Medical Entomology Unit
Vector Control Services
Ministry of Public Health
Guyana

Aedes Insecticide Resistance Surveillance Protocol

Mr. Govindra Punu
Biologist

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Introduction

The establishment of *Aedes* mosquito in Guyana has raised public health concerns in the ten administrative regions where conditions are suitable for their existence. Even though rainfall may affect abundance and productivity of breeding sites, the preference for artificial water containers means that they do not have to rely on rainfall for the existence of larval development sites (Jansen and Beebe 2010). Additionally, the preference for feeding and resting indoors makes them less susceptible to the effects of climatic factors which could influence their distribution.

At present, all Guyanese are at risk of contracting Dengue, Chikungunya and Zika. Recently, studies were conducted in Georgetown to determine the risk of transmission in the city using the Breteau Index tool. This research (unpolished data) has revealed regional epidemic risks and transmission risks in most areas.

Dengue, Chikungunya and Zika in Guyana

Guyana is endemic to Dengue; over the past five years, much emphasis has been placed on elimination of mosquito foci by larval source management and adulticiding. Nevertheless, the incidence of dengue cases continues to be of concern along the coastal plain where Region # 4, with almost half the population of the country has repeatedly reported a high burden of dengue cases.

The outbreak of Chikungunya started in Guyana in 2015 and that of Zika began in 2016; both have been characterized as explosive and debilitating illnesses. Their outbreaks were classified as short and explosive with cases having plateaued since then (Vector Control Services, Guyana, 2018).

Seasonal variation of cases

In Guyana, climatic conditions may not be the biggest influence on the incidence of Dengue cases. The Vector Control Services in Guyana noted no correlation between mean temperature and dengue cases; however, it was noted that peak rainfall periods (May/June) were followed by an increase in the incidence of cases. Having established a link between the incidence of dengue cases and rainfall, it is noted that environmental conditions do influence the incidence of cases as well (Vector Control Services, Guyana, 2018).

Vectors and Entomological Indicators

At present there is limited data on entomological monitoring and surveillance for vector control in Guyana. The medical entomology unit is now establishing itself and is at the stage of gathering baseline data. The main constraints to conducting adequate monitoring and surveillances activities are:

- Lack of a national framework for entomological monitoring and surveillance;
- Inadequate human resources to carry out entomological monitoring and surveillance at regional and central levels; and,
- Absence of a well-equipped medical entomology laboratory in the country

Limited access to the necessary infrastructural facilities has resulted in there being limited entomological monitoring. The generation of sufficient local evidence to support planning for vector control intervention becomes more difficult. Additionally, the Vector Control Services/Ministry of Public Health has not monitored adequately insecticide susceptibility of vectors in the ten administrative regions; this affects the informed selection of insecticides.

Recent entomological investigations (Unpublished Data) indicate that among the *Aedes* mosquitos, *aegypti* dominates all districts of the capital, Georgetown. Surprisingly no *Aedes albopictus* mosquitoes were detected. Entomological indicators such as house, container, and breteau indices were analysed. Our main aim is to reduce and or maintain the risk status of all districts to a level below 5% using breteau Index calculations. These studies have shown transmission risks are higher than 20% in almost all the study districts of Georgetown.

Surveillance and Conventional Vector Control Interventions

Guyana is divided into ten administrative regions. Each of these regions is autonomous and is only guided by policies created at the central levels. The Administrative head of each region is referred to as the Regional Executive Officer and he is supported by a team of programme heads inclusive of the Regional Health Officer, the Regional Education Officer, the Regional Engineer among others. The Regional Health Officer heads the Regional Health Programme. The Regional Health Programme has critical components such as a vector control unit and an environmental health unit. The head of the Environmental Health Unit is referred to as the Environmental Health Officer. This Officer is responsible for conducting house-to-house inspections and supervising the implementation of entomological activities at the regional level with support from other regional staff. There is room for improvement in channelling to central level about Flavi-viral occurrences and the dissemination of entomological reports.

At the central level, a vector control team conducts inspections to identify and treat positive *Aedes* breeding sites in and around Georgetown, disseminates educational materials, investigates cases and carries out larvae and adult mosquito control activities. There is a need for regional teams to conduct regular countrywide surveillance activities, under the leadership of the central team.

