Title

Scented Sugar Baits Enhance Detection of St. Louis Encephalitis and West Nile Viruses in Mosquitoes in Suburban California

Journal

Journal of Medical Entomology, 55(5)

ISSN

0022-2585

Authors

Steiner, Cody D

Riemersma, Kasen K

Stuart, Jackson B

et al.

Publication Date

2018-08-29

DOI

10.1093/jme/tjy064

Journal of Medical Entomology, 55(5), 2018, 1307–1318
doi: 10.1093/jme/tjy064
Advance Access Publication Date: 27 April 2018
Research Article

Scented Sugar Baits Enhance Detection of St. Louis Encephalitis and West Nile Viruses in Mosquitoes in Suburban California

Cody D. Steiner,[1,*] Kasen K. Riemersma,[1,*] Jackson B. Stuart,[1] Anil Singapuri,[1] Hugh D. Lothrop,[2] and Lark L. Coffey[1,3]

[1]Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, One Shields Avenue, 5327 VM3A Davis, CA 95616, [2]Coachella Valley Mosquito and Vector Control District, Riverside County, CA, and [3]Corresponding author, e-mail: lcoffey@ucdavis.edu

*These authors contributed equally to this article.

Subject Editor: Harry Savage

Received 20 December 2017; Editorial decision 3 April 2018

Abstract

Scented sugar baits deployed in California deserts detected early West Nile virus (WNV) transmission by mosquitoes, representing a potential improvement to conventional arbovirus surveillance that relies heavily on infection rates in mosquito pools. In this study, we expanded deployment of scented sugar baits into suburban Sacramento and Yolo (2015, 2016) and Riverside Counties (2016), California. The goal of the study was to determine whether scented sugar baits detect WNV and St. Louis encephalitis virus (SLEV) concurrent with mosquito infections in trapped pools in areas of high human density. Between 8 and 10% of sugar baits were WNV RNA positive in both study years across the three counties. In Riverside County, where SLEV re-emerged in 2015, 1% of sugar baits were SLEV positive in 2016. Rates of sugar bait positives were at least 100 times higher than infection rates in trapped mosquitoes in the same districts. The prevalence of sugar bait positives varied temporally and did not coincide with infections in mosquitoes collected at the same sites each week. WNV RNA positive sugar baits were detected up to 2 wk before and after concurrent surveillance detected infection in mosquito pools at the same sites. Sugar baits also detected WNV in Riverside County at locations where no WNV activity was detected in mosquito pools. Sugar baits generated between 0.8 and 1.2 WNV positives per $1,000 and can be more economical than carbon dioxide baited traps that produce 0.8 positives per $1,000. These results indicate that the sugar bait approach enhances conventional arbovirus surveillance in mosquitoes in suburban California.

Key words: arbovirus surveillance, mosquito, West Nile virus, St. Louis encephalitis virus

Arbovirus surveillance in many regions of the world conventionally includes trapping mosquitoes and testing them for viruses. In California, conventional surveillance includes detection of mosquito-bird-mosquito transmitted West Nile (WNV) and St. Louis encephalitis viruses (SLEV, both *Flaviviridae*, *Flavivirus*) that cause encephalitis in humans. Mosquito traps used in California include modified Centers for Disease Control (CDC) traps baited with dry ice that emit carbon dioxide to attract host-seeking female mosquitoes and draw them into a holding container using downdraft fans (Newhouse and Siverly 1966), as well as gravid traps consisting of tubs of water with organic matter to attract ovipositing females that are drawn into a container using updraft fans (Reiter et al. 1986, Cummings 1992). Although highly used, both trap types have drawbacks. These include the high cost of dry ice or propane to produce carbon dioxide, the requirement for batteries to operate the fans, the need to carry heavy equipment and jugs of water to trap sites, and the requirement for facilities and expertise for identifying and processing mosquitoes before extraction of viral RNA from pools for molecular diagnosis. Due to these requirements, traps are often only present at a given site one night per week, restricting surveillance to a fraction of each month, and potentially leading to missed arbovirus activity in mosquitoes. Furthermore, conventional mosquito surveillance involves collecting and testing whole mosquito carcasses for arbovirus infection, which potentially overestimates transmission intensity because not all infected mosquitoes are capable of transmitting (Coffey et al. 2014). Pooling can also

underestimate infection, especially when arbovirus circulation intensities are high, due to implicit assumptions of one positive mosquito per pool (Gu et al. 2003, Condotta et al. 2004).

A newer sugar baited surveillance method based on research that showed *Culex* vectors feed frequently and repeatedly on fructose in nature (Reisen et al. 1986), now in widespread use in Australia (Hall-Mendelin et al. 2010, van den Hurk et al. 2014, Flies et al. 2015) and piloted in desert regions of California in 2011 (Lothrop et al. 2012), circumvents limitations inherent to carbon dioxide baited and gravid traps. Another advantage is *Culex* mosquitoes that transmit SLEV and WNV require sugar throughout their gonotrophic cycles, thereby feeding more frequently on sugar than on blood from hosts (Foster 1995), potentially increasing arbovirus detection. Sugared wicks or cards containing nucleic acid preservatives placed in the field are tested for the presence of arbovirus RNA deposited by sugar-feeding mosquito vectors. Importantly, sugar-baited tracking of arboviruses documents virus transmission by mosquitoes instead of infection and is less limited by conventional economical and logistical trapping constraints since arbovirus RNA is deposited into wicks that are inexpensive and easy to transport and deploy in high numbers, without the requirement for a power source.

Sugar-baited surveillance in some areas of Australia currently couples honey-soaked cards placed in traps with or without a carbon dioxide lure. Cards are first tested for arbovirus RNA, indicating transmission, sometimes for multiple arboviruses at once (Hall-Mendelin et al. 2010, van den Hurk et al. 2014, Flies et al. 2015, Johnson et al. 2015). If arbovirus positive cards in traps are detected, mosquitoes in traps are not screened, saving time and energy required for processing and testing mosquito pools, which is only conducted if cards test negative. Here we attempted to further simplify the sugar baited detection approach by eliminating the trap, thus deploying sugar baits coupled with an odorant lure.

An earlier study that employed sugar baits to detect arbovirus activity in California showed that in some locations, WNV RNA positive sugar baits were detected before concurrent surveillance measures of infection from mosquito pools or transmission from sentinel chicken seroconversions in the Coachella Valley (COAV) desert in Riverside County (Lothrop et al. 2012). However, only 1% (1/90) of sugar baits deployed in suburban regions of California tested WNV RNA positive in that study, possibly due to an abundance of naturally available sugar sources in flowers in suburbia that outcompeted the sugar wicks, or the small sample size of suburban baits. Given that most of the 40 million inhabitants of California live in suburban or urban areas, surveillance must serve those regions to best estimate human risk for arbovirus infections. Therefore, the purpose of this study was twofold: 1) refine the sugar baited surveillance approach in California developed by Lothrop et al. by making baits easier to construct at lower cost, and 2) to extend use of sugar baited surveillance into suburban areas of California. Our results show that sugar-baited surveillance detects WNV transmission up to 2 wk earlier and 2 wk later in California than conventional carbon dioxide and gravid traps. SLEV activity in sugar baits was also detected in COAV. Arbovirus activity was rarely detected by sugar baits and conventional traps at the same locations and times, suggesting that neither approach alone estimates true arbovirus activity. Cost analyses of our sugar bait approach compared to trapping methods revealed sugar baits to be up to 50% more cost-effective than conventional methods for WNV detection. The results from these studies indicate that sugar baits may serve as an economical complement to conventional trap-based surveillance for arboviruses in mosquitoes in California.

Materials and Methods

Sugar Bait Construction

New, lower-cost sugar baits, compared to polyvinyl chloride (PVC) pipes used previously that required custom construction (Lothrop et al. 2012), were designed using materials purchased from Ace Hardware, United States, including Plantation Products Peat Pot Strips, Tool Time Caulking Caps, and steel baling wire. A 2-ml microcentrifuge tube (Eppendorf, North America, Hauppauge, NY) was filled with 2 ml of sucrose solution, made by dissolving grocery store grade sugar in sterile water, and was plugged with a 1.5 cm piece of Richmond Cotton Dental Wick (Charlotte, NC), then inserted into the caulking cap (Fig. 1). To test stability of the sugar bait and pot cover in the field, three baits were deployed for 2 wk at ca. 30°C at 1 m height hanging from tree branches in suburban Yolo County and monitored daily to ensure structural integrity during continuous outdoor deployment. Although no rainfall occurred during the trial, we mimicked rain by soaking the peat pot in water every 2 d; this had no destructive effect on the pot. The caulking caps would loosen the pot cover during handling over time. To circumvent this issue, a smaller PVC pipe than originally used in the 2011 study was engineered and used as replacement for the caps at the COAV sites in 2016. Bait tubes were filled with 40% sucrose solution (C&H pure cane sugar,

Fig. 1. Photographs of sugar baits. Sugar bait devices were constructed by threading baling wire through a caulking cap and feeding it through the hole of a seedling starter pot. A 2-ml plastic tube (Eppendorf) was filled with ~2 ml of a 40% sucrose and tap water solution and plugged with a cotton dental wick, and this bait tube was inserted into the open end of the caulking cap. Once deployed, the sugar soaked cotton wick was exposed for sugar seeking mosquitoes to access for 6 to 7 d. The image on the right compares the peat pot cover used in SAYO Counties to the small PVC cover used in the COAV.

