



Indira Gandhi
National Open University
School of Sciences

BZYET-143 INSECT VECTORS AND VECTOR BORNE DISEASES

VOL

2

MEDICALLY IMPORTANT DIPTERANS AND IVM

BLOCK 3

MEDICALLY IMPORTANT INSECT VECTORS-II	7
--	----------

BLOCK 4

MEDICALLY IMPORTANT INSECT VECTORS-II AND IVM	65
--	-----------



ignou
THE PEOPLE'S
UNIVERSITY

BZYET-143
INSECT VECTORS
AND VECTOR BORNE
DISEASES

Block

3**MEDICALLY IMPORTANT INSECT VECTORS-II**

UNIT 10**Dipterans as Disease Vectors-I (*Anopheles* Mosquitoes) 7**

UNIT 11**Dipterans as Disease Vectors-II (*Culex* Mosquitoes) 28**

UNIT 12**Dipterans as Disease Vectors-III (*Aedes* Mosquitoes) 45**

Course Design Committee

Prof. M.S. Nathawat
Former Director, School of Sciences
IGNOU, Maidan Garhi, New Delhi-110068

Prof. S. S. Hasan (Retd.)
School of Sciences, IGNOU
Maidan Garhi, New Delhi-110068

Dr. R.S. Sharma (Retd.)
Head, Centre of Medical Entomology and Vector
Control, National Centre for Disease Control,
Ministry of Health and Family Welfare, 22, Sham
Nath Marg, Delhi-110054

Dr. S.K. Sagar
Associate Professor in Zoology
Swami Shraddhanand College, Alipur Village
University of Delhi, Delhi- 110036

Dr. H.S. Pawar
Scientist D, NIMR
Sector 8, Dwarka, New Delhi-110077

Dr. Ranjana Saxena
Associate Professor in Zoology
Dyal Singh College, Lodhi Road, New Delhi-110003

Prof. Neera Kapoor
School of Sciences, IGNOU
Maidan Garhi, New Delhi-110068

Block Preparation Team

Dr. H.S. Pawar
Scientist D, NIMR, Sector 8, Dwarka
New Delhi-110077 (Units 10 to 12)

School of Sciences

Prof. Neera Kapoor (Units 10 to 12)

Course Coordinator	:	Prof. Neera Kapoor
---------------------------	---	---------------------------

Course Editor	:	Prof. Sarita Kumar Professor in Zoology Acharya Narendra Dev College, Kalkaji New Delhi-110009
----------------------	---	---

Production

Mr. Hemant Kumar
SO (P), MPDD, IGNOU

Acknowledgement:

- Prof. Neera Kapoor and Mr. Ajit Kumar, Suggestions for figures and Cover Design.
- Mr. Vikas Kumar, JAT for word processing and CRC preparation.

December, 2021

©Indira Gandhi National Open University, 2021

ISBN:

All rights reserved. No part of this work may be reproduced in any form, by mimeograph or any other means, without permission in writing from Indira Gandhi National Open University.

Further information on Indira Gandhi National Open University courses may be obtained from the University's office at Maidan Garhi, New Delhi-110 068 or IGNOU website www.ignou.ac.in.

Printed and published on behalf of Indira Gandhi National Open University, New Delhi by the Registrar, MPDD, IGNOU.

Printed at:

BLOCK 3: MEDICALLY IMPORTANT INSECT VECTORS-II

Vectors are living organisms that can transmit infectious diseases between humans or from animals to humans. Many of these vectors are blood sucking insects. Mosquitoes are the best known disease vectors. Others include certain species of ticks, flies, sandflies, fleas, bugs etc. In Volume 1 you have studied about some medically important insect vectors like ticks, fleas, bugs, lice. In Volume 2, that comprises Block 3 and Block 4 you will study about mosquitoes, flies and sandflies (dipterans) as major insect vectors and diseases borne by them. You will also study about IVM (Integrated Vector Management) as the best control option for these vectors. Vector-borne diseases are illnesses caused by pathogens and parasites in humans. Block 3 discusses the mosquito borne diseases. Mosquitoes are major insect vectors for transmission of many fatal diseases.

Mosquitoes are vectors of malaria, filariasis, and some arboviral infections. Female mosquitoes may feed on a variety of mammals, birds, and reptiles, each species having its own preferences for a particular source of blood. Many species of mosquitoes bite people, but only some of them are vectors of disease. Although all mosquitoes lay their eggs either in water or on the moist surfaces at the water's edge, each species has a particular ecological niche, and their breeding sites can be very specific. Mosquito disease vectors are divided into two groups, the anophelines and the culicines, which can be readily distinguished by characters that can be seen by the naked eye.

Block 3 comprises 3 units, Unit 10 discusses about *Anopheles* mosquitoes and diseases caused by them. All malaria is transmitted by *Anopheles mosquitoes* which fly up to 2-3 km from breeding site. Only older females (2 weeks or more) can transmit malaria. Transmits four species of malaria (*ovale*, *malariae*, *vivax*, and *falciparum*). *Falciparum* malaria is the most important as it is particularly dangerous to those without acquired immunity. In any one area of the world malaria is mainly transmitted by one or two *Anopheles* species. Control programmes are highly specific to the species of mosquito involved in transmission. If a National Malaria Programme is not being undertaken then specialist advice from an experienced entomologist will be needed to ascertain the likely effectiveness of a control programme.

The term 'arbovirus' is used to describe any virus transmitted by an arthropod (insect, mite or tick). There are over 400 arboviruses but only a hundred of these have been shown to cause clinical symptoms in humans. Those which cause major diseases are well studied, at least in some areas. These include dengue and dengue haemorrhagic fever (DHF), yellow fever, and Japanese Encephalitis (JE). These diseases and the causative mosquito vectors are discussed in Units 11 and 12. Dengue, DHF yellow fever, Chikungunya and Zika are usually transmitted by *culicine* mosquitoes belonging to the *Aedes* genus. These are discussed in Unit 11. *Aedes* breeds in water held in man-made containers, such as water storage jars, pots, tin cans, and old car tyres. It is nearly always associated with human habitats and mainly bites humans.

Dengue is endemic throughout the tropics but tends to occur in periodic epidemics. Dengue itself is fairly benign but it may be associated with dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS), which are severe, often fatal diseases. Yellow fever, in its severe form, is also often fatal. Effective vaccines are available against yellow fever and these should be used as the first preventive measure. Control of *Aedes* vectors is practicable in an urban environment but is usually impossible in a forested area, where the mosquitoes breed in dispersed natural water pools in leaf axils or tree holes. Unlike malaria or arboviral

infections that may be transmitted by a single bite of a single mosquito, filarial infections require repeated inoculations of the infective larvae, perhaps hundreds per year, before the worms are present in sufficient number to produce the symptoms of disease.

Bancroftian filariasis (elephantiasis) is caused by the parasitic worm *Wuchereria bancrofti*. The disease is widespread in the tropics, especially in urban environments, and is a chronic disease that may take several years to manifest itself after infection. In urban environments the vector *Culex quinquefasciatus* is the most important vector. *Culex* is characterised by the presence of distinct parivilli. *Culex* adults can mate only in darkness. It is stout mosquito with strong pair of wings.

Brugian filariasis (elephantiasis) is caused by the parasitic worm *Brugia spp.* It is found in India and South Asia, predominantly in rural situations. It is a chronic disease that may take several years to manifest itself after infection. It is usually transmitted by night-biting mosquitoes *Mansonia spp.* You will discuss about these diseases in Unit 12. In Block 4, you will study about flies (houseflies and sandflies), diseases caused by them and their control measures. IVM is discussed in the last unit of this course.

Objectives

After studying this block you will be able to:

- identify the features, life cycle and behavior of *Anopheles* mosquitoes,
- explain the epidemiology of the diseases spread by the *Anopheles* species as vectors,
- describe the preventive and control measures of disease transmitted by the vector, *Anopheles*,
- explain the life cycle of *Culex* mosquito,
- explain the diseases transmitted by *Culex* species, recognize their symptoms and suggest the remedial measures,
- discuss the disease epidemiology and affecting factors,
- describe the various preventive and control measures of *Culex* mosquitoes,
- explain the life cycle of *Aedes* mosquito,
- know about the diseases transmitted by *Aedes* species, recognize their symptoms and discuss the remedial measures,
- discuss the disease epidemiology and affecting factors, and
- describe the various preventive and control measures of *Aedes* mosquitoes.

You are advised to go through the Annexures given at the end of the Block and the Volume. They provide comprehensive overview of the Units.

UNIT 10

DIPTERANS AS DISEASE VECTORS-I (*ANOPHELES* MOSQUITOES)

Structure

10.1 Introduction

Objectives

10.2 Biology

Eggs

Larvae

Pupae

Adults

Duration of Life Cycle

Behavior

10.3 Diseases

Life Cycle of Malaria Parasite

Symptoms

Diagnosis

Treatment

10.4 Prevention and Control

Traditional Methods of Control

Modern Approaches in Control

10.5 Summary

10.6 Terminal Questions

10.7 Answers

10.1 INTRODUCTION

In the previous unit you have studied about the different types of vectors, mode of transmission of diseases and their relationships with the hosts. In the present unit you will learn the biology of the mosquito-borne diseases, their prevention and various control measures. As you are aware that mosquitoes are slender, long-legged insects that are easily recognised by long proboscis and presence of scales on most parts of the body. The mosquito larvae are, however, without legs. They have a distinct head bearing mouth brushes and antennae, bulbous thorax that is wider than the head and abdomen, and a slender abdomen with posterior anal papillae and either a pair of respiratory openings (subfamily Anophelinae) or an elongate respiratory siphon (subfamily Culicinae) borne at the end.

There are approximately 3,500 species of mosquitoes grouped into 41 genera under family Culicidae and order Diptera. Meigen (1818) classified **culicidae** family according to presence of two wings. The subfamily Anophelinae comprises three genera, and Culicinae consists of 110 genera segregated into 11 tribes (Fig. 10.1).

