Butte County Mosquito and Vector Control District

POLICY MANUAL

POLICY TITLE: **Vector and Vector-Borne Disease Risk Assessment and Response**
POLICY NUMBER: **4050**

4050.1 Vector and Vector-Borne Disease Risk Assessment and Response

4050.1.1 On July 14, 2010, the Butte County Mosquito and Vector Control District's Board of Trustees approved the use of the California Department of Public Health's (CDPH) and the Mosquito and Vector Control Association's California Mosquito-Borne Virus Surveillance and Response Plan (see Appendix #1). The District will follow the most current CDPH California Mosquito-Borne Virus Surveillance and Response Plan, as referenced in Appendix #1.

4050.1.2 The California Mosquito-Borne Virus Surveillance and Response Plan (Plan) was developed to meet several objectives. Specifically, the Plan;

4050.1.2.1 Provides guidelines and information on the surveillance and control of mosquito populations and mosquito-borne viruses in California, including WNV, SLE, and WEE;

4050.1.2.2 Incorporates surveillance data into risk assessment models;

4050.1.2.3 Prompts surveillance and control activities associated with virus transmission risk level;

4050.1.2.4 Provides local and state agencies with a decision support system; and

4050.1.2.5 Outlines the roles and responsibilities of local (District) and state agencies involved with mosquito-borne virus surveillance and response.

4050.1.3 The Plan provides statewide guidelines, but can be modified to meet local or regional conditions.

4050.1.4 The District utilizes and follows the most current provisions of the Plan to establish risk assessments and response based on the Plan's criteria and categories of risk.

4050.1.5 The District reserves the right to modify the Plan's guidelines and provisions as needed to meet the needs of the District and local conditions.

California Mosquito-Borne Virus Surveillance & Response Plan

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California Mosquito-Borne Virus Surveillance and Response Plan

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Objectives

The California Mosquito-Borne Virus Surveillance and Response Plan was developed to meet several objectives. Specifically, the Response Plan:

- Provides guidelines and information on the surveillance and control of endemic mosquito-borne viruses in California, including West Nile, St. Louis encephalitis, and western equine encephalitis viruses;
- Incorporates surveillance data into risk assessment models;
- Prompts surveillance and control activities associated with virus transmission risk level;
- Provides local and state agencies with a decision support system; and
- Outlines the roles and responsibilities of local and state agencies involved with mosquito-borne virus surveillance and response.

This document provides statewide guidelines but can be modified to meet local or regional conditions. For response to non-endemic mosquito-borne viruses, see Guidance for Surveillance of and Response to Invasive *Aedes* Mosquitoes and Dengue, Chikungunya, and Zika in California.

Introduction

California has a comprehensive mosquito-borne disease surveillance, prevention, and control program that has monitored mosquito abundance and mosquito-borne virus activity since 1969 (Reeves et al. 1990). Previous guidelines for surveillance and interagency responses have been published by the California Department of Public Health (Walsh 1987) and the Mosquito and Vector Control Association of California (Reisen 1995). Since the discovery of West Nile virus (WNV) in New York in 1999, WNV rapidly spread westward and had been detected in all 48 contiguous states of the United States by 2004. The detection of West Nile virus (WNV) in New York, a virus not previously recognized in the Western Hemisphere, prompted a review and enhancement of existing California guidelines to ensure that they were appropriate for WNV. California remains vulnerable to the introduction of other highly virulent mosquito-borne viruses of public and veterinary health concern, including Japanese encephalitis, dengue, Zika, chikungunya, yellow fever, Rift Valley fever, and Venezuelan equine encephalitis viruses. It is critical that local and state agencies are prepared to respond effectively if an existing or introduced virus is detected to safeguard the health of both humans and animals. This document outlines an enhanced surveillance and response program for mosquito-borne viruses in the State of California. Its contents represent the collective effort of the California Department of Public Health (CDPH), the Mosquito and Vector Control Association of California (MVCAC), and the University of California at Davis (UCD).

Background

Arboviruses (**ar**thropod-**bo**rne) are a group of viruses transmitted by mosquitoes. There are 15 known mosquito-borne viruses in California; however, only WNV, St. Louis encephalitis virus (SLEV), and western equine encephalitis virus (WEEV) have caused significant human disease. Since its first introduction to CA in 2003, WNV continues to seriously impact the health of humans, horses, and wild birds throughout the state. From 2003 to 2024, there have been 8,247 WNV human cases with 406 deaths. Additionally, 1,401 cases of WNV were reported in horses in CA during the same period. Since the reemergence of SLEV in California in 2015, 63 human cases of SLEV disease have also been identified. There have been no reported human cases of WEEV disease in California since 1986 and equine WEEV cases were last observed in 1997. Consequently, the California Arbovirus Surveillance Program emphasizes monitoring and providing early detection of WNV, SLEV, and WEEV activity.

WNV, SLEV, and WEEV are viruses that are maintained in wild bird-mosquito cycles and do not depend upon infections of humans or domestic animals to persist. Surveillance and control activities focus on this maintenance cycle, which primarily involves *Culex* mosquitoes, such as the western encephalitis mosquito, *Culex tarsalis*, and preferred blood meal hosts such as crows, jays, house finches, house sparrows, and other passerines. Mosquito species, such as *Cx. tarsalis*, *Cx. pipiens, Cx. quinquefasciatus*, and *Cx. stigmatosoma*, are important in the transmission cycles of WNV and SLEV in both urban and suburban areas. Immature stages of mosquitoes (called larvae and pupae) can be found in various aquatic sources throughout CA. Most water sources, ranging from clean to highly polluted waters, are associated with irrigation of agricultural crops or urban wastewater. Additional species such as *Aedes vexans* and *Cx. erythrothorax* may also be important bridge vectors (i.e., bird to mammal).

Mosquito control is the most practical method of protecting the human population from arbovirus infection. There are no specific treatments or cures for diseases caused by these viruses, and presently there are no vaccines that have been approved for human use. West Nile virus also kills a wide variety of native and non-native birds. Vaccines for WNV and WEEV are available to protect horses from severe neurological disease caused by these viruses and for a limited number of endangered and captive bird species. Mosquito-borne disease prevention strategies must be based on a well-planned integrated pest management (IPM) program that uses near real-time surveillance to detect problem areas, focus control, and evaluate operational efficacy. The primary components of an IPM program include education, surveillance, and mosquito control.

Education

Residents, farmers, and wetland managers can play an important role in reducing the number of adult mosquitoes by eliminating standing water that may support the development of immature mosquitoes. For instance, individuals can help by properly disposing of discarded tires, cans, or buckets; emptying plastic and other swimming pools when not in use; and unclogging blocked rain gutters around homes and businesses. Bird baths and any standing water allowed to remain should be drained and

refilled at least once per week to prevent mosquito development. Farmers and ranchers can be instructed to use irrigation practices that do not allow water to stand for extended periods, while wetland managers or duck club owners can work with mosquito control agencies to determine optimal flooding schedules. Public education programs that encourage the public to curtail outdoor activities during peak mosquito biting times, use insect repellents, and wear long-sleeved clothing can help reduce exposure to mosquitoes. Clinical surveillance is enhanced through education of the medical and veterinary communities to recognize the symptoms of WNV, SLEV, and WEEV, and to request appropriate laboratory tests. Public health officials need to be alerted if mosquito-borne virus activity has been detected in an area, particularly if activity is elevated and widespread.

Surveillance

Surveillance includes monitoring, visualization, and analysis of data on climatic factors, immature and adult mosquito abundance, and arboviral testing in humans, mosquitoes, sentinel chickens, dead birds, and horses. For zoonotic viruses such as WNV, surveillance of the mosquitoes and vertebrate hosts (e.g., birds) that transmit the virus is particularly important as an early warning for disease risk in humans. Surveillance must focus not only on mosquito-borne viruses known to exist in California, but also be sufficiently broad to detect newly introduced viruses. This is especially important since the recent establishment of the globally important arboviral vectors, *Ae. aegypti* and *Ae. albopictus,* in California.

Climate Variation

California’s Mediterranean climate provides ideal opportunities for forecasting mosquito abundance and arbovirus activity because most precipitation occurs in winter, as rain at lower elevations or as snow at higher elevations. Spring and summer temperatures then influence the rate of snow melt and runoff, mosquito population growth, the frequency of blood feeding, the rate of virus development in the mosquito, and therefore the intensity of virus transmission. In the past, WEEV outbreaks have occurred in the Central Valley when wet winters were followed by warm summers, whereas SLEV and WNV outbreaks have been linked to warm, dry conditions that lead to large populations of urban *Culex* mosquitoes. Although climate variation may forecast conditions conducive to virus amplification, a critical sequence of events is required for amplification to reach outbreak levels.

Mosquito Abundance

Mosquito abundance can be estimated through collection of immature and adult mosquitoes. The immature stages (larvae and pupae) can be collected from water sources where mosquitoes lay their eggs. A long-handled ladle (“dipper”) is used to collect water samples and estimate the number of immature mosquitoes per "dip." Most local mosquito control agencies have technicians search for new sources and inspect known habitats for mosquitoes on a 7 to 14-day cycle. These data are used to direct control operations. Maintaining careful records of immature mosquito occurrence and abundance, developmental stages treated, source sizes, and control effectiveness can be useful for estimating the expected size of future adult populations.

Adult mosquito abundance is a key factor contributing to the risk of virus transmission. Monitoring the abundance of adult mosquito populations provides important information on the size of the vector population as it responds to changing climatic factors and control efforts. Four adult mosquito sampling methods are currently used for *Culex* in California: New Jersey light traps, carbon dioxide-baited traps, gravid female traps, and resting adult mosquito collections. The advantages and disadvantages of these sampling methods, and guidelines for the design, operation, and processing of the traps have been discussed in Guidelines for Integrated Mosquito Surveillance (Meyer et al. 2003) and are summarized in **Appendix A**.

Mosquito Infections

Virus activity in the environment can be monitored by collecting and testing adult mosquitoes for virus infection. The current mosquito surveillance system is designed to detect and measure levels of infection for WNV, SLEV, and WEEV. Surveillance efforts should emphasize the testing of *Cx. tarsalis*, the primary rural vector of WNV, SLEV, and WEEV, as well as *Cx. quinquefasciatus* and *Cx. pipiens,* which are important urban vectors of WNV and SLEV. Additionally, it is important to test *Cx. stigmatosoma*, a highly competent but less widely distributed vector of WNV and SLEV that feeds on birds and may be important in enzootic transmission where abundant.

Mosquito testing typically begins early in the season during springtime and, with adequate trapping and testing effort, provides an early warning of virus activity. Testing adult mosquitoes for infection is also one of the best methods to detect newly introduced or emerging mosquito-borne viruses. Female mosquitoes are trapped, usually using carbon dioxide-baited or gravid traps, identified to species, and counted into groups (pools) of ≤ 50 females each for testing at the Davis Arbovirus Research and Training (DART) laboratory at UC Davis or by local agencies that pass annual proficiency panel tests. Procedures for submitting and processing mosquitoes for virus testing are detailed in **Appendix B**.

Avian Infections

Arboviral transmission can be detected within bird populations by 1) using caged chickens as sentinels and bleeding them routinely to detect development of antiviral antibodies (seroconversion) and 2) testing dead wild birds reported by the public for WNV.

Chickens mount an immune response to WNV, SLEV, and WEEV infection but do not develop sufficiently high viremia to infect mosquitoes, thus making them excellent sentinels for virus activity. Frequent serological testing of strategically placed flocks of sentinel chickens provides an effective method to monitor encephalitis virus transmission in an area, particularly as a surrogate for human disease risk because laboratory confirmation of human cases often arrives too late to effectively influence mosquito control decisions. Because chickens are continuously available to host-seeking mosquitoes, they are not subject to the night-to-night variations associated with mosquito trapping, and their fixed, permanent locations provide a specific spatial indication of transmission when seroconversions occur. In California, flocks of 6-10

chickens that were previously unexposed to arboviruses are placed in locations where mosquito abundance is known to be high or where there is a history of virus activity. Each chicken is bled every two weeks by pricking the comb and collecting blood onto a filter paper strip. The blood is tested at the CDPH Vector-Borne Disease Section for antibodies to WNV, SLEV, and WEEV. Agencies that conduct their own in-house testing should send positive samples to CDPH for confirmation. Sentinel housing, bleeding instructions, and testing protocols are provided in **Appendix C**. Information detailing surveillance site registration for participating vector control agencies is found in **Appendix D.**

Unlike WEEV and SLEV, WNV frequently causes mortality in North American birds, especially those in the family Corvidae (e.g., crows, ravens, magpies, and jays). Dead bird surveillance was initiated by CDPH in 2000 to provide early detection of WNV. Dead bird surveillance has been shown to be one of the earliest and most cost-effective indicators of WNV activity where susceptible bird species are abundant and local agencies promote this program. Dead birds are reported by the public to CDPH's dead bird call center (1-877-WNV-BIRD) or via the California West Nile virus website. Dead birds that meet criteria for species and condition are collected by local agencies for WNV testing. Agencies collect an oral sample by swabbing the oropharyngeal cavity of the bird and pressing the swab onto an RNA preservation card, which safely preserves nucleic acids. The cards are shipped to DART for WNV RNA testing by RT-qPCR. Local agencies may also test dead birds in-house using RT-qPCR provided they have passed an annual proficiency panel. The communication and testing algorithm for the dead bird surveillance program is detailed in **Appendix E**.

