Chikungunya: Vector Surveillance and Control in the United States

Division of Vector-Borne Diseases

CENTERS FOR DISEASE CONTROL AND PREVENTION

Table of Contents

-Overview
-Objectives
-Vector Surveillance and Control Recommendations
- ***-****Before mosquito season*
- *-Beginning of mosquito season*
- *-Single or several suspected/confirmed imported/locally acquired cases*
- *-Outbreak; clusters of suspected or confirmed cases*

-Specimen Collection and Types of Traps
- *-Ovitraps*
- *-Adult mosquito trapping*

-Mosquito-Based Surveillance Indicators
- *-Immature stage survey indices*
- *-Eggs per ovitrap per week*
- *-Female adults per sticky trap per week*
- *-Adult infection rates*

-Handling of field-collected adult mosquitoes
-Limitations to mosquito-based surveillance
-Vector control
- ***-****Control of immature stages*
- *-Control of adult mosquitoes*

-References

Overview

The prevention or reduction of chikungunya virus (CHIKV) transmission is completely dependent on the control of mosquito vectors and limiting human-vector contact. Several different mosquito species are involved in the transmission of CHIKV in Africa (Jupp and McIntosh 1988) and in the islands of the western Pacific Ocean (Savage et al. 2015). However, the principal epidemic vectors for CHIKV worldwide are *Ae. aegypti* (Jupp and McIntosh 1988) and *Ae. albopictus* (Hawley 1988) and the CDC recommendations for the surveillance and control of CHIKV in the United States specifically target these two species.

Mosquito-based surveillance is an integral component of an integrated vector management program. Mosquito-based surveillance for CHIKV is very similar to that of DENV; determining vector presence and estimating abundance are the primary tools for quantifying human risk. The principal functions of a mosquito-based CHIKV surveillance program are to:

- Determine presence or absence of *Ae. aegypti* and *Ae. albopictus* in the geographic area
- Identify what types of containers are producing mosquitoes and their relative importance
- Develop detailed maps for larval sites if *Ae. aegypti* or *Ae. albopictus* are detected in an area
- Collect mosquito population data and identify geographic areas of high abundance (high-risk)
- Monitor the effectiveness of vector control efforts
- Collect data on mosquito infection rates during outbreaks to incriminate vectors

CHIKV transmission ecology varies regionally, and surveillance practices vary among programs (e.g., number and type of traps, frequency of sampling etc.). Developing useful thresholds requires consistent effort to assure the surveillance indices and their association to human risk is comparable over time, and requires mosquito surveillance and human disease incidence data from several transmission seasons to produce useful predictive indicators.

Detection of CHIK cases precedes detection of CHIKV in mosquito vectors therefore vector control specialists depend on healthcare providers and local health department's reports to track CHIKV activity. Timely response to CHIKV activity requires constant communication between public health departments, healthcare providers and vector control specialists. Effective vector-based CHIK prevention involves initiating control measures such as source reduction and larvicide treatments before the beginning of the mosquito season, and adult reduction measures such as adulticide treatments following detection of CHIKV activity. Containment, a combination of procedures to prevent CHIKV from spreading, may be initiated whenever a suspected/confirmed imported or locally acquired case is detected. During outbreaks a combination of containment and large-scale vector control may be used to minimize vector-human contact.

Objectives

The primary objective of this document is to provide guidance for mosquito-based surveillance, prevention and response to CHIKV in the United States. This document is intended for state and local public health officials and vector control specialists.

Vector Surveillance and Control Recommendations

Before mosquito season

Response:

- Conduct education campaigns focusing on reducing or eliminating larval habitats for the mosquito vectors *Ae. aegypti* and *Ae. albopictus*
- Conduct surveys to determine abundance, distribution and type of containers; large numbers of containers may translate into high mosquito abundance and high risk
- Initiate source reduction – remove any water holding containers
- Cover or modify immovable and large containers to prevent water accumulation
- Treat containers that can't be covered, moved, or modified with long lasting larvicide

Beginning of mosquito season

Response:

- Continue education campaigns focusing on reducing or eliminating larval habitats for the mosquito vectors *Ae. aegypti* and *Ae. albopictus*
- Distribute information about chikungunya and about personal protection measures
- Initiate CHIKV vectors surveys to:
    - determine presence or absence of *Ae. aegypti* and/or *Ae. albopictus*
    - estimate relative abundance of the vectors
    - determine distribution
    - develop detailed vector distribution maps
    - evaluate the efficacy of source reduction and larvicide treatments
- Continue/maintain source reduction efforts; containers are continually discarded
- Initiate adult sampling to determine or confirm areas of high adult mosquito abundance
- Initiate preventive adult control to reduce adult populations targeting areas of high mosquito abundance
- Concentrate control efforts around places with high mosquito density

Single or several suspected/confirmed imported/locally acquired cases

Response:

- Containment education campaigns aimed at preventing or minimizing contact between vectors and suspected or confirmed human cases
- Inform the public of the risk and persuade them to use repellents, use window screens, use air conditioning, prevent mosquitoes from entering houses, kill mosquitoes found indoors
- Initiate containment to minimize the spread of CHIKV into the neighborhood to include preventive adult control 100–200m around the imported case's home:
    - Outdoor residual and spatial insecticide treatments, repeated as necessary to reduce vector abundance
    - Initiate/maintain adult sampling to estimate adult mosquito abundance and evaluate effectiveness of insecticide treatments
- Maintain source reduction efforts:
    - facilitate community pickup of discarded containers
    - apply residual insecticide treatments to immovable containers

Outbreak; clusters of suspected or confirmed cases

Response:

- Divide the outbreak area into operational control areas where control measures can be effectively applied to all buildings and public areas within a few days and repeated as needed to reduce mosquito density
- Conduct door-to-door inspections and mosquito control in an area-wide fashion (reach >90% coverage of the control area within a week)
- Identify and treat or remove containers producing mosquitoes
- Organize clean-up campaigns targeting disposable containers (source reduction), including large junk objects that accumulate water (broken washing machines, refrigerators, toilets) in buildings, public areas, etc.
- Combine outdoor spatial or residual spraying with source reduction and larviciding (including residual spraying of container surfaces and adjacent mosquito resting areas)

Specimen Collection and Types of Traps

Ovitraps: Ovitraps are small metal, glass or plastic containers, usually of a dark color, containing water and a substrate (wood, seed germination paper, cloth, plant gel) where female mosquitoes lay their eggs. Ovitraps can be used to detect the presence of gravid *Ae. aegypti*, *Ae. albopictus* and a wide variety other gravid females in the genus *Aedes* (Fay et al. 1966, Reiter et al. 1991, Mackay et al. 2013). Ovitraps take advantage of the fact that gravid *Ae. aegypti*, *Ae. albopictus* and many other *Aedes* females lay their eggs in artificial containers. Adequate sampling requires regular (weekly) trapping at fixed sites throughout the community that are representative of the habitat types present in the area. However, ovitraps should not be deployed in the field for more than a week at a time because they could become larval sites and begin producing adult mosquitoes. However, some ovitraps are specifically designed not to produce mosquitoes (Chan et al. 1977; Barrera et al. 2013).

Ovitraps have the advantages of being inexpensive, easily deployed, and not invasive (they can be placed outside of houses, not requiring entry homes). A small number of ovitraps is usually enough to determine vector presence; less than 100 ovitraps can reliably estimate abundance in a large urban neighborhood (Mogi et al. 1990). Typically, one ovitrap is placed per city block. Lastly, ovitrap data is easy to analyze; it is usually expressed as the percentage of positive ovitraps (ovitraps with eggs). The mean number of eggs per ovitrap can be used to estimate adult mosquito abundance.

Interpreting ovitrap data may sometimes need caution, because ovitraps compete with naturally occurring larval habitats and the estimates from oviposition surveys may not accurately reflect the abundance of gravid females under some conditions. For example, oviposition indices may be skewed after source reduction campaigns when gravid females find fewer suitable habitats and lay large proportions of eggs in the ovitraps (Focks 2003) confounding the evaluation of control efforts. Some degree of training in microscopy may be needed for accurate egg counting especially when there is debris on the oviposition surfaces. Lastly, the collected eggs need to be hatched and reared out in the laboratory and the larvae or adults identified to species, which may require trained personnel.

Immature stage (larvae and pupae) surveys: Because of a wide variety in type, size and shapes of the containers, there is no standard equipment for sampling the immature stages of container breeding mosquitoes. If the container is large enough such as a 55 gallon barrel a dipper or net may be used. However, the common containers are small cans, tires etc., and usually the entire contents are emptied onto a tray or a pan and the immature stages picked out using a dropper. The immature stages are usually reared out in the lab and identified to species.

Adult mosquito trapping: *Aedes aegypti* and *Ae. albopictus* are not efficiently captured by the most commonly used mosquito traps, such as the CDC miniature light trap, CDC gravid trap, or the New Jersey light traps. There

are several fan-operated traps designed to capture *Ae. aegypti* adults, which take advantage of the propensity of this species to be attracted to dark objects (Fay 1968, Fay and Prince 1970, Wilton and Kloter 1985, Freier and Francy 1991). The Fay-Prince trap has been the most widely used but it is heavy and bulky, making it difficult to use in sufficient numbers to obtain reliable estimates of mosquito abundance. Currently the most commonly used adult traps for *Ae. aegypti* and *Ae. albopictus* are BG Sentinel Traps, and a variety of gravid traps such the CDC-Autocidal Gravid Ovitrap (CDC-AGO) (Mackay et al. 2013, Barrera et al. 2014).