Domino Foods, Inc., Iselin, NJ) and placed in their peat pots. The outside of the pot was sprayed with Plumeria Fragrance Mist (Bath and Body Works, Reynoldsburg, OH) to serve as a floral attractant. In 2016 in the Sacramento and Yolo (SAYO) Mosquito and Vector Control Districts, 300 ul of raw honey (Trader Joe's, Monrovia, CA) mixed into 2 ml of 40% sucrose was added to the cotton wicks.

Field Deployment of Carbon Dioxide Baited and Gravid Traps

Sugar-baited surveillance was conducted in parallel with carbon dioxide baited and gravid mosquito trapping in SAYO in 2015 and 2016 and in COAV in 2016. In SAYO, Heavy Duty EVS Carbon Dioxide Mosquito Traps, (product #2801A, Bioquip Products, Rancho Dominguez, CA) without light bulbs, with a fan powered by four D-cell batteries, and containing 3 pounds of pelleted dry ice per trap per night were used. Gravid traps in SAYO consisted of Gravid Mosquito Traps (product #2800, Bioquip Products, Rancho Dominguez, CA) without bulbs run on a 6 volt 12 amp hour sealed lead acid battery, without carbon dioxide as a lure, that contained infusion water made in a 1 gallon container by combining 4 liters of warm water, 4-g ground alfalfa pellets, 3-g ground hog chow (Purina, Largo, FL), and 0.5 g Brewer's Yeast (Swanson, Fargo, ND). Water was allowed to steep for at least 1 wk prior to deployment. Both carbon dioxide and gravid traps in SAYO were set 1 d per week between 9:00 a.m. and 2:00 p.m. and retrieved before 12:00 p.m. the following day at the same locations were sugar baits were deployed. At most sites, two carbon dioxide and one gravid trap were present.

In COAV, carbon dioxide traps were constructed by the Coachella Valley Mosquito and Vector Control District Staff from a coupler for a 3-inch acrylonitrile butadiene styrene drain pipe where the internal diameter of the coupler was 3.5 inches. Fans and 6-volt motors were from John W. Hock, Co. No bulbs were used. Traps were powered by 6-volt lead acid gel batteries and set with 3 pounds of dry ice per trap night contained in a rectangular Styrofoam shipping container. Gravid traps operated in COAV were the same as in SAYO except the infusion recipe was as follows: 2.5 gallons of warm water were combined with 2.5 tablespoons of ground guinea pig chow and 0.75 tablespoons of Brewer's Yeast, then aged for 5 d. Vector control staff in COAV used one carbon dioxide trap operated bi-weekly or one gravid trap operated one night weekly per site, deployed in the afternoon and retrieved the following morning.

Field Deployments of Sugar Baits

We conducted sugar-baited surveillance in SAYO in 2015 and 2016 and in COAV in 2016. Sugar baits were deployed concurrently and at the same locations as carbon dioxide-baited and gravid traps. Because arbovirus activity was not detected using sugar-baited surveillance in suburban COAV in the 2011 study (Lothrop et al. 2012), we biased our 2015 study locations in SAYO to focus on sites with highest historical WNV activity based on mosquito surveillance data from 2011 to 2014 (California Vectorborne Disease Surveillance System 2017). Locations of high activity were identified using Getis-Ord hotspot analysis z-scores in ArcGIS 10.0 software. Schedules of weekly conventional trap deployments, inclusions of rural, suburban, or desert sites, and ease of access for mosquito control personnel were also considered. In 2016, sugar baits were deployed at all sites in the entire SAYO District where carbon dioxide and gravid traps were used. Deployment sites in COAV, selected by district staff, did not cover all trapping sites in the entire district but instead focused on suburban trapping sites and areas near the Salton Sea where arbovirus activity has historically been detected each summer.

Sugar baits were hung from trees at 1.5–2 m above the ground and 5–20 m away from carbon dioxide traps to minimize cross-attraction between the carbon dioxide and the floral lure on the sugar bait. At COAV sites, sugar baits were placed within 10 m of the trap. While traps were deployed at each site 1 night/wk, sugar baits remained at each site for six or seven continuous days and nights in SAYO and COAV, respectively, at which point bait tubes were retrieved and replaced. At SAYO sites, sugar baits were not deployed during the 24 h that carbon dioxide traps were deployed in order to prevent cross-attraction between the carbon dioxide and floral lure (Gillies and Wilkes 1969).

Arbovirus Detection

Sugar baits retrieved after deployment were stored at −80°C until tested for arbovirus RNA. The cotton wick was agitated with a pipette or vortexed to access 50 ul of sucrose solution. Any bait that was retrieved fully desiccated was recorded as 'dry' and was rehydrated with 200 µl MagMax lysis buffer solution. RNA was extracted from the sucrose solutions in baits according to manufacturer's recommendations using an Applied Biosystems MagMax 96-well Viral RNA kit and the AM1836 DW200 STD program on a MagMax Express 96-Deep Well Magnetic Particle Processor (ThermoFisher, Waltham, MA). For sugar baits deployed in 2016, because SLEV reemerged in the COAV in 2015 (White et al. 2016), we tested for both WNV and SLEV. The arbovirus RNA extracted from sugar baits was amplified in 96-well plates using Taqman One-Step Virus kits (ThermoFisher), and Applied Biosystems ViiA 7 real-time PCR machines (ThermoFisher) using established primers and probes (Brault et al. 2015) that were used in singleplex to test for either WNV or SLEV. Serially diluted WNV or SLEV of known plaque forming unit (PFU) concentrations were included in each plate to verify that the limit of detection of each run was similar and to serve as positive controls. Cycle threshold (Ct) values are represented as WNV or SLEV PFU derived from the standards. Based on the cut-off for an arbovirus positive mosquito pool in conventional testing (Brault et al. 2015), any Ct value lower than 40 was considered SLEV or WNV RNA positive. Non-deployed sugar baits spiked with RNA-free water and processed in parallel with field deployed sugar baits, and quantitative reverse transcription PCR (qRT-PCR) reactions with RNA-free water only were included in each qRT-PCR plate as negative controls. If either type of negative control produced a WNV or SLEV Ct value less than 40, all sugar bait samples tested on the same plate were excluded from further analysis; this occurred only once out of 43 total qRT-PCR plates in a RNA-free water template control that yielded a Ct of 38. Most sugar baits in the study were tested in multiple replicates; of the arbovirus positives, 15% were tested in just one replicate, 65% were tested in duplicate and 20% were tested in triplicate. When a sugar bait tested in replicate wells yielded a detectable Ct for one or more replicate but an undetectable Ct for other replicate(s), the sugar bait was classified as arbovirus positive based on the low rate of false positives and the rationale that low viral RNA levels could be real (see Results section on spiked sugar baits). The mean number of PFU equivalents detected each sugar bait was generated. The PFU values of replicates with undetectable Cts were reported as 0.

Data Analyses

Data points obtained were binary qRT-PCR results from a sugar bait as the dependent variable, and binary qRT-PCR results from mosquito pools tested from traps at the same locations and weeks as sugar baits, termed 'site-weeks'. Odds ratios, McNemar's tests, and mixed-effects regression models (SAS version 9.4) were used

to investigate associations between the arbovirus prevalence determined from mosquito traps versus sugar baits. Additionally, the moisture status of a sugar bait ('dry' with no visible remaining liquid, or 'wet' with remaining visible liquid), the presence of fungus or mold growth, evidenced as discoloration of the bait with the naked eye (binary), site classification (rural, suburban, desert), mosquito species trapped during the site-week (none/mixed, *Culex tarsalis,* or *Culex pipiens spp.*), the type of trap that was positive (carbon dioxide, gravid), and mosquito abundance (continuous) were investigated as covariates using a mixed-effects logistic regression model. Since sugar baits do not capture mosquitoes, the identity of mosquito species and their abundance in traps were obtained from carbon dioxide and gravid traps at the same sites where sugar baits were deployed. Maps were generated using ArcMap 10.3.1 (Esri). City boundaries and roadway shape files were obtained from the TIGER database (United States Government 2017). Surveillance data from the SAYO and COAV Districts were obtained with permission (California Vectorborne Disease Surveillance System 2017). Surveillance sites were geolocated onto maps. The total number of WNV RNA positive sugar baits and traps were plotted by detection week to determine arbovirus activity in mosquitoes over time. Abundance data, defined as the number of females per species per trap night were obtained from the same traps where WNV RNA data from mosquito pools was collected.