Equine Infections

Currently, WNV or WEEV equine disease is no longer a sensitive indicator of epizootic activity in California because of widespread vaccination efforts in horses, donkeys, and mules. Nevertheless, confirmed horse cases can indicate that WNV or WEEV has amplified to levels where tangential transmission has occurred and the risk to humans is elevated in that region of the state. Numerous infectious and non-infectious causes, including other mosquito-borne viruses, can contribute to encephalitis and neurologic signs in horses. Testing of equine specimens for these possible etiologies is available through the California Animal Health and Food Safety Laboratory (CAHFS). Complete information on specimen collection and submission is available on the California Department of Food and Agriculture (CDFA) website. See **Appendix F**.

Human Infections

Local mosquito control agencies need information from the detection and reporting of human infections to rapidly plan and implement emergency control activities to prevent additional infections. However, human arboviral cases are an insensitive surveillance indicator for viral activity because most persons who become infected develop no or mild symptoms. Among individuals who do become ill, it may take up to two weeks for symptoms to appear, followed by additional time until the case seeks healthcare, samples are tested, and local health authorities investigate and report the case. In 2002, a regional public health laboratory network was established to enhance human WNV testing and surveillance efforts throughout the state. This network consists of the

CDPH Viral and Rickettsial Disease Laboratory (VRDL) as well as county public health laboratories that conduct WNV testing. Healthcare providers are encouraged to submit specimens from suspect WNV cases to their local public health laboratories. Specimens from patients with encephalitis may also be submitted directly to VRDL, which offers diagnostic testing for many agents known to cause encephalitis, including WNV and other arboviruses. VRDL also works with commercial laboratories to confirm additional suspect WNV cases.

In accordance with Title 17 of the California Code of Regulations (Sections 2500 and 2505), healthcare providers and laboratories are required to report positive test results for WNV, SLEV, and WEEV in humans to the local health department with jurisdiction in the area where that patient resides. Positive arbovirus test results are investigated by local health department officials to determine whether a patient meets the clinical and laboratory criteria for diagnosis of arboviral disease. If so, the local health department collects demographic and clinical information on the patient using a standardized form and reports these data to the state health department. The local health department also determines whether the infection was acquired locally, imported from a region outside the patient's residence, or acquired by a non-mosquito route of transmission such as blood transfusion or organ transplantation. **Appendix G** details the protocol for submission of specimens to the regional public health laboratory network for WNV testing. For more information regarding guidelines and protocols for the investigation and submission of human WNV infection please refer to West Nile and St. Louis Encephalitis Viruses in California: Guidelines for Human Testing, Surveillance, and Reporting. The national surveillance case definitions for WNV, SLEV, and WEEV infections can be found in **Appendix H**. For information on *Aedes*-transmitted diseases, such as Zika, dengue, and chikungunya, please refer to Guidance for Surveillance of and Response to Invasive *Aedes* Mosquitoes and Dengue, Chikungunya, and Zika in California.

Mosquito Control

Arboviral disease risk is mitigated through larval and adult mosquito control and is the most proven public health method to protect people from mosquito-borne disease. Mosquito control in California is conducted by approximately 80 local agencies, including mosquito and vector control districts, county environmental and health departments, and county agriculture departments. Pesticides generally used for larval and adult mosquito control in California are described in **Appendix I**. Additional considerations regarding adult mosquito control in urban areas are described in **Appendix J**.

Agencies that apply pesticides directly to a body of water in the United States, or where deposition may ultimately enter a water of the United States, must obtain a National Pollutant Discharge Elimination System (NPDES) permit for Biological and Residual Pesticide Discharges to Waters of the United States from Vector Control Applications (Vector Control Permit). Agencies must comply with provisions of the permit. Please refer to the Vector Control Permit for a list of vector control pesticides that may be applied to waters of the United States, unless the receiving water has an existing impairment from a pesticide with the same active ingredient. Please review the

California State Water Resources Control Board listing of impaired water bodies (303d list) prior to applying any pesticide.

Larval Control

Adult female mosquitoes are capable of transmitting pathogens, serving as biting nuisances, and ultimately, producing subsequent generations of mosquitoes. To prevent the emergence of adult mosquitoes, mosquito larval and pupal control methods should be target-specific. As such, most mosquito and vector control agencies in California target the immature stages rather than the adult stage of the mosquito. Larval and pupal mosquito control has three key components: environmental management, biological control, and chemical control.

Environmental management techniques can be used to reduce the availability of habitat suitable for immature mosquitoes. These methods may include water management, such as increasing the water disposal rate through evaporation, percolation, recirculation, or drainage. Water may also be managed by laser-leveling of fields, which minimizes pooling at low spots, allows even distribution of irrigation water, and precludes standing water for long periods. Controlled irrigation or the careful timing of wetland flooding for waterfowl can reduce mosquito production or limit emergence to cooler seasons of the year when virus activity is unlikely. Vegetation management may also be used to reduce larval habitat, as emergent vegetation provides food and refuge for mosquito larvae. Vegetation management strategies include the periodic removal or thinning of vegetation, restricting growth of vegetation, and controlling algae.

Biological control uses natural predators, parasites, or pathogens to reduce immature mosquito numbers. Mosquitofish, *Gambusia affinis*, are the most widely used biological control agent in California. These fish are released annually in a variety of habitats such as rice fields, small ponds, and canals.

Chemicals that control mosquito larvae and pupae are known as larvicides. There are several larvicides that are highly specific and thus have minimal impact on non-target organisms. These include microbial control agents and insect growth regulators. Microbial control agents such as *Bacillus thuringiensis israelensis* (Bti), *Bacillus sphaericus*, and spinosad prevent larval mosquitoes from developing. Insect growth regulators, such as methoprene, can also prevent immature mosquitoes from developing into adults. Other larvicides, such as surface films and chemical products, are less specific and may impact non-target organisms. Surface films are very effective against both larvae and pupae, but may suffocate other surface-breathing aquatic insects. Organophosphate pesticides can be used for larval control but are used infrequently because of widespread resistance within mosquito populations and their impact on non-target organisms and the environment.

Adult Control

When larval control is not possible, or more immediate control measures are needed, adult mosquito control may be required to suppress populations of infected mosquitoes and interrupt virus transmission. Adult mosquito control products may be applied using ground-based equipment, fixed wing airplanes, or helicopters. Products applied in

ultralow volume (ULV) formulations and dosages include organophosphates (e.g., malathion and naled), pyrethroids (e.g., resmethrin, sumithrin, and permethrin), and pyrethrins (e.g., Pyrenone crop spray). Factors to consider when selecting an adulticide include: 1) efficacy against the target species or life cycle stage, 2) resistance status, 3) pesticide label requirements, 4) availability of pesticide and application equipment, 5) environmental conditions, 6) cost, and 7) toxicity to non-target species, including humans.

For more information about mosquito control please see Best Management Practices for Mosquito Control in California.

Response Levels

The California Mosquito-Borne Virus Surveillance and Response Plan was developed to provide a semi-quantitative measure of virus transmission risk to humans that could be used by local mosquito control agencies to plan and modulate control activities. Independent models are presented for WNV, SLEV, and WEEV to accommodate the different ecological dynamics of these viruses (Barker et al. 2003). WNV and SLEV are closely related, require similar environmental conditions and are transmitted by the same *Culex* vectors. Seven surveillance factors are measured and analyzed to determine the level of risk for human infection and thereby gauge the appropriate response level:

1. Environmental or climatic conditions (e.g., snowpack, rainfall, and temperature)
2. Adult *Culex* vector abundance
3. Virus infection rate in *Culex* mosquito vectors
4. Sentinel chicken seroconversions
5. Fatal infections in birds (WNV only)
6. Infections in humans
7. Proximity of detected virus activity to urban or suburban regions (WEEV only)

Each factor is scored on an ordinal scale from 1 (lowest risk) to 5 (highest risk). The mean score calculated from these factors corresponds to a response level as follows: normal season (1.0 to 2.5), emergency planning (2.6 to 4.0), and epidemic (4.1 to 5.0). **Table 1** provides a worksheet to assist in determining the appropriate rating for each of the risk factors for each of the three viruses. **Appendix K** shows sources of data useful for the calculation of risk in Table 1. Surveillance data can be managed and risk level calculated in time and space using the CalSurv Gateway, a web-based data management system maintained by DART and utilized by California vector control agencies.

Risk calculations should be applied within a defined area, typically encompassing a local mosquito and vector control district. Use of smaller spatial units (e.g., city boundaries) is ideal due to spatial variation in virus activity and the need to define potential target areas for mosquito control at finer spatial scales. Decisions about the appropriate spatial scale for risk calculations should consider the balance between (1) the desire to assess risk at a scale fine enough to target mosquito control, and (2) the need to ensure that there is adequate surveillance information available in each area to support the risk calculations. Due to spatial variation in the distributions of humans and

the dominant vector species, *Cx. tarsalis* and the *Cx. pipiens* complex, separate calculation of risk for urban and rural areas is encouraged where applicable.

For surveillance factor 2 (vector abundance), abundance is expressed as a percentage of normal by comparing the current level for an area to the average over the previous five years for the same area and two-week period. The mosquito virus infection rate (surveillance factor 3) should be calculated using the most recent data (prior two-week period) and expressed as the minimum infection rate (MIR) per 1,000 female mosquitoes tested. Alternatively, when infection rates are high, they may be calculated using maximum likelihood estimates (Hepworth and Biggerstaff 2017), which account for varying numbers of specimens in pools and the possibility that more than one mosquito could be infected in each positive pool. For WNV and SLEV, risk may be estimated separately for *Cx. tarsalis* and the *Cx. pipiens* complex because these species generally have different habitat requirements and therefore spatial distributions (e.g., rural vs. urban).

WEEV, SLEV and WNV differ in their response to ecological conditions. WEEV activity has historically been highest during El Niño conditions of wet winters, above-normal run-off and flooding, cool springs, and increased *Cx. tarsalis* abundance. Historically, WEEV spillover into a secondary *Aedes*-rabbit cycle was common in the Central Valley, but this virus spillover has not been detected for more than 25 years. In contrast, SLEV and perhaps WNV activity appear to be greatest during La Niña conditions of drought and hot summer temperatures because SLEV and WNV transmission risk increases when temperatures are above normal. Abundance and infection of the *Cx. pipiens* complex are included in both SLEV and WNV risk estimates because these mosquito species are important vectors, particularly in suburban/urban environments. The occurrence of dead bird infections is included as a risk factor in the WNV calculations. For surveillance factors 4–6 (chickens, dead birds, and humans), the specific region is defined as the area within the agency's boundary and the broad region includes the area within 150 miles (~241 km) of the agency's boundary.

Proximity of virus activity to human population centers is considered an important risk factor for all three viruses of public health concern. The risk assessment model in Table 1 accommodated this in two different ways. WEEV transmitted by *Cx. tarsalis* typically amplifies first in rural areas and may eventually spread into small and then larger communities. A risk score was included to account for where virus activity was detected. WNV and SLEV may be amplified concurrently or sequentially in rural and urban cycles. The rural cycle is similar to WEEV and is transmitted primarily by *Cx. tarsalis*, whereas the urban cycle is transmitted primarily by members of the *Cx. pipiens* complex. If the spatial distributions of key *Culex* species differ within an area (e.g., rural vs. urban), it may be advantageous to assess risk separately by species for abundance and infection rates in *Cx. tarsalis* and the *Cx. pipiens* complex. This would result in two estimates of overall risk for the areas dominated by each species.

Each of these surveillance factors can differ in impact and significance according to time of year and geographic region. Climate is used prospectively to forecast risk during the coming season. Climatic factors provide the earliest indication of the potential for increased mosquito abundance and virus transmission and constitute the only risk factor measured in many areas from the start of the calendar year through mid-spring

when enzootic surveillance commences. Other factors that may inform control efforts as the season progresses are typically, in chronological order: mosquito abundance, infections in non-humans (e.g., dead birds for WNV, mosquitoes, and sentinel chickens), and infections in humans. Enzootic indicators measure virus amplification within the *Culex*-bird cycle and provide current assessments (nowcasts) of risk, whereas human infections document tangential transmission and are the outcome measure of forecasts and nowcasts. Response to the calculated risk level should consider the time of year (e.g., epidemic conditions in October would warrant a less aggressive response compared to epidemic conditions in July because cooler weather in late fall will contribute to declining risk of arbovirus transmission).

The ratings listed in **Table 1** are benchmarks only and may be modified as appropriate to the conditions in each specific region or biome of the state. Calculation and mapping of risk have also been enabled by tools for local agency use included in the CalSurv Gateway. Roles and responsibilities of key agencies involved in carrying out the California Mosquito-Borne Virus Surveillance and Response Plan are outlined in **Key Agency Responsibilities**.

