforce MHC regulations accordingly. We thank Marco Metzger and Renjie Hu of the State Department of Public Health, Vector Borne Disease Section, Ontario office for their consultation. Lastly, thanks to Carrie Fogarty and Robert Cummings of the Orange County Mosquito and Vector Control District for the preliminary tests of fleas collected from the subject properties.

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Filarial Infection of Mosquitoes in Lake County, California

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ABSTRACT Filarial parasites are a type of nematode that can infect humans and animals. These parasites, including *Dirofilaria immitis* (dog heartworm) and *Setaria yehi* (deer body worm), require mosquito vectors for transmission between hosts. According to the American Heartworm Society, the Lake County region has a higher incidence of dog heartworm than much of the state. Additionally, deer body worm is prevalent in young deer in this area. The goal of the current study was to take steps to determine which mosquito species are important in transmitting these parasites, particularly dog heartworm, by (1) determining the prevalence of filarial parasites in Lake County mosquitoes and (2) evaluating the vector competence of *Culex tarsalis* for *D. immitis* in the laboratory. To determine prevalence, mosquitoes were collected from April to November in 2014 and tested by PCR using two sets of primers. One primer set targeted cytochrome c oxidase I *(COI)* and detected a wide range of filarial parasites; the other targeted the gene for 5s rRNA and was specific to *D. immitis*. Of 1,008 total mosquito pools (total number of specimens= 31,508), filarial parasites were detected in 23 mosquito pools: *Aedes increpitus* (n=1, MIR= 4.61), *Aedes sierrensis* (n=3, MIR= 20.13), *Anopheles franciscanus* (n=1, MIR= 0.55), *Anopheles freeborni* (n=7, MIR= 3.76), *Culex stigmatosoma* (n=3, MIR= 1.24), and *Culex tarsalis* (n=8, MIR= 0.39). Additional mosquito species were tested, but none were positive for filarial parasites. Positive pools *D. immitis* (n=6), S. yehi (n=9), *Splendidofilaria sp.* (n=4), and unidentified filarial nematode (n=4). To evaluate vector competence of *Cx. tarsalis*, 48 colony and 48 field-caught mosquitos were fed a *D. immitis*-infected bloodmeal and were tested for transmission. Fourteen days post feeding, the mosquitos were decapitated, but no infective L3 larvae were observed. After testing the abdomens and thoraces separately for infection by PCR, 6.3% of the colony abdomens and 4.2% colony thoraces were infected. Higher rates of infection were found in the field-caught *Cx. tarsalis*, with 20.8% of the abdomens and 22.9% of the thoraces found to be positive. Additional transmission tests will be conducted with longer post-feeding times to better observe vector competence of *Culex tarsalis*, and future work will explore vector competence in other mosquito species. Based on this preliminary data, several species, including *Cx. tarsalis*, are potential vectors of filarial parasites in California.

Plague in South Lake Tahoe

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In August 2015, a California ground squirrel carcass collected from a U.S. Forest Service Lake Tahoe Basin Management Unit South Shore recreation area tested positive for plague bacteria. Follow-up assessments, including rodent and flea surveillance, indicated an increased risk for human plague transmission. In response, recreation areas were temporarily closed, and staff from the California Department of Public Health and County of El Dorado Vector Control implemented flea control measures to help decrease the potential transmission of plague to humans. To reduce the number of fleas within recreation areas, rodent burrows were treated with DeltaDust® insecticide. Carpeted bait stations, treated with Suspend® SC insecticide, were placed in areas with high human activity. Post-treatment assessments indicated the number of fleas per rodent had decreased significantly.

Bionomics and Vector Potential of *Culex thriambus* Mosquitoes in Lake County, California

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Culex tarsalis, members of the *Culex pipiens* complex, and *Culex stigmatosoma* are competent and efficient laboratory vectors of WNV and are repeatedly implicated in the maintenance and amplification of the virus in California. Mosquito control and surveillance activities are focused on these key *Culex* species, however, comparable studies on other *Culex* vectors such as *Culex thriambus* are lacking. While considered a relatively rare, riparian species in California, its role in the transmission of WNV may be underappreciated as adults are infrequently collected. Recently, a large population of larval and adult *Cx. thriambus* was detected in Lake County, California. The present study reports results of field and semi-natural studies on aspects of the bionomics and WNV potential for *Cx. thriambus* mosquitoes in Lake County, including relative abundance and seasonality, overwintering and mating, host selection, and infection rates in mosquito pools.

Utilization of American Crows by host-seeking *Culex* mosquitoes

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ABSTRACT: American Crows (crows) are exquisitely sensitive to West Nile virus (WNV) and often succumb to infection. The presence of dead crows has become a hallmark of WNV activity and is often utilized as a viral surveillance tool. Despite the frequency with which crows are infected with WNV, they are often found to be under-utilized by host-seeking *Culex* mosquitoes in bloodmeal identification studies. To better understand this discordance, bloodfed *Culex tarsalis* and *Culex pipiens* complex females were collected within known distances of nesting crows. The nests were closely monitored so that crow occupancy periods and developmental stages of each nest (nesting building, egg incubation, chicks in nest, and chicks fledged) could be established. When *Culex* mosquitoes were collected within 50m of a crow nest, they were more likely to have fed on crows than if they had been collected >50m from a crow nest. This result indicates that crow movement patterns may bias bloodmeal identification studies away from the detection of crow bloodmeals, if bloodfed mosquitoes are collected >50m from nighttime crow roosting or nesting locations. Thus the lack of crow bloodmeals in previous studies may not have been a function of *Culex* host preference but rather crow proximity.

West Nile virus present in a winter roost of American Crows *(Corvus brachyrhynchos)*: examining the importance of horizontal transmission[1]

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AIM AND SCOPE

Following its initial emergence in North America, West Nile Virus (WNV) generated great interest among ecologists, public health professionals, and managers due to its impact on avian, equine, and human communities (Komar 2003; Eidson et al. 2001; Azad and Thomas 2004). Though much effort has been put into understanding WNV transmission, there is still a great deal that we do not fully understand regarding transmission dynamics. This is particularly true with regard to persistence across the winter season at temperate latitudes. Cold winter temperatures hinder viral replication within mosquito vectors and, perhaps more importantly, vector activity is quite low in the winter when *Culex* mosquitoes (competent WNV vectors; Molaei et al. 2006) tend to be quiescent (Reisen et al. 2006; Nelms et al. 2013a, b). American crows are known for their importance as WNV sentinels due to their relatively high susceptibility to the disease causing agent (Komar et al. 2003). Previous studies suggest that abundance and activity of these highly social, synanthropic birds might play an important role in amplification and transmission of WNV (Reisen et al. 2006a; Lothrop et al. 2008; Hartley et al. 2012). During the late fall and winter months American crows form large, nocturnal roosts (Gorenzel and Salmon 1995) in which it is likely that there are opportunities for transmission of WNV via fecal-oral mechanisms. Furthermore, researchers have documented collection of WNV positive American crow carcasses in the winter (Dawson et al. 2007). The aim of our study was to elucidate mechanisms by which WNV persists overwinter within one of these large American crow roosts in the Sacramento Valley of California. We examined seasonal patterns of crow roost density and *Culex* mosquito activity, WNV infection rates in crow carcasses collected within Davis, CA, and the prevalence of WNV-positive feces within the Davis crow roost across two winter seasons. We also assessed the opportunity for fecal-oral transmission of WNV among wild American crows by determining seasonal rates of feather fecal-staining. Using these data we tested two hypotheses: that WNV infection occurs within winter crow roosts when mosquito vector activity is low, and that WNV infection occurs repeatedly through fecal-oral horizontal transmission. Furthermore, we made the prediction that if WNV was found to be present sans mosquito vector activity, there would be sufficient evidence for horizontal transmission within the roost site.

MATERIALS AND METHODS

The large American crow roost covered a roughly 400 m^2 area and was located on the University of California, Davis campus in Yolo County, California. We collected crow roost density data, crow carcasses, and fecal samples while the roost was formed (in fall and winter) from January 2013 through August 2014. To supplement these data, we also collected fecal samples at additional winter crow roosts located within Yuba City (Sutter County, CA) and Sacramento (Sacramento County, CA) during March of 2014. We quantified crow roost density at Davis via visual surveys conducted 1-3 times per week throughout the roosting season. Visual surveys included approximation of the number of crows roosting within each occupied tree throughout the roost. A single observer conducted most of these surveys (77%), and each survey was concluded roughly 30 minutes before sunrise, when crows begin to leave the roost. Furthermore, we measured the percentage of birds with conspicuous fecal stains (binary variable) on their plumage at staging areas, locations at which crows leaving the roost group, within 200m of the roost from October 2013 through May 2014. Using a spotting scope, a single observer conducted fecal stain surveys 2-6 times per month as crows left the roost at sunrise. We examined the relationship between fecal staining and local crow abundance using a generalized linear model, with the proportion of stained birds (weighted by the number of birds observed) as the response and the recent number of birds counted at the roost as the predictor.

1 *This research has been published: Hinton et al. 2015.*

From 13 February – 1 March 2013 we collected 495 fresh fecal samples, and from October 2013 – March 2014 we collected 414 samples from underneath the Davis overwintering roost (total of 909). In March 2014, we also collected 120 and 90 fecal samples from overwintering roosts in Yuba City, CA and Sacramento, CA, respectively. Additionally, we collected 263 samples from large dawn/dusk staging groups in Davis in August and September 2013. We used sterile cotton-tipped swabs to collect fresh samples (considered 'fresh' if still moist at the time of collection). Collection occurred within approximately one hour of sunrise. We labeled swabs individually and stored the samples in 1-mL aliquots of viral transport media to be tested for WNV RNA (described below). Roughly 1-3 times per week, throughout the study period, we searched for crow carcasses along a set route which lead through the Davis roost. We also received reports of local dead birds through our web-based carcass reporting system in Davis. We collected a total of 62 American crow carcasses and stored them at -20°C until we were able to test for WNV. The California Animal Health and Food Safety lab tested eight of our carcasses as part of the Dead Bird Program (http://westnile.ca.gov/report_wnv.php). From the remaining 54 crow carcasses, we collected kidney samples and cloacal swabs (if viable) for WNV RNA testing by RT-PCR. We report threshold cycle scores (C_t) for these samples. The C_t score is a relative measure of the concentration of target sequence in a qRT-PCR reaction. We compared C_t scores using a paired t-test. We also used a linear regression to analyze changes in kidney C_t scores over time throughout the study period. C_t scores were log transformed to meet assumptions of equal variances and normality. Finally, we also compared percentages of WNV positive carcasses before and during the winter roosting period with a McNemar's test for marginal homogeneity using a continuity correction.

We also trapped mosquitoes in Davis from October 2013 to August 2014 in order to assess seasonal mosquito activity within and near the Davis overwintering roost. We conducted mosquito trapping once per week using three distinct trapping methods within 8 specific sites. We set traps between 1200-1600h at four within-roost sites and at four comparison locations that were between 2500-800m away from the roost. We then collected traps the following morning (30-60 minutes after sunrise) and identified captured mosquitoes to species. Throughout the study we captured low numbers of *Culex pipiens* L. and *Cx. tarsalis* Coquillet and, since either might contribute to WNV transmission, included both species in our estimates of local mosquito activity. After capture and identification, we pooled *Culex* mosquitoes and stored them at -80°C prior to WNV RNA testing by qRT-PCR. We compared mean *Cx. tarsalis* and *Cx. pipiens* as well as combined *Culex* capture between roost and non-roost sites using Wilcoxon Signed Rank tests with a continuity correction; additionally, we log transformed mosquito capture data to meet assumptions of equal variances and normality.

MAIN CONCLUSIONS

In the current study we report WNV infection in crow carcasses collected throughout the year, including winter. Fifty percent (31/62) of the carcasses we collected tested positive for WNV RNA; 72% (13/18) of the summer carcasses were WNV positive, whereas 41% (18/44) of the winter-roost period carcasses tested positive, a significant difference (χ^2=6.26, df=1, p=0.012). Overall, *Culex* mosquito activity was quite low in winter and all mosquitoes collected during these months tested negative for WNV. Though we did capture some *Culex* host-seeking during the winter, and therefore it is possible that mosquitoes may have contributed to WNV transmission to crows at the winter roost, we do not think it likely that winter infections were driven by recent bites from infected mosquitoes. In WNV positive carcasses from which we were able to collect both kidney samples and cloaca swabs, 59.2% (16/27) had WNV RNA in both samples, and the concentration of WNV RNA was significantly higher (C_t scores were significantly lower) in the kidneys than in the cloaca (paired t-test: t=-5.26, df=14, p=0.00012). This suggests that WNV-infected crows were likely shedding virus in their feces, though at low concentrations. Furthermore, in mid-winter the percentage of birds observed near the roost with fecal staining on their plumage reached 57.5% (and percentage significantly increased with local crow abundance; ±SE = 0.74 ± 0.07, z (23) = 11.12, $p < 0.0001$). Given our results, we conclude that consistent opportunities did exist for fecal-oral transmission at the overwintering roost. Despite these clear opportunities, this study provided no evidence of WNV amplification overwinter. Additionally, we provide no direct evidence to suggest that fecal-oral transmission in overwintering crows was occurring frequently. Though there was active WNV transmission during the preceding summers, none of the 1,119 feces collected from three roosts over two winters contained detectable WNV RNA.

It is possible that our sampling efforts were insufficient to detect low levels of bird-bird transmission that might have occurred through the winter. We did detect a 2.3% prevalence of WNV among the 88 fecal samples taken under a staging group of crows in the late summer, when infected mosquitoes were active and the crow roost was relatively small (~300 birds). Although we cannot rule out low levels of bird-to-bird transmission overwinter, it does not appear to maintain or amplify WNV above late-summer levels; given an expectation of a 2.3% prevalence (similar to the prevalence found in Dawson et al. 2007), the probability of finding no positives among all 1119 winter samples across both years was <0.0000001 (Binomial Approximation via Poisson distribution). An alternative explanation for WNV infection observed among winter crow carcasses is the persistence of WNV within birds that survive initial summer infection. In our study, kidney C_t scores increased significantly as the winter progressed (r^2=0.46, t=4.268, df=21; p=0.00034). Previous work has correlated elevated C_t scores with WNV persistence rather than acute infection (Reisen et al. 2013). Therefore, mortality of persistently infected birds, as opposed to those with newly acquired acute infection, may have been a major cause of WNV cases observed in winter during our study.

Despite our lack of detection of WNV among American Crow fecal samples throughout two winters, the presence of WNV among crows during the winter season suggests that winter roost sites still hold potential as important locations for vernal amplification of WNV once mosquito activity increases in the spring (just before and as the roost begins to dissipate). Prior studies provide evidence to suggest that mosquitoes feed on crows during the spring and that crow infections lead to additional mosquito infection and following outbreaks (Nielsen and Reisen 2007; Campbell et al. 2013). We strongly encourage researchers to examine the source of infection for winter cases of WNV infection among crows as they tend to maintain high density near or within urban centers. Viral persistence, WNV survival rates among crows, and the reliability of high C_t scores as an indicator for persistent infection are a few issues that we think need to be addressed. Doing so will allow us to gain a greater understanding for the role that crows play in WNV persistence over winter and amplification in the spring which in turn would serve to inform surveillance response and WNV management programs.

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The role of communal crow roosts in West Nile virus overwintering

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INTRODUCTION

The American Crow (*Corvus brachyrhynchos*; hereafter "crow") is a highly competent host for West Nile virus (WNV; family *Flaviviridae*, genus *Flavivirus*), developing extremely high viremias and exhibiting high mortality following infection (Komar et al. 2003, McLean 2004, Kipp et al. 2006, Bowen and Nemeth 2007). Particularly in winter, crows spend nights at communal roosts consisting of thousands of birds flocking together. This is likely to increase contact rates between individuals compared to other non-communally roosting North American avian species. Direct WNV transmission between crows in the absence of mosquitoes has been reported (McLean et al. 2001, Komar et al. 2003), crows in a communal roost were frequently stained with feces of other crows (Hinton et al. 2015), feces of infected crows can have high titers of WNV (Kipp et al. 2006), and dead crows positive for WNV have been recovered during cold periods when mosquitoes are not actively feeding (Dawson et al. 2007, Wheeler et al. 2014, Hinton et al. 2015). Taken together, this evidence makes it seem plausible that communal crow roosts could sustain WNV infection through the winter in the absence of vector transmission, thereby acting as a reservoir of the virus.

In this paper, we constructed a model for bird-to-bird WNV transmission to study the potential for a large communal crow roost to sustain WNV during winter in the absence of mosquito-bird transmission.

METHODS

Our study population consisted of the winter bird community on the campus of the University of California, Davis, because it is the site of a large and well-documented crow roost that harbors around 10,000 birds between November and March (Hinton et al. 2015), and estimates were available for the numbers of other bird species each winter since 2009.

We constructed a deterministic, continuous-time model with 3 groups of birds as hosts for WNV defined by their potential contributions to bird-to-bird transmission: crows, raptors, and other birds. Crows and raptors were compartmentalized as susceptible individuals (S; uninfected), exposed (E; infected but not infectious), acute infectious (I; infected and infectious to other birds, including shedding virus through feces), latent (L; retains virus in organs but does not shed virus; however, infectious if fed upon), and recovered (R; birds that survive WNV infection and remain immune). Other birds were susceptible, but could not get infected, because they do not prey or feed upon carrion. The compartments and infection mechanisms are shown in Figure 1. Dead crows (D), raptors and other birds were also tracked, and they were removed through scavenging or decomposition. Raptors prey upon other birds and crows; crows scavenge on dead raptors and other birds. No birth occurred because we considered only late fall and winter seasons. Model parameterization was accomplished using the estimates from previous biology studies and experimental infection studies in crows and in restricted raptor and other bird species. Parameter values for the raptor and other groups were weighted according to the number of individuals by each of the species that constituted these groups.

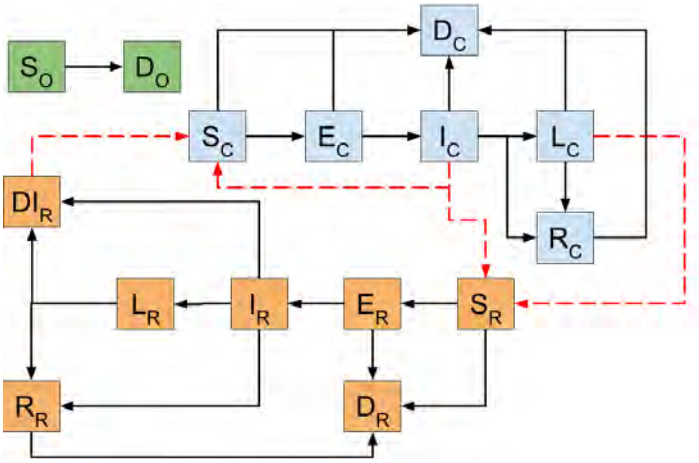

Figure 1. Modeled Crow (C), Raptor (R) and Other birds (O) compartments according to WNV infection and transition among these compartments (black arrows). Red arrows indicate sources of infection to susceptible C or R individuals.

To understand the impact of model parameter values that cannot be known with certainty, we explored realistic ranges. We modeled the dynamics of infection in the bird community using ranges of adequate contact rate among crows between 0.01 and 2 crows per crow per day, and transmission probabilities (given adequate contact for WNV transmission) between 0.01 and 1 transmissions per adequate contact, implying transmission rates between 0.001 and 2 per crow per day. Crow-crow transmission was modeled as frequency-dependent. On Nov 1, we introduced 1-100 exposed crows to the bird community to simulate the range of possible scenarios from a single-infected bird returning from migration to many infected birds carrying over infections into winter following the peak WNV transmission season. We varied the number of crows scavenging together at the same carcass (1-10) and the rate of raptor predation on crows (0.05 - 0.25% of natural crow mortality rate).

RESULTS AND DISCUSSION

The main results showed that the system is strongly influenced by the number of infected crows that carry virus into the modeled winter season starting on November 1st, with larger numbers of infected crows accelerating the wave of transmission. However, transmission within the bird community was not sensitive to the number of crows communally scavenging per dead bird or the proportion of raptors feeding upon crows.

Transmission rates between crows > 0.15 per crow per day appeared to be unrealistic, because simulations generated an outbreak that depleted the crow population, and generated an unrealistic number of dead birds. Interestingly, lower transmission rates (> 0.03 – 0.15) yielded a number of infectious and dead crows that were plausible in a natural outbreak, leaving most of the population alive and susceptible, but sustaining a limited number of infectious and latent (chronically infected) crows by the end of the simulated winter period (March 31st). Very low rates (0.001 - 0.03), did not trigger an outbreak, and WNV died out in the bird community before reaching the end of winter when horizontal bird-mosquito-bird transmission would become more plausible.

In conclusion, transmission rate values that were plausible in nature were capable of maintaining infectious and chronically infected crows through the winter, supporting the hypothesis that crow roosts might constitute an overwintering reservoir for WNV, even in the absence of winter mosquito feeding. Future models should consider effects of herd immunity, the migration of crows from the winter roost at the end of our study period, and the likelihood that mosquitoes, once the warm season begins, could initiate vernal WNV transmission among mosquitoes and birds after feeding on infectious crows.

ACKNOWLEDGMENTS

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Fighting an Unpredictable Foe: An Overview of Two Consecutive Years of Record West Nile Virus Outbreaks, 2014 – 2015, in Orange County, California

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ABSTRACT: Orange County, California, experienced two consecutive years of record West Nile virus (WNV) activity during 2014 and 2015, with 264 human cases (9 deaths) and 92 cases (8 deaths), respectively. The Orange County Mosquito and Vector Control District (District) had learned from ten years of previous WNV activity to focus its mosquito larvicidal control program, public outreach, and surveillance efforts spatially and temporally to well-defined, disease prone areas during periods of heightened virus activity. Despite these directed efforts, the severity of the WNV 2014 – 2015 outbreaks could not be sufficiently mitigated by the District's intensified larvicidal control strategies. In both years, the District mobilized for an elevated response plan utilizing area-wide adult mosquito control (adulticiding) when surveillance indicators exceeded epidemic thresholds; however, the plan was never implemented. This paper will discuss some of the significant challenges the District faced when presented with expanding its WNV suppression program to include aerial adulticiding over a highly urbanized environment.

INTRODUCTION

Recognizing temporal and spatial patterns of West Nile virus (WNV; family *Flaviviridae*, genus *Flavivirus*) activity by monitoring viral infection rates in mosquitoes and birds can be helpful in predicting WNV transmission to humans. Since the arrival of WNV in Orange County (County), California, during 2003, the Orange County Mosquito and Vector Control District (District) has employed a year-round, integrated arboviral surveillance program composed of monitoring WNV seroprevalence rates in free-ranging wild birds, testing dead birds and mosquitoes for WNV infection, and monitoring veterinarian and physician reports for WNV cases in animals and humans. In addition, the District has partnered with the Orange County Health Care Agency (OCHCA) through a Memorandum of Understanding (MOU) (Orange County Board of Supervisors 2005) that allows the District to map putative exposure sites of human cases for cluster analysis (Nguyen et al. 2015).

The District's mosquito control program consists largely of ground-based applications of larvicides (various commercial formulations of *Bacillus thuringiensis* subsp. *israelensis*, *Lysinibacillus sphaericus*, *Saccharopolyspora spinosa*, and lightweight oils) to known breeding sources in approximately 1,200 km (750 mi.) of street gutters, 530 km (330 mi) of poorly-draining flood control channels, 5,000 neglected swimming pools, 69,000 drop inlets to underground storm water drainage systems, and a variety of small peridomestic breeding sources. The District applies EPA-approved, public health insecticides to kill adult mosquitoes (adulticiding) via truck-mounted, ultra-low volume (ULV) space sprays in open, unpopulated areas only, primarily at three wetland locations. Mosquito fish (*Gambusia affinis*), source reduction practices, and public education are also used wherever possible to minimize mosquito production. *Culex quinquefasciatus* Say is the most abundant mosquito species breeding in both peridomestic and large water conveyance sources in Orange County (Schreiber et al. 1989, Reisen et al. 1990, Nguyen et al. 2010, Krueger et al. 2015); adults of this species are collected routinely in gravid traps (Cummings 1992) and are considered moderately-competent vectors of WNV (Goddard et al. 2002).

Since introduction of the virus, the annual numbers of reported human WNV cases have oscillated through periods of low-to-high extremes, ranging from only 1 case in 2010 (no deaths) to 356 cases (17 deaths) during two consecutive years of heightened activity from 2014 – 2015 (OCHCA 2016) (Figure 1).

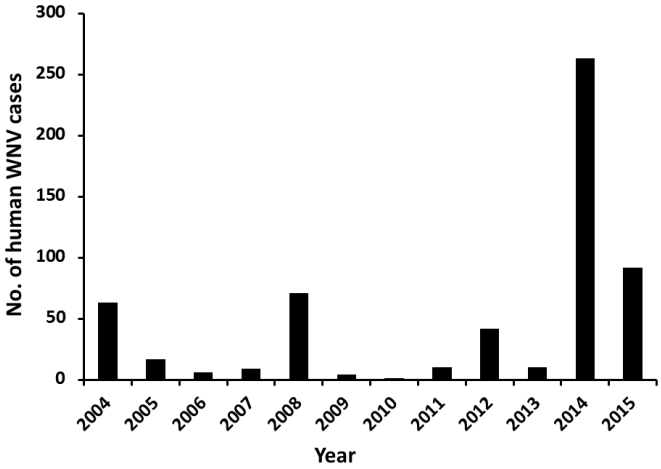

Figure 1. Annual human WNV cases, 2004 – 2015.

The reasons for these annual variations in WNV human cases and viral recrudescence are largely unclear and were never predicted in Orange County, or in other areas of the U.S. (Petersen and Fischer 2012). The District has found that none of the standard surveillance measures (WNV infection rates in dead birds and mosquitoes, mosquito abundance, and seroprevalence levels in

free-ranging wild birds) were useful in effectively forecasting elevated WNV activity beyond a relatively short duration after collection of the data. Routine monitoring of local weather conditions, such as amounts of winter precipitation and trends in daily temperatures (OC Weather 2015), also have not been particularly insightful.

Unfortunately, the lack of predictability of WNV epidemics and the inability to distinguish between a low and high WNV active year, early in the mosquito season, has limited the District's ability to respond with timely and accurately focused larval-based mosquito control measures to mitigate WNV amplification in mosquitoes and prevent human disease during epidemic years. The severity of the 2014 and 2015 WNV disease outbreaks could not be sufficiently alleviated by the District's intensified larviciding control strategies. In both years, the District mobilized for an elevated response centered on an area-wide adult mosquito control (adulticiding) campaign when surveillance indicators exceeded epidemic thresholds; however, the plan was never implemented. This paper will discuss some of the challenges the District faced when presented with expanding its WNV suppression program from ground-based larvicidal applications to include aerial adulticiding over a highly urbanized environment.

MATERIALS AND METHODS

Study Area: Orange County, California, is the third most populous county in the state of California, with an estimated population of 3,169,776 people living in a largely metropolitan area of 2,047 km^2 (790.6 mi.^{2}) along the Pacific Ocean; the population density is 1,548 people/km^2 (4,009 people/mi.^{2}) county-wide (US Census Bureau 2015). Approximately two-thirds of the population resides in the northwestern part of the County, where the population density increases to 17,376 people/km^2 (45,000 people/mi^2) in the central cities of Anaheim, Garden Grove, and Santa Ana (US Census Bureau 2015). The landscape of north Orange County is relatively flat, rising only about 3 m/km (3 ft./1000 ft.) in elevation from the sea to a height of 45 m (150 ft.), approximately 15 km (9.3 mi) inland (Orange County Facts and Figures 2015).