Spike Experiment

Given that relatively low levels of WNV or SLEV RNA were expected to be deposited onto the baits since mosquitoes expectorate relatively low doses of flaviviruses (Vanlandingham et al. 2004, Colton et al. 2005, Hall-Mendelin et al. 2010, Lothrop et al. 2012), we performed an additional sensitivity and stability study to augment information provided by Lothrop et al. in which they established the technique for use in California (Lothrop et al. 2012). We spiked 100, 10, 1, or 0.1 PFU of WNV or SLEV in 10 ul onto individual sugar baits that were either wet or dry. Prior to spiking virus, wet baits were filled with 1 ml of a 40% sucrose solution to represent a field-deployed bait that had not fully desiccated, while dry baits contained only enough (ca. 300 ul) 40% sucrose solution to hydrate the cotton wick and allow for desiccation. The wet and dry baits were further divided into 'incubation' or 'no incubation' treatment groups, thus creating four treatment groups—Dry/Incubation, Dry/No Incubation, Wet/Incubation, and Wet/No Incubation. The incubation baits were enclosed in a chamber at 37°C and ca. 50% humidity for 7 d to simulate field conditions, while the no incubation baits were immediately frozen at −80°C following virus spiking. Baits from all four treatment groups were spiked and processed in parallel with three baits per group except for SLEV one PFU Dry/Incubate which was performed in duplicate due to mishandling of the third replicate. A negative control sugar bait was spiked with 10 ul spiked with water for each treatment group. Seven days

Table 1. Descriptive statistics for three sugar bait evaluations detecting West Nile virus in SAYO Counties in 2015, and Sacramento, Yolo, and Riverside Counties in 2016

California County	Year	Number of sites	Weeks	Baits per site	Total baits	WNV RNA positive baits (%)	Number of site-weeks	WNV RNA positive site-weeks (%)
Sacramento / Yolo	2015	9	16	5	715	55 (8%)	143	44 (31%)
Sacramento / Yolo	2016	22	22	1	467	41 (9%)	467	41 (9%)
Riverside	2016	18	25*	3	860	88 (10%)	296	62 (21%)

The number of sites baited and the number of baits placed per site varied over time and location to compare the efficiency of multiple deployment methods. A site-week was defined as the collection of all of the baits deployed at a single site for a 6-d period. WNV RNA positivity status was determined by detectable WNV RNA by qRT-PCR in sugar bait. Percentages represent number of positives divided by the total number of baits deployed. The asterisk shows that the although the study duration was 25 wk, but data was only collected for 22 of the 25 wk due to an intermediate 3-wk interruption in bait deployment.

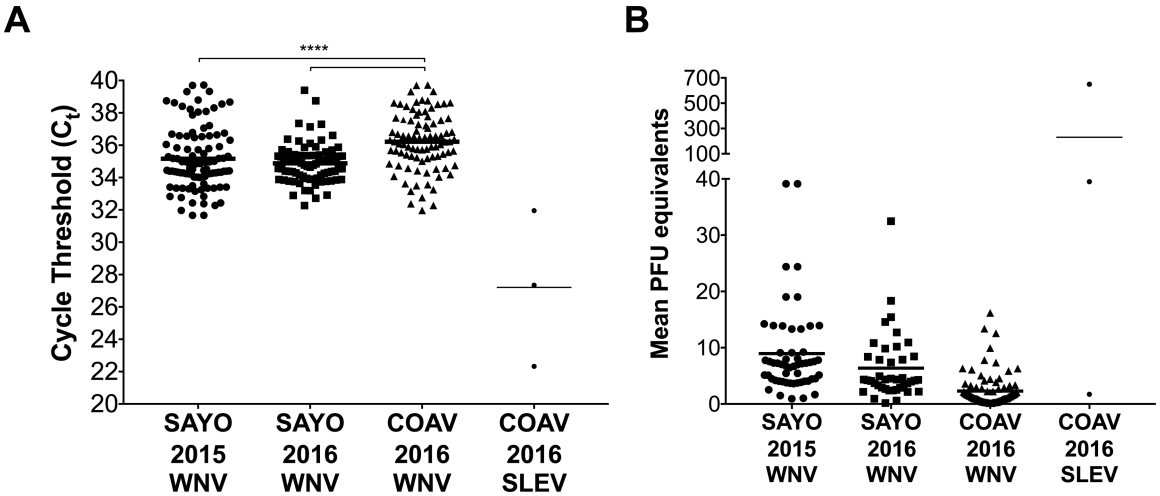

Fig. 2. Sugar bait WNV and SLEV positives in SAYO Counties and COAV, Riverside County in 2015 and 2016 represented as (A) Ct and (B) mean PFU equivalents. Each dot in panel A represents a sugar bait qRT-PCR replicate with a detectable WNV or SLEV Ct. Baits with undetectable Cts above 40 are not shown. Each dot in panel B represents the mean PFU equivalents detected for a single sugar bait. Horizontal lines show mean values for the entire group. The four asterisks above parentheses show groups that are statistically significantly different (one-way ANOVA, $P < 0.0001$).

post-spike, the frozen sugar baits were thawed and all were processed together to extract viral RNA as described above for field-deployed sugar baits. Sugar baits had therefore been thawed 0 or 1 times with the exception of the WNV baits that were immediately frozen after spiking which were thawed two times due to a technical problem with RNA extraction the first time that required a replicate extraction. In total, an additional eight extraction controls (virus RNA-free water) were interspersed and processed with the bait samples to detect cross-contamination during viral RNA extraction. Viral RNA detection and quantification by qRT-PCR was then completed for all spiked bait samples, negative-spike controls, extraction controls, and six negative qRT-PCR controls (water instead of RNA extractions) using the same methods as for field-deployed baits, as described above.

Results

Field Detection of WNV and SLEV in Sugar Baits

A total of 2,042 sugar baits, comprising 715 in SAYO in 2015, 467 in SAYO in 2016, and 860 in COAV in 2016, were deployed in this study (Table 1). The sugar baited surveillance tool was successful in detecting WNV RNA in summer 2015 and 2016 in the SAYO District and SLEV and WNV in the COAV District in summer 2016 (Table 1).