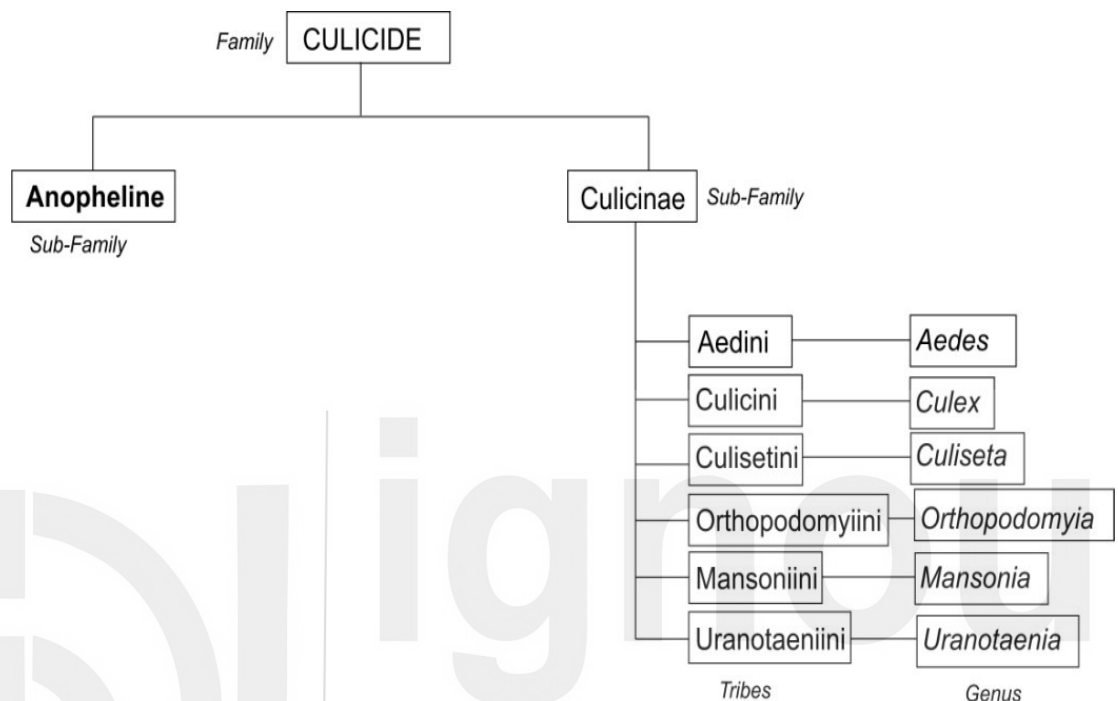


Fig. 10.1: Classification of Family Culicidae.

O'nyong'nyong fever. ONNV (O'nyong nyong Virus), a mosquito borne Alphavirus, is transmitted through the bite of **Anopheles funestus** and **Anopheles gambiae**, Vectors in Africa. ONNV fever is characterised by arthralgia, similar to chikungunya fever.

Anophelines are distributed in the temperate and tropical regions of the world, except the polar regions, extreme colder regions and higher altitudes. As described by J. W. Meigen (1818), *Anopheles* mosquito has about 460 species of which only 100 species are reported to be involved as vectors. Among these, females of only 30-40 species are reported to transmit the malarial pathogen, *Plasmodium* species. Some species of *Anopheles* can also serve as the vectors of canine heartworm, *Dirofilaria immitis*; the filariasis-causing worm, *Wuchereria bancrofti* and *Brugia malayi*; and viruses such as the alpha virus that causes **O'nyong'nyong fever**. An association of brain tumour incidence and malaria suggests that *Anopheles* might transmit a virus or other agent that could cause a brain tumour.

Objectives

After having read this unit you should be able to:

- ❖ identify the features, life cycle and behavior of *Anopheles* mosquitoes,
- ❖ explain the epidemiology of the diseases spread by the *Anopheles* species as vectors, and
- ❖ describe the preventive and control measures of disease transmitted by the vector, *Anopheles*.

10.2 BIOLOGY

Like all mosquitoes, anophelines go through four stages in their life cycle: egg, larva, pupa, and adult. The first three stages are aquatic and last for 7-14 days, depending on the species and the ambient temperature. The adult stage is winged and terrestrial. The adult females can live up to a month (or more in captivity) but generally do not live more than 1-2 weeks in nature.

10.2.1 Eggs

Adult females of *Anopheles* lay 50-200 eggs per oviposition. The eggs are laid after complete embryonic growth. Eggs are laid singly directly on water (Fig. 10.2) and are 5-8 mm in size. These are unique in having floats on the lateral sides which help the eggs to float on the water surface (Fig. 10.3) and are also an important tool in identifying the sibling species. For example, identification of bioforms of *An. stephensi* is based on number of ridges present on the float of eggs. The mysorensis bioform (Life form) has less than 14 egg ridges, while the bioforms with 15-16 ridges are classified as intermediate bioforms and if the ridge count is more than 16 in *An stephensi*, they are categorised as Type bioform.

The freshly laid eggs of *Anopheles* are white in colour while they turn to dark black after coming into contact with light and external environment due to change in the chitinous structure. Eggs are not resistant to drying and hatch within 2-3 days, although hatching may take up to 2-3 weeks in colder climates.

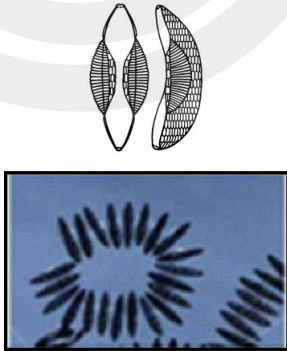
	<i>Anopheles</i>	<i>Culex</i>	<i>Aedes</i>
Eggs	 • They have floats • They are thus laid on the surface of water • Singly laid • Single oviposition	 • They form egg raft they stick together • They are in cluster • Single oviposition	 • They are laid out of surface water just above the water lining of containers • Singly laid • Multiple ovipositioning

Fig. 10.2: Difference in the eggs of different genera of mosquitoes.

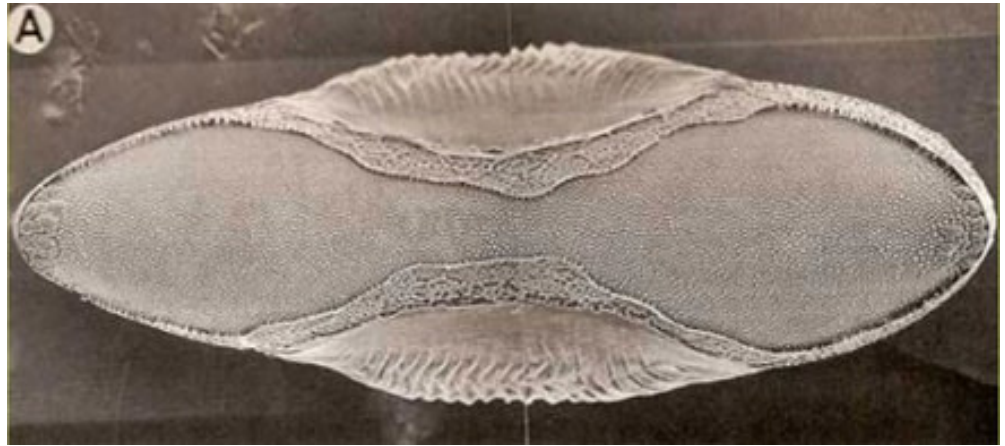


Fig. 10.3: An enlarged egg of the common malaria mosquito, *Anopheles* (size 5-8 mm) with floats on the lateral sides.

10.2.2 Larvae

Mosquito larvae, commonly called wrigglers, have a well-developed head with mouth brushes used for feeding, a large thorax, and a slender 9-segmented abdomen (Fig. 10.4). They are apodous and thus, are devoid of legs.

In contrast to other mosquitoes, *Anopheles* larvae lack a respiratory siphon on their abdominal end. Instead, they breathe through spiracles located on the 8th abdominal segment. The larvae depend upon the atmospheric air for breathing and thus they need to come to the surface frequently. Thus for effective breathing, they are positioned parallel to the surface of the water.

The larvae feed on algae, bacteria and other microorganisms present in the surface micro-layer. They dive below the surface only when disturbed. Larvae swim either by jerky movements of the entire body or through propulsion with the mouth brushes. They develop through 4 stages, called instars, and finally they metamorphose into pupae. At the end of each instar, the larvae molt, shedding their exoskeleton, or skin, to allow for further growth. The larval development is temperature-dependent. Under optimum conditions, it is approximately 8-10 days.

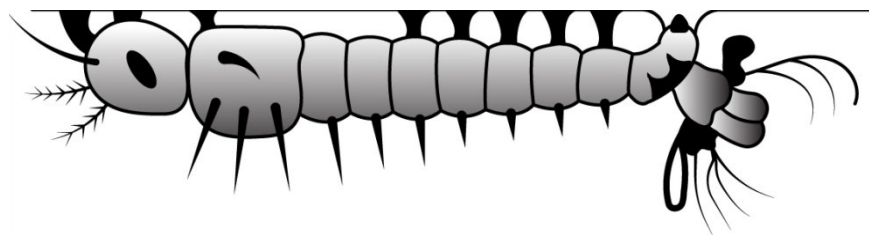


Fig. 10.4: *Anopheles* Larva. Note the position, parallel to the water surface.

The *Anopheles* larvae occur in a wide range of habitats but most species prefer clean, unpolluted water. They have been found in fresh- or salt-water marshes, mangrove swamps, rice fields, grassy ditches, edges of streams and rivers, and even small, temporary rain pools. Many species prefer habitats with vegetation whereas others prefer habitats that have none. Some breed in open, sun-lit pools while others are found only in shaded breeding sites in forests. A few species breed in tree holes or the leaf axils of some plants (Fig. 10.5).

	Anopheles	Culex	Aedes
Larvae			
	•Remain horizontal to surface of water •Can be found open ponds, containers, cooler, tanks which have clean water	•Angled hanging to the surface of water •They are mostly found in dirty water rice fields etc	•Hang at large angle on the surface of water •Can be found open containers, coolers, all surface containers which have clean water

Fig. 10.5: Differences in the larvae of different genera of mosquitoes.

10.2.3 Pupae

The pupa of *Anopheles* is comma-shaped when viewed from the side and is called tumbler (Fig. 10.6). The head and thorax are merged into a cephalothorax and the abdomen curves around underneath. Just like larvae, pupae breathe atmospheric air and thus, come to the water surface frequently. They breathe through a pair of respiratory trumpets present on their cephalothorax.