Table 1. Mosquito-Borne Virus Risk Assessment

WNV Surveillance Factor	Assessment Value	Benchmark	Assigned Value	
1. **Environmental conditions** High-risk environmental conditions include above-normal temperatures with or without above-normal rainfall, runoff, or snowpack.	1	Avg daily temperature during prior 2 weeks ≤ 56°F		
	2	Avg daily temperature during prior 2 weeks 57–65°F		
	3	Avg daily temperature during prior 2 weeks 66–72°F		
	4	Avg daily temperature during prior 2 weeks 73–79°F		
	5	Avg daily temperature during prior 2 weeks > 79°F		
			Cx tars	*Cx pip*
2. Relative abundance of adult female *Culex tarsalis* and *Cx. pipiens* complex mosquitoes* Determined by trapping adults, enumerating them by species, and comparing numbers to those previously documented for an area for the prior 2-week period.	1	Vector abundance well below average (≤ 50%)		
	2	Vector abundance below average (51–90%)		
	3	Vector abundance average (91–150%)		
	4	Vector abundance above average (151–300%)		
	5	Vector abundance well above average (> 300%)		
3. Virus infection rate in *Cx. tarsalis* and *Cx. pipiens* complex mosquitoes* Tested in pools of ≤ 50 females. Test results expressed as minimum infection rate per 1,000 mosquitoes tested (MIR) for the prior 2-week period.	1	MIR = 0		
	2	MIR = 0.1–1.0		
	3	MIR = 1.1–2.0		
	4	MIR = 2.1–5.0		
	5	MIR > 5.0		
4. Sentinel chicken seroconversion Number of chickens in a flock that develop antibodies to WNV during the prior 2-week period. If more than one flock is present in a region, number of flocks with seropositive chickens is an additional consideration. Recommend 6 - 10 chickens per flock.	1	No seroconversions in broad region		
	2	One or more seroconversions in broad region		
	3	One or two seroconversions in a single flock in specific region		
	4	More than two seroconversions in a single flock or two flocks with one or two seroconversions in specific region		
	5	More than two seroconversions per flock in multiple flocks in specific region		
5. Dead bird infection Number of birds that have tested positive for WNV during the prior 3-month period. This longer time period reduces the impact of zip code closures during periods of increased WNV transmission.	1	No positive dead birds in broad region		
	2	One or more positive dead birds in broad region		
	3	One positive dead bird in specific region		
	4	Two to five positive dead birds in specific region		
	5	More than five positive dead birds in specific region		
6. Human cases Do not include this factor in calculations if no cases are detected in region.	3	One or more human infections in broad region		
	4	One human infection in specific region		
	5	More than one human infection in specific region		
			Cx tars	*Cx pip*
Response Level / Average Rating: Normal Season (1.0 to 2.5) Emergency Planning (2.6 to 4.0) Epidemic (4.1 to 5.0)		**TOTAL**		
		AVERAGE		

*Calculation of separate risk values for *Cx. tarsalis* and the *Cx. pipiens* complex may be useful if their spatial distributions (e.g., rural vs. urban) differ within the assessment area.

SLEV Surveillance Factor	Assessment Value	Benchmark	Assigned Value	
1. **Environmental conditions** High-risk environmental conditions include above-normal temperatures with or without above-normal rainfall, runoff, or snowpack.	1	Avg daily temperature during prior 2 weeks ≤ 56°F		
	2	Avg daily temperature during prior 2 weeks 57–65°F		
	3	Avg daily temperature during prior 2 weeks 66–72°F		
	4	Avg daily temperature during prior 2 weeks 73–79°F		
	5	Avg daily temperature during prior 2 weeks > 79°F		
			Cx tars	*Cx pip*
2. **Relative abundance of adult female *Culex tarsalis* and *Cx. pipiens* complex mosquitoes*** Determined by trapping adults, enumerating them by species, and comparing numbers to those previously documented for an area for the prior 2-week period.	1	Vector abundance well below average (≤ 50%)		
	2	Vector abundance below average (51–90%)		
	3	Vector abundance average (91–150%)		
	4	Vector abundance above average (151–300%)		
	5	Vector abundance well above average (> 300%)		
3. **Virus infection rate in *Cx. tarsalis* and *Cx. pipiens* complex mosquitoes*** Tested in pools of ≤ 50 females . Test results expressed as minimum infection rate per 1,000 mosquitoes tested (MIR) for the prior 2-week collection period.	1	MIR = 0		
	2	MIR = 0.1–1.0		
	3	MIR = 1.1–2.0		
	4	MIR = 2.1–5.0		
	5	MIR > 5.0		
4. **Sentinel chicken seroconversion** Number of chickens in a flock that develop antibodies to SLEV during the prior 2-week period. If more than one flock is present in a region, number of flocks with seropositive chickens is an additional consideration. Recommend 6 - 10 chickens per flock.	1	No seroconversions in broad region		
	2	One or more seroconversions in broad region		
	3	One or two seroconversions in a single flock in specific region		
	4	More than two seroconversions in a single flock or two flocks with one or two seroconversions in specific region		
	5	More than two seroconversions per flock in multiple flocks in specific region		
5. **Human cases** Do not include this factor in calculations if no cases are detected in region.	3	One or more human cases in broad region		
	4	One human case in specific region		
	5	More than one human case in specific region		
			Cx tars	*Cx pip*
Response Level / Average Rating: Normal Season (1.0 to 2.5) Emergency Planning (2.6 to 4.0) Epidemic (4.1 to 5.0)		**TOTAL**		
		AVERAGE		

*Calculation of separate risk values for *Cx. tarsalis* and the *Cx. pipiens* complex may be useful if their spatial distributions (e.g., rural vs. urban) differ within the assessment area.

WEEV Surveillance Factor	Assessment Value	Benchmark	Assigned Value
1. **Environmental conditions** High-risk environmental conditions include above normal rainfall, snowpack, and runoff during the early season followed by a strong warming trend.	1	Cumulative rainfall and runoff well below average	
	2	Cumulative rainfall and runoff below average	
	3	Cumulative rainfall and runoff average	
	4	Cumulative rainfall and runoff above average	
	5	Cumulative rainfall and runoff well above average	
2. **Relative abundance of adult female *Culex tarsalis* mosquitoes** Determined by trapping adults, enumerating them by species, and comparing numbers to averages previously documented for an area for the prior 2-week period.	1	*Cx. tarsalis* abundance well below average (≤ 50%)	
	2	*Cx. tarsalis* abundance below average (51–90%)	
	3	*Cx. tarsalis* abundance average (91–150%)	
	4	*Cx. tarsalis* abundance above average (151–300%)	
	5	*Cx. tarsalis* abundance well above average (> 300%)	
3. **Virus infection rate in *Cx. tarsalis* mosquitoes** Tested in pools of ≤ 50 females. Test results expressed as minimum infection rate per 1,000 mosquitoes tested (MIR) for the prior 2-week collection period.	1	*Cx. tarsalis* MIR = 0	
	2	*Cx. tarsalis* MIR = 0.1–1.0	
	3	*Cx. tarsalis* MIR = 1.1–2.0	
	4	*Cx. tarsalis* MIR = 2.1–5.0	
	5	*Cx. tarsalis* MIR > 5.0	
4. **Sentinel chicken seroconversion** Number of chickens in a flock that develop antibodies to WEEV during the prior 2-week period. If more than one flock is present in a region, number of flocks with seropositive chickens is an additional consideration. Recommend 6 - 10 chickens per flock.	1	No seroconversions in broad region	
	2	One or more seroconversions in broad region	
	3	One or two seroconversions in a single flock in specific region	
	4	More than two seroconversions in a single flock or two flocks with one or two seroconversions in specific region	
	5	More than two seroconversions per flock in multiple flocks in specific region	
5. **Proximity to urban or suburban regions** (score only if virus activity is detected) Risk of outbreak is highest in urban areas because of high likelihood of contact between humans and vectors.	1	Virus detected in rural area	
	3	Virus detected in small town or suburban area	
	5	Virus detected in urban area	
6. **Human cases** Do not include this factor in calculations if no cases found in region or in agency.	3	One or more human cases in broad region	
	4	One human case in specific region	
	5	More than one human case in specific region	
<u>Response Level / Average Rating:</u> Normal Season (1.0 to 2.5) Emergency Planning (2.6 to 4.0) Epidemic (4.1 to 5.0)		**TOTAL**	
		AVERAGE	

General suggestions for applying the risk assessment model locally

- Use a consistent time period for environmental conditions, adult mosquito abundance, mosquito infection rates, and human cases, and use this same period of time for all estimates. If you use a time frame that differs from the prior two-week period defined in the risk assessment, such as the prior month, use the same time period for all other relevant estimates. Note that sentinel chicken seroconversions may require special consideration to account for bleeding schedules, and dead bird data should consider areas with no dead bird surveillance, either on a permanent or temporary basis (e.g., ZIP code closures).
- If you have multiple trap types in your surveillance program, determine the vector abundance anomaly for each trap type and species and use the most sensitive trap type's value in the risk assessment.
- When determining the vector abundance anomaly, there should be at least two years (preferably five) of historical data to provide a comparative baseline for each trap type. Ideally, the prior years should use the same or very similar trap locations, be contiguous, and immediately precede the time period being evaluated.

Risk assessment as implemented by the CalSurv Gateway

- Due to privacy concerns and delays in detection and reporting, human cases are not part of CalSurv's risk assessment.
- Risk estimates based on mosquito abundance and infection rates are calculated separately for the key mosquito taxa, *Culex tarsalis* and the *Cx. pipiens* complex.
- The risk assessment model is also implemented as an online calculator for use by local vector control agencies that allows user definition of locations, date ranges, and other criteria.
- Maps showing the positive collections of various virus-tested mosquitoes, dead birds, and sentinel chickens over time in California can be visualized at VectorSurv Maps.

Characterization of Conditions and Responses for State and Local Agencies

Level 1: Normal Season

Risk rating: 1.0 to 2.5

Conditions
• Cool to moderate seasonal temperatures (< 65°F) • *Culex* mosquito abundance at or below five-year average (key indicator = adults of vector species) • No virus infection detected in mosquitoes • No seroconversions in sentinel chickens • No infected WNV-positive dead birds • No human cases

Response
• Conduct routine public education (eliminate standing water around homes, use personal protection measures) • Conduct routine mosquito and virus surveillance activities • Comply with National Pollutant Discharge Eliminations System (NPDES) permit if applying pesticides to waters of the United States • Conduct routine mosquito control with emphasis on larval control • Inventory pesticides and equipment • Evaluate pesticide resistance in vector species • Ensure adequate emergency funding • Release routine press notices • Send routine notifications to physicians and veterinarians • Establish and maintain routine communication with local office of emergency services personnel; obtain Standardized Emergency Management System (SEMS) training

Level 2: Emergency Planning

Risk rating: 2.6 to 4.0

Conditions
• Temperature above average (66–79°F) • Adult *Culex* mosquito abundance greater than 5-year average (150% to 300% above normal) • One or more virus infections detected in *Culex* mosquitoes (MIR < 5 per 1,000 tested) • One or more seroconversions in single flock or one to two seroconversions in multiple flocks in specific region • One to five infected WNV-positive dead birds in specific region • One human case in broad or specific region • WEEV detected in small towns or suburban area

Response
• Review epidemic Response Plan • Enhance public education (include messages on the signs and symptoms of encephalitis; seek medical care if needed; inform public about pesticide applications if appropriate) • Enhance information to public health providers • Conduct epidemiological investigations of cases of equine or human disease • Increase surveillance and control of mosquito larvae • Increase adult mosquito surveillance • Increase number of mosquito pools tested for virus • Conduct or increase localized chemical control of adult mosquitoes as appropriate • Contact commercial applicators in anticipation of large-scale adulticiding • Review candidate pesticides for availability and susceptibility of vector mosquito species • Ensure notification of key agencies of presence of viral activity, including the local office of emergency services

Level 3: Epidemic Conditions

Risk rating: 4.1 to 5.0

Conditions
• Temperature well above average (> 79°F) • Adult vector population extremely high (> 300% above normal) • Virus infections detected in multiple pools of *Culex tarsalis* or *Cx. pipiens* mosquitoes (MIR > 5 per 1,000 tested) • More than two seroconversions per flock in multiple flocks in specific region • More than five infected WNV-positive dead birds and multiple reports of dead birds in specific region • More than one human case in specific region • WEEV detection in urban or suburban areas

Response
• Conduct full-scale media campaign • Alert physicians and veterinarians to expect cases • Conduct active human case surveillance with outreach to the medical community • Conduct epidemiological investigations of cases of equine or human disease • Continue enhanced larval surveillance and control of immature mosquitoes • Broaden geographic coverage of adult mosquito surveillance • Accelerate adult mosquito control as appropriate by ground and/or air • Coordinate the response with the local Office of Emergency Services or if activated, the Emergency Operation Center (EOC) • Initiate mosquito surveillance and control in geographic regions without an organized vector control program • Determine whether declaration of a local emergency should be considered by the County Board of Supervisors (or Local Health Officer) • Determine whether declaration of a “State of Emergency” should be considered by the Governor at the request of designated county or city officials • Ensure state funds and resources are available to assist local agencies at their request • Determine whether to activate a Standardized Emergency Management System (SEMS) plan at the local or state level • Continue mosquito education and control programs until mosquito abundance and enzootic virus activity is substantially reduced and no additional human cases are detected

For more detailed information on responding to a mosquito-borne disease outbreak, please refer to: Operational Plan for Emergency Response to Mosquito--Borne Disease Outbreaks, California Department of Public Health (supplement to California Mosquito-Borne Virus Surveillance and Response Plan).

Key Agency Responsibilities

Local Mosquito and Vector Control Agencies

- Acquire and interpret local climate and weather data.
- Monitor abundance of immature and adult mosquitoes.
- Collect and submit mosquito pools to DART or local laboratories for testing.
- Maintain sentinel chicken flocks, collect blood samples and send samples to CDPH VBDS for testing.
- Pick up suitable dead birds and collect and submit oral swab samples to DART or local laboratories for WNV testing.
- Report weekly mosquito and dead bird results that are tested in-house to the CalSurv Gateway.
- Conduct routine control of immature mosquitoes.
- Comply with NPDES permit if applying pesticides to waters of the United States.
- Conduct control of adult mosquitoes when needed.
- Educate public on mosquito avoidance and reduction of mosquito breeding sites.
- Coordinate with local Office of Emergency Services personnel.
- Communicate regularly with neighboring agencies.

Mosquito and Vector Control Association of California

- Coordinate purchase and disburse payment for sentinel chickens.
- Receive, track, and disburse payment for mosquito surveillance expenses.
- Coordinate surveillance and response activities among member agencies.
- Serve as spokesperson for member agencies.
- Establish liaisons with press and government officials.

