Module 3

Part A - Malaria Vectors

Learning Objectives :

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

- Malaria - Disease transmission
- Vectors of Malaria
- Life Cycle of Anopheles Mosquito
- Morphological Characters of AnophelesMosquito
- External Anatomy of Adult AnophelesMosquito
- External Anatomy of Anopheleslarva
- Distinguishing Characters between Anopheline, Culex and Aedes
- Malaria Vectors: Biology & Taxonomic Characteristics

Malaria

Malaria is an acute febrile illness caused by *Plasmodium parasites,* which are spread to people through the bites of infected female *Anopheles* mosquitoes. There are 5 parasite species (*P.vivax, P.falciparum, P.malariae, P.ovale and P.knowlesi*) that cause malaria in humans, and 2 of these species – *P. falciparum and P. vivax – pose the greatest threat. P. falciparum* is the deadliest malaria parasite. According to the latest World malaria report, there were 241 million cases of malaria in 2020 compared to 227 million cases in 2019. The estimated number of malaria deaths stood at 627 000 in 2020 - an increase of 69 000 deaths over the previous year. Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected female Anopheles mosquitoes. It is preventable and curable.

About 95% population in India resides in malaria-endemic areas and 80% of malaria reported in the country is confined to areas consisting of 20% of the population residing in tribal, hilly, difficult and inaccessible areas. India contributes about 70% of malaria in the South East Asian Region of WHO. The high-burden populations are ethnic tribes living in the forested pockets of the states like Orissa, Jharkhand, Madhya Pradesh, Chhattisgarh and the North Eastern states which contribute to the bulk of morbidity and mortality due to malaria in the country. The cases have consistently declined from 2.09 million to 0.19 million from 2001 to 2020. Similarly, Pf cases have declined from 1.0 to 0.12 million cases during the same period. Less than 2000 deaths were reported during all the years within this period with a peak in 2006 when an epidemic was reported in NE States. The country's SPR has declined from 2.31 to 0.19 and its SFR has declined from 1.11 in 2001 to 0.12 in 2020. During 2021, a total of 161753 malaria cases were reported in the country with 90 deaths. In the year 2022 (till June), there is a report of 46809 malaria cases and 6 deaths as per the NCVBDC reports.

Malaria is a vector-borne and rains-related seasonal disease. India's geographic position and varied climatic conditions help and provide favour for the transmission of malaria. The relationship between rainfall, temperature and humidity and the presence of mosquito breeding habitats in different eco-epidemiological settings provide strong linkages in this regard. Shallow water pools, pits, rivulet pools, irrigation channels and projects may lead to the breeding of mosquitoes. Malaria consequent on such undertakings is called "man-made malaria", particularly in urban areas. The main variables of the human

elements that influence malaria epidemiology include the following: age, sex, race, socioeconomic development, housing, population mobility, occupation, human habits and immunity.

Vectors

Mosquito is an insect with two wings which belongs to:

Order: Diptera

Family: Culicidae

Sub Family:

A) Culicinae
B) Anophelinae
C) Toxorhynchitinae

These are characterized by distinct needle-shaped mouthparts consisting of the proboscis. The proboscis is used by the female mosquito for sucking blood. Anopheles mosquitoes most frequently occur in tropical and subtropical regions and are also found in temperate climates and even in the Arctic zone during the summer. Anopheles are not usually found at an altitude above 2,000-2,500 meters. Out of 58 species of anophelines in India, 9 species are proven vectors of malaria. *An. culicifacies, An. fluviatilis, An. minimus, An. dirus (baimaii), An. stephensi* and *An. sundaicus* (epiroticus) have been considered as primary vectors of malaria whereas three species are secondary vectors viz. An. annularis, *An. philippinensis* and *An. varuna* being of local importance in the transmission of the disease. *An. stephensi* is mainly involved in the transmission of malaria in urban areas whereas the remaining vectors play their role in rural areas. The distribution of major vector species of malaria is shown in Figure 1.

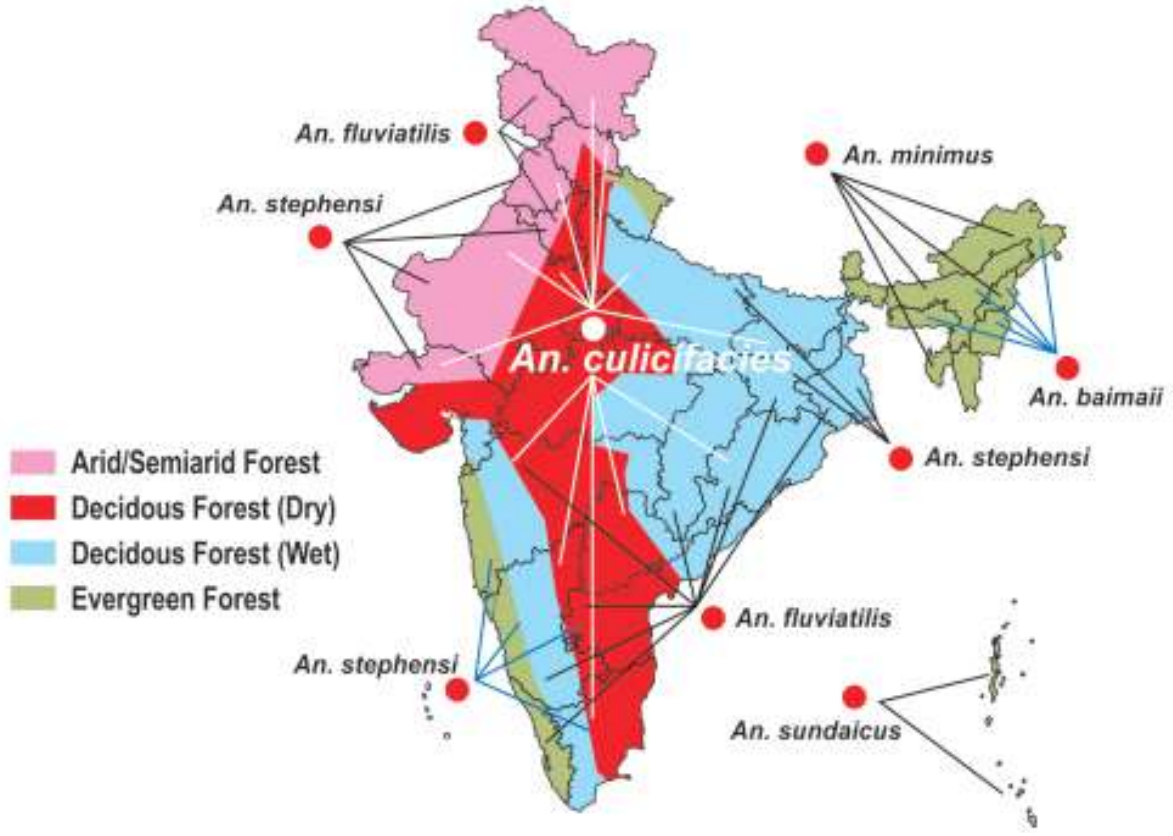


Figure 1.The distribution of major vector species in India

The following anophelines have been incriminated as vectors transmitting malaria.

- *Anopheles culicifacies*
- *Anopheles baimaii (An. dirus)*
- *Anopheles fluviatilis*
- *Anopheles minimus*
- *Anopheles stephensi*
- *Anopheles sundaicus*
- *Anopheles annularis*
- *Anopheles jeyporiensis*
- *Anopheles philippinensis*
- *Anopheles nivipes*
- *Anopheles varuna*
- *Anopheles maculatus*

(WHO 2007; Bhattacharyya et al. 2010; Rao 1984)

Out of the six primary vector species of anopheles, *An. culicifacies* is the main rural vector contributing 60-80% of total malaria. *An. stephensi* is the main urban vector. *An. fluviatilis* is the vector found in the foothill areas breeding in slow-moving streams rivulets. *An. minimus* and *An. baimai* are the main vectors of malaria in the northeastern states. *An. sundaicus* plays a role as the main vector in the coastal areas of the Andaman and Nicobar Islands. The other above-mentioned species may play the role of secondary vectors in the transmission of malaria. There are about 490 species of *Anopheles* mosquitoes including sibling species reported worldwide. Approximately 60-70 species worldwide can transmit malaria and of these, about 30 are vectors of major importance. Some anophelines prefer to bite animals and only rarely transmit malaria parasites to humans.

Life-cycleofanophelinemosquitoes

The life cycle of mosquitoes has four distinct stages: egg, larva, pupa and adult (Figure 2). The time taken for the various stages to develop depends on temperature and nutritional factors, with development more rapid at higher temperatures.

