# Module 5

# Dengue/ Chikungunya (CHK)/ Zika – Vectors

Learning Objectives :

At the end of the module, the participant will be able to understand:

- Dengue/ Chikungunya (CHK)/ ZIKA Disease
- Vectors and its Biology
- Aedes aegypti
- Aedes albopictus
- Life Cycle
- Morphological differences between Ae. Aegypti, Ae. Albopictus and Ae. vittatus
- Vector Surveillance
- Entomological Indicators
- Adult Vector Control
- Larval Vector Control

## Introduction

Dengue Fever and dengue haemorrhagic fever (DF/ DHF) are caused by dengue viruses belonging to the genus Flavivirus, family Flaviviridae. There are four antigenically related, but distinct dengue virus serotypes (DEN-1, DEN-2, DEN-3 and DEN-4), all of which can cause DF/ DHF. All four serotypes have humans as the vertebrate host and *Aedes (Stegomyia) aegypti (L.)*, and *Ae. albopictus* as invertebrate hosts.

A global pandemic of DF began in South East Asia after Second World War and has intensified greatly over the last 10-15 years. More than 2.5 billion people are at risk of infections in over 100 countries worldwide. There are probably tens of millions of cases each year and at least five hundred thousand cases of DHF with a mortality of about five per cent in most countries. In many countries of South East Asia, DHF has become a leading cause of hospitalization and death among children; it is continuing to spread geographically within the region and its incidence is increasing.

The importance of *Aedes aegypti* as the principal transmitter of dengue was confirmed beyond doubt in 1916 in Australia by Cleland et al. Many details of dengue transmission by *Ae.aegypti* as well as the incrimination of a second vector, *Ae. albopictus*, were reported in an extensive series of human volunteer experiments carried out in the Philippines in the 1920 by US Army researchers.

## Chikungunya

Chikungunya is a mosquito-borne viral disease that causes fever and severe joint pain. The disease was first recognized in 1952 during an outbreak in southern Tanzania. It is a ribonucleic acid (RNA) virus that belongs to the alphavirus genus of the family Togaviridae. The name “chikungunya” derives from a word in the Kimakonde language of southern Tanzania, meaning “to become contorted”, and describes the stooped appearance of sufferers with joint pain (arthralgia).

Chikungunya is transmitted to humans by the bites of infected female mosquitoes. Most commonly, the mosquitoes involved are *Aedes aegypti* and *Aedes albopictus*. These two species can also transmit other mosquito-borne viruses,

including dengue. They bite throughout daylight hours, although there may be peaks of activity in the early morning and late afternoon.

The disease occurs in Africa and Asia, although imported cases have been recorded in the WHO European Region and the Region of the Americas. Over 2 million cases have been reported since 2005.

## Zika

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## Yellow Fever

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### *Aedes aegypti*

**Distribution, Ecology and Bionomics**

Aedes aegypti is strongly believed to have originated from Africa where they spread to the Western hemisphere in the seventeenth century, to the Mediterranean basin in the eighteenth century, to tropical Asia in the nineteenth Century and finally to the Pacific Islands in the late nineteenth and beginning of the twentieth centuries.

**Eggs**

The eggs are approximately 1 mm long and pale white, turning to an intensely black colour within a short time. They are elongated/ oval-shape and under the microscope appear somewhat cigar-shaped, with one end rather thicker and more abruptly tapered than the other (Figure 14). Fertilized eggs are deposited singly on the moist walls of the containers and the embryo develops within a few hours. The eggs are capable of withstanding desiccation for longer periods of upto 1 year. Females prefer to lay eggs in the afternoon on a surface with a high degree of dark colour, roughness and water absorption. During oviposition, the females often deposit eggs in several containers in the households.