California Department of Public Health

- Provide and maintain Vector Control Technician Certification program.
- Maintain a WNV information and dead bird reporting call center, 1-877-WNV-BIRD, and a WNV website.
- Provide supplies for sentinel chicken diagnostic specimens.
- Test sentinel chicken blood for antiviral antibodies.
- Coordinate surveillance for human infections and conduct epidemiological investigations of suspect cases of human disease.
- Coordinate and oversee testing and acquisition of human specimens for virus and antiviral antibodies.
- Distribute a weekly bulletin summarizing surveillance test results.
- Report weekly environmental and human surveillance data to the CDC arboviral surveillance system (ArboNET).
- Immediately notify local public health officials when evidence of virus activity is found.
- Coordinate and participate in a regional emergency response in conjunction with California Office of Emergency Services.

- Provide oversight to local jurisdictions without defined vector-borne disease control program.
- Maintain inventory of antigens, antisera, and molecular assays to detect exotic viruses in human specimens.
- Provide confirmatory laboratory testing for local agencies.

University of California, Davis

- Conduct research on arbovirus surveillance, transmission of mosquito-borne pathogens, and mosquito ecology and control.
- Test mosquito and dead bird samples for endemic and exotic arboviruses.
- Provide an annual proficiency panel to local agencies that conduct in-house testing on birds and/or mosquitoes for WNV, SLEV, and WEEV to ensure quality control for local laboratory results.
- Maintain an interactive website for management and dissemination of data on mosquito-borne virus surveillance and control.
- Maintain inventory of antigens, antisera, and viruses to detect the introduction of exotic viruses in mosquitoes.
- Provide confirmation for tests done by local or state agencies.

California Department of Food and Agriculture

- Notify veterinarians and veterinary diagnostic laboratories about WNV and WEEV testing available at CAHFS.
- Educate the general public and livestock managers about the need to monitor and report equine and ratite encephalitides.
- Facilitate equine sample submission from veterinarians.
- Conduct investigations of confirmed WNV and WEEV equine cases.
- Notify CDPH regarding WNV or WEEV positive equines.

California Animal Health and Food Safety Laboratory

- Test equine and other animal specimens for evidence of WNV or other arbovirus infection.

Local Health Departments and Public Health Laboratories

- Test human specimens for WNV and other arboviruses.
- Refer human specimens to CDPH for further testing as needed.
- Conduct epidemiological investigations of cases of human disease.
- Notify local medical community, including hospitals and laboratories, if evidence of viral activity is present.
- Pick up suitable dead birds and collect and submit oral swab samples to DART for WNV testing.
- Participate in emergency response.
- Report WNV and other arboviral infections to CDPH.
- Conduct public outreach and education.

California Office of Emergency Services

- Coordinate the local, regional, or statewide emergency response to epidemic conditions in conjunction with CDPH via the Standardized Emergency Management System (SEMS).
- Serve as liaison with the Federal Emergency Management Agency (FEMA) in the event that a federal disaster has been declared.

United States Centers for Disease Control and Prevention

- Provide consultation to state and local agencies in California if epidemic conditions exist.
- Provide national surveillance data to state health departments.
- Provide diagnostic consultation.

State Water Resources Control Board

- Review NPDES permit applications and respond in a timely manner.
- Review vector control pesticides registered by the California Department of Pesticide Regulation for inclusion on the Vector Control NPDES permit.

Appendix A: Guidelines for Adult Mosquito Surveillance

The objective of Appendix A is to standardize mosquito sampling and reporting procedures to provide comparable and interpretable abundance measures among collaborating mosquito control agencies in California. Appendix A summarizes information from Integrated Mosquito Surveillance Program Guidelines for California that have been adopted by the Mosquito and Vector Control Association (MVCAC) (Meyer et al. 2003). The MVCAC guidelines recommend stratifying the use of different sampling methods in rural, small town, and urban environments for each of the major biomes of California and provide a listing of target vector and nuisance mosquito species. The stratified sampling approach monitors vector populations and virus activity in rural enzootic foci, agricultural or suburban amplification sites, and densely populated urban centers to provide estimates of early, eminent, and current epidemic risk. Specific sampling methods for invasive *Aedes* have been summarized in the document Guidance for Surveillance of and Response to Invasive *Aedes* Mosquitoes and Dengue, Chikungunya, and Zika in California.

The four sampling methods currently used by mosquito control agencies are:

1) New Jersey (American) light trap (Mulhern 1942);
2) CO_2-baited trap, such as CDC/EVS style (Newhouse et al. 1966; Sudia and Chamberlain 1962);
3) Gravid trap (Cummings 1992; Reiter 1983);
4) Adult resting collections (Loomis and Sherman 1959).

Collection location sites should be geocoded and registered using the CalSurv Gateway. Studies comparing trap design and for surveillance purposes have been published (Reisen et al. 2000; Reisen et al. 2002). These guidelines describe:

1) A comparison of the sampling methods
2) Equipment design
3) Operation
4) Specimen processing
5) Data recording and analysis
6) Data usage

Advantages and Disadvantages of Mosquito Sampling Methods

New Jersey Light Trap

Pros	Cons
• All female gonotrophic states and males collected • Minimal collection effort (can be run nightly without service) • Long history of use in California	• Selective for phototactic nocturnally active mosquitoes • Ineffective in the presence of competing light sources • Sorting time excessive because of other insects in traps • Specimens dead; less useful for virus detection • Collects relatively fewer specimens

CDC/EVS CO_2 Trap

Pros	Cons
• Samples biting population • Collects large numbers of virus vector species • Specimens are alive and suitable for virus detection • Without light, collects mostly mosquitoes and reduces sorting time • Battery operated, portable	• Collects >50% newly emerged females that have never blood fed, implying lower probability of infection • Must be set and picked-up daily • Dry ice cost may be high and availability can be a problem • Does not collect males or bloodfed and gravid females

Gravid Trap

Pros	Cons
• Primarily collects females that have bloodfed and digested a blood meal; may have higher infection rate than CO_2 trap • Specimens are alive and suitable for virus detection • Effective for *Culex quinquefasciatus and Cx. pipiens* in urban habitats • Bait is inexpensive, consisting of water and organic matter • Battery operated, portable	• Collects only foul-water *Culex* (mostly *Cx. pipiens* complex) • Bait has an objectionable odor • Must be set and picked-up daily

Resting Catches

Pros	Cons
• All female reproductive stages collected (unfed, bloodfed, and gravid) • Minimal equipment needed • Specimens are collected alive and suitable for virus detection • Bloodfed and gravid specimens can be tested to improve sensitivity of virus surveillance	• Standardization is difficult due to: 1. Variable shelter size and type 2. Variable collector efficiency • Labor intensive; difficult to concurrently sample many sites

New Jersey (American) Light Trap (NJLT)

Operation

At a minimum, one trap should be located in each principal municipality of a district, or have a density of about one trap/township (36 mi^2). Correct placement of the NJLT is a critical factor in its performance as an effective surveillance mechanism for measuring the relative abundance of phototactic mosquitoes. Place the traps at a height of six feet. This can be done by using a metal stand, or by hanging the traps from tree limbs or roof eaves. These distances should maximize attractancy over a 360-degree radius. The trap should be placed on the leeward side of a structure or tree line to decrease the influence of wind on trap catch.

Traps should be kept away from smoke or chemical odors that may be repellent to the mosquitoes, and placed away from street and security lights that may diminish attractancy of the trap bulb. Traps should also be kept away from buildings in which animals are housed and should not be in the immediate vicinity of sentinel flocks to minimize attractancy competition; however, a trap should be placed approximately 100–200 feet from each sentinel chicken flock when possible to link abundance with seroconversions.

Traps should be operated from week 14 to week 44 of the calendar year for districts north of the Tehachapi Mountains and all year long for districts south of the Tehachapi. Ideally, the traps should run consecutively for four to seven nights before the collection is retrieved (Loomis and Hanks 1959). The trap should be cleaned thoroughly at each visit with a brush to remove spider webs or any other debris that may hinder airflow through the trap. A regular cleaning schedule should be maintained during the trapping season to maintain trap efficiency.

Processing

Adult mosquitoes from the NJLT collection should be sorted from the other insects in a white pan before being identified and counted at 10x magnification under a dissecting microscope. Counting aliquots or subsamples of all specimens should be discouraged

because vector species may comprise only a small fraction of the total mosquito collection.

CDC style CO_2-baited trap

Operation

Carbon dioxide-baited traps are effective traps for monitoring mosquito abundance and capturing mosquitoes for virus testing. Increasing the density of traps can improve the accuracy of population and infection rate estimates (Healy et al. 2015). For standardized trap placement during population and virus infection rate monitoring, traps should be suspended from a 6-foot tall standard pole approximately 4 feet above ground level. To enhance catch size and increase sampling sensitivity, the host-seeking patterns and preferred sources of the target species are essential for determining the best CO_2-baited trap placement locations. Some examples include:

- *Cx. tarsalis* primarily bloodfeed on birds and seek bloodmeals along vegetative borders and tree canopies where birds roost and nest.
- *Cx. erythrothorax* are best collected within wetland areas near dense stands of tules and cattails.
- *Anopheles freeborni* and *Cx. tarsalis* can be collected in large, open breeding sources such as rice fields usingCO_2-baited traps hung on standards on the upwind side of the mosquito source.
- *Aedes melanimon* and *Ae. nigromaculis* are mammal feeders and typically seek hosts over open fields.

When used for arbovirus surveillance, traps should be operated at different locations to enhance geographical coverage and surveillance sensitivity. Labor and time constraints determine the extent of sampling. When used to monitor population abundance, traps should be operated weekly or biweekly at the same fixed stations. Temperature, wind speed, wind direction, and rainfall should be recorded because these factors affect catch size. The mini light may be removed because it attracts other phototactic insects that may hinder sorting and/or damage female mosquitoes in the collection container. Place the CO_2-baited trap within a 100–200 ft radius of the sentinel flock site, but no closer than 100 feet from the flock. Do not place the trap in immediate proximity to the sentinel chicken flock, as it will compete with exposure of the sentinel birds and lessen their exposure to arboviruses.

Processing

Mosquitoes collected for arbovirus surveillance should be processed according to the procedures outlined in Appendix B. Mosquitoes collected for population monitoring should be anesthetized in a well-ventilated area or under a chemical hood using triethylamine, identified to species under a dissecting microscope, counted, pooled, and immediately frozen at -80°C or on dry ice for later virus testing. Only whole live mosquitoes should be used for virus testing. Any dead or dried specimens should be counted and discarded.

Reiter/Cummings gravid traps

Trap design and components

The Reiter/Cummings gravid trap consists of a rectangular trap housing (plastic toolbox) with an inlet tube on the bottom and an outlet tube on the side or top. The rectangular housing is provided with legs to stabilize the trap over the attractant basin containing the hay-infusion mixture (Cummings 1992). The oviposition attractant consists of a fermented infusion made by mixing hay, Brewer's yeast, and water. The mixture should sit at ambient temperature for a minimum of three to four days prior to use to allow fermentation and increase attractancy. New solutions should be made at least biweekly to maintain consistent attractancy.

Operation

The Reiter/Cummings gravid trap is primarily used in suburban and urban residential settings for surveillance of gravid females in the *Cx. pipiens* complex. As for CO_2-baited traps, increased trapping density will result in increased certainty for estimates of mosquito abundance and infection rates (Healy et al. 2015). Gravid traps are placed on the ground near dense vegetation that serves as resting sites for gravid females. Specimens may be retrieved on a one to three-day basis.

Processing

Cx. pipiens complex females collected with the gravid trap for arbovirus surveillance should be retrieved daily and the protocol for mosquito pool submission as outlined in Appendix B should be followed. For population monitoring of the *Cx. pipiens* complex, collections may be retrieved every third day. The females are killed, identified, and counted before being discarded. Autogenous females also may be attracted to the gravid trap.

Adult resting collections

Trap design and operation

A flashlight and mechanical aspirator can be used to collect adult mosquitoes resting in habitats such as shady alcoves, buildings, culverts, or spaces under bridges. Highest numbers usually are collected at humid sites protected from strong air currents. Adults resting in vegetation may be collected using a mechanical sweeper such as the Arbovirus Field Station (AFS) sweeper (Meyer et al. 1983). For quantification, time spent searching is recorded, and abundance expressed as the number collected per person-hour.

Red boxes can be used standardize resting adult collections spatially. Red boxes are boxes of varying dimensions that are painted red and fitted with a screen door. The largest adult mosquito catches are made in semi-permanent walk-in red boxes which measure 4'x4'x6' (Meyer 1985). Smaller 1'x1'x1' foot boxes typically collect fewer specimens but are readily portable. The entrance of the walk-in red box should be left open, draped with canvas, or closed with a plywood door. The canvas or plywood door should have a 1 or 2 ft gap at the bottom to allow entry of mosquitoes, while affording

some protection from the wind and decreasing the light intensity within the box. The box entrance should not face eastward into the morning sun or into the prevailing wind direction.

Processing

Mosquitoes should be anesthetized with triethylamine, identified under a dissecting microscope, sorted by sex and female gonotrophic status (i.e., empty or unfed, blood fed, or gravid), and counted. Females may be counted into ten pools of approximately 50 females per site per collection date for virus monitoring (Appendix B). Only living females should be used for arbovirus surveillance. Data on gonotrophic status may indicate population reproductive age as well as diapause status.

Data recording and analysis

Counts from NJLT, EVS, and gravid traps and information on pools submitted for testing or tested locally should be entered directly in electronic format through the CalSurv Gateway (https://gateway.calsurv.org). Data import from local or proprietary data systems is available. For comparisons of abundance over time, space, or collection methods, refer to Bidlingmeyer (1969).