The county's climate is maritime Mediterranean, with mild winter temperatures and warm, dry summers moderated by easterly winds from the Pacific Ocean. The mean annual temperature and precipitation are 18.1° C (64.6° F) and 345 mm (13.6 inches), respectively; on average, measurable rainfall occurs on only 22 days per year (OC Weather 2015). Mean daily temperatures are above the minimum threshold [14.3° C (57.7° F)] needed to support the extrinsic incubation period of WNV in mosquitoes throughout the year (Reisen et al. 2006).

Human WNV Cases: The 2005 MOU with the OCHCA allows for the sharing of relevant human data with the District. These data include place of residence (street address) and date of disease onset for WNV cases.

Mosquito Trapping: As part of the District's expanded arboviral surveillance program since 2004, mosquitoes have been collected weekly from approximately 80 – 100 gravid and CO_2-baited Encephalitis Virus Surveillance (EVS) (Rohe and Fall 1979) traps placed primarily in the densely populated areas [ca. 1450 km^2 (560 mi^2)] of Orange County. Mosquito trap density has increased to approximately 7 traps/100 km^2 with the expansion of the surveillance program, similar to trap densities reported by Healy et al. (2015). Captured mosquitoes were identified by species, sexed, enumerated, and pooled for testing by real-time TaqMan singleplex RT-qPCR (ABI 7300, Applied Biosystems, Foster City, CA) using WNV- specific primers (Lanciotti et al. 2000). Results with cycle thresholds (Ct) < 30 were considered WNV-positive (Reisen et al. 2013); values from 30 - 40 were retested with primer/probes for the WNV NS1 region and confirmed WNV-positive at Ct values < 40 (Shi et al. 2001).

Statistical Analysis: The WNV outbreak years (2004, 2008, 2012, 2014, 2015) and non-outbreak years (2005 – 2007; 2009 – 2011; 2013) were analyzed separately. For the five outbreak and seven non-outbreak years, mosquito infection rates and known WNV illness onset dates for human cases (N = 515 and 58, respectively) were calculated by individual disease weeks across the years. Although a total of 531 human WNV cases (26 deaths) were reported during the five outbreak years, 16 cases were reported to the District by month only and were not included in the weekly analysis. Of the 59 reported WNV cases (no deaths) that occurred during the seven non-outbreak years, only 1 case (2010) lacked a reported disease onset week. Human WNV cases were summed and averaged by onset week separately for outbreak and non-outbreak years.

WNV mosquito infection rates [Maximum Likelihood Estimate (MLE), number of infected mosquitoes/1000] for female *Cx. quinquefasciatus* were calculated weekly for disease weeks 20 – 50 (mid-May to mid-December) using PooledInfRate, version 6.0 software (Biggerstaff 2009) and compared with human WNV case onset data. Pool sizes were similar during outbreak and non-outbreak years, averaging 29.8 and 29.6 female *Cx. quinquefasciatus*, respectively. Because of the relatively small numbers of other mosquito species collected, no others were evaluated.

Dead Bird Surveillance: Dead birds were collected in response to reports from the public and through cooperation with various animal control agencies. Dead birds suitable for necropsy were processed (Krueger et al. 2012) and samples of kidney tissue were tested by real time RT-qPCR as described previously.

Wild Bird Serosurveillance: The District's wild bird serosurveillance program has focused primarily on the house finch (*Haemorhous mexicanus*) and house sparrow (*Passer domesticus*), since they are widely abundant in the County (GBBC 2015), are considered moderately competent hosts of WNV (Komar et al. 2003, Reisen et al. 2005), and are frequently fed-upon by mosquitoes in southern California (Molaei et al. 2010, Thiemann et al. 2012). Wild birds were trapped on alternate weeks in 4 - 8 modified Australian crow traps (McClure 1984) at sites used to sample the adult mosquito population. Newly captured birds were banded (USGS-issued bands for native birds; District-issued bands for non-native birds), aged, sexed (if possible), bled, held briefly for wound healing, and released. Blood samples (0.2 ml) were taken

from the jugular vein using a 1.0 ml insulin syringe with a 28-g needle (BD Products, Franklin Lakes, NJ). The quantity of blood taken from each bird was less than the recommended maximum (1% of bird body weight, Gaunt and Oring 1999) for house finches (average weight = 21 g) and house sparrows (average weight = 28 g), respectively (Cornel Lab of Ornithology 2016). Blood was dispensed into a 1.8 ml field diluent solution (PBS), kept cool, and returned to the District's laboratory for processing. Serology was performed using a blocking ELISA with a baculovirus-Kunjin epitope NS1 recombinant antigen and the specific West Nile anti-NS1 monoclonal antibody 3.1112G (Hall et al. 1995, Jozan et al. 2003). During 2008 – 2015, blood samples were taken from approximately 500 – 750 birds per year.

RESULTS

The District tested a total of 577,930 *Cx. quinquefasciatus* females in 19,435 mosquito pools from 2004 – 2015. During the 5 outbreak years, MLEs for female *Cx. quinquefasciatus* were highest at week 31 (MLE = 27.8), declined slightly, and rose again at week 34 (MLE = 25.9); in both instances, these maxima preceded peaks of human case onsets by 2-3 weeks (Figure 2).

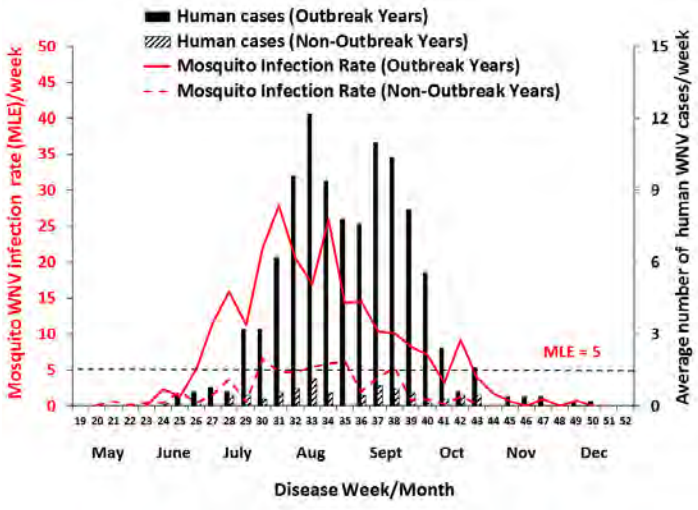

Figure 2. West Nile virus infection rates in mosquitoes and human WNV case onset by weeks.

Elevated WNV infection rates in *Cx. quinquefasciatus* arose in early July and stayed high through disease week 43 in late October (Fig. 2). A summation of average weekly human WNV case onsets showed a bi-modal distribution during these outbreak years, with case onsets at their highest during weeks 33 and 37, despite declining infection rates in mosquitoes (Fig. 2).

During the seven non-outbreak years, mosquito infection rates rarely exceeded the putative epidemic threshold (MLE = 5) (Kramer 2008) for weeks 30 – 38 (Fig. 2). The average weekly human WNV disease onsets during the non-outbreak years followed the same temporal pattern as the outbreak years, with case onsets at their highest during weeks 33 and 37.

No difference was seen in seasonal (May – October) temperatures between outbreak [21.6° C (70.9° F)] and non-outbreak years [21.2° C (70.1°F)]. However, the average annual rainfall was less in the five outbreak years [238 mm (9.37 ins.)] than in the seven non-outbreak years [314 mm (12.36 ins.)]. The lowest WNV active year (2010; 1 human case, Fig. 1) was also an *El Niño* year, with 684 mm (26.93 ins.) of rainfall (UC IPM 2015).

During outbreak years, more dead birds were received and tested (598.8/yr.) compared to non-outbreak years (328.9/yr.). Approximately 52.9% (1,584/2,994) of the sampled dead birds tested WNV-positive during outbreak years, compared to only 22.3% (514/2,302) during non-outbreak years.

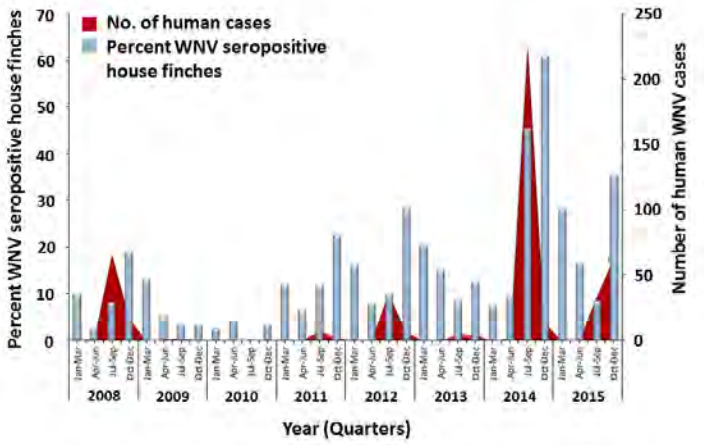

Figure 3. WNV antibody seroprevalence rates in house finches and human WNV cases by quarter of year, 2008 - 2015.

As shown in Figure 3, WNV seropositive rates in house finches rose in parallel with human WNV cases and reached their highest levels at the end of the year during outbreak years. Preceding the 2014 outbreak, WNV seropositive rates in house finches were < 10%, but increased to approximately 60% from October – December (Fig. 3). WNV-seropositive rates exceeded 20% prior to the 2015 levels, and rose again as the 2015 epidemic receded. [House sparrow data are not presented here because of smaller, less consistent sampling size].

DISCUSSION

Understanding the interplay among a variety of abiotic and biotic factors responsible for WNV persistence and rapid amplification in Orange County has been challenging for District staff. Some factors contributing to WNV persistence and epizootic transmission in the County may include: 1) the region's relatively mild Mediterranean climate, which supports year-round production of the primary vector, *Cx. quinquefasciatus* (Schreiber et al. 1989), year-round WNV amplification in mosquitoes (Reisen et al. 2006, Hartley et al. 2012), and years with below-average rainfall and possibly, drought-induced epidemics (Johnson and Sukhdeo 2013); 2) urbanization, which can create habitats conducive to high relative abundances of WNV competent,

urban-adapted avian hosts and mosquitoes (Blair 2004, LaDeau et al. 2013); 3) the capacity of several WNV-competent avian hosts to act as possible overwintering reservoirs of the virus (Wheeler et al. 2012); 4) difficulty in controlling mosquito production in underground storm drain systems in flat landscapes (Su et al. 2003) and other cryptic sources; 5) low socioeconomic conditions (Reisen et al. 2008, Harrigan et al. 2010); and 6) high human population densities in close proximity to mosquito breeding sources (Liao et al. 2014), which may contribute to an increase in the bird-vector-human contact rate (Wilcox and Gubler 2005). Predicting levels of WNV activity in Orange County well-ahead of the mosquito season based on this suite of interplaying factors is currently beyond the District's capability.

Because the District collects and tests mosquitoes weekly, mosquito infection results were obtained within several days of collection. One observed characteristic during the 2014 - 2015 outbreak years was the high intensity of mosquito activity in relatively small geographical areas. Following early detections, WNV-positive mosquito pools appeared to spread outwardly from the initial foci. In contrast, human WNV case reports from the OCHCA lagged by 3 - 4 weeks after illness onset and were found to occur in areas where WNV-positive mosquitoes had been previously detected. On average, the discovery of WNV-positive mosquitoes in a given geographic area preceded human case onsets by 2-3 weeks; hence, weekly trapping and testing of mosquitoes proved to be the most accurate spatial and temporal indicator of impending WNV infection in humans, as has been observed previously in Arizona and Colorado (Colborn et al. 2013, Kilpatrick and Pape 2013). This analysis also tends to support maintaining the epidemic threshold for the mosquito infection rate at 5.0 (MLE), in that relatively few human WNV cases occurred during periods when MLEs remained at or below this value (Fig. 2).

Earlier studies by Liao et al. (2014) and Nguyen et al. (2015) failed to show any predictive patterns regarding the locations and timing of WNV-positive dead birds in relation to the distribution and temporal onset of human WNV cases in Orange County. The dead bird program has been considered the least sensitive of the District's WNV surveillance systems. Varying levels of public interest and participation by animal control agencies may have contributed to inconsistencies in the program over the years, as has also been observed statewide (Foss et al. 2015).

The District's free-ranging wild bird serosurveillance program was also disappointing in its predictive power. Previous work by Kwan et al. (2012) suggested that once seroprevalence or "herd immunity" exceeded 25% in the house finch and house sparrow populations, the number of human WNV cases would remain low during the following year; conversely, once seroprevalence declined below 10%, the chances of a resurgence in human cases would increase. The District also observed similar trends in its wild bird data in previous years (Cummings et al. 2009). Hence, low WNV activity was expected in 2015 due to relatively high levels of WNV seroprevalence in surviving birds from the 2014 outbreak (ca. 30%, Oct. 2014 – April 2015, Fig. 3) and the hypothesized result of reduced viral transmission associated with natural avian herd immunity (Kwan et al. 2012).

While a second year of heightened WNV activity was not considered likely based upon high house finch seroprevelance rates at the end of 2014, the recruitment of immunologically-naïve, hatching-year birds in the spring/early summer of 2015 seemed sufficient to precipitate rapid WNV amplification within the same year (Fig. 3). Previous studies had suggested that a gradual, multi-year reduction in WNV seroprevalence due to high house finch and house sparrow reproduction and natural death of older, immune birds was needed before WNV recrudescence would return (Cummings et al. 2009, Kwan et al. 2012). Additionally, because of the diversity of birds found infected with WNV in Orange County and California (37 species, Krueger et al. 2009; 169 species, Foss et al. 2015, respectively), other WNV-competent species could be secondary contributors to viral amplification in a region when WNV-antibody seroprevalence rates are high in key hosts (Wheeler et al. 2012). Morgan et al. (2015), however, determined that the frequency of early season WNV seroconversions in free-ranging house finches was predictive of the County's 2014 WNV outbreak in a retrospective review of the District's wild bird data. Future study will attempt to validate this observation.

District access to the street addresses of human WNV cases has allowed for the creation of a "heat map" that has profiled areas in the county at high risk for WNV transmission to humans (i.e., disease hotspots) (Fig. 4). This "heat map" was built on the assumption that WNV infection of humans occurred predominately at home or within the immediate area of reported case address (Gibney et al. 2012, Krueger et al. 2015). These data were analyzed with spatial analysis tools (Nguyen et al. 2015) and have been useful in directing the District's surveillance and WNV control efforts to recognized disease hotspots, where approximately 93% of all human WNV cases (N = 590) occurred within the County (Fig. 4). These WNV hotspots may be the result of the local ecology of *Cx. quinquefasciatus* mosquitoes, topographic and demographic factors, and the interaction between other abiotic and biotic environmental conditions supportive of WNV amplification.

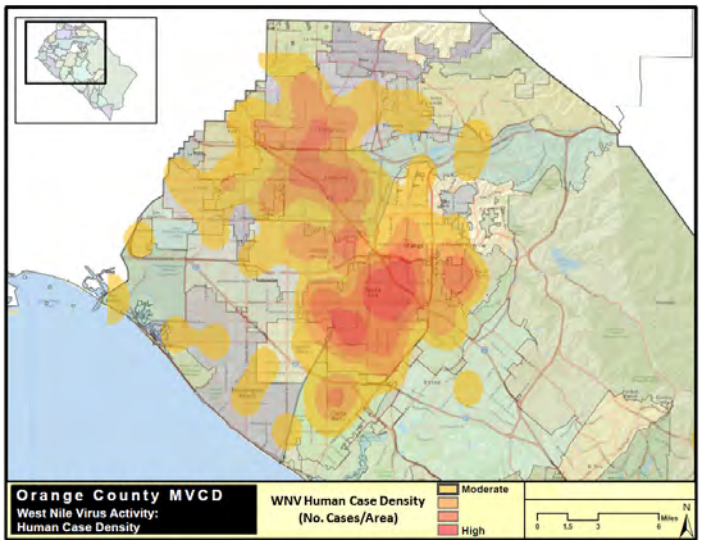

Figure 4. Map of human WNV disease hotspots in Orange County, Calif. 2004 - 2015

During the WNV epidemic years of 2014 – 2015, the District proposed mitigating the rapid rise in human WNV cases through truck-mounted and aerial adulticiding applications of EPA-approved, public health insecticides in areas with high mosquito WNV infection rates during August and September (Fig. 4). However, this recommendation was met with strong opposition from outspoken members of the public and some elected officials. In September, 2014, at the height of the WNV outbreak, the District's attempt at truck-mounted adulticiding applications in neighborhoods of Santa Ana with high WNV activity was canceled due to unfavorable meteorological conditions (i.e., lack of an inversion layer and insufficient nighttime wind speed), threats of violence from some residents, and opposition from the city council. In response to the cancelation, several city council members expressed their concerns about the District's adulticiding plans and gratitude that the District did not spray (Santa Ana City Council Meetings 2014), even though Santa Ana experienced 81 WNV cases with 3 deaths during 2014 (OCHCA 2016) for a relatively high case incidence rate of 24.1/100,000 people. In a scenario similar to the District's experience, Tedesco et al. (2010) examined how local anti-adulticiding policies contributed to higher human WNV disease rates in unsprayed versus adulticide-treated areas during an outbreak in Chicago, Ill., in 2002.

The economic and public health benefits of area-wide mosquito adulticiding to arrest large-scale WNV disease outbreaks has been quantified in several studies in Sacramento County, Calif. (Carney et al. 2008, Barber et al. 2010, Macedo et al. 2010). Together, these studies have demonstrated the miniscule health risk associated with aerial applications of adulticides, high efficacy (6×) at reducing the number of human WNV cases in treated versus untreated areas, and economic benefits in health expenditures in stopping a large-scale WNV outbreak. In 2014, for example, Sacramento County had enzootic WNV surveillance indicators similar to those of Orange County: 294 vs. 431 WNV-positive dead birds, and 487 vs. 499 WNV-positive mosquito pools, respectively (CDPH 2014). However, because of its aerial spray program, Sacramento County ended 2014 with just 10 human cases (0.69 cases/100,000 population) vs. Orange County's 264 human cases (8.5 cases/100,000 population) (CDPH 2014), resulting in a WNV incidence rate 12.4 times lower than Orange County's.

WNV remediation efforts would be more effective in limiting illness and death associated with human infection if conducted at the onset of enzootic amplification rather than after occurrence of human cases (Carney et al. 2008). The Center for Disease Control and Prevention (CDC 2013) and California Department of Public Health (CDPH 2015) recommendations emphasize that larviciding alone may not be sufficient to break local virus transmission cycles and reduce the risk of human infection. In such situations, adulticiding may be the only practical control technique available to reduce the density of adult mosquito populations quickly to lower the risk of WNV transmission to humans (CDC 2013).

Convincing the public and elected officials of the inherent advantages of containing WNV outbreaks through mosquito adulticiding is a primary objective of the District. The District is fully engaged with local city and county staff and political representatives in informing them of the importance of pro-active mosquito suppression when surveillance indicators in a specified area, such as high WNV infection rates in mosquitoes, are indicative of an impending outbreak. It is the District's intent to avoid a repeat of 2014 when Orange County unfortunately led all California counties in the number of WNV cases.

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Surveillance for Mosquito-borne Encephalitis Virus Activity in California, 2015

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ABSTRACT: In 2015, the California surveillance program for mosquito-borne encephalitis virus activity tested humans, dead birds, mosquitoes, and sentinel chickens to detect arbovirus activity. West Nile virus (WNV) activity was elevated and detected throughout several counties, and for the first time since 2003, St. Louis encephalitis virus (SLEV) activity was detected in one county. A total of 860 human WNV infections were reported, and elevated WNV enzootic activity was detected among dead birds, mosquitoes, and sentinel chickens. Enzootic SLEV activity was detected in Riverside County via mosquito and sentinel chicken surveillance.

INTRODUCTION

The California Arbovirus Surveillance program is a cooperative effort of the California Department of Public Health (CDPH), the University of California Davis Arbovirus Research and Training laboratory (DART), 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 2015, the surveillance program components 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) Monitoring mosquito abundance and testing mosquitoes for the presence of SLEV, WNV, western equine encephalomyelitis virus (WEEV), and other arboviruses as appropriate.
(3) Serological monitoring of sentinel chickens for SLEV, WEEV, and WNV antibodies.
(4) Reporting and WNV diagnostic testing of dead birds.
(5) Monthly reporting of arbovirus test results to ArboNET, the national arbovirus surveillance system.
(6) Weekly reporting of arbovirus activity in the CDPH Arbovirus Surveillance Bulletin and on the California WNV website: www.westnile.ca.gov.
(7) Data management and reporting through the CalSurv Gateway, the California arbovirus surveillance system.

West Nile virus activity was reported in 41 (71%) out of 58 counties in California (Table 1), whereas SLEV activity was detected in one county (Riverside).

Table 1. Infections with West Nile Virus in California, 2015. Includes asymptomatic infections detected through blood bank screening. NT = None tested

County	Humans	Dead Birds	Mosquito Pools	Sentinel Chickens
Alameda	0	19	16	0
Alpine	0	NT	NT	NT
Amador	0	NT	NT	NT
Butte	58	38	94	37
Calaveras	0	NT	NT	0
Colusa	1	3	NT	10
Contra Costa	1	11	8	18
Del Norte	0	0	NT	NT
El Dorado	0	4	NT	NT
Fresno	13	3	108	NT
Glenn	21	4	21	10
Humboldt	0	2	NT	NT
Imperial	1	NT	NT	NT
Inyo	0	NT	NT	NT
Kern	12	1	135	NT
Kings	0	3	144	NT
Lake	2	5	31	4
Lassen	0	NT	NT	NT
Los Angeles	313	103	294	138
Madera	4	1	21	NT
Marin	1	3	0	0
Mariposa	0	NT	NT	NT
Mendocino	2	NT	NT	NT
Merced	2	3	8	23
Modoc	0	NT	NT	NT
Mono	0	NT	NT	NT
Monterey	0	0	NT	0
Napa	1	7	0	NT
Nevada	2	10	NT	4
Orange	97	83	576	NT
Placer	1	21	52	8
Plumas	0	NT	NT	NT
Riverside	141	82	158	22
Sacramento	6	103	164	2
San Benito	0	0	NT	0
San Bernardino	60	25	291	36
San Diego	44	355	46	12
San Francisco	0	0	0	NT
San Joaquin	2	18	208	NT
San Luis Obispo	0	0	0	NT
San Mateo	0	23	5	0
Santa Barbara	0	3	1	0
Santa Clara	8	231	20	5
Santa Cruz	0	0	0	0
Shasta	3	16	48	22
Sierra	0	NT	NT	NT
Siskiyou	1	NT	NT	NT
Solano	1	10	6	7
Sonoma	0	11	12	0
Stanislaus	13	14	84	9
Sutter	2	16	54	36
Tehama	7	NT	NT	18
Trinity	0	2	NT	NT
Tulare	15	23	528	NT
Tuolumne	0	0	NT	NT
Ventura	6	26	0	11
Yolo	9	61	173	6
Yuba	10	6	23	11
State Totals	**860**	**1,349**	**3,329**	**449**

HUMAN DISEASE SURVEILLANCE

Serological diagnosis of human infection with WNV and other arboviruses was performed at the CDPH Viral and Rickettsial Disease Laboratory (VRDL) and 10 local county public health laboratories. Local laboratories tested for WNV using an IgM or IgG immunofluorescence assay (IFA) and/or an IgM enzyme immunoassay (EIA). Specimens with inconclusive results were forwarded to the VRDL for further testing with a plaque reduction neutralization test (PRNT) or reverse transcriptase-polymerase chain reaction (RT-PCR) assay. Additional WNV infections were identified through testing performed at blood donation centers.

County	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2015 incidence per 100,000 person-years	Ten-year incidence per 100,000 person-years
Alameda	1	0	1	0	1	0	2	0	1	0	0.00	0.04
Alpine	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Amador	0	0	0	0	0	1	0	0	0	0	0.00	0.28
Butte	31	16	6	2	1	3	10	24	24	53	23.63	7.58
Calaveras	0	0	1	0	0	0	0	0	0	0	0.00	0.22
Colusa	4	2	1	0	0	0	3	2	3	1	4.61	7.37
Contra Costa	8	3	4	5	4	3	4	5	5	1	0.09	0.38
Del Norte	0	0	0	0	0	0	0	0	0	0	0.00	0.00
El Dorado	2	0	1	1	0	1	0	1	0	0	0.00	0.32
Fresno	11	17	3	13	23	9	24	8	43	8	0.82	1.64
Glenn	12	7	1	0	2	1	7	9	10	19	66.14	23.67
Humboldt	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Imperial	1	3	0	0	0	0	1	0	1	1	0.55	0.38
Inyo	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Kern	49	140	2	18	15	18	25	25	11	11	1.26	3.59
Kings	1	7	2	3	1	1	3	1	4	0	0.00	1.54
Lake	2	0	0	0	0	0	1	0	1	2	3.08	0.92
Lassen	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Los Angeles	13	36	156	20	4	58	163	151	253	286	2.82	1.12
Madera	0	2	0	1	7	2	3	3	3	4	2.57	1.60
Marin	1	0	0	0	0	0	0	2	0	1	0.39	0.15
Mariposa	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Mendocino	0	2	0	0	0	0	0	0	1	2	2.25	0.56
Merced	4	4	1	4	1	1	13	0	1	1	0.38	1.13
Modoc	2	0	0	0	0	0	0	0	0	0	0.00	2.13
Mono	1	0	0	0	0	0	0	0	0	0	0.00	0.68
Monterey	0	0	0	1	0	0	1	0	0	0	0.00	0.05
Napa	1	1	0	0	0	0	0	1	0	0	0.00	0.21
Nevada	1	0	0	0	0	0	0	0	0	2	2.04	0.31
Orange	6	9	71	4	1	10	42	10	263	92	2.92	1.61
Placer	8	4	6	0	3	1	12	6	7	0	0.00	1.27
Plumas	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Riverside	4	17	62	3	0	7	19	35	14	127	5.50	1.25
Sacramento	15	25	13	0	12	4	29	11	10	4	0.27	0.84
San Benito	0	0	0	0	0	0	0	0	0	0	0.00	0.00
San Bernardino	3	4	36	2	5	4	33	13	21	54	2.57	0.83
San Diego	1	15	35	4	0	0	1	0	11	42	1.30	0.34
San Francisco	0	0	0	0	1	0	1	1	0	0	0.00	0.04
San Joaquin	8	10	12	10	6	5	13	8	9	2	0.28	1.15
San Luis Obispo	1	0	0	0	0	0	0	0	0	0	0.00	0.04
San Mateo	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Santa Barbara	0	0	1	0	0	1	0	1	0	0	0.00	0.07
Santa Clara	5	4	1	0	0	1	0	2	10	8	0.42	0.16
Santa Cruz	0	0	0	0	0	1	0	0	0	0	0.00	0.04
Shasta	4	9	1	0	0	0	1	1	2	3	1.68	1.18
Sierra	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Siskiyou	0	0	0	0	0	0	0	0	0	1	2.22	0.22
Solano	8	1	1	0	0	0	2	1	5	1	0.23	0.44
Sonoma	0	1	0	0	0	0	0	0	0	0	0.00	0.02
Stanislaus	11	21	17	14	12	11	26	17	33	13	2.44	3.29
Sutter	12	3	0	0	0	0	8	10	8	2	2.08	4.48
Tehama	6	4	4	0	0	1	4	5	4	5	7.77	5.13
Trinity	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Tulare	6	10	5	4	12	11	7	5	21	13	2.81	2.03
Tuolumne	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Ventura	3	1	0	0	0	0	7	2	1	6	0.71	0.24
Yolo	27	2	1	2	0	0	10	6	15	8	3.82	3.39
Yuba	5	0	0	1	0	3	4	13	6	10	13.50	5.67
Total WNV Cases	**278**	**380**	**445**	**112**	**111**	**158**	**479**	**379**	**801**	**783**	**2.02**	**1.01**
Asymptomatic Infections	14	29	53	17	20	18	48	54	91	77		
Total WNV infections	**292**	**409**	**498**	**129**	**131**	**176**	**527**	**433**	**892**	**860**	**2.22**	**1.12**

Table 2. Reported West Nile virus human cases by county of residence, and year, California, 2006-2015

A total of 783 symptomatic and 77 asymptomatic infections with WNV were reported in 2015, which was a 3.6% decrease compared to 2014 (Table 2). Of the 783 clinical cases, 585 (75%) were classified as West Nile neuroinvasive disease (i.e. encephalitis, meningitis, or acute flaccid paralysis) and 198 (25%) were classified as West Nile fever. Case-patients were residents of 31 counties and 482 (62%) were male. Incidence was highest (66.1 cases per 100,000 persons) in Glenn County (Table 2, Figure 1). The median ages for West Nile fever and neuroinvasive cases were 58 years (range, 9 to 94 years) and 61 years (range, 5 months to 98 years), respectively. The median age of the 53 WNV-associated fatalities was 75 years (range, 44 to 98 years). Dates of symptom onset ranged from March 19 – December 4, with the peak occurring on week 35 (August 30 –September 5).