After two days, the dorsal surface of the cephalothorax of pupa splits and the adult mosquito emerges. The duration, however, can vary depending upon environmental conditions

The duration of development from an egg to adult varies considerably among different species and is strongly influenced by ambient temperature. *Anopheles* can develop from the egg to adult in as little as 5 days but usually take 10-14 days in tropical conditions.

10.2.4 Adults

Like all insects, body of adult anophelines is divided into 3 sections: head, thorax and abdomen. The head is specialized for acquiring sensory information and feeding. It contains eyes and a pair of long, many-segmented antennae. The antennae are important for detecting host odours as well as odours of breeding sites where females lay eggs. Males possess highly feathery plumose antennae while females have less hairy pylose antennae. Mouth parts are piercing and sucking type. These are characterized by an elongate, forward-projecting proboscis used for feeding, and two sensory palps. The thorax is 3-segmented and specialized for locomotion. It contains three pairs of legs and a pair of forewings. Hind wings are reduced to club-shaped structures called halteres which are used for balancing. The abdomen is slender and specialized for food digestion and egg development.

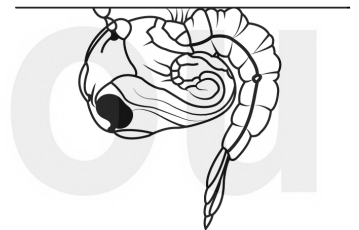


Fig. 10.6: *Anopheles* Pupa.

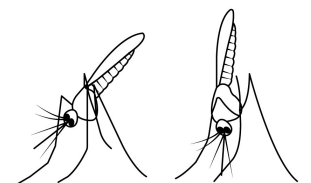
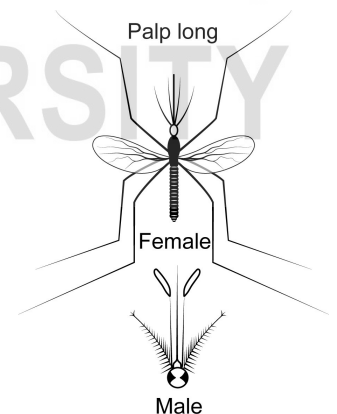


Fig. 10.7: *Anopheles* Adults. Bottom figures show their typical resting position.

Anopheles mosquitoes can be distinguished from other mosquitoes by the palps, which are as long as the proboscis, and by the presence of discrete blocks of black and white scales on the fore margin of their wings (Fig. 10.7). Such wings are known as dappled wings. Adult *Anopheles* can also be identified by their typical resting position at an angle of 45° rather than sitting parallel to the surface.

Adult mosquitoes usually mate within 2-3 days after their emergence from the pupal stage. In most species, the males form large swarms, usually around dusk, and the females fly into the swarms to mate.

Male adults live for about a week, feeding on nectar and other sources of sugar. Females also feed on sugar sources for energy but they also require a blood meal for the development of eggs. The abdomen of females expands considerably after a blood meal. The blood is digested over time serving as a source of protein for the production and maturation of eggs. The maturation of eggs depends on the temperature but usually takes 2-3 days in tropical conditions. Once the eggs are fully developed, the female lays them and resumes host seeking. The cycle repeats itself until the female dies. As discussed earlier, females can survive up to a month (or longer in captivity) but generally do not live longer than 1-2 weeks in nature. Their chances of survival depend not only on temperature and humidity, but also on their ability to successfully obtain a blood meal while avoiding host defenses.

	Anopheles	Culex	Aedes
Adults	 •Sits at 45° angle •Wings have spots •Brownish to black in appearance •Feeds in evening to morning hours	 •Sits in hunchback •Wings have no spots •Mostly Brownish •Feeds in evening to morning hours	 •Sits in hunchback •Wings have no spots •Ornamented with stripes and spots •Feeds in Day mostly

Fig. 10.8: Difference in the Adults of different genera of mosquitoes.

10.2.5 Duration of Life Cycle

Life cycle of *Anopheles* mosquito comprises four major stages as discussed above i.e. eggs, larvae, pupae and adults (Fig. 10.9).

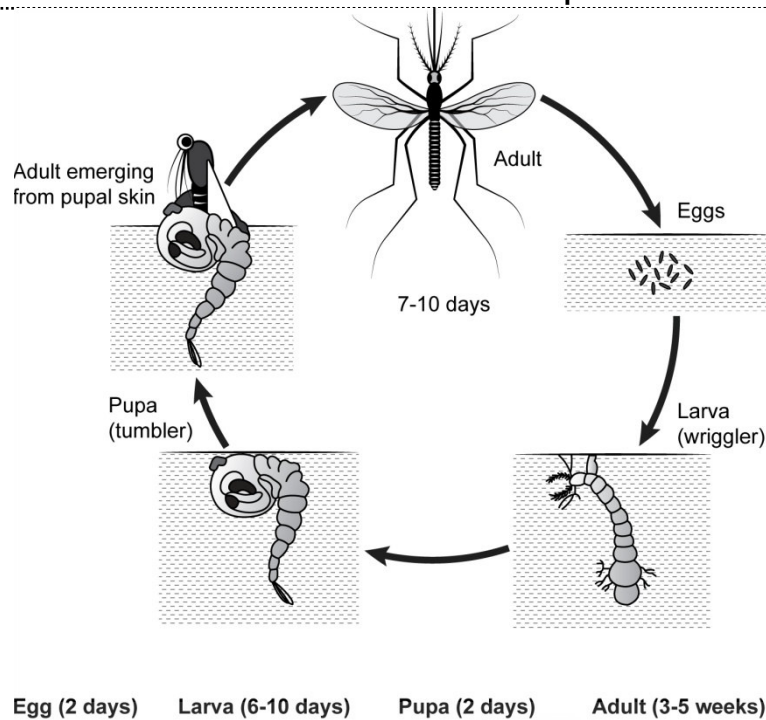


Fig. 10.9: Life cycle of *Anopheles* mosquito.

The length of the aquatic life cycle of *Anopheles* varies from 7-14 days under optimal conditions of water temperature 25-27°C, while adults can live for 1-2 months. Rise in temperature decreases the duration of life cycle. However, temperature higher than 36-40°C destroys a number of immature stages hampering the life cycle. Similarly, atmospheric temperature also affects longevity of the adult form.

10.2.6 Behavior

Mosquito behavior like flying, feeding, biting preference, resting and oviposition (egg laying), impact life cycle of *Anopheles*. These studies are useful for planning any kind of control for specific type of *Anopheles* species.

Flying

Flight activity of malarial mosquito peaks a short period after dark. For remainder of the night, they exhibit limited flight for blood and at dusk the start searching for the resting sites (Carpenter et al. 1946). Flight range is usually regarded as less than one mile under normal conditions, but this species is capable of much longer flights as demonstrated by mark and recapture studies (Carpenter and LaCasse 1955). Mosquitoes can fly up to several kilometers! They can reach far off places by taking shelter in motor vehicles, ships and aircraft.

A study on *An. gambiae* showed that the average speed of species was around 1 km/h. The maximal flight distance taken by sugar-fed females was 9 km, while blood-fed females could travel for 10 km and starved females just below 3 km. In some *Anopheles*, the maximal flight distances were 10-12 km when sugar-fed, 4.5 km when blood-fed, and below 3.5 km when starved, with an average speed of 1.3 km/h. Flight performances consisted of 1-4 h intervals of continuous flights, but mainly of bouts shorter than an hour, randomly distributed during the long flight trials.

Feeding

The mosquito adults have host seeking ability through vision, olfactory sensation and thermal source detection. Typically, both male and female mosquitoes feed on nectar and plant juices, but females have mouthparts adapted for piercing the skin of animal hosts and sucking their blood as ectoparasites. As discussed earlier, **females need blood proteins for the development and maturation of eggs**. (That is why they suck blood) In a few species, the females can produce more eggs after a blood meal. Males are dependent only on the nectar and the sugary plant juices throughout their life.

Biting Preference

Most *Anopheles* mosquitoes are crepuscular (active at dusk or dawn) or nocturnal (active at night). The *Anopheles* sp. which prefers to feed on animals are referred to as **zoophilic** in nature whereas those who like to bite humans more are referred to as **anthropophilic**. This biting behavior is very important for an Anopheline species to become an efficient vector. Different *Anopheles* species also show preference for biting outdoors or indoors. Some feed indoors (**endophagic**), while others feed outdoors (**exophagic**). The biting time and host are highly significant to determine the control methods.

Resting

Resting is a significant behavior of mosquitoes. After emergence, adults rest on the surface of water for some time and on the wall of container. Similarly, after taking blood meal, mosquitoes require adequate rest as they become heavy and need to digest blood. Resting helps in the development of eggs which takes 2-3 days depending upon the temperature.

After feeding on blood, mosquitoes may prefer to rest indoors (**endophilic**), or rest outdoors (**exophilic**), though this can differ regionally based on the local vector ecotype, vector chromosomal makeup, housing type and local microclimatic conditions. This behavior is very important for mosquito control. Indoor residual sprays (IRS) are effective against endophilic mosquitoes whereas in case of outdoor resting mosquitoes, the house sprays may not be so useful.

Ovipositioning Behavior (Egg Laying)

Anopheles mosquitoes breed in natural water collections, normally clean water (Fig. 10.10). The species, such as *An. fluviatilis*, like to breed in slow flowing streams while species like *An. sundaicus* prefer to breed in brackish water. The breeding of *Anopheles* increases dramatically in the rainy season when water collects in bottles, tins, tender coconut shells, buckets, tyres etc., that are thrown out in the open and provide ample breeding ground. Apart from these, ponds, water tanks, paddy fields etc., also act as breeding grounds. Construction sites provide ample breeding places for them— water used for curing on the concrete slabs, water collected in tanks, water collected in and around the construction site owing to blockage of water drains – all these help in breeding. It is very important to destroy these water collections or to keep them properly covered to prevent breeding.



Fountains



Cement Tank



Pits



Stone Quarry



Cement tanks



Ponds

Kamrup - *An. minimus* breeding placesFig. 10.10: Some of the breeding places for *Anopheles*.