The BG Sentinel Trap: The BG Sentinel Traps use a combination of attractive visual cues and creation of convection currents that mimic those created by a human body. They have the advantage of being collapsible and light. BG-Sentinel traps are more effective capturing *Ae. aegypti* than CDC backpack aspirators, and also collect adult females in all physiological states (Maciel-de-Freitas et al. 2006, Williams et al. 2006, Ball and Ritchie 2010). These traps are also effective for collecting *Ae. albopictus* (Meeraus et al. 2008, Bhalala and Arias 2009, Farajollahi et al. 2009, Obenauer et al. 2010). The efficiency of BG traps can be increased by baiting them with lures (e.g., CO_2, BG-Lure®).

Gravid female traps: There are a number of recently developed traps that use similar principles of attraction to ovitraps; attract and capture gravid females. These traps either use funnels (Gomes et al. 2007, Eiras et al. 2014) or sticky boards (Mackay et al. 2013, Chadee et al. 2010, Barrera et al. 2014) to prevent captured mosquitoes from escaping. The advantage of gravid traps is that they are considerably cheaper and easier to operate.

Mechanical aspirators: Several aspirator devices may be used to collect resting mosquitoes for CHIKV monitoring. Collecting resting mosquitoes provides a good representation of vector population structure since un-fed, gravid and blood-fed females (as well as males) may be collected (Service 1992). Since resting populations typically provide samples that are representative of the population they will also provide more representative CHIKV infection rates. Handheld or backpack mechanical aspirators can be used to remove mosquitoes from natural resting harborage or artificial resting structures (e.g., wooden resting boxes, red boxes, fiber pots and other similar containers) (Service 1992). Aspirators are particularly useful for collecting *Ae. aegypti* indoors and the data obtained from this collecting technique provide more representative mosquito abundance per unit area (e.g., per house, master bedroom, etc.). Sampling indoors can be standardized such as aspirating for 15 minutes per house, etc., but frequently there are large variations in number of mosquitoes collected per house, therefore, this technique requires sampling large numbers of houses in short periods of time. (e.g., 200 houses per neighborhood). Due to the wide variety of resting sites and the low density of resting mosquitoes in most locations, sampling resting populations especially outdoors is difficult to standardize, labor intensive, require trained personnel and sufficient sample sizes are often difficult to obtain.

Landing –biting counts: This is one of the oldest techniques used to capture host-seeking daytime biting mosquito species such as *Ae. aegytpi* and *Ae. albopictus.* This is a highly effective technique for detecting and quantifying dengue and CHIKV vectors. The CDC does not recommend this technique especially in areas with ongoing arbovirus transmission due to health risks to the field staff. Other limitations include variations among collectors both in attracting and collecting specimens and it is labor intensive. A tent trap has been recently developed which protects collectors from mosquito bites (Casas-Martinez et al. 2013).

Mosquito-Based Surveillance Indicators

Similar to dengue surveillance, data derived from mosquito surveillance primarily estimates mosquito abundance and the estimates are used to indicate levels of risk. The indices derived from those data vary in information content, ability to be compared over time and space, and association with CHIKV transmission levels and levels of human risk. The indicators that have commonly been used can be broadly divided into 1) immature stage (larvae and pupae) survey indices, 2) Eggs per ovitrap per week, 3) Female mosquitoes per sticky gravid trap per week, and 4) adult infection rates (IR).

Immature stage survey indices

Larval surveys (*Stegomyia* indices): Larval surveys usually involve identifying all or most of the immature mosquitoes found in every container (or a representative sample of containers) in the target area, home(s) community, neighborhood etc. Every water-holding container is inspected and categorized as positive (contains larvae) or negative otherwise (no larvae). The second and less used method is single-larva surveys (Sheppard 1969) where only a single larva is identified from each container. The container indices below are computed form survey data.

- House Index (HI; percentage of houses with at least one positive container)
- Container Index (CI; percentage of all containers with water that are larva positive), and
- Breteau Index (BI; number of positive containers per 100 houses; (Connor et al. 1923, WHO 2009)).

CHIKV infection thresholds (larval abundance indices) have to be determined by each local vector control program for each location; state or national wide thresholds should be used with caution. It was proposed that a House Index of < 5% (Soper 1967), a Container Index of <10% (Connor et al. 1923), or a Breteau Index of <5 (Brown 1977) were protective against yellow fever, and that HI of <1% suppressed dengue transmission (Pontes et al. 2000). Such thresholds may not apply to all locations and to all arboviruses.

