Chapter 6

Sampling Design and Mosquito Trapping for Surveillance of Arboviral Activity

Luís E. Paternina and Juan David Rodas

Abstract

Mosquitoes are the most important vectors for arboviral human diseases across the world. Diseases such as Dengue Fever (DF), West Nile Virus (WNV), Yellow Fever (YF), Japanese Encephalitis (JE), Venezuelan Equine Encephalitis (VEE), and St. Louis Encephalitis (SLE), among others, have a deep impact in public health. Usually mosquitoes acquire the arboviral infection when they feed on viremic animals (birds or mammals), so their infection can be detected along the year or in short periods of time (seasons). All of this depends on the frequency and seasonality of the encounters between viremic animals and vectors.

With the convergence of several phenomena like the increasing traveling of human populations, globalization of economy and more recently the global warming, the introduction of nonendemic arbovirus into new areas has become the current scenario. As examples of this new social and environmental frame we can mention the outbreak of West Nile Virus in North America in the late 1990s and more recently the outbreaks of chikungunya and Zika virus in the Americas. The present chapter deals with one of the first steps in the development of research studies and diagnosis programs, the surveillance of arboviruses in their vectors, the sampling design and mosquito trapping methods. The chapter also includes some important considerations and tips to be taken into account during the mosquito fieldwork.

Key words GIS, Mosquitoes, Sampling, Trapping methods, Arbovirus, Surveillance

1 Introduction

Global warming is already extending the geographical range of mosquitoes and ticks that harbor and transmit arboviruses, resulting in outbreaks of dengue fever and yellow fever in new locations [1]. Here we describe the use of geographic information systems (GIS) in sampling design for vector surveillance, and we describe collection and processing of insect vectors in fieldwork conditions.

1.1 Sampling Design for Vector Surveillance Using Geographic Information Systems (GIS)

The search of arboviruses through mosquito sampling is usually performed as a result of the notification of viral infection in humans or animals in endemic or enzootic areas. However, in areas where arboviruses have not yet been detected in mosquitoes, a random

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and uniform sampling design helps to systematize the study of arbovirus and vector distribution.

This methodology should reduce systematic errors in vector sampling and maximize the chances of finding arboviral agents in nature using a spatially variable approach. A wide variety of software exists for GIS analyses to help with the spatial design of sampling. The best known of these is ArcGis (property of ESRI Company); however, free software from the Open Source Geospatial Foundation (OSGeo) such as GRASS GIS and QGIS are also available.

Here, we focus on the software QGIS, which is now widely used among private and official institutions around the world as an alternative to ArcGis software. The increasing popularity of QGIS lies in its free distribution under a General Public License (GPL) and the availability of hundreds of plugins for geo-processing, geospatial statistics, and handling and analysis of processed layers and remote sensor images.

The random or uniform sampling design is meant to make a relatively unbiased sampling of vector/reservoir populations in a defined zone. Both methodologies allow the creation of points over the area of interest where the trapping of vectors will be carried out. It is important to emphasize that these methodologies are popular in ecological analyses in order to establish comparisons between areas or search for specific events in wildlife populations, but it is uncommon in investigations of zoonotic diseases.

The planning of trap distribution for collections of zoonotic vectors is an important element in the surveillance of hemorrhagic fevers caused by arboviruses and roboviruses [2, 3]. Unfortunately, examples of successful random or uniform sampling designs for the surveillance of arboviral diseases are not very common in the literature, because they require a large economic and logistic effort. However, there are several examples that show how a good sampling design can provide answers to very important questions on the dynamics of hemorrhagic diseases. One very successful example of the sampling design for arboviral surveillance is the work of a Brazilian team that studies the dengue fever vector [4]. They found strong spatial-temporal correlations between the abundance of *Aedes aegypti* females with cluster of cases in Belo Horizonte (Minas Gerais, Brazil) using a uniform sampling design based on city blocks [4]. Another example is the surveillance of arboviruses in well characterized areas or Australia using modern techniques of sampling with monitoring of frequent vector population [5–7].

It is important to mention that GIS is not only useful for sampling design purposes or for the retrospective study of disease patterns, it is also used for real time surveillance of arboviral diseases and for modeling of disease and forecasting [2, 8–10]. For example, the Georgia Department of Public Health sponsors the Arboviral Query and Mapping Tool website. This platform provides information

about arboviral activity by years in this part of the USA. In Australia for example, the New South Wales Arbovirus Surveillance Program provides weekly reports about arbovirus activity (http://medent.usyd.edu.au/arbovirus/) with detailed environmental information and mosquitoes/arbovirus detection.