Insecticide Resistance Monitoring

In Guyana, larviciding and adulticiding are major control initiatives used to suppress the mosquito population. The country has been using Malathion for more than ten years for adulticiding. In addition Temephos 5% has been used to conduct larviciding activities for more than 10 years also.

No routine insecticide resistance monitoring has been done over the past ten years. The entomology unit has received training from CARPHA in 2016 and USAID in 2017 on how to conduct insecticide resistance tests. At present, the country does not have adequate staff and facilities to support routine insecticide resistance monitoring.

There is urgent need for routine studies on insecticide susceptibility in the ten administrative regions of Guyana with respect to the effectiveness of Temephos used for larviciding and Malathion as an adulticide.

Conventional Vector Control Interventions

Larviciding

- Temephos 5% sand granular

Adulticiding

- Malathion 96% ULV

Others

- Indoor Residual Spraying – Fendona

Infrastructure and Human Resources for Vector Control

There is no entomological unit set up in the different regions of Guyana. Currently there is a team of twenty-seven field staff at central location, Georgetown and Environmental Health Officers at regional locations to assist with vector control activities during routine house to house sanitary inspections. The Environmental Health Officers and field staff have the responsibility of implementing measures aimed at the prevention and control of the breeding of mosquitoes in the ten administrative regions, a task which is significantly challenging.

The limited entomological practices in the different regions results from there being personnel with limited capacities to collect and identify the larval and adult stages of mosquitoes. The effort is focused at present on equipping the Environmental Health unit in every region with basic knowledge on medical entomology and how to collect samples which can be analysed in Georgetown by the central Entomology team.

At central level we are equipped with the materials and equipment for basic entomological investigations as well as knowledge on how to perform insecticide resistance tests using the WHO and CDC standards.

Rationale

Flavi-virus infections such as Zengue, Chikungunya and zika continue to be an important

public health problem in Guyana. Due to the lack of effective vaccines and therapeutic treatments, the primary strategy to control the transmission of disease is to reduce vector-human contact. This includes reducing vector populations, applying physical control methods (destroying potential breeding sites), chemical control methods (insecticides and repellents) and biological control measures (fishes and tadpoles).

Frequent use of insecticides such as fendona, Malathion and Temephos can result in resistance of certain strains of mosquitoes to these insecticides; therefore, it is imperative that surveillance and monitoring be performed effectively in localities of high transmission of disease to manage and reduce insecticide resistance.

The entomology component of the arbovirus system complements evidence-based decision making. Routine entomological surveillance countrywide can define where and which vector-control strategies are needed such as spatial insecticide spraying. This protocol helps by having a plan to monitor insecticide resistance in the *Aedes aegypti* populations and to adopt an evidence-based rotation strategy of insecticide groups used in a national vector control program.

Goal

To contribute in a sustained way the improvement of the entomological surveillance programs, supporting the implementation of a consensual strategy for the monitoring and management of insecticide resistance in the main disease vectors in Guyana.

Aim

To accompany PAHO/WHO member countries in the Network for Surveillance and Management of Resistance to Insecticides in the development and implementation of a regular surveillance of the susceptibility to insecticides of disease vectors, in order to develop national and regional strategies which ensure effective and rational use of insecticides used to control these diseases.

Expected Results

- Determine the population of Aedes vector predominant in each site

- Determine the susceptibility/resistance to adulticides in each site in *Aedes aegypti* and *albobictus* in Guyana
- Determine the efficacy to larvicides in each site in *Aedes aegypti* and *albobictus* in Guyana
- Determine the occurrence of arbovirus transmission in *Aedes* captured for resistance/susceptibility surveillance.

Methodology

Ovitraps will be installed across the country and eggs will be collected every week. In the laboratory, the eggs will mature into mosquitoes, at which point multiple tests will be performed.

First, in order to understand what occurs with mosquito populations in endemic areas, biological tests, *i.e* the CDC's bottle bioassay, will allow for the detection and characterization of resistance to insecticides. The CDC bottle bioassay relies on time mortality data, which are measures of the time it takes an insecticide to penetrate a vector, penetrate its tissues, get to the target site, and act on that site. Additionally, the WHO insecticide resistance testing standard will be adopted.

Second, the mosquitoes will undergo virology testing in order to define the transmission of arboviruses countrywide.