Pupal surveys: Pupal surveys are based on the assumption that pupal productivity is a better estimate of the adult population than the traditional indices (HI, CI, and BI) or larval counts (Focks 2003). Pupal surveys can also identify the types of containers that produce the majority of adult mosquitoes and these are targeted for enhanced surveillance and control (Focks and Chadee 1997, Nathan and Focks 2006). Pupal surveys usually involve sampling large numbers of houses and containers to obtain reliable estimates (Reuben et al. 1978, Barrera et al. 2006). However, several methods have been developed to guide sample size requirements for pupal surveys (Alexander et al. 2006, Barrera et al. 2006, Barrera et al. 2009). Commonly used pupal survey indices are:

- Pupal House Index (PHI; percentage of houses with at least one pupa positive container)
- Pupal Container Index (PCI; percentage of all containers with water that are pupa positive), and
- Pupal Breteau Index (PBI; number of pupa positive containers per 100 houses; (Connor et al. 1923, WHO 2009).

As in the case of larval surveys, pupal surveys for CHIKV transmission thresholds (pupal abundance indices) have to be determined by each local vector control program for each location. Currently there is no information on pupal indices and CHIKV transmission however some models show that it takes between 0.5 and 1.5 *Ae. aegypti* pupae per person to sustain dengue virus transmission at 28˚C in a human population with 0 – 67% immunity (Focks et al. 2000). Since CHIKV-specific data is lacking, this may provide a starting point for evaluating CHIKV risk.

Eggs per ovitrap per week. Although no specific threshold values have been established for CHIKV, absence of dengue hemorrhagic fever cases in Thailand was noted when the densities of eggs of *Ae. aegypti* per ovitrap per week was less than two (Mogi et al. 1990). Also, although using a different ovitrap, DENV transmission occurred in Taiwan when the density of eggs per house (2 ovitraps/house) was around two (Wu et al. 2013).

Female adults per sticky trap per week. Sticky gravid used for *Ae. aegypti* surveillance during a dengue outbreak in Australia, indicated that a density of two or more females per trap per week was associated with dengue cases, whereas a density of less than one female per trap per week was a safe level (Ritchie et al. 2004). *Aedes*

aegypti densities associated with confirmed human CHIK cases and presence of the virus in mosquitoes in Puerto Rico varied between 0.9 and 1.9 female per trap per day using the CDC AGO trap. In this study, absence of CHIKV is the mosquito at the location of a confirmed human case was 0.7 females per trap per day (CDC, unpublished).

Adult infection rates

In the past, *Aedes aegypti* and *Ae. albopictus* surveillance has relied heavily on immature indices because until recently it has been difficult to monitor adult mosquito abundance. However, currently the BG Sentinel Trap and a variety of gravid traps make it possible to accurately estimate adult mosquito abundance and to track infected mosquitoes.

Tracking adult infected mosquitoes may help establish entomological infection rate thresholds for CHIKV transmission similar to WNV, SLEV, EEEV etc. The infection indices used are the same as those used for other arboviruses, Minimum Infection Rate (MIR), Maximum Likelihood Estimates of the Infection Rate (MLE), and Vector Index (VI) (CDC 2013). However, adult mosquito infection rates cannot be used to predict outbreaks in CHIKV surveillance programs because of the very limited data on CHIKV infection rates and prevalence of human infections. Data obtained in DENV surveillance programs show that in some cases elevation in mosquito infection rates precede outbreaks or increased transmission (Chow et al. 1998, Mendez et al 2006) but not in others (Chen et al. 2010). The mixed results make it difficult to establish threshold mosquito infection rates for human infections and outbreaks for DENV. However, these studies used different mosquito collection methods and there is a chance data obtained from BG and gravid traps may improve abundance and infection rate estimates, and provide timely risk assessment.

Handling of field-collected adult mosquitoes

Since virologic surveillance relies on identifying CHIKV in the collected mosquitoes through detection of viral proteins, viral RNA, or live virus, efforts should be made to handle and process the specimens in a way that minimizes exposure to conditions (e.g., heat, successive freeze-thaw cycles) that would degrade the virus.

- Optimally, a cold chain should be maintained from the time mosquitoes are removed from the traps to the time they are delivered to the processing laboratory, and through any short-term storage and processing.
- Transport mosquitoes from the field in a cooler either with cold packs or on dry ice. Sort and identify the mosquitoes to species on a chill-table if available.
- If CHIKV screening is not done immediately after mosquito identification and pooling, the pooled samples should be stored frozen, optimally at -70◦C, but temperatures below freezing may suffice for short-term storage.

Mosquitoes are generally tested in pools of 50 and only female mosquitoes should are tested in routine CHIKV surveillance programs. CHIKV can be detected in mosquito pools by using RT-PCR assays (Lanciotti et al. 2007, Laurent et al. 2007, Ummul Haninah et al. 2010, Savage et al. 2015) or by isolating the virus in cell culture.

