

ENTOMOLOGICAL SURVEILLANCE FOR PROTECTING PUBLIC HEALTH FROM MOSQUITO-BORNE DISEASES

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Vector-borne diseases account for more than 17% of all infectious diseases. Among the vectors, mosquitoes are the deadliest ones. Since 2014, major outbreaks of dengue, malaria, chikungunya, yellow fever and Zika have afflicted populations, claimed lives and overwhelmed health systems in many countries (WHO, 2017). More than half of the world's population live in areas where this mosquito species is present. Sustained mosquito control efforts are important to prevent outbreaks from these diseases (WHO, 2019). It is a well-known fact that the mosquito-borne disease control and prevention largely relies upon lowering the contact rate between human and vector and killing the vector. A proper Integrated Mosquito Management programme uses various techniques to reduce mosquito numbers and surveillance provides the data on which those actions apply. In fact, with the help of surveillance throughout the year, we can predict the chance of a disease outbreak and can deploy management strategies to prevent the havoc.

Mosquito Surveillance is the routine monitoring of both larval and adult mosquito populations over the course of an entire mosquito season based on the life cycle of the vector in interest. Through Surveillance we can monitor the fluctuation in the mosquito population in an area, can identify the major species in that area, can detect mosquito-borne diseases and can find out the efficiency of control measures based on the mosquito numbers. Surveillance is usually done by dividing an area such as a village, town or industrial facility into different zones. Within each zone, an experienced field technician collects mosquito larvae from standing water sources using standardized techniques, while they set adult mosquito traps for adult sampling (Markowski, 2015). The procedure is as follows:

Larval Sampling

Locate The Breeding Source: First, we have to find the mosquito breeding places which includes all the water sources in that locality. Different mosquito genera prefer different source of water like freshwater, dirty and polluted water, artificial collection of water and water containing certain aquatic vegetation. We can use geographical maps, land use board maps, watershed maps, soil maps, aerial photos and Geographic Information System-Global Positioning System (GIS-GPS) etc. Any place which can retain a pool of water is targeted. In rural area, we have low-lying vegetated areas, marshes, ponds, riverbeds and backwaters, irrigated fields and pastures, wells, areas of poorly drained soils and in urban areas; overhead tanks, ditches, street gutters, drainage systems, swimming pools, unused public toilets, cesspools, and any large or small natural and artificial containers at home or other building.

Monitoring Larval Habitats: The monitoring is started after locating the sources. Monitor daily or weekly or biweekly depending on the season and study. The dipping method is often used for collecting mosquito larvae. The standardized equipment is a “Dipper”, a white enamel or plastic cup attached to a 3-4 feet long wooden dowel. A simple ladle can also be used. Dip it in the breeding places (edges of swamps, ditches, streams, rice fields other bodies of waters) at an angle of 45° (Srivastava and Dhariwal, 2016). The larval density is assessed in terms of average larval density per dip. A minimum of 10 dips per acre is mandatory. Collect the larvae for further identification and species determination. The larvae are stored in glass vials containing 70-80% ethanol for preservation. No more than 20 larvae should be placed in a 50 ml single vial because the water contained in the bodies of the larvae will significantly dilute the concentration of ethanol and jeopardise preservation (EFSA, 2018).

Netting using larval nets (fine-meshed (≤ 0.5 mm) aquatic net (aquarium net) and sieve) and pipetting (from tree holes etc.) are also methods for collecting larvae. For the collection of *Mansonia* larvae, a one-foot square bottom tin/wooden tray is kept over floating vegetation and the number of plants is counted. The plants are then removed to an enamel tray with water and the plants are then well shaken to disentangle the *Mansonia* larvae from the roots. Then the number of larvae and the number of plants are counted and the average number of larvae and pupae per plant estimated.

Adult Sampling

Several sampling methods are available for adult mosquito surveillance which can be used alone or together as per necessity. Broadly it can be classified as collecting techniques and trapping techniques.

Collecting techniques include

- a) **Sampling Resting Population:** Most of the mosquitoes feed at dawn and dusk, and a few hours into dark except some that will feed both day and night like *Aedes*. When they take rest, they are found indoor in cool and dark corners like house ceilings, amongst thatch and cobwebs, on the underside of shelves, amongst clothing and other hanging articles, cattle sheds, pet houses, etc. When outdoor, mosquitoes are seen resting in bushes, shrubs, cracks and crevices of walls, under bridges, culverts and in tree holes, etc. (Srivastava and Dhariwal, 2016). These mosquitoes are collected and counted using a vacuum aspirator. Sampling the resting adults usually provides a representative sample of the population *viz.* males, newly hatched adults, unfed as well as fed and gravid females. When looking for natural resting places, this method is tedious, so we can provide artificial resting places and collect them.
- b) **Human Landing Collection (Hlc):** This method is the simplest and most authentic one, as human beings are directly posed as bait, but ethical issues are to be taken care of. The collector or the person, on whom the mosquito sits for feeding, should be healthy. He has to stand for 2-5 min (up to 15 min in some cases) in an area where there is peak mosquito activity, at peak time, preferably between sunset and one to two hours after sunset. The mosquitoes are collected from his body using a vacuum aspirator. Landing counts must be done in a standard, consistent manner, with collections made at the same time of day, in the same place, for the same amount of time, using the same collector. Animal bite catches can also be used for collection. This is done by removing mosquitoes directly from a tethered animal host with an aspirator or inside a drop-net and also using a suction trap baited with small animals.