As the GIS technologies become more common because of the globalization of computational mobile systems (cell phones, tablets, and personal computers), these tools can be a great source of data for monitoring mosquitoes for hemorrhagic fever surveillance programs across the world.

1.2 Preferred Collection Methods

For purposes of arbovirus surveillance and detection in mosquitoes, scientists take advantage of the common knowledge about the positive phototaxis and CO_2-tropism shown by insects, their ecology and habitat preferences [11]. Consequently, mosquito collection is usually performed with light traps (such as the CDC Light Traps or Shannon Light traps) or the traditionally used "oral aspirators" for the search of insects in their resting places. However, using these two generic strategies only a small fraction of the fauna of insects that may be involved in transmission cycles of arboviral agents can be studied [12, 13].

Since many of the insect-borne viral agents that cause hemorrhagic diseases have genetic material based on RNA [13], insects must be kept alive until they arrive at the laboratory and are either processed immediately or preserved in the cold chain. In this way, we can guarantee the integrity of the genetic material for detection and further isolation and characterization of the arboviral agent [5, 14, 15]. In the mosquito-trapping scheme, the collection method is particularly important, since this determines the amount and richness of the mosquito community that is being sampled [16]; this is why there are many different strategies for trapping insects.

Several factors influence mosquito trapping in terms of quantity (number of mosquitos/night) or quality (richness or diversity of the caught fauna), the choice of the collection method is particularly important because is one of the determining factors of the diversity of samples for the future arboviral testing. Here we present a synopsis of the main categories of trapping methods with some notes about their use in field-work (Table 1). In spite of the many methods available for mosquito trapping, the CDC Light Trap remains as the standard and is one of the most widely used methods because it allows the collection of a considerable amount of mosquitoes during the night by using the positive phototaxis exhibited by many groups of mosquitos. Nevertheless this positive phototaxis can be variable among different groups of mosquitoes. Here, we describe the standard collection of mosquitoes in field work, presenting CDC Light traps guidelines for surveillance of arboviruses with successful results in the Old World and in the Americas [5, 17–21].

Table 1
Evolution of mosquito trapping methods commonly used for the surveillance of arboviruses

Methods	Environment	Main purpose	Performance and notes	References
Oral aspirators	Indoor/outdoor	Resting and blood-seeking mosquitoes	Low performance, time-consuming and uneven population sampling of unfed and fed mosquitoes.	[11, 25]
Animal-Baited Trap	Outdoor	Blood-seeking female	Low performance.	[11, 26]
Light Traps	Indoor/outdoor	Mainly blood-seeking and blood-fed mosquitoes	High performance, uneven population sampling.	[11, 17–19, 21, 28]
CO_2-baited Light Traps	Indoor/outdoor	Mainly blood-seeking and blood-fed females	Very high performance, uneven population sampling.	[11, 21, 27–30]
Resting Site Traps (RST)	Outdoor mainly	Mainly blood-fed and gravid females	High performance, more even population sampling because of the relative unbiased method.	[30–32]
Passive Box Traps (PBT)	Outdoor mainly	Mainly blood-fed and gravid females	High performance, more even population sampling because of the relative unbiased method.	[23, 27]
CO_2-baited PBT	Outdoor mainly	Mainly blood-fed and blood-seeking females	High performance, uneven population sampling because of the relative biased method.	[23, 33]
Honey-FTA® Card in PBT	Outdoor mainly	Blood-fed and blood-seeking females	High performance, more even population sampling because of the relative unbiased method.	[7, 23, 24]

A typical arbovirus life cycle vectored by mosquitoes begins when the mosquito feeds on viremic mammals or birds. Because of this, blood-fed and nonnulliparous females are usually the preferred target of the sampling methods [13]. These guidelines are applicable to many arboviral agents transmitted by mosquitoes. Nevertheless, not all mosquitoes are equally attracted by the CDC Light Trap, and sometimes the use of a bait (CO_2 and other attractants) and oral aspirator collection at resting places would be helpful in the trapping of mosquitoes of exotic species.

The systematic search for blood-fed and gravid females has facilitated the detection and discovery of multiple arboviral agents. New methods have been developed specifically for capture and surveillance of other zoonotic agents [6, 22]. As an example, in recent years we have seen the monitoring of Japanese Encephalitis Virus using Passive Box Trapping (PBT) in Australia. This trapping system allows the evaluation of infection by arboviral agents in the captured mosquito population, and an estimation of vector abundance [23].