Limitations to mosquito-based surveillance:

- Currently available information adult infection rates and larval/pupal indices may not predict risk for human infection.
- Larval/pupal surveys may miss cryptic habitats and fail to provide accurate data on the relative abundance of the vector species.
- Larval/pupal indices may not be correlated to adult mosquito abundance.

- Adult sampling has traditionally relied on aspirator collections which are labor intensive, require well trained personnel, and are subjective (inconsistent).
- Developing useful thresholds requires consistent effort to assure the surveillance indices and their association to human risk is comparable over time, and requires mosquito surveillance and human disease incidence data from several transmission seasons to produce useful predictive indicators.

Vector control

Control of immature stages

An important step in CHIKV control operations is identifying the types and abundance of containers producing mosquitoes and their productivity. Different containers require specific control measures that depend on the nature of the container and how it is used. There are five general types of containers producing CHIKV vectors:

- Phytotelmata (eg. treeholes, leaf axils etc.)
- Non-essential or disposable containers (food and drink containers, tires, broken appliances).
- Useful containers (water-storage vessels, potted plants and trivets, animal drinking pans, paint trays, toys, pails, septic tanks)
- Cavities in structures (fence poles, bricks, uneven floors and roofs, roof gutters, air-conditioner trays)
- Outdoor underground structures (storms drains, water meters, public wells, septic tanks)

Commonly used control methods

Environmental sanitation: This is the permanent elimination of containers producing CHIKV vectors such as establishing reliable supplies of piped water, municipal refuse recycling programs (glass, metal, and plastic), used-tire recycling operations replacing septic tanks with sewerage, etc.

Larvicides: This is the use of chemicals and/or biological agents to kill or prevent development of mosquito immature stages. There are a number of agents that can be used to control mosquito production in containers:

- Chemical larvicides
- Biological larvicides: These include products containing *Bacillus thuringiensis* var. *israelensis* (B.t.i.), Insect Growth Regulators (IGR's), juvenile hormone analogs (Methoprene, Pyriproxyfen), Chitin synthesis inhibitors (Diflubenzuron, Novaluron) and Spinosad. Biological larvicides have little or no impact on non-target organisms and do not accumulate in the environment.
- Monomolecular films and Oils. These products spread on the water surface forming a thin film that causes suffocation of immature mosquitoes by preventing gas exchange.

Evaluation of the effectiveness of pre-adult mosquito control may be accomplished by comparing the presence / absence and abundance of immature stages in treated containers before and after treatment or by comparing treated and untreated areas (Chadee 2009).

Biological control: A variety of aquatic predators may be used especially in large containers. These include carnivorus copepods and larvivorous fish (*Gambusia affinis*). However, biological control may not be practical especially since most CHIKV vectors develop in small containers.

Control of adult mosquitoes

Chemical control: Chemical control of adult mosquitoes includes space spraying, residual spraying, barrier spraying, and using attractive toxic baits.

- Barrier spraying of residual insecticides on external walls of houses and vegetation has been effectively used to reduce exposure to exophilic mosquito species (Anderson et al. 1991, Perich et al. 1993, Cilek 2008), including *Ae. albopictus* (Trout et al. 2007).
- Residual insecticides are used on surfaces that adult mosquitoes frequently visit and land on, such as walls ceilings, discarded containers, vegetation, curtains, covers for water-storage vessels, lethal ovitraps oviposition strips etc. There is evidence that indoor residual spraying (IRS) is particularly effective for controlling *Ae. aegypti* (Chadee 1990) primarily due to its indoor resting behavior. However, there are concerns about continuous insecticide exposure for the residents and currently, no residual insecticides are registered in the US for indoor control of adult mosquitoes.
- Space spraying of insecticides is carried out by backpack, truck- or air-craft mounted equipment.
- Attractive toxic sugar baits have been shown to reduce adult populations of *Ae. albopictus* in Florida (Naranjo et al. 2013, Revay et al. 2014). Eugenol (a component of clove oil) and boric acid have been tested as toxicants in these studies. It is not clear whether these baits would work against *Ae. aegypti* in tropical urban areas because it has been reported that females of this species do not commonly consume sugars (Costero et al 1998).

Using insecticide to control adult mosquitoes should always include insecticide resistance monitoring and management. Insecticide resistance has been demonstrated in almost every class of insecticide, including microbial pesticides and IGRs (Brogdon and McAllister 1998). Insecticide resistance usually leads to significant reduction in the susceptibility of insect populations which renders insecticide treatments ineffective and it is inheritable. Insecticide resistance may be monitored using bioassays in larvae and adult mosquitoes (WHO 2009, Brogdon and McAllister 1998b) (http://www.cdc.gov/malaria/resources/pdf/fsp/ir_manual/ir_cdc_bioassay_en.pdf).

Physical control (non-insecticidal mosquito traps): Gravid females mosquitoes can be lured to traps baited with an oviposition medium and captured using sticky glue while attempting to lay eggs (CDC autocidal gravid ovitrap; AGO trap;(Mackay et al. 2013, Barrera et al. 2014).