Data usage

Mosquito collections from some or all four adult sampling methods collectively can be used to:

1. Assess control efforts.
2. Monitor arbovirus vector abundance and infection rates.
3. Compare mosquito abundance from collections with the number of service requests from the public to determine the tolerance of neighborhoods to mosquito abundance.
4. Determine proximity of breeding source(s) by the number of males present in collections from the NJLTs and red boxes.
5. Determine age structure of females collected by CO_2 traps and resting adult collections; such data are critical to evaluating the vector potential of the population.

Appendix B: Procedures for Processing Mosquitoes

1. Collect live mosquitoes and return them immediately to the laboratory. Collections should be kept humid during transport with moist toweling to prevent desiccation. Females should be offered 5–10 % sucrose if held overnight or longer before processing.

2. Anesthetize mosquitoes with cold, carbon dioxide, or triethylamine (TEA). TEA is recommended because specimens are permanently immobilized with minimal mortality and no loss of virus titer (Kramer et al. 1990). TEA should be used either outdoors, or under a chemical hood. Collections can be anesthetized outdoors using a few drops of TEA, the specimens transferred to petri dishes, and then taken into the laboratory for processing. If refrigerated and kept humid, mosquitoes will remain alive in covered Petri dishes for one or two days without additional anesthesia. If mosquitoes are frozen before processing, sorting to species and enumeration must be done on a chill table to prevent virus loss.

3. Sort mosquito collections to species under a dissecting microscope at 10X to ensure correct identification and to make sure that extraneous mosquito parts (i.e., legs, wings) or other small insects (e.g., chironomids or *Culicoides*) are not inadvertently included in the pools. Count and discard dead and dried mosquitoes. Pools are comprised of up to 50 females of each vector species from each collection site counted into individual polystyrene vials with snap caps containing two 5 mm glass beads. Recommended sampling effort is ten pools of 50 females of each species from each site per week to detect minimum infection rates (MIRs) ranging from 0 to 20 per 1,000 females tested. Vials with pools should be labeled sequentially each year with the pool number and year after the agency code (e.g., KERN-1-25, where 25 refers to the year of collection (2025). Number pools consecutively starting with “1” for each calendar year within your agency.

   Data on each pool can be entered directly in electronic format through the CalSurv Gateway. **Pools must be accompanied by a Mosquito Pool Submission Form (generated using the CalSurv Gateway) and can only be tested from registered surveillance sites.** Surveillance sites should be registered online.

   Register the surveillance site code for each pool in the CalSurv Gateway that consists of a designated four-letter agency code followed by six digits to identify the site (e.g., KERN000001). Pool numbers do not need to follow the ordering of site codes (e.g., pool #1 may be from KERN000001, pool #2 may be from KERN000004, pool #3 may be from KERN000003, etc.).

4. Freeze pools immediately at -80°C either on dry ice in an insulated container or in an ultra-low temperature freezer. Pools should be shipped overnight, frozen on dry ice or blue ice (8 blue ice packs minimum) to DART for testing by real-time multiplex RT-PCR. Agencies will receive an automated email notification that

results have been entered into the CalSurv Gateway as well as a summary of positive pools; additionally, positive pools will be reported weekly in the California Arbovirus Surveillance Bulletin. Each pool is screened for West Nile virus (WNV), St. Louis encephalitis virus (SLEV), and western equine encephalomyelitis virus (WEEV) by a multiplex RT-qPCR assay. Positive pools with Ct scores >35 are confirmed by singleplex RT-qPCR with a different set of virus species-specific primers and probes. Invasive *Aedes* mosquitoes and other mosquito species can also be tested for chikungunya, dengue, and Zika viruses upon request by a separate multiplex RT-qPCR. Pools from selected areas are also screened for additional viruses using Vero cell culture with isolates identified by genetic sequencing. Care must be taken not to allow pools to defrost during storage or shipment, because each freeze-thaw cycle may result in a decrease in viral titer; all virus will be lost if the specimens sit at room temperature for extended periods.

Address mosquito pool shipments to:

when shipping via GLS:
ATTN: Anil Singapuri
UC Davis, Dept. of PMI
1285 Veterinary Medicine Mall
Building: Vet Med 3A, Room 3336
Davis, CA. 95617

when shipping via FedEx or UPS
ATTN: Anil Singapuri
Vet Med 3A, Room 3336
1285 Veterinary Medicine Mall
University of California, Davis
Davis, CA 95616

5. Local agencies that conduct their own testing must complete and pass an annual proficiency panel distributed in early spring, for the results to be reported by CDPH.

Appendix C: Procedures for Maintaining and Bleeding Sentinel Chickens

1. Procure hens in early spring (as notified by MVCAC), when the chickens are 14–18 weeks of age to ensure minimal mortality during handling. Hens at this age have not yet begun to lay eggs, but they should have received all vaccinations and have been dewormed.
2. **Recommended housing for chickens.** Flocks of 6–10 sentinel chickens can be housed in a 3Wx6Lx3H foot coop framed with 2x2 and 2x4 inch construction lumber and screened with no smaller than 1x1 inch welded wire. It is critical that the wire mesh be large enough to allow the mosquitoes to easily enter the coop and the coops be placed in locations with a history of arbovirus transmission and/or high mosquito abundance. The site and band numbers located at each coop must be registered online. Coops should be at least two feet off the ground to reduce predator access, facilitate capture of the birds for bleeding, and allow the free passage of the feces through the wire floor to the ground. A single, hinged door should be placed in the middle of the coop, so that the entire coop is accessible during chicken capture. After construction, the lumber and roof should be protected with water seal. A self-filling watering device should be fitted to one end of the coop and a 25 lb. feeder suspended in the center for easy access. In exchange for the eggs, a local person (usually the homeowner, farm manager, etc.) should check the birds (especially the watering device) and remove the eggs daily. If hung so the bottom is about four inches above the cage floor and adjusted properly, the feeder should only have to be refilled weekly (i.e., 100 lb. of feed per month per flock of ten birds). Therefore, if proper arrangements can be made and an empty 55-gallon drum provided to store extra feed, sentinel flocks need only be visited biweekly when blood samples are collected.
3. Band each bird in the web of the wing using metal hog ear tags and appropriate pliers. This band number, the date, and site registration number must accompany each blood sample sent to the laboratory for testing.
4. Bleed each hen from the distal portion of the comb using a standard lancet used for human finger "prick" blood samples. The bird can be immobilized by wedging the wings between the bleeder's forearm and thigh, thereby leaving the hand free to hold the head by grabbing the base of the comb with the thumb and forefinger. Use alcohol swabs on comb before bleeding. Blood samples are collected on half-inch wide filter paper strips, which should be labeled with the date bled and wing band number. The comb should be "pricked" with the lancet and blood allowed to flow from the "wound" to form a drop. Collect the blood by touching the opposite end of the pre-labeled filter paper strip to the wound. **The blood must completely soak through a ¾ inch long portion of the strip.** Place the labeled end of the strip into the slot of the holder (or "jaws" of the clothes pin) leaving the blood-soaked end exposed to air dry.
5. Attach the completely dry filter paper strips to a 5x7 inch card in sequential order, from left to right by stapling the labeled end towards the top edge of the card, and

leaving the blood-soaked end free so that laboratory staff can readily remove a standard punch sample. Write the county, agency code, site, and date bled onto the card and place it into a Ziploc plastic bag (only one card per bag). It is important that the blood samples do not become dirty, wet, or touch each other. Chicken Samples must be accompanied by a "SENTINEL CHICKEN BLOOD" form outside the Ziploc bag. Do not staple the form to the bag.

Samples from each collection date can be placed into a mailing envelope and sent to:

California Department of Public Health
Vector-Borne Disease Section, G164 Attn: ARBO
850 Marina Bay Parkway
Richmond, CA 94804

Specimens will be tested within 1–3 days upon receipt by the laboratory.

6. In the laboratory, a single punch is removed from the blooded end of the paper and tested for West Nile virus (WNV), St. Louis encephalitis virus (SLEV), and western equine encephalomyelitis virus (WEEV) IgG antibodies using an enzyme immunoassay (Patiris et al. 2008; Taketa-Graham et al. 2010). Positive specimens are confirmed with an indirect fluorescent antibody test and/or a western blot. Samples yielding inconclusive results are tested further by cross-neutralization tests. Agencies will receive an automated email notification that results have been entered into the CalSurv Gateway. Additionally, positive chickens will be reported in the weekly California Arbovirus Surveillance Bulletin.

Appendix D: Registration of Agencies and Surveillance Sites

1. Participation of agencies

Agencies interested in participating in the statewide surveillance program for mosquito-borne viruses should place orders for sentinel chicken testing through the California Department of Public Health (CDPH). Agencies will be billed in advance for the number of samples to be tested. Mosquito pool testing by the UC Davis Arbovirus Research and Training (DART) laboratory will be billed through the Mosquito and Vector Control Association (MVCAC).

Agencies are responsible for registering and maintaining updated information for their sites online at the CalSurv Gateway.

2. Registration of sentinel flock sites and wing band numbers

Agencies must use the unique band numbers assigned to their district by CDPH each year. Prior to submitting any sentinel chicken blood samples to CDPH, each agency must ensure that each **flock site** and accompanying band numbers are registered online at the CalSurv Gateway. CDPH will only test samples if they are accompanied by the "SENTINEL CHICKEN BLOOD" form for each flock site, which includes the registered agency code, the registered site code (assigned by local agency), the wing band numbers assigned to that site, and date bled. **Also, the form should indicate any changes made and match the sample card exactly.**

3. Registration of mosquito sampling sites

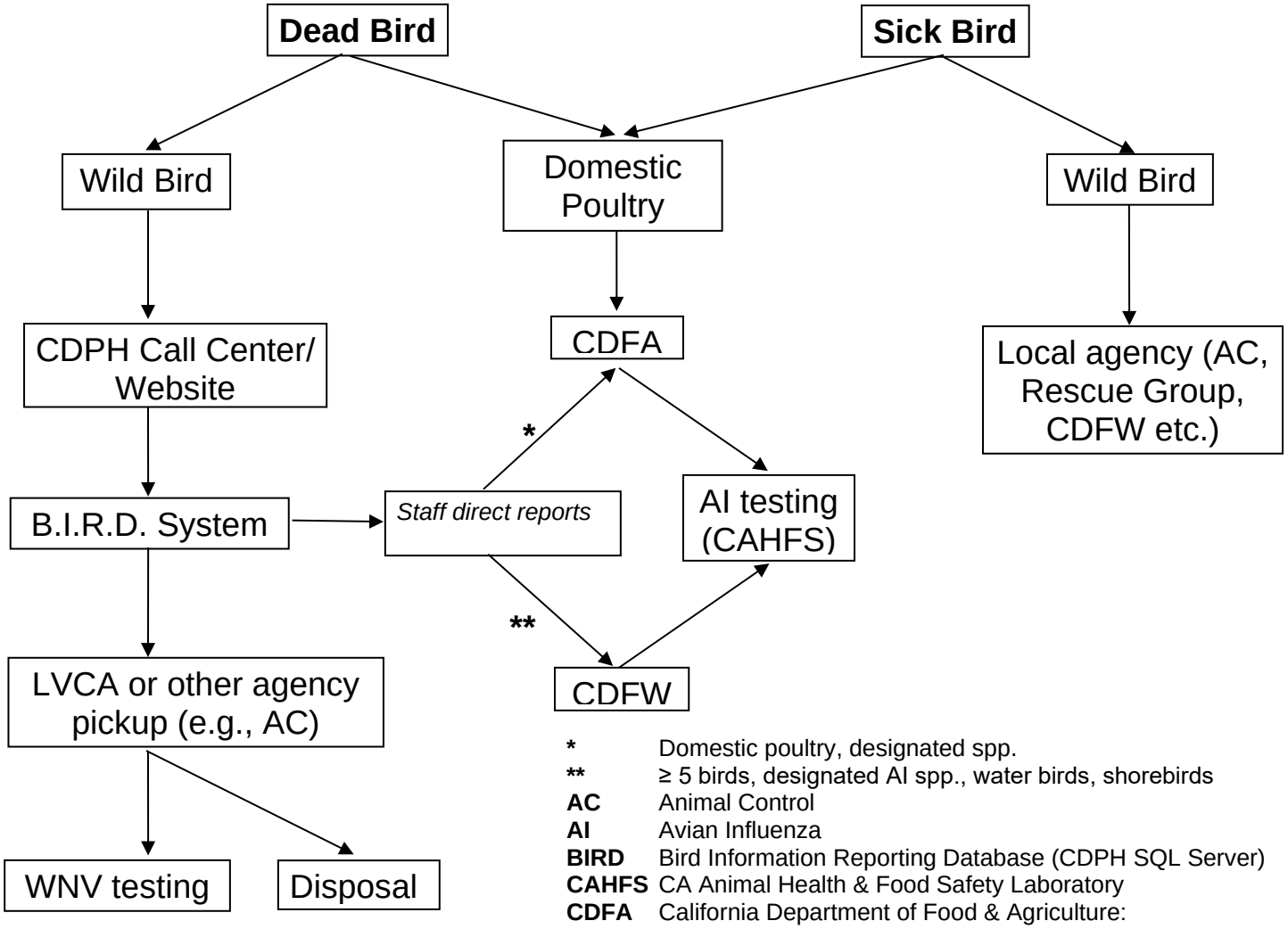
Registration of **new** sites used for collection of mosquitoes for virus testing may be accomplished by accessing the CalSurv Gateway. Since 2010, the CalSurv Gateway has included enhanced spatial capabilities that allow users the option of directly entering geographic coordinates for sites or interactively selecting the location using a new Google Maps-based interface. The laboratory will test the pools provided that adequate information is provided on the "MOSQUITO POOL SUBMISSION" form including your agency code, site code, and geographic coordinates.