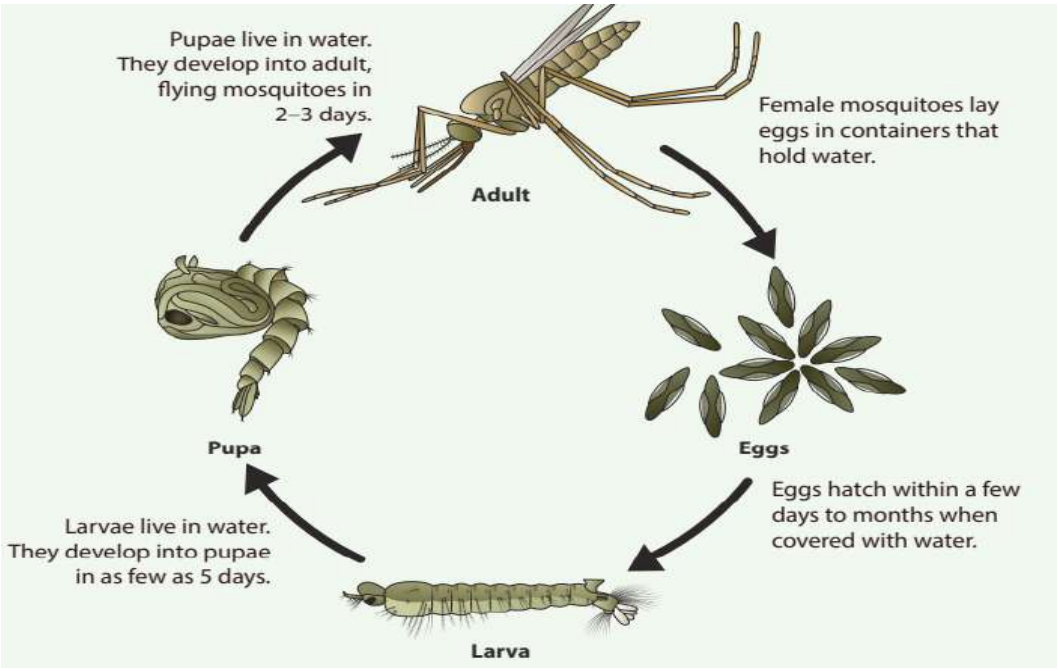


Figure 2.Life-cycle of an Anopheles mosquito

Eggs

A female anopheline mosquito normally mates only once in its lifetime and usually requires a blood meal after mating before the eggs can develop. Blood meals are generally taken every 2–3 days before the next batch of eggs is laid. About 100–150 eggs are laid on the water surface during oviposition. Oviposition sites vary from small hoof-prints and rain pools to streams, swamps, canals, rivers, ponds, lakes, rice fields, and sometimes even dirty water. Each species of mosquito prefers a particular type of habitat for oviposition.

Under the most favourableconditionsinthetropics, the average life spanoffemaleanopheline mosquitoes is 3–4 weeks.Afemale mosquito continues tolay eggs throughout life and most willlay 1–3 batches of eggs, though some may layas many as 7 batches.

Larva

Alarvahatchesfromtheeggafter 1–2 day sand generally floats below and paralleltothewater surface,whereitbreathesair.Itfeedsbyfilteringfoodparticlesfromthewater. When disturbed, the larva quickly swims down wards but soon needs to returnt othe surface to breathe.

There are four larval stages or instars. The small larva emerging from the egg is called the first instar. After 1–2 days it sheds its skin and becomes the second instar, followed by the third and fourth instars at further intervals of about two days each. The larva remains in the fourth instar stage for 3-4 more days before changing to become a pupa. The total time spent in the larval stage is generally 8-10 days at normal tropical water temperatures. At lower temperatures, the aquatic stages take longer to develop. Depending on the species, larvae may be found in small pools, fresh water, rice land, drains, ditches, running water with shade, brackish water, salt water, streams, ponds, lakes, marshes, wells, water containers, discarded tin cans, discarded tyres and hoof-prints.

Pupa

The pupa undergoes a major transformation, from living in water to becoming a flying adult mosquito. The pupa is shaped like a comma. It stays under the surface and swims down when disturbed. The pupae do not feed. The pupal stage lasts for 2-3 days after which the skin splits. The adult mosquito then emerges and rests temporarily on the water's surface until it flies.

Adult

Mating take splace soon after the adult emerges from the pupa. The female usually mates only once because sufficients permare received froma single mating for all subsequent egg batches. Normally the female takes the first blood-meal only after mating, but some times the first blood-mealis taken by young virgin females. The first batch of eggs develop safter one or two blood- meals (depending on the species) while successive batches usually require only one blood-meal.

The feeding and resting habits of mosquitoes are of great importance invector control programmes and they must be well understood. Most anopheline mosquitoes bite at night. Some bites hortly after sun set while others bite later, around mid night or the early morning. Some mosquitoes enter houses to bite and are described as being end ophagic; others bite mostly out door sandare called exophagic.

After taking a blood-meal the mosquitous ually rests for a short period. Mosquitoes that entera house usually rest on a wall, under furniture or on clothes hanging in the house and are said to beendophilic. Mosquitoes that bite out doors usually rest on plants, inholes, in trees or on the ground or in other cool dark places and are termedexophilic.

Host preferences are different for different species of mosquitoes. Some mosquitoes prefer to take blood from humans rather than animals and are described as being anthropophagic while others take only animal blood and are known as zoophagic. Those who prefer to take human blood are the most

dangerous as they can transmit infection in human populations. The adults can be found on vegetation, on solid surfaces in sheltered places, on the banks of streams and ditches, holes in rocks, culverts, cracks, caves, animal burrows, on the trunk of trees and on termite mounds.

External Characteristics of Mosquito

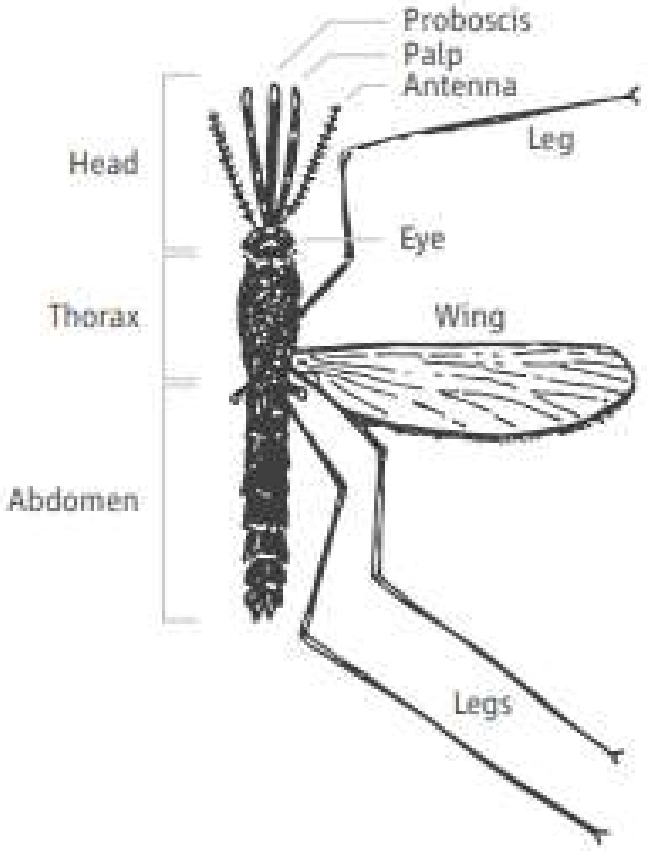


Figure 3.Body Parts of a Mosquito

Description

Mosquitoes can be recognized by the characteristics listed below.