Repellents: CDC recommends the use of products containing active ingredients which have been registered by the U.S. Environmental Protection Agency (EPA) for use as repellents applied to skin and clothing. EPA registration of repellent active ingredients indicates the materials have been reviewed and approved for efficacy and human safety when applied according to the instructions on the label. For more details go to http://www.cdc.gov/westnile/faq/repellent.html

References

Alexander N, Lenhart AE, Romero-Vivas CME, Barbazan P, Morrison AC, Barrera R, et al. Sample sizes for identifying the key types of container occupied by dengue-vector pupae: the use of entropy in analyses of compositional data. Annals of Tropical Medicine and Parasitology. 2006;100:S5-S16.

Anderson AL, Apperson CS, Knake R. Effectiveness of mist-blower applications of malathion and permethrin to foliage as barrier sprays for salt marsh mosquitoes. J Am Mosq Control Assoc. 1991 Mar;7(1):116-7.

Ball TS, Ritchie SR. Evaluation of BG-sentinel trap trapping efficacy for *Aedes aegypti* (Diptera: Culicidae) in a visually competitive environment. J Med Entomol. 2010 Jul;47(4):657-63.

Barrera R, Amador M, Clark GG. Use of the pupal survey technique for measuring *Aedes aegypti* (Diptera: Culicidae) productivity in Puerto Rico. The American journal of tropical medicine and hygiene. 2006 Feb;74(2):290-302.

Barrera R, Amador M, Clark GG. Sample-size requirements for developing strategies, based on the pupal/demographic survey, for the targeted control of dengue. Annals of tropical medicine and parasitology. 2006 Apr;100 Suppl 1:S33-S43.

Barrera R. Simplified pupal surveys of *Aedes aegypti* (L.) for entomologic surveillance and dengue control. The American journal of tropical medicine and hygiene. 2009 Jul;81(1):100-7.

Barrera R, Amador M, Acevedo V, Caban B, Felix G, Mackay A. Use of the CDC Autocidal Gravid Ovitrap to Control and Prevent Outbreaks of<I>Aedes aegypti</I>(Diptera: Culicidae). Journal of medical entomology. 2014;51(1):145-54.

Barrera R, Mackay AJ, Amador M. A novel autocidal ovitrap for the surveillance and control of *Aedes aegypti*. Journal of the American Mosquito Control Association. 2013; 29: 293-296.

Bhalala H, Arias JR. The Zumba mosquito trap and BG-Sentinel trap: novel surveillance tools for host-seeking mosquitoes. J Am Mosq Control Assoc. 2009 Jun;25(2):134-9.

Brogdon WG, McAllister JC. Simplification of adult mosquito bioassays through use of time-mortality determinations in glass bottles. J Am Mosq Control Assoc. 1998 Jun;14(2):159-64.

Brown AWA. World wide surveillance of Aedes aegypti. Academic Press; 1977. p. 271-316.

Casas-Martinez M, Orozco-Bonilla A, Munoz-Reyes M, Ulloa-Garcia A, Bond JG, Valle-Mora J, et al. A new tent trap for monitoring the daily activity of *Aedes aegypti* and *Aedes albopictus*. Journal of Vector Ecology. 2013 Dec;38(2):277-88.

CDC. 2013. West Nile Virus in the United States: Guidelines for Surveillance, Prevention, and Control. 2013. 4th Edition. http://www.cdc.gov/westnile/resources/pdfs/wnvGuidelines.pdf

Chadee DD. Methods for evaluating Aedes aegypti populations and insecticide treatment in a town of Trinidad, West Indies. Bol Oficina Sanit Panam. 1990 Oct;109(4):350-9.

Chadee DD. Impact of pre-seasonal focal treatment on population densities of the mosquito *Aedes aegypti* in Trinidad, West Indies: A preliminary study. Acta Tropica. 2009;109(3):236-40.

Chadee DD, Ritchie SA. Oviposition behaviour and parity rates of Aedes aegypti collected in sticky traps in Trinidad, West Indies. Acta Tropica. [Article]. 2010 Dec;116(3):212-6.

Chan KL, No SK, Tan KK. An autocidal ovitrap for the control and possible eradication of *Aedes aegypti.* Southeast Asian J Trop Med Pub Hlth. 1977; 8(1): 56-62.

Chen CF, Shu PY, Teng HJ, Su CL, Wu JW, Wang JH, et al. Screening of dengue virus in field-caught *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) by one-step SYBR green-based reverse transcriptase-polymerase chain reaction assay during 2004-2007 in Southern Taiwan. Vector borne and zoonotic diseases (Larchmont, NY). 2010 Dec;10(10):1017-25.