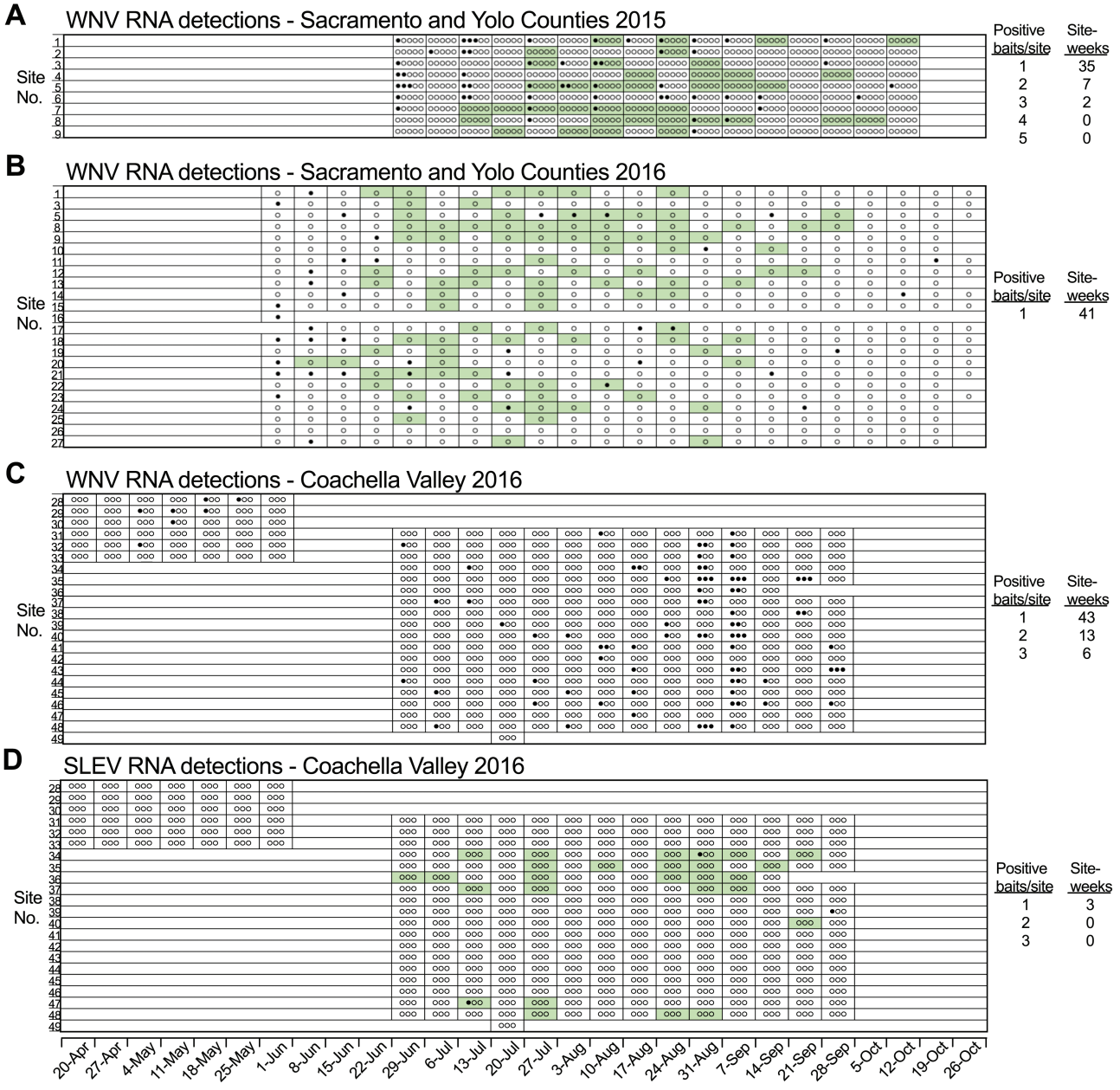

Fig. 3. Temporal and spatial comparison of West Nile virus detections in sugar baits compared to traps in SAYO Counties in (A) 2015, (B) 2016, and (C) COAV, 2016 and (D) St. Louis encephalitis virus in COAV, 2016. Sites are plotted by week. The number of circles inside each rectangle shows the number of sugar baits deployed per site per week. A black circle shows a WNV or SLEV detection in a bait; unfilled white circles show baits that did not contain detectable WNV or SLEV. Green shading shows WNV or SLEV detection in carbon dioxide or gravid traps at each site and week. The absence of green shading in panel C shows that no WNV was detected in traps at the same sites sugar baits were deployed in COAV in 2016.

The WNV Cts for sugar baits ranged from 31 to 39 with a mean of 35.4 for both SAYO 2015 and 2016 and 36.5 for COAV 2016 (Fig. 2A). WNV Cts for SAYO 2015 versus SAYO 2016 were not significantly different (one-way ANOVA, $P = 0.50$), while WNV Cts for SAYO 2015 and SAYO 2016 were both significantly lower than those from COAV 2016 (one-way ANOVA, $P < 0.0001$). The WNV PFU equivalent (Fig. 2B) in baits ranged from 0.1 to 39, corresponding to approximately 100–39,000 WNV genomes based on the estimation that 1,000 genomes generate one WNV PFU (Grubaugh et al. 2017). SLEV RNA was not detected in sugar baits in SAYO, consistent with an absence of SLEV activity in trapped mosquitoes, bird seroconversions, or human cases in 2015 or 2016 (California Department of Public Health 2017a). The SLEV Cts for the three positive sugar baits in 2016 in COAV ranged from 22 to 32 with a mean of 27 (Fig. 2A). The SLEV PFU equivalent (Fig. 2B) in baits ranged from 2 to 650, corresponding to approximately 2,000–650,000 SLEV genomes at an estimated ratio of 1,000 SLEV genomes:1 SLEV PFU. Dried baits from the field were significantly associated with WNV RNA detection (Chi-squared test, $P < 0.0001$), an observation partially replicated in the laboratory spike experiment described below.

In SAYO, 8% of sugar baits deployed over the course of the study in 2015 and 9% in 2016 were WNV RNA positive. In 2015 and 2016, 31 and 9%, respectively, of site-weeks in SAYO were WNV sugar bait positive. In COAV, 10% of sugar baits deployed in 2016 were WNV RNA positive. In 2016, 21% of site-weeks in COAV were WNV RNA positive (Table 1). By comparison, minimum infection rates (MIRs) in mosquitoes for the entire COAV or SAYO regions in both years were lower than rates of sugar bait positivity. A total of 337 of 7,005 mosquito pools, representing 127,700 individual mosquitoes, tested WNV positive in all of SAYO in 2015, representing a MIR of 2.6 mosquitoes per 1,000 (lower estimate: 2.3, upper 2.9). In 2016, 714 of 8,245 pools, representing 159,258 individual mosquitoes, tested WNV positive, yielding a MIR of 4.4 (lower estimate: 4.2, upper: 4.9). In COAV in 2016, 19 pools out of 4,644, representing 148,446 individuals, were WNV positive, producing a MIR of 0.1 (lower estimate: 0.07, upper: 0.2) (California Vectorborne Disease Surveillance System 2017).

In SAYO in 2015 (Fig. 3A) 12 sites and 2016, six sites (Fig. 3B) yielded both WNV RNA positive sugar baits and WNV RNA positive mosquito pools captured in carbon dioxide and gravid traps over the same week. These data indicate that both methods can detect WNV activity in the same areas, but most often do not within the same week. In COAV 2016, while all the sites yielded at least one WNV RNA sugar bait positive during the study period, none of the carbon dioxide and gravid traps at the same sites where sugar baits were deployed contained WNV RNA positive mosquitoes (Fig. 3C). In COAV in 2016, 92 pools out of 4,644, representing 148,446 individuals were SLEV positive, producing a MIR of 0.61 (lower estimate: 0.5, upper: 0.75) (California Vectorborne Disease Surveillance System 2017)

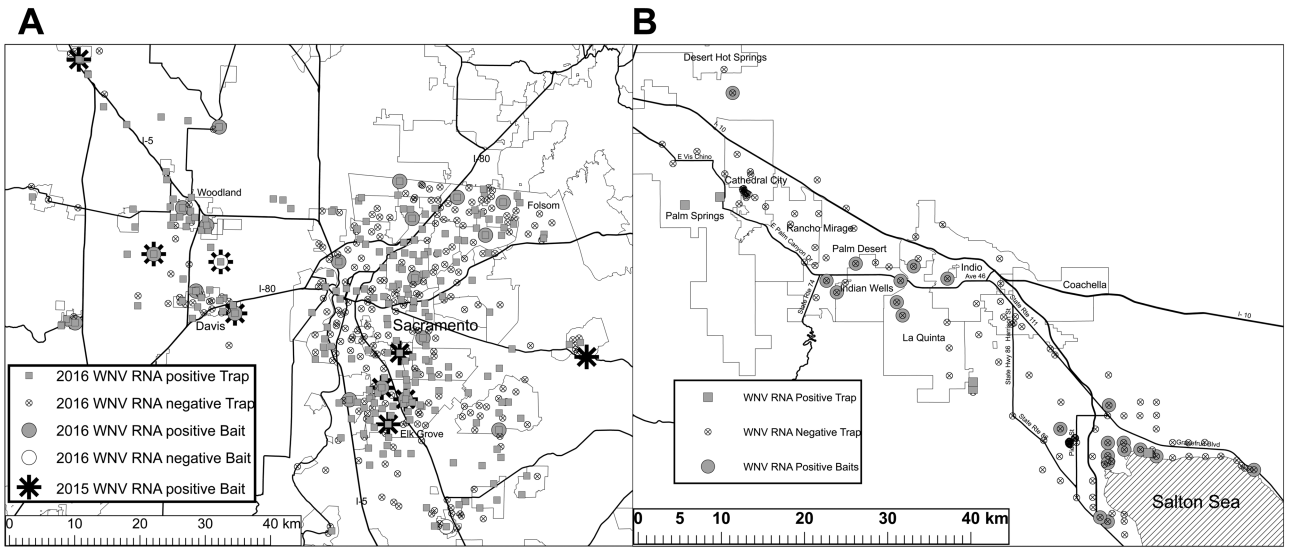

Fig. 4. (A) Maps of SAYO Counties and (B) COAV, Riverside County, California showing WNV RNA detections by sugar baits and traps in 2015–2016. Sites indicated as being WNV RNA positive had at least one mosquito pool or sugar bait test positive during the season. Sites labeled as WNV RNA negative did not test positive by either approach for the duration of the study. Eleven sites in the southern tip of Sacramento County are not pictured.