The body has the following characteristics:

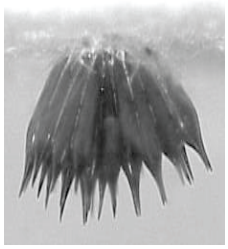
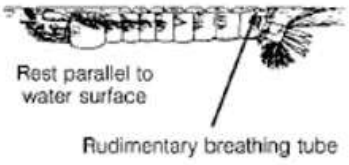
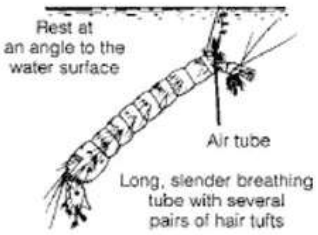
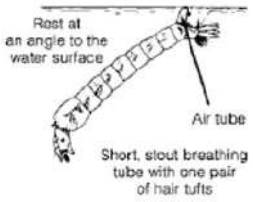
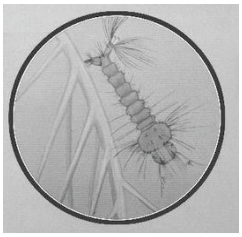
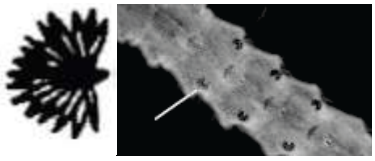
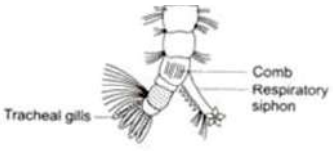
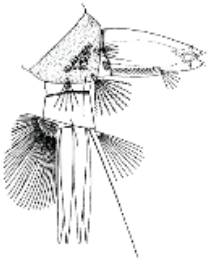
- The body is divided into three sections - head, thorax and abdomen
- The head has one pair of antenna, and a pair of compound eyes
- The thorax has three pairs of legs

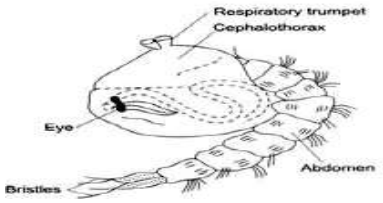
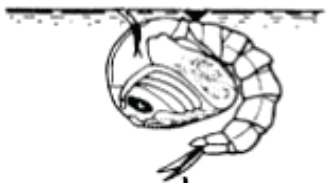
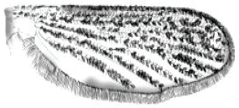
Class Insecta includes severalorders; mosquitoes belong to the order Diptera. Insects in this order have the following characteristics:

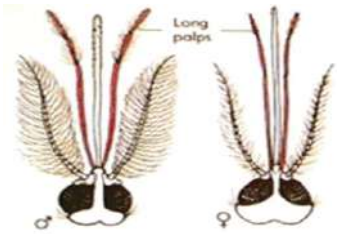
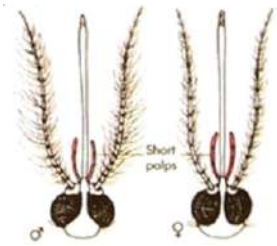
- The thorax has one pair of visible wings
- The hind wings, which are vestigial, are small movable filaments known as halters, which are mainly used for balance

Distinguishing Characteristics in Stages of Life Cycle among Different Vectors

The main taxonomic characteristics which differentiate among vector species of anopheline and culicine are shown below.

Anopheline	Culicine		
	Culex	**Aedes**	**Mansonia**
Eggs			
 Eggs laid singly, are boat shaped with lateral air floats	 Eggs laid in cluster with no lateral floats	 Eggs are cigar shaped with no lateral floats a laid either on the ground or above the water line	 Eggs are laid in star shaped clusters on the undersurface of leaves of aquatic plants
Larva			
 Floats horizontally parallel to water surface exclusively surface feeder Very active with swift movement	 Hangs down at an angle of 45° to the water surface with head downwards Whip-like or 'figure C' snake like movement	 Hangs down at angle of 45° to water surface with head downwards Characteristic 'S' shape or 'figure 8 ' movement, which involves all parts of the body	 Larvae are attached to the rootlets of aquatic plants by their siphon tubes. They obtain their air supply from these rootlets
 Presence of palmate/ float hair on abdominal segments	No palmate hair	No palmate hair	No palmate hair
 No siphon tube i.e., rudimentary breathing tube	 respiratory siphon tube is long slender/ narrow 3 times as long as wide with several pairs of hair tufts	 Siphon/ breathing tube dark in color short, stout i.e. about twice as long as wide with one pair of hair tufts	 Siphon is characte-ristically modified for piercing plant tissue

Pupa			
 Short broad breathing/ respiratory trumpet, distally expanded	 Paddle at 9th segment	 Long narrow breathing trumpet, tubular	 Pupae are attached to rootlets of aquatic plants by their siphon tubes
Adult ***Resting***			
 Rests inclined at an angle of 45° to the surface, probosics and body in same straight line	 Rests parallel to the surface, probosics and body at an angle to on another	 Rests parallel to the surface, probosics and body at an angle to one another	 Rests parallel to the surface, probosics and body at an angle to one another
Wings			
 Generally spotted with white and dark scales	 Generally unspotted with only dark scales	 Dark scales with brown/ black color	 Dark brown and pale scales; scales broad with cutoff tip
Scutellum			
 Half-moon shaped with a uniform row of hairs along the margin	 Trilobed, with three bunches of hair on the lobes	 Trilobed, with three bunches of hair on the lobes	 Trilobed, with three bunches of hair on the lobes
Abdomen and legs			
 Abdomen completely or largely devoid of scales	 Uniform layer of over lapping flat white and dark scales on the abdomen	 Black and white basal band on abdomen and legs	 Big, black or brown mosquitoes with sparkling on their wings and legs

Palpi			
			
 Probosics dark and palp has white rings. Palpi long nearly equal to probosics. Antennae shorter than probosics **Male:** Palpi dub-shaped at the distal ends. Antennae plumose(many feathery hairs) **Female:** Palpi long slender. Antennae pilose (few spidery hairs)	 Probosics and palp dark and short. Antennae about same size as probosics **Male:** Palpi usually longer than probosics long, pointed and bent. Antennae plumose (many feathery hairs) **Female:** short stub like reduced. Antennae pilose (few spidery hairs)	Probosics is dark and palps have silvery white scales at the tip **Male:** Palpi usually longer than probosics long, pointed and bent. Antennae plumose (many feathery hairs) **Female:** Palpi short stub like reduced. Antennae pilose (few spidery hairs)	Palps not more than 1/3rd as long as probosics "club-like" **Male:** Palpi usually longer than probosics long, pointed and bent. Antennae plumose (many feathery hairs) **Female:** Palpi short stub like reduced. Antennae pilose (few spidery hairs)

Source: Operational Manual for Integrated Vector Managemnt, 2016.

Part B - Biology - Malaria Vectors

Anopheles culicifacies

1. Distribution: Widely distributed in India.
2. Breeding places: Breeds in rainwater pools & puddles, borrow-pits, river bed pools, irrigation channels, seepages, rice fields, wells, pond margins, sluggish streams. Extensive breeding encountered following monsoon.

3. Resting habits: Rests during daytime in human dwellings and cattle-sheds.
4. Biting time: Biting is throughout night. Peak biting is from 19.00 to 04.00 hrs.
5. Feeding habits: Zoophilic species but with high density, it feeds on human.
6. Flight range: About 1-3 kms.

Taxonomic Characteristics

1. Size Smaller to Medium
2. Sitting posture is like Culex
3. Palpi : Apical pale band and pre-apical dark band nearly equal
4. Wing: 3rd vein dark

Anopheles stephensi

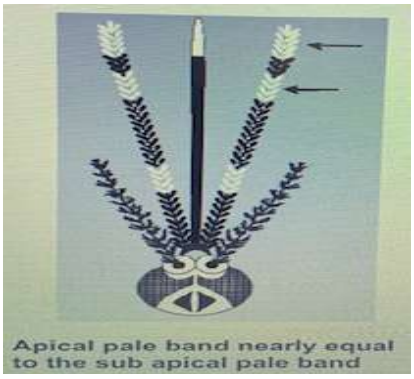
1. **Distribution:** Distributed throughout India except at higher altitudes.

2. **Breeding Places:** Breeds in wells, overhead, water tanks, cisterns, rain water collections in roof gutters, peri-domestic containers and water storage tanks like Tankas.

3. **Resting Habits:** Rests in human dwellings and cattle-sheds. Outdoor resting in wells and underground cement tanks is also observed.
4. **Biting Time:** Peak biting is generally from 22.00 to 24.00 hrs. Biting varies from area to area and seasonally.
5. **Feeding Habits:** Indiscriminate feeder and bites both human and animals.
6. **Flight Range:** Limited flight range in the urban areas but in rural areas the flight range may be upto 3 kms.

Taxonomic Characteristics

- Size Smaller to Medium
- Palpi: Apical and sub-apical pale band are equal
- Dark and legs speckled



Anopheles fluviatilis

1. **Distribution:** Widely distributed in foothills of peninsular & Northeast India.