Site selection

General inclusion criteria

- Reported cases of Flavi- viruses during the previous year
- Vector control interventions have been carried out at the site in the 5 last years

The sites include urban and rural areas as shown in the table below.

District	**Localities**	
	Urban	**Rural**
Barima Waini	Mabaruma	
Pomeroon- Supernaam	Anna Regina	
Essequibo Island West Demerara	Vreed En Hoop	

Demerara Mahaica	Georgetown	
Mahaica- Berbice	Fort Wellington	
East Berbice Correntyne	New Amsterdam	
Cuyuni Mazaruni	Bartica	
Potaro- Siparuni	Mahdia	
Upper Takatu- Upper Essequibo	Lethem	
Upper Demerara Berbice	Linden	

Sample collection in each site

No previous studies were conducted on insecticide resistance in Guyana with the use of Ovitraps. This has resulted in the following:

- No data being available on the number of Ovitraps in Urban Areas; No data being available on the number of Ovitraps in Rural Areas

Personnel Required

It is recommended that each vector control unit has one to two persons responsible for ovitrap installation and reading. Given that each field worker will read approximately 20 ovitraps each day from 10 blocks (see Appendix 1 for more details), a total of 100 ovitraps can be read each week by a single field worker. The table below describes the minimum amount of personnel required at each vector control unit.

District	Number of Ovitraps (Sum of Urban and Rural)	Personnel Required
Barima Waini	100	5-10
Pomeroon- Supernaam	100	5-10
Essequibo Island West Demerara	100	5-10
Demerara Mahaica	100	5-10
Mahaica- Berbice	100	5-10
East Berbice Correntyne	100	5-10
Cuyuni Mazaruni	100	5-10
Potaro- Siparuni	100	5-10
Upper Takatu- Upper Essequibo	100	5-10
Upper Demerara Berbice	100	5-10
Total	**1000**	**50-100**

It is also recommended that a supervisor be assigned for all the ovitrap installation procedures. The supervisor will conduct monitoring and fill the Supervision Form in Appendix 1.

Ovitrap Installation

The Mexican guidelines for installation of ovitraps will be used as described in Appendix 1.

Equipment and materials

Appendix 1 describes all the necessary equipment and materials required for the implementation of this protocol.

Testing in the Laboratory

Once the data has been collected, samples will be delivered to the designated lab space where they will undergo testing using the CDC bottle assay and WHO standards. It is

recommended that field workers visit the Entomological Unit in a foreign entity to see how the ovitraps are handled post-collection.

Information System

Data collection will flow from the sentinel sites to the vector control unit. Weekly collection will be sent from each site to the Entomological Unit in Georgetown. The samples will be packed and accompanied by the required forms. The forms are included in Appendix 1.

Monitoring of Field Work

In order to ensure the best quality of surveillance, monitoring of the field work should be conducted on a weekly basis. A systematic report (see Appendix 1) will be filled out by the supervisor to assess and improve worker's performance. All staff must be aware that they will be monitored and the forms should be shared with the Director, the Coordinator of the Aedes Program and the Biologist of the Vector Control Services in Georgetown.

References

- Guyana, Ministry of Health. Integrated Management Strategy for Dengue Prevention and Control in Guyana. Georgetown: Ministry of Health; 2011.
- Jansen CC, Beebe NW. The dengue vector *Aedes aegypti*: what comes next? Microbes Infect. 2010 Apr;12 (4):272-9.

Appendix 1: Ovitraps Installation Guidelines

Contents

Ovitraps staff will read a minimum of 20 Ovitraps or 5 blocks per item per day (100 Ovitraps per week). Once the surveillance of Ovitraps has started, the monitoring is not to be interrupted

For the installation of Ovitraps it will be necessary to:

1. Have exclusive personnel for Ovitrap installation, reading, counting and weekly activity tracking.
2. Store and cut the F-1600 pellon paper in an exclusive area, free of any type of insecticide and/or contaminants such as fungi.
3. Staff responsible for reading Ovitraps should avoid both exposure and handling of all insecticides.