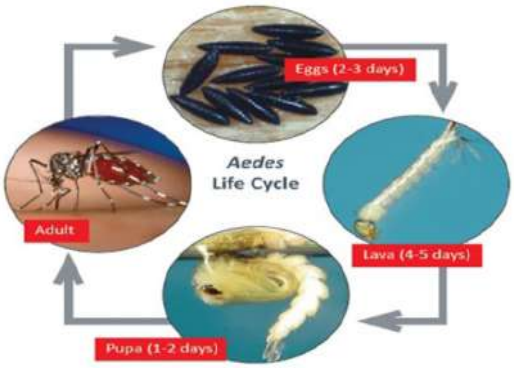


**Figure 14.Life Cycle of Dengue Vector**

**Larval Habitats**

*Aedes aegypti* predominantly breeds in desert coolers, water storage tanks, clay pots and a varied variety of other receptacles used for storing water (Figure 15). Breeding has also been observed in flower vases, pans/ trays used for collecting water from fridges, used tyres, unused wells, broken cans and other items put to disuse by the community. The larval development is completed within a week but this span can be shortened and or prolonged depending on the environmental conditions.


**Figure 15.Breeding Habitats for Dengue Vector**

**Larvae and Pupae**

Larvae hang almost vertically at the water's surface and swim with a distinct looping movement. When disturbed they swim to the bottom of the container. The larvae feed on particles of organic matter present on the bottom or sides of containers by pharyngeal filtration of minute particles using fan-like brushes. The larvae also browse on the bottoms of sides or the containers, detaching matter from the surface over which they are gliding.

*Aedes larvae* can be distinguished by the naked eye from most other genera. The siphon is shorter than in other culicines (it is lacking in anopheles). After hatching from the egg, the larvae undergo three successive moults. The fourth ecdysis or pupation gives rise to the pupa, which does not feed but actively swims and floats. Duration of the larval stage is 7–9 days at 25°C and that of the pupal is 2–3 days at the same temperature.

**The Adult mosquito**

The adult mosquito, after emergence, rests on the walls of the breeding site for a few hours to allow the exoskeleton and wings to harden. Approximately 24 hours after emergence both sexes can mate and females can take a blood meal. These two activities often take place simultaneously since both males and females are attracted towards vertebrate hosts due to body odours. Wing beat frequency of the unfed females and the sound generated may also be attractive to the males. Generally, once inseminated, the females do not mate again.

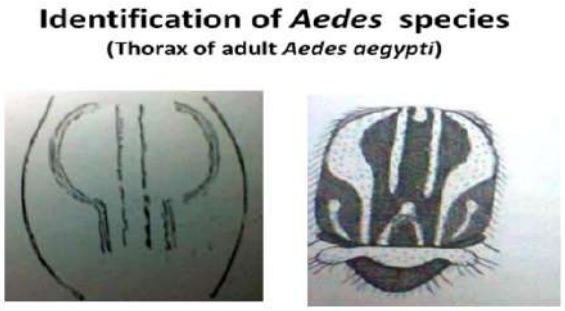


**Figure 16.Identification of *Aedes aegypti (Adult)***

The adult *Aedes aegypti* can be easily identified with sauce shaped (white) structure on the thorax region of adult mosquito (Fig. 16). Biting is observed in two peaks after dawn and dusk each lasting for about 1 hour with a maximum flight range of about 100 metres. The 'maximum biting' activity takes place at 20–28°C with relative humidity ranging from 60–90%. At 16°C and below they do not feed and at lower temperatures they die. The most frequent mode of dispersal is through eggs and larvae. Adults rest in human dwellings in dark and undisturbed sites. The average life span of the adult male & female is 3–6 and 08–15 days respectively.

***Aedes albopictus***

*Aedes albopictus* is primarily an Asian species that until recently remained restricted to the Indian sub-continent, South East Asia, China, Japan, and Indonesia islands in the Indian ocean and since the beginning of this century the Hawaiian islands. In the last 10 years, however, the species has spread as a result of the intercontinental shipment of used tyres to the USA. Brazil, Mexico, Guatemalan, EI Salvador, Colombo, Bolivia, Nigeria New Guinea and even to South of Europe (Albania and Italy). *Aedes albopictus* is primarily a species found in forested areas and where there is natural vegetation. The species has also now adapted to urban environments. *Aedes albopictus* is also prevalent in more northern latitudes than in *Ae. Aegypti*. Besides tree holes, plant axils, cut bamboo stumps and opened coconuts which constituted the original habitats, *Ae. Albopictus l*arvae now use outdoor artificial, containers such as water storage barrels and trash receptacles. This diversity of larval habits explains the abundance of this species in rural areas as well as in peri-urban localities and parks in cities. Nonetheless in the centre of large cities, it is usually replaced by *Ae. Aegypti* which is better adapted to urban ecosystem.