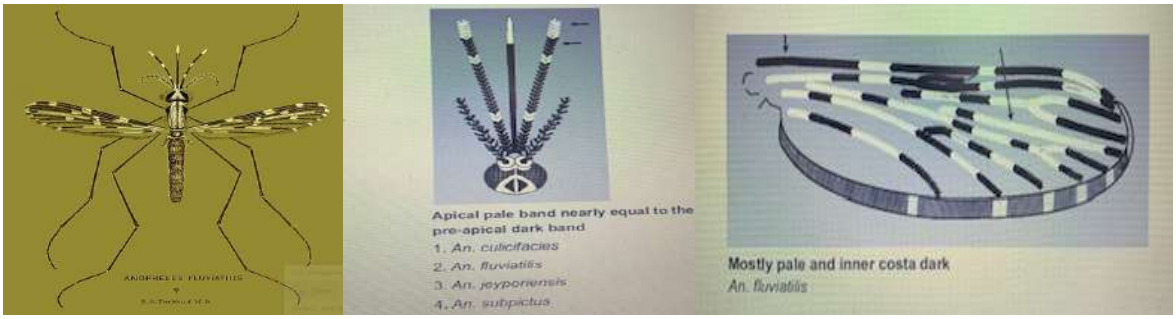
2. **Breeding Places:** Breeds in slow running streams, seepages and irrigation channels; also recorded from rice fields and shallow wells. During heavy rains the breeding of *An.fluviatilis* is often flushed

out.

3. Resting habits: Rests indoors in human dwellings and cattle-sheds.
4. Biting time: Generally enters houses at dusk and completes feeding before midnight with peak from 09.00 to 11.00 hrs.
5. Feeding preferences: This species is in general highly anthropophilic; may be mainly zoophagic in northern India.
6. Flight range: Limited flight range.

Taxonomic Characteristics

- Size Smaller to Medium
- Palpi : Apical pale band and pre-apical dark band nearly equal
- Wing: 3rd vein Pale
- No Fringe spot on Vein 6

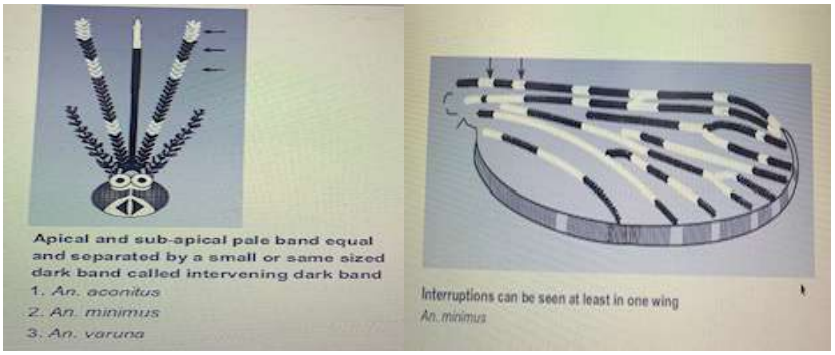


Anopheles minimus

1. **Distribution:** Distribution is restricted to the north-eastern states.

2. **Breeding Places:** Breeds in shaded slow running streams with grassy margins, swamps, ditches; occasionally found to breed in borrow-pits, rice fields and seepage from flowing water.

3. **Resting Habits:** Rests in houses and cattle-sheds, preferring to rest on the lower portions of walls.
4. **Biting Time:** Peak biting activity occurs from 18.00 to 19.00 hrs outdoors and 24.00 to 02.00 hrs indoors. Biting time may vary area and season wise.
5. **Feeding Habits:** A highly anthropophilic species.
6. **Flight Range:** Normally 0.5 km but can disperse upto 2 kms.



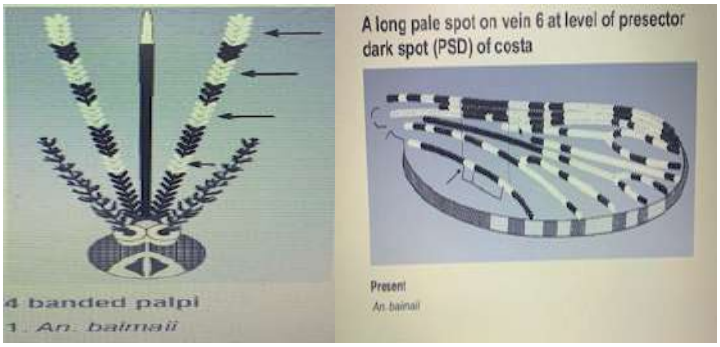
Anopheles baimai (dirus)

1. **Distribution:** Restricted to forested areas of Northeastern states.

2. **Breeding Places:** Breeds in pools, rain-water collections in deep forest and forest fringes, stream margins with decaying organic matter.

3. **Resting Habits:** Enters human dwellings to bite and rest but has a tendency to leave houses soon after blood meal.
4. **Biting Time:** The peak biting activity is from 22.00 to 02.00 hrs.
5. **Feeding Habits:** High preference for human blood but also bites monkey, other primates and cattle.
6. **Flight Range:** Flight range varies from 1 to 2.5 km in forests.

Taxonomic Characteristics

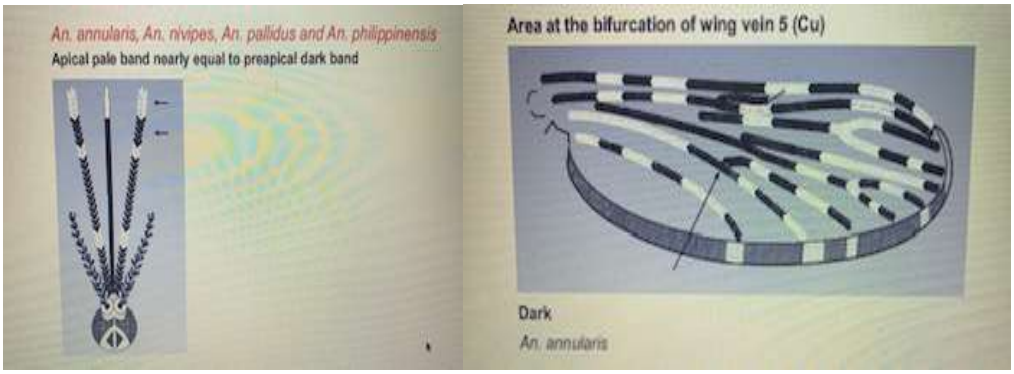


Anopheles annularis

1. **Distribution:** Widely distributed across country except Andaman and Nicobar and Lakshadweep islands.
2. **Breeding Places:** Breeds in still waters with abundant vegetation in a variety of water bodies; also breeds in wells, tanks, borrow-pits, rice fields,lakes and stream margins with vegetation.
3. **Resting Habits:** During day time rests in houses, cattle-sheds and mixed dwellings, and also rests outdoors in small numbers.
4. **Biting Time:** Peak biting activity takes place from 22.00 to 24.00 hrs.
5. **Feeding Habits:** A zoophilic mosquito; biting on man is infrequent.
6. **Flight Range:** Normally upto 1 km.

Taxonomic Characteristics

- Size Smaller to Medium
- Palpi : Apical pale band and pre-apical dark band nearly equal
- Wing: 3rd Vein Pale and Black
- 5th vein bifurcation Dark
- Pale Fringe spot on Vein 6



Anopheles sundaicus

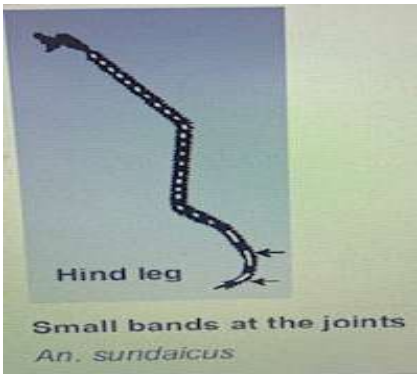
1. **Distribution:** Reported from coastal Orissa, Andhra Pradesh and West Bengal in 1950s but currently restricted to Andaman and Nicobar Islands.

2. **Breeding Places:** Breeds in brackish water pools with algae, margins of mangroves and lagoons and swamps. *An.sundaicus* can tolerate salinity levels from 0.08 to 2.6 percent and pH from 7.7 to 8.5.

1. **Resting Habits:** Rests indoors in human-dwellings, cattle-sheds and mixed dwellings.
2. **Biting Time:** Throughout night but peak biting is from 20.00 to 02.00 hrs.
3. **Feeding Habits:** An opportunistic feeder, prefers to bite man.
4. **Flight Range:** About 1-3 kms.