Recording the geographic coordinates of all surveillance sites allows users to filter data spatially for analysis, and the locations are used to generate computer maps that show all registered sites and test results. As part of a collaborative effort, the DART laboratory hosts real-time maps. Local agencies can log in on the mapping website or the CalSurv Gateway to access more detailed maps and enhanced analysis tools.

Appendix E: Procedures for Testing Dead Birds

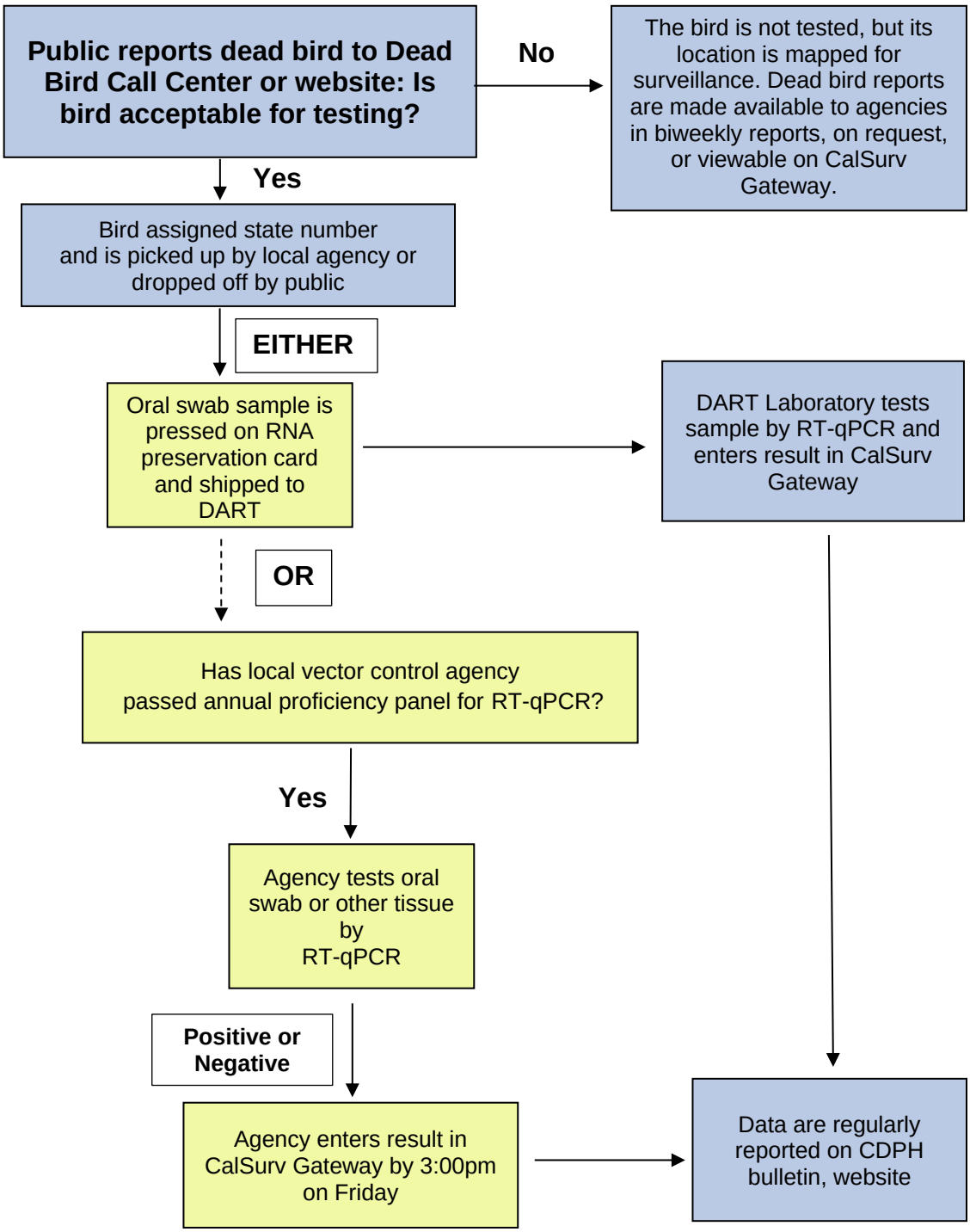
In 2000, the California Department of Public Health (CDPH) initiated a dead bird surveillance program in collaboration with other public agencies. The public is notified about the program through the media and outreach materials, and it is important for local agencies to publicize the need to report dead birds to ensure that the system will be effective. Dead birds are reported to CDPH or data entered electronically through the CalSurv Gateway. An oral swab sample is taken from the bird, pressed on an RNA preservation card, and sent to the UC Davis Arbovirus Research and Training (DART) laboratory for West Nile virus (WNV) RNA detection via RT-qPCR. Overviews of the dead bird reporting and testing algorithms are provided below.

Sick/Dead Bird Reporting Protocol for Public and Local Agencies



*	Domestic poultry, designated spp.
**	≥ 5 birds, designated AI spp., water birds, shorebirds
AC	Animal Control
AI	Avian Influenza
BIRD	Bird Information Reporting Database (CDPH SQL Server)
CAHFS	CA Animal Health & Food Safety Laboratory
CDFA	California Department of Food & Agriculture: California Bird Flu Hotline: **1-866-922-BIRD**
CDFW	California Department of Fish & Wildlife Investigations Lab phone: **1-916-358-2790** website: https://www.wildlife.ca.gov
CDPH	California Department of Public Health West Nile virus & Dead Bird Line: **1-877-WNV-BIRD** website: https://westnile.ca.gov
LVCA	Local Vector Control Agency

Dead Bird Testing Protocol



DART = Davis Arbovirus Research and Training laboratory, UC Davis
VBDS = Vector-Borne Disease Section, California Department of Public Health

VBDS or DART

Local Agencies

Dead Bird Reporting and Sample Submission Instructions for Local Agencies

California West Nile Virus (WNV) Dead Bird Surveillance Program
California Department of Public Health (CDPH)
Division of Communicable Disease Control

Local vector control agencies should direct calls from the public about dead birds to the **CDPH West Nile Virus and Dead Bird Call Center at 1-877-WNV-BIRD (968-2473) or the online report page at the CDPH West Nile Virus website. Crows, ravens, magpies, jays, and raptors are especially vulnerable to WNV, but most bird species may be accepted for testing as well (doves, quails, and pigeons are rarely tested).** A field guide to birds of Western North America is recommended to help identify birds to species, and pictures and descriptions of common birds can be found on the West Nile Virus website.

The WNV and Dead Bird Call Center will be staffed **8:00am–4:30pm, Monday–Friday (5 days a week from mid-April to mid-October).** Reports can also be made on the WNV website year-round or after hours via voicemail prompts during the April to October season. CDPH staff will assess the suitability of the dead bird for testing, work with the resident to secure the bird, and contact the agency if the carcass is approved for pickup. Agencies may call directly **(510-412-4601)** to coordinate bird pickups with call center operators. Local agencies can call this number to receive a dead bird number and submission form for birds they intake directly prior to sampling and/or testing.

Agencies also can obtain a dead bird number directly in the CalSurv Gateway: input a new carcass report and select “Submitted” from the “Status” dropdown menu. CalSurv will assign a number to the dead bird.

Agencies are authorized under an agreement between CDPH and the California Department of Fish & Wildlife to pick up dead birds, which include local mosquito and vector control districts, environmental health departments, and other designated agencies. Dead tree squirrels may also be picked up but will no longer be tested in the program. To test tree squirrels for WNV, the Center for Animal Health and Food Safety (CAHFS) offers a fee-based testing service.

Collect fresh carcasses. Badly decomposed, runover, or scavenged carcasses are of limited diagnostic value. Signs that a bird has been dead for too long (over 48 hours) are the presence of maggots; an extremely lightweight carcass; missing eyes; skin discoloration; skin or feathers that rub off easily; strong odor; or a soft, mushy carcass. However, some agencies will accept older carcasses for other tissue or maggot sampling.

If the carcass is found to be unacceptable upon pick-up (e.g., an unaccepted species or badly decomposed specimen), agencies can collect the carcass, double bag it and

dispose of it in a secure garbage can or dumpster, and call or email CDPH (email: arbovirus@cdph.ca.gov) if the carcass will no longer be submitted. The status of the dead bird may also be updated in CalSurv.

Once the dead bird is collected, the agency will collect an oral swab sample for an RNA preservation card (please see protocol below); cards are then mailed to the UC Davis Arbovirus Research and Training (DART) laboratory for WNV testing. There is no fee for testing, but agencies must purchase the RNA preservation cards and swabs.

To ensure safety when handling carcasses, please follow these instructions:

Dead Bird Oral Swab Sampling Procedure

Materials needed:

- Biosafety cabinet or N95 respirator masks
- Refrigerator to store RNA preservation cards
- **RNA preservation cards** (specifically, RNASound™ cards). Order online from FortiusBio (www.fortiusbio.com). Packages of 25 ($140) or 10 ($60.20) are available. (Once cards arrive, store in the refrigerator, and note the expiration date on the silver pouch. These cards are fine to use up to 18 months past the expiration date. Order as needed annually.)
- Individually-wrapped polyester swabs such as Fisher brand catalog no. 22-029-682
- Disposable nitrile or latex gloves
- Lab coat
- Small metal spatula
- Permanent ink pen or pencil
- Shipping envelopes (business size, FedEx, or other)

Methods:

1. Note on storage via refrigeration and freezing: It is recommended to refrigerate carcasses until ready for swabbing in lieu of maintaining at room temperature. RNA preservation cards must also be stored in the refrigerator until use. Freezing dead birds is only recommended if you cannot swab the bird for several days after collection (more than 3 days), as it will require many hours for the carcass to thaw before it can be swabbed.

2. Clean and disinfect biosafety cabinet or prepare for outdoor sampling and gather needed supplies. Dead birds should be handled in a Class II biosafety cabinet within a laboratory (WNV can be aerosolized). If it is not possible to work in a biosafety cabinet, work should be conducted outside while wearing an N95 respirator.

3. Put on disposable gloves. Partially unwrap the disposable swab. Open the bag containing the bird to expose the head. With gloved hands, pry open the beak (a metal spatula may help with this) and put swab into the mouth. Aggressively swab the mouth and oropharyngeal cavity (throat).

4. Press and roll the swab onto the target area of the RNA preservation card (over the two perforated discs). The sample may be dry and may even be colored with some blood; this is fine. Make sure to label the card with the dead bird number (i.e. 24-####) assigned to the bird by the WNV call center or obtained in CalSurv.

5. Discard the swab into the bag containing the dead bird. Double bag, knot the bag, and dispose in the trash. If you sample birds at the place of collection, the resident may dispose of the carcass or you may do it for them (residents usually appreciate the removal of the bird). Agencies conducting in-house testing may dispose of WNV-negative birds in the trash. **However, WNV-positive carcasses must be disposed as biohazardous wasted (incinerate).**

6. Wipe the inside of cabinet and metal spatula used for opening the beak with a fresh solution of 10% bleach, followed by 70 to 100% ethanol or isopropyl alcohol and change gloves after each bird. Cavicide™ is a product which kills viruses without corroding stainless steel and may also be used.

7. Allow cards to dry in biosafety cabinet or a cool place for 2 hours. Make sure the dead bird number corresponding to the dead bird is written on the front flap of each card. Seal RNA preservation cards back into their small individual bags with desiccation packet. Once used, the cards do not need to be stored in the refrigerator but kept at room temperature. **However, they should be tested within 10 days of sample taken.**

8. Place cards in an envelope for shipment. **IMPORTANT:** Include an inventory list of bird numbers corresponding to RNA preservation card samples in each shipment, or a printout of each dead bird report submitted by an operator.

   Shipping options:

   a. Add to weekly mosquito pool shipment. Seal all cards with card inventory list in a Ziplock bag and place in mosquito box. The cold temperatures of the mosquito boxes are fine for these cards but they should be protected from moisture.

   - Or -

   b. Ship batches of cards via overnight delivery (FedEx, GLS). Ship on Monday for fastest turnaround times during the testing season.

   - Or -

c. Regular U.S. Postal Service mail is accepted; however, paying additional for tracking or shipping in a larger, more conspicuous envelope is recommended to help avoid lost packages.

9. To be notified when the cards have arrived at the lab, change the status of each dead bird in CalSurv to “submitted”. Upon receipt, DART will update the status of the card in CalSurv to “received.”

10. Upon receiving WNV positive test results, telephone the resident who reported the positive dead bird to let them know the bird was positive for WNV and deliver risk prevention information if needed. Some agencies also opt to call residents when the test result is WNV-negative. Or, if you have an agreement with CDPH that they will make the call, staff at the call center will inform residents whose birds tested **positive** each Monday.

Ship cards using the addresses below:

when shipping via GLS:
ATTN: Anil Singapuri
UC Davis, Dept. of PMI
1285 Veterinary Medicine Mall
Building: Vet Med 3A, Room 3336
Davis, CA. 95617

when shipping via FedEx or UPS
ATTN: Anil Singapuri
Vet Med 3A, Room 3336
1285 Veterinary Medicine Mall
University of California, Davis
Davis, CA 95616

For agencies conducting in-house testing by RT-qPCR:

Once agencies pass the annual proficiency panel, agencies may conduct in-house testing. Results can be entered directly in CalSurv Gateway. Agencies conducting in-house testing must dispose of any WNV-positive birds as biohazard waste (incinerate) in compliance with **the California Health and Safety Code.** Negative birds can be placed in a double bag and disposed of in a secure garbage can or dumpster.