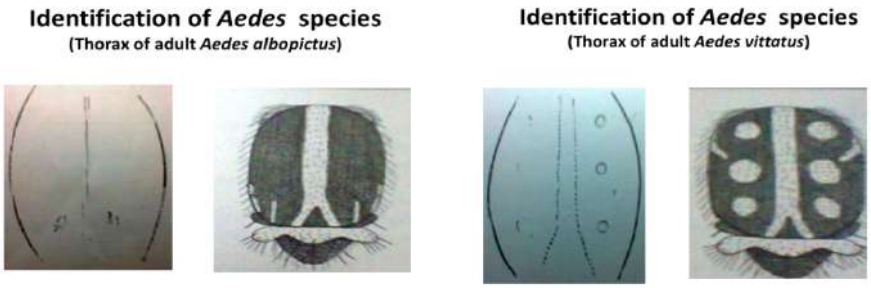


**Figure 16.Identification of *Aedes albopictus* and Aedes vittaus (Adult)**

Adults of *Ae.albopictus* are easily distinguished from those of *Ae. aegypti* by distinct markings on the dorsum of the mesonotum. *Ae.albopictus* can be easily distinguished from *Ae. aegypti* with the presence of a straight slit line (white) between the thorax, while in the *Ae. vittaus* three dots on both sides of the straight slit are present (Figure 16). Ae. albopictus received its nickname, "tiger mosquito" in South East Asia due to its median silvery line extending from the head to the dorsum and its distinct tarsal bands. It is considered an aggressive and a colourful mosquito (Figure 17).

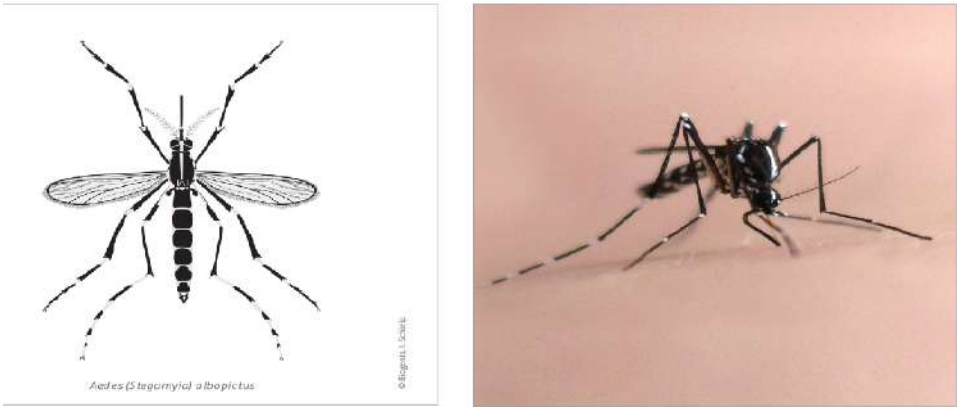

**Figure 17.Adult *Aedes albopictus* (Morphological character on thorax)**

## Larval Biology and Habitats

Larvae can be easily distinguished, under a dissecting microscope, from those of *Ae. aegypti* by the different shapes of the comb scales and pectin teeth in the terminal segments. *Aedes albopictus* also has four ventral brushes in contrast to the five which are found in *Ae. aegypti.* Larvae are similar in their swimming action and behaviour.

*Aedes aegypti* inhabits domestic premises while *Ae. albopictus* remains outdoors exhibiting two biting peaks one in the early morning and the other in the late afternoon.

## Ecology and Seasonal History

The eggs of *Ae.albopictus*, as also those of other Stegomyia, can withstand long periods of dryness. Its duration of the life cycle, from oviposition to emergence of adults may range from slightly over one week to six or seven weeks. It has been suggested that *Ae. albopictus* has a lower fecundity and longer life cycle than *Ae. aegypti,* thus giving the latter species a biological advantage.