Table 2. Concurrent SLEV RNA detections by sugar baits and traps in COAV, 2016

COAV 2016	Trap SLEV RNA positive	Trap SLEV RNA negative	Total
Sugar bait SLEV RNA positive	2	1	3
Sugar bait SLEV RNA negative	25	268	293
Total	27	269	296
OR (95% CI): 21.4 (1.9, 244.8)		*P*-value: <0.0001	

Units are reported in site-weeks. Site-weeks for each region and week contained a different number of sugar baits. Only traps in COAV where sugar baits were deployed are included. *P*-values are from McNemar's tests for paired ORs.

Spatial and Temporal Field Detection of WNV and SLEV in Sugar Baits

While both sugar baits and traps detected WNV activity at geographically distant sites in SAYO (Fig. 4A), most WNV activity in COAV was located northwest of the Salton Sea (Fig. 4B); spatial patterns in both areas parallel activity hotspots for both viruses in previous years (California Department of Public Health 2017a).

Three sugar baits out of 296 deployed (1%) in COAV District were SLEV RNA positive in summer 2016 (Table 2, Fig. 5), over a period of almost 2 mo (Fig. 5B). Sugar bait activity was concentrated north of the Salton Sea (Fig. 5A), an area where both WNV and SLEV activity have historically been high (California Department of Public Health 2017a). The detection of just three SLEV positive sugar baits in COAV 2016 contrasts with the 27 SLEV positive mosquito pools from traps at the same study sites (Table 2, Fig. 5) and 92 positive pools in traps over the entire summer in all of COAV. In contrast with WNV activity that was detected at most sampling sites by only one of the two detection methods, two of the three SLEV detections on sugar baits occurred at the same sites SLEV RNA positive mosquito pools were trapped during the week of 31 August 2016 (Fig. 3D). Also contrasting with results from WNV, the first seasonal SLEV positive in COAV detected by sugar baits was 3 wk later than SLEV positives detected in carbon dioxide and gravid traps (Fig. 5B; SLEV data from those traps is not included since those were not locations where sugar baits were deployed).

WNV RNA detections over time in SAYO or COAV oscillated weekly using sugar bait or trapping approaches (Fig. 6A–C). Sugar baits detected WNV RNA earlier at both study sites than carbon dioxide and gravid traps in 2015 and 2016. In the SAYO District in 2015, sugar baits detected WNV 2 wk earlier than traps (Fig. 6A) and in 2016 sugar baits detected WNV 1 wk earlier (Fig. 6B). Sugar baits also detected WNV activity for 5 wk longer (although discontinuously in the fourth week) than traps in SAYO in 2016 (Fig. 6B). In COAV, none of the traps at the sites where sugar baits were also deployed yielded WNV RNA detections during the study period (Fig. 6C). SLEV trap positives at other sites

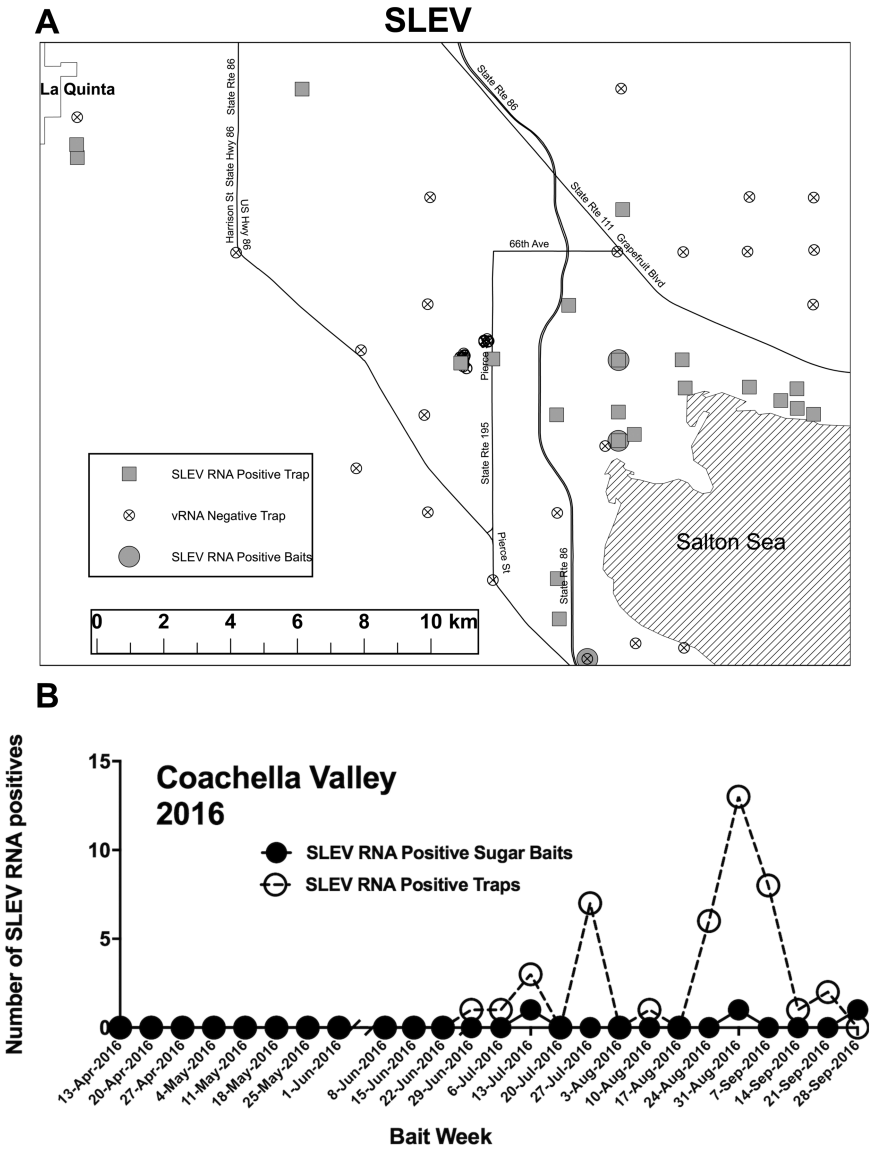

Fig. 5. SLEV RNA sugar bait and trap positives represented in COAV, Riverside County, California represented (A) geographically on a map of the area and (B) temporally from April–September, 2016. The discontinuous line in COAV shows the 3 wk when sugar baits were not deployed.

in COAV occurred starting the week after (11 May 2016, data not shown) the first sugar bait detection (4 May 2016) and were last detected the week of 14 September 2016, 2 wk earlier than the last sugar bait positive was detected the week of 28 September 2016. In SAYO in 2015 and COAV in 2016, there was no association between positive WNV RNA detections in a sugar bait versus a trap at the same site and week ($P = 0.25$, McNemar's test, mixed-effects logistic regression). Furthermore, in SAYO in 2016, there was a strong negative association between the two detection methods ($P < 0.001$, McNemar's test, mixed-effects logistic regression, Table 3).

Cost Analysis of Sugar Bait Versus Trap Based Surveillance

Based on costs incurred by the SAYO District, 0.8 WNV RNA positive detections are achieved per $1,000 USD spent using conventional carbon dioxide and gravid traps (Healy et al. 2015). Sugar baits achieved between 0.8 and 1.2 positives per $1,000 for the 2015 and 2016 SAYO and COAV studies described here (Table 4). These costs were calculated using materials priced for the University of California, Davis with $20 for laboratory testing of one sugar bait and $40 for a trap where two pools per trap are on average tested (Supplementary Table 1). The placement of one sugar bait at each site provided the highest cost effectiveness, generating 1.2 positives per $1,000. However, deploying three baits per site increased the positive site-weeks from 9 to 21%, at a cost of 0.9 WNV RNA positives per $1,000. The data from these cost analyses therefore indicate that there is a trade-off between the added cost of deploying more sugar baits at each site and the frequency of WNV RNA detection in sugar baits.