SAQ 1

For each sentence choose the appropriate word given in the parentheses.

- i) Eggs are laid singly directly on the surface of (land/water) by adult females of *Anopheles*.
- ii) Mosquito larvae breathe through (gills/spiracles) located on the 8th abdominal segment.
- iii) The pupa of mosquito is (round/comma) shaped when viewed from the side.
- iv) Male mosquitoes live for about a week, feeding on the (blood/nectar of flower).
- v) Mosquitoes can develop from egg to adult in (5/10) days.
- vi) *Anopheles gambiae* can fly distances of (3.5/9 Km).
- vii) The *Anopheles* species with a preference to feed on animals is referred to as (Anthrophilic/Zoophilic).

10.3 DISEASES

Malaria is an *Anopheles*-borne disease caused by the *Plasmodium* parasite (A protist). Symptoms are of fever, chills, and flu-like illness. If the patients are left untreated, they may develop severe complications and even die. About 3 billion population of world is under the threat of malaria. In 2019, approximately 229 million cases of malaria were reported worldwide with 409,000 deaths. Children, especially in the African Region, are more vulnerable to the disease. In 2020, India experienced 181831 malaria cases and 63 deaths. About 1,700 cases of malaria are diagnosed in the United States each year. The vast majority of cases in the United States are reported in travellers and immigrants returning from malaria-affected countries, such as sub-Saharan Africa and South Asia.

Malaria has been a major public health problem in India. Intermittent fever in the diseases and high incidence during the rainy season, coinciding with agriculture, sowing and harvesting, was first recognized by Romans and Greeks who associated it with swampy areas. They postulated that intermittent fevers were due to the 'bad odour' coming from the marshy areas and thus gave the name 'malaria' ('mal'=bad + 'air'). Despite the causative organism known today, the name has stuck to this disease.

Malaria is caused by different species of *Plasmodium*; *Plasmodium vivax* (*P. vivax*), *Plasmodium falciparum* (*P. falciparum*), *Plasmodium malariae* (*P. malariae*) and *Plasmodium ovale* (*P. ovale*). Among these, *P. vivax* and *P. falciparum* are commonly reported from India. Recently *Plasmodium knowlesi*, which causes malaria in primates has been reported in humans. *Anopheles* species is called disease vector as infective bite of the mosquito transmits the pathogen from infected to healthy person. Man develops disease after 10 to 14 days of being bitten by an infective mosquito. Inside the human host, the parasite undergoes a series of changes as a part of its complex life cycle. The parasite completes its life cycle in liver cells (pre-erythrocytic schizogony) and red blood cells (erythrocytic schizogony). Infection with *P. falciparum* is the deadliest form of malaria.

10.3.1 Life Cycle of Malaria Parasite

The infection of malaria starts in human beings, when a female *Anopheles* bites a healthy human being to suck the blood and injects her saliva containing "sporozoites" of *Plasmodium*. A sporozoite travels in the bloodstream and enters the liver where it invades a liver cell. It matures into a "schizont" (mother cell) which produces 30000–40000 "merozoites" (daughter cells) within six days by multiple fission. The merozoites burst out of the liver cells and either re-infect liver cells or invade red blood cells (RBCs). In RBCs, the merozoite transforms into a trophozoite within two days and starts feeding on the haemoglobin breaking it down into haem pigment and globin protein. The trophozoite gradually develops into a schizont through a series of stages and 8–24 new merozoites burst out from the red blood cell. The ruptured cell releases toxic pigment (haem converts to haemozoin) which causes chills and fever. The released merozoites invade new red cells. *Plasmodium* can prevent the destruction of an infected red blood cell in the spleen (the organ where old

and damaged red cells are destroyed) by sending adhesive proteins to the cell membrane of the RBC. The proteins stick the red blood cell to the walls of small blood vessels. This poses a threat to the human host since the clustered red blood cells might block the circulatory system.

A merozoite, sometimes develops into a "gametocyte", the stage that enters the mosquito. The merozoites form two kinds of gametocytes: male gametocytes (microgametes) and female gametocytes (macrogametes). They get ingested by a mosquito, while sucking the blood. The gametocytes mature into gametes and fuse inside the mosquito's midgut to form "zygotes" which then elongate and develop into "ookinetes". The motile ookinetes penetrate the midgut wall and a cyst is formed around them forming "oocysts". The cysts undergo meiotic division and eventually release sporozoites. These migrate into the salivary glands from where they get injected into human blood. **The development of *Plasmodium* inside a mosquito takes about two weeks and makes a mosquito capable to transmit the disease. *Plasmodium* cannot complete its life cycle at temperatures below 20 °C (Fig. 10.11).**

The life cycle of *Plasmodium* in liver cells is called **exoerythrocytic cycle**, while inside blood cells it is termed as **erythrocytic cycle**. On the other hand, the life cycle completed in mosquito is named as **sporogonic cycle**.

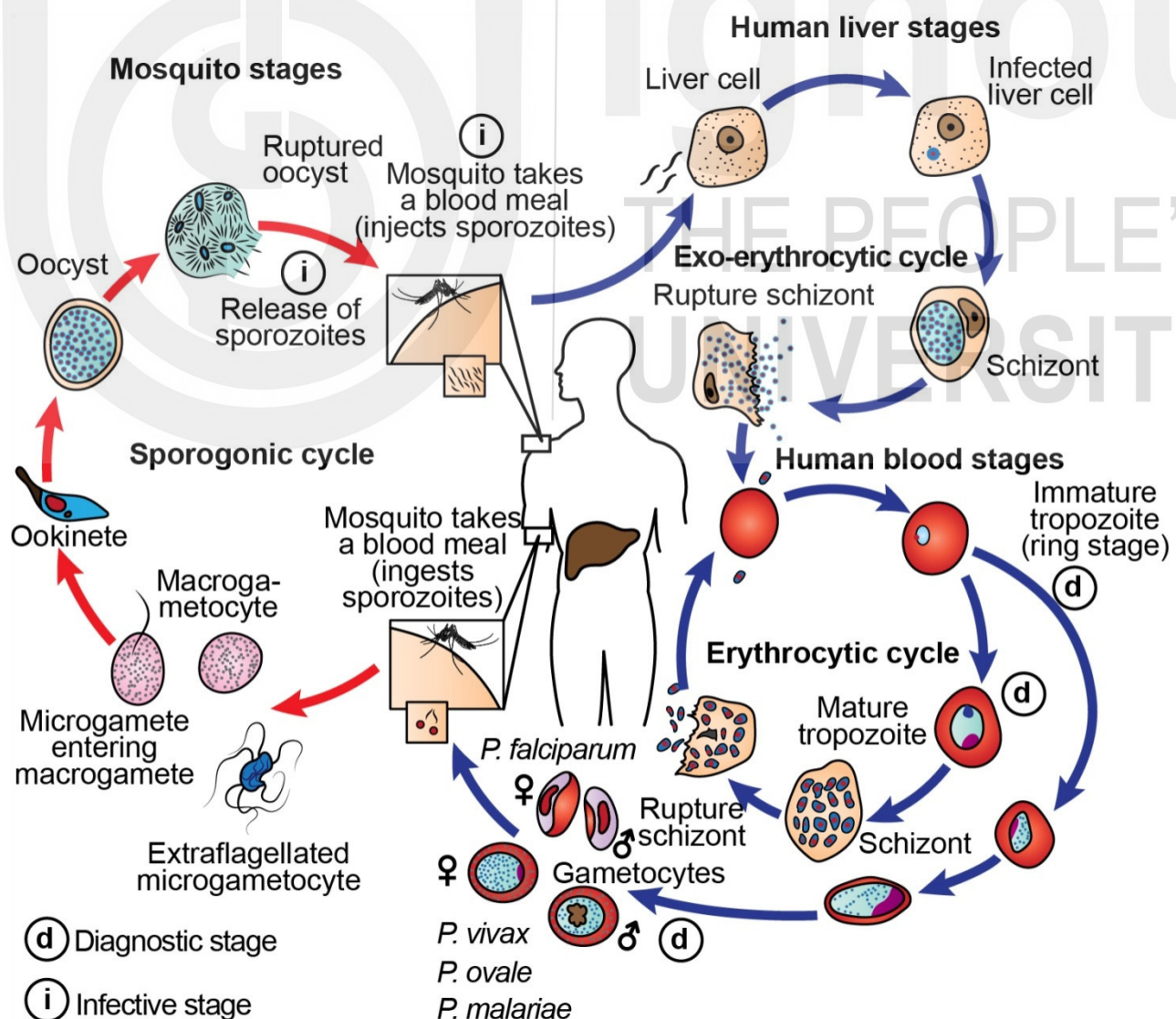


Fig. 10.11: Life cycle of malaria parasite (courtesy CDC).

10.3.2 Symptoms

After being bitten by an infected mosquito, symptoms usually begin in the human beings within 10–30 days. Malaria can be uncomplicated or severe. Symptoms of uncomplicated malaria might include; Chills, diarrhea, fever, headaches, muscle pain, nausea, sweating, vomiting and weakness.

Populations, most at risk are young children, pregnant women, people living with HIV, people affected with humanitarian emergencies and non immune travellers moving into endemic area. The poorest of the poor in vulnerable communities, living in remote rural areas with limited access to health facilities, suffer the most.

Some less noticeable manifestations of malaria are enlargement of the spleen (Splenomegaly) or liver (Hepatomegaly), increased breathing frequency, mild anemia and mild jaundice (yellowish coloration in the eye whites and skin).

The disease, however, can turn into severe malaria, if there are serious organ failures or abnormalities in the bloodstream or body metabolism. Symptoms of severe malaria include; breathing difficulties, coma, confusion, death, focal neurologic signs, seizures and severe anemia. Some less noticeable manifestations of severe malaria are abnormalities in blood coagulation, presence of haemoglobin in the urine, high acidity of the blood, hypoglycemia (low blood glucose), hypotension (low blood pressure) and kidney failure. During pregnancy, malaria can lead to premature baby delivery or delivery of a low-birth-weight baby. The infant can get the parasite from the mother and develop the disease. In small children, involvement of central nervous system (cerebral malaria) can cause blindness, deafness, speech difficulty, paralysis and trouble with movements.