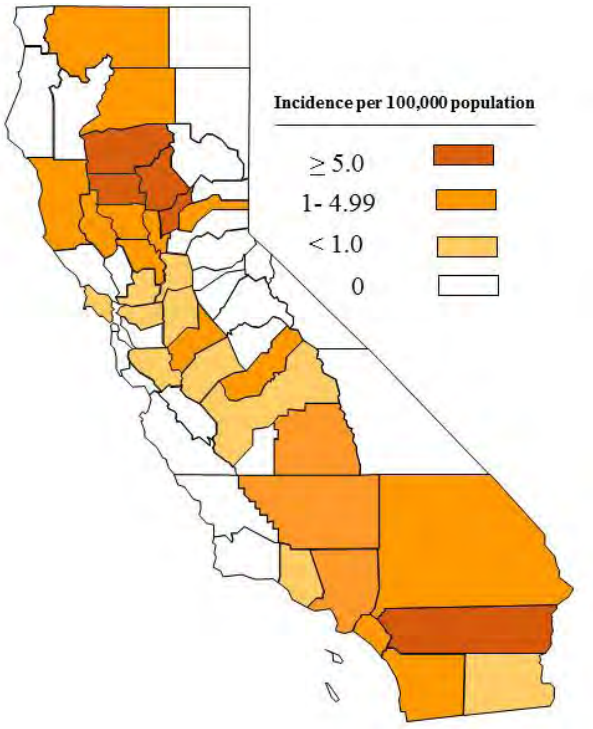

Figure 1. Incidence of human cases of West Nile virus in California, 2015.

MOSQUITO SURVEILLANCE

Mosquito testing was performed at DART and 14 local mosquito and vector control agencies. DART tested mosquitoes for WNV, SLEV, and WEEV using a multiplex real-time (TaqMan) reverse transcriptase-polymerase chain reaction (qRT-PCR). Eleven local agencies tested mosquitoes for WNV only using qRT-PCR or a commercial rapid assay-RAMP® (Rapid Analyte Measurement Platform, Response Biomedical Corp). Three local agencies tested for WNV, SLEV, and WEEV by qRT-PCR. A total of 39,711 mosquito pools were tested from 35 counties: 23,140 pools (652,717 mosquitoes) were tested for WNV, SLEV, and WEEV, whereas 16,571 pools (422,032 mosquitoes) were tested for WNV only. Four pools (48 mosquitoes) of *Ae. aegypti* were also tested for dengue and chikungunya viruses at DART by a separate qRT-PCR.

West Nile virus was detected in 3,329 mosquito pools from 29 counties (Table 3), and SLEV was detected in 38 mosquito pools from the Coachella Valley in Riverside County. Statewide,

the annual minimum infection rate (MIR-defined as the minimum number of infected female mosquitoes per 1,000 tested) of WNV in all mosquitoes tested was 3.1. During the peak transmission period (July – September) the statewide MIR in *Culex* mosquitoes was as high as 4.9 and 13 counties reported MIRs greater than 5.0, the epidemic threshold value (Fig. 2 and Fig. 3) (California Department of Public Health).

County	No. mosquitoes tested	No. mosquito pools tested	WNV + pools	No. flocks	No. chickens	No. WNV positive flocks	WNV + sera
Alameda	4,824	388	16	2	10	0	0
Butte	18,604	392	94	7	46	7	37
Calaveras	0			1	10	0	0
Colusa	0			1	10	1	10
Contra Costa	20,942	603	8	5	50	3	18
Fresno	38,564	928	108	0			
Glenn	3,143	63	21	1	10	1	10
Kern	22,038	584	135	0			
Kings	19,825	549	144	0			
Lake	17,100	660	31	2	12	2	4
Los Angeles	87,141	2,566	294	48	311	27	138
Madera	8,782	349	21	0			
Marin	1,800	105	0	1	6	0	0
Merced	8,723	374	8	8	48	8	23
Monterey	0			2	22	0	0
Napa	4,516	214	0	0			
Nevada	0			4	21	2	4
Orange	159,997	5,179	576	0			
Placer	32,618	2,103	52	2	11	2	8
Riverside	145,746	4,720	158	15	130	6	22
Sacramento	70,098	4,568	164	2	10	2	2
San Benito	0			1	10	0	0
San Bernardino	51,882	2,277	291	9	72	7	36
San Diego	5,256	352	46	3	21	2	12
San Francisco	75	7	0	0			
San Joaquin	77,933	3,147	208	0			
San Luis Obispo	192	5	0	0			
San Mateo	1,125	204	5	3	30	0	0
Santa Barbara	8,885	222	1	5	50	0	0
Santa Clara	8,429	620	20	8	56	2	5
Santa Cruz	3,284	186	0	2	20	0	0
Shasta	16,522	551	48	7	62	4	22
Solano	6,935	207	6	3	36	3	7
Sonoma	15,205	595	12	2	12	0	0
Stanislaus	83,830	2,285	84	2	16	2	9
Sutter	9,503	249	54	6	42	6	36
Tehama	0			3	30	3	18
Tulare	57,449	1,853	528	0			
Ventura	1,327	32	0	5	48	2	11
Yolo	57,602	2,437	173	2	10	2	6
Yuba	4,854	137	23	2	14	2	11
Total	**1,074,749**	**39,711**	**3,329**	**164**	**1,236**	**96**	**449**

Table 3. Results of mosquito and sentinel chicken testing for West Nile virus, California, 2015.

West Nile virus was identified from six *Culex* species *(Cx. erythrothorax, Cx. pipiens, Cx. quinquefasciatus, Cx. stigmatosoma, Cx. tarsalis, and Cx. thriambus)* and *Culiseta incidens* (Table 4); positive pools were collected from March 18 – December 10, with the peak occurring on week 30 (July 26 – August 1). St. Louis encephalitis virus was identified only from *Cx. tarsalis* pools, which were collected from July 8 – October 6 around the north and west shores of the Salton Sea in Riverside County. Four pools were positive for both SLEV and WNV.

***Culex* species**	**Pools**	**No. mosquitoes**	**WNV +**	**MIR**
Cx. erraticus	1	39	0	0.0
Cx. erythrothorax	1,490	56,224	17	0.3
Cx. pipiens	9,755	204,556	470	2.3
Cx. quinquefasciatus	14,267	427,866	1,988	4.6
Cx. restuans	1	18	0	0.0
Cx. stigmatosoma	835	12,331	43	3.5
Cx. tarsalis	12,542	353,833	805	2.3
Cx. territans	1	9	0	0.0
Cx. thriambus	115	427	4	9.4
unknown	3	51	0	0.0
All *Culex*	**39,010**	**1,055,354**	**3,327**	**3.2**

***Anopheles* species**	**Pools**	**No. mosquitoes**	**WNV +**	**MIR**
An. franciscanus	8	47	0	0.0
An. freeborni	218	5,880	0	0.0
An. hermsi	11	255	0	0.0
An. punctipennis	4	11	0	0.0
All *Anopheles*	**241**	**6,193**	**0**	**0.0**

***Aedes* species**	**Pools**	**No. mosquitoes**	**WNV +**	**MIR**
Ae. aegypti	4	48	0	0.0
Ae. dorsalis	9	65	0	0.0
Ae. melanimom	1	10	0	0.0
Ae. squamiger	8	59	0	0.0
Ae. taeniorhynchus	11	486	0	0.0
Ae. vexans	17	599	0	0.0
Ae. washinoi	16	677	0	0.0
All *Aedes*	**66**	**1,944**	**0**	**0.0**

Other species	**Pools**	**No. mosquitoes**	**WNV +**	**MIR**
Culiseta incidens	267	6362	2	0.3
Culiseta inornata	24	254	0	0.0
Culiseta particeps	20	546	0	0.0
Psorophora columbiae	1	11	0	0.0
Unknown	82	4,085	0	0.0
All other	**394**	**11,258**	**2**	**0.2**

Table 4. Mosquitoes tested for West Nile virus, California, 2015.

CHICKEN SEROSURVEILLANCE

In 2015, 35 local mosquito and vector control agencies in 31 counties maintained 164 sentinel chicken flocks (Table 3). Blood samples were collected from chickens every other week and tested for antibodies to WNV, SLEV, and WEEV by an EIA at the CDPH Vector-Borne Disease Section Laboratory (VBDS). Presumptive positive samples were confirmed by IFA or western blot. Samples with inconclusive results were tested by PRNT at the VRDL.

A total of 449 seroconversions to WNV were detected among 96 flocks in 22 counties (Figure 4, Tables 1 and 3), and nine SLEV seroconversions were detected among two flocks near the Salton Sea in Coachella Valley, Riverside County. Statewide, 37% of all sentinel chickens seroconverted to WNV (Figure 5), the highest proportion ever. Seroconversions to WNV occurred from June 4 – November 18, with the peak occurring on week 35 (August 30 – September 5). The first and last SLEV seroconversions occurred August 17 and November 9, respectively.

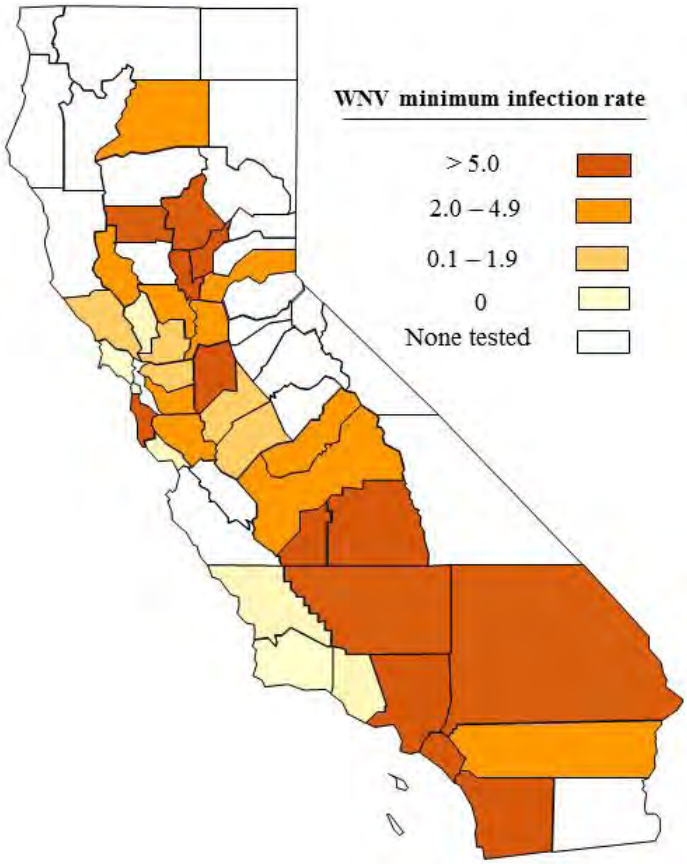

Figure 2. West Nile virus minimum infection rate of *Culex* mosquitoes, by county, California, July – September, 2015. Minimum infection rate defined as the minimum number of infected female mosquitoes per 1,000 tested.

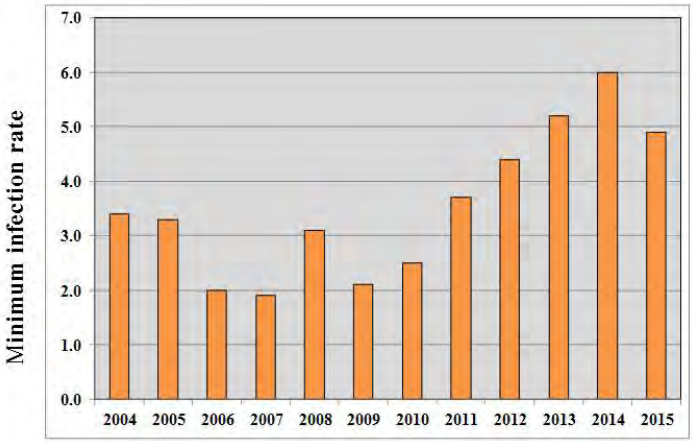

Figure 3. Statewide minimum infection rate of West Nile virus in *Culex* mosquitoes in California, July – September, 2004 – 2015. Minimum infection rate defined as the minimum number of infected female mosquitoes per 1,000 tested.

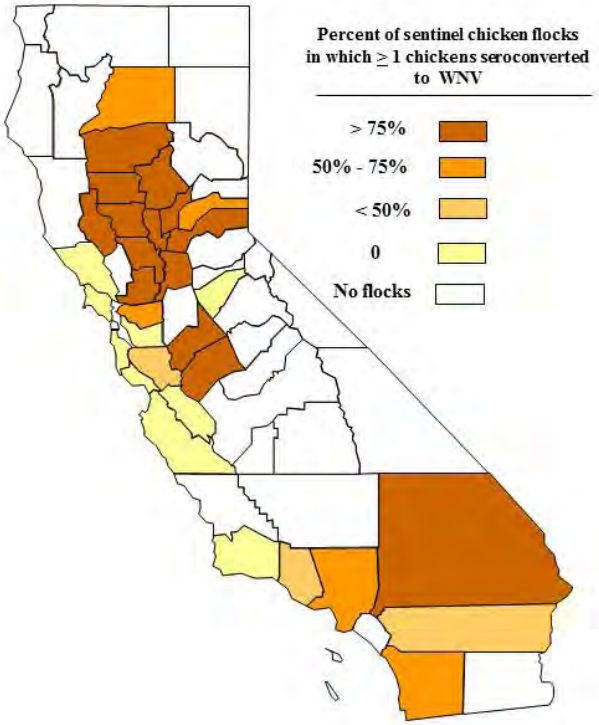

Figure 4. West Nile virus seroconversions in sentinel chicken flocks, by county, California, 2015.

DEAD BIRD SURVEILLANCE

In 2015, the WNV Dead Bird Hotline and website received 10,850 dead bird reports from the public in 56 counties (Table 5). Oral swabs or tissue samples from dead bird carcasses were tested either at DART by qRT-PCR or at one of 15 local agencies by qRT-PCR or RAMP. Of the 3,244 carcasses deemed suitable for testing, WNV was detected in 1,349 (42%) carcasses from 37 counties; 1,297 by qRT-PCR and 52 by RAMP (Figure 6, Tables 1 and 5). Statewide, a lower proportion of birds tested positive for WNV compared to the last three years, but the infection prevalence exceeded 50% in 14 counties (Figures 6 and 7, Table 5). Positive birds were collected almost every month of the year, from February 3 – December 29, with the peak occurring on week 35 (August 30 – September 5). The first and last WNV positive birds were an American crow and red-shouldered hawk, collected from Alameda and San Diego counties, respectively.

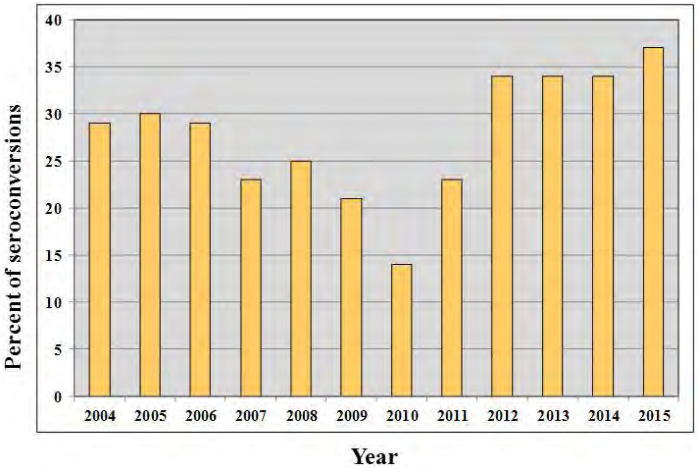

Figure 5. Percentage of sentinel chickens that seroconverted to West Nile virus in California, 2004-2015.

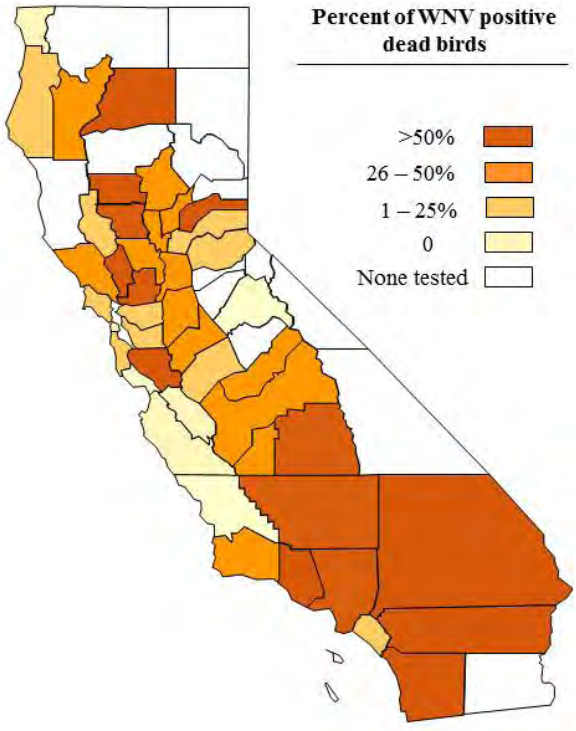

Figure 6. West Nile virus infection prevalence in dead birds, by county, California, 2015.

County	Reported	Tested	Positive	(%)
Alameda	494	82	19	(23.2)
Alpine	0			
Amador	14	0		
Butte	322	82	38	(46.3)
Calaveras	18	0		
Colusa	8	4	3	(75.0)
Contra Costa	914	49	11	(22.5)
Del Norte	3	1	0	
El Dorado	136	26	4	(15.4)
Fresno	186	10	3	(30.0)
Glenn	16	5	4	(80.0)
Humboldt	33	9	2	(22.2)
Imperial	3	0		
Inyo	3	0		
Kern	58	1	1	(100)
Kings	20	7	3	(42.9)
Lake	54	21	5	(23.8)
Lassen	6	0		
Los Angeles	1,096	164	103	(63.8)
Madera	14	3	1	(33.3)
Marin	170	14	3	(21.4)
Mariposa	9	0		
Mendocino	20	0		
Merced	114	13	3	(23.1)
Modoc	7	0		
Mono	9	0		
Monterey	37	5	0	
Napa	77	11	7	(63.6)
Nevada	55	17	10	(58.8)
Orange	217	422	83	(19.8)
Placer	205	191	21	(11.0)
Plumas	4	0		
Riverside	358	110	82	(74.6)
Sacramento	1,045	363	103	(28.4)
San Benito	15	2	0	
San Bernardino	264	41	25	(61.0)
San Diego	438	550	355	(64.6)
San Francisco	109	15	0	
San Joaquin	279	68	18	(26.5)
San Luis Obispo	41	1	0	
San Mateo	626	158	23	(14.6)
Santa Barbara	71	8	3	(37.5)
Santa Clara	1,390	357	231	(64.7)
Santa Cruz	125	43	0	
Shasta	99	26	16	(61.6)
Sierra	0			
Siskiyou	5	0		
Solano	177	14	10	(71.4)
Sonoma	281	36	11	(30.6)
Stanislaus	318	44	14	(31.8)
Sutter	102	43	16	(37.2)
Tehama	27	0		
Trinity	10	6	2	(33.3)
Tulare	119	44	23	(52.3)
Tuolumne	11	2	0	
Ventura	254	36	26	(72.2)
Yolo	310	124	61	(49.2)
Yuba	54	16	6	(37.5)
Totals	**10,850**	**3,244**	**1,349**	**(41.6)**

Table 5. Dead birds reported, tested, and positive for West Nile virus, California 2015.

CONCLUSIONS

In 2015, 783 human WNV disease cases were reported from 31 counties, which was the third highest number of cases ever reported since the virus was first introduced to California in 2003. Notably, 585 neuroinvasive disease cases and 53 fatalities were reported. The proportion of WNND cases among all reported cases was 75%, suggesting that up to 41,000 non-neuroinvasive cases may have occurred, but were not clinically diagnosed, laboratory confirmed, and reported (Centers for Disease Control and Prevention, 2010). The annual incidence rate for the state was 2.0 cases per 100,000 persons. Counties with the highest case incidence included Glenn, Butte, Yuba, and Tehama in the northern region, and Riverside County in the southern region (Figure 1). The highest numbers of cases were reported from Los Angeles and Riverside counties (Table 2).

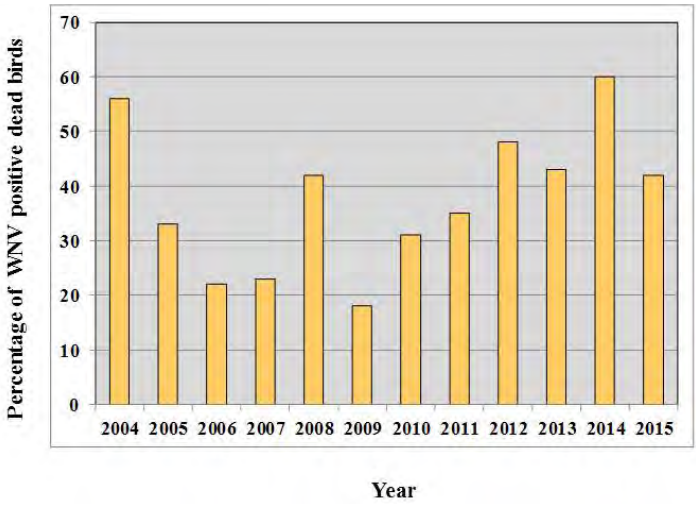

Figure 7. Percentage of WNV infection in dead birds in California, 2004-2015.

Surveillance data from dead birds, mosquitoes and sentinel chickens documented high levels of WNV virus activity in southern California and in several north central valley counties, areas which subsequently reported a higher incidence of human disease compared to other regions. St. Louis encephalitis virus re-emerged in the Coachella Valley (Riverside County) after 11 consecutive years of no documented activity throughout the state. The disappearance of SLEV was notable, because prior to the introduction of WNV in 2003, SLEV activity had been detected in California every single year since 1969. Furthermore in 2015, an SLEV outbreak occurred in Maricopa County, Arizona, with 19 human cases reported, which suggests the virus may be re-emerging in the southwestern United States. No human SLE cases were reported in California during 2015, but positive mosquito pools and sentinel chicken seroconversions were detected along the northern and western shores of the Salton Sea. Interestingly, WNV was also detected in this area, demonstrating that the two similar flaviviruses can co-circulate in a given area.

Although the ecological surveillance data documented WNV activity throughout the year, most WNV detections occurred from June through October, with peak activity at the end of August. The rise in human WNV infections followed the rise in ecological indicators, which highlights the importance of early environmental surveillance to determine the level of risk for WNV transmission and to direct mosquito control efforts. In addition, the re-discovery of SLEV in California demonstrates the continued need for surveillance for other arboviruses. Ongoing drought conditions and record high temperatures in California may alter the ecology of endemic arboviruses and contribute to the establishment of new mosquito-borne diseases.

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Greater Los Angeles County Vector Control District Agency Update 2015

Truc Dever, Greater Los Angeles County Vector Control District

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ABSTRACT: The Greater Los Angeles County Vector Control District has been battling invasive *Aedes* species mosquitoes since 2011. The trials and tribulations of invasive *Aedes* surveillance and control led the District to explore new control strategies and options. In 2015, the District partnered with University of Kentucky researchers to conduct a Wolbachia-infected male mosquito release pilot program for the control of *Aedes albopictus* in the San Gabriel Valley. This presentation will cover initial results and lessons learned from this pilot program and the impact of invasive *Aedes* control on the District and its resources.

Santa Clara County Vector Control District 2015: A Year in Review

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Santa Clara County Vector Control is somewhat unique in California by having prevention and control programs for both mosquito and other vectors plus a wildlife education program. In 2015, West Nile virus (WNV) activity in the county was less than the2014 peak activity, with 237 dead bird, 20 mosquito pool, 5 sentinel chicken, and 8 human WNV positives. Fogging operations to reduce WNV infected mosquitoes were down from 19 in 2014 to 10 in 2015, but mosquito and virus activity in 2015 persisted into late October. In 2015, the agency hosted its first Open House during West Nile virus Prevention Week. Further outreach activities were the addition of tick bite and West Nile virus prevention videos at DMV offices, a CBS video online, mosquito fogging outreach to the homeless, and a complete rewriting of district informational handouts.

Analysis of the 2015 Program Year for the San Joaquin County Mosquito and Vector Control District

Eddie Lucchesi, Manager, District Staff, and Board of Trustees

San Joaquin County Mosquito and Vector Control District, 7759 South Airport Way, Stockton, CA 95206

ABSTRACT: In the 2015 mosquito control year the District was faced with many challenges that included a large abundance of vector mosquito species and the associated increased prevalence of West Nile Virus (WNV) activity, drought conditions and its effects on mosquito control, removal of sludge build up from the bottoms of the District's mosquito fish rearing ponds that had accumulated over a period of twenty years, and the laborious effort to sell District surplus real property. This paper will discuss the comprehensive activities the District faced during the 2015 program year. Steps were taken to sell the District's former northern office location. Enhanced control efforts were employed to reduce a record number of vector mosquito species and to minimize the threat of WNV transmission to our human population. Accumulated sludge was mechanically removed from the ponds at the District's White Slough mosquito fish rearing facility to reduce fish mortality during seining operations. The District also was faced with drought-related conditions that presented unforeseen challenges related to deteriorated water quality and the necessity to educate our public that extraordinary mosquito abundance and virus activity could occur, even though counter intuitive to what most people would expect during a dry year. This paper takes into account the actions of management, staff, and the Board of Trustees to successfully accomplish the desired resolutions during the 2015 Program Year.