1. Equipment and Materials

Equipment

- Magnifying glass (10X)
- Lamp
- Stereoscopic Microscope
- 4 digits Manual Counter
- Grid for counting in quadrants

Materials

- Container for Ovitraps: the container should be cylindrical, plastic, black and one litre in size. The container provided might need to be drilled at a height of 8 cm from the base to the opening of the container. This depends on the climatic conditions of each region (in areas that are dry, hot and lack rain, the hole is not necessary) (Fig. 1).
- Pellon paper (F-1600): The pellon paper will be 12 cm wide by 35 cm long.
- Label with the identification data of the Ovitraps
- Adhesive labels 100 mm x 50 mm.
- #2 Pencil
- Two-colour pencils (blue and red) to draw the quadrants in case the grid paper is not available
- White typing sheet
- Plastic bags
- Brown paper bags
- 1.5-Liter Plastic bottle for carrying water in areas where the supply is limited or the supply is very irregular
- 2-Liter Bottle or more (for the moist chamber)
- Fibre to clean the walls of the container
- Clipboard

2. Field Work

3.1 Locating the Sampling Area

It is necessary to know the geography of the region where the work will be done; graphic sketches should be used to mark the areas in which Ovitraps are used. House number and vacancy status should be included in the sketches. The area can be selected in two ways:

1. If the geographical area can be presented in the form of plain, Ovitraps will be placed within every 4 to 6 blocks
2. Natural elevation from hills or mountains can also be used to identify location for Ovitraps. Ovitraps should be placed every 2 or 3 blocks or as the area permits. The blocks should not be next to each other.

Once the five blocks have been selected, 20 Ovitraps will be placed per block either at the front or back of the house/unit. Depending on the shape of the block, it is preferred that one Ovitrap is placed on each side of the block. If this is not possible, 2 or 4 can be placed on a single side but with a distance of 50 meters from each other. The site where the Ovitraps is placed should be marked in a sketch.

In sectors where the blocks are irregular, with 2 or 3 houses and historical background of Dengue, Chikungunya and Zika disease, the installation will be done with the Ovitraps Installation in Schools (*See Appendix 1*).

3.2 Installation

Upon arrival at the house, the person installing the Ovitraps will present himself/herself as an employee of the Ministry of Public Health, Vector Control Unit, Entomology Unit. The head of the family should be informed about the work to be done. Personnel will clearly explain that a weekly visit will be made to check the position of the Ovitraps and to collect samples. He should also state that the Ovitraps will remain at the house for approximately one year and that the Ovitraps should not be tampered with.

In case the area has a high percentage of closed or uninhabited residences, a public message should be used to inform the population about the sampling. The message should include that the Ministry of Public Health has selected the area for Ovitraps installation. It should also solicit the cooperation of the population to not tamper with any of the equipment.

3.3 Development

Step 1. Write basic data on the corner of the fabric pellon paper F-1600 with a pencil: date, Ovitraps number, neighborhood or locality, reading number, and in red the number of eggs. Do not use the entire fabric pellon paper to record the data so as to facilitate the counting of eggs and their reuse. Note that the fabric pellon paper can be washed for its reuse.

Step2. Place the fabric pellon inside the plastic container. The pellon must completely cover the circumference or inside of the container. Clean water is added to the height of the suggested hole at 8cm. Do not add chlorine or larviciding.

3.4 Placement and location at the house

Place the Ovitraps, preferably between plants and away from other potential breeding sites since this may pose competition (tires, batteries, drums, various boys, etc.); in a shaded or dark place, out of the reach of children and pets, at a height of a meter and a half tall and avoiding direct sunlight. Record the corresponding data in the E-OV1 and E-OV2 Forms (*See Appendix 2 and 3*). Finally, on the outside of the house mark the acronym OV and corresponding Ovitraps number.

3. Examination

The visit takes place weekly. This time period is ideal for ovipositor of pregnant females. **The fabric pellon has to be changed carefully so the eggs do not stick to your fingers.**

4.1 Counting eggs

Removing pellon paper from the water of the same Ovitraps (to activate embryogenesis) has to be done very carefully. To not lose eggs, drain the excess water, if the amount is more than 100 eggs, use a magnifying glass 10x, preferably with grid 2x 2cm to perform the count at the collection area. The sampling will be done by placing the grid with quadrants under the pellon paper so as to determine the density and coverage of eggs in each quadrant. Other counting techniques can also be used, such as enclosing the eggs by groups and recording the amount. Divide the pellon paper and record the number by divided fraction. Avoid double counting an egg where the larva has hatched. This might require the support of a stereoscopic microscope.