Appendix F: Procedures for Testing Equines

The California Department of Food and Agriculture (CDFA) has primary responsibility for investigation of West Nile virus (WNV) in equids. Veterinarians and diagnostic laboratories are required to report cases of WNV and other equine encephalomyelitides to CDFA (California Food and Agriculture Code §9101; Title 9 California Code of Regulations §161.4(f)).

Each spring, CDFA sends information on the California West Nile Surveillance Program to approximately 1,200 veterinarians, animal health branch personnel, and other interested parties. The mailing includes case definitions for equine WNV and instructions for collection and submission of specimens for diagnostic testing. Specimen submission is coordinated through the California Animal Health and Food Safety Laboratory System (CAHFS) and other laboratories or individual veterinarians. Equine serum and cerebrospinal fluid are tested by CAHFS using the IgM-capture ELISA. Equine neurologic tissue specimens are also sent to CAHFS for microscopic examination and, as indicated by clinical findings, forwarded to the USDA National Veterinary Services Laboratories (NVSL) for further arbovirus testing. All fatal cases of equine encephalitis should also be evaluated for rabies at the local or state public health laboratory.

Additional information on WNV for veterinarians, horse owners, and ratite owners is available from CDFA, Animal Health Branch (916) 900-5002, and at the CDFA website. Information on submission of laboratory samples is available from CAHFS (530) 752-8700 and at the CAHFS website.

Appendix G: Protocol for Submission of Specimens from Humans

West Nile virus (WNV) testing within the regional public health laboratory network (i.e., the California Department of Public Health Viral and Rickettsial Disease Laboratory (CDPH VRDL) and participating local public health laboratories) is recommended for individuals with the following symptoms, particularly during WNV season, which typically peaks between July and October in California:

Neuroinvasive disease

- Meningitis, encephalitis, acute flaccid paralysis, or other acute signs of central or peripheral neurologic dysfunction, as documented by a physician, AND
- Absence of a more likely clinical explanation. Other clinically compatible symptoms of arboviral disease include headache, myalgia, rash, arthralgia, vertigo, vomiting, paresis and/ or nuchal rigidity.

Non-neuroinvasive disease

- Fever (chills) as reported by the patient or a health-care provider, AND
- Absence of neuroinvasive disease, AND
- Absence of a more likely clinical explanation. Other clinically compatible symptoms of non-neuroinvasive arboviral disease include headache, myalgia, rash, arthralgia, and vomiting.

Recommended Specimen for Collection

- Serum: 3-5 mL of whole blood in a red top or serum separator tube OR
- Cerebrospinal fluid (CSF): 1 mL CSF in a sterile collection tube
- Whole blood: 3-5 mL in EDTA (lavender/purple top) tube
- Urine: 0.1-5 mL clean catch
- **CSF, EDTA whole blood, and/or urine samples *must be* accompanied by a serum sample**

If WNV is highly suspected and acute serum is negative or inconclusive, request:

- 2^{nd} serum (convalescent): $\geq$ 2cc serum collected 7-10 days after acute serum

Contact your local health department for instructions on where to send specimens.

Appendix H: West Nile Virus Surveillance Case Definition

Infections with West Nile virus (WNV), St. Louis encephalitis virus (SLEV), and western equine encephalomyelitis virus (WEEV) are reportable to local health departments under Title 17 of the California Code of Regulations. Local health departments should report human infections to the California Department of Public Health (CDPH). Blood and organ donors testing positive for WNV through screening should also be reported to CDPH, regardless of clinical presentation.

Case Definition for Neuroinvasive and Non-neuroinvasive WNV, SLEV, and WEEV
NOTE: This definition is for public health surveillance purposes only. It is not intended for clinical diagnoses.

Symptomatic Cases (adapted from 2015 CSTE case definition)

Clinical criteria for diagnosis

Neuroinvasive disease
- Meningitis, encephalitis, acute flaccid paralysis, or other acute signs of central or peripheral neurologic dysfunction, as documented by a physician, AND
- Absence of a more likely clinical explanation.

Non-neuroinvasive disease
- Fever or chills as reported by the patient or a healthcare provider, AND
- Absence of neuroinvasive disease, AND
- Absence of a more likely clinical explanation.

Case classification

Note: This classification changes after an environmental detection of SLEV. Contact CDPH for clarification regarding all suspect human cases of SLEV.

Confirmed: A case that meets the above clinical criteria and one or more of the following laboratory criteria for a confirmed case:
- Isolation of virus from, or demonstration of specific viral antigen or nucleic acid in tissue, blood, CSF, or other body fluid, OR
- Four-fold or greater change in virus-specific quantitative antibody titers in paired sera, OR
- Four-fold or greater difference in virus-specific quantitative antibody titers demonstrated via PRNT, OR
- Virus-specific IgM antibodies in CSF and a negative result for other IgM antibodies in CSF for arboviruses endemic to the region where exposure occurred.

Probable: A case that meets the above clinical criteria and the following laboratory criteria:
- Virus-specific IgM antibodies in serum but with no other testing.

Presumptive Viremic Donors (asymptomatic)

Asymptomatic infections with WNV, which are generally identified in blood donors, but also in organ donors, are also reportable. Blood donors who test positive for WNV may not necessarily be ill, nor will they initially have positive IgM or IgG antibody test results. Local health departments should report blood donors who meet the following criteria for being a presumptively viremic donor to CDPH:

A presumptively viremic donor (PVD) is a person with a blood donation that meets at least one of the following criteria:

a) One reactive nucleic acid amplification (NAT) test with signal-to-cutoff (S/CO) ≥ 17
b) Two reactive NATs

Additional serological testing is not required. Local health departments should follow up with the donor two weeks after the date of donation to assess if the patient subsequently became ill. If the donor did become ill as a result of WNV infection, the disease incident should be reclassified as “West Nile virus – Non-neuroinvasive” or “West Nile virus – Neuroinvasive,” depending on the individual’s clinical symptoms. Similarly, organ donors testing positive for WNV should also be reported to CDPH and receive public health follow-up by the local health department.

Appendix I: Compounds Approved for Mosquito Control in California

Pesticide label rates and usage vary from year to year and geographically; consult the product label, your County Agricultural Commissioner, and the California Department of Fish and Wildlife before application. Examples of products containing specific active ingredients are provided below, but this list is not exhaustive, nor does it constitute product endorsement. For more information on pesticides and mosquito control, please refer to the Environmental Protection Agency website.

It is recommended that agencies test their mosquitoes for resistance to pesticides at least once per year to ensure that their control efforts remain effective. Identifying resistance in mosquito populations can allow for the development of alternative control strategies and prevent the overuse of pesticides, which can lead to further resistance and the potential for negative impacts on non-target organisms. For more information on insecticide resistance and management tools please refer to the CDC Bottle Bioassay webpage.

Larvicides:

1. *Bacillus thuringiensis* subspecies *israelensis* (Bti: e.g., Aquabac 200G, VectoBac® 12AS, Teknar SC)
   Use: Approved for most permanent and temporary bodies of water.
   Limitations: Only works on actively feeding stages. Does not persist well in the water column.

2. *Bacillus sphaericus* (Bs: e.g., VectoLex® FG)
   Use: Approved for most permanent and temporary bodies of water.
   Limitations: Only works on actively feeding stages. Does not work well on all species. May persist and have residual activity in some sites.

3. Spinosad (e.g., Natular™ G30)
   Limitations: Effective against all larval stages and moderately effective against pupal stage. Toxic via ingestion and contact. Some formulations approved for use in OMRI certified organic crops.

4. IGRs (Insect Growth Regulators)
   a. (S)-Methoprene (e.g., Altosid® Pellets)
      Use: Approved for most permanent and temporary bodies of water.
      Limitations: Works best on older instars. Some populations of mosquitoes may show some resistance.

5. Larviciding oils (e.g., CocoBear® Larvicidal Oil)
   Use: Ditches, dairy lagoons, floodwater. Effective against all stages, including pupae.
   Limitations: Consult with the California Department of Fish and Game for local restrictions.

Adulticides:

1. Organophosphate compounds

    a. Malathion (e.g., Fyfanon® ULV MOSQUITO)
    Use: May be applied by air or ground equipment over urban areas, some crops including rice, wetlands.
    Limitations: Paint damage to cars; toxic to fish, wildlife and bees; crop residue limitations restrict application before harvest.
    b. Naled (e.g., Trumpet® EC)
    Use: Air or ground application on fodder crops, swamps, floodwater, residential areas.
    Limitations: Similar to malathion.

2. Pyrethrins (natural pyrethrin products: e.g., Merus® 3.0, Evergreen®)
    Use: Wetlands, floodwater, residential areas, some crops.
    Limitations: Do not apply to drinking water, milking areas; may be toxic to bees, fish, and some wildlife. Some formulations with synergists have greater limitations.

3. Pyrethroids (synthetic pyrethrin products containing deltamethrin, cyfluthrin, permethrin, resmethrin, sumithrin or etofenprox: e.g., Suspend® SC, Tempo Ultra WP, Aqua-Reslin®, Anvil® 10+10 ULV, Zenivex E20, and Duet – which also contains the mosquito exciter prallethrin)
    Use: All non-crop areas including wetlands and floodwater.
    Limitations: May be toxic to bees, fish, and some wildlife; avoid treating food crops, drinking water or milk production.

Appendix J: Adult Mosquito Control in Urban Areas

Adult mosquito control via ultralow volume (ULV) application is an integral part of an integrated mosquito management program. This Response Plan recommends the consideration of adult mosquito control to disrupt local virus transmission cycles and reduce the risk of human infection. The following provides guidelines for local agencies considering ground or aerial ULV control of adult mosquitoes. Agencies should ensure they are complying with National Pollutant Discharge Elimination System (NPDES) permit requirements.

Preparatory steps for aerial application contracts

- Send out request for proposals (RFP) to commercial applicators well in advance of any potential need for treatment. Specify required equipment and abilities in the RFP such as: 1) application equipment capable of producing desired droplet spectrum and application rate, 2) aircraft availability time frames (remember FAA requires 2-engine aircraft for applications over urban areas), and 3) the demonstrated ability to apply the chosen product to the target area in accordance with label requirements.
- Outline the desired capabilities and equipment within the RFP such as: 1) onboard real time weather systems, and 2) advanced onboard drift optimization and guidance software.
- Determine in advance whether the vector control agency or contractor will secure and provide pesticides. If the contractor will supply the pesticide, verify their knowledge of and ability to comply with regulations regarding the transport, use, and disposal of all pesticide and containers.
- Enter into a contingency contract with the commercial applicator.
- Consider acquiring non-owned, multiple engine aircraft insurance with urban application endorsement for added protection.
- Determine product and application rate to be used, along with a contingency plan. The product choice may be subject to change depending on product availability, the determination of resistance, labeling restrictions, environmental conditions, or other unforeseen factors.

Preparatory steps for ground-based applications

- Ensure that application equipment has been properly calibrated and tested for droplet size and flow rate. The vector control agency should have enough equipment, operators, and product available to finish the desired application(s) between sunset and midnight, or within 2-3 hours pre-sunrise (or when mosquitoes are demonstrated to be most active) to maximize efficacy.
- Ensure that vehicles are equipped with safety lighting and appropriate identifying signs; use sufficient personnel.
- Contact local law enforcement and provide them with locations to be treated and approximate time frames.

- Consider using lead and trailing vehicles particularly if the area has not been treated before and personnel are available.

Implementing an aerial application contract

- Contact commercial applicator and determine availability.
- Review long-term weather forecasts. Ideally applications should be scheduled during periods of mild winds to avoid last minute cancellations.

  Contractor should:

  - Contact Local Flight Standards District Office (FSDO) for low flying waiver.
  - Arrange for suitable airport facilities.
  - Contact local air traffic control.
  - Locate potential hazards prior to any application and implement a strategy to avoid those hazards during the application – often in darkness.
  - Provide equipment and personnel for mixing and loading of material (if previously agreed upon in contract).
  - Register with applicable County Agricultural Commissioner’s office.

  Vector control agency should:

  - Delineate treatment block in a GIS format and send to contractor.
  - Identify areas that must be avoided during an application and include detailed maps of those areas to contract applicators (e.g., open water, registered organic farms, any area excluded by product label).
  - Send authorization letter to FSDO authorizing contractor to fly on the agency's behalf; contractor should provide contact information and assistance.
  - Send map of application area and flight times / dates to local air traffic control; contractor should provide contact information and assistance.
  - Consult with County Agricultural Commissioner’s office. Commissioner's office can provide guidance on contacting registered beekeepers and help identify any registered organic farms that may need to be excluded from application.
  - If vector control agency is providing material, ensure adequate quantity to complete mission and that the agency has means to transport material.

Efficacy evaluation for aerial or ground based application

- Choose appropriate method(s) for evaluating efficacy of application
  - Determine changes in adult mosquito population via routine or enhanced surveillance.

    - Conduct three-day pre and post-trapping in all treatment and control areas.
    - Set out bioassay cages with wild caught and laboratory reared (susceptible) mosquitoes during application.
- Ensure adequate planning so surveillance staff are available and trained, equipment is available, and trap / bioassay cage test locations are selected prior to application.
- Ensure efficacy evaluation activities are timed appropriately with applications.
- Enlist an outside agency such as CDPH and/or university personnel to help evaluate efficacy of application as appropriate.

Actions at time of application

- Confirm application rate with contractor.
- Confirm treatment block.
- Coordinate efficacy evaluations.

Public notification

Notification to the public prior to a mosquito control pesticide application by a vector control agency signatory to a Cooperative Agreement with CDPH, or under contract for such agency is not a legal requirement in California (California Code of Regulations – Title 3: Food and Agriculture: Division 6. Pesticides and Pest Control Operations: Section 6620a). However, public notification of a pending adult mosquito control is recommended as early as possible prior to the treatment event.