Later modifications of this methodology allowed the inclusion of FTA® cards (nucleic acid-binding paper) soaked with honey in the Passive Box Trap (Honey-FTA PBT) in order to get a very high performance passive trap for mosquitoes with a preservation system for arboviral RNA. In this particular case the FTA® is used to bind the nucleic acid (RNA or DNA) when the arbovirus-infected mosquito regurgitates the virus while it is consuming the honey [7, 24]. Then, the FTA cards are used for detection and characterization of the viral RNA obtained from mosquitoes. This FTA-PBT coupled technology must be evaluated in some others scenarios for the validation of the methodology; however, this trapping method represents a major breakthrough in arboviral surveillance for the identification of active risk transmission in endemic conditions, and for the research of arboviral agents in new areas. Further reading about other mosquito-sampling methods (past and current methodologies) are available from the classical text in medical entomology, Mosquito Ecology [11].

2 Materials

2.1 Use of Geographic Information Systems (GIS) in Surveillance Design

1. Laptop or desktop computer: we do not need an expensive computer; just make sure that your equipment is good enough to work with videos or images.
2. QGIS software: a copy of the software can be freely downloaded from http://qgis.org/es/site/forusers/download.html.
3. Shapefile: a shapefile (.shp, is a vector file) of your study area that will be helpful in order to limit the number and the extent of the sampling design.

4. Point Sampling Tool plugin for QGIS: this tool will generate points in the shapefile space according to our needs, in random or uniform ways.

2.2 Trapping Mosquitoes in Fieldwork

1. CDC Light Traps: CDC Light Traps are commercially available in many entomological stores, as an example you can obtain traps at Bioquip (Cat. 2836BQ). Traps are commonly provided with one collection bag, you will need one bag per trapping night for each CDC Light Trap.
2. Forceps: straight, curved, and featherweight forceps are commercially available.
3. Chill table: commercially available, it is better to have a portable unit in order to facilitate the transportation of the equipment.
4. GPS: Precise equipment is required; the technical specifications of the equipment tell you the error in the estimation of the spatial position.
5. Plastic microtubes: 1.5 or 2 mm polypropylene microtubes are commercial available and are used to store individual insects.
6. RNALater (optional): Use this reagent only if you are not planning to use liquid nitrogen for preservation of mosquitoes. This is highly recommended when you are collecting mosquitoes in remote locations.

3 Methods

3.1 Use of GIS to Design Surveillance Strategy

1. Install the stand-alone version of the latest QGIS from their official website; this may take a few minutes after the download of the software (*see* **Note 1**).
2. Once you have installed the software, in the "Complement" menu of the main panel of QGIS, select "Manage and install complements". In the new panel, please look for the "Point Sampling Tool" and select it for installation (*see* **Note 2**).
3. Import a shapefile of your study area into QGIS; we use the shapefile of US counties (scale 500k, 1:500.000) obtained from the Cartographic Boundary Shapefiles Database of the USA Census Bureau (https://www.census.gov/geo/maps-data/data/cbf/cbf_counties.html). Once you have imported your shapefile into QGIS, the software will ask you about the Coordinate Reference System (CRS); we use for this example the WGS84 (also known as EPSG: 4326). After this you will see your data (in our case the US counties map) on the main screen of QGIS.
4. To generate the points over the study area, go to "Vector" menu, then to "Research Tools" submenu and finally, to