10.3.3 Diagnosis

Malaria is usually diagnosed by examining a blood sample under a microscope. The test kits are also available that detect antigens of *P. falciparum* in the patient's blood. These immunologic tests, known as **rapid diagnostic tests** (RDTs), can detect two different kinds of malaria antigens, one of *P. falciparum* and the other found in all the four *Plasmodium* species. RDTs usually give results in about 20 minutes. It is a good alternative to microscopy in the absence of reliable microscopic diagnosis. However, RDT might not detect infections, if there are not enough malaria parasites in the patient's blood. In that case, a negative RDT result can be followed by microscopy. If a patient with positive RDT result is not responding to treatment, the *Plasmodium* species should be diagnosed with microscopy for appropriate medication.

Diagnosis of malaria can be challenging for many reasons. Many health workers in developing countries are insufficiently trained and supervised. The microscopes and reagents might be of poor quality and the supply of electricity might be unreliable. Storage of blood samples and delayed testing until a qualified person is available to perform the microscopy, results in incorrect diagnosis. Many malaria endemic communities do not have the proper diagnostic tools such as microscopes and RDT kits.

10.3.4 Treatment

Malaria can lead to deaths in severe manifestations, though most deaths occur in rural areas. Quick progression from illness to death can be prevented by fast and effective medication.

When treating a malaria patient, the following should be taken into account:

- a) Age and weight of the person to give the correct amount of medication.
- b) Drug allergies or other medications taken by the patient.
- c) Place of Infection and *Plasmodium* species responsible for infection to decide the drug.

Species, such as *P. falciparum* and *P. vivax* have become resistant (in some areas) to many antimalarial drugs. For example, **chloroquine-resistant** strain of *P. falciparum* has spread to most endemic areas.

Listed below are some drugs that are usually recommended by national malaria control programmes. They might not be effective in many parts of the world due to drug-resistant strains.

- Artemisinin-containing combination treatments (for example, artemether-lumefantrine, Artesunate-amodiaquine)
- Atovaquone-proguanil
- Chloroquine
- Doxycycline
- Mefloquine
- Quinine
- Sulfadoxine-pyrimethamine.

Primaquine, is used as an adjunct drug against certain *Plasmodium* species. It is active against the dormant liver forms, which is rare/nonexistent in *P. falciparum*. Primaquine is not recommended for people who are deficient in glucose-6-phosphate dehydrogenase enzyme or for pregnant women.

Patients who have uncomplicated malaria can take treatment from a nearby hospital and rest at home. In emergency cases, rectal **artesianate drug** can be given as a first line treatment (if they cannot be treated orally). Patients with severe malaria can be hospitalized for many days. Treating all people simultaneously in a population, though can prevent major malaria epidemics, but unfortunately it can also increase drug resistance in the parasite and cause complications in those who are deficient in glucose-6-phosphate dehydrogenase.

Humans living in areas where malaria is common can become partially immune. Travellers, young children, women having their first or second pregnancy and those who have weak immunity due to other diseases (such as AIDS) have little to no immunity against malaria. In malaria endemic areas, pregnant women are recommended to eat iron and folate supplements to prevent anemia, get a curative dose of an antimalarial drug at least twice during pregnancy (starting from the second trimester) and sleep under an insecticide-treated bed net (ITNs).

SAQ 2

Read the following sentences and write True (T) or False (F).

- i) Malaria is a disease caused by *Anopheles* mosquito.
- ii) The vector for malaria is *Culex* species.
- iii) The infection of malaria starts when a female mosquito injects sporozoites of *Plasmodium* sp. present in her saliva into a human skin.
- iv) *Plasmodium* cannot complete its life cycle at temperature below 20°C.
- v) During pregnancy malaria can lead to premature baby delivery.
- vi) Most malarial deaths occur in urban areas.

10.4 PREVENTION AND CONTROL

The best way to prevent malaria is to avoid mosquito bites. The mosquitoes that carry the pathogen usually bite during early hours of night. *Anopheles* mosquito can be controlled at the larval as well as adult stage.

10.4.1 Traditional Methods of Control

Mosquito larvae can be eliminated before they reach adulthood. Rainfall forms water puddles where mosquitoes lay their eggs which hatch into aquatic larvae and they develop into adults in a few days. Draining or removal of small puddles can reduce the *Anopheles* population. Chemical insecticides can also be applied in the water but might harm the environment and other organisms living in that habitat.

Other measures which can be taken against larvae are:

- Insect growth regulators (IGRs)
- Cover or spray the water surface with oil, such as kerosene, petroleum etc. which suffocates the aquatic larvae
- Use larvicides, such as temephos in domestic water
- Introduce larvivorous fishes, like *Gambusia*; naiads of dragonfly and mayfly; and cyclopoid copepods in water bodies which feed on the mosquito larvae
- Add toxins from the bacterium *Bacillus thuringiensis* var. israelensis (*Bti*), or *Bacillus sphaericus* in water

Additional personal protection and other methods used against mosquito adults include:

- Use glass windows (a well-constructed house).
- Clear off bushes and shrubs where mosquitoes can breed.
- Fumigate the houses and neighbouring areas with insecticides, such as pyrethroids, malathion, etc. to kill the mosquito adults.

- Sleep under a mosquito net/insecticide treated mosquito net.
- Use mosquito repellent on exposed skin.
- Kill adults by spraying insecticides inside the human dwellings.
- Use mosquito mats, coils and anti-mosquito cream to repel the mosquitoes from biting.
- Cover exposed parts of the body with thick and white or light-coloured clothing.

10.4.2 Modern Approaches in Control

(a) Bed-Nets

Insecticide-Treated Nets (ITNs): These are simple mosquito nets that are usually made of polyester but sometimes cotton, polyethylene, or polypropylene is used. These are treated with an insecticide, generally pyrethroids which have low health risks to humans but are toxic to mosquitoes at low doses. Pyrethroids do not rapidly wear off, unless exposed to sunlight or washed.

These nets require 're-dipping' to restore the insecticide every 6-12 months. Re-dipping involves soaking the nets in a bucket of insecticide solution and then hanging them up to dry.

Simple mosquito nets: These are nets without any insecticide treatment. They do not kill but prevent mosquito bite and local transmission.

LLINs: Long lasting insecticide nets (LLINs): These are used in malaria-endemic areas for mosquito control and prevention of bites. The insecticide is cleverly bound within the fibres that make up the netting and is 'slowly released' over a 4-5 years period. Hence, it is called 'long lasting'.

Insecticide-treated nets, therefore, provide two levels of protection. First, as a mechanical barrier against the bites of malaria-carrying mosquitoes and second, as a means of killing mosquitoes on contact with the insecticide. The mosquitoes are drawn to the nets by the odour of the person sleeping beneath it and are killed as soon as they land on the nets. The 'knock down' or killing of the mosquito protects the person sleeping under the net. Though, nets can develop small holes over time yet even with a few holes, they still remain 90 to 95% effective, and as and when the mosquito lands on the net it is knocked down and killed (Fig. 10.12).



Fig. 10.12: Long lasting Insecticide treated Nets.

These nets are safe for children as the quantity of insecticide, a child might ingest by licking their hands after touching the net, is small enough not to cause any harm. The small amount that is transferred to the tiny mosquito, however, is enough to kill it.

Modified Nets: Some of Anophelines are developing resistance to LLINs, therefore, new combination nets are now tested for use. Piperonylbutoxide (PBO) (a synergist) + Variant LLINs is one such net targeting pyrethroid-resistant vector populations.

(b) Indoor Residual Spray (IRS)

Indoor residual spray refers to the deposition of insecticide on the wall of house. Most of the insecticides having residual effect are sprayed indoors. When mosquitoes after taking blood meal rest in the house, they pick up sufficient insecticide particles sprayed on the walls and other indoor surfaces of the house. As a result, the longevity of mosquito reduces so much that it does not survive.

In areas where the vectors are strongly endophilic, i.e. they tend to rest indoors, IRS of human dwellings can give very effective control. Vectors that are exophilic, i.e. they tend to rest outdoor but tend to feed or rest indoors briefly, can also be effectively controlled by indoor residual spraying. In areas where vectors are strongly exophilic and/or exophagic, i.e. they rest and bite outdoors, other control methods, such as use of insecticide-treated mosquito nets or exterior space spraying (for emergency control), are practiced.

Table 10.1 presents a few insecticides, their formulations and dosages used during spray. Different types of sprayers are used depending upon the area to be sprayed and chemical used. Nowadays, new type of pumps with control flow valve are being used with improved tip of spray nozzle to avoid choking and erosion (Fig. 10.13).

Table 10.1: Insecticide formulations and dosages for IRS (<https://nvbdcp.gov.in/>).

S. No.	Name of Insecticide	Preparation of suspension in water	Dosage per sq. metre of active ingredient	Residual effect in weeks	Area to be covered by 10 L of suspension to get correct dosage
1.	DDT 50% WP	1 kg/10 L	1 g	10-12	500 sqm
2.	Malathion 25% WP	2 kg/10 L	2 g	6-8	250 sqm
3.	Deltamethrin 2.5% WP	400 g/10 L	20 mg	10-12	500 sqm
4.	Cyfluthrin 10% WP	125 g/10 L	25 mg	10-12	500 sqm
5.	Lambda-cyhalothrin 10% WP	125 g/10 L	25 mg	10-12	500 sqm
6.	Alpha-cypermethrin 5% WP	250g/10 L	25 mg	10-12	500 sqm
7.	Bifenthrin 10% WP	125g/10 L	25 mg	10-12	500 sqm

(c) Attractive Toxic Sugar Baits (ATSB)

Attractive-toxic sugar baits (ATSBs) are considered a new vector control paradigm based on “attract and kill” approach. The concept is based on the fact that mosquitoes use plant sugars as an energy source; and the successful feeding helps in high survival rate and reproductive fitness of mosquito.

The sugar feeding behavior of mosquito is tapped to formulate ATSBs by combining a concentrated sugar-based food source, an olfaction stimulant and a systemic insecticide. Vectors searching for **natural sugar sources** are diverted and attracted to baits. Vectors **ingest a toxin orally** as they feed on and are killed.