Chow VTK, Chan YC, Yong R, Lee KM, Lim LK, Chung YK, et al. Monitoring of dengue viruses in field-caught *Aedes aegypti* and *Aedes albopictus* mosquitoes by a type-specific polymerase chain reaction and cycle sequencing. American Journal of Tropical Medicine and Hygiene. 1998;58(5):578-86.

Cilek JE. Application of insecticides to vegetation as barriers against host-seeking mosquitoes. J Am Mosq Control Assoc. 2008 Mar;24(1):172-6.

Connor ME, Monroe WM. Stegomyia indices and their value in yellow fever control. American Journal of Tropical Medicine and Hygiene. 1923;3:9-19.

Costero A, Attardo GM, Scott TW, Edman JD. An experimental study on the detection of fructose in *Aedes aegypti*. J Am Mosq Control Assoc. 1998 Sep;14(3):234-42.

Eiras AE, Buhagiar TS, Ritchie SA. Development of the gravid *Aedes* trap for the capture of adult female container-exploiting mosquitoes (Diptera: Culicidae). J Med Entomol. 2014 Jan;51(1):200-9.

Farajollahi A, Kesavaraju B, Price DC, Williams GM, Healy SP, Gaugler R, et al. Field efficacy of BG-Sentinel and industry-standard traps for Aedes albopictus (Diptera: Culicidae) and West Nile virus surveillance. Journal of Medical Entomology. 2009 July;46(4):919-25.

Fay RW, Eliason DA. A preferred oviposition site as a surveillance method for *Aedes aegypti*. [not specified]. Mosquito News. [Journal article]. 1966;26(4):531-5.

Fay RW. A trap based on visual responses of adult mosquitoes. Mosquito News. 1968;28:1-7.

Fay RW, Prince WH. A modified visual trap for *Aedes aegypti*. [not specified]. Mosquito News. [Journal article]. 1970;30(1):20-3.

Focks DA, Chadee DD. Pupal survey: An epidemiologically significant surveillance method for *Aedes aegypti*: An example using data from Trinidad. American Journal of Tropical Medicine and Hygiene. [Article]. 1997 Feb;56(2):159-67.

Focks DA. A Review of Entomological Sampling Methods and Indicators for Dengue Vectors. Geneva, Switzerland2003.

Freier JE, Francy DB. A duplex cone trap for the collection of adult *Aedes albopictus*. Journal of the American Mosquito Control Association. [Journal article]. 1991;7(1):73-9.

Gomes ADC, Da Silva NN, Bernal RTI, Leandro ADS, De Camargo NJ, Da Silva AM, et al. Specificity of the Adultrap for capturing females of *Aedes aegypti* (Diptera: Culicidae). [Portuguese]. Revista da Sociedade Brasileira de Medicina Tropical. 2007 Mar;40(2):216-9.

Hawley WA. The biology of *Aedes albopictus*. Journal of the American Mosquito Control Association. 1988; Suppl 1: 1-40.

Jupp PG, McIntosh BM. Chikungunya virus disease. In: Monath TP , editor. The arboviruses: epidemiology and ecology vol. II. Boca Raton, FL: CRC Press; 1988. p. 137-157.

Lanciotti RS, Kosoy OL, Laven JJ, Panella AJ, Velez JO, Lambert AJ, Campbell GL, 2007. Chikungunya virus in US travelers returning from India, 2006. Emerg Infect Dis 13: 764–767.

Laurent P, Le Roux K, Grivard P, Bertil G, Naze F, Picard M, Staikowsky F, Barau G, Schuffenecker I, Michault A. Development of a sensitive real-time reverse transcriptase PCR assay with an internal control to detect and quantify chikungunya virus. Clin Chem. 2007 Aug;53(8):1408-14.

Maciel-de-Freitas R, Eiras AE, Lourenco-de-Oliveira R. Field evaluation of effectiveness of the BG-Sentinel, a new trap for capturing adult *Aedes aegypti* (Diptera: Culicidae). Mem Inst Oswaldo Cruz. 2006 May;101(3):321-5.

Mackay A, Amador M, Barrera R. An improved autocidal gravid ovitrap for the control and surveillance of *Aedes aegypti*. Parasites & vectors. 2013;6(1):225.

Meeraus WH, Armistead JS, Arias JR. Field comparison of novel and gold standard traps for collecting *Aedes albopictus* in Northern Virginia. J Am Mosq Control Assoc. 2008 Jun;24(2):244-8.

Mendez F, Barreto M, Arias JF, Rengifo G, Munoz J, Burbano ME, et al. Human and mosquito infections by dengue viruses during and after epidemics in a dengue-endemic region of Colombia. American Journal of Tropical Medicine and Hygiene. 2006;74(4):678-83.