Taxonomic Characteristics

- Palpi : Apical pale band and pre-apical dark band nearly equal
- Femora Tibia & Tarsi(first) spotted and banded
- Remaining Tarsi Black



Anopheles philippinensis

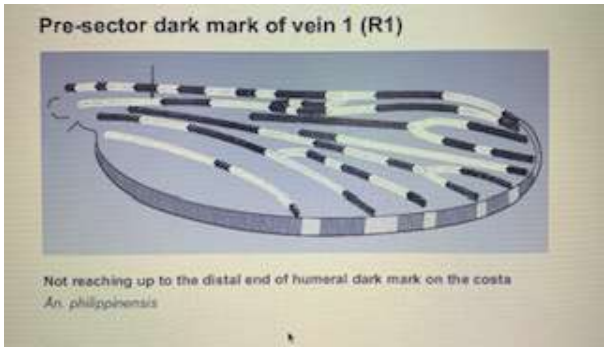
1. **Distribution:** Distributed in West Bengal, North Eastern Andaman and Nicobar Islands.

2. **Breeding Places:** Breeds in tanks, swamps, ditches, rice fields, pools, leaf axils, shaded lake margins, inundated drains & water bodies with generally good growth of vegetation.
3. **Resting Habits:** Rest in cattle-sheds and human dwellings.

1. **Biting Time:** Biting outdoors and indoors throughout night with two biting peaks from 20.00 to 22.00 and 02.00 to 04.00hrs.
2. **Feeding Habits:** Predominantly zoophagic but also bites man.
3. **Flight Range:** Normally upto 0.8 km.

Taxonomic Characteristics

- Two tarsal segment completely white
- Femora and Tibia not speckled
- Wing: Vein 1 with Pre sector dark mark & 5th vein Pale
- Not much scales on abdomen as in pucherrimus

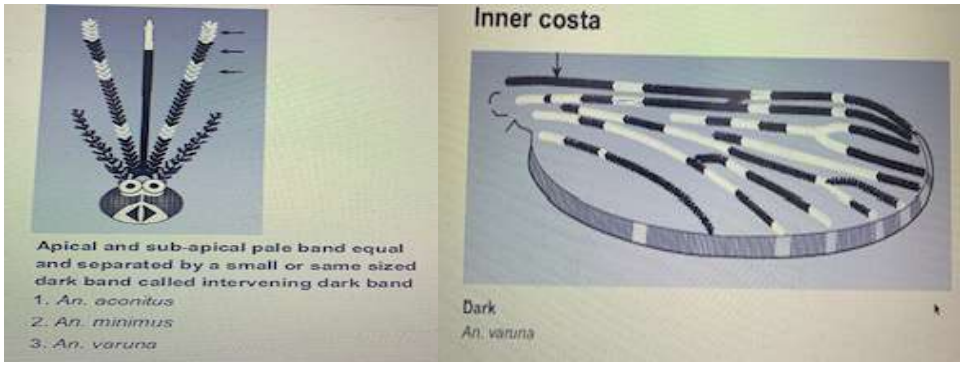


Anopheles Varuna

1. **Distribution:** Distributed widely in the country from north east plains, peninsular India, and the Lakshadweep islands.
2. **Breeding Places:** Breeds in rain water pools, tanks, ponds, rice fields, drains, irrigation channels, wells and slow moving streams with plenty of shade provided by overhanging vegetation.
3. **Resting Habits:** Rests indoors during daytime in human dwellings, cattlesheds and mixed dwellings. Rests outdoors near stream banks.
4. **Biting Time:** Throughout night, but peak biting is from 24.00 to 02.00 hrs.
5. **Feeding Habits:** Resting habits may differ from area to area.
6. **Flight Range:** About 1 km.

Taxonomic Characteristics

- Size Smaller to Medium
- Palpi: Apical pale band and pre-apical dark band nearly equal
- Wing: Inner Costa Dark



Part C - Vector Surveillance Methods and Entomological Indicators

Learning Objectives :

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

- Mosquito Surveillance
- Surveillance Methods: Objectives
- Adult Mosquito Collection Methods
- Larval Mosquito Collection Methods
- Entomological Indicators and its significance

Mosquito Surveillance

Entomological Surveillance

Surveillance for vectors is important in determining the distribution, population density, larval habitats, and insecticide resistance to prioritizing vector control in terms of time and space. These data will enable the selection and use of the most appropriate vector control tools and can be used to monitor their effectiveness. There are several methods available for the detection and monitoring of larval and adult populations. The selection of appropriate sampling methods depends on surveillance objectives, levels of infestation, and availability of resources.

Collection of Adult Mosquitoes

The collection of adult mosquitoes is made for:

1. **Qualitative Studies:** To study the prevalence, distribution, behaviour of different mosquito species in different macro and micro environmental conditions.
2. **Qualitative Studies:** To study the vector relative density and abundance, longevity, infectivity, impact of anti- vector measures on the vector population, impact on the transmission. Several methods for sampling of mosquitoes are available which are undertaken alone or in combination with others depending on objective of survey.

Hand Collection of Mosquitoes

Principles and Objective of the Method

Mosquitoes feeding on host species or resting on different surfaces (indoor and outdoor) can be collected by a test tube or sucking tube. Such a collection would yield information about their resting habits, feeding habit, ovipositing habits, relative density – vectorial capacity and for carrying out susceptibility tests, precipitin tests, etc.

Collection of Mosquitoes

Adult mosquitoes in indoor situations should be searched in dark corners of houses, ceilings, amongst thatch and cobwebs, on the underside of shelves, amongst clothing and other hanging articles with the help of torchlight. A large number of mosquitoes may be collected from sheds used for cattle, horses and pigsties, etc.

1. **By Aspirator Tube or Sucking Tube**

This is the most widely used and convenient method for mosquito collection. An aspirator tube is generally having a length of 30-45 cm (internal diameter, 8-12 mm) and is made up of glass or plastic tubing. A piece of mosquito netting is fixed over a short piece of smaller-diameter rubber tuning, which is inserted into the end of the larger tubing. A 50 cm long rubber tubing is slipped over the end of glass tubing provided with mosquito netting (Figure 1(a)). The resting of the feeding mosquito on being detected with a torch light can be sucked in gently, unless to worker keeps sucking or closes the end of the tube with a finger or cotton plug, the captured mosquitoes are liable to fly out (Figure 1(b)).

Figure 1.(a) Aspirator Tube (b) Mosquito Collection

2. **For outdoor collection**, mosquitoes sheltering under overhanging banks of streambeds, in cracks, in banks or walls, under bridges, culverts and in tree holes should be thoroughly searched in the area.
3. **By Test Tube:** Test tubes without rims and having a length of about 100 mm (20 mm diameter) are used for the collection of mosquitoes. After locating a mosquito with a torch light, hold a test tube in the middle and brings its mouth slowly over the insect; then move the tube slightly to dislodge the mosquito, slide the hand up the tube and quickly place a finger over the open end and plug it with cotton (Figure 2).

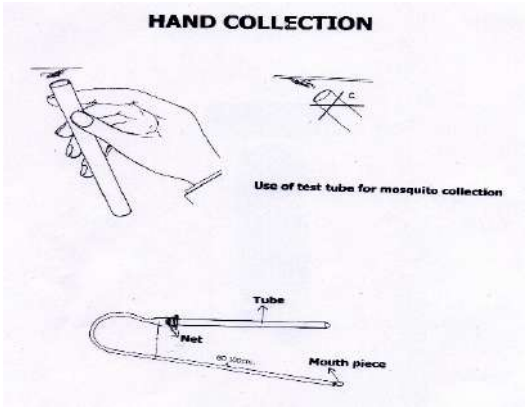


Figure 2.Hand Collection

4. **Catches off Baits:** Mosquitoes are collected directly off the human or animal baits using a sucking tube while they land on the host to bite or while biting a human or an animal host. This method is one of the most important for collecting partially or entirely exophilic mosquitoes. Mosquitoes may also be collected while resting in the vicinity of the bait, either before or after feeding.
5. **Hand net Catches:** A small hand net about 15 cm in diameter, made of fine mosquito netting and provided with a long handle is being used to catch adult mosquitoes resting in human and animal habitations in large numbers. The usual procedure is to gently spray the hut with a non-toxic oil (Rosella or citronella oil) paying attention to cracks and crevices. The disturbed mosquitoes are collected by sweeping the net.
6. **Spray Sheet Collection:** The method is applied during the daytime, usually early in the morning

between 06.30 and 10.00 hours, depending on the situation and objective. All occupants, animals and easily removable objects like foodstuff, drinking water, furniture, etc. are first removed from the structures where the collection is to be made. All doors and windows should be closed and the floor of the hut should be covered with a white sheet. The hut space was then sprayed with an ordinary hand pump containing 0.1 per cent pyrethrum in kerosene oil @ 15-30 m1/1000 cu. ft. After filling the room with insecticide mist, the collector leaves the hut and closes the doors. Ten minutes after the spray, the doors are opened and the sheets are lifted with four corners and brought outside in daylight (Figure 3). The mosquitoes are collected with entomological forceps and transported to the laboratory. The mosquito thus collected can be used for dissection of malaria / filarial parasites, ovarian age grading and precipitin test, etc.