Shasta MVCD: 2015 Year in Review

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ABSTRACT: During the 2015 Program year, the Shasta Mosquito and Vector Control District (SMVCD) continued efforts in the control and surveillance of vectors and vector borne diseases. SMVCD was first formed in 1919 and covers approximately 1,100 square miles in Northern California charged with the mission "To protect the public's health from vector-borne disease and nuisance, through a comprehensive mosquito and vector control program focused on innovation, experience and efficiency." Full time staffing levels remain at 15, with five seasonal employees hired annually. In 2015, the District conducted normal larval surveillance and control, ground-based Ultra Low Volume (ULV) adult mosquito control applications, biological control, physical control activities and outreach. The District is now in the third year of our indoor *Gambusia affins* rearing and is seeing positive outcomes like the ability to plant fish earlier and a more stable supply of fish for the season. SMVCD's physical control program consists of vegetation management projects and ditching projects, in addition to plan approval on new housing developments within the District boundaries. Our tick program collected 299 pools of *Ixodes pacificus*, of which 7 samples were positive for *Borrelia burgdorferi* and 12 samples were positive for *Borrelia miyamotoi*. SMVCD manages 44 fixed Encephalitis Virus Surveillance (EVS) traps every week and sets additional 10-15 "floater" EVS traps as needed. The District continued to see mosquito abundance above the 5 year average and West Nile virus (WNv) positives greater than average in 2015. In 2016 the District plans to begin a laboratory remodel and expansion, expand the invasive *Aedes* surveillance and increase our operational research capacity.

ACKNOWLEDGEMENTS: I would like to thank the staff and board of the Shasta Mosquito and Vector Control District for providing the support and hard work to make 2015 a successful year.

Mosquito Control in Rice at Placer MVCD: a Five Year Review

Joel Buettner, General Manager

Placer Mosquito and Vector Control District

The challenges of managing the risk to the public posed by mosquitoes associated with rice agriculture in California have been present since the first rice seed was planted. Over the past five years, the Placer Mosquito and Vector Control District has conducted a progressive and adaptive assessment of mosquito control techniques on approximately 15,000 acres of conventional and organic rice in western Placer County. Among the challenges faced were difficulty in assessing larval abundance in rice fields, immigration of mosquitoes from areas outside District boundaries, cost of treatment, and spatially interspersed organic and conventional rice fields. The guiding principles used to make control decisions during this period were 1) minimize risk to people, 2) focus control on the time, place and mosquito life stage which are most vulnerable to the technique utilized, and 3) maximize the use of resources for the most benefit. These principles, paired with a foundation of sound integrated pest management, allowed us to evaluate methods and metrics for determining treatment thresholds and timing, optimization of larvicide program, and efficacy and optimization of aerial adulticide operations.

West Nile Virus Surveillance in San Mateo County, California: 2013 – 2015

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ABSTRACT: San Mateo County Mosquito and Vector Control District updated its program to detect and operationally respond to West Nile Virus (WNV) during the past 3 years. In response to WNV activities, intensive larval habitat inspection, reduction and treatment, and ground ULV applications for adult control in selected areas were conducted during the active transmission season. During the last 3 years, 8,433 samples consisting of birds, mosquitoes, and squirrels were collected from 21 incorporated and unincorporated cities of San Mateo County and resulted in 72 positive detections of WNV. This paper discusses the WNV activities and the efficacy of adulticide applications in 2013 – 2015.

INTRODUCTION

The San Mateo County Mosquito and Vector Control District (SMCMVCD) comprises approximately 448 square miles with 750,000 residents within its borders. Eastern San Mateo County is mostly populated with urban/suburban habitats and has a variety of residential mosquito larval habitats. The western part of the county is less populated with a mostly rural environment. SMCMVCD was created to provide mosquito control to county residents and protect them from vector-borne diseases. The District's WNV response plan encompasses disease surveillance, public outreach and education, and larval and adult mosquito surveillance and suppression programs. An important element of the plan is accurate and timely surveillance to help guide district efforts during the WNV season. The district employs an integrated arboviral disease surveillance system including sero-surveillance (sentinel chickens), mosquito trapping and testing of four important vectors of WNV (*Culex pipiens, Cx. tarsalis, Cx. erythrothorax* and *Cx. stigmatosoma*), collecting and testing dead birds and squirrels, and monitoring California Department of Public Health (CDPH) reports for WNV infections in animals and humans. WNV activity in California began in 2003 and was limited to few southern counties. In 2004 and 2005 activity quickly spread in every county of the state. The current paper discusses the WNV activity in San Mateo County during the previous three years, describes the detection of WNV by various surveillance methods, and discusses differences among 2013, 2014 and 2105.

MATERIALS AND METHODS

Dead birds/Squirrel Surveillance: Dead birds or squirrels were reported throughout the year by the public via California Vector-Borne Diseases section (VBDS) dead bird hotline. Corvids, including the American crow and common raven, were sampled by oral swabs using Dacron-tipped disposable swabs. Other eligible bird species were sampled by bilateral intraocular cocktail (Lim et al. 2013). Samples were tested for the presence of WNV by reverse transcriptase polymerase chain reaction (RT-PCR). Dead squirrels were sent to California Animal Health and Food Safety (CAHFS) laboratory for necropsy and later testing by qRT-PCR by the Center for Vector borne Diseases (CVEC). When a bird or squirrel tested positive, the district set 20 EVS traps within a half mile radius of the positive bird or squirrel location (Figure 1). Address and GPS coordinates were recorded for each animal so that the data could be mapped later.

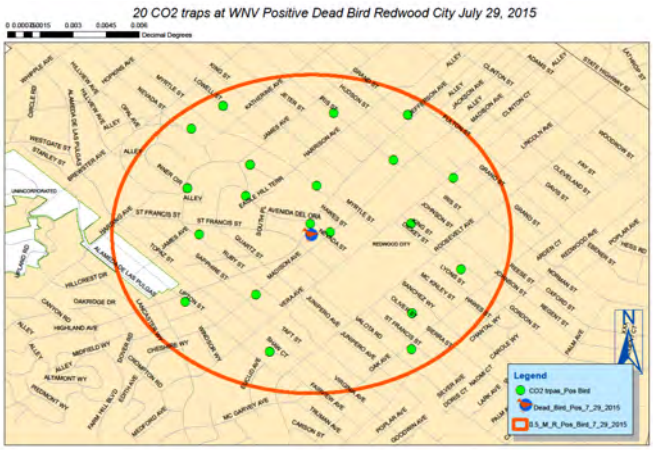

Figure 1. 0.5 Mile radius of one positive WNV dead bird with 20 CO_2 traps in Redwood City, San Mateo County

Mosquito Surveillance: Mosquitoes were collected weekly from a total of 40 EVS/CO_2 traps operated throughout the district. An additional 20 EVS traps as recommended by Gu and Novak (2004) were placed proximal to recent WNV positive detections in birds or squirrels (Figure 1). Adult mosquitoes were euthanized using trimethylamine, sorted under a dissecting microscope, and prepared for testing for WNV RNA by RT-PCR. When WNV-infected mosquitoes were detected, the district used a ground Ultra-Low-Volume (ULV) truck-mounted application to control adult mosquitoes.

Sentinel Chicken Flocks: The district maintained three sentinel flocks of ten chickens each in open air cages that allowed easy access by mosquitoes. The chickens were bled on a biweekly schedule established by the California Department of Public Health (CDPH) starting in April and ending in November. Chickens were bled onto filter paper which was shipped to CDPH for enzyme immunoassay (EIA) testing for SLEV, WEEV and WNV antibodies (Figure 2).

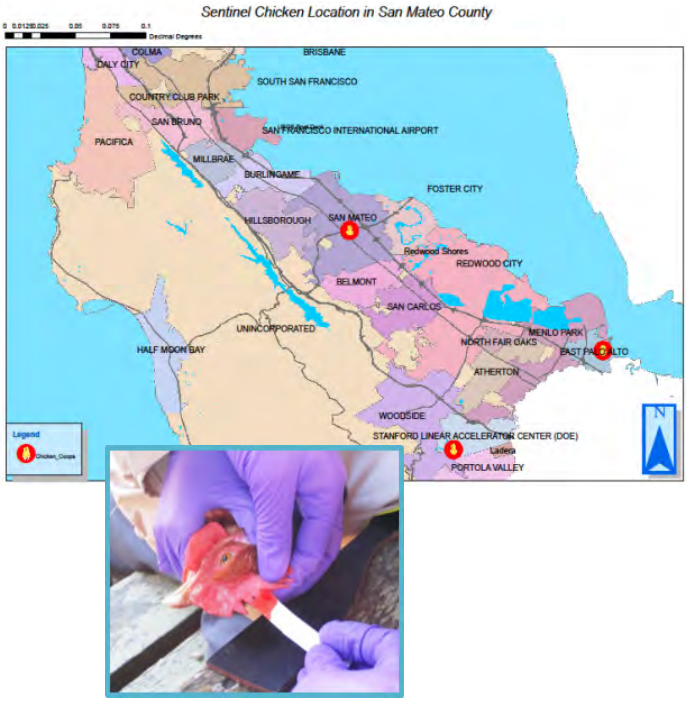

Figure 2. Sentinel Chicken flocks location in San Mateo County, biweekly bleeding

Adulticide: When surveillance detected WNV-infected mosquitoes, the district used ground ULV truck mounted adulticide applications to control adult mosquitoes. Two adulticides Pyrenone 25-5 and Zenivex E4were used as control for positive WNV mosquitoes. Areas were delineated based on points consisting of WNV positive mosquito detections and a circle with a 0.5 mile radius was plotted using the buffer feature of ArcGIS (Figure 3).

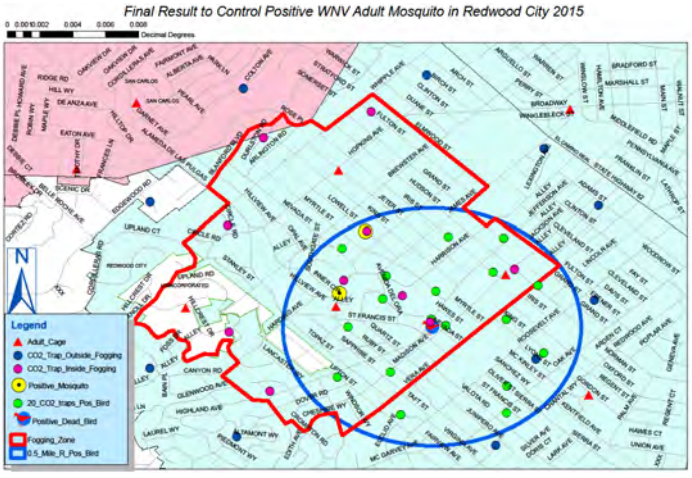

Figure 3. Positive WNV mosquito, fogging area, adult cages, and per/post CO_2 traps

Fogging Procedure: To evaluate the efficacy of the ULV technique on adults in the target area the district used sentinel adult mosquitoes in cages (Rathburn et al. 1969). The body of the cage is constructed from PVC rings. An inner ring is 6″ inside diameter with 2″ wide wall thickness. A ¾″ hole is punched in the center of the inner ring. Nylon net approximately 18x18 mesh is cut to size and held over the ends of the wide ring using adhesive glue (Figure 4). About 25 *Cx. pipiens* female mosquitoes 6 to 8 days old from laboratory colonies were used in each exposure cage. Mosquitoes are gently aspirated into the cage and the hole is plugged with cork which had raisons attached to the cork as a sugar source. Cages were attached to the cross arm of a 2m (6ft) PVC post (Figure 4). All cages were placed at the target area two hours before the fogging, and were transferred to the lab after fogging to count the number of live mosquitoes in each cage. In the field, temperature, relative humidity, wind velocity, and wind direction were recorded. The total of 8 adult mosquito cages were set for each fogging, 4 cages outside the fogging zone served as controls and 4 cages inside the treated zone. Efficacy was calculated based on Abbott's formula. To calculate the mosquito population reduction as a result of fogging, the district set up 20 EVS traps inside and outside the spray zone before and after treatment. These mosquitoes also were pooled and tested for WNV by RT-PCR. The percentage reduction in adult mosquito was calculated using the formula of Mulla et al. (1971).

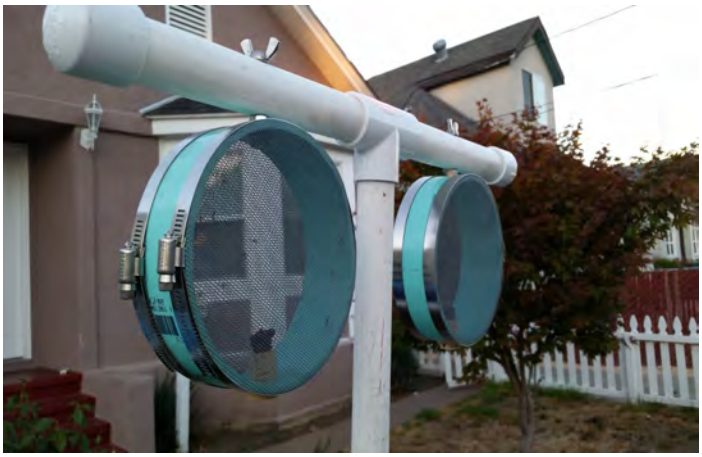

Figure 4. Sentinel adult cages attached to PVC

Upon receiving test results for WNV, mosquito, bird, squirrel and sentinel chicken data were entered into the database and exported to a geographic information system (GIS; ArcGIS 10.2, ESRI, Redland, CA) for assessment of WNV risk level, printing for website and outreach maps, staff work assignments, and ultimately designation of adulticide application zones (Figure 3).

Data statistical analysis: The results of mosquito bioassay cages were reported as percentage mortality. In cases where the mortality in the control of adulticidal tests ranged from 5 – 20%, the observed percentage mortality (efficacy of materials) was corrected by Abbott's formula (Abbott 1925) as applied by Mulla et al. (1971):

% Mortality (M) = (X – Y) / (100 – Y)*100

X = % Mortality at treatment

Y = % Mortality at control

To calculate the percentage reduction in pre and post treatment traps we used Mulla's Formula, where:

% Reduction = 100 – [(C1 x T2)/(C2 x T1)] X100

C1 = Pre-treatment density in control sites

C2 = Post-treatment density in control sites

T1 = Pre-treatment density in treated sites

T2 =Post-treatment density in treated sites

RESULT AND DISCUSSSION

The SMCMVCD first detected WNV in July 2004 and by the end of year 14 dead birds and one squirrel tested positive. In 2013 five of 66 dead birds tested positive for WNV, and the number positive was highest in June and August. In 2014 and 2015 WNV activity was higher than 2013, and total of 183 and 224 dead birds were tested of which 21 and 22 dead birds were positive for WNV, respectively (Figure 5).

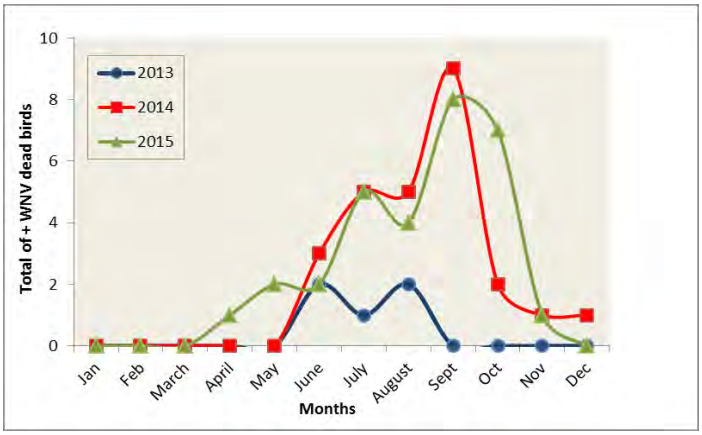

Figure 5. Total number of positive dead birds per month in San Mateo County (2013 – 215)

The abundance of *Culex* mosquitoes during the 2013 season peaked in May with 28.1 females/trap night compared to the 2014 and 2015 season when abundance peaked in August with 29.5 and 27.3 females/trap night, respectively. Table 1 summarizes the numbers of mosquitoes collected, mosquito pools tested, pools positive for WNV and the minimum infection rate per year (MIR) during three years surveillance. In June 2013 the first mosquito pool tested positive for WNV, in 2014 mosquito pools in July and August had higher number virus detections compared to 2015. During the three years of surveillance, the highest numbers of mosquito infections were detected during the month of August (Figure 6). The percentage of WNV positive pools in *Cx. tarsalis* was higher than *Cx. pipiens* in all three years of surveillance (Table 1).

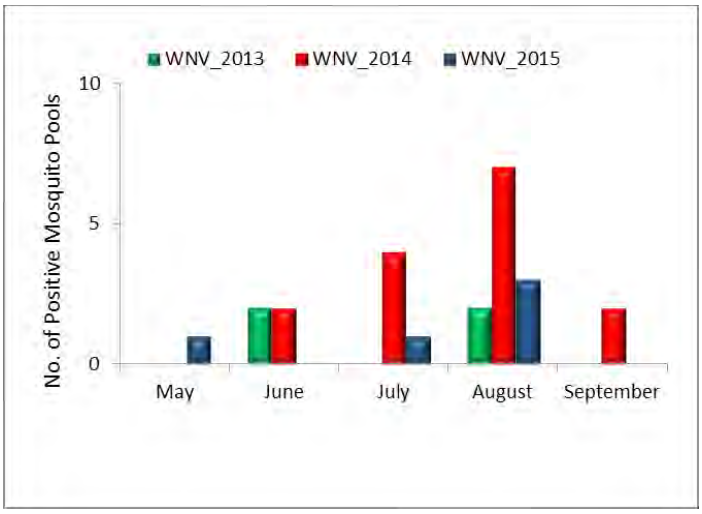

Figure 6. Number of WNV positive mosquitoes per month in San Mateo County (2013 – 2015)

Three flocks of sentinel chickens were maintained within the county as part of the California statewide encephalitis virus surveillance program, but none of the chickens seroconverted to WNV during these three years.

	2013	2014	2015
Culex spp.*	2088	3522	2100
Cx. pipiens	1878	3189	1840
Cx. tarsalis	197	316	240
Cx. stigmatosoma	0	11	12
Cx. erythrothorax	13	6	8
Pools Tested	458	705	387
Positive Pools	4	15	5
Cx. pipiens	2 (0.11%)	11 (0.35%)	2 (0.11%)
Cx. tarsalis	2 (1.02%)	4 (1.27%)	3 (1.25%)
MIR**	1.9	4.3	2.3

* Four Culex spp. Tested.

** Minimum infection rate.

Table 1. MIR and number of mosquito tested for WNV in San Mateo County (2013 – 2015)

During 2013 to 2015 in District used adulticide applications 14 times in designated WNV active areas.Response time for applications for adult mosquito suppression averaged 1 to 4 days. Table 2 showed the efficacy of materials (sentinel mortality) and mosquito population reduction (EVS trap results) using the application of Pyrenone 25-5 or Zenivex E4 adulticides.

In summary during the last three years, 8,433 samples of birds, mosquitoes, and squirrels were tested from 21 cities and unincorporated regions of San Mateo County and 72 were positive for WNV. The ULV application achieved the reduction of the mosquito populations in the treated areas. American crows and western scrub jays comprised about half and one-third of the

total positive dead birds, respectively. When surveillance detected WNV-infected mosquitoes, the district implemented a series of control measures such as warning and educating the public about risk and the need to exercise personal preventive measures, identifying and treating hot spots of infected mosquitoes, and applying adulticides by ULV truck mounted equipment.

Material	Date	City	Efficacy*	% Reduction**
Pyrenone 25-5	September 05	Menlo Park	N/A	86.31%
Pyrenone 25-5	June 19	San Mateo	82.91	77%
Zenivex E4	July 21	San Mateo	97.20	98.88%
Zenivex E4	July 21	Portola Valley	90.23	99.01%
Zenivex E4	July 28	San Mateo	95.06	78.59%
Zenivex E4	July 30	San Mateo	96.41	99.51%
Zenivex E4	August 11	Menlo Park	93.63	86.47%
Zenivex E4	August 19	S. San Francisco	99.54	77.90%
Zenivex E4	August 24	E Menlo Park	N/A	98.00%
Zenivex E4	September 02	Redwood City	98.74	88.79%
Zenivex E4	September 18	Foster City	99.49	90.72%
Zenivex E4	August 02	Menlo Park	94.56	93.18
Zenivex E4	August 05	Redwood City	77.21	74.72
Zenivex E4	September 02	Menlo Park	96.71	81.45

* Efficacy was calculated based on Abbott's formula.

** Formula of Mulla et al. 1971.

Table 2. Efficacy of ULV adulticide and mosquito reduction in San Mateo County

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Comparison of RNA Extraction Methods for Detection of Virus in Mosquitoes

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ABSTRACT: The isolation of viral RNA from mosquitoes and its analysis using quantitative reverse transcription polymerase chain reaction (qRT-PCR) is becoming increasingly important for arbovirus surveillance and evaluating risk reduction practices. We evaluated the quantity and quality of West Nile virus (WNV), Saint Louis encephalitis virus (SLEV), and Western equine encephalitis virus (WEEV) RNA that was isolated in the presence of adult mosquitoes using two commercial kits (RNeasy Mini Kit columns (Qiagen) and MagMAX Viral RNA Isolation Kit (Thermo Fisher Scientific)). The RNA that was eluted from the MagMAX Kit contained a brown-colored precipitate that increased with the number of mosquitoes that were included in the sample, as measured by the absorbance at 525 nm. Clarification of RNA eluted with the MagMAX Kit using centrifugation reduced the absorbance of the RNA solution relative to the unclarified samples, with the greatest reductions of 5- and 8-fold for samples containing 25 or 50 mosquitoes, respectively. However, there was no significant difference in the concentration of RNA that was detected before and after clarification. The clarified samples produced significantly lower cycle threshold (Ct) values when the RNA was assayed for WNV, SLEV and WEEV RNA using qRT-PCR. The Ct values generated from samples isolated using the MagMAX Kit were significantly lower than those of samples isolated with RNeasy Mini Kit columns (Ct difference of 1.01 +/- 0.28, range of 0.542 – 1.882), suggesting that MagMAX method may be more sensitive for use in arbovirus surveillance by vector control agencies.

INTRODUCTION

Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) is employed to analyze low quantities viral RNA that is isolated from mosquitoes for estimating the prevalence of arboviruses and intensity of infection in mosquitoes (Brault et al. 2015). Two solid phase extraction technologies predominate for isolating RNA: silica gel membranes (GM) or silica conjugated to magnetic particles (MP). Both technologies employ relatively simple methods that rapidly yield nucleic acid for use in qRT-PCR assays. Comparison of these technologies showed that when RNA was isolated from West Nile virus (WNV)-infected mosquitoes using MP and analyzed using qRT-PCR, lower cycle threshold (Ct) values were produced relative to duplicate samples isolated using GM (Fang et al. 2010), demonstrating that MP is more sensitive than GM. However, the effect of mosquito number in a sample, and the relative quantity or quality of the isolated RNA has not been evaluated in a study that compares GM- and MP-based methods. Herein, we compared arbovirus RNA isolated in the presence of multiple numbers of adult mosquitoes per sample (0, 1, 5, 10, 25, or 50) using GM from the RNeasy Mini Kit (Qiagen) to the RNA eluted from MP in the MagMAX Viral RNA Isolation Kit (Thermo Fisher Scientific). The quantity and purity of the isolated RNA was evaluated, as was its suitability for use in multiplex TaqMan-based qRT-PCR assays that detect arboviruses.

METHODS

Adult mosquitoes were collected using CDC EVS CO_2 traps, *Culex erythrothorax* separated on a chill table (BioQuip Products, Compton, CA) using a dissection microscope, and frozen at -80 ^oC until use. *Cx. erythrothorax* mosquitoes, inactivated virus (WNV, SLEV and WEEV; provided by the Davis Arbovirus Research and Training Lab, Davis CA) and a glass bead were added to lysis buffer included with the RNeasy Mini Kit (Qiagen, Hilden, Germany) or MagMAX Viral RNA Isolation Kit (Thermo Fisher Scientific, Waltham, MA). Tubes containing 0, 1, 5, 10, 25 or 50 mosquitoes were homogenized with each lysis buffer using a 'bead beater' (45 s; Biospec Products, Bartlesville, OK) to assess the impact of RNA isolation method and number of mosquitoes in a tube (n = 3 replicates per treatment). Samples were subsequently centrifuged (21,000 x g, 1 m) and the RNA isolated from the supernatant using RNeasy columns with a vacuum manifold (Qiagen) followed by centrifugation for the final wash and RNA elution, or using the MagMAX Kit with a MagMAX Express instrument (Applied Biosystems, Foster City, CA), as described by the manufacturers. Identical sample and RNA elution volumes were used for each sample. Half of each eluted RNA sample was subsequently clarified with centrifugation to remove residual precipitates from the isolated RNA (21,000 x g, 4 m). The optical density at 525 nm, the ratio of absorbance at 260 and 280 nm, and RNA concentration of the samples were measured using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific), before and after clarifying. Triplex TaqMan qRT-PCR was used to assess the relative quantity of WNV, SLEV and WEEV in each sample. Briefly, 2 µl or 10 µl of each elution was added to TaqMan Fast Virus 1-Step Master Mix (Thermo Fisher Scientific; final volume of 20 µl), with the TaqMan primers and probes described in Brault et al. (2015); primer and probe concentrations were for WNV: 600 nM primers, 150 nM probe (FAM-QSY); SLEV: 800 nM primers, 200 nM probe (VIC-QSY); WEEV: 900 nM primers, 250 nM probe (ABY-QSY)), and analyzed using a QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific) using the following amplification protocol: 48.0 ^oC for 15 m, 95 ^oC for 30 s, and 40 cycles of 95 ^oC for 3 s with 60.0 ^oC for 30 s.