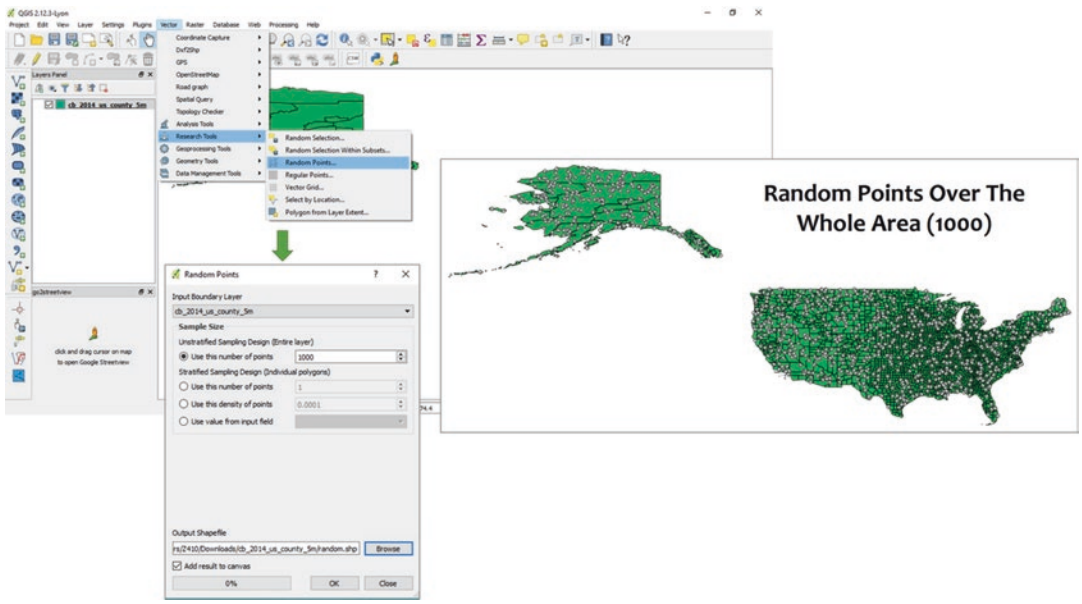

Fig. 1 Sampling points generated using an unstratified sampling design in QGIS 2

"Random Points". Using this procedure you can generate sampling points in two different ways: (1) random points over the area or (2) evenly distributed points over the study area. The selection of a random or uniform sampling algorithm depends on the purpose of the vector sampling (Fig. 1).

5. For the random generation of points you will need to provide the number of points to be created, this number depends on the number of trapping stations that you can use, e.g., 100 mosquito traps, 300 Sherman traps (for rodents), and 150 tomahawks (for rodents). Then, once you have provided the number or points in the "Unstratified Sampling Design", the software will make a new layer (vector layer) containing the points required.
6. One can also "Stratify" the number of points that cover a shapefile, meaning that one can create a certain number of points according to each hierarchy of the shapefile (country, state, cities) using the first option under the "Stratified Sampling Design" submenu. In this particular case, we want 1 point for each polygon inside our US shape file (Fig. 2).
7. To create a uniform sampling design, one must select the "Regular Points" option in the "Research Tools" submenu. These settings will produce a desired number of points evenly distributed in your shape file. One can set the number of points for convenience or get an unknown number of points by choosing the "spacing point" option.
8. You are now ready to distribute traps and plan your collections.