4. Reinstalling the ovitrap

Wash and scrub the walls of the container using the fiber. **This will prevent larvae, pupae, and /or Imagos to grow.** Fill the container with water using the same procedure and amount previously described and places a pellon paper (new or recycled) completely covering the inside.

If the Ovitraps is not found, if it is turned over, without pellon paper or without water, it should be relocated somewhere else in the house. The inhabitants should be kindly asked to take care of it. If these events are repeated more than twice, as in the case of closed home for two consecutive weeks, you should relocate to another house, preferably on the same side of the block.

Before the end of the day, the personnel working with the Ovitraps should return to the closed homes so as to read the Ovitraps found there and change the pellon paper. It is important to note that each entomological brigade can establish regional strategies to reduce the percentage of closed houses, as each of these closed houses translates to a potential breeding site for *Aedes Aegypti* or *Aedes Albopictus* in an area at risk.

When the housing is closed and the next week pellon paper is positive, the data is ignored and the number of eggs will not be registered on the system. However, it does this alternate registration was carried by local data bases, for further analysis since it is not known if positivity is last week or the week worked.

5. Handling of the Pellon paper With Eggs

At the end of counting the total number of eggs observed, on the pellon paper, record the total number of eggs followed by the Ovitraps number, for example OVl 010/530. Then fold keeping the side with the eggs in the inside so as to prevent the loss of eggs. All papers should be folded individually, not collectively. You can spray or dip the pellon paper in the same water used in the Ovitraps, carefully holding it at the corner to prevent the release or lose of eggs. The pellon paper will be placed in containers, which function as humid chambers and provide suitable environmental conditions for the pellon paper while the entomologist completes his/her work. If the pellon papers are still damp upon completing the area, they can be hung by a string like a close line, at room temperature (26-28 °C) under the shade for 1-2 days. They should be hung from a corner, keeping the eggs on the inside, aided by a fastener/clip (never place under the sun or on trucks). The negative pellon papers are returned to the bioassay laboratory for its recycling, after it they have been washed and dried under the sun (no more than 2 times).

6. Storage

Each dry pellon paper and folded with the egg on the inside should be placed inside a brown paper bags, and properly labeled (District, City, Sector, Block, Registration date, Epidemiologic Week, Number of revision, ovitrap number, collector, number of eggs, temperature and humidity). The bag should be placed in an area free from insecticides, moisture, and predatory animals (mainly ants).