(d) Biological Control

The biological control focuses on the use of agents that act as pathogens or parasites impacting their survival or as predators and kill them.

For example, larvivorous fishes, like *Gambusia* and *Poecilia*; naiads of dragonfly and mayfly; and cyclopoid copepods are used in water bodies. Different bacterial pathogens, such as *Bacillus thuringiensis* var. israelensis (*Bti*), or *Bacillus sphaericus* are also used to kill larvae. Other biological agents are Mermitid Nematode (*Romanomermis culicivorax*), Notonectid bug, *Ambylospora* (Protozoa), *Coelomomyces* (Fungus) and NPV (Nuclear Polyhedrosis Virus).

Wolbachia, an intracellular bacterium, has been used to reduce mosquito fitness. Infected females can pass the bacteria on to their offspring in their eggs, and thus the maternally inherited endosymbiont infects the next generation. The uninfected females when mate with *Wolbachia*-infected males produce no progeny (Cytoplasmic incompatibility) (Fig. 10.14). Also, mosquitoes develop refractoriness to *Plasmodium*.

However, if females are infected with *Wolbachia*, whether they mate with infected or uninfected males, there is no reduction in the number of offspring. Infected females thus have a strong selective advantage.

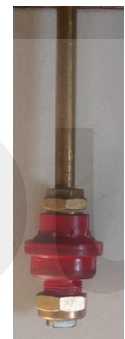


Fig. 10.13: Improved IRS sprayer and Nozzle with control flow valve.

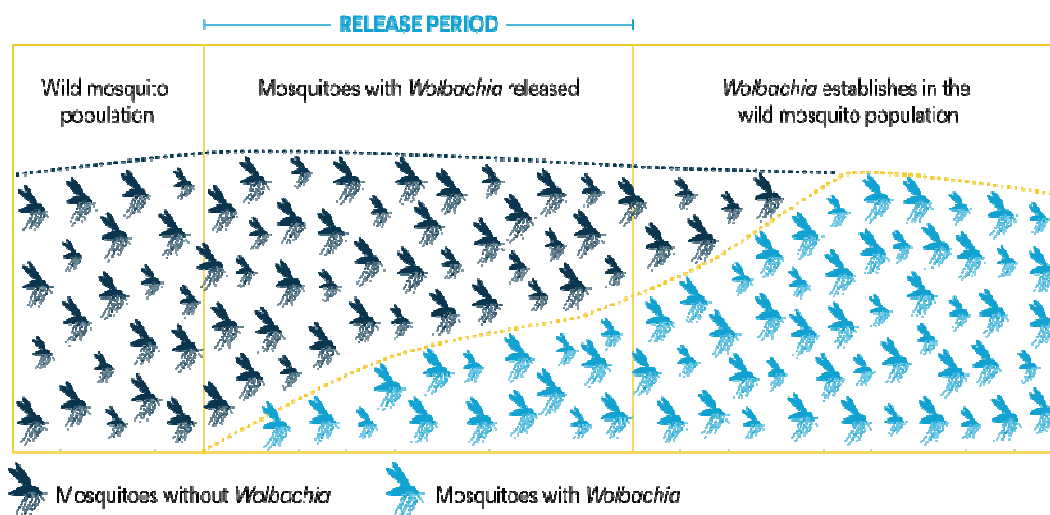


Fig.10.14: Mosquito control with *Wolbachia*.

Until recently it was thought that *Wolbachia* did not naturally infect *An. gambiae*, however, populations of *An. coluzzi* and *An. gambiae*, naturally infected with a strain of *Wolbachia*, have been discovered in Burkina Faso, West Africa.

The exciting prospect of using a *Wolbachia*-based strategy to control malaria transmission by anopheline mosquitoes attracted the attention of vector biologists. However, the creation of stably transfected populations of these mosquitoes has proved technically difficult. But once established in Anophelines it can be of great productivity. *Wolbachia* trials are going on in India as a novel control approach.

(e) Genetic Approaches

Mosquitoes can be genetically modified to impair their ability to transmit the malaria parasite.

Combined SIT and IIT: The sterile insect technique (SIT), incompatible insect technique (IIT) or a combination of the two could be used to suppress mosquito populations. Male mosquitoes are sterilised either by the application of irradiation (SIT) or infected with a bacterium, *Wolbachia* (IIT), or both. These are then released into the target population. These compete with the fertile males to mate with females and result in suppression of next generation (Fig. 10.15).

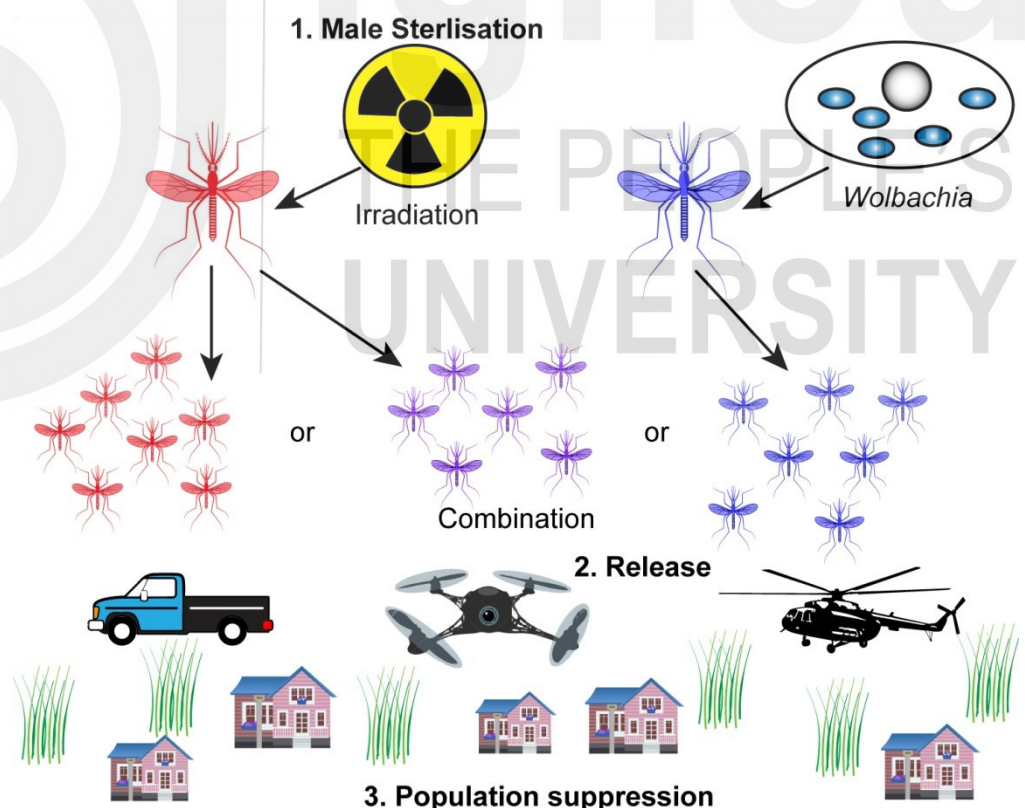


Fig. 10.15: Combined Male Sterilization Technique using Radiation and *Wolbachia*.

CRISPR/Cas – mediated Control: The recently developed CRISPR/Cas9-based genome editing tools for *Anopheles* mosquitoes provide new and promising opportunities for developing malaria control strategies through gene deletion to achieve complete agonist inactivation.

For example, gene editing can disrupt male-determining gene in the mosquitoes resulting in the production of males with feminized genitalia. Gene inactivation, obtained by CRISPR/Cas9 genome editing can reduce susceptibility to *Plasmodium* and mosquito fitness.

Other measures: Apart from these, introduction of chromosomal translocations and aberrations, such as deletion, inversion, etc.; and use of cytoplasmic incompatibility for producing hybrid sterility have been used as control measures in many *Anopheles* sp.

(f) RS & GIS

Remote sensing (RS) and Geographical Information system (GIS) are the tools initiated with satellite launching and advancement in Computer technology. These are now used to get information of large to very small areas as per the need and the geographical attributes which are confounding to malaria. .

- This helps to map the malaria transmission by mapping of breeding site and geo-spatial distribution of malaria using satellite information /image.
- GIS software combines ground collected data on mosquito-borne diseases and population epidemiology with the geospatial data through which mapping of risk areas and needs for control measures can be enumerated.
- Risk assessments/predictions of diseases can be done which helps to plan and formulate control strategies.
- Change in land use pattern and climates can be assessed that can impact disease transmission.
- Intensive control activities to reach the elimination target.
- Regular surveillance of vectors for changes in prevalence of vector species and their behavioral aspects.
- Regular monitoring of insecticide resistance.
- Identify the knowledge gap and generate data to fill it.
- Test new tools for their efficacy and their suitability in different ecosystems the vector species are occupying.

SAQ 3

Match Column A and Column B.

Column A	Column B
i) Long lasting Insecticide nets (LLINs)	a) Parasitizes or kills larvae
ii) Indoor Residual spray (IRS)	b) CRISPR/Cas-9
iii) Attractants	c) Mapping of breeding site
iv) Biological Control	d) Searching for natural sugar sources get diverted

v) Genetic Approaches	e) Deposition of insecticide on the wall of the house
vi) Remote sensing of Geographical Information System	f) Insecticide is bound with the fibres

10.5 SUMMARY

Let us summarise what we have learnt so far:

- Human malaria is transmitted by female of the genus *Anopheles*. It has 460 species, of which 30-40 species are reported to transmit pathogen, *Plasmodium* species.
- Anophelines comprise four stages in their life cycle: egg, larva, pupa and adult. The first three stages are aquatic and last for 7-14 days, depending on the species and the ambient temperature. The adult female *Anopheles* mosquito acts as vector for malaria. It can live upto a month (or more in captivity) but generally does not live more than 1-2 weeks in nature.
- Malaria is a mosquito-borne disease caused by a *Plasmodium* parasite. Symptoms of the disease consists of fever, chills and flu-like illness. If left untreated, humans may develop severe complications and die.
- Anopheles* mosquitoes feed during the night. Thus, use of simple bed nets or pyrethroid-treated bed nets prevent their bites. There are several traditional and modern approaches used in the control of mosquitoes. These include use of biological and microbial agents, chemical insecticides, net screens, gene editing, cytoplasmic incompatibility, SIT, sugar baits, remote sensing, etc.