Figure 3.Spray Sheet Total Catch Method

7. **Trap Collection:** Traps are used extensively for collecting mosquitoes which are flying in search of food, shelter or egg-laying sites or due to some external unfavourable influences, whether natural (wind, change of humidity, temperature and light) or produced by a human being like smoke, insecticides, ventilation, etc. Some of the important traps used for the collection of adult mosquitoes are window traps, Magoon trap, malaise, light traps, etc.
8. **Window Trap:** These are the most widely used for mosquito collection in malaria programs. They are placed in the path of incoming or outgoing flying mosquitoes and are used without any attractant (Figure 4). The window trap consists of a wooden frame, a cube of six sides of one foot each, five sides of which are closed with mosquito nettings whiles to the sixth side a deep conical funnel of netting or provided. The frame of the trap should fit exactly into the window frame of the house so that no space is left to escape from it or the open areas around the window trap should be plugged with cotton or cloth etc. Window trap collections give information on the circulation of mosquitoes in different physiological conditions from outside to inside and vice versa.

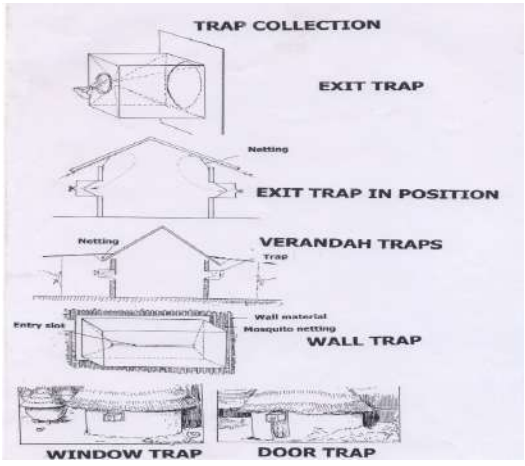


Figure 4.Window Trap

9. **Magoon Trap:** These are essentially portable/detachable temporary wooden huts, in which the upper half of the standing wooden panels in fitting with wire gauze netting and an entry slit about 2 cm wide and V-shaped in appearance is provided all around (Figure 5). A convenient size of the trap is 8 m x 8 m and it should be high enough for the collector to stand up inside. The roof of the trap should be sufficiently slanting to shed water. The trap is baited with a calf, goat or some other animal in the evening and a large number of mosquitoes can be collected the next morning in a single catch. The placement of the trap and choice of bait is very important to sample different populations of mosquitoes. The various parts of the trap can be dismantled and bolted together again, facilitating its easy transportation from one area to another area.

Figure 5.Magoon Trap

10. **Light Trap:** The basic principle of the light trap is that the mosquito attracted at night to the bright electric light enters under the hood of the trap where they are exposed to a strong downward air current produced by a fan operated by an electric motor. The mosquitoes are collected in a holding cage attached to them. Light traps have mostly been used for collecting outdoor flying mosquitoes. This trap is good for the routine sampling of the culicine mosquito population and the study of culicine vectors of virus diseases. Figure 7 shows the construction of CDC type of light trap.

Figure 7.CDC Light Trap

Larval Mosquito Collection

To identify the preferred breeding sites, it is essential to be systematic and check all possible breeding places, even those that are difficult to reach. This will indicate the type of site most likely to harbour the larvae of anopheline mosquitoes.

Objectives

- To establish the breeding habits of different species.
- To establish the geographical distribution of the vectors.
- To establish the active breeding places.
- To study the development of aquatic stages.

- To evaluate the impact of anti-larval measures on the larval density.
- To collect samples of larvae for rearing adults for taxonomic studies or biological observation (bioassay/susceptibility tests).

Equipment

Dipper, larval net, large tray, pipette, specimen tubes (vials), 70% alcohol solution, coon wool, a pencil, and safety match or lighter. If live specimens are required for insecticide testing, larger boles or a wide-mouthed vacuum flask will also be needed.

Larval Collection Method

1. Dipping
2. Netting
3. Pipetting

Dipping

The dipping method is the most frequently used for collecting mosquito larvae. The collecting equipment viz. Enamel bowl, frying pan or ladle (Figure 8) should be immersed in the breeding places (edges of swamps, ditches, streams, rice fields other bodies of water) at an angle of 450.

The surface water will flow into the larval container automatically along with the larvae if any. If the dipper is immersed too slowly the larvae are disturbed and go to the bottom. There should be an interval of 2-3 minutes between each dip to allow stage III IV larvae and pupae to come to the surface again.

In case the surface should be agitated to cause the larvae to sink, clear away the vegetation and then wait for 3-4 minutes for the larva to come to the surface and collect them with a dipper. The larval density is assessed in terms of average larval density per dip.

Figure 8.Dipping Method

Netting

Larvae may be collected from large stretches of water along the edge of streams, ponds, wells, and other large water bodies. A larval net (Figure 9) consists of a ring of iron frame, 20-25 cm in diameter, to which a nylon/muslin cloth net is attached, measuring about 10 cm long. A long wooden handle is attached to the ring. For collecting larvae, the net is held at an angle of 300 and skimmed rapidly through the surface water near emerging or floating vegetation. The net is inverted and washed out in a bowl of water and the larvae are collected with a pipette.

The density is measured in terms of density per larval net. The usual pond net devoid of a handle and provided with a nylon string attached to four points on the iron ring at equal distances is used for collecting larvae from wells. Join the four pieces of string in such a way that the ring forms an angle of 300 and attach this to a rope tied with it. While collecting larvae from a well, put a small weight in the net to keep its bottom under the water's surface. The net is moved around the border of the well two-three times, it is then withdrawn, and inverted in a white enamel bowl containing water. The larvae are collected by pipette. The density is measured in terms of larvae per well net.

Figure 9.Netting Method

Pipetting

Small pipettes or small spoons may be used for collecting larvae from the shallow breeding sites like hoof prints, etc.

Entomological Indicators and its Significance

The important entomological indicators are vector density, vector longevity, susceptibility to insecticide, sporozoite rate and man-mosquito contact. An understanding of entomological parameters with their significance is important in the study of the dynamics of vector-borne diseases. These parameters are useful in the assessment of the intensity of transmission in an area as well as the assessment of anti-vector measures, the proper understanding of which depends on the integration of values obtained under individual parameters. The important entomological parameters used in the Programme are given as under:

Adult Mosquito Density

Per Man Hour Density

Method

This index is calculated for each vector species and total anophelines. The index is calculated from the daytime hand collection made by the Insect collectors. Tube Aspirators flashlight technique is commonly used for collecting the mosquitoes.

This is measured as follows:

$$\frac{\text{No. of mosquitoes (male and female) collected}}{\text{No. of collectors x time spent for search per hour}}$$

Significance: This parameter is useful to know:

- Mosquito fauna of the area
- Seasonal prevalence of mosquitoes and vectors
- Resting habits, both in-doors and out-doors
- Impact of vector control measures

Pyrethrum Spray Catch

Method

This method involves the collection of indoor resting mosquitoes on a white cotton sheet after knock-down by space spraying of Pyrethrum solution in a closed room. Pyrethrum solution is prepared by mixing one part of 2% pyrethrum extract with 19 parts of Kerosene oil and sprayed in suitable rooms

whose openings (windows and doors) can be temporarily closed. The above pyrethrum solution can be sprayed at the rate of ½ ounce per 1,000 sq. ft in the pucca room and one ounce per 1,000 sq. ft in the thatched room. The knock-down mosquitoes are collected, identified and recorded. After making such collections in 3-4 rooms, the indoor resting mosquitoes per room on average may be calculated.

Significance: This parameter is useful to know:

- Mosquito/ vector prevalence in an area of low indoor resting densities especially in places where indoor hiding mosquitoes are not easily detected through hand collection method.
- When large number of mosquitoes are required for dissection.

Out-door Collection (PMHD) through Trapping Device

- **Method:** Hand collection of mosquitoes in outdoors are made through trapping device like pit shelters, box shelters etc.
- **Significance:** This parameter indicates dispersal of mosquito population in space and time.

Mosquito Collection on Animal

- **Method:** Hand collection with the help of aspirator tube and flash light from the animals in the proximity of human dwellings is done during the usual hours.