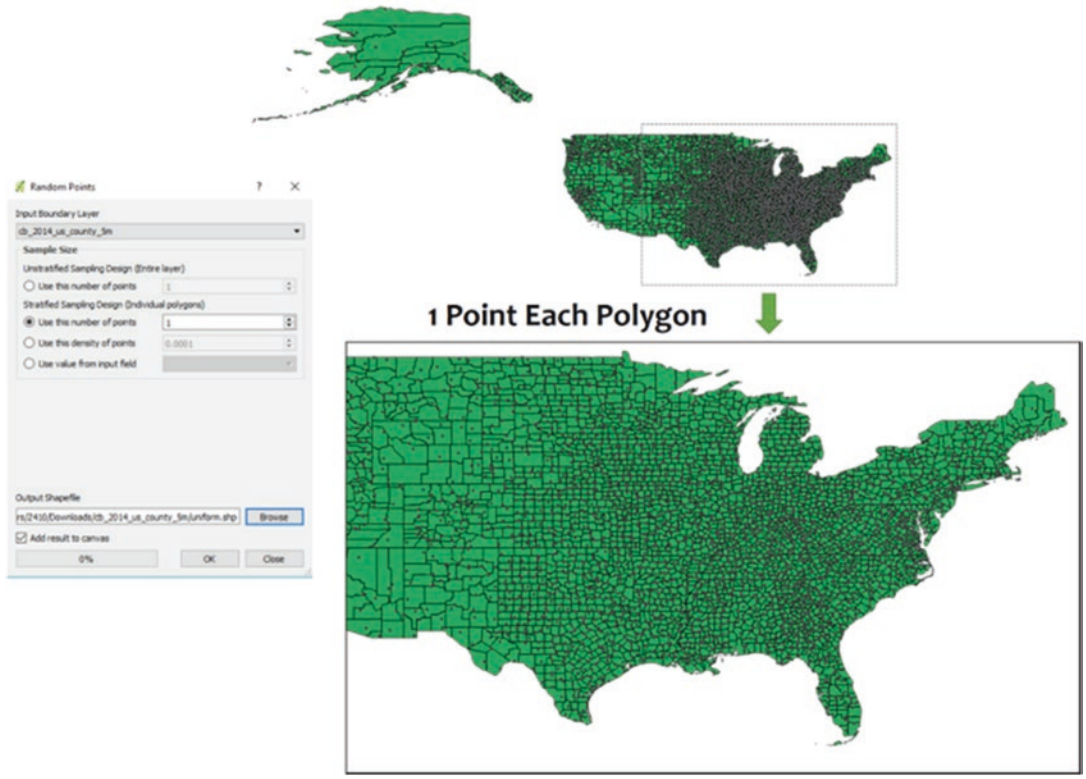

Fig. 2 Stratified sampling points design in QGIS 2.12

3.2 Field Trapping of Mosquitos

1. Select the points for the installation of light traps using the criteria of your convenience (canopy density, distance to forest patch, and distance to rivers, among many other criteria) and taking into consideration the ecology of the mosquito community that you want to sample. The trapping grid must be defined before the fieldwork, the trapping points can be randomly distributed over the study area or following a trapping grid defined by the scientist (lineal, radial, and small windows, among others) (*see* **Note 3**) (Fig. 3).
2. Make sure that the installed traps are at least 1.5 m above the ground level. Take into consideration that mosquito community may have a vertical stratification and at this ground level the canopy mosquitoes are not typically collected (*see* **Note 4**).
3. Usually the sampling of mosquitoes takes from 18:00 to 6:00, after this time detach the collection bag and close the open side. The bags must be handled carefully to avoid any possible damage to mosquitoes. Once you have collected the bag, you must replace it the collection bag with a new one (*see* **Note 5**).

Fig. 3 Examples of trapping sites using two light trap methodologies. (**a**) A CDC trap installed on a secondary tropical dry forest. (**b**) Shannon trap installed in a secondary tropical dry forest

4. Carefully place the mosquitoes of a single collection bag in one (or several) Petri dish. Each petri dish will be placed on the chilling plate and checked under the stereomicroscope in order to sort the mosquitoes according to species (or genera if you have trouble in the species identification process) using the proper taxonomic keys. A very useful and interactive mosquito identification key is provided by the Walter Reed Biosystematic Unit (http://www.wrbu.org/VecID_MQ.html) (*see* **Note 6**).
5. Each pool of sorted insects must be gently transferred to microtubes with RNALater (or empty cryovials if you are using a liquid nitrogen tank for mosquito preservation and transport). The pool size may vary between 2 and 50 insects, it depends entirely of the mosquito abundance and general size of the insects, some insects can be small as *Uranotaenia* (Culicinae, Uranotaeniini) or as large as *Mansonia* (Culicinae, Mansoniini) (*see* **Note 7**).
6. If you are not planning to do RNA extraction immediately, save your samples at −80 °C until the performance of the nucleic acid extraction protocol. The samples must be preserved in cold chain in order to guarantee the quality and integrity of viral RNA for detection and characterization purposes.

4 Notes

1. This software is completely free (free software policy of the Open Source Initiative) and it is very intuitive. QGIS was created by the Open Source Geospatial Foundation (OSGeo), there are documentation and support (commercial and community-based support) for its use in Linux, Windows, and

Mac. You can get further information about the software at http://www.qgis.org.

2. There are thousands of additional plugins for every task in QGIS; so, feel free to navigate among the plugins if you have other needs to cover.
3. The prior definition of trapping scheme (random trap distribution or in a predefined grid) is important for a good spatial representation of the studied area. Mark the spatial position of every CDC light trap with your GPS, this will allow you to locate quickly your traps in the field and allow you to determine if there are ecological patterns of mosquito and arboviral distribution in a posterior analysis. The accuracy of the GPS device normally range from ~10 ft to 3 in. Select GPS equipment according to your needs and budget.
4. Usually the batteries that power your trap must be protected from the rain and wild animals, use a blanket or a battery bag (or a plastic battery box) to protect it from any damage.
5. Collection of mosquitoes must be done preferentially over at least 3 days, it is preferable longer sessions (more than 3 days) of trapping in order to get a better representation of the mosquito community. Make sure that you have enough batteries for the fieldwork, always test the battery power between trapping nights.
6. The sorting and proper identification of mosquitoes is critical for the understanding of transmission cycles of arboviral agents. The identification of mosquitoes is usually a challenging task, and it is more challenging in field conditions, currently the identification of mosquitoes related with the transmission of arboviral agents can be done also through the analysis of mitochondrial Cytochrome B (CytB) or Cytochrome c Oxidase I (COI) DNA sequences.
7. If you are not planning to do RNA extraction immediately, save your samples at −80 °C until the performance of the nucleic acid extraction protocol. The samples must be preserved in cold chain in order to guarantee the quality and integrity of viral RNA for detection and characterization/isolation purposes.

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