RESULTS

Visual inspection of the eluted RNA solutions from the MagMAX system showed a brown precipitate that was absent from the samples eluted from the RNeasy columns (not shown). To evaluate the extent of brown precipitate in the eluted RNA samples, the absorbance at λ_{max} (525 nm) was measured for each, before and after clarifying the samples. The absorbance of the 25 and 50 mosquitoes per tube samples were significantly greater for those isolated with MagMAX than the RNeasy column samples (0.507 +/- 0.030 for MagMAX samples and 0.01667 +/- 0.0025 for RNeasy column samples containing 50 mosquitoes; Paired t tests, $P < 0.011$). There was no significant difference in the absorbance of eluted RNA for samples that contained 0, 1, 5 or 10 mosquitoes (Unpaired t tests, $P > 0.05$; not shown). Using centrifugation to clarify the RNA solutions that were eluted using the MagMAX Kit significantly reduced their absorbance by an average of 0.327 +/- 0.056 for samples with 25 mosquitoes, and 0.413 +/- 0.020 for those containing 50 mosquitoes (8.39-fold and 5.37-fold reductions, respectively; Two-way ANOVA, $P < 0.0001$). We next sought to determine whether the presence of the brown precipitate affected RNA concentration or quality. There was no significant difference in RNA concentration before and after clarification of the same sample (Two-way ANOVA, RNeasy $P = 0.826$, MagMAX $P = 0.879$; not shown). For samples containing equivalent numbers of mosquitoes and isolated using RNeasy columns or MagMAX, there was no significant difference in the concentration of eluted RNA when the samples contained 0 to 10 mosquitoes (Multiple paired t tests, $P > 0.1$; not shown). However, when there were 25 or 50 mosquitoes in a sample, there was significantly less RNA in samples isolated using RNeasy columns relative to those from MagMAX (Unpaired t test, $P < 0.01$), with 45 % less RNA in the elutions from samples that contained 50 mosquitoes (not shown). RNA quality, as measured by the ratio of absorbance at 260 and 280 nm, was high for all samples (260/280 = 2.15 ± 0.147; not shown). The amplification efficiency of WNV, SLEV and WEEV in each sample, before and after clarification, was subsequently evaluated using triplex qRT-PCR (2 µl and 10 µl of each RNA sample was analyzed). Because differences in the Ct values among treatments were similar for WNV, SLEV, and WEEV Taqman assays, only results for WNV are described. The Ct values from TaqMan assays with 10 µl of eluted RNA were significantly lower relative to assays with 2 µl of RNA (Two-way ANOVA, $P < 0.001$; Figure 1). Similarly, the Ct values for samples assayed after clarification were significantly lower compared to those analyzed with TaqMan before clarification (Two-way ANOVA, $P < 0.001$; Figure 1). Within an isolation method (e.g. clarified MagMAX samples), the Ct values of samples containing no mosquitoes were always higher than samples that contained mosquitoes (Figure 1). Moreover, the Ct values from samples that did not contain mosquitoes were significant outliers of the others within an isolation method (Grubb's test, $P < 0.05$), with the exception of samples isolated using MagMAX and not clarified (Grubb's test, $P > 0.05$). Within each isolation method, there was no significant difference in the Ct values for samples containing 1, 5, 10, 25 or 50 mosquitoes (Multiple t tests, $P > 0.1$). The Ct values from samples isolated using MagMAX were significantly lower than those from RNA isolated using RNeasy columns, even when samples that did not contain mosquitoes were excluded from the analysis (Two-way ANOVA, $P < 0.0001$; Figure 1).

DISCUSSION

Clarification of the eluted RNA using centrifugation did not affect RNA concentration, but did reduce the quantity of brown precipitate in the eluted RNA samples, and improved the detection of arbovirus RNA using qRT-PCR. Increasing the quantity of eluted RNA in the qRT-PCR assay from 2 µl to 10 µl improved the sensitivity for detecting arbovirus RNA. Inclusion of 1 mosquito in the sample improved arbovirus detection, suggesting that cellular biomolecules such as mRNA may interact with arbovirus RNA to enhance its isolation or detection with qRT-PCR. Increasing the number of mosquitoes in a sample tube (*i.e.* mosquito pool) from 1 to 50 did not significantly affect the amplification of WNV, SLEV or WEEV in the qRT-PCR assay. Because the Ct values from RNA samples isolated using RNeasy columns were higher than the Ct values for samples isolated using MagMAX magnetic beads, use of the later method may increase the sensitivity of arbovirus detection in mosquitoes.

ACKNOWLEDGEMENTS

We thank Dr. Jan Washburn (Alameda County Mosquito Abatement District) for discussions of the project design and data analysis, and Dr. Gregory White (Coachella Valley Mosquito and Vector Control District) for guidance on the primer and probe concentrations used for the qRT-PCR assays.

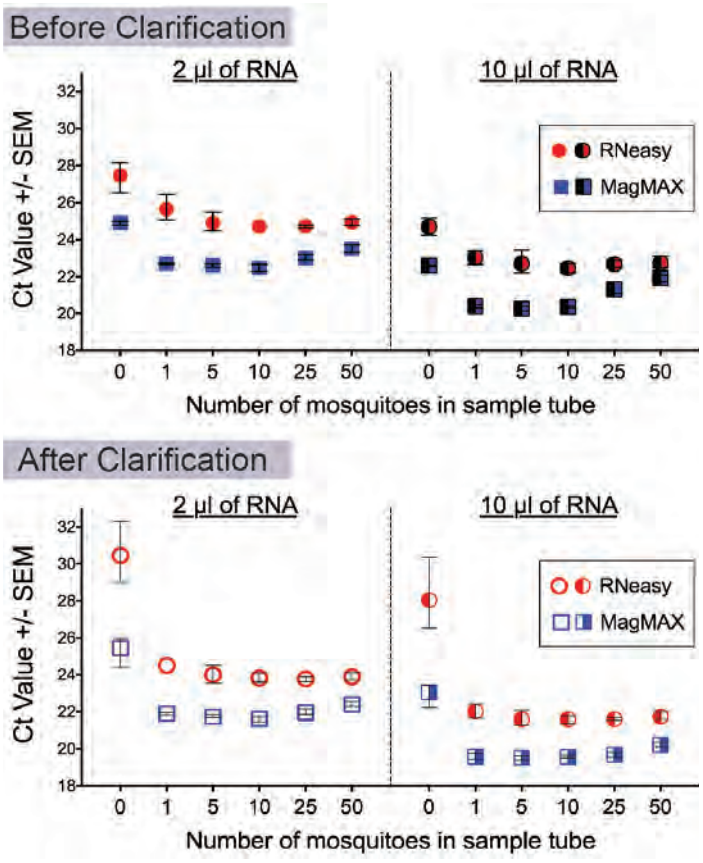

Figure 1. Triplex qRT-PCR (TaqMan) amplification of WNV using 2 µl or 10 µl of eluted RNA that was isolated using MagMAX or RNeasy columns, before and after clarification using centrifugation (top and bottom, respectively). A similar distribution of Ct values was observed for amplification of WEEV and SLEV (not shown). When the RNA samples were clarified using centrifugation, there was a significant reduction in Ct values from the TaqMan assays (Two-way ANOVA, $P < 0.001$), suggesting that clarifying extracted RNA samples with centrifugation may increase the sensitivity of the TaqMan assay for arboviruses isolated from mosquitoes. TaqMan assays with 10 µl of RNA had significantly lower Ct values relative to those with 2 µl of RNA (Two-way ANOVA, $P < 0.001$), suggesting that use of the highest volume of eluted RNA in the TaqMan assay may enhance detection of arboviruses. When the number of mosquitoes in a sample was increased from 1 to 50, there was no significant difference in Ct values within each isolation method (Multiple t tests, $P > 0.1$), suggesting that isolating arbovirus RNA from up to 50 mosquitoes in a single tube does not negatively affect the TaqMan assay. RNA isolated using MagMAX produced significantly lower Ct values in the arbovirus TaqMan assays relative to RNA isolated using RNeasy columns (Two-way ANOVA, $P < 0.0001$), suggesting that use of MagMAX may be more sensitive in screening mosquitoes for arbovirus infection.

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Mosquito Trap Modifications for Improved Utility in Abundance Monitoring

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ABSTRACT: Mosquito traps for monitoring mosquito abundance may need to be placed at sites that are prone to being disturbed by natural changes in the environment, such as increased wind or human activity. Moreover, desirable trapping locations may lack structures strong enough to support heavy objects such as the CDC EVS CO_2 or Faye-Prince traps. To address these limitations, we manufactured and deployed supports for ovi-cup traps that are resistant to tipping and sturdy supports from which weighty CDC EVS CO_2 or Faye-Prince traps can be suspended that can be driven into the soil or placed on to hard surfaces. . Use of these supports have diversified the locations in which traps have been successfully placed and improved the rates of successfully retrieving traps that contain mosquitoes, thereby enhancing mosquito surveillance activities.

INTRODUCTION

Monitoring mosquito abundance relies upon traps that remain where they are placed and do not tip or are affected by natural or anthropogenic disturbances that allow captured mosquitoes to escape (e.g wind, shifting soil, or mechanized leaf blowers). CDC EVS CO_2 traps containing dry ice can have a mass of over 3 kg, whereas Faye-Prince traps baited with CO_2 released from sublimating dry ice can weigh twice as much. Moreover, best placement sites for collecting mosquitoes with suspended CDC EVS CO_2 or Faye-Prince traps may not be near to structures that can support such weight (e.g., in a marsh or the open field of a cemetery). Additionally, obtaining permission from property owners or managers to use available supports for suspending heavy traps may not be granted. Ovi-cup traps for monitoring oviposition events by invasive *Aedes* species have a much lower mass (typically less than 0.5 kg), but most have relatively narrow bases that are unstable and prone to tipping, resulting in the contents of the trap being lost. Moreover, ovi-cup traps are often placed at sites for monitoring mosquito abundance where people are most active, thereby increasing the potential for the ovi-cup traps to be disturbed. We designed and deployed easy to manufacture supports for suspended traps and ovi-cup traps that have substantially increased stability.

METHODS

Various hand-held and consumer-grade electric tools were used to manufacture the mosquito trap supports. The ovi-cup trap supports were made from 1.9 cm aluminum channel that was cut into 30 cm long stakes with one end blunted and the other cut to a 45 degree angle. The ends were deburred to remove sharp edges. The blunt end was drilled with two parallel holes, 2.5 cm apart, and a 1.3 L capacity drink cup holder affixed using sheet metal screws. A hammer or shanked boot was used to drive the ovi-cup trap support into the soil, and the ovi-cup trap subsequently placed into the holder (Figure 1, left).

Two types of support were produced for suspending CDC EVS CO_2 and Prince-Faye traps: one for inserting the trap support into the soil (i.e., an in-ground trap support) and another for placing it atop of hard surfaces (i.e., an on-ground trap support). The base of the on-ground trap support was a 2.54 cm inner diameter (ID) anchor flange mounted to a plumber's tape-reinforced wooden plank (45 cm x 30 cm x 5 cm), weighted with sand bags. The base of the in-ground trap support was a 60 cm length of 2.54 cm ID pipe welded to a 1.25 cm diameter iron rod having a right angle bend that was used to place the rod into the soil with the strike of a shanked boot. Inserted into each base was a 150 cm length of electrical conduit (1.9 cm ID). A 90 cm length of electrical conduit (1.25 cm ID) was bent to produce a 60 cm high pole having a 30 cm horizontal extension with a downward facing hook affixed. A CDC EVS CO_2 or Fay-Prince trap was suspended from the hook near the apex of the support, and the pole inserted into the 150 cm long base pole to produce the assembled trap.

RESULTS

Unsupported ovi-cup traps are relatively unstable under typical field conditions. Within two days, one quarter or more of the ovi-cup traps we placed were tipped and the water drained. The ovi-cup trap support substantially improved the stability of the trap, resulting in less than one in twenty being tipped after 4 days in the field. The supports for suspended CDC EVS CO_2 and Fay-Prince traps have afforded the opportunity for trap placement at sites that were otherwise unavailable because of unsuitable structures *in situ* that could support the weight of the trap.

DISCUSSION

The use of ovi-cup trap supports has resulted in much higher proportion of the oviposition substrates from the traps being recovered successfully and returned to the lab for analysis, thereby increasing the efficiency of the efforts placed into surveillance for invasive species of *Aedes* mosquitoes. Use of

the in-ground supports for suspended CDC EVS CO_2 traps has opened up opportunities for surveillance of *Culex erythrothorax* emerging near tule in marsh habitats. On-ground supports have increased the ease of mosquito surveillance at sites with extensive concrete ground surfaces such as urban nurseries and waste water treatment plants.

Figure 1. Ovi-cup support (left), on-ground support with suspended Faye-Prince Trap (center), and in-ground support with suspended CDC EVS CO_2 trap (right).

West Nile Virus Surveillance at Northwest Mosquito and Vector Control District during 2013 and 2014

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ABSTRACT: District laboratory staff continued its arbovirus surveillance in 2013 and 2014. West Nile Virus activity was detected in mosquito pools (17 of 550), wild bird seroconversions (3 of 198), dead bird submissions (13 of 37) and sentinel chicken seroconversions (10 of 500). *Culex quinquefasciatus* was the most frequently pooled mosquito (191 of 550), yielding the majority of positive pools (11 of 17). Of the wild birds tested, the brown-headed cowbird constituted all WNV seroconversions (3). Most of the positive dead birds (11 of 13) were American crows. Sentinel chicken samples totaled 500, with 10 seroconversions from 10 birds. Our district area had more viral detections in 2013 compared to 2014. There were 18 human cases of WNV in 2013 and seven in 2014.

INTRODUCTION

The Northwest Mosquito and Vector Control District (NWMVCD) has been carrying out mosquito surveillance and control services in the cities of Norco, Corona, Eastvale, Jurupa Valley, Mira Loma, Lake Elsinore, Riverside, Canyon Lake, and several adjoining unincorporated communities for over 40 years. The NWMVCD service area encompasses approximately 310 square miles with over 900,000 residents living in urban, sub-urban and rural areas, with many living in close proximity to wetlands and/or riparian habitats. West Nile virus (WNV) was first detected within the district boundaries in 2003. Since then WNV has been detected every year from mosquito pools, dead birds, and/or chicken serum samples. To monitor arbovirus activity, mosquitoes were collected using the encephalitis virus surveillance (EVS) traps, gravid mosquito traps, and resting box traps. Arbovirus surveillance was conducted for WNV, St. Louis encephalitis (SLEV) and western equine encephalomyelitis (WEEV) viruses by testing pooled female mosquitoes, blood sera from sentinel chickens and wild birds, and testing dead birds submitted to the District. In this paper, we present and discuss data generated in various facets of the District's West Nile Virus Program carried out in 2013 and 2014.

MATERIALS AND METHODS

Carbon Dioxide Baited Traps

Host-seeking female mosquitoes were monitored using the Northwest-Dever modified Encephalitis Virus Surveillance traps (Williams et al. 2009) operated sporadically throughout the calendar year from a variety of locations. Trapping was conducted to survey for arboviruses and to monitor abundance of mosquitoes, especially *Culex erythrothorax* Dyar and *Cx. tarsalis* Coquillett from known trouble spots. All mosquitoes collected in EVS traps were anesthetized with triethylamine (TEA) and sorted by species and sex and enumerated. Pools consisting of 10 to 50 mosquitoes per species were shipped overnight on dry ice to the University of California Davis Center for Vectorborne Diseases (CVEC) for testing by multiplex (qualitative reverse transcriptase polymerase chain reaction) (Brault et al. 2015). Pools of female *Anopheles hermsi* Barr & Guptavanij, *Cx. erythrothorax*, *Cx. quinquefasciatus* Say, *Cx. stigmatosoma* Dyar, *Cx. tarsalis*, *Cx. thriambus* Dyar, *Aedes vexans* (Meigen) *Culiseta incidens* (Thomson) and *Cs. particeps* (Adams) were submitted.

Gravid Traps

The Reiter/Cummings gravid female traps (Cummings 1992) were the inspiration for designing our district modified down draft gravid traps (Fig. 1). These traps were set in rural and suburban areas from February to September in 2013 and July through November in 2014 and were baited with an infusion that contained 4.0 grams of alfalfa per gallon of water. All mosquitoes collected were anesthetized with TEA and sorted by species and sex and enumerated. Female mosquitoes were pooled and tested for virus.

Resting Boxes

Walk-in style resting red boxes were placed at established sites and monitored from January through November (Meyer 1987). Sites were located in proximity to the Santa Ana River and were checked bi-weekly. Mosquitoes were collected using an in-house designed battery powered aspirator. The resting boxes were used specifically to target and obtain blood engorged and gravid mosquitoes. All mosquitoes collected in resting boxes were anesthetized with TEA and enumerated before sorting by species and sex. Female mosquitoes were pooled and tested for virus.

Figure 1. In-house modified down draft gravid trap and components: Collection capsule is placed underneath reservoir dish (where the gravid attractant is placed) with center piece removed (5-inch diameter hole) and replaced with ABS cuff and clip for capsule attachment, inside of 5-gallon bucket. This cuff is conduit for air flow and functions to keep the gravid attractant contained. The bucket has an 8 x 8 in^2 hole on one side to allow access to the clip and cuff. The collection housing has pegs that lock into the cuff clips. The motor capsule attaches in the same fashion and contains a battery lead for the Lithium ion battery pack.

Larval Collections

Larval samples were collected from February through December in 2013 and every month in 2014. Sources known to produce mosquitoes were checked with regularity by technicians. When mosquito breeding was detected, a sample was collected using a dipper and a sample vial. A data tag is placed on the collection lid indicating date, source type, address, number mosquitoes per dip, mosquito stages present, collector, and district zone. The sample is then submitted to the laboratory for identification and data processing.

Sentinel Chicken Flocks

In 2013 six sentinel chicken flocks, comprised of three white leghorn birds each, were maintained at different locations throughout the District. In 2014 seven flocks were comprised of three Rhode Island Red birds each. Blood samples were collected biweekly from April through November. The samples were placed on Nuboto filter-paper strips, air dried and submitted to the California Department of Public Health - Viral and Rickettsial Disease Laboratory in Richmond for testing by an enzyme-linked immunosorbent assay (Patiris et al. 2008).

Wild Birds

In 2013 and 2014 wild birds were bled at three locations. Birds were trapped in modified Australian Crow traps (McClure 1984). Traps were checked every day during the bird breeding season (March 15 to September 15) to ensure that all migratory bird species were held no longer than 24 hours. The birds were identified to species, sexed, and bled. All blood samples came from brown-headed cowbirds (*Molothrus ater* Boddaert) and house sparrows (*Passer domesticus* Linnaeus) obtained from modified Australian crow traps operated by Santa Ana Watershed Association (SAWA) and the Orange County Water District (OCWD). These two agencies operated the Least Bell's Vireo (*Vireo bellii pusillus* Audubon) Conservation Project and possessed the proper permits to operate the traps and to salvage brown-headed cowbirds. Brown-headed cowbirds are collected in vireo habitat, because they are brood parasites. All brown-headed cowbirds and house sparrows caught in these traps were salvaged according to the project protocol. Bird blood samples (0.1 - 0.2 ml from each bird) were collected from the jugular vein with a 1-ml insulin syringe fitted with a 28 gauge, ½ inch needle. Each sample was dissolved in 0.9 ml of 0.75% bovine serum albumin/PBS (phosphate-buffered saline) diluent and submitted to the Orange County Vector Control District Laboratory for SLE and WEE antibody testing by serum hemagglutination inhibition as described by Gruwell at al. (2000). All samples were also tested for antibodies specific to WNV by a blocking enzyme-linked immunosorbent assay (Jozan et al. 2003).

Dead Birds

Through the participation of NWMVCD with the CDPH Dead Bird Surveillance Program, dead birds reported to the District were picked up and submitted to the CVEC for WNV testing in 2013. Additional samples were processed at NWMVCD and tested at OCMVCD. In 2014 all dead birds collected were swabbed. The swab sample was then placed on RNA sound cards and then shipped to CVEC for testing by qRT-PCR.

RESULTS

Mosquito Surveillance

EVS CO_2-baitedTraps

Over the two year period, a total of 413,427 female mosquitoes were collected in the Northwest-Dever modified EVS traps during 1,375 trap-nights, resulting in 309.6 mosquitoes per trap-night. *Culex erythrothorax* was the predominant species collected (358,224) at 337.4 and 279.2/trap-night for 2013 and 2014, respectively. The highest number/trap-night (923.3) for

this species occurred in June 2013 (Fig. 2) with monthly trap-night counts above 100 for all months except January, February, and December of 2013 and January 2014. *Culex tarsalis* was the second most abundant mosquito (34,104) with a trap-night count of 114.0 in September 2013 and 97.8 in September 2014. *Culex quinquefasciatus* had its highest trap-night count of 10.4 in September 2013, but showed higher abundance of 13.7 and 39.7/ trap-night in October and November, 2014, respectively.

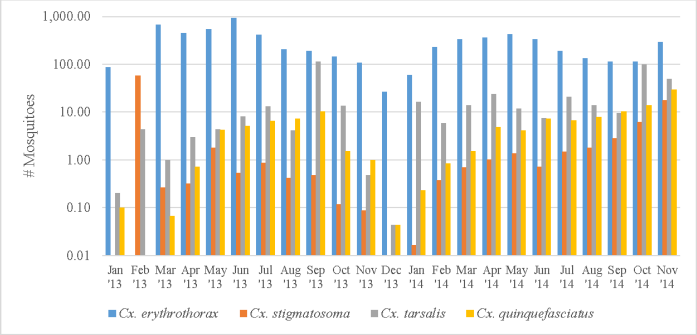

Figure 2. Number of *Culex* species/trap-night/month collected in Northwest-Dever modified EVS traps during 2013-2014.

Gravid Traps

There were 64 gravid trap mosquito collections (trap nights) in 2013, and 61 in 2014. In 2013, 735 females were collected. In 2014 577 females were collected. *Culex quinquefasciatus* represented 90% of the total trap catches for both years. Additional species included *Cx. stigmatosoma* (gravid females) and *Cx. tarsalis* (non-gravid females).

Resting Boxes

There were 123 collections from resting boxes in 2013 and 96 in 2014 resulting in 1,820 and 1,658 female mosquitoes, respectively. The majority of the mosquitoes collected belonged to *Culex* spp. (87.58% in 2013 and 95.48% in 2014); the remainder included *Cs. incidens* and *An. hermsi*. Of the *Culex* species *Cx. quinquefasciatus* was most prevalent, followed by *Cx. tarsalis*, *Cx. stigmatosoma, Cx. erythrothroax* and *Cx. thriambus* (Fig. 3).

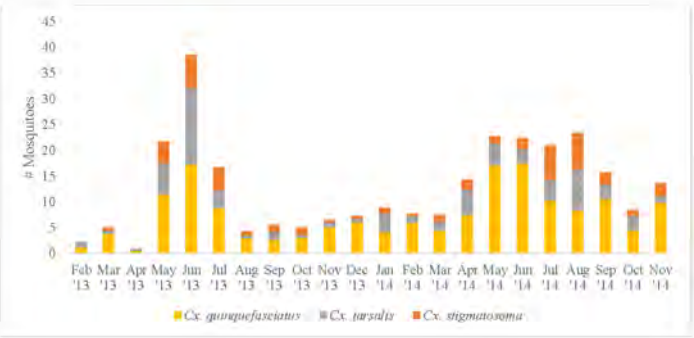

Figure 3. Monthly average number of *Culex* species collected nightly from resting boxes from February 2013 through November 2014.

Larval collections

Of 1,165 larval samples in 2013 and 2014, approximately 13,000 larvae were identified each year (13,007 in 2013 and 12,961 in 2014), of which *Cx. quinquefasciatus* was the most numerous species. For thoroughness sake, the entire contents of each sample were identified rather than a sub-sample. Technicians were strongly encouraged to record the average number of larvae seen per dip across the entire site, although this was not always recorded. Contrasting sharply to our adult collections in which *Cx. erythrothorax* was predominant, *Cx. quinquefasciatus* appeared as the most numerous species in our larval samples. *Culex quinquefasciatus* accounted for 54.0% (7,023/13,007) and 72.96% (9,450/12,240) of the larvae identified in 2013 and 2014, respectively. Other frequently collected species included *Cx. tarsalis* and *Cx. stigmatosoma*, which were also collected throughout most of the year. Technicians did bring back more of the elusive *Cx. erythrothorax* larvae than expected. Fig. 4 depicts the monthly frequency of the primary *Culex* species collected. Over a two year period, approcimately 50 *Cx. thriambus* and 100 *Cx. restuans* larvae were collected mainly in spring (May) and fall (Sept-Nov). Also collected were larvae of *Culiseta incidens*, *Cs. particeps*, *Cs. inornata* and *Anopheles hermsi*. Rarer finds included *An. punctipennis* (one in each year*), Aedes sierrensis* in 2013 and *Ae. washinoi* in 2014.

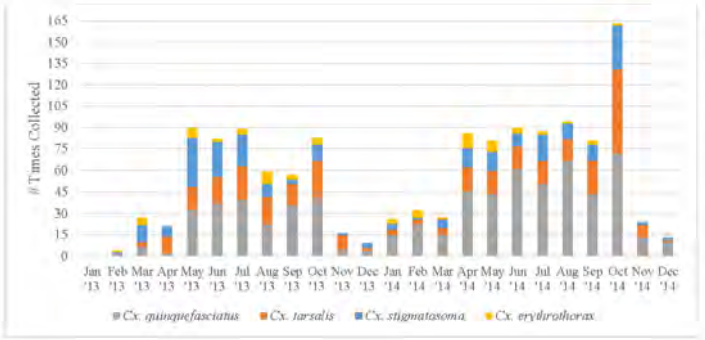

Figure 4. Total number of *Culex* species collected by dipping at all habitat types (treatment wetland, pools, ditches, BMPs, pool/ spas, curb & gutter, flood control channel, etc.) in northwestern Riverside County, 2013-2014.

West Nile Virus Surveillance

Mosquito Pools

A total of 550 mosquito pools comprising 16,316 mosquitoes were submitted to CVEC for testing (Table 1). Of the positive pools, 65% were *Cx. quinquefasciatus*. Sixty-five percent (65%) of the positive pools came from EVS traps (5 pools of *Cx. quinquefasciatus,* 5 pools of *Cx. tarsalis* and one pool of *Cx. erythrothorax*), 23.5% from gravid traps (*Cx. quinquefasciatus*), and lastly 11.8% (two pools of *Cx. quinquefasciatus*) from resting box traps. The majority of these pools were collected in close proximity to the Santa Ana River. The first positive pool detections of 2013 and 2014 occurred in June and July, respectively. The latest pool detection during 2013 and 2014 was in September and August, respectively.

Table 1. Number of mosquito pools submitted to the UC Davis - Center for Vector-borne Diseases tested for Saint Louis encephalitis, western equine encephalomyelitis and West Nile viruses during 2013 and 2014. Data shown include number of pools followed (by number mosquitoes) and number of pools testing positive for WNV. All pools tested negative for St. Louis and western equine encephalomyelitis.

2013 Mosquito Pools							
Month	*Culex tarsalis*	*Culex quinquef.*	*Culex stigsmato.*	*Culex erythroth.*	*Culiseta incidens*	Other[a]	Total
Jan.	0	0	0	0	0		**0**
Feb.	1(1)	0	0	0	0		**1(1)**
Mar.	2(4)	2(12)	0	0	0		**4(16)**
Apr.	0	0	0	0	0		**0**
May	2(5)	3(20)	2(11)	1(17)	0	1(2)	**9(55)**
Jun.	9(197)	11(191)1	8(44)	1(5)	0		**29(437)1**
Jul.	27(754)1	21(334)1	13(77)	15(750)	0	1(1)	**77(1916)2**
Aug.	12(265)	27(537)3	6(15)	7(314)	0	3(10)	**55(1141)3**
Sep.	68(3271)1	21(631)3	2(12)	9(450)	1(23)		**101(4387)4**
Oct.	6(213)	9(101)	2(6)	0	0		**17(320)**
Nov.	1(4)	5(50)	0	1(9)	0		**7(63)**
Dec.	0	0	0	0	0		**0**
Total	**128(4714)2**	**99(1876)8**	**33(165)**	**34(1545)**	**1(23)**	**5(13)**	**300(8336)10**
2014 Mosquito Pools							
	Culex tarsalis	*Culex quinquef.*	*Culex stigsmato.*	*Culex erythroth.*	*Culiseta incidens*	Other[b]	Total
Jan.	5(167)	3(22)	2(5)	0	1(50)		**11(244)**
Feb.	1(50)	3(48)	0	0	1(50)		**5(148)**
Mar.	1(50)	1(9)	0	0	1(48)		**3(107)**
Apr.	6(149)	7(152)	2(14)	1(50)	0		**16(365)**
May	7(134)	13(307)	4(16)	4(200)	0		**28(657)**
Jun.	0	4(118)	2(9)	0	0		**6(127)**
Jul.	22(1333)2	11(255)	5(63)	17(850)1	0		**55(2501)3**
Aug.	24(802)1	32(640)3	2(42)	24(1197)	0		**82(2681)4**
Sep.	11(274)	18(491)	8(123)	6(255)	0	1(7)	**44(1150)**
Oct.	0	0	0	0	0		**0**
Nov.	0	0	0	0	0		**0**
Dec.	0	0	0	0	0		**0**
Total	**77(2959)3**	**92(2042)3**	**25(272)**	**52(2552)1**	**3(148)**	**1(7)**	**250(7980)7**
Grand Total	**205(7673)5**	**191(3918)11**	**58(437)**	**86(4097)1**	**4(171)**	**6(20)**	**550(16,316)17**

[a]Includes pools from *An. hermsi* , *Cs. particeps* , and *Cx. thriambus*

[b]Includes one pool from *Ae. vexans*

Sentinel Chickens

Blood samples from our sentinel chickens tested positive for WNV in 2013 and 2014. A total of 500 blood samples were submitted and tested. Ten out of 39 chickens from three of seven sentinel flocks throughout the District had seroconversions (Fig. 5). The first infected chickens in 2013, with a probable seroconversion date of August 6, were identified in two flocks, near riparian corridors. One additional flock became infected on November 13, located just east of the Santa Ana River. The WNV activity in 2014 was first detected in our chickens August 28, and continued into September as shown by two additional seroconversions of chickens at the aforementioned flocks on September 8.