## Vector Surveillance and Epidemiological Interpretation

The main purpose of vector surveillance is to generate entomological data to assist entomologists and other health authorities concerned with its control in the following:

- Identifying the major breeding sources of potential and actual vectors in domestic and peridomestic environments;
- Division of areas according to density measurements to allow earmarking of high-risk areas and areas with high Aedes densities and plotting of vector distribution and DHF cases on maps to decide on priorities for control measures;
- Determining seasonal fluctuations of the populations of vector, to ensure alertness during peak period;
- Providing information for prevention of outbreaks of DHF by relating vector indices to other epidemiological information, so that action for control of the disease can be taken in time;
- Determining any changes in vector density, distribution and/ or vectorial capacity to ensure proper strategies for control; and
- Generating data on susceptibility/ resistance of vectors to insecticides.

## Surveillance Tools

1. **Aspirator Suction Tube:** It uses simple suction force to collect the resting adult mosquitoes. It has a 30–45 cm long collection tube made up of 0.5" OD polycarbonate plastic or glass. And 80-95cm suction silicone tube (Figure 18). A nylon mesh screen is placed below the collection tube to prevent the entry of mosquitoes, and dusts to the mouth.

**Figure 18.Aspirator Suction Tube**

2. **Mechanical Aspirator:** It uses suction pressure generated by the motor to trap the resting adult mosquitoes in the collection tube. It has a detachable inlet and collection tube with battery-powered aspirator (Fig.19). A nylon mesh screen is placed below the collection tube.Dimension of the handle - length x diameter = 200 x 49 mm.

- Dimension of inlet tube - length x internal diameter = 153 x 9 mm.
- Dimension of collection tube - length x internal diameter = 50 x 28 mm.
- It is powered by two D-cell batteries.


**Figure 19.Mechanical Aspirator**

3. **Prokopack Aspirator:** It consists of blower-cum-suction fan, a chip-based controller, a telescopic handle, a battery (4–12v with 6.5–12 Ah), and a utility bag to carry the items (Figure 20). Adult mosquitoes are trapped due to suction force in the mesh screened collection cups which is placed in the collection side of the chip-based controller. The angle and speed of the fan are adjustable.


**Figure 20.Prokopack Aspirator**

4. **BG sentinel Trap:** The trap uses visual and olfactory cues (e.g. CO2, BG-Lure®- lactic acid, carporic acid). The diameter is 36 cm (14 inches), the height is 40 cm and it is collapsible. It has a blue fabric container with a white lid and a black entrance funnel in the middle. BG-Lure® was placed in the opening adjacent to the middle black funnel (Fig. 21). Inside, there is a small electrical fan that sucks air through the trap and draws any approaching mosquitoes into a catch bag. The BG-Sentinel trap requires a large 12 V batteries or mains power for operation.


**Figure 21.BG sentinel trap**

The methods of vector surveillance presently recommended by the WHO DHF advisory Committee are those of landing or resting rates for adult mosquitoes as well as larval indices such as the House Index and/ or Breteau Index. The traditional larval indices have several shortcomings. Although it has been shown that there is a strong correlation between the different types of larval index and it is possible to have conversion factors between House, Breteau and container indices, the significance of these indices in terms of abundance of adult mosquitoes and actual man-mosquito contact,

and thus their use as tools for forecasting outbreaks or mapping areas of risk, may vary from one area to another. The larval indices give a reasonable distribution and degree of the relative prevalence of *Ae. aegypti*.

The other factors which govern epidemics of DHF are those of vector competence, the virulence of the virus and the immunity status of the population as a whole. If vector surveillance is to be sued to delineate areas of transmission risk, forecast epidemics and plan intensive control measures, any such surveillance should be carried out in conjunction with the assessment of these other factors.

## Epidemiological Interpretation of Surveillance Data

It is quite challenging to cut off the density figure for *Ae. aegypti* or Ae. albopictus at which no transmission will occur. However, the usefulness and significance of an estimated threshold density cannot be over-emphasized. Entomologists and health officers should try to work out, in their localities, possible threshold vector densities required for transmission of dengue hemorrhagic fever. Constant monitoring of this index through routine surveillance enables epidemiologists to predict the trend of the disease and vector control officers to intensify control efforts when the suspected critical level is reached. In actual practice, there are, unfortunately, situations where low vector indices are not maintained and where the disease remains endemic on a year-round basis.