10.6 TERMINAL QUESTIONS

- Describe the life cycle of *Anopheles* mosquito.
- Explain the epidemiology of malarial parasite.
- Discuss the preventive and control measures of *Anopheles* mosquito.

10.7 ANSWERS

Self Assessments Questions

- i) Water, ii) Spiracles, iii) Comma, iv) Nectar, v) 5 days, vi) 9 Km, vii) Zoophilic.
- i) F, ii) F, iii) T, iv) T, v) T, vi) F.
- i) f, ii) e, iii) d, iv) a, v) b, vi) c.

Terminal Questions

1. Refer to Section 10.2.
2. Refer to Section 10.3.
3. Refer to Section 10.4.



ignou
THE PEOPLE'S
UNIVERSITY

DIPTERANS AS DISEASE VECTORS-II (*CULEX* MOSQUITOES)

Structure

11.1	Introduction	National Filaria Control Programme (NFCP)
	Objectives	
11.2	Life Cycle of <i>Culex</i>	Elimination of Lymphatic Filariasis (ELF)
	Adult	11.6 Japanese Encephalitis (JE)
	Eggs	Pathogenicity
	Larvae	Diagnosis
	Pupae	Treatment
11.3	Behavior	11.7 Prevention and Control
11.4	<i>Culex</i> -borne Diseases	Anti-Larval Measures
11.5	Filariasis	Anti-Adult Measures
	Epidemiology	Protection against Mosquito Bites
	Causative Agent	11.8 Summary
	Life Cycle of the Parasite – <i>Wuchereria Bancrofti</i>	11.9 Terminal Questions
	Pathogenicity	11.10 Answers
	Diagnosis	
	Treatment	

11.1 INTRODUCTION

In the previous unit you have studied about the mosquito-borne diseases transmitted by *Anopheles* species. The present unit is focused on the diseases (pathogens) transmitted by another mosquito species, *Culex*. The distribution of *Culex* ranges from the tropics to cool temperate regions, but does not extend to the extreme northern latitudes where only *Aedes* and *Ochlerotatus* mosquito species are found.

Culex sp. are frequently referred to as “nuisance mosquitoes”. In India, *Culex quinquefasciatus* is the most commonly found domestic species which inhabits closer to the human population. Other prevalent species include *Cx. pipiens*, *Cx. fatigans*, *Cx. tritaeniorhynchus*, *Cx. tarsalis* and *Cx. gelidus*.

Diseases (pathogens) carried by one or more species of *Culex* mosquitoes vary in their dependence on the species of vector. Some are rarely and only incidentally transmitted by *Culex* species; but *Culex* and closely related genera of culicine mosquitoes, if established in a particular region, readily support perennial epidemics of certain major diseases. Arbovirus infections transmitted by various species of *Culex* include **West Nile virus**, **Japanese Encephalitis (JE)**, **St. Louis encephalitis**, and, **Western and Eastern equine encephalitis**. It is believed that *Culex* species might have role in Zika virus transmission. Nematode infection, filariasis, is transmitted by *Cx. quinquefasciatus* in India, though in other countries it can spread by other mosquitoes and blood-sucking flies. Although the *Culex* mosquito is not a primary vector for prevalent mosquito-borne diseases such as malaria, dengue and Chikungunya, the illnesses transmitted by them can also pose serious health threats to human beings.

Objectives

After you have read this unit you should be able to:

- ❖ explain the life cycle of *Culex* mosquito,
- ❖ explain the diseases transmitted by *Culex* species, recognize their symptoms and suggest the remedial measures,
- ❖ discuss the disease epidemiology and affecting factors, and
- ❖ describe the various preventive and control measures of *Culex* mosquitoes.

11.2 LIFE CYCLE OF CULEX

Mosquitoes are holometabolous insects. Life cycle of all mosquitoes are alike and passes through four stages, i.e. egg, larva, pupa, and adult. The first three stages are aquatic and last for 7-10 days, depending on the species and the ambient temperature. *Culex* adults can mate only in the darkness. They form swarms after sunset which is a natural mating behavior. The sound produced by a female mosquito attracts the flying males and copulation occurs during flight. After mating, the female mosquito selects some stagnant water of low depth for laying eggs.

11.2.1 Adult (Fig. 11.1)

Culex was described by Linnaeus in 1758. It is a brown-coloured mosquito, the size of which ranges from 4–10 mm (0.2–0.4 in). Adults are usually unicolor, but some species of the subgenus *Culex* have wings without scales, markings on the legs and pale spots on the wings similar to *Anopheles*.



Fig. 11.1: *Culex* mosquito.

Culex adults are characterized by the presence of distinct pulvilli and the absence of pre-spiracular setae and post-spiracular setae. The adults sit parallel to the surface unlike *Anopheles* which sits at an angle of 45°. They have long hairy antennae, short maxillary palps and long proboscis. The proboscis, thorax and wings are darker than the body. Tip of the abdomen is blunt and covered with broad scales. Males can be distinguished from the females in having feathery antennae and large palps.

11.2.2 Eggs (Fig. 11.2)

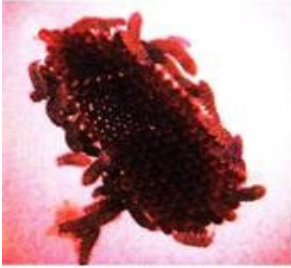


Fig. 11.2: Egg raft of the *Culex* mosquito

Adult female lays 150-300 eggs per oviposition. Eggs are laid on the surface of fresh or stagnant water. Water sources include puddles, barrels, horse troughs, ditches, ornamental ponds, unmaintained swimming pools and marshy areas.

Eggs are held together by adhesion forces and form a raft. It helps the eggs to float on the surface of water by trapping air bubbles. The eggs are brownish in colour and cigar-shaped, tapering at one end. Eggs are normally laid at night; they hatch after 24-48 hours into free swimming larvae.

11.2.3 Larvae (Fig. 11.3)



Fig. 11.3: Larva of *Culex* hanging at an angle from the water surface (Peter J. Bryant).

Culex larva is also called as wriggler because it exhibits a wriggling movement in water. The body is divided into head, thorax and abdomen. Head bears a pair of antennae, a pair of compound eyes, a pair of simple eyes and mouth parts bearing mandibles and maxillae. Feeding brushes are present around the mouth.

Thorax is large, unsegmented and abdomen is 9-segmented. Abdomen is without palmate hairs unlike *Anopheles*. Eighth abdominal segment bears a long tubular respiratory siphon at the tip of which lies a spiracle for aerial respiration while four tracheal gills are located in the 9th segment for aquatic respiration. Larva is bottom feeder but wriggles up frequently for taking atmospheric oxygen through respiratory siphon. At rest, larva hangs at an angle to the surface of water with its head downwards.

The larvae spend most of their time feeding on algae, bacteria, and other microorganisms. They occur in a wide range of habitats but most species prefer polluted water or water with high biological waste and open drains. The species, *Cx. tritaeniorhynchus*, prefers to breed in rice fields, grassy ditches, the edges of streams and rivers, and small, temporary rain pools.

11.2.4 Pupae (Fig. 11.4)

Culex pupa is comma-shaped and non-feeding stage. It swims actively and also called as tumbler. The head and thorax are merged into a cephalothorax and the abdomen is curved giving it the characteristic comma shape. The abdomen is long and 9-segmented. The abdominal 9th segment bears a pair of paddles for swimming and a pair of tracheal gills for aquatic respiration. Palmate hairs are present on all abdominal segments.

A pair of large respiratory trumpets for aerial respiration is present on cephalothorax. Inside the pupal case, developing adult structures can be observed. Within 2-7 days as a pupa, the dorsal surface of the cephalothorax splits and the adult mosquito emerges.

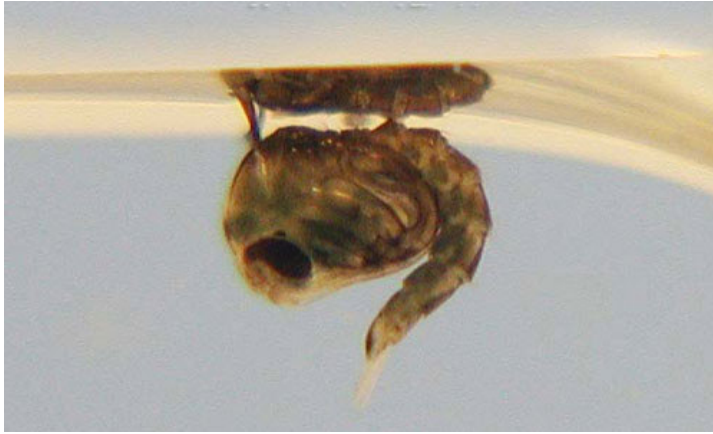


Fig. 11.4: Pupa of *Culex* (Source: Michelle Cutwa-Francis, University of Florida).

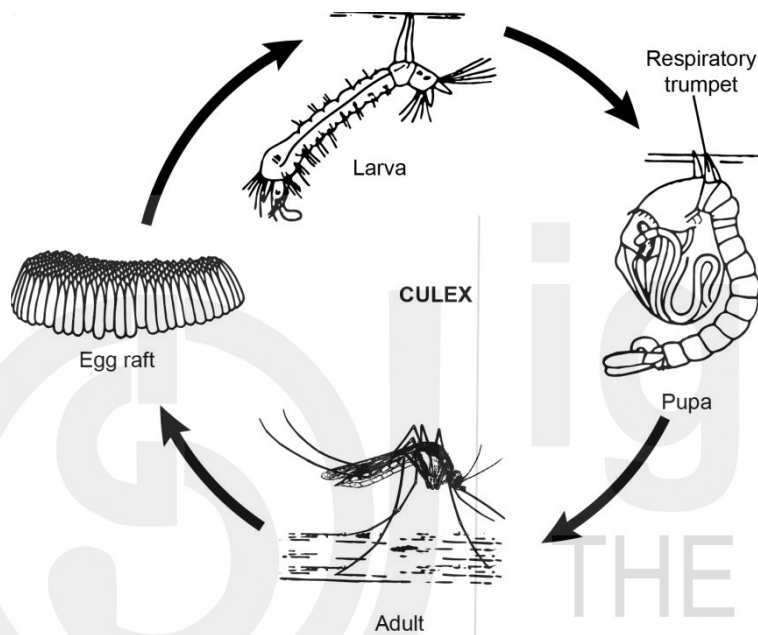


Fig. 11.5: Life cycle of *Culex* mosquito.