- **Significance**

a) This parameter indicated preference of mosquitoes for animals, or human blood meals.
b) If mosquito has predilection for animals, the latter may be used as zoo-prophylactic.

Larval Density

- **Method:** The larval collection is done with the help of a standard ladle, net well net etc. The common approach is to work out per dip density of larvae. Commonly a minimum of four dips are applied in each breeding place at different points and the number of larvae on average per dip is determined.

- **Significance:**

a) This parameter is used in understanding mosquito breeding habits.
b) This indicator is used in assessing the anti-larval measures in urban areas.

Man Mosquito Contact

Man Biting Rate

- **Method:** Mosquitoes are collected from the human baits in the night during usual sleeping hours. The index is determined either indoors or outdoors usually from 6 P.M. to 6 A.M. Hourly collections are recorded. No. of mosquitoes collected per night on each bait becomes the parameter.

- **Significance**

a) This parameter helps in understanding the vectorial potency and quantum of man mosquito contact in space and time.
b) This indicates differential man feeding propensity.
c) Helps in understanding the site of man-vector contact.
d) Helps in deciding whether indoor residual insecticidal spraying is advisable. To understand animal biting rate, collection may be done on animal baits.
e) Helps in understanding endophagy or exophagy.

Human Animal Bait Traps

- **Method:** Animal or human volunteers may be put as bait in traps like bait night traps, Magoon traps, steer bait traps etc. The mosquitoes are collected by hand in the early morning and density per trap per night may be calculated.

- **Significance**

a) This indicator helps in understanding differential feeding on human or animals.
b) This gives an idea about site of man-mosquito contract.
c) Helps in understanding man biting rate and vectorial potency in space and time.

24 Hours Survival Rate

- **Method:** Exit window traps are fixed in the houses and adult females caught in them are put in the holding chambers and observed for 24 hours for survival/ mortality. The number of mosquitoes caught in the trap is placed individually in small vials for this purpose.

- **Significance**

a) This indicator gives an idea about the impact of residual insecticidal spray.
b) This gives an indication of inherent population characteristic of daily mortality.

Seasonal Infectivity of Vectors/ Sporozoite Rate

- **Method:** Mosquitoes collected from known positivecases and nearby houses are dissected in 0.68% saline for salivary gland dissection. The glands so obtained are to be examined under Compound Microscope. The sporozoite rate (infectivity rate) may be calculated as follow:

$$\text{Sporozoite rate} = \frac{\text{No. of mosquitoes found with sporozoites}}{\text{No. of mosquitoes dissected.}} \times 100$$

- **Significance:** The significance of this parameter is:

a) To incriminate the suspected vector/ re-incriminate known vectors
b) To evaluate the impact of control measures
c) To estimate transmission season
d) To understand the vectorial potency of vectors

Oocyst Rate

- **Method:** Mosquitoes are dissected for the gut, which may be examined under low power microscope for the presence of oocyst on the gut wall. The rate may be calculated as follows:

$$\frac{\text{No. of mosquitoes found with oocyst}}{\text{No. of mosquitoes dissected}} \times 100$$

It will be informative to know the number of oocyst per gut for the positive mosquitoes.

- **Significance**

a) Determining transmission season
b) Evaluating the impact of vector control measures

Parity Rate

Method

a) **Detinova Technique:** Mosquitoes are dissected for ovaries. After water on the slide around the ovary is nearly half dry, the ovary is covered with glycerin and examined under binoculars to observe whether tracheolar endings were coiled (skeins) or straightened. The parous females i.e. those who have taken bloodmeals microscope once and digested it will not show skeins. For this dissection preferably the females caught during the human bait collections be utilised. The parous rate is calculated as follows:

$$\frac{\text{No. of Mosquitoes parous}}{\text{No. of mosquitoes dissected}} \times 100$$

b) **Polovodova Technique:** Here also ovary of the mosquito is dissected as above and the follicular dilatations in the ovariole stalk are observed under a microscope and the number of dilatation on knots is counted. Each dilatation represents the happening of one egg-laying.

The rate is calculated as under:

$$\frac{\text{No. of mosquitoes with follicular dilatations}}{\text{No. dissected}} \times 100$$

- Significance

This parameter helps in:

- Determining the physiological age of the mosquito
- Evaluating the impact of anti-vector measures
- Determining the structure of vector population

Bio Assay Test

Contact Bio-assay

- **Method:** Mosquitoes are exposed to a sprayed wall surface for a standard period (usually 30 minutes) as per the standard WHO method and mortality is recorded at the end of 24 hours holding period.

- **Significance**

This parameter helps in:

a) Determining the residual efficacy of insecticide on different surfaces and in different durations of time.
b) Evaluating the quality of insecticidal spraying

Aerial Bio-assay

- **Method:** Mosquitoes from unsprayed areas/ lab bred are kept in small mosquito cage and later suspended in the middle of a sprayed room. The mortality in the mosquito is observed after 24 hours and the cage is taken out of the room.

a) The caged mosquitoes may be placed outside the house at suitable place for assessing the efficacy of fogging.

- **Significance**

a) Understanding the vapour or air-borne effect of insecticide on mosquitoes.
b) Assessing efficacy of thermal/ cold fog at varying distances and heights.

Mosquito Vector Susceptibility to Insecticide

Determining Susceptibility Status of Adult Mosquitoes

- **Method:** Blood-fed female adult mosquitoes are exposed to diagnostic doses of insecticides for the standard time of 1 hour in case of DDT 4%, Dieldrin 4%, Malathion 5%, Propoxure 1% and synthetic pyrethroids (Deltamethrin 0.05%, Lambda-cyhalothrin 0.05%, Cyfluthrin 0.15%). The mosquito mortality is recorded at the end of 24 hours holding period. If the control mortality is recorded between 5 to 20%, the test mortality may be corrected by the following formula (Abbot's).

$$\text{Corrected mortality} = \frac{\text{\% test mortality - \% control mortality}}{\text{100 - \% control mortality}} \times 100$$

If the control mortality is more than 20% test is to be discarded.

- **Significance**

a) For establishing base line susceptibility level of vectors to different insecticides and subsequently to monitor general susceptibility levels at different time intervals.
b) For suggesting change in current control methods or suggesting alternative control measures.

Susceptibility Test of Mosquito Larvae

- **Method:** The fourth instar larvae are exposed to diagnostic concentration of larvicide in water and mortality are recorded after 24 hours. Tests are carried out with the help of WHP Test Kit and mortality, if need be, corrected applying Abbot's formula.

- **Significance**

a) To establish baseline susceptibility level of vectors to different insecticides before these insecticides are put to usage and subsequently monitor any change in susceptibility level in course of time.
b) To change the existing control measures or to suggest alternative control methods.

Anthropophilic Index

- **Method:** Blood meal samples from freshly fed females are collected on filter papers and the human blood index is determined either by precipitin test or gel-diffusion test or any other method and the index is calculated as under:

$$\text{HBI} = \frac{\text{No. of mosquitoes showing source of human blood}}{\text{No. of mosquito blood meals tested}} \times 100$$

- **Significance**

This is important in understanding:

a) The feeding preference and biting frequency of mosquito on man.
b) Change in feeding behavior of mosquitoes.

Abdominal Condition of Female Mosquito

- **Method:** The mosquitoes collected during early morning hours are examined for abdominal conditions, like fully fed, unfed, semi-gravid and gravid and then recorded. The percentage of each type may be worked out.

$$\text{e.g. fullyfed} = \frac{\text{No. of mosquitoes with FF abdomen}}{\text{No. of mosquito blood meal tested}} \times 100$$

- **Significance**

a) To determine composition of mosquito population.
b) To assess impact of spraying.

Duration of Gonotrophic Cycle

- **Method**

a) Direct observations on known fed mosquitoes in cages
b) Estimated ratio of blood fed and gravid and semi-gravid in morning pyrethrum space spray collection.

- Significance

This parameter helps in making estimation of vectorial capacity of the mosquitoes.

Part D - Vector Management

Learning Objectives :

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

- Principle of Integrated Vector Management
- Components of Vector Management
- Adult Vector Control
- Larval Vector Control
- Biological Vector Control
- Environmental Vector Control
- Adulticides and larvicides

Vector Management

Vector management is a cornerstone of malaria control and it remains the most generally effective measure to prevent malaria transmission and therefore is one of the strategic approaches to malaria control.

Objectives:

- To protect individual people against infective malaria mosquito bites.
- To reduce the intensity of local malaria transmission at community level by reducing the longevity, density and human-vector contact of the local vector mosquito population.