Detection of WNV and SLEV on Spiked Sugar Baits

The study by Lothrop et al. determined that as little as 10 PFU could be detected on spiked sugar baits (testing of lower doses was not attempted) (Lothrop et al. 2012). We performed an additional laboratory study to evaluate the sensitivity of WNV or SLEV RNA

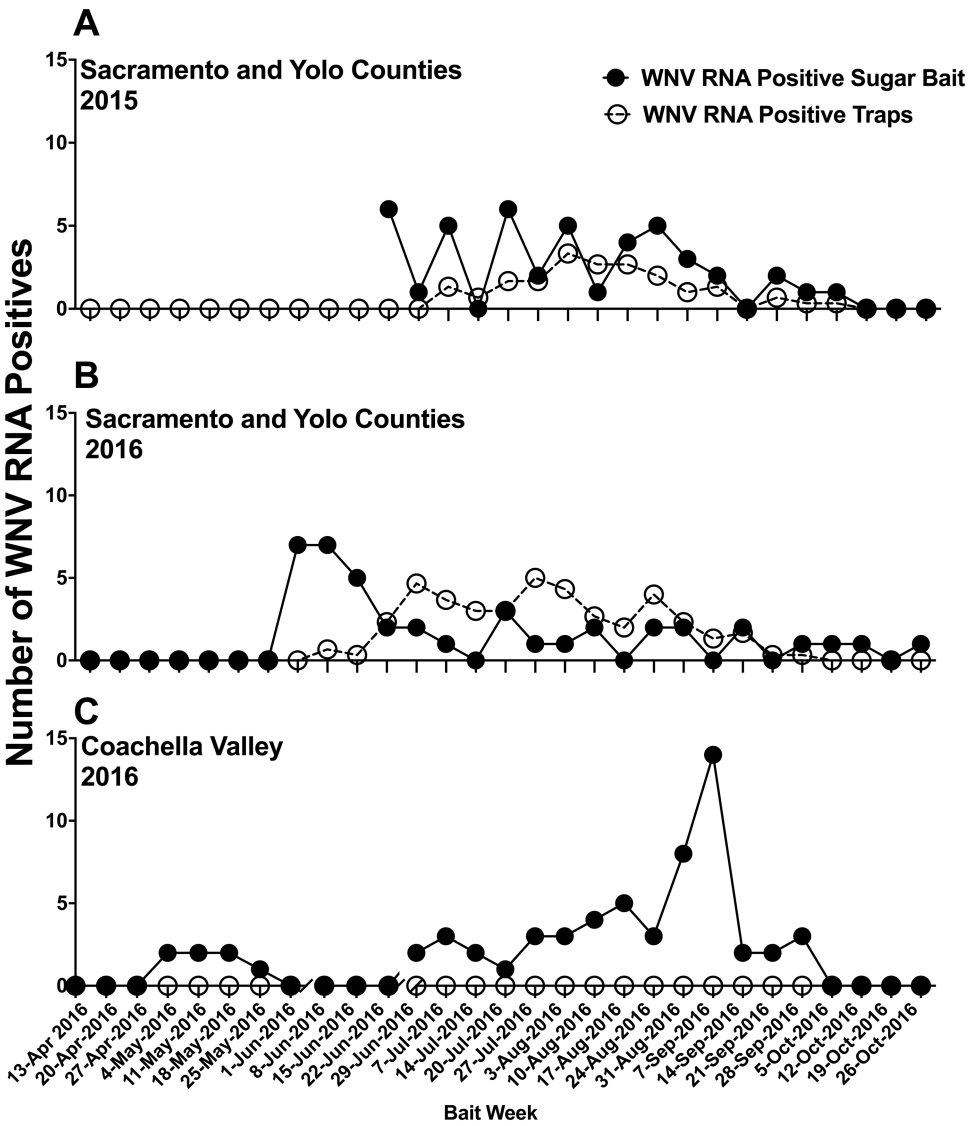

Fig. 6. WNV RNA sugar bait and trap positives at sites where both approaches were used by week in SAYO Counties in (A) 2015 and (B) 2016 and (C) COAV, Riverside County, 2016. A week with both WNV RNA positive traps and sugar baits does not necessarily indicate that the positives were detected at the same site. The discontinuous line in panel C shows the 3-wk period when sugar baits were not deployed. No WNV RNA was detected in mosquitoes at the same sites as sugar baits in COAV in 2016.

Table 3. Concurrent WNV RNA detections by sugar baits and traps in Sacramento, Yolo, and Riverside counties

Study location, year		Trap WNV RNA positive	Trap WNV RNA negative	Total	OR (95% CI)	*P*-value
SAYO, 2015	Sugar bait WNV RNA positive	12	32	44	0.48 (0.23, 1.06)	0.25
	Sugar bait WNV RNA negative	43	56	99		
	Total	55	88	143		
SAYO, 2016	Sugar bait WNV RNA positive	8	33	41	0.93 (0.41, 2.08)	<0.0001
	Sugar bait WNV RNA negative	88	336	424		
	Total	96	369	465		
COAV, 2016	Sugar Bait WNV RNA positive	0	62	62	n/a	n/a
	Sugar Bait WNV RNA negative	0	234	234		
	Total	0	296	296		

The units for all values given in the table are site-weeks. Site-weeks for each region and time contained a different number of sugar baits. *P*-values are from McNemar's tests for paired odds ratios. The 2016 COAV data do not meet the assumptions of the test due to '0' values; hence the n/a (not-applicable) designation.

Table 4. Costs of WNV RNA detections in carbon dioxide or gravid traps or sugar baits per $1,000 spent

	Traps or baits per site			
	3 traps	5 baits	3 baits	1 bait
% WNV RNA positive site-weeks	17%	31%	21%	9%
WNV RNA positives per $1,000	0.8	0.8	0.9	1.2
$ per positive	1,265	1,224	1,083	840

Trap data from Healy et al. (Healy et al. 2015).

detections on sugar baits using methods employed for this study and to determine if our different methods for processing wet versus dry field-deployed baits and the 1-wk deployment significantly impact viral RNA detection. We detected viral RNA in 100% (24/24) of baits spiked with 100 PFU of WNV or SLEV, 83% (20/24) spiked with 10 PFU, 26% (6/23) spiked with one PFU, and 17% (4/24) spiked with 0.1 PFU across all treatment groups (Fig. 7A and B). Incubation at 37°C for 7 d reduced the amount of viral RNA detected. For the 100 PFU WNV spike samples, significantly higher levels of WNV PFU equivalents were detected in the Dry/No Incubation baits than the Wet/Incubation baits (Kruskal–Wallis test, $P = 0.04$, Fig. 7A). Likewise, for the 10 PFU SLEV spike samples, significantly higher levels of SLEV PFU equivalents were detected in the Dry/No Incubation baits than the Wet/Incubation baits (Kruskal–Wallis test, $P = 0.04$; Fig. 7B). No statistically significant differences were observed between other treatment groups at the other spike titers. Additionally, when comparing detection of viral RNA instead of the quantity of viral RNA detected, no statistically significant differences were observed between wet and dry baits, or between incubation and no incubation baits (chi-squared tests, and a Fisher's exact test for SLEV dry vs. wet, $P > 0.05$; Fig. 7C).

The amount of spiked WNV or SLEV recovered on sugar baits was a small fraction of the amount of deposited virus. For wet baits, RNA was extracted from ca. 5% of the total volume of sucrose solution, whereas for dry baits the volume of eluent (200 ul) was insufficient to fully rehydrate the dry wick. For WNV, the percent of deposited virus recovered in wet baits ranged from 0 to 7.8% with a mean of 1.1%, and in dry baits ranged from 0 to 5.4% with a mean of 1.2%. For SLEV, wet baits ranged from 0 to 1.6% with a mean of 0.4%, and dry baits ranged from 0 to 57% with a mean of 6.4%. Inefficient recovery of viral RNA on spiked baits can likely be attributed in large part to the RNA extraction methodologies employed, with RNA degradation having a small effect as well, as seen by slightly higher levels in RNA recovery in the no incubation baits.

We also used our spike experiment to address the use of single, double, or triple qRT-PCR replicates for WNV or SLEV detection with our field-deployed baits. All baits in our spike experiments were tested in triplicate by qRT-PCR, allowing us to evaluate the probability of false negatives had we employed single or double replicates instead. For all WNV spike titers, we detected viral RNA in 0 qRT-PCR replicates in 16/24 baits, one replicate in 2/24 baits, two replicates in 1/24 baits, and three replicates in 5/24 baits. Thus, the probability of falsely identifying a WNV positive bait as negative is 0.07 if one qRT-PCR replicate was employed, and 0.03 if two qRT-PCR replicates were employed. For all SLEV spike titers, we detected viral RNA in 0 qRT-PCR replicates in 7/23 baits, one replicate in 3/23 baits, two replicates in 6/23 baits, and three replicates in 7/23 baits. As such, the probability of falsely identifying a SLEV positive bait as negative is 0.17 if one qRT-PCR replicate was employed, and 0.04 if two qRT-PCR replicates were employed. Therefore, while three qRT-PCR replicates are best at detecting true WNV or SLEV RNA positives, these results suggest that the false negative rate for single or double replicate testing was relatively low. Regarding false positives, none of the negative control sugar baits that were spiked with water and processed with virus-spiked baits ($n = 8$), extraction control samples ($n = 8$), nor qRT-PCR negative controls ($n = 6$) were WNV or SLEV RNA positive, suggesting that false positives due to cross-contamination in the laboratory are unlikely to have occurred. Together, these results from our spike studies suggest that our differential methods for processing wet versus dry baits and the week-long field deployment had minimal effects on our ability to detect WNV and SLEV in sugar baits, although dry baits did allow slightly greater quantities of WNV and SLEV to be detected.