7. Shipping

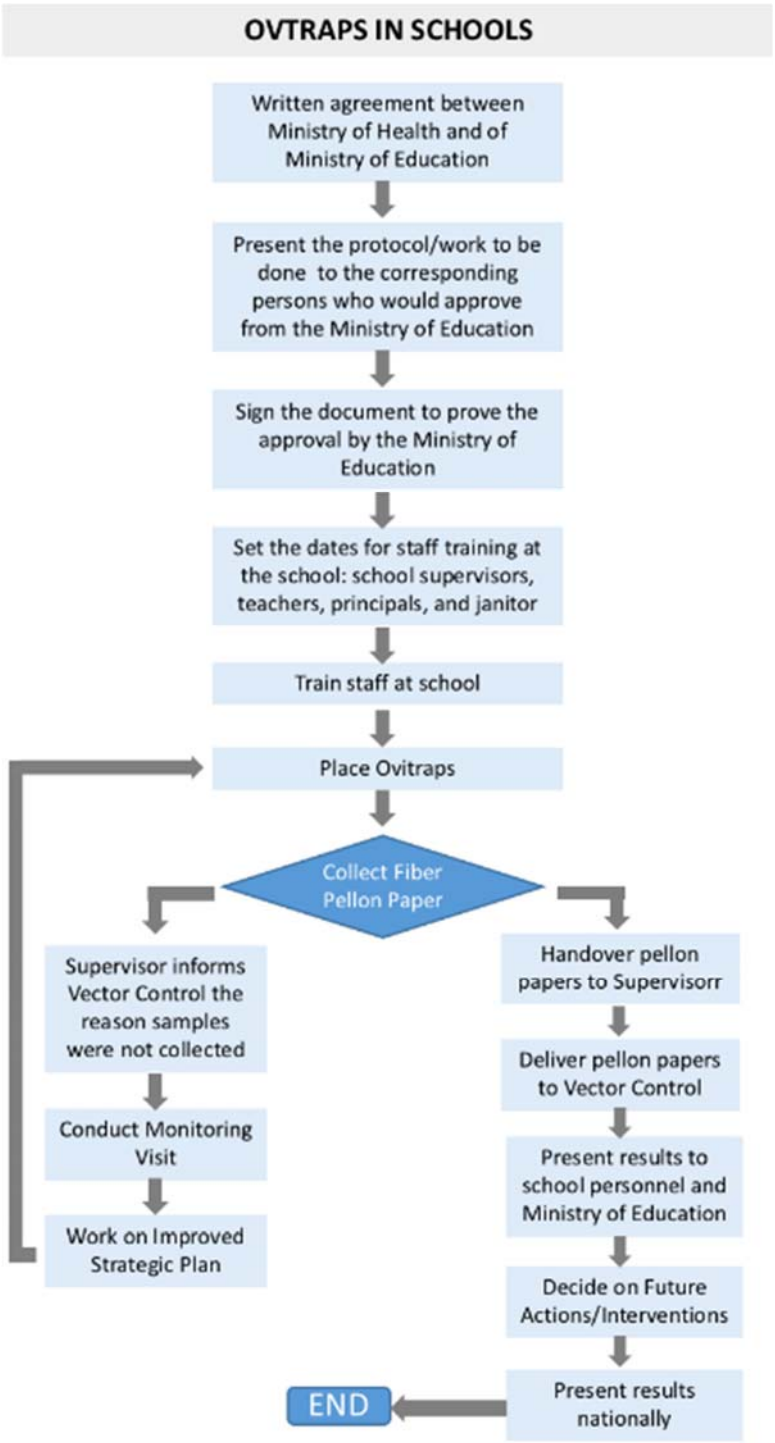
Figure 13. Filling the label for proper storage.

Pellon paper with eggs will be sent to the Central Entomology Unit in Georgetown to determine resistance of larvicides and adulticides used in the vector control program. At the units, the pellon papers have to be verified with respect to their viability before hatching, by observing them under the stereomicroscope; viable eggs will be counted microscopically excluding dried, broken and / or unhatched giving the following qualitative parameters:

- None: 0 viable eggs
- Poor: Less than 15% viable eggs
- Low: up to 35% viable eggs
- Good: 36% to 79% viable eggs
- Optimal: 80 to 100% viable eggs

A report with information on the percentage of viable eggs will be done.

Appendix 2: Ovitrap Installation in Schools



Appendix 3: E-OV 1 Form

Entomological Surveillance of Ovitraps
E-OV1 Form

District:_____ Municipality: _____
Area: _____ Health Jurisdiction: _____
Study Start Date: _____

Ovitrap Number	Sector Number	Block Number	Address	Location of the Ovitrap	Reading Number: ( ) Date: Number of Eggs	Reading Number: ( ) Date: Number of Eggs	Reading Number: ( ) Date: Number of Eggs	Reading Number: ( ) Date: Number of Eggs	Reading Number: ( ) Date: Number of Eggs

Observations: _____
Name and Signature of Worker: _____
Name and Signature of Supervisor: _____

The form is divided into three components and should be completed every time a reading is completed.

Basic Data: District, municipality, and date Ovitraps was installed

Ovitraps Data: Ovitraps number, block number, house address and clear description of the location of the Ovitraps (include color of wall, “on the terrace” etc…)

Data collection: Reading Number, Date Readings were made, and number of eggs.

Appendix 4: E-OV 2 Form

Entomological Surveillance of Ovitraps
For Use of the Coordinator (E-OV 2 Form)

District:_____ Municipality:_____

Area:_____ Health Jurisdiction:_____

Study Start Date:_____

#	Social Security of Applicant	Sector #	Homes			Ovitraps						Total		
			Functional	Closed	Unwilling	Installed	Collected	Destroyed	Without Water	Lost	Lost Touch	Read	Positive	Negative
1														
2														
3														
4														
5														
6														
7														
8														
9														
10														
11														
12														
13														
14														
15														

Observations: _____

Name and Signature of Field Worker: _____

Name and Signature of Supervisor: _____