## Methods of Survey

Surveillance of vectors is an essential step in the planning of control measures and their evaluation, and in studies to determine the risk of an outbreak of dengue/ DHF. Surveys areas are also necessary for studying the ecology and distribution of vectors.

Surveys enable information concerning the presence of vectors, their frequency of occurrence, their abundance and distribution in time and space their movements including migration, and their establishment in other areas to be obtained. These surveys also assist in the stratification of areas where outbreaks of dengue fever can occur. Vector surveillance should be routine and includes the monitoring of ecological and epidemiological parameters from both the virological and entomological points of view. The objectives of these surveys have been summarized as follows:

- To pinpoint high-risk areas (areas with high vector density and high disease endemicity) through the plotting of vector distribution and DHF cases on maps, so that these areas can serve as priority areas for control during both normal and epidemic conditions;
- To detect, through routine surveillance, any changes in vector density, distribution or other epidemiological parameters relating to the vectorial capacity of the vectors;
- To determine the seasonal population fluctuations of the vectors so that special emphasis can be given to the maintenance of control and alertness during peak periods, and
- To determine the major breeding places in domestic environments so that source reduction or elimination, with public reduction or elimination, with public participation, can be carried out through health education campaigns and law enforcement.

## Larval Surveys

Due to the nature of the larval habitats and the ease with which larvae can be collected, larval surveys are commonly used for Aedes species and involve the collection of larvae or pupae. The immature stages are collected from water-holding containers found both inside and outside houses. Information concerning the locality, date of survey, precise location and classification of the container or source is carefully recorded. Receptacles which are negative for immature stages on examination are also recorded. The exact format of such records will vary from place to place depending on the type of breeding site and the purpose of the survey. Larval surveys may be of three types:

- Those concerned with all larvae, or a number of larvae, from all positive containers;
- Those concerned with a single larva from each positive container, and visual.

To carry out the above, which results in a saving of time for field workers, detailed information about the composition of the *Aedes* species breeding in the containers should be available. Visual larval surveys and the one larva per container survey are only accurate when one species, eg. *A.aegypti*, is found, as is the case in some urban areas.

## Larval Indices

The commonly used larval indices are as follows:

House or premises index

This is the percentage of houses or premises with one or more habitats positive for *A. aegypti* or related species. It is calculated as follows:

$$\text{House or premises index} = \frac{\text{No. of infested houses}}{\text{No. of inspected houses}} \times 100$$

$$\text{Container index} \quad \text{Percentage of containers Infested} = \frac{\text{No. of infested containers}}{\text{No. of inspected containers}} \times 100$$

In examining the containers, only those which have water in them are counted.

Breteau index

Originally this index was used in connection with A. aegypti, and

$$= \frac{\text{No. of infested containers}}{\text{No. of inspected houses}} \times 100$$

The *Breteau* index is generally considered the best of the commonly used indices (such as the House or premises Index and the Container Index) since it combines dwellings and containers and is more qualitative and of more epidemiological significance.

The House Index is the most frequently used and understood. It also involves less labour because, when the first positive container is located in a house, there is no need to proceed further. This index does not take into account the number of positive containers in an infested house. The House Index gives an idea of the percentage of houses positive for vector breeding and hence the percentage of the population at risk. If the index is high, transmission occurs easily to neighbouring houses, and if the index is low transmission occurs less rapidly.

The Container Index, although not so useful from an epidemiological point of view, is a useful comparative figure, especially when the evaluation of control measures is being carried out.

## Adult Surveys

Since only adult mosquitoes are involved in disease transmission and control measures may be directed against adults, it is important to carry out surveys for adult mosquitoes as well. In such surveys, the mosquitoes are collected by aspirators either while they are resting or when they are at the bait. The dengue vectors are generally anthropophagic, hence animal baits are not used. The following adult indices are generally measured.

- **House Density Index:** The number of female mosquitoes per house, or the number of females per house per unit of time.
- **Biting Rate Index:** The number of female mosquitoes taken at bait per unit of time.
- **Net Index:** The number of female mosquitoes caught per man hour of collection by net.