Discussion

The results from the current study demonstrate that sugar-baited surveillance can be used to detect WNV and SLEV transmitted by mosquitoes in suburban California, however, the effectiveness of this technique is dependent on the setting. In 2015 and 2016 in SAYO counties, sugar-baited WNV detection was observed both weeks earlier and weeks later than WNV detected by conventional

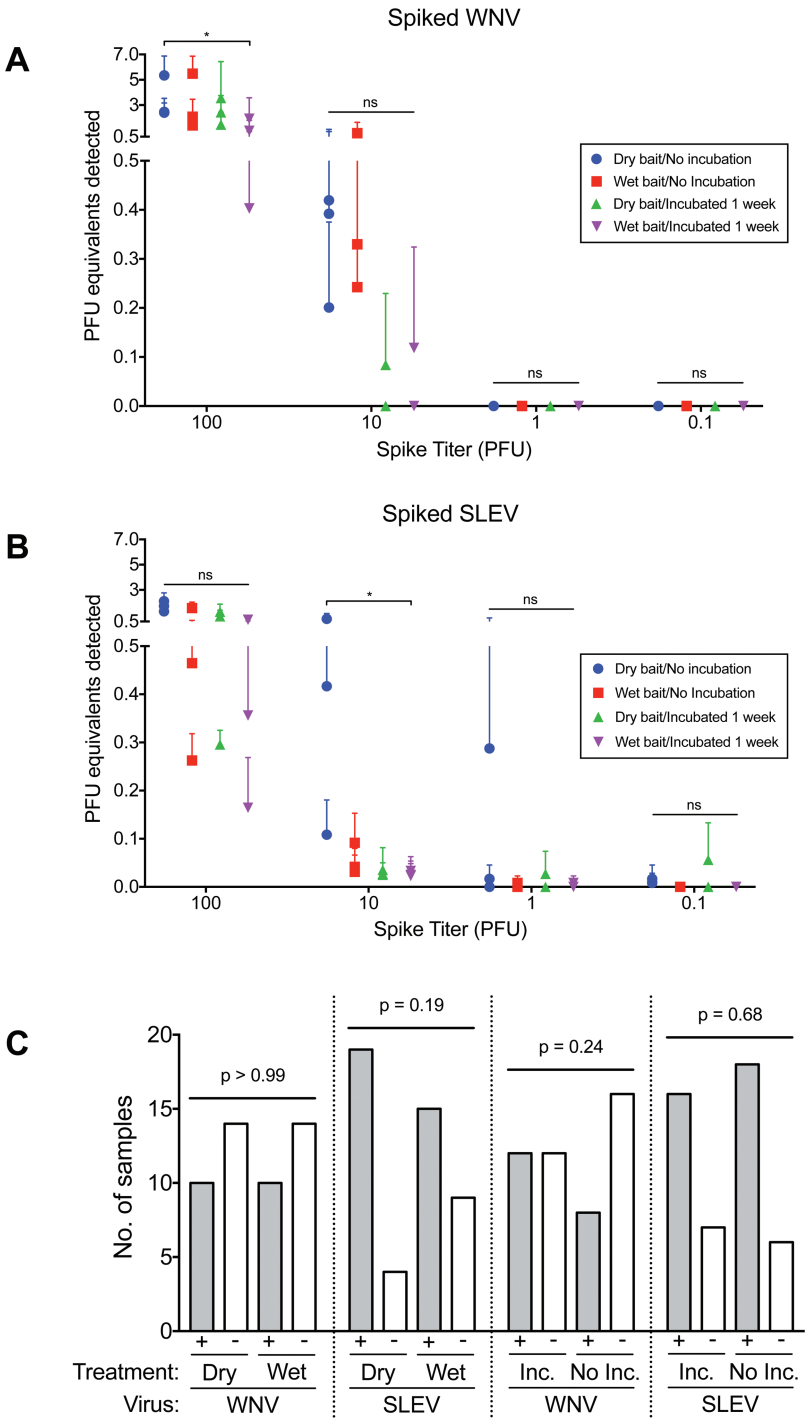

Fig. 7. Laboratory experiment of (A) WNV and (B) SLEV spiked on sugar baits processed to simulate conditions for field-deployed sugar baits and (C) WNV and SLEV detection compared between treatment groups. Each dot in panels A and B represents a single spiked sugar bait. Error bars indicate standard deviations of three qRT-PCR replicates for each wick. In panel C, incubation is abbreviated to 'Inc.'. Single asterisks above brackets in panels A and B show groups that are statistically significantly different (Kruskal–Wallis test, $P < 0.05$), 'ns' denotes groups that are not significantly different (Kruskal–Wallis test, $P > 0.05$).

surveillance in mosquitoes collected in carbon dioxide and gravid traps. The rates of WNV sugar bait positives ranged 8–10% in SAYO and COAV Districts in 2015 and 2016. In 2016, 1% of sugar baits in COAV District were SLEV positive. This study is the first use of sugar-baited surveillance to detect SLEV circulating in California. In COAV, sugar baits detected WNV at sites where traps detected no WNV positive mosquito pools. Although there was not a temporal association between sugar bait and trap positives, both approaches detected SLEV in the same area of the COAV in summer 2016. Additionally, sugar-baited detection was less expensive than conventional trap-based surveillance in California. While traps in California yield 0.8 WNV detections per $1,000 spent (Healy et al. 2015), the sugar baits produced slightly more detections, 0.8–1.2, for the same cost, depending on how many baits were deployed at each site. For future studies using a sugar baited approach, if WNV circulation intensities and minimum mosquito infection rates are

similar to this study, one bait per site would detect WNV at a cost of 1.2 positives per $1,000.

The observation that sugar baits and carbon dioxide or gravid trap WNV RNA detections were not associated in time or showed a negative association raises new questions. Mosquito attractants including carbon dioxide that mimic hosts are more effective than those simulating plant odors, except for females digesting a bloodmeal (Reisen et al. 1986, Kline et al. 1990). Therefore, the gonotrophic status of female mosquitoes at a site should favor one method over the other. The discordance in sugar bait SLEV and WNV detections at the same sites and times compared to carbon dioxide and gravid traps, especially for SLEV in COAV 2016 where about 10 times more SLEV activity was detected in traps compared to baits, indicates that trapping methods detect more arbovirus activity in mosquitoes than sugar baits but that neither approach detects all arbovirus activity in mosquitoes in California.

Our observation that dried field-deployed baits were significantly more likely to be WNV RNA positive than wicks still wet with sucrose solution may reflect a methodological difference in the way viral RNA was extracted. Dried baits were rehydrated completely with lysis buffer, whereas the remaining sucrose solution, possibly not from the end of the wick containing arbovirus RNA, was used from wet wicks. This difference in sensitivity between dry and wet wicks was partially supported by our laboratory spike experiment, in which we observed a slight increase in arbovirus RNA quantity detected, but no differences in overall detection.

A major drawback of the sugar-baited approach is that it does not capture mosquitoes, precluding identification of vector species transmitting WNV or SLEV into baits. We acknowledge this disadvantage, especially considering that mosquito abundance calculated from traps is one measure that features in the California Mosquitoborne Virus Response Plan (California Department of Public Health 2017b). However, in California where the two primary SLEV and WNV vector species, *Culex tarsalis* (Coquilett, 1896, Diptera: Culicidae) and *Culex quinquefasciatus* (Say, 1823, Diptera: Culicidae), are well established (Reisen et al. 1992a; Reisen et al. 1992b; Reisen et al. 2004; Reisen et al. 2005, 2006) and represent approximately 99% of mosquito infections with both viruses annually (California Vectorborne Disease Surveillance System 2017), these are likely to be the species transmitting into sugar baits. To verify this possibility, in parallel with studies deploying sugar baits inside of traps, we are presently adapting a method developed in the laboratory to detect and identify the species of mosquitoes based on mosquito DNA that is also deposited on sugar baits during feeding (Grubaugh et al. 2017). Detecting mosquito DNA on sugar baits will also circumvent the inability to identify whether arbovirus RNA negative baits were not fed on by mosquitoes or whether they were fed on by non-transmitting vectors.

In summary, this study demonstrates successful application of low-cost sugar baits for detection of arbovirus transmission in suburban and urban areas in California, albeit with incongruent spatiotemporal WNV and SLEV detections in mosquitoes collected in carbon dioxide and gravid traps. We therefore propose the sugar-baited approach as a cost-effective complement to carbon dioxide and gravid traps. Sugar baits may be especially useful when surveillance needs to span large regions with less frequent site visits, where weeklong sugar bait deployments could halve travel expenses to and from surveillance sites given that a new bait could be placed when a deployed bait is retrieved.