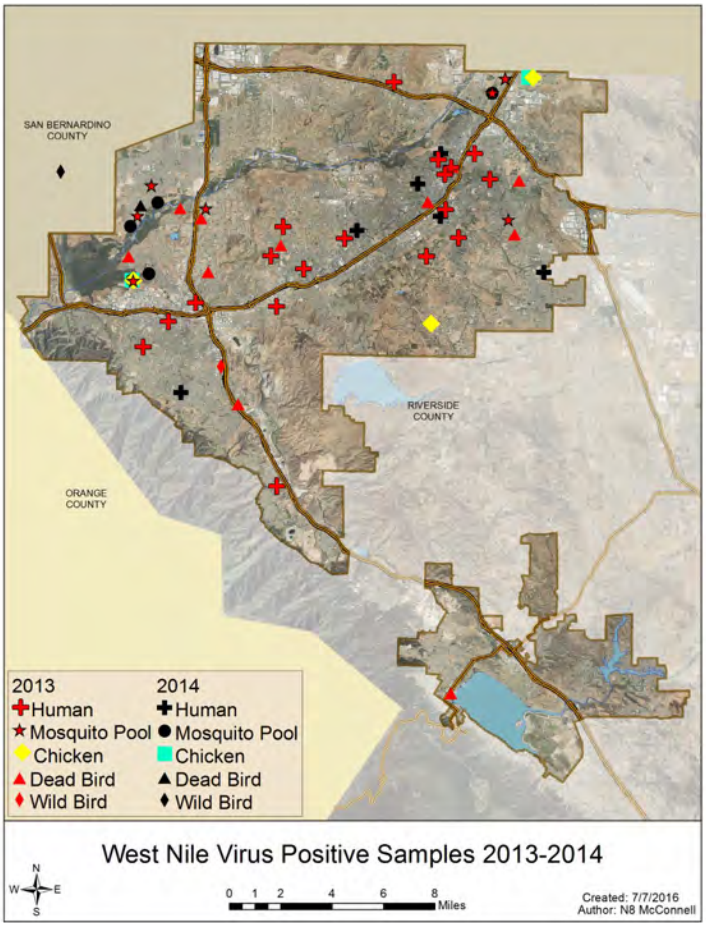

Figure 5. Map of NWMVCD showing West Nile virus positive samples collected at various sites during 2013-2014.

Wild Birds

In 2013 and 2014 combined, 198 wild birds were sampled including three brown-headed cowbirds which tested antibody positive for WNV (Fig. 4). The first two positive birds were bled on May 29, 2013, and an additional bird with detectable antibody was bled on December 29, 2014. A total of 57 birds were bled in 2013 and an additional 141 birds were bled in 2014. The three sites sampled were the Orange County Water District office in the Prado Basin (six bleeding events), Chino Dairy located in Chino (four bleeding events), and Northwest Mosquito and Vector Control District (seven bleeding events). Both positive birds were found at our District site.

Dead Birds

A total of 37 dead bird samples in the form of carcass, kidney or oral swabs on RNA sound card were submitted between March of 2013 and November of 2014. Of the 13 WNV-positive birds, 11 were American crows collected from June through September of both years. Three of these WNV-positive American crows were processed in-house and tested by Orange County Mosquito and Vector Control; the remainder were tested at the CVEC laboraory. The two remaining dead bird detections came from a house finch and an American goldfinch.

DISCUSSION

Culex erythrothorax and *Cx. tarsalis* dominated our adult mosquito surveillance collections, whereas *Cx. quinquefasciatus* was most abundantly found in our larval collections. Other adult *Culex spp*. were collected using a variety of collection methods, notably resting boxes and gravid traps. *Culex erythrothorax* was most prevalent along the Santa Ana River and Temescal Wash, particularly riparian and wetland areas which were planted with cattails (*Typha* spp.), tules and bulrush (*Schoenoplectus* spp.). These areas have a heavy residential component, particularly because many of the historical dairies and open land have been developed into housing tracts within the last 14 years. Development along the Santa Ana River, and canyons along the Temescal Wash have a variety of mitigation areas with habitat conducive to mosquito breeding. These mitigation areas are usually designated for parks and recreation, habitat restoration, Endangered Species Act designation, and/or water quality improvement. Multiple land uses are typically accompanied by multiple jurisdictions that further complicated mosquito management activities. The remnant *Cx. quinquefasciatus, Cx. stigmatosoma* and *Cx. tarsalis* populations from the dairies seemed to persist in or near these renovated environments as can be seen from both larval and adult collections. Larval collections from abandoned swimming pools frequently yield high numbers of *Cx. stigmatosoma* as well as *Cx. tarsalis* and *Cx. quinquefasciatus*. In addition, these areas seemed to focus WNV amplification as the earliest detected WNV-positive mosquito pools, dead birds, live birds and sentinel chicken seroconversions were associated with these riparian habitats.

The City of Riverside had considerable WNV activity. This area is relatively new to our district, being added in the spring of 2013. Three positive dead birds were recovered in areas that had the highest occurrence of WNV positive humans. *Culex quinquefasciatus* was the most abundant mosquito trapped in the city of Riverside, with many breeding sources coming from backyards and flood channels above and below the ground. Corona also had a human case cluster and contains similar breeding habitat to that of Riverside.

ACKNOWLEDGMENTS

We thank CDPH / CVEC, as well as the biologists of SAWA Least Bell's Vireo Conservation Project and OCWD for their cooperation in maintaining and supplying brown-headed cowbirds to test for the WNV antibodies. We gratefully acknowledge the assistance of Bob Cummings for his invaluable support in our wild bird and dead bird surveillance program. We thank Martine Jozan and Carrie L. Fogarty of OCMVCD for testing of avian blood sera for arboviruses.

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Tickborne Disease in Lake County, CA 1995-2015

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ABSTRACT: Lake County is a rural county in the coastal foothills of Northern California; oak savannah, conifer and mixed oak forests and chaparral dominate the landscape. The pursuits of farming, natural resource management and outdoor recreation overlap human activitites and tick environments. The Lake County Vector Control District began tickborne disease surveillance and outreach efforts during the 1990's. Human-embedded *Ixodes pacificus* submitted by the public are tested at the Sonoma County Public Health lab for Lyme disease; in 2015, 22 ticks were tested and one was positive. Three species of tick are regularly collected by flagging: *Dermacentor variablilis*, *D. occidentalis*, and *Ixodes pacificus*. *Ixodes pacificus* pools including all life stages were tested for *Borrelia burgdorferi* sensu lato (3.65% positive) and *B. miyamotoi* (2.08% positive). Pools of *D. occidentalis* in all life stages have been tested for *Rickettsia philipii*, the etiologic agent of Pacific Coast tick fever beginning in 2008; the index case patient was diagnosed in Lake County that year. No infection was detected in the twelve adult *D. variabilis* tested for *Rickettsia rickettsia*. Incidence of Lyme disease, Rocky Mountain spotted fever, and Pacific Coast tick fever between 1995 and 2013 was low in Lake County, but higher than the California state-wide incidence for the same eighteen-year period.

INTRODUCTION

Lake County covers 1,329 square miles in the Coastal Foothills of Northern California and is home to 64,184 residents. It is a rural county with a population density of approximately 49 residents per square mile. The county is centered around Clear Lake, which covers 68 square miles. This natural lake basin sits at an elevation of 1,300 feet and contains one of the oldest lakes in North America. Geological activity in the region maintains an unstable lake bottom which drops at approximately the same rate as sediment accumulates. Snow Mountain is the southern entry-point to the Coastal Mountain Range, and at 7,000 feet, it is the highest point in Lake County. Public lands in the county include portions of the Mendocino National Forest to the north and the Berryessa Snow Mountain Monument and Cow Mountain Wilderness to the east and west, respectively. Other public areas include the Clear Lake State Park, the Anderson Marsh State Historic Park, and many other smaller county and city parks. Many wild areas and abundant wild life make Lake County a beautiful place to live and recreate.

The Lake County Vector Control District (District) provides mosquito control and mosquito and tickborne disease surveillance for all of Lake County. The typical lifestyle in Lake County provides residents, workers, and visitors with ample opportunities to encounter ticks around their homes, during recreational activities, and at work. There are many ways that area residents may be exposed to tick bites around the home: by ticks infesting their pets, in natural landscaping around the house or adjacent areas, by maintaining firebreaks and handling firewood. Popular recreational activities such as hiking, dog walking, hunting, trail riding, and bird watching also present risks of exposure to ticks. Likewise, a number of local occupations expose employees to tick bite: firefighting, law enforcement, ranching, landscaping, wood cutting, land clearing, firebreak inspection, and farming. This human-tick interface creates a public health risk; the District works to identify local ticks and tickborne diseases and provides that information to residents and their healthcare providers. This paper will review recorded tickborne disease cases and ticks tested for pathogenic bacteria in Lake County.

RESULTS

Human Cases of Tickborne Disease in Lake County

Between 1995 and 2013, sixteen confirmed cases of tickborne disease in Lake County have been reported to the California Department of Public Health (CDPH) (Table 1). Three additional cases of infection likely occurred in Lake County, but were not included in this total. In one case, the patient was a Lake County resident with a clinically compatible illness that was not confirmed. In two other cases, non-residents likely contracted the disease in Lake County, but the cases were recorded within their county of residence. For epidemiological purposes, confirmed human cases of disease in California are reported in their county of residence regardless of where they were most likely exposed to the etiologic agent.

Cases of human tickborne disease are reported as disease incidence, which is defined as the number of infections per 100,000 residents during the surveillance period. Lyme disease, Rocky Mountain spotted fever and Pacific Coast tick fever were reported from Lake County. The incidence of each disease was higher in Lake County than from California as a whole. Lake County's most commonly reported tickborne disease was Lyme disease (LD), with an incidence of infection of 0.89. During the same period, California's reported incidence of infection was 0.25. The incidence of Pacific Coast tick fever (PCTF) was 0.41 for Lake County and 0.002 for California. While the incidence of PCTF for this eighteen-year period was lower than that of LD, the incidence may be artificially low given that PCTF was only recognized as a human pathogen in 2008 (Shapiro et al. 2010). The incidence of Rocky Mountain spotted fever (RMSF) was 0.08 in Lake County and 0.002 in California. No cases of human granulocytic anaplasmosis (HGA) or babesiosis were reported

from Lake County during this period; however, cases did occur elsewhere in California.

Disease	Human Cases (Incidence)	
	Lake County	California
Lyme disease	11 (0.89)	1,711 (0.25)
Rocky Mountain spotted fever	1 (0.08)	13 (0.002)
Pacific Coast tick fever	5* (0.41)	14 (0.002)
Human granulocytic anaplasmosis	0	8 (0.001)
Babesiosis	0	4 (0.0005)

Table 1. Number of confirmed select tickborne diseases and incidence of disease for Lake County and California for 1995-2013. Incidence is defined as the number of cases per 100,000 population over the eighteen years reported. Cases are reported for county of residence which may differ from county of exposure. Data are provide by the California Department of Public Health.

Tick Collections: From the Field and the Public

The District conducts tick surveillance at select locations within Lake County and receives ticks submitted for identification by the public. Ticks are collected from the field by flagging. This technique utilizes a one-meter square piece of white flannel material attached to a wooden dowel. The "flag" is then moved along brush and grass to sample for ticks. Three species of tick are commonly encountered during surveillance efforts and by the public: *D. variabilis*, *D. occidentalis*, and *I. pacficus*.

Dermacentor variabilis

We have no records of collecting *Dermacentor variabilis* Say subadults. Adults have been collected by flagging and have also been collected by hand, questing on natural and man-made materials. These ticks are most abundant in grasslands, particularly near water.

California-collected *D. variabilis* may be infected with the bacteria *Rickettsia rickettsia,* the causative agent of RMSF (Grewal et al. 2014). Rocky Mountain spotted fever was first found to be pathogenic to people in the 1890's. Since the District began collecting and testing ticks, twelve adult *D. variabilis* have been tested for Spotted Fever Group *Rickettsia* (SFGR) and all were negative.

Dermacentor occidentalis

This species has been collected at a majority of the sampling sites in Lake County generally in low numbers throughout the year, but are most numerous in the spring months. Subadult *D. occidentalis* ticks have been collected sporadically.

Subadults of this tick may vector PCTF. Field collected *D. occidentalis* were first tested for SFGR in 2008, the year that PCTF was characterized. Samples also were submitted in 2011, 2012, 2013, and 2014. Subadult and adult ticks were either submitted individually or as a pool, so results are reported as the percentage of samples that were positive. In total 458 ticks were tested in 235 samples and of these, six (2.55%) were positive for SFGR but were not characterized further. Ten samples (4.26%) were positive for *R. rhipicephali* and twenty-four samples (10.21%) were positive for *R. philipii*.

Ixodes pacificus

This species is the most frequently collected in Lake County, but is rarely taken in July, August, and September.

Ixodes pacificus is known to vector three bacteria in California. *Borrelia burgdorferi* sensu stricto (s.s.) is the etiologic agent of LD which was first described in 1976 and was detected in California in 1979 (Steere et al. 1979). A related bacteria, *B. miyamotoi*, causes human disease which was first described in 2011 and has since been found in areas of the United State where *B. burgdorferi* s.s. is endemic (Krause et al. 2013). *Anaplasma phagocytophilum* is the etiologic agent of HGA which was first documented in the United States in 1994 and has subsequently been found in ticks from California (Kramer et al. 1999). A small number of Lake County *I. pacificus* adults and nymphs were tested for *A. phagocytophilum* in 1998 and all were negative for the bacteria. Testing for *B. miyamotoi* began in 2007 and ticks were submitted for testing to the Sacramento-Yolo Mosquito and Vector Control District, the United States Army, and the California Department of Public Health (CDPH)-Vector-Borne Disease Section. Although *B. burgdorferi* s.s. is the causative agent of LD, test results are reported as *B. burgdorferi* sensu lato (s.l.) which includes *B. burgdorferi* s.s. along with other closely related *Borrelia* spp. found in California. This is necessary because the specificity of bacterial identification varied among the laboratories used to detect the presence of *Borrelia* spp.

Field collected *I. pacificus* from Lake County were first tested for LD in 2002. Samples included here have been submitted from the following years: 2002, 2005, 2007-2009, 2011-2013. A total of 1,610 ticks were submitted and tested for the presence of *Borrelia* spp. and *B. burgdorferi* s.l., either as individuals or as a pool. The 876 samples included all life stages of *I. pacificus*. Two samples (0.23%) were positive for *Borrelia* spp. but were not further characterized. Thirty-two samples (3.65%) were positive for *B. burgdorferi* s.l.

A total of 1,507 ticks were tested for *B. miyamotoi* in 864 samples. Of those, *B. miyamotoi* was detected in eighteen samples (2.08%). One male *I. pacificus* was found to be co-infected with *B. miyamotoi* and *B. burgdorferi* s.l.

Public Outreach

The District does not apply acaricides to reduce the abundance of ticks. Our efforts to control tickborne disease in Lake County are focused on public outreach. We provide CDPH "Ticks in California" cards at outreach events and at the District. This is a popular reference card that includes information about how to safely remove a tick, District contact information, and tick identification tips for the three tick species discussed above.

The District provides other informational brochures about ticks and tickborne diseases at its office and at outreach events. The District's website (www.lcvcd.org) has additional information on tick biology and links to the CDPH and Centers for Disease Control and Prevention (CDC) tick resource pages, and to Tick Encounter, a University of Rhode Island project.

We also encourage members of the public to submit ticks to the District for identification. Ticks that have fed on a person may be brought in by the resident or submitted by the Lake County Department of Public Health and local physicians. *Ixodes pacificus* are mailed to the Sonoma County Public Health Lab to determine if *B. burgdorferi* can be identified in the specimen. The District began this program during the 1996 tick season. The tick season begins in the fall and continues through the following summer. No ticks have been submitted during the month of September. Since the 2009-10 season there has been an increase in the number of ticks submitted (Figure 1), with a high of twenty-two ticks submitted during the 2014-15 season. Since 1996, a total of 128 ticks have been submitted for testing; the sex and life stage were not recorded for 13 and all of the remaining were nymphs and adult females. Two females have been positive for *B. burgdorferi* to date, one each submitted during the 2014 and 2015 seasons.

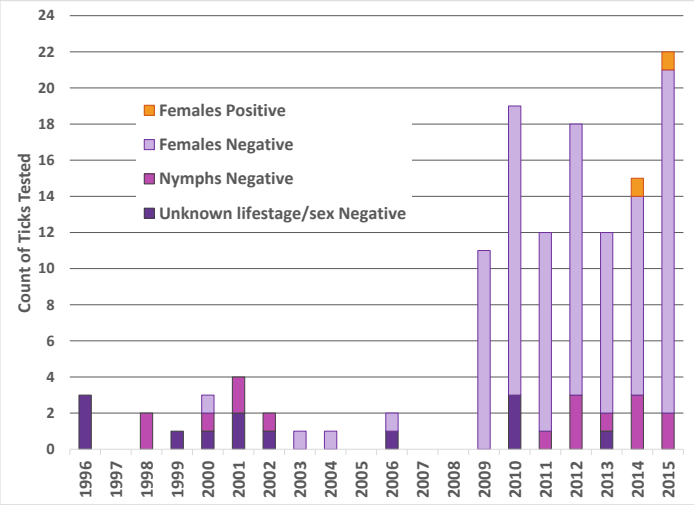

Figure 1. Ticks that have fed on county residents may be submitted to the Lake County Vector Control District in Lakeport, CA for species identification. One hundred twenty-eight *I. pacificus* were tested for the presence of *Borrelia burgdorferi*, the etiologic agent of Lyme disease at the County of Sonoma Department of Health Services Public Health Laboratory from 1996 through 2015. The year shown refers to the tick season ending in October of that year, e.g., 1996 includes all ticks submitted from November 1995 through October 1996.

Summary

Tickborne diseases, whether well-known, such as LD, or emerging, such as PCTF, present a risk to public health. The incidences of infection of LD, PCTF, and RMSF in Lake County are low, but above the statewide average. Lake County ticks have been found infected with *B. burgdorferi* s.l., *B. miyamotoi,* and *R. philipii.* Risk of tick bite in Lake County is high because lifestyle and tick habitat commonly overlap.

The District continues to provide information on the prevalence and distribution of pathogen-harboring ticks within the county. Standardized testing methods that characterize *B. burgdorferi* s.s. specifically and include a wider array of tickborne agents would make the District's tickborne disease surveillance program more useful to residents and healthcare providers. Effective collection methods targeting subadult *D. occidentalis* need to be developed so that greater numbers can be collected and tested for the presence of *R. philipii*. Continued public participation in the embedded tick testing program provides an opportunity for District staff to talk to people about tick-bites and tickborne disease prevention. It also assists District staff in identifying new tick surveillance sites. The District would like to expand its outreach program so that tick bite prevention and knowledge of tickborne diseases may become common sense in Lake County.

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The initial detection and establishment of invasive *Aedes aegypti* in California, 2013

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ABSTRACT: *Aedes aegypti* is the primary vector of dengue, chikungunya, Zika, and yellow fever viruses. While not endemic to the USA, these viruses are frequently detected in returning travelers to California. This report describes the invasion and establishment of *Ae. aegypti* in California. The invasive mosquito was initally detected in June 2013 in Madera County in response to a resident service request with a complaint of day-biting mosquitoes. *Aedes aegypti* were subsequently detected in other California counties during routine and enhanced mosquito surveillance activities, as well as in response to resident service requests. Enhanced surveillance in *Ae. aegypti* infested areas includes standard and novel adult mosquito traps, ovitraps, and resident yard inspections. Enhanced *Ae. aegypti* control activities include public outreach and education, sanitation/source reduction, and adult and larval chemical control. Novel detections most frequently were in response to resident service requests indicating the importance of media releases and public education. The mode of introduction of *Ae. aegypti* to California remains unknown. From 2013-2015, ten California counties, Alameda, Fresno, Imperial, Kern, Los Angeles, Madera, Orange, San Diego, San Mateo, and Tulare, have had detections of *Ae. aegypti*.

INTRODUCTION

Aedes aegypti (L.) is the primary vector of dengue and yellow fever viruses worldwide (Christophers 1960), and is a competent vector of chikungunya and Zika viruses (Turrell et al. 1992, Chouin-Carneiro et al. 2016). This species lives in close association with humans and oviposits dissication resistant eggs in peridomestic water sources (Christophers 1960, Womach 1993, Harrington et al. 2001, Harrington et al. 2005). *Aedes aegypti* has a broad worldwide distribution throughout most tropical and subtropical latitudes (Kraemer 2015a, b). In the United States, this species is found in the southeastern states, as well as Texas, New Mexico, and Arizona (Morlan and Tinker 1965, Eisen and Moore 2013, Kraemer 2015b). While numerous quarantine interceptions of both the larval and adult stages have occurred in California (Herms 1947), until 2013 *Ae. aegypti* had failed to establish populations within the state.

HISTORIC *AEDES AEGYPTI* DETECTIONS

The earliest documented detections in California occurred in 1910 in San Diego and on Angel Island in the San Francisco Bay at a former US Bureau of Immigration inspection and detention facility (Theobald 1910, Kumm 1931). In addition, in 1979 a single fourth instar larval *Ae. aegytpi* was collected during routine surveillance in a marshland adjacent to the San Francisco International airport in San Mateo County (Jewell and Grodhaus 1984); the area was consequently treated extensively with organophosphate insecticides. The most recent historically documented detection occurred in 1987 where a single dead *Ae. aegypti* larva was identified in a shipment of tire casings arriving from Florida at a tire depot in South San Francisco, San Mateo County (Jewell and Schoeppner 1987).

CONTEMPORARY *AEDES AEGYPTI* DETECTIONS

Beginning in 2013, *Ae. aegypti* was detected in several urban areas of California (Table 1, Figure 1). The mode of introduction of *Ae. aegypti* into California remains unknown, but genetic analyses indicate that some California populations are most closely related to populations from the southeastern USA (Gloria-Soria et al 2014). Response activities post-detection have included the issuance of press releases to increase public awareness, development and distribution of public education materials, enhanced mosquito surveillance (Table 2), and intensive control (Table 2) efforts targeting both immature and adult life stages.

Local vector control districts in counties that detected *Ae. aegypti* maintained surveillance throughout the winter season. These efforts included the use of CDC Autocidal Gravid Ovitrap (CDC-AGO) (Mackay et al. 2013) and ovitraps at fixed sites. BioGents Sentinel traps (BGS) were commonly deployed in response to resident service requests throughout the infestation zones. This paper describes details of initial *Ae. aegypti* detections in California, as well as surveillance and control efforts in the attempt to eradicate this invasive mosquito.

Table 1. Chronology of detection events of *Aedes aegypti* in California, 2013-2015.

Date of detection	Location: County (City)	Latitude, Longitude		Initial detection tool
2013				
June 6	Madera (Madera)	36.9614	-120.0608	Resident service request
June 20	Fresno (Clovis)	36.8253	-119.7031	Resident service request
July 8	Madera (Parkwood)	36.9269	-120.0447	Routine arbovirus surveillance (CO_2 EVS trap)
July 11	Madera (Madera Ranchos)	36.9311	-119.8815	Routine arbovirus surveillance (CO_2 EVS trap)
August 6	San Mateo (Menlo Park)	37.4528	-122.1833	Enhanced surveillance (ovitrap)
October 11	Fresno (Fowler)	36.6333	-119.6833	Routine arbovirus surveillance (CDC gravid trap)
October 15	Fresno (Fresno)	36.7500	-119.7667	Resident service request (captured indoors)
October 28	San Mateo (Atherton)	37.4586	-122.2000	Enhanced surveillance (inspections)
2014				
January 13	Madera (Madera)	36.9614	-120.0608	Resident service request (captured indoors)
January 23	San Mateo (Menlo Park)	37.4528	-122.1833	Enhanced surveillance (inspections, ovitrap, CDC-AGO)
March 7	Fresno (Clovis)	36.8253	-119.7031	Enhanced surveillance (CDC-AGO)
May 20	Madera (Madera Acres)	37.0192	-120.0669	Enhanced surveillance (BGS, CO_2 EVS trap)
June 12	San Mateo (Atherton)	37.4586	-122.2000	Enhanced surveillance (inspections)
August 8	Tulare (Exeter)	36.2942	-119.1428	Routine arbovirus surveillance (CDC gravid trap)
August 11	Kern (Arvin)	35.2092	-118.8283	Enhanced surveillance (CDC-AGO)
August 19	Madera (Madera Ranchos)	36.9311	-119.8815	Enhanced surveillance (ovitrap)
August 22	Madera (Parkwood)	36.9269	-120.0447	Enhanced surveillance (CO_2 EVS trap)
September 10	Fresno (Fresno)	36.7500	-119.7667	Enhanced surveillance (ovitrap)
October 7	San Diego (San Diego)	32.7150	-117.1625	Service request/consultation (captured indoors)
October 7	Los Angeles (Commerce)	34.0006	-118.1547	Service request/consultation (captured indoors)
October 8	Los Angeles (Pico Rivera)	33.9889	-118.0892	Routine arbovirus surveillance (CDC gravid trap)
October 29	San Diego (Chula Vista)	32.6278	-117.0481	Resident service request (captured indoors)
2015				
January 9	Imperial (Andrade)	32.7258	-114.7258	Enhanced surveillance (BGS)
January 27	San Mateo (Menlo Park)	37.4528	-122.1833	Enhanced surveillance (ovitrap)
February 3	Imperial (Calexico)	32.6789	-115.4989	Enhanced surveillance (BGS)
February 6	Fresno (Clovis)	36.8253	-119.7031	Resident service request (captured indoors)
February 19	San Diego (Escondido)	33.1247	-117.0808	Service request (captured indoors)
February 25	San Diego (Chula Vista)	32.6278	-117.0481	Resident service request (captured indoors)
April 21	Orange (Anaheim)	33.8361	-117.8897	Resident service request (captured indoors)
April 21	Madera (Madera)	36.9614	-120.0608	Enhanced surveillance (CDC-AGO)
April 22	Los Angeles (Commerce)	34.0006	-118.1547	Enhanced surveillance (inspections)
April 30	Alameda (Hayward)	37.6689	-122.0808	Service request/consultation (captured indoors)
July 13	Tulare (Exeter)	36.2942	-119.1428	Enhanced surveillance (ovitrap)
July 17	San Diego (San Ysidro)	36.9949	-121.5024	Routine arbovirus surveillance (CO_2 EVS trap)
July 27	Imperial (Heber)	32.7308	-115.5297	Enhanced surveillance (ovitrap)
July 29	Madera (Parkwood)	36.9269	-120.0447	Enhanced surveillance (CO_2 EVS trap)
August 4	Fresno (Fresno)	36.7500	-119.7667	Enhanced surveillance (CDC gravid trap)
August 4	Imperial (El Centro)	32.8000	-115.5667	Enhanced surveillance (BGS)
August 6	Kern (Arvin)	35.2092	-118.8283	Enhanced surveillance (CDC-AGO)
August 12	Los Angeles (East Los Angeles)	34.0226	-118.1670	Enhanced surveillance (inspections)
August 17	Imperial (Imperial)	32.8475	-115.5692	Enhanced surveillance (BGS)
August 19	Los Angeles (Los Angeles)	34.0500	-118.2500	Enhanced surveillance (inspections)
August 28	San Diego (Bonita)	32.6583	-117.0353	Resident service request (CO_2 EVS trap)
September 1	Los Angeles (Maywood)	33.9872	-118.1855	Routine arbovirus surveillance (CDC gravid trap)
September 1	Madera (Madera Ranchos)	36.9311	-119.8815	Enhanced surveillance (CDC-AGO)

Table 2. Surveillance and control elements used by California vector control agencies to detect and control invasive *Aedes aegypti*.