These indices are good for domestic or intradomiciliary species, especially the House Density Index. This is obtained by collecting all adult mosquitoes or collecting indoor resting mosquitoes during a unit of time eg. 15 minutes, using an aspirator (mouth or battery powered) torch light or hand sweep-nets. Adult *A. aegypti* are generally found resting on hanging objects, such as clothing, shady and dark corners on the wall, and furniture in bedrooms and other rooms. These results are expressed as the number collected per man hour or the number collected per hour or the number collected per house. The mosquitoes are identified as to species and sex. Catching stations can be selected at random or,

when specific or comparative studies are to be made, fixed catching stations can be employed. Too frequent collection can reduce the population and affect the purpose of the survey.

### Biting or Landing Catches

*Aedes* mosquitoes can also be collected on human baits. The worker can collect mosquitoes from his own body (exposed legs) or that of his helper. Generally, 15 to 20 minutes are spent in each room and an aspirator is used to collect all mosquitoes landing or biting. Based on the peaks of activity of Aedes, the collection should be carried out between 09.00 and 11.00 hours. The collector(s) should sit in a dimly-lit room and expose the legs up to the knees, removing shoes and socks. Male mosquitoes are very often collected in this kind of catch, but they are counted when calculating the biting or landing index.

To determine the best time to carry out these catches, it is appropriate to do some 12–14 hour catches, from before dawn until dusk. Should comparisons between the seasons or between pre- or post-spraying be required, all the conditions should be standardized.

Vector Control: The control of the dengue vector is to be planned and executed as per the IVM Strategy with the main focus on source reduction at the aquatic stage and other options of space spray for knockdown of adult mosquitoes as per details given below (Figure 22).

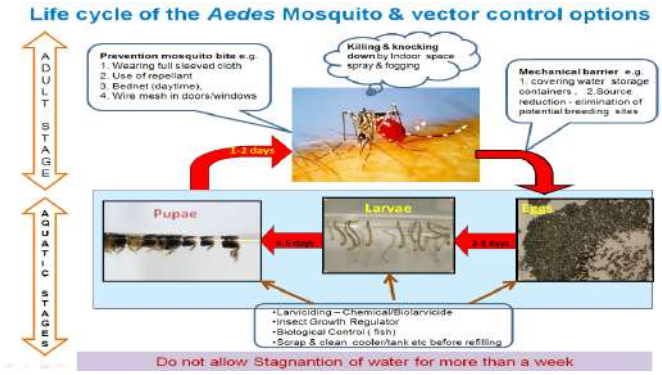


**Figure 22.Vector Control Options for Dengue Vector**

### Molecular Xenomonitoring

In the present scenario of dengue/ CHK transmission, it has been found that the entomological indices i.e. HI, CI and BI do not stand true for the prediction of any outbreak situations as per the threshold values indicated by WHO or different studies. There have been instances when there are reports of outbreaks with low entomological indices for dengue vectors in a given area. Therefore, molecular xenomonitoring needs to be done carefully to establish an early warning signal for dengue outbreaks.

Xenosurveillance (XS) is generally used when pathogens are screened in bloodmeals of mosquitoes where no replication occurs, and is defined as a surveillance technique that makes use of the haematophagous behaviour of some arthropods to survey vertebrates for the presence of pathogens. Xenosurveillance is a method that utilizes mosquitoes as sampling devices to search for genetic signatures of pathogens in vertebrates.

Molecular xenomonitoring (MX), the detection of pathogen DNA/ RNA in a vector as a proxy of human infection, was developed after significant advances in laboratory methods over the past two decades and can transform VBD surveillance. Molecular xenomonitoring (MX) is a disease surveillance technique that involves the collection and testing of hematophagous insects (such as mosquitoes, flies or ticks) to detect the DNA or RNA of a pathogen or parasite of human or animal health importance. In dengue/ CHK/ Zika, the threshold levels of entomological indices (CI, HI, BI) were holding a good few years back to give early warning signals for disease outbreaks. However, different studies held in different countries give different perspectives of entomological indices and do not stand fit unless validated at the field level. Hence, the time has come when the xenosurveillance tool can be of great help in the identification of viral activity among the mosquito population at a given point in time and thus, helping in planning the appropriate preparedness and control interventions.