Mogi M, Choochote W, Khamboonruang C, Suwanpanit P. Applicability of presence-absence and sequential sampling for ovitrap surveillance of *Aedes* (Diptera: Culicidae) in Chiang Mai, Northern Thailand. Journal of Medical Entomology. 1990;27:509-14.

Naranjo D, Qualls W, Mueller G, Samson D, Roque D, Mueller G, et al. Evaluation of boric acid sugar baits against *Aedes albopictus* (Diptera: Culicidae) in tropical environments. Parasitology research. 2013;112(4):1583-7.

Nathan MB, Focks DA. Pupal/demographic surveys to inform dengue-vector control. Annals of Tropical Medicine and Parasitology. 2006;100:S1-S3.

Perich MJ, Tidwell MA, Dobson SE, Sardelis MR, Zaglul A, Williams DC. Barrier spraying to control the malaria vector Anopheles albimanus: laboratory and field evaluation in the Dominican Republic. Med Vet Entomol. 1993 Oct;7(4):363-8.

Pontes RJS, Freeman J, Oliveira-Lima JW, Hodgson JC, Spielman A. Vector densities that potentiate Dengue outbreaks in a Brazilian city. American Journal of Tropical Medicine and Hygiene. 2000;62(3):378-83.

Reiter P, Amador MA, Colon N. Enhancement of the CDC ovitrap with hay infusions for daily monitoring of *Aedes aegypti* populations. Journal of the American Mosquito Control Association. [Journal article]. 1991;7(1):52-5.

Reuben R, Das PK, Samuel GD, Brooks GD. Estimation of daily emergence of *Aedes aegypti* (Diptera: Culicidae) in Sonepat, India. Journal of Medical Entomology. 1978;14:705-14.

Revay E, Mueller G, Qualls W, Kline D, Naranjo D, Müller G, et al. Control of *Aedes albopictus* with attractive toxic sugar baits (ATSB) and potential impact on non-target organisms in St. Augustine, Florida. Parasitology research. 2014;113(1):73-9.

Ritchie SA, Long S, Smith G, Pyke A, Knox TB. Entomological investigations in a focus of dengue transmission in Cairns, Queensland, Australia, by using the sticky ovitraps. J Med Entomol. 2004 Jan;41(1):1-4.

Savage HM, Ledermann JP, Yug L, Burkhalter KL, Marfel M, Hancock WT. Incrimination of *Aedes* (*Stegomyia*) *hensilli* Farner as an Epidemic Vector of Chikungunya Virus on Yap Island, Federated States of Micronesia, 2013. Am J Trop Med Hyg. 2015 Feb 4;92(2):429-36.

Service MW. Importance of ecology in *Aedes aegypti* control. Southeast Asian J Trop Med Public Health. 1992 Dec;23(4):681-90.

Sheppard PM, Macdonald WW, Tonn RJ. A new method of measuring the relative prevalence of *Aedes aegypti*. Bulletin of the World Health Organization (WHO). 1969;40(3):467-8.

Soper FL. *Aedes aegypti* and yellow fever. Bull World Health Organ. 1967;36(4):521-7.

Staples JE, Breiman RF, Powers AM. Chikungunya fever: an epidemiological review of a re-emerging infectious disease. Clin Infect Dis. 2009 Sep 15;49(6):942-8.

Trout RT, Brown GC, Potter MF, Hubbard JL. Efficacy of two pyrethroid insecticides applied as barrier treatments for managing mosquito (Diptera: Culicidae) populations in suburban residential properties. J Med Entomol. 2007 May;44(3):470-7.

Ummul Haninah A, Vasan SS, Ravindran T, Chandru A, Lee HL, Shamala Devi S. Development and evaluation of a one-step SYBR-Green I-based real-time RT-PCR assay for the detection and quantification of Chikungunya virus in human, monkey and mosquito samples. Trop Biomed. 2010 Dec;27(3):611-23.

Williams CR, Long SA, Russell RC, Ritchie SA. Field efficacy of the BG-Sentinel compared with CDC Backpack Aspirators and CO2-baited EVS traps for collection of adult *Aedes aegypti* in Cairns, Queensland, Australia. Journal of the American Mosquito Control Association. [Article]. 2006 Jun;22(2):296-300.

Wilton DP, Kloter KO. Preliminary evaluation of a black cylinder suction trap for *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). Journal of Medical Entomology. [Journal article]. 1985;22(1):113-4.

Wu HH, Wang CY, Teng HJ, Lin C, Lu LC, Jian SW, et al. A Dengue Vector Surveillance by Human Population-Stratified Ovitrap Survey for Aedes (Diptera: Culicidae) Adult and Egg Collections in High Dengue-Risk Areas of Taiwan. Journal of Medical Entomology. [Article]. 2013 Mar;50(2):261-9.

World Health Organization. Dengue guidelines for diagnosis, treatment, prevention and control : new edition. Geneva: World Health Organization; 2009.