Surveillance elements	Control elements
CO_2-baited EVS trap (EVS)	Sanitation
CDC gravid traps (gravid)	Public and medical community education
BioGents Sentinel trap (BGS)	Barrier treatments using adulticides with residual activity
CDC Autocidal Gravid Ovitrap (CDC-AGO)	Larvicides
Ovitraps	Truck-mounted ultra-low volume adulticide applications
Property inspections	Truck-mounted low volume larvicide applications
Service requests	Water crystals or sand for permanent containers
	Novel adult control methods (i.e., auto-dissemination augmented by males; *Wolbachia*)

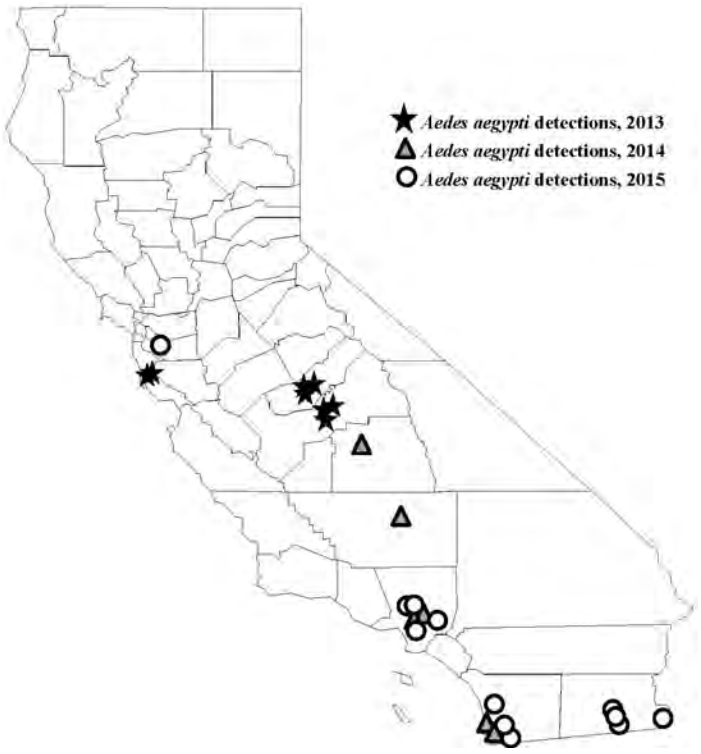

Figure 1. *Aedes aegypti* detections in California, 2013-2015.

Madera County

The county of Madera is located in the California Central Valley and has a hot semi-arid climate. *Aedes aegypti* was initially detected on June 6, 2013 in response to a service request from a resident in the city of Madera that complained of day-biting mosquitoes. During inspection of the property, Madera County Mosquito and Vector Control District (MVCD) personnel did not observe immature mosquitoes and per standard procedures, a CO_2-baited EVS trap (EVS) was placed in the backyard overnight; the three adult specimens collected that night were confirmed as *Ae. aegypti* females by the California Department of Public Health (CDPH). Follow-up surveillance with a BGS trap the following day at the same property detected 33 males and 17 females in a single trap night, indicative of an active adult population. Enhanced adult and larval surveillance revealed that this exotic species was present throughout the city of Madera.

As part of routine arbovirus surveillance, a single adult female was collected in Parkwood (4 miles south of the city of Madera) on July 8, 2013 and four adult *Ae. aegypti* were collected in Madera Ranchos (15 miles east of the city of Madera) on July 11, 2013. Enhanced surveillance at these sites, using BGS, CDC-AGO and ovitraps resulted in subsequent mosquito detections. The last detection in Madera County in 2013 occurred on December 15 and was a single female adult collected in a CDC-AGO trap located in the city of Madera, approximately 1 mile from the initial detection location.

In 2014, Madera MVCD received two separate resident service requests in mid-January complaining of mosquitoes biting indoors. In both cases, the resident captured adult *Ae. aegypti* from a bedroom. These homes were located within a few blocks of the initial 2013 detection site. No *Ae. aegypti* were discovered during the follow-up inspections of yards and interiors of these properties or as part of the enhanced surveillance throughout the city of Madera during the winter months. In 2014, the first *Ae. aegypti* in Madera County associated with enhanced trap deployment was a single female found in a BGS trap in early April. Most 2014 detections occurred within the city of Madera, and within the known 2013 infestation area.

Fresno County

On June 20, 2013, technicians from the Consolidated Mosquito Abatement District (MAD) responded to a routine service request from a resident in the city of Clovis. The city of Clovis is located 30 miles south of Madera, off the Highway 99 corridor. District personnel did not observe immature mosquitoes during the inspection of the property, and per standard procedures EVS and CDC gravid traps were placed in the backyard overnight. Three adult females from the EVS trap and one adult male from the gravid trap were confirmed as *Ae. aegypti*. Subsequent enhanced surveillance in 2013 indicated that *Ae. aegypti* had become well-established throughout Clovis.

Also within the jurisdictional limits of Consolidated MAD, a single detection, with no indication of an established population, occurred in the city of Fowler (15 miles south along Highway 99) in mid-October 2013. Additionally, in mid-October 2013, Fresno MVCD collected a single adult female from inside a house within the city limits of Fresno in response to a resident service request. No other *Ae. aegypti* were collected within the Fresno MVCD district boundaries despite public outreach and employing enhanced surveillance methods with ovitraps and CDC-AGOs. The final detection in Fresno County in 2013 was a single female collected from a CDC-AGO trap checked on December 3 in Clovis.

In early March 2014, Consolidated MAD detected three adult female mosquitoes in the city of Clovis over a 10-day period in CDC-AGO traps, further indicating that *Ae. aegypti* successfully overwintered in California. On September 10, 2014, Fresno MVCD reported that 29 eggs from an ovitrap placed at a business that sells garden pots in the city of Fresno were *Ae. aegypti*. Upon inspection of the business, *Ae. aegypti* larvae were collected from stagnant water in decorative fountains, and employees complained of being bitten by adults and seeing many mosquitoes indoors. *Aedes aegypti* activity throughout the 2014 season continued to be very active in the county of Fresno.

San Mateo County

In 2001, *Ae. albopictus* was imported to California from China via lucky bamboo and distributed to many nurseries around the state, including one in San Mateo County (Linthicum et al 2003). Although *Ae. albopictus* was believed to have been eradicated from most areas of California where it had been detected, ongoing detections have been made in Los Angeles County since 2011 (Zhong et al. 2013). As such, beginning in April 2013, San Mateo MVCD placed ovitraps at cemeteries and nurseries located throughout the county. On August 9, 2013, San Mateo County MVCD discovered three *Aedes* eggs in an ovitrap placed in a Menlo Park cemetery; a single adult male emerged and was confirmed as *Ae. aegypti* by CDPH. Enhanced surveillance in the adjacent residential neighborhood within a 0.2 mile radius of the cemetery included the deployment of 72 ovitraps, 60 EVS, 8 CDC-AGO, and 5 BGS traps, along with extensive door-to-door yard inspections. Those efforts resulted in the collection of 6 adults from CDC-AGO and BGS traps, 4 positive ovitraps, and 14 properties positive for *Ae. aegypti* larvae. Menlo Park is an affluent city located in the San Francisco Bay area with a mild climate. In 2013, the extent of the infestation appeared to be limited to a 0.5 square mile area around the Menlo Park cemetery with one infested property in the adjacent town of Atherton. The last 2013 collection in San Mateo County occurred on October 26 in a CDC-AGO.

In late January 2014, San Mateo MVCD detected *Ae. aegypti* activity in an area proximate to the previously infested Menlo Park cemetery. The four positive collections included one dead larva found in a previously treated flower pot, one larva in an ovitrap, one ovitrap with 11 eggs, and one adult female in a CDC-AGO. The total infestation zone in 2014 was approximately 0.5 square miles larger than the area infested in 2013 and included more properties in the adjacent town of Atherton.

ENHANCED SURVEILLANCE

Despite 2014 being the third driest year on record (USGS 2016), humid microhabitats created through extensive yard watering likely facilitated the establishment of *Ae. aegypti* in California. Larval habitats found on infested properties included small amounts of water in bird baths and planter saucers, non-functioning fountains, childrens toys, sprinkler heads, and litter. There were also instances of *Ae. aegypti* breeding in small amounts of water found in cryptic and unusual sites, such as an abandoned hot tub, sump drains, and rain gutters.

In addition to comprehensive property inspections, enhanced surveillance included the use of BGS traps in response to any resident complaining of day-time biting mosquitoes. Within a jurisdiction, EVS CO_2 traps continued to be placed at fixed sites for routine mosquito-borne virus surveillance (primarily for *Culex* and West Nile virus), and throughout an infestation zone, strategically placed ovitraps, BGS, and CDC-AGOs were also deployed. Most vector control agencies implemented a buffer zone strategy, whereby intensified surveillance and resident outreach occurred within a 0.2 mile radius of a site where *Ae. aegypti* of any life stage was collected.

Although most trap types have captured adult *Ae. aegypti* in known infested areas, it remains unclear which surveillance element works best for detections at novel sites in California. Most vector control agencies in areas adjacent to known *Ae. aegypti* populations have deployed CDC-AGOs and ovitraps due to the ease of use and low cost, respectively. In response to the general increased alert levels in California for this invasive *Aedes* species and related *Ae. albopictus*, vector control agenices are not only enhancing surveillance with novel traps, but also are utilizing the current extensive mosquito-borne virus surveillance system already in place. In addition, CDPH developed specific guidance for invasive *Aedes* surveillance and response in California (CDPH 2016).

Using enhanced surveillance methods and with the working knowledge that *Ae. aegypti* was established in multiple California sites, vector control districts were watchful for this invasive mosquito species. In 2014, *Ae. aegypti* mosquitoes persisted in Fresno, Madera, and San Mateo counties, and were newly detected in Kern, Los Angeles, San Diego, and Tulare counties. In 2015, *Ae. aegypti* mosquitoes persisted in all the previously detected counties along with new detections in Alameda, Imperial, and Orange counties. (https://www.cdph.ca.gov/HealthInfo/discond/Documents/AedesDistributionMap.pdf).

ENHANCED CONTROL

Enhanced control methods for combating *Ae. aegypti* have been different than the approaches used to control *Culex* vectors in response to West Nile virus activity. In particular, there has been a focus on sanitation through property inspections and the removal of possible breeding sources, along with extensive public outreach. Larviciding and adulticiding have supplemented source clean-up. A variety of larvicidal materials have been used, including methoprene, *Bacillus thuringiensis* subsp. *israelensis,* and spinosad.

Some vector control agencies initially implemented truck-mounted ultra-low volume applications of adulticides, but this approach has had limited success. The use of adulticides as residual barrier treatments on home exterior walls and vegetation in backyards was a more effective use of adulticide applications. The materials used included lambda-cyhalothrin, bifenthrin, and pyrethrin.

Local vector control agencies in areas with confirmed *Ae. aegypti* infestations were notified by their local health department of *Aedes*-borne arbovirus cases. In 2014, 137 human cases of chikungunya (68 confirmed, 69 probable) from 21 counties and 132 human cases of dengue (25 confirmed, 104 probable) from 21 counties were reported to CDPH. All dengue and chikungunya cases had travel histories to areas considered endemic or with a current outbreak (Porse et al. 2015). The vector control response to these cases typically included enhanced mosquito surveillance and control in the vicinity of the case patient's residence and neighborhood. Invasive *Aedes* mosquitoes collected during follow-up surveillance to a human case were generally submitted to the Davis Arbovirus Research and Training (DART) Laboratory at the University of California, Davis where they were tested for West Nile, St. Louis encephalitis, Western equine encephalitis, dengue, and chikungunya viruses (Table 3).

Table 3. *Aedes aegypti* from California tested for dengue, chikungunya, and West Nile viruses, 2013-2015.

Year	Location: County (City)	Individuals (pools) N	Results [1] DENV	CHIKV	WNV
2013	Fresno (Clovis)	214 (13)	NEG	NEG	NEG
2014	Fresno (Clovis)	1479 (45)	NEG	NEG	2 pools positive
2014	Madera (Madera)	231 (13)	NEG	NEG	NEG
2014	Los Angeles (Pico Rivera)	19 (2)	NEG	NEG	NEG
2015	Madera (Madera)	20 (1)	NEG	NEG	NEG
2015	Fresno (Fresno)	27 (2)	NEG	NEG	NEG

[1] DENV, dengue virus; CHIKV, chikungunya virus; WNV, West Nile Virus; NEG, all pools negative. Tests conducted at UC Davis on mosquito pools ranging from 1-50 individuals.

PREVENTION AND RESPONSE RECOMMENDATIONS

In response to the detection of *Ae. albopictus* in southern California in 2001 (Linthicum et al. 2003, Madon et al. 2002) and again in 2011 (Zhong et al. 2013), CDPH and local vector control agencies designed response activities to contain and potentially eradicate invasive mosquitoes. Due to similar biologies, many of the same recommendations are being implemented in response to the detections of *Ae. aegypti* (CDPH 2016). These response actions include: 1) notification of the local health department and neighboring vector control agencies upon invasive *Aedes* detection; 2) preparation of media release (in conjunction with the local health department) requesting the public to contact vector control if they detect day-biting *Aedes* mosquitoes on their property; 3) enhancement of adult and larval mosquito surveillance through the use of standard and novel trapping methods; 4) enhancement of mosquito control in infested areas through sanitation, larviciding, and barrier chemical applications; 5) assessment of potential alternative control methods (i.e., area-wide truck-mounted low-volume larvicide treatments, release of *Wolbachia*-infected mosquitoes, auto-dissemination of pyriproxyfen); and 6) distribution of education materials that target both general public and medical communities (https://www.cdph.ca.gov/HealthInfo/discond/Documents/2016InvasiveAedesSurveillanceandResponseinCA.pdf).

DISCUSSION

Upon detection of *Ae. aegypti* in 2013, the overall objectives were to determine the extent of the infestations, control and possibly eliminate these mosquito populations, and ultimately attempt eradication of this invasive species from California. However, as of 2015, with detections of *Ae. aegypti* in ten counties (https://www.cdph.ca.gov/HealthInfo/discond/Documents/AedesDistributionMap.pdf) and the possible expansion of the known infestation areas, eradication is likely no longer feasible (Morrison et al. 2008). The winter of 2013-2014 brought multiple consecutive days and nights of sub-freezing temperatures throughout California, especially in the Central Valley (NOAA 2014). Although the outcomes of a California winter on *Ae. aegypti* survival in the field were unpredicatable (Christophers 1960), it appears that *Ae. aegypti* successfully survived the winter and is now established in some areas of California. While the risk for autochthonous mosquito transmission of dengue, chikungunya, and Zika viruses remains low, several critical factors such as established populations of the primary vector and importations of these viruses in infected travelers do currently exist in California.

ACKNOWLEDGEMENTS

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Operational Impacts of Full Vector Reorganization

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ABSTRACT: In March 2015, the Coachella Valley Mosquito and Vector Control District implemented major changes to the way its Operations Department was organized. Prior to reorganization, separate departments existed for mosquito and imported fire ant work. Field technicians were rotated between the programs on a two-year cycle, with a need to re-train staff on protocols as part of the rotation. The reorganization eliminated separate departments and incorporated imported fire ant work into the field technicians' weekly responsibilities. This poster summarizes the benefits and disadvantages of this reorganization.

BACKGROUND

Prior to 2015, the Coachella Valley Mosquito and Vector Control District's Operations Department was organized into separate, vector specific departments that were supervised and operated independently. Increasing Mosquito and Red Imported Fire Ant work load in urban areas and expanding marsh areas due to Salton Sea shrinkage required a change in workforce allocation. Meeting the changing workload requirements had to be accomplished by improving field work efficiency. In late 2014 the District decided to merge Operations Departments and Supervisors into a "Full Vector" program to maximize field technician versatility and efficiency.

The Operations Department was organized into two separate departments. The Mosquito Department was composed of 8 urban zones, 3 agricultural zones, and 2 Salton Sea shoreline zones. The department had two Field Supervisors – one who specialized on Urban Mosquitoes and one who oversaw the work of the Rural group (the agricultural and Salton Sea shoreline zones). Zone technician responsibilities included conducting mosquito service requests, inspecting and treating neglected pools, and mosquito larval surveillance and treatments in their zone. Technicians also spent two days each week from October through March conducting mosquito surveillance and treatments at duck clubs near the Salton Sea twice each week. The Red Imported Fire Ant (RIFA) Department was composed of 8 technicians whose responsibilities included service requests and inspection and treatment of large scheduled properties (golf courses, homeowners' associations, schools, and parks). The Field Supervisor of this Department oversaw the scheduling of the large properties and the service requests.

Beginning March 1, 2015, technicians were assigned to 21 new zones – 15 urban zones, 3 agricultural zones, and 3 Salton Sea shoreline zones (Figure 1). Zone technicians were assigned to the 3 Field Supervisors so technicians from each habitat type (urban, agricultural, or Salton Sea) reported to each supervisor. Zone technician responsibilities included mosquito service requests, neglected pools, mosquito larval surveillance and treatments, and RIFA service requests. Technicians were given the ability to schedule RIFA service requests from homeowners within their zone instead of the Field Supervisor scheduling the inspection and treatment of the property. To conduct the treatment of the large properties for fire ants, each Field Supervisor's team was scheduled one day each week to make treatments. Inspections and treatments for duck clubs were reduced to once each week, because 21 technicians were now available instead of the 13 in the previous Mosquito Department.

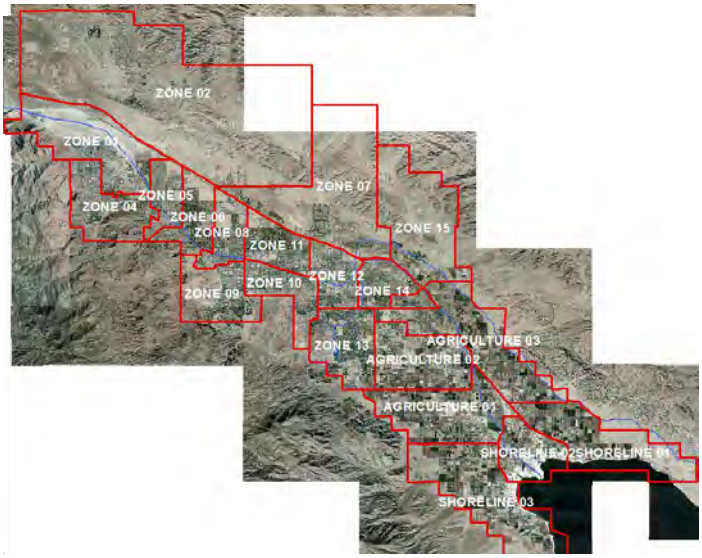

Figure 1. Coachella Valley technician work zones. The size of each zone varies and was calculated due to mosquito inspections and treatments, red imported fire ant service requests, and neglected pool work from 2014.

RESULTS

As expected, the most significant change that resulted from the reorganization was in the number of larval mosquito treatments. An increase in personnel in urban zones resulted in smaller zones which led to a 36% increase in larval mosquito treatments in residential habitats (Figure 2). More personnel in shoreline zones resulted in 47% more larval mosquito treatments in Salton Sea habitats (Figure 2). While there was a small decrease in the number of treatments made for red imported fire ants in 2015 compared with previous years (Figure 3), it was not a dramatic reduction.

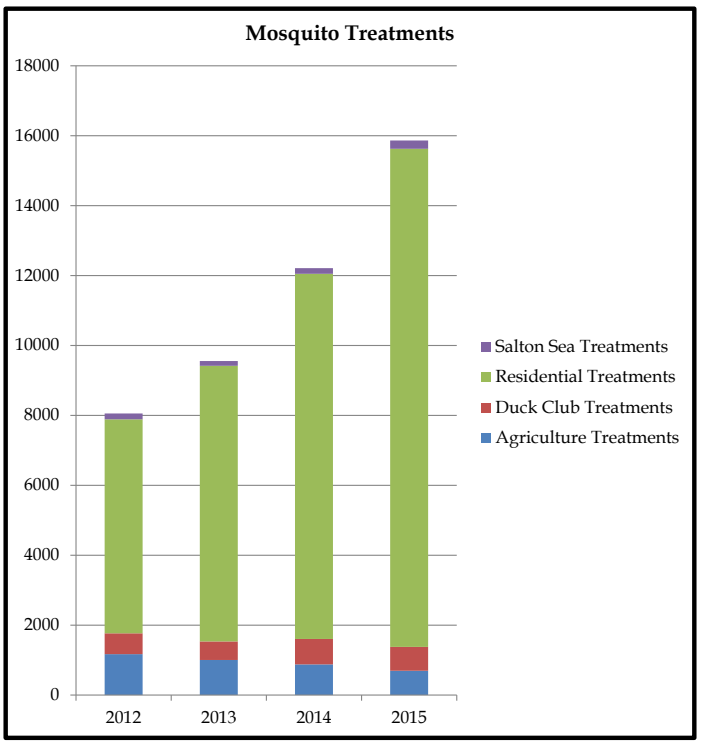

Figure 2. Number of mosquito treatments each year by habitat.

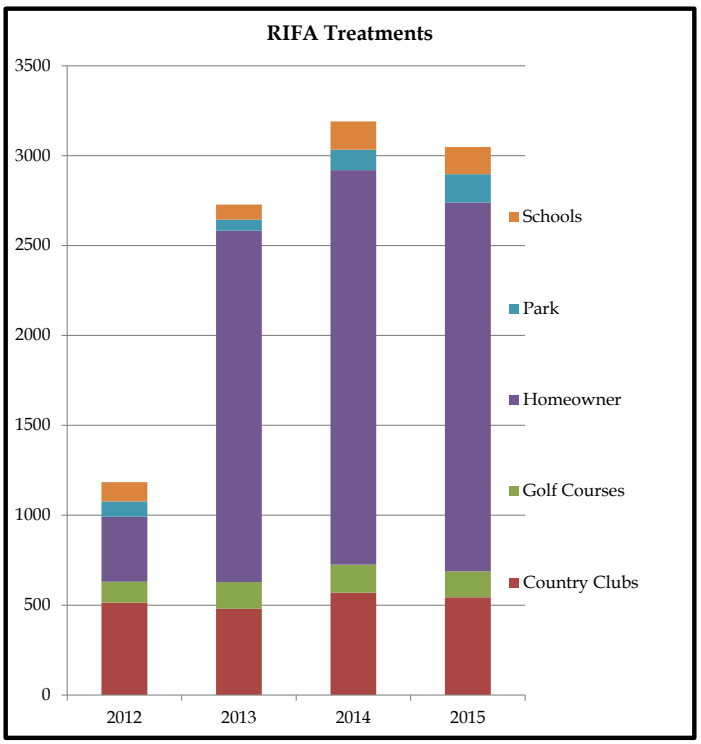

Figure 3. Number of red imported fire ant (RIFA) treatments each year by habitat.

In previous years, the District received a large number of service requests for fire ant inspection and treatment in the fall, often leading to a backlog where the resident would wait several weeks between the initial call and the time a technician could get to the property. Although there is no official record of how long it would take, there is a feeling that there was a reduction in the time between when the request was initiated and when the property was inspected (Figure 3).

In 2015, the District detected West Nile virus in mosquitoes within an urban area for a sustained period of time. The change to Full Vector allowed the Operations Department to have greater flexibility in responding to this problem as more staff members were available to move to the area of concern. This allowed for quicker inspections and treatments, which may have contributed to the low number of human cases detected in the Coachella Valley in 2015.

The change to Full Vector did contribute to some additional equipment costs, as there was a need to outfit all staff with the ability to treat both mosquitoes and fire ants. Additional time was spent in the winter ensuring that all technicians were knowledgeable about protocols for both mosquitoes and fire ants.

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Using Established Work Standards to Evaluate Field Technician Performance

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ABSTRACT: The Coachella Valley Mosquito and Vector Control District has established Field Technician work standards to set the level of expected work performance. In order to expedite and ensure uniformity of Field Supervisor's annual Field Technician employee evaluations, supervisors use a data gathering and reporting program developed at the District.

BACKGROUND

In March 2015, the Coachella Valley Mosquito and Vector Control District reorganized its Operations Department to a Full Vector program (Avalos et al. 2016). The elimination of specialized, vector specific departments and technicians also meant that Field Supervisors were managing all aspects of vector control operations. To ensure uniform evaluations of technicians, a program was developed to help Supervisors rate and evaluate Field Technicians in achieving their work standards, vector control knowledge, and adherence to District policy and procedures in a uniform manner. Work standards have been developed for each position at the District based upon the Job Description and the work that was being completed by staff members in that role. For the Technician position, these standards were developed using averages of work performed in different habitats over several seasons. The Work Standards were developed in 2013 and revised in 2015 with the re-organization of the Operations Department.

THE PROGRAM

The program consists of four forms that are web based and field accessible (Figure 1). Two forms are used by Supervisors when accompanying technicians in the field (Technician Ride-Along Form and Service Request Form), one is specific to larval treatment follow-up evaluation (Mosquito Follow-up Form), and the Supervisor's Notes form is used to document situations not covered by the other forms. A specific form can be selected depending on the activities the Supervisor is observing. The rating categories are designed to highlight work standards and District policies. A Supervisor can rate the performance for that task from 1 to 4, where a ranking of 4 indicates a mastery of the skill while 1 indicates no knowledge of how to perform the skill. Each item also allows for the entry of notes that the Supervisor can review.

