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

Interim guidance for entomologists

World Health Organization

WHO/ZIKV/VC/16.2

Acknowledgements: This document was developed by staff from the WHO Department of Control of Neglected Tropical Diseases (Raman Velayudhan, Rajpal Yadav) and Global Malaria Programme (Abraham Mnzava, Martha Quinones Pinzon), Geneva.

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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.

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

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

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

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

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

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.