Entomological surveillance for *Aedes spp.* in the context of Zika virus

Interim guidance for entomologists

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Introduction

Entomological surveillance of *Aedes* mosquitoes is used for operational (and research) purposes to determine changes in geographical distribution, for monitoring and evaluating control programmes, for obtaining relative measurements of the vector population over time, and for facilitating appropriate and timely decisions regarding interventions. There are a number of methods for monitoring vectors (mostly *Aedes aegypti*) of arboviral diseases. However, the selection and use of a method requires a clear understanding of the surveillance objectives, the availability of skills and resources, and in some instances the level of infestation.

Surveillance may serve to identify areas of high-density infestation or periods of increasing mosquito populations. In areas where the vector is no longer present, entomological surveillance is critical in order to rapidly detect new introductions, before they become widespread and difficult to eliminate. Monitoring of the susceptibility of the vector population to insecticide should also be an integral part of any programme that uses insecticides. Separate guidance on monitoring and managing insecticide resistance in *Aedes* populations can be found at http://www.who.int/csr/resources/publications/zika/insecticide-resistance.

This document describes selected sampling methods that can be used to conduct surveillance of *Aedes* mosquitoes, pupae and oviposition. It is intended for qualified entomologists at national and sub-national level who are responsible for the surveillance of local *Aedes* populations.

Sampling larvae and pupae

For reasons of practicality and reproducibility, the most common survey methodologies employ larval (active immatures, including pupae) sampling procedures rather than egg or adult collections. The basic sampling unit is the house or premise, which is systematically searched for water-holding containers.

Containers are examined for the presence of mosquito larvae, pupae, and larval and pupal skins. Depending on the objectives of the survey, the search may be terminated as soon as aedine larvae are found, or continued until all containers have been examined. Laboratory examination is usually necessary to confirm the species. The following three indices are commonly used to record *Aedes* infestation levels:

- House (premise) index: percentage of houses infested with larvae and/or pupae.

$$\frac{\text{Infested houses X 100}}{\text{Houses inspected}} \quad \text{(HI)}$$

- Container index: percentage of water-holding containers infested with larvae or pupae.

$$\frac{\text{Containers positive X 100}}{\text{Containers inspected}} \quad \text{(CI)}$$

- Breteau index: percentage of positive containers in inspected houses.

$$\frac{\text{Number of positive containers X 100}}{\text{Houses inspected}} \quad \text{(BI)}$$

The house index has been used most widely for measuring population levels, but does not take into account the number of positive containers or the productivity of those containers. Similarly, the container index provides information only on the proportion of water-holding containers that are positive. The Breteau index establishes a relationship between positive containers and houses and is considered to be the most informative, but also does not consider container productivity. Nevertheless, in the course of gathering the basic information to calculate a Breteau index, it is possible (and highly desirable) to also obtain a profile of the characteristics of the larval habitat by recording the various container types either as potential or actual sites of mosquito production (e.g. the number of positive drums per 100 houses, the number of positive tyres per 100 houses). These data are particularly relevant for focusing larval control efforts on the management or elimination of the most common habitats and to orientate educational messages for community-based initiatives (2).

It should be noted that larval indices are a poor indication of adult production. For instance, the rate of emergence of adult mosquitoes from rainwater drums is likely to differ markedly from the rate from discarded cans or house plants, yet larval survey will register these only as positive or negative. Thus for localities with similar larval indices but different container profiles, adult abundance and hence transmission potentials may be quite different.

Pupal/demographic surveys

If the types of containers with the highest rates of adult mosquito emergence are known in a community, they can be selectively targeted for source reduction (e.g. elimination) or other vector control interventions to optimize the use of limited resources (3). A pupal/demographic survey is an operational research tool to identify these most epidemiologically important types of containers.

Unlike the traditional *Stegomyia* (*Aedes*) indices described above, pupal/demographic surveys measure the total number of pupae in different classes of containers in a given community. Such surveys are far more labour-intensive than the larval surveys previously described, and are not envisaged for routine monitoring of *Aedes* populations. The collection of demographic data enables the calculation of the ratio between the numbers of pupae (a proxy for adult mosquitoes) and persons in the community. There is growing evidence that (3), together with other epidemiological parameters such as dengue serotype-specific sero-conversion rates and temperature, it is possible to determine the level of vector control needed in a specific location to inhibit virus transmission. This remains an important area for research, with potential for public health application.

Passive collection of larvae and pupae

Funnel traps have been used for sampling *Aedes* species and other container-breeding organisms in sites with poor or difficult access, such as wells (4). The funnel trap is comprised of a weighted funnel attached to a bottle that inverts on entry to and exit from a water surface where it floats. The device collects organisms such as fish, copepods, mosquitoes, ostracods and tadpoles as they return to the surface. Calibration of the device, using known numbers of *Aedes* larvae, enables the size of the larval population to be estimated (5). In some locations the device has focused attention on the importance of subterranean habitats and harbourages during winter or in dry conditions (6). The funnel trap captures a lower proportion of pupae because they are less active than larvae.

Quantification of the funnel trap allows results to be compared with larval counts in other containers and estimates to be made of the relative importance of the various types of containers. However, there is no way to relate funnel trap captures to the risk of transmission because there is no direct relationship between larval densities and density-dependent larval survival.

Sampling adult mosquito populations

Adult mosquito sampling can provide valuable data for studies of seasonal population trends or evaluation of adulticiding measures. However, results are less reproducible than those obtained from sampling immature stages. The methods for collecting adult mosquitoes also tend to be labour-intensive and depend heavily on the collector's proficiency and skill. Backpack battery-operated aspirators and baited traps may also be useful to estimate adult mosquito densities.