The Technician Ride-Along Form (Figure 1) covers the correct use of equipment, storing chemical, and organization of work vehicle; appropriate selection of pesticide (product) and rate; using personal protection equipment; entering data into the Mobile system; demonstrating appropriate knowledge of products, modes of action, and product rotation; demonstrating appropriate knowledge of vector surveillance, habitats, behavior, and seasonality; and managing zone work. The Service Request Ride-Along Form (Figure 2) is used when the Field Supervisor accompanies a Technician conducting a Service Request. The Supervisor evaluates the technician on having an organized and accessible set of paperwork and informational brochures; appearing presentable in uniform; interaction with the residents; conducting a thorough inspection and treatment; using appropriate personal protection equipment; selecting an appropriate pesticide and application rate; entering data into the Mobile system; and securing equipment and chemical after the treatment. Using the Mosquito Follow-up Form (Figure 3), the Field Supervisor evaluates the Technician on inspections and treatments the Technician has previously made. The Form allows for scores to be entered on choice of pesticide and application rate; entry of data into the Mobile system; choice of application equipment; use of personal protection equipment; product efficacy; and product rotation.

USE

Along with forms listed above, the Technician Activity Report (Figure 4) allows the Field Supervisor to see a summary or the work that a Technician has conducted in a defined period of time. The Field Supervisor uses this report in their monthly discussions with the Technician to discuss how well the Technician is meeting the Work Standards. This allows the Technician to know how well they are performing in the job and what areas could be improved prior to the annual evaluation. Because the items are less subjective, it allows for greater uniformity of evaluation of performance across different Field Supervisors. The Field Supervisors have had discussions as the forms and report were being developed to ensure that they were rating staff equivalently.

A separate report is being developed that will compile individual question scores on the Ride-along and Follow-up forms and tie them to specific categories in the employees annual performance evaluation; making those evaluations more uniform and consistent between different supervisors. The program will undergo continued improvement as Field Supervisors provide feedback on potential additional forms and information that can be added to the reports.

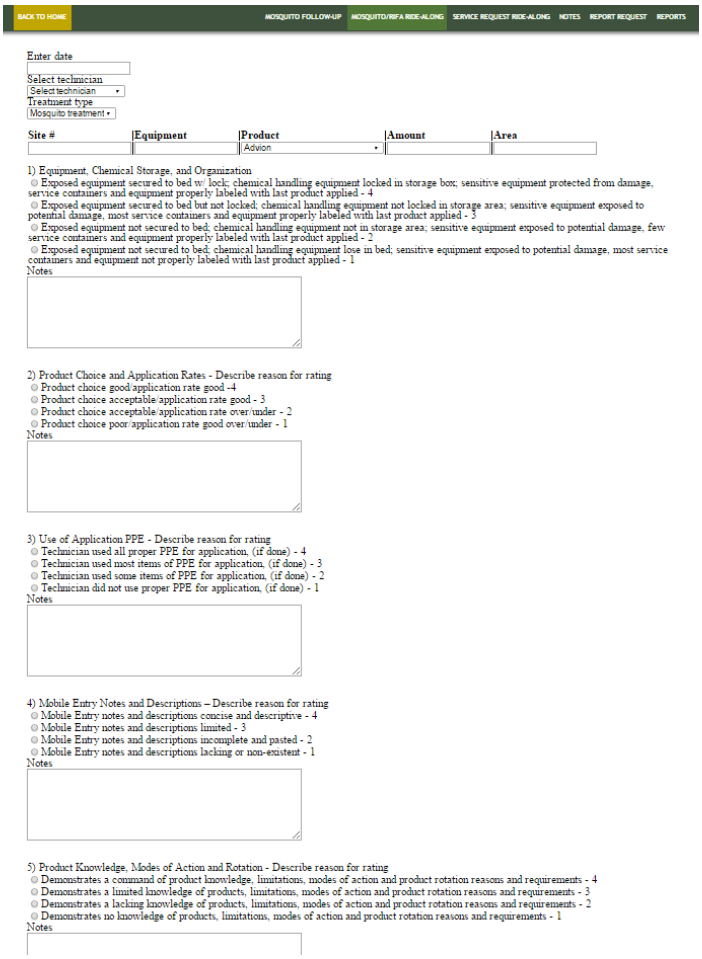

Figure 1. Example of a form used in the online system. The form shown is the Technician Ride-Along Form, which allows the Field Supervisor to score the Technician on their knowledge and use of appropriate procedures for equipment, product choice, personal protection equipment, and Mobile data entry system, among others.

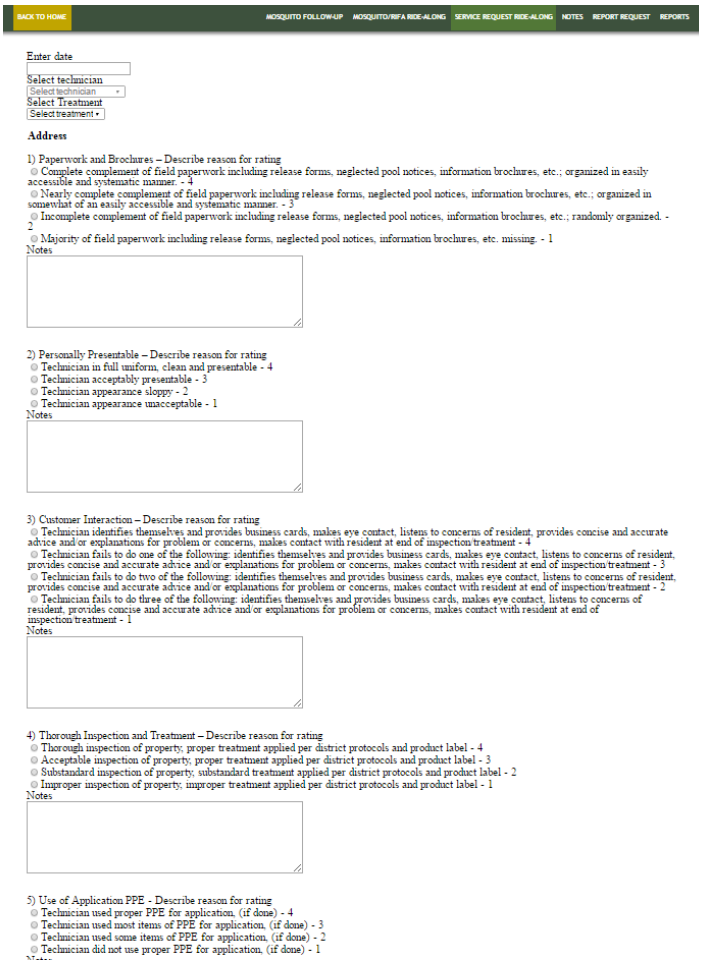

Figure 2. The Service Request Ride-Along Form, which allows the Supervisor to evaluate the Technician on presentation and interaction with the resident during a Service Request visit.

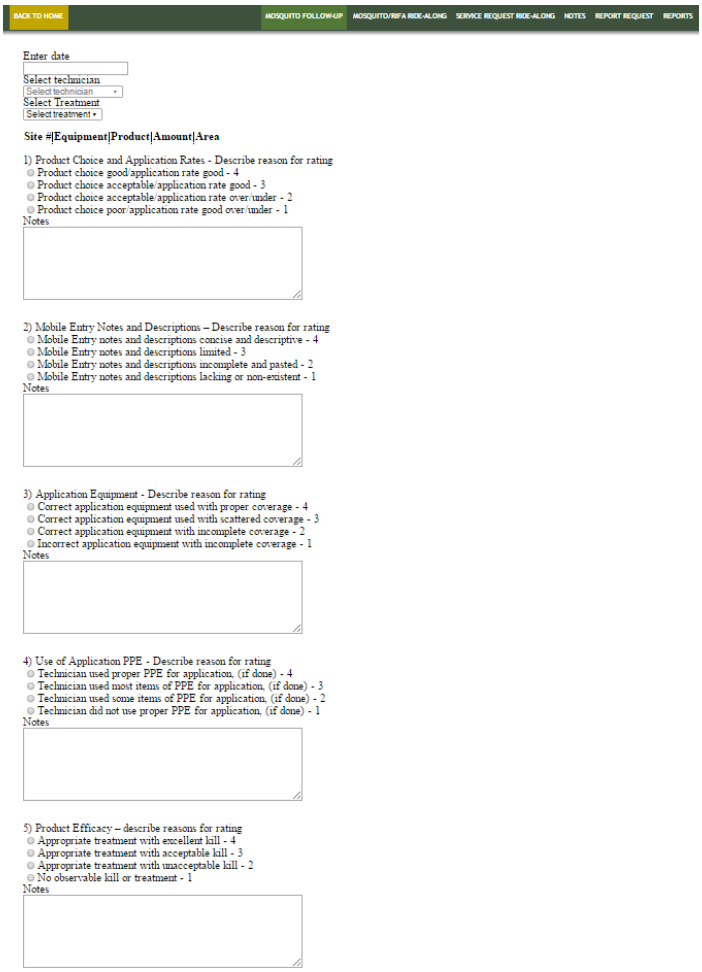

Figure 3. The Mosquito Follow-up Form which allows for a Field Supervisor to evaluate an application made by a Technician, ensuring that the product choice

Coachella Valley Mosquito and Vector Control District
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Technician Activity Report

Technician: Jeremy Wittie

From: 02/01/2015

To: 01/31/2016

Rifa Treatment	**197**
Type	**Count**
Homeowner	157
Country Club/HO	24
Park	8
School	5
Golf Course	3

Rifa Inspection	**219**
Type	**Count**
Homeowner	170
Country Club/HO	29
Golf Course	14
Park	3
School	3

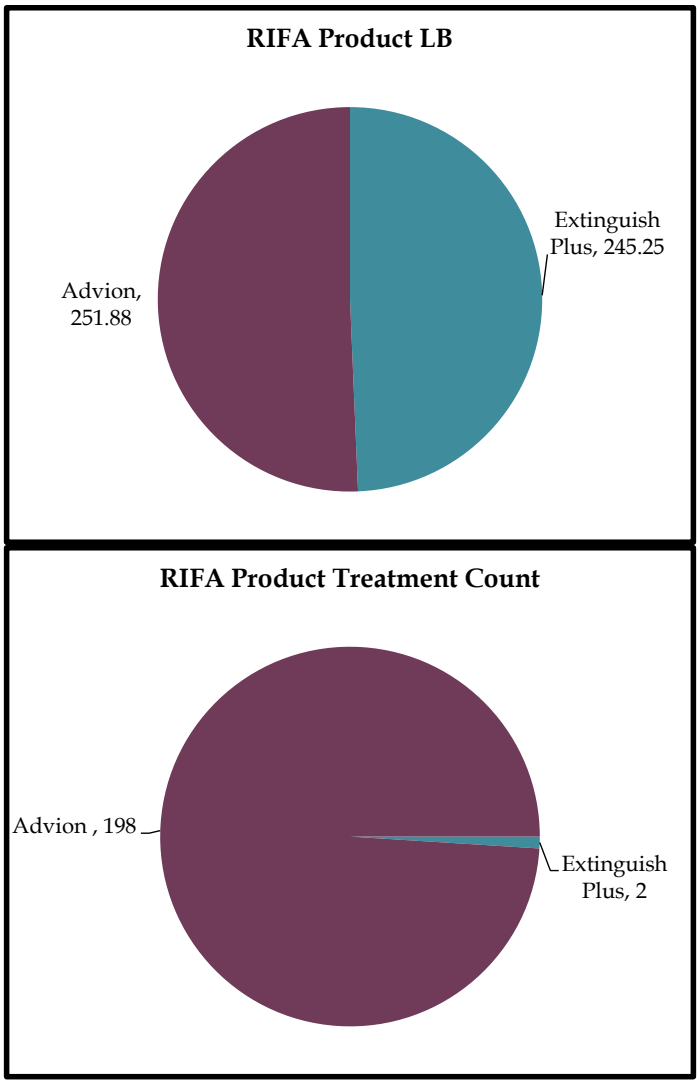
Vector Treatment	**1810**
Type	**Count**
Residential	1745
Duck Club	42
Agriculture	22
Salton Sea	1

Vector Inspection	**3082**
Type	**Count**
Residential	3015
Duck Club	52
Agriculture	13
Salton Sea	2

Lab Sample	**67**
Type	**Count**
Duck Club	37
Residential	21
Agriculture	9

Larval Surveillance	**1416**
Type	**Count**
Residential	1404
Agriculture	11
Duck Club	1

Service Request	**235**
Type	**Count**
Rifa	190
Neglected Pool	13
Mosquitoes	12
Bees	8
Standing Water	4
Fly/EyeGnat	3
Mosquitofish	3
Other	1
Rodents	1



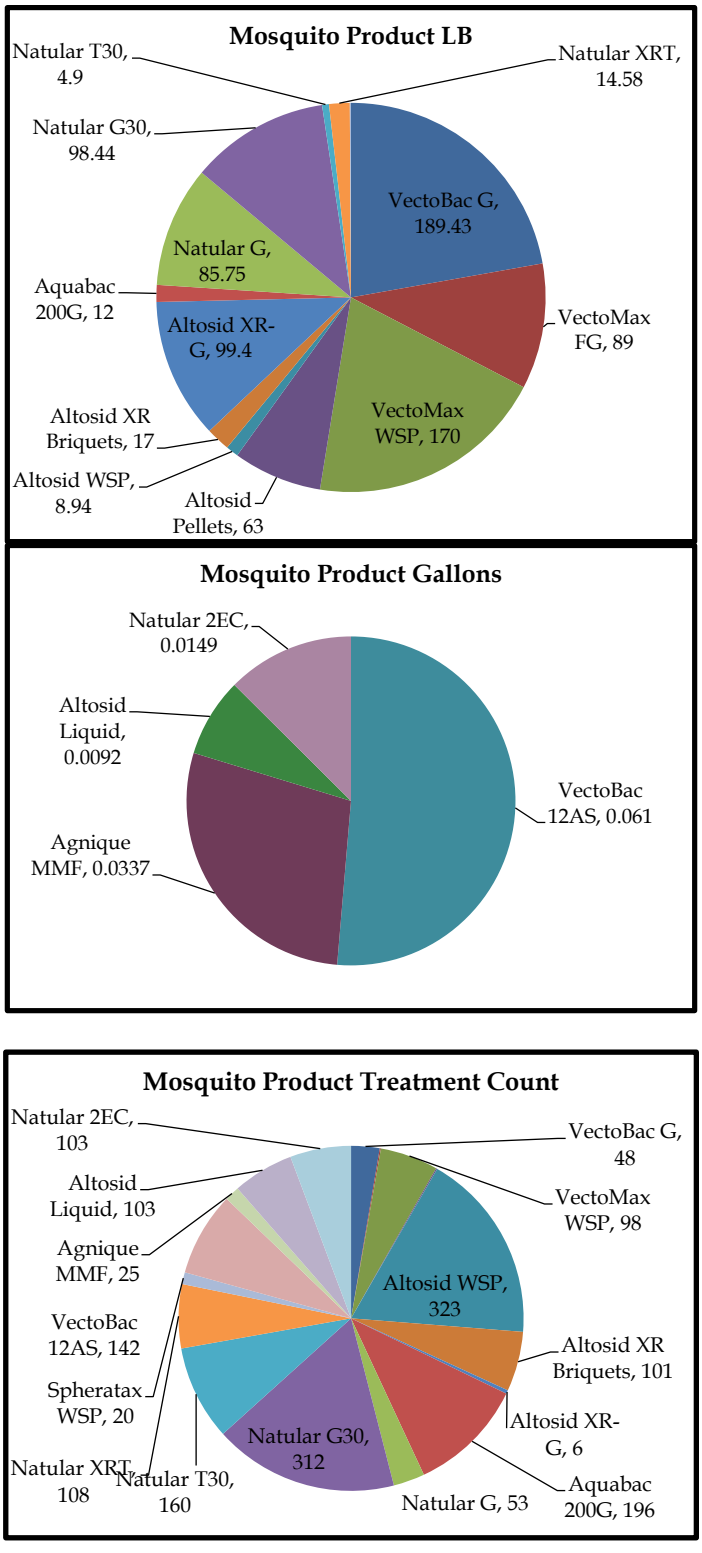


Figure 4. Print-out of the Technician Activity Report. Based on the entries that the technician makes into the Mobile Application, counts and product use are tallied. The graphs that follow are also produced by the Technician Activity Report, allowing supervisors to check for product use and rotation.

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The Impact of Using Backpack LV Sprayers and Hand Held ULV Foggers to Manage *Aedes albopictus* in the San Gabriel Valley, Los Angeles County, California

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ABSTRACT: Since *Aedes albopictus* was found in our district in Sep 2011, attempts at eradication have failed, resulting in increases in both its abundance and distribution. We used Stihl® SR 450 backpack sprayers (sprayers) to apply VectoBac® WDG and Colt 4 hand held foggers (foggers) to apply Duet® for the past two years to manage the infestation of *Ae. albopictus* and relieve residents from mosquito bites. Here we analyze data from 2014 to determine the effectiveness of this strategy as a means of suppressing populations and reducing biting nuisance. Eggs collected from oviposition cups and larvae from peridomestic sources were counted at each site. Adults gathered weekly from CDC- Autocidal Gravid Ovitraps (AGO) and BioGents (BG) Sentinel traps were counted. Although we reduced the number of adults, larvae, and eggs that were present, this approach was labor intensive, and the impact was short-lived. The effectiveness of this strategy may be enhanced if it is a component of an integrated program to manage vectors. Even though sprayers and foggers only provided short term suppression, they were helpful tools in temporarily reducing the risk of being bitten by mosquitoes that could potentially transmit pathogens.

INTRODUCTION

Aedes albopictus is a container breeding species. Not only is it opportunistic and a relentless biter, but it is considered a competent vector of at least 23 arboviruses including chikungunya, dengue, and Zika viruses (Chouin-Carneiro *et al*., 2016, Moore and Mitchell 1997). This species was found in Los Angeles County in 2001 in the cities of Monterey Park, El Monte, Alhambra, and the City of Industry, but was believed to be eradicated (Linthicum *et al*., 2003). *Aedes albopictus* was rediscovered on 2 Sep 2011 in a mobile home park in the south-central region of the city of El Monte (Fujioka *et al.*, 2012). The city is in the jurisdiction of the San Gabriel Valley Mosquito and Vector Control District, which provides services for 23 cities and unincorporated county areas of the San Gabriel Valley in Los Angeles County. The majority of the 54,390 hectares in the district is urbanized; there are over 300,000 individual parcels (Fujioka *et al.*, 2012). Multiple agencies attempted to eradicate the latest infestation, but unfortunately *Ae. albopictus* spread exponentially from one to 13 cities in three years (Wekesa *et al.,* 2014, Brisco *et al.,* 2015) and to 17 cities in five years (Ruedas *et al.,* 2016) covering 11,672 hectares in 2014 and 26,421 hectares of the District in 2015. As the area infested by *Ae. albopictus* continues to increase, so does the concern about its ability to transmit viruses and the need for practical and effective control methods. The current attention to Zika virus has created an even greater sense of urgency to these issues.

As the area infested by *Ae. albopictus* expanded from 2011-2013, the District attempted to design a cost-effective program to relieve residents from the biting nuisance from this species. Unlike our traditional surveillance and control program, this approach focused primarily on preventing a nuisance and secondarily on reducing the potential for disease transmission.

MATERIALS AND METHODS

All treatments were in the cities of El Monte and Duarte both of which have established populations of *Ae. albopictus*. Properties where *Ae. albopictus* are found typically have yards with shade and vegetation, gardens or a high number of potted plants, bromeliads, or accumulated debris. Sites with these conditions have perpetual sources of standing water and a relatively cool and humid microclimate which is ideal for *Ae. albopictus.*

We applied pesticides to infested and adjacent properties. Infested properties were defined as those sites where samples of eggs, larvae, and/or adult *Ae. albopictus* were collected. Sampling conducted for two weeks prior to treatment provided an estimate of infestation. Eggs were gathered from oviposition cups (ovicups). To determine larval count, sources were surveyed before each application. Adults were collected using a single BioGents (BG) Sentinel traps or CDC-Autocidal Gravid Ovitraps (CDC-AGO traps). One of each trap type was placed on a property with the homeowner's consent. Residents were instructed to remove containers that could hold water prior to treatment. Mosquito samples were collected for 2 weeks pre-treatment and 8 weeks post-treatment. We compared the number of eggs, larvae, and adult samples that were collected pre- and post-treatment to determine the impact of the back pack sprayer and handheld fogger applications.

We applied the larvicide VectoBac® WDG (37.4% *Bacillus thuringienses* var. *israelensis* (*Bt*i) at 7 fl. oz. per acre with a Stihl® SR450 back pack sprayer (low volume, 60 -120 um droplet size)(sprayer), and the adulticide Duet® (1% prallethrin, 5% sumithrin, 5% piperonyl butoxide) at 1.25 fl. oz per acre, 1:3 ratio of concentrate to mineral oil, with a Colt 4 handheld fogger (ultra-low volume, 20 - 40 um droplet size). Treatment protocols were larvicide alone or larvicide and adulticide. Larvicide alone was used at properties with a high number of eggs and larvae but few adults, and a combination of larvicide and adulticide was applied

at sites when all stages of *Ae. albopictus* were present in high numbers.

The properties were monitored for eight weeks post-treatment. If adult *Ae. albopictus* were found on the treated property upon follow up, the property was re-treated. Pesticide applications and the impact on abundance of *Ae. albopictus* was determined by regression analysis of the abundance of all stages of the target mosquito over a 10 week period, 2 weeks pre- and 8 weeks post-treatment. Only sites (n) that were monitored continuously were included in the analysis. Other than the two weeks monitored pre-treatment, untreated control comparisons were not used in this study. The untreated controls were not considered due to the ethical concerns associated with *Ae. albopictus* bites and potential for disease transmission.

RESULTS

Applying pesticides with sprayers and foggers to control *Ae. albopictus* in infested properties produced a consistent impact on its abundance measured in the counts of eggs, larvae, and adults collected over an eight week post-treatment period. To determine the impact of pesticide treatment, sites with eggs and larvae of *Ae. albopictus* received larvicide treatments only, and those with high counts of eggs, larvae, and adults received both larvicide and adulticide treatments. The properties treated with VectoBac® WDG alone showed a significant reduction in *Ae. albopictus* larvae ($r = -0.55$, $p < 0.05$) immediately after treatment (week 0), although the number rebounded two weeks later, the re-treatment further reduced the larval counts. These reductions were easily sustained with additional re-treatments on week 8 (Figure 1). The number of adults also showed an immediate decline after treatments ($r = -0.60$, $p < 0.05$), but rebounded four weeks later. Re-treatment reduced both larval and adult counts further, and these reductions were easily sustained with additional re-treatments on week 8 (Figure 1).

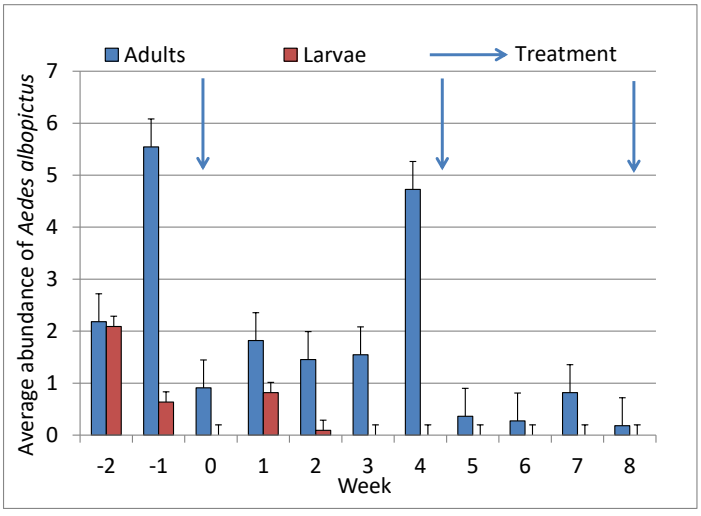

Figure 1. The average number of larvae and adult *Ae. albopictus* (+ SE) collected at sites (n =11) treated with VectoBac® WDG.

This same trend occurred when a combination treatment of Duet™ and VectoBac® WDG was applied. This combination treatment was applied multiple times due to the rebound effect that was observed in adults and larval counts. The adult counts declined immediately after treatment during week 0 but rebounded by week 2 post-treatment necessitating a second treatment. A similar rebound effect, observed at week 6, exactly 3 weeks after the second treatment. The last observed rebound was reversed by another combination application at week 8 (Figure 2). Number of eggs collected at sites during the study period before treatment declined by 60% at week 8 post-treatment ($n=25$, $r = -0.98$, $p < 0.05$, Figure 2). This decline seemed sustainable although it may have mirrored the adult mosquito counts (Figure 2).

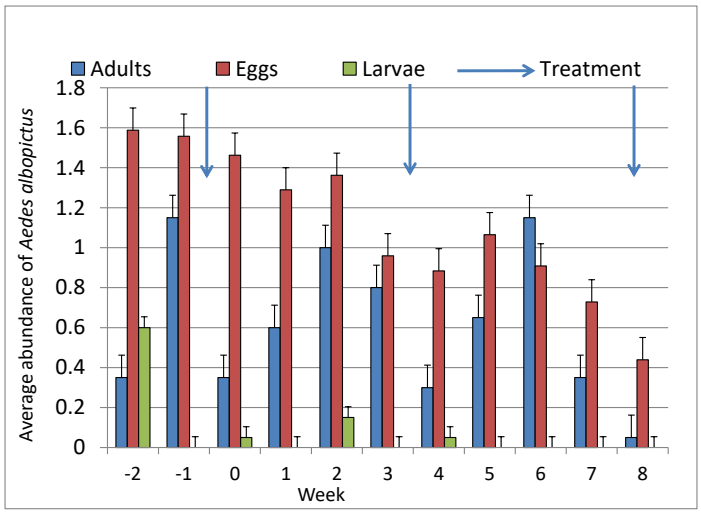

Figure 2. The average number of eggs (log10), larvae and adult *Ae. albopictus* (+ SE) collected at sites (n = 25) treated with VectoBac® WDG and Duet®.

DISCUSSION

Our protocol of using sprayers and foggers to combat invasive *Aedes* generated promising results. Adulticides applied using ULV cold aerosol fogger or thermal space sprays are considered as the only effective means of providing relief from mosquito bites when *Ae. albopcitus* densities are at peak abundance (CDC 2013). Applying adulticides combined with low volume (LV) applications of VectoBac WDG® larvicides (Wekesa *et al.,* 2015) may arrest a mosquito-transmitted disease outbreak by targeting mosquitoes at several stages of development.

Although the impact of treatments in this study were short-lived, they were effective in providing a significant reduction of *Ae. albopictus* biting nuisance. This was documented when the adult populations plummeted shortly after treatments. This observation is important because the *Bt*i in VectoBac® WDG is known to cause mortality of adult mosquitoes when applied as an aqueous suspension (Klowden *et al.*, 1983, Zahiri and Mulla, 2005). Whether VectoBac® WDG was applied in combination with Duet™ or alone, it decreased the number of larvae and also appeared to measurably impact adult mosquitoes

(see Figure 1 and 2). If the applications of both products were made over a larger area, they may have limited migration of *Ae. albopictus* into the treated area and the impact could have been more dramatic. The challenge this approach faces is similar to the door-to-door inspections; it is labor intensive and relies on residents' cooperation. Despite this, we think that simultaneously adulticiding and larviciding combined with other integrated pest management approaches may significantly reduce *Ae. albopictus* populations. Additional investigation is warranted to quantify the impact of VectoBac® WDG on adult *Ae. albopictus*.

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