Vector control methods vary considerably in their applicability, cost and sustainability of their results. They target the adult mosquito and/ or its larvae.

Integrated Vector Management (IVM)

Mosquitoes play a vital role in the transmission of vector-borne diseases. Anopheline species that transmit malaria are freshwater post-monsoon related species and rest indoor situations. Culicine species of mosquitoes breed in polluted water bodies and are responsible to transmit lymphatic filariasis and JE. Aedes aegypti is a vector for the transmission of Dengue, Chikungunya and Zika and is a small container breeder.

The management of mosquito vectors involves the exact mapping of the breeding potential areas and endemicity of a particular disease in a given area.

Key Elements of IVM Strategy (WHO GVCR 2017-2030)

1. Evidence-based decision-making
2. Integrated approaches
3. Collaboration within the health sector and with other sectors
4. Advocacy, social mobilization, and legislation
5. Capacity-building

Under the concept of IVM, there are many tools / options available and recommended appropriately for vector control. The following methods for Integrated vector management can be used.

1. Reducing human-vector contact: a). Insecticide-treated mosquito nets b). Improved housing c). Repellents and mosquito coils
2. Larval control : a). Source reduction b. Biological Control (Larvivorous fish) c). Larviciding
3. Adult mosquito control : a). Indoor residual spraying b). Use of LLINs c) . Space spraying

Reducing Human-vector Contact

Personal Protection

- **Protective Clothing:** Clothing reduces the risk of mosquito biting if the cloth is sufficiently thick or loosely fitting. Long sleeves and trousers with stockings protect the arms and legs, the preferred sites for mosquito bites. Schoolchildren should adhere to these practices whenever possible.
- **Mats, Coils and Aerosols:** Household insecticidal products, namely mosquito coils, electric vaporizer mats and liquid vaporizers, pyrethrum space spray and aerosols have been used extensively for personal protection against mosquitoes.
- Repellents are a common means of personal protection against mosquitoes and other biting insects. These are broadly classified into two categories, natural repellents and chemical repellents. Essential oils from plant extracts are the main natural repellent ingredients, i.e. citronella oil, lemongrass oil and neem oil. Chemical repellents such as DEET (N, N-Diethyl-m-Toluamide) can protect Ae. albopictus, Ae. aegypti and anopheline species for several hours.
- **Insecticide-treated Mosquito Nets and Curtains:** Insecticide-treated mosquito nets (ITMN)/LLINs are used under programme since many years in high malarious areas. Though LLINs have limited utility in dengue control due to day biter vector, it can be effectively utilized to protect infants and night workers who sleep during day. Impregnated curtains can be used as mosquito nets are not used by all in every area due to weather conditions.

Source Reduction and Environmental Management

It involves any change that prevents or minimizes vector breeding thereby reducing human-vector contact. The major environmental management methods, used for the control of the immature stages of dengue vectors, through the removal of hyacinth and de-weeding/ desilting in the marshy pools of water bodies are summarized below:

Figure 10.Source Reduction

- **Improved Water Supply:** If deficient and irregular piped water supply is inadequate and available only at restricted hours or at low pressure, the storage of water in varied types of containers is encouraged, thus leading to increased Aedes breeding. The majority of such containers are large and heavy (e.g. storage jars) and can neither be easily disposed of nor cleaned. It is, therefore, essential that potable water supplies be delivered in sufficient quantity, quality and consistency to reduce the necessity and use of water storage containers that serve as the most productive larval habitats.
- **Mosquito-proofing of Overhead Tanks/ Cisterns/ Underground Reservoir/ Wells**: These structures should be mosquito-proofed either with tight lid or with proper mesh.

Biological Control

The application of biological control agents against the larval stages of mosquitoes used under programme are mainly fish or bacteria.

Fish: Larvivorus fishes (Gambusia affinis and Poecilia reticulata) have been extensively used for the control of An. stephensi and/ or Ae. aegyptiin large water bodies or large water containers in many parts of countries (Figure 11).

Figure 11(a).Gambusia affinis

Figure 11(b).Poecillia reticulata

Gambusia Affinis

The larvivorous efficiency of Gambusia is due to following characters:

- A single full grown fish eats about 100 to 300 mosquito larvae per day.
- Gambusia is a surface feeder, hence it is suitable for feeding on both anophelines and culicines.
- It frequents the margins of the water container, pond or other ground water collections, except where there is dense vegetation at the margins of the water body.
- It is small and inedible.
- It can tolerate salinity.
- It can withstand transportation and does not require any specialized equipment or containers.
- It survives in new places (water bodies) and multiplies easily. After release when it becomes well established in a water body, the fish can survive in good numbers for years and does not require constant care.

Poecillia Reticulata

A single fish eats about 80 to 100 mosquito larvae in 24 hours. Therefore, it is comparatively less efficient than Gambusia affinis.

- It is a surface feeder
- Negotiates margins of ponds more easily
- It is highly carnivorous and parents or older brood may eat up their own young ones. Therefore, a fair amount of weeds is required in the water so that young ones can hide and survive
- Tolerates handling and transportation very well
- Does not require specialized equipment for transportation
- Survives and reproduces when introduced into new water bodies. Once well established
- it can be found in the habitat even after many years.

The Hatchery for larvivorous fish can be established in:

- A Natural water body
- A special hatchery

The Natural Water Body

Criteria for selecting a water body for a fish hatchery are:

- It should be a permanent water body.
- Depth of water should be at least 1.5 metre or more.

- Water should be confined and without big natural outlet.
- The minimum size of water body should be at least 5 m X 4 m. The water body of 10m X 5m can support 50000 fish.
- It should be free from other carnivorous fish.
- Water should not be contaminated by chemical or other harmful substances.
- Easily accessible for daily or periodic inspection and for collection of fish.
- De-weeding in ponds and shallow water bodies and cleaning of margins should be carried out periodically.

Transportation of Fish

- The fish are best transported in small containers of up to 40 litres, such as plastic buckets and jerry cans, or in strong plastic bags, half filled with water from the rearing pond.
- Fish should be transported in water at ambient temperatures and should not be exposed to direct sunlight. The containers should have sufficient openings to allow flow of air.
- Take polythene bag of 3 -5 litre capacity.
- Fill it with 1.5 lit. of water.
- Introduce the fish in the bag till the total volume of water + fish is two litres.
- Bubble the oxygen in bag from O2 cylinder or from air pump.
- Close the mouth of bag with a string leaving sufficient space at the top.
- Put the bag in a thermocol container and close the mouth of container.
- The container can be transported for a period of 24 hours without further filling oxygen If the period of tran-port is more than 24 hours then arrange for change of water and oxygenate.

Collection of Fishes

- Fishes are collected with help of netting, which is fitted on a circular iron ring of 60 to
- 90 cm diameter with a wooden handle.
- Sufficient quantity is collected by repeated dips.
- Collection in bucket or drum till they are packed for transportation.

Precaution during Transportation

- Fish do not tolerate sudden temperature changes.
- Preferably the fishes should not be given any food for 10.12 hours period prior to packing for transportation.

Release of Fish

- To release the fish in a water body, measure the perimeter of water body.
- Release the fishes at the rate of 5.10 fish per linear meter.
- If the larval density is high more fish up to 20 can be released.

Precautions during Release of Fish

- Fishes should be released in the morning hours or in the evening.
- Before releasing ensure that the temperature of water both in container and in the larval habitat is more or less same.
- Clean out dense vegetation from the water body.
- Ensure that water body is free from predators of larvivorous fishes.

Where to Use Fish

- Fish should be preferably introduced in all unused wells in the rural and peri-urban areas before the high mosquito breeding season to maximize impact. However, the fish may be used in such wells even if the seeding has been delayed.
- Fresh water bodies in rural areas such as stagnant ponds, slow moving streams quarry pits, large borrow pits, margins of ponds should be targetted apart from wells. Such places should be surveyed and numbered to facilitate subsequent monitoring of impact.

- In open mosquito breeding sites or rice fields, the fishes need to be protected from pesticides applied to crops, when used in rice fields.

Bacteria: Two species of endotoxin-producing bacteria are recommended under the programme which are *Bacillus thuringiensis* serotype H-14 and *Bacillus sphaericus*. These are effective mosquito control agents and do not affect nontarget species. Bt.H-14 is most effective against *An. Stephensi* and Ae. aegypti, while Bs is the most effective against *Culex quinquefasciatus* which breeds in polluted water.

The details of the chemical methods for malaria vector control are dealt in a separate module. Insecticides and Larvicides: Formulations and dosages.