Basic notification steps

- Provide notification of pending application as early as possible.
- Post clearly defined treatment block map online or through appropriate media outlet.
- Post product label and material safety data sheet (MSDS) online or through appropriate media outlet.
- Post and/or have available scientific publications regarding the efficacy of aerial or ground based applications (as appropriate), including effects on non-target organisms and risk-assessments.

Public relations considerations

- Ensure staffing is adequate to handle a significant increase in phone calls.
- Ensure website capability is adequate to handle a rapid increase in visitors.
- Train personnel answering phones to address calls from citizens concerned about personal and environmental pesticide exposure.

- Ensure adequate follow-through for calls related to sporting events, concerts, weddings, and other outdoor events that may be scheduled during the application and within the treatment block.

Appendix K: Websites Related to Arbovirus Surveillance in California

Website	URL	Available information
California West Nile Virus Website	https://westnile.ca.gov	Up to date information on the spread of West Nile virus throughout California, personal protection measures, online dead bird reporting, bird identification charts, mosquito control information and links, clinician information, local agency information, public education materials.
California Department of Public Health	https://www.cdph.ca.gov	Use search box to find information on mosquitoes, mosquito-borne diseases, or other vectors and diseases.
Davis Arbovirus Research and Training Laboratory at UC Davis	https://dart.ucdavis.edu	Information on mosquito and arbovirus surveillance in California and related research.
Mosquito and Vector Control Association of California	https://www.mvcac.org	News, membership information, event calendars, and other topics of interest to California's mosquito control agencies.
California Vector-Borne Disease Surveillance Maps	https://maps.vectorsurv.org	Maps showing locations of arbovirus activity and detections of invasive mosquitoes.
California Data Exchange Center	https://cdec.water.ca.gov	Water-related data from the California Department of Water Resources, including historical and current stream flow, snowpack, and precipitation information.
UC IPM Online	https://ipm.ucanr.edu/	Precipitation and temperature data for stations throughout California; also allows calculation of degree-days based on user-defined data and parameters.
National Weather Service – Climate Prediction Center	https://www.cpc.ncep.noaa.gov	Short-range (daily) to long-range (seasonal) temperature and precipitation forecasts. Also provides El Niño-related forecasts.

Website	URL	Available information
California Agricultural Statistics Service	https://www.nass.usda.gov/Statistics_by_State/California	Crop acreage, yield, and production estimates for past years and the current year's projections. Reports for particular crops are published at specific times during the year – see the calendar on the website.
State Water Resources Control Board	https://www.waterboards.ca.gov/water_issues/programs/npdes/pesticides/	National Pollutant Discharge Elimination System (NPDES) permit for vector control information.
US Environmental Protection Agency – Mosquito Control	https://epa.gov/mosquitocontrol	Describes the role of mosquito control agencies and products used for mosquito control.
US Centers for Disease Control and Prevention – West Nile Virus	https://www.cdc.gov/west-nile-virus/about/index.html	Information on the transmission of West Nile virus across the United States, viral ecology and background on WNV, and personal protection measures in various languages, nationwide statistics, maps, and data.
UC Davis Veterinary Medicine – Guide to Poultry Health	https://www.vetmed.ucdavis.edu/poultry-health	Information and instructional videos on chicken handling and bleeding techniques.

Appendix L: Reference List

Barker, C. M., W. K. Reisen, and V. L. Kramer. 2003. California State Mosquito-borne Virus Surveillance and Response Plan: A retrospective evaluation using conditional simulations. Am. J. Trop. Med. Hyg. 68: 508-518.

Bidlingmeyer, W.L. 1969. The use of logarithms in analyzing trap collections. Mosq. News 29:635-640.

Council of State and Territorial Epidemiologists (CSTE). Arboviral Diseases, Neuroinvasive and Non-neuroinvasive 2015 Case Definition. CSTE Position Statement 14-ID-04. Atlanta, CSTE, June 2014

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

Healy, J.M., W.K. Reisen, V.L. Kramer, M. Fischer, N. Lindsey, R.S. Nasci, P.A. Macedo, G. White, R. Takahashi, L. Khang, C.M. Barker. 2015. Comparison of the efficiency and cost of West Nile virus surveillance methods in California. Vector-Borne and Zoonotic Diseases 15:147-155.

Hepworth, G., Biggerstaff B.J. 2017. Bias Correction in Estimating Proportions by Pooled Testing. Journal of Agricultural, Biological and Environmental Statistics 22(4), 602-614.

Kramer, L.D., Presser S.B., Houk E.J., Hardy J.L. 1990. Effect of the anesthetizing agent triethylamine on western equine encephalomyelitis and St. Louis encephalitis viral titers in mosquitoes (Diptera:Culicidae). J. Med. Entomol. 27:1008-1010.

Loomis, E.C. and S.G. Hanks. 1959. Light trap indices of mosquito abundance: a comparison of operation for four and seven nights a week. Mosq. News 19:168-171.

Loomis,E.C., Sherman E.J. 1959. Comparison of artificial shelters and light traps for measurement of *Culex tarsalis* and *Anopheles freeborni* populations. Mosq. News 19:232-237.

Meyer, R. P., W. K. Reisen and Vector and Vector-borne Disease Committee. 2003. Integrated mosquito surveillance guidelines. Mosq. Vector. Contr. Assoc. Calif.

Meyer, R.P., W.K. Reisen, B.R. Hill, and V.M. Martinez. 1983. The “AFS sweeper”, a battery powered backpack mechanical aspirator for collecting adult mosquitoes. Mosq. News 43:346-350.

Mulhern. T.D. 1942. The New Jersey mechanical trap for mosquito surveys. NJ Ag. Exp. Sta. Circ. 421:1-8.

Newhouse. VF, Chamberlain RW, Johnston Jr JG, Sudia WD. 1966. Use of dry ice to increase mosquito catches of the CDC miniature light trap. Mosq. News 26:30-35.

Patiris. PJ, Oceguera LF, III, Peck GW, Chiles RE, Reisen WK, Hanson CV. 2008. Serologic diagnosis of West Nile and St. Louis encephalitis virus infections in domestic chickens. Am. J. Trop. Med. Hyg. 78:434-441.

Reeves, W. C., M. M. Milby and W. K. Reisen. 1990. Development of a statewide arbovirus surveillance program and models of vector populations and virus transmission. pp.: 431-458. *In:* W. C. Reeves, (ed.) Epidemiology and control of mosquito-borne arboviruses in California, 1983-1987 Sacramento, Calif. Calif. Mosq. Vector Control Assoc., Inc.

Reeves, W.C. 1990. Epidemiology and control of mosquito-borne arboviruses in California, 1943-1987. California Mosquito Vector Control Association, Sacramento.

Reisen, W. K., B. F. Eldridge, T. W. Scott, A. Gutierrez, R. Takahashi, K. Lorenzen, J. DeBenedictis, K. Boyce, and R. Swartzell. 2002**.** Comparison of dry ice-baited CDC and NJ light traps for measuring mosquito abundance. J. Am. Mosq. Control Assoc. 18: 158-163.

Reisen, W. K., R. P. Meyer, R. F. Cummings, and O. Delgado. 2000. Effects of trap design and CO2 presentation on the measurement of adult mosquito abundance using CDC style miniature light traps. J. Am. Mosq. Control Assoc. 16: 13-18.

Reisen, W.K. 1995. Guidelines for surveillance and control of arbovirus encephalitis in California. pp. 1-34 in: Interagency guidelines for the surveillance and control of selected vector-borne pathogens in California. California Mosquito Vector Control Association, Inc., Sacramento.

Sudia, W.D., Chamberlain R.W. 1962. Battery-operated light trap, an improved model. Mosq. News 22:126-129.

Taketa-Graham M., Powell Pereira J.L., Baylis E., Cossen C., Oceguera L., Patiris P., Chiles R., Hanson C.V., Forghani B. 2010. High throughput quantitative colorimetric microneutralization assay for the confirmation and differentiation of West Nile Virus and St. Louis encephalitis virus. Am. J. Trop. Med. Hyg. 82:501-504.

Walsh, J.D. 1987. California's mosquito-borne encephalitis virus surveillance and control program. California Department of Health Services, Sacramento.

Appendix M: Additional Resources

Barr, A.R., T.A. Smith, M.M. Boreham, and K.E. White. 1963. Evaluation of some factors affecting the efficiency of light traps in collecting mosquitoes. J. Econ. Entomol. 56:123-127.

Danforth, M.E., R.E. Snyder, E.T. Lonstrup, C.M. Barker, and V.L. Kramer. 2022. Evaluation of the effectiveness of the California Mosquito-Borne Virus Surveillance & Response Plan. PLOS Negl Trop Dis. 16(5): e0010375.

Eldridge, B.F. 2000. The epidemiology of arthropod-borne diseases. pp. 165-185 in B. F. Eldridge and J. Edman, Eds. Medical entomology: A textbook of public health and veterinary problems caused by arthropods. Kluwer Academic Publications. Dordrecht, the Netherlands.

Eldridge, B.F. 2000. Surveillance for arthropod-borne diseases. pp. 515-538 in B. F. Eldridge and J. Edman, Eds. Medical entomology: A textbook on public health and veterinary problems caused by arthropods. Kluwer Academic Publications. Dordrecht, Netherlands.

Eldridge, B.F. 1987. Strategies for surveillance, prevention, and control of arbovirus diseases in western North America. Am. J. Trop. Med. Hyg. 37:77S-86S.

Foss, L, Feiszli T., Kramer V.L., Reisen W.K., Padgett K. 2023. Epidemic versus endemic West Nile virus dead bird surveillance in California: Changes in sensitivity and focus. PLoS ONE 18(4): e0284039.

Foss, L, Reisen W.K., Fang Y., Kramer V., Padgett K. 2016. Evaluation of nucleic acid preservation cards for West Nile Virus testing in dead birds. PLoS ONE; 11(6): e0157555.

Hui, L.T., S.R. Husted, W.K. Reisen, C.M. Myers, M.S. Ascher, V.L. Kramer. 1999. Summary of reported St. Louis encephalitis and western equine encephalomyelitis virus activity in California from 1969-1997. Proc. Calif. Mosq. Vector Control Assoc. 67: 61-72.

Komar, N., S. Langevin, S. Hinten, N. Nemeth, E. Edwards, D. Hettler, B. Davis, R. Bowen, and M. Bunning. 2003. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. Emerg. Infect. Dis. 9: 311-322.

Meyer, R.P. 1996. Mosquito surveillance and sampling methods *in* The Biology and Control of Mosquitoes in California (S. Durso, Ed.). Calif. Mosq. and Vector Control Assoc., Inc. Sacramento

Mulhern, T.D. 1953. Better results with mosquito light traps through standardizing mechanical performance. Mosq. News 13:130-133.

Nemeth, N.M., Thomsen B.V., Spraker T.R., Benson J.M., Bosco-Lauth A.M., Oesterle P.T., et. al. 2011. Clinical and pathologic responses of American crows *(Corvus brachyrhynchos)* and fish crows *(C. ossifragus)* to experimental West Nile virus infection. Vet Pathol. 48(6): 1061–1074.

Padgett, K.A, W.K. Reisen, N. Kahl-Purcell, Y. Fang, B. Cahoon-Young, R. Carney, N. Anderson, L. Zucca, L. Woods, S. Husted, V.L. Kramer. 2007. West Nile virus infection in tree squirrels (Rodentia: Sciuridae) in California, 2004-2005. Am. J. Trop. Med. Hyg. 76: 810-813.

Pfuntner, A.P. 1979. A modified CO_2-baited miniature surveillance trap. Bull. Soc. Vector Ecol. 4:31-35.

Reeves, W.C. 2000. The threat of exotic arbovirus introductions into California. Proc. Calif. Mosq. Vector Control Assoc. 68: 9-10.

Reisen, W. K., H. D. Lothrop, R. E. Chiles, M. B. Madon, C. Cossen, L. Woods, S. Husted, V. L. Kramer, J. D. Edman. 2004. West Nile Virus in California. Emerg. Infect. Dis.8: 1369-1378.

Reisen, W.K., R.P. Meyer, S.B. Presser, and J.L. Hardy. 1993. Effect of temperature on the transmission of western equine encephalomyelitis and St. Louis encephalitis viruses by *Culex tarsalis* (Diptera: Culicidae). J. Med. Entomol. 30: 151-160.

Reiter, P. 1987. A revised version of the CDC gravid mosquito trap. J. Am. Mosq. Control Assoc. 3:325-327.

Reiter, P. 1983. A portable, battery-powered trap for collecting gravid Culex mosquitoes. Mosq. News 43:496-498.

Snyder, R.E., Cooksey G., Kramer V., Jain S., Vugia D.J. 2021. West Nile virus Associated Hospitalizations in California, 2004-2017. Clin Infect Dis. 73(3):441-447.

Snyder, R.; Feiszli T., Foss L., Messenger S., Fang Y., Barker C.M., Reisen W.K., Vugia D.J., Padgett K.A., Kramer V.L.. 2020. West Nile virus in California, 2003-2018: A persistent threat. PLoS Negl Trop Dis. 14(11): e0008841.

Theophilides, C. N., S. C. Ahearn, E. S. Binkowski, W. S. Paul, K. Gibbs. 2006. First evidence of West Nile virus amplification and relationship to human infections. Int. J. Geogr. Inf. Sci. 20:1:103-115.

Theophilides, C. N., S. C. Ahearn, S. Grady, M. Merlino. 2003. Identifying West Nile virus risk areas: The Dynamic Continuous-Area Space-Time System. Am. J. Epidemiol. 157:843-854.