Resting collections

During periods of inactivity, adult *Aedes* typically rest indoors, especially in bedrooms and dark places such as clothes closets and other hidden sites. Resting collections involve the systematic searching of these sites with the aid of a flashlight and the capture of adults using mouth- or battery-powered aspirators and hand-held nets. Backpack aspirators powered by rechargeable 12-volt batteries have proven to be an efficient and effective alternative means of collecting resting adult mosquitoes in and around human habitation. Following a standard collection routine, densities are recorded as the number of adult mosquitoes per house (females, males, or both) or the number of adults collected per hour of effort. Where infestation levels are low, the percentage of houses positive for adults is sometimes used.

Sticky trap collections

Various sticky trap devices have been used for sampling adult *Aedes*. They may be designed to be visually attractive, odour-baited, or both, or may simply be located at constricted access points through which adult mosquitoes pass (e.g. points of exit and entry from subterranean habitats such as keyholes in service manhole covers in roads). Age and viral infection have been determined in adult mosquitoes collected with sticky traps, though mainly in research contexts.

Sampling oviposition populations

Oviposition traps

These devices, also known as "ovitraps", constitute a sensitive and economical method to detect the presence of *Aedes* in situations where infestations are low and larval surveys are generally unproductive (e.g. when the Breteau index is < 5). They have proved especially useful for the early detection of new infestations in areas where the mosquito has been previously eliminated. For this reason, oviposition traps are useful for surveillance at international ports of entry which, in accordance with international sanitary codes, should be kept free of vector foci.

The standard ovitrap is a wide-mouth 0.5 litre glass jar painted black on the outside and equipped with a hardboard or wooden paddle that is clipped vertically to the inside with a roughened side facing inwards. The jar is partially filled with clean water and is appropriately placed in a rain-sheltered site – usually outdoors and close to habitation.

Ovitraps are usually serviced weekly and the paddles are examined for the presence of *Aedes* eggs. The percentage of positive ovitraps provides the simplest index of infestation levels. In more detailed studies, the eggs on each paddle are counted and the mean number of eggs per ovitrap calculated. Ovitraps with plant germination paper as a substrate for egg deposition can also be used. For accurate interpretation, field records must indicate the location of each ovitrap and its condition at the time of servicing. If a trap is flooded, dry, missing, or overturned, the data should be discarded.

Ovitraps are inexpensive and can be installed and serviced over large areas relatively quickly. They can also be used by people without specialized training.

While ovitraps can be used to monitor changes in oviposition activity over time, comparisons between areas are not reliable because the availability of larval habitats in which females can lay eggs will differ. Similarly, it can be misleading to monitor and interpret ovitrap data over time in a given area where vector control interventions, including source reduction measures, may be carried out.

Tyre section larvitraps

Tyre section larvitraps of various designs have also been used to monitor oviposition activity, with the simplest of these being a water-filled radial section of a tyre. A prerequisite for any tyre section larvitrap is that the water can be visually inspected in situ, or that the contents can be readily transferred to another container for examination.

Tyre larvitraps differ functionally from ovitraps in that water level fluctuations caused by rainfall induce the hatching of eggs, and it is the larvae that are counted rather than the eggs deposited on the inner surfaces of the trap. The usefulness of tyre section larvitraps as an alternative to ovitraps for early detection of new infestations and surveillance of low-density vector populations has been well demonstrated. (1)

Operational priorities for surveillance

In the context of Zika virus, the following operational priorities for entomological surveillance may be considered.

A. Countries without *Aedes*

- Enhance surveillance of mosquitoes at border areas.
- Monitor imported goods (e.g. used tyres, plants), from countries endemic with / receptive to *Aedes,* by quarantine measures to avoid entry of invasive species of mosquitoes. Ovitraps can be used for this surveillance.
- Implement vector surveillance and control at points of entry – as per the International Health Regulations (2005) – emphasising non-chemical interventions such as source reduction.

B. Countries with *Aedes*, but no evidence of Zika virus circulation

- Establish sentinel surveillance of *Aedes* and collect data regularly. If any increase in *Aedes* density is detected, promptly target breeding sites with source reduction in a radius of 400 metres, and community awareness activities.
- Ensure placement of contingency stocks of nationally approved insecticides and equipment to respond to potential outbreaks of arboviruses.
- Develop adequate capacity, skills and equipment for control, and ensure availability of funds to respond to potential outbreaks of arboviruses.
- Identify local areas with high densities of *Aedes.*
- Prioritise the most productive breeding sites and target control measures.
- Aim for zero breeding sites in low-density areas and prevent expansion of the vectors to other areas by rapid control activities in the vicinity.
- Maintain constant monitoring of vector density through surveillance programmes. All efforts must be made to maintain vector density at a low level.

C. Countries with *Aedes* and evidence of Zika virus circulation

- Establish sentinel surveillance of *Aedes* and collect data regularly. Surveillance data should reflect trends and impact of control measures.
- Develop adequate capacity, skills and equipment for control, and ensure availability of funds to manage the outbreak.
- Identify local areas with high density of *Aedes.*
- Prioritise the most productive breeding sites and target control measures.
- Encourage community involvement to target smaller breeding sites in and around houses once a week.
- In the event of a large outbreak, enhance control to include targeted adult control measures such as fogging, along with larval control measures.
- Develop key messages for communication to the community. Target messages for schools and other community groups and organisations to support the campaign.

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