Supplementary Material

Supplementary data are available at *Journal of Medical Entomology* online.

Acknowledgments

We would like to thank the personnel at the Sacramento-Yolo Mosquito and Vector Control District and the Coachella Valley Mosquito and Vector Control Districts for contributing their time, efforts, and feedback towards the deployment of sugar baits in the field. Bill Reisen and Jay Nicholson provided constructive criticism of manuscript drafts. K.K.R. was supported by a Ruth L. Kirschstein National Research Service Award, funded by the National Institutes of Health (T32 OD O11147). This study was supported by the Coachella Valley Mosquito and Vector Control District Grants to L.L.C. in 2015–2017, the Mosquito Research Foundation, and start up funds from the School of Veterinary Medicine, University of California, Davis to L.L.C. This publication also was supported by Cooperative Agreement Number U01 CK000516-01, funded by the Centers for Disease Control and Prevention. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the Centers for Disease Control and Prevention or the Department of Health and Human Services. *Author Contributions*: Conceptualization: L.L.C., C.D.S. and H.D.L. Methodology: L.L.C., C.D.S., H.D.L., J.B.S., and K.K.R. Investigation: C.D.S., A.S., H.D.L., K.K.R., J.B.S. Writing-Review and Editing: C.D.S., K.K.R., L.C., H.D.L. Visualization: K.K.R., J.B.S., and C.D.S. Supervision and Project Administration: L.L.C. Funding Acquisition: L.L.C.

References Cited

Brault, A. C., Y. Fang, and W. K. Reisen. 2015. Multiplex qRT-PCR for the detection of Western equine encephalomyelitis, St. Louis encephalitis, and West Nile viral RNA in mosquito pools (Diptera: Culicidae). J. Med. Entomol. 52: 491–499.

California Department of Public Health. 2017a. California West Nile Virus Website. http://westnile.ca.gov.

California Department of Public Health. 2017b. California Mosquito-borne Virus Surveillance & Response Plan. http://westnile.ca.gov/resources.php.

California Vectorborne Disease Surveillance System. 2017. California vector-borne disease surveillance system archives. http://www.calsurv.org/.

Coffey, L. L., A. B. Failloux, and S. C. Weaver. 2014. Chikungunya virus-vector interactions. Viruses. 6: 4628–4663.

Colton, L., B. J. Biggerstaff, A. Johnson, and R. S. Nasci. 2005. Quantification of West Nile virus in vector mosquito saliva. J. Am. Mosq. Control Assoc. 21: 49–53.

Condotta, S. A., F. F. Hunter, and M. J. Bidochka. 2004. West Nile virus infection rates in pooled and individual mosquito samples. Vector Borne Zoonotic Dis. 4: 198–203.

Cummings, R. 1992. Design and use of a modified Reiter gravid mosquito trap for mosquito-borne encephalitis surveillance in Los Angeles County, California. Proc. Pap. Annu. Conf. Calif. Mosq. Control Assoc. 60: 170–176.

Flies, E. J., C. Toi, P. Weinstein, S. L. Doggett, and C. R. Williams. 2015. Converting mosquito surveillance to arbovirus surveillance with honey-baited nucleic acid preservation cards. Vector Borne Zoonotic Dis. 15: 397–403.

Foster, W. A. 1995. Mosquito sugar feeding and reproductive energetics. Annu. Rev. Entomol. 40: 443–474.

Gillies, M. T., and T. J. Wilkes. 1969. A comparison of the range of attraction of animal baits and of carbon dioxide for some West African mosquitoes. Bull. Entomol. Res. 59: 441–456.

Grubaugh, N. D., J. R. Fauver, C. Rückert, J. Weger-Lucarelli, S. Garcia-Luna, R. A. Murrieta, A. Gendernalik, D. R. Smith, D. E. Brackney, and G. D. Ebel. 2017. Mosquitoes transmit unique West Nile virus populations during each feeding episode. Cell Rep. 19: 709–718.

Gu, W., R. Lampman, and R. J. Novak. 2003. Problems in estimating mosquito infection rates using minimum infection rate. J. Med. Entomol. 40: 595–596.

Hall-Mendelin, S., S. A. Ritchie, C. A. Johansen, P. Zborowski, G. Cortis, S. Dandridge, R. A. Hall, and A. F. van den Hurk. 2010. Exploiting mosquito sugar feeding to detect mosquito-borne pathogens. Proc. Natl. Acad. Sci. U. S. A. 107: 11255–11259.

Healy, J. M., W. K. Reisen, V. L. Kramer, M. Fischer, N. P. Lindsey, R. S. Nasci, P. A. Macedo, G. White, R. Takahashi, L. Khang, et al. 2015. Comparison of

the efficiency and cost of West Nile virus surveillance methods in California. Vector Borne Zoonotic Dis. 15: 147–155.

van den Hurk, A. F., S. Hall-Mendelin, M. Townsend, N. Kurucz, J. Edwards, G. Ehlers, C. Rodwell, F. A. Moore, J. L. McMahon, J. A. Northill, et al. 2014. Applications of a sugar-based surveillance system to track arboviruses in wild mosquito populations. Vector Borne Zoonotic Dis. 14: 66–73.

Johnson, B. J., T. Kerlin, S. Hall-Mendelin, A. F. van den Hurk, G. Cortis, S. L. Doggett, C. Toi, K. Fall, J. L. McMahon, M. Townsend, et al. 2015. Development and field evaluation of the sentinel mosquito arbovirus capture kit (SMACK). Parasit. Vectors. 8: 509.

Kline, D. L., W. Takken, J. R. Wood, and D. A. Carlson. 1990. Field studies on the potential of butanone, carbon dioxide, honey extract, 1-octen-3-ol, L-lactic acid and phenols as attractants for mosquitoes. Med. Vet. Entomol. 4: 383–391.

Lothrop, H. D., S. S. Wheeler, Y. Fang, and W. K. Reisen. 2012. Use of scented sugar bait stations to track mosquito-borne arbovirus transmission in California. J. Med. Entomol. 49: 1466–1472.

Newhouse, V. F., and R. E. Siverly. 1966. St. Louis encephalitis virus from mosquitoes in southwestern Indiana, 1964. J. Med. Entomol. 3: 340–342.

Reisen, W. K., R. P. Meyer, and M. M. Milby. 1986. Patterns of fructose feeding by *Culex tarsalis* (Diptera: Culicidae). J. Med. Entomol. 23: 366–373.

Reisen, W. K., M. M. Milby, S. B. Presser, and J. L. Hardy. 1992a. Ecology of mosquitoes and St. Louis encephalitis virus in the Los Angeles Basin of California, 1987–1990. J. Med. Entomol. 29: 582–598.

Reisen, W. K., R. P. Meyer, M. M. Milby, S. B. Presser, R. W. Emmons, J. L. Hardy, and W. C. Reeves. 1992b. Ecological observations on the 1989 outbreak of St. Louis encephalitis virus in the southern San Joaquin Valley of California. J. Med. Entomol. 29: 472–482.

Reisen, W., H. Lothrop, R. Chiles, M. Madon, C. Cossen, L. Woods, S. Husted, V. Kramer, and J. Edman. 2004. West Nile virus in California. Emerg. Infect. Dis. 10: 1369–1378.

Reisen, W. K., Y. Fang, and V. M. Martinez. 2005. Avian host and mosquito (Diptera: Culicidae) vector competence determine the efficiency of West Nile and St. Louis encephalitis virus transmission. J. Med. Entomol. 42: 367–375.

Reisen, W. K., Y. Fang, and V. M. Martinez. 2006. Effects of temperature on the transmission of West Nile virus by *Culex tarsalis* (Diptera: Culicidae). J. Med. Entomol. 43: 309–317.

Reiter, P., W. L. Jakob, D. B. Francy, and J. B. Mullenix. 1986. Evaluation of the CDC gravid trap for the surveillance of St. Louis encephalitis vectors in Memphis, Tennessee. J. Am. Mosq. Control Assoc. 2: 209–211.

United States Government. 2017. United States Census Bureau. https://www.census.gov/geo/maps-data/.

Vanlandingham, D. L., B. S. Schneider, K. Klingler, J. Fair, D. Beasley, J. Huang, P. Hamilton, and S. Higgs. 2004. Real-time reverse transcriptase-polymerase chain reaction quantification of West Nile virus transmitted by *Culex pipiens quinquefasciatus*. Am. J. Trop. Med. Hyg. 71: 120–123.

White, G. S., K. Symmes, P. Sun, Y. Fang, S. Garcia, C. Steiner, K. Smith, W. K. Reisen, and L. L. Coffey. 2016. Reemergence of St. Louis Encephalitis Virus, California, 2015. Emerg. Infect. Dis. 22: 2185–2188.