Trapping techniques include

- a) **Light Traps (UV And Incandescent Light Traps):** The basic principle of the light trap is that the mosquitoes attracted to the electric light, enters under the hood of the trap where they get exposed to a strong downward air current produced by a fan-operated by

an electric motor. These mosquitoes are collected in a holding cage attached to it (Srivastava and Dhariwal, 2016). Traps are placed in areas away from other competent light sources (including moonlight, street and house lights) suspended about six feet above the ground, in open areas near trees and shrubs. It shall be placed at a distance of 30 feet or more from buildings. It shall not be affected by strong winds and sources of smoke or fumes. A block of dry ice (CO₂) wrapped in several layers of newspaper or in a padded envelope suspended above the trap is the common attractant used. The trap shall be operated from just before dark until just after daylight.

CDC miniature light traps, New Jersey Light Traps, BG-Sentinel traps, etc. are commonly used. BG-Sentinel traps are designed to attract *Aedes aegypti* and *Aedes albopictus* with a specific chemical lure (BG-Lure or Sweetscent). Their effectiveness can be increased by baiting the trap (e.g. a mouse in a cage) or by adding a carbon dioxide source which makes the trap attractive to a wide range of mosquito species (e.g. *Culex pipiens* and *Anopheles plumbeus*). There are other kinds of traps like Window trap and Magoon trap which do not need a light source.

- b) **Gravid Traps/ Oviposition Traps:** They selectively sample blood-fed females. These traps consist of a black bucket/cup filled with water, hay or an infusion of dead leaves and are designed to collect gravid females that fed at least once and need to oviposit (EFSA, 2018). The CDC gravid mosquito trap is one example where it attracts gravid females searching for a place to lay their eggs (oviposit) and collects them in a net similar to the miniature light trap. Since the mosquitoes collected in this trap have already fed at least once, these individuals are more likely to be infected.

Conservation of Specimens

It is very important to conserve them in the right manner while transporting them to the laboratory for further processing, especially if they are sent to taxonomists for identification.

Larvae

- a. For immediate morphological or genetic identification: collect in vials with 70–80% ethanol.
- b. For morphological identification after further development: collect in vials/small containers with water taken from its breeding place for rearing L1-L3 larvae to L4 larvae (which are identified with higher reliability) or for keeping the larvae until adult emergence.

Adults

Tightly closed sampling nets with mosquitoes are transported to the laboratory for further processing (if possible in dry-ice containers)

- c. For morphological identification (females, males): males in vials with 70–80% ethanol (for genitalia); females pinned as soon as possible in insect boxes (if not possible, keep frozen; then pin in the lab).
- d. For blood meal analysis (freshly blood-fed females): abdomen squashed on filter paper (ELISA and/or PCR) or in vials with 70–80% ethanol (PCR detection + gene sequencing).
- e. For pathogen detection (females): frozen or in vials with 70–80% ethanol (depending on the pathogen and subsequent techniques, do not use ethanol if virus detection is foreseen), collected every day.
- f. For detection of insecticide resistance gene (e.g. knockdown resistance) (females and males): in vials with 70–80% ethanol.

Specimen Submission for Identification

When specimens are submitted for taxonomic identification, Date and time of day of collection, name and phone number of collector and detailed information on locality/habitat must be included with the specimens.

Dengue Vector Sampling

Flight range studies suggest that most female *Ae. aegypti* may spend their lifetime in or around the houses where they emerge as adults and fly an average of 400 metres. For adult surveillance, human biting catches are not recommended for dengue vectors. Sentinel sites are established in areas endemic or epidemic for dengue. These are surveyed at least monthly during the dengue season. As per the guidance of WHO, the following indices are used to monitor *Aedes* population for dengue virus transmission (Sanchez *et al.*, 2006), (Abdalmagid and Alhusein, 2008), (Erlanger *et al.*, 2008)

Larval Surveys

1. House index (HI): percentage of houses infested with larvae and/or pupae.
2. Container index (CI): percentage of water-holding containers infested with larvae or pupae.

3. Breteau index (BI): number of positive containers per 100 houses inspected.

Pupal Surveys

1. Pupae index (PI): number of pupae per 100 houses inspected.

Conclusion

Comprehensive public health measures encompassing disease surveillance, vector surveillance and control measures with support from all sectors of the community are required to combat the old and newly emerging vector-borne diseases. Rather than relying on emergency response, monitoring and surveillance throughout the year allow timely detection of changes in abundance and species diversity of vectors providing valuable knowledge to health authorities, scientific community and entities that can manage vector populations below the threshold level, reducing their impact on public health. The short-term and long-term changes in the population are predicted by correlating the population dynamics of the species and the weather conditions. It is also important to understand the fluctuation in vector population according to the seasonal activity, to improve our control programs (Mohiddinet *al.*, 2015). The vectors collected via field sampling can also be used to identify new deadly viruses using advanced technologies in different laboratories around the world.

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