The duration from egg to adult varies considerably among species and is strongly influenced by ambient temperature and moisture conditions. Mosquitoes can develop from egg to adult in as little as 7 days but usually take 10-14 days in tropical conditions (Fig. 11.5).

11.3 BEHAVIOR

Behavior of mosquito is highly significant to assess its pest status. It comprises the swarming, biting, flight and egg laying habits.

Swarming: Among various species of mosquitoes, *Culex* mosquitoes form large swarms for mating in the evening that are visible from the very far off distances.

This swarming behavior is a kind of mating behavior of *Culex* mosquito which is performed during the flight.

Biting: Most of the *Culex* species are zoophilic in nature (like to bite animals). They enter the house at dusk and reach maximum density by midnight. These nocturnal insects are highly troublesome and irritating ones. Legs, particularly below the knee are the preferred biting sites.

During day, adults rest in the darker regions of their habitat. Both males and females feed upon the plant juices, though females also bite vertebrates and suck the blood once every 2 to 3 days for the development of their eggs.

Flight: The *Culex* has a strong pair of wings. They can fly up to eleven kilometers while sometimes the flight may be limited due to easy availability of host. They can also migrate to very long distance as the air drag may take them very far off places.

Oviposition (egg laying): After three days of mating and blood feeding, adult females lay eggs for which they search safe water bodies containing high amounts of biological waste or nutrients (Fig. 11.6). They, then lay eggs in the form of a boat-shaped raft of 150-300 eggs. *Culex* are the only mosquitoes which lay eggs in rafts. A female can lay 5-6 egg rafts in her life time. Mostly, the eggs are laid in the night when the water surface is least disturbed.



Rice Field



Shallow Ditches at Field

(a) *Breeding sites of Culex tritaeniorhynchus, Culex vishnui and Culex pseudovishnui*



(b) *Dirty stagnated water breeding site for Culex quinquefasciatus.*

Fig. 11.6: Breeding Sites of *Culex* mosquitoes.

SAQ 1

Read the following question and tick (✓) mark the correct option.

- i) Egg laying habit of mosquitoes is known as
 - a) Ovitrap
 - b) Oviposition
 - c) Ova-positioning
 - d) None of the above
- ii) At rest, *Culex* larvae
 - a) remain horizontal to the surface of water.
 - b) remain at the bottom of water container.
 - c) hang at an angle with the surface of water.
 - d) hang out side around the water container.

11.4 CULEX-BORNE DISEASES

Culex is a vector of several diseases prevalent in human beings and vertebrates (Table 11.1). In context with the Indian perspective, *Culex* species transmit primarily two diseases, **Filariasis** and **Japanese encephalitis**. Let us study about the transmission and symptoms of *Culex*-borne diseases.

Table 11.1: Diseases spread by *Culex* species.

Vector Species	Disease	Pathogen
<i>Culex pipiens</i> <i>Cx. quinquefasciatus</i>	Filariasis	<i>Wuchereria bancrofti</i>
<i>Cx. tarsalis</i> , <i>Cx. quinquefasciatus</i> , <i>Cx. stigmatosoma</i> , <i>Cx. thriambus</i> , <i>Cx. pipiens</i>	West Nile Virus (WNV)	<i>Alphavirus</i>
<i>Cx. tarsalis</i> , <i>Cx. pipiens complex</i> , <i>Cx. quinquefasciatus</i>	St. Louis Encephalitis (SLE)	<i>Flavivirus</i>
<i>Cx. tritaeniorhynchus</i> , <i>Cx. vishnui</i> and <i>Cx. pseudovishnui</i>	Japanese Encephalitis (JE)	<i>Flavivirus</i>

11.5 FILARIASIS

Filariasis has been a major public health problem in India, only next to malaria. The disease was recorded in India as early as 6th century B.C. by the famous Indian physician, Susruta in his book *Susruta Samhita*. In 7th century A.D.,

Madhavakara described signs and symptoms of the disease in his treatise '*Madhava Nidhana*' which holds good even today. In 1709, Clarke called elephantoid legs in Cochin as Malabar legs.

11.5.1 Epidemiology

Filariasis is confined to tropical and sub-tropical regions and prevalent mainly in developing countries. It affects over 120 million people in 73 countries including India, West Indies, Southern China, Japan, South America, West and Central Africa, Pacific Islands and Korea. It is almost absent from Europe, North America and polar regions.

Lymphatic filariasis is often associated with urbanization, industrialization, illiteracy, poverty and poor sanitation. Migration of people has not only favored the spread of filariasis in new areas but also led to the extension of filariasis into areas where filariasis was not so prevalent.

Climate is an important factor in the epidemiology of filariasis. Regions which are damp and moist and have stagnant water round the year provide a good breeding ground for the mosquitoes. In India, it is chiefly distributed in the Southern area especially along the sea coast affecting more than hundred million people. Indigenous cases have been reported from about 256 districts in 21 states/Union Territories. These are Andhra Pradesh, Assam, Bihar, Chhattisgarh, Goa, Jharkhand, Karnataka, Gujarat, Kerala, Madhya Pradesh, Maharashtra, Orissa, Tamil Nadu, Telangana, Uttar Pradesh, West Bengal, Pondicherry, Andaman & Nicobar Islands, Daman & Diu, Dadra & Nagar Haveli and Lakshadweep.

The North-Western States/UTs namely Jammu & Kashmir, Himachal Pradesh, Punjab, Haryana, Chandigarh, Rajasthan, Delhi and Uttaranchal and; North-Eastern States namely Sikkim, Arunachal Pradesh, Nagaland, Meghalaya, Mizoram, Manipur and Tripura are known to be free from indigenously acquired filarial infection.

11.5.2 Causative Agent

Three nematode parasites which cause lymphatic filariasis (LF) in human are *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. Of these, only *Wuchereria bancrofti* and *Brugia malayi* are found in India. In mainland India, *Wuchereria bancrofti*, transmitted by the ubiquitous vector, *Culex quinquefasciatus*, has been the predominant infection contributing to 99.4% of the problem in the country. The infection is prevalent in both urban and rural areas. The worm is not found in any other mammal and thus, is not a zoonotic disease. The worm is mainly found where *Culex* mosquitoes are found; in and around temporary pools or standing water.

Brugia malayi infection is primarily spread by *Mansonia (Mansonioides) annulifera* and *M. (M). uniformis*. The disease has been reported earlier from some rural areas in seven states viz., Kerala, Orissa, Tamil Nadu, Andhra Pradesh, Madhya Pradesh, Assam and West Bengal. However, its prevalence is now reportedly restricted to rural areas of Kerala and the infection disappeared in some pockets in other states.

11.5.3 Life Cycle of the Parasite – *Wuchereria Bancrofti*

Wuchereria bancrofti is a pseudocoelomate and cylindrical nematode. The body is covered with a thick cuticle. Man is the definitive host i.e. where the mature adult male and female parasites mate and produce microfilariae whereas the mosquito is the intermediate host (Fig. 11.7).

The adult parasite worms, male and female, live in the lymph vessels and lymph nodes by making nest in the dilated lymphatics, particularly in the groin regions. The adult worms survive for about 5-8 years and sometimes for as long as 15 years.

Adults are elongated, hair-like, cylindrical worms with a curved body. Both the ends of worm are tapering with head end terminating in a slightly round swelling. They are often creamy-white in colour, though sometimes they look transparent. Generally, males and females live coiled together and it is very difficult to separate them. Copulation between male and female adults takes place in the lymph glands of man. The females of *Wuchereria bancrofti* are **ovo-viviparous**. They liberate numerous, as many as 50,000, active embryos, called **juveniles** or **microfilariae**.

The discovery of microfilariae (mf) in the peripheral blood was made first by Lewis in 1872 in Calcutta (Kolkata).

Microfilariae are covered with a transparent sheath which is larger than the size of their body. The sheathed microfilariae begin to appear in the blood circulation in six months to one year after infection (prepatent period). They first enter the main lymphatic trunks from where they find their way into the circulating blood. They move with the blood stream and ultimately migrate to reside in the deeper blood vessel.

The sexual cycle of the parasite takes place in the human host, where the adult worms ultimately die. The life cycle of the parasite is cyclo-developmental in the vector where the parasites do not multiply. The microfilariae need a lower temperature for their development. Thus, they need mosquito for their development. If they are not sucked by mosquitoes, they die in human body. The average life span of microfilariae in human body is approximately 70 days.

You are aware that *Culex* is a nocturnal feeder, thus, to be sucked by them microfilariae exhibit **nocturnal periodicity** which coincides with the biting activity of the vector. During day time they reside in the large and deeper blood vessels of various organs, such as lungs, kidneys, heart and large arteries. However, during night, they appear in the peripheral blood vessels, especially between 10 pm to 4 am, to be sucked by *Culex*.

Microfilariae, (when picked up by the mosquito during blood meal) undergo development in mosquitoes to form infective larvae which usually takes about 10 to 14 days. The ingested microfilariae first shed their sheaths and penetrate the stomach wall. They then migrate to the muscles of the thorax and develop there without multiplication. The slender and tiny microfilariae (124 μ - 290 μ long) transform into immobile and inactive sausage-shaped larva (L1). It is thick and has a spiky tail formed by the cuticle. The larva grows rapidly in length and breadth after their first moult to become L2 or pre-infective larva (225 μ - 330 μ long), which is recognized by the presence of one or two papillae at its caudal end and a short tail. The L2 stage metamorphose into L3 which is highly motile and infective. It is slender and thread like, measuring about 1500-2000 microns in length.

When the infective mosquito feeds on another human host, the infective larvae are deposited at the site of mosquito bite. The larvae attracted by the warmth of human body invade the skin either through the puncture or on their own and enter the lymphatic system. In the human host, the infective larvae undergo two moults and develop into adult male and female worms. The adult worms survive for about 5–8 years or sometimes as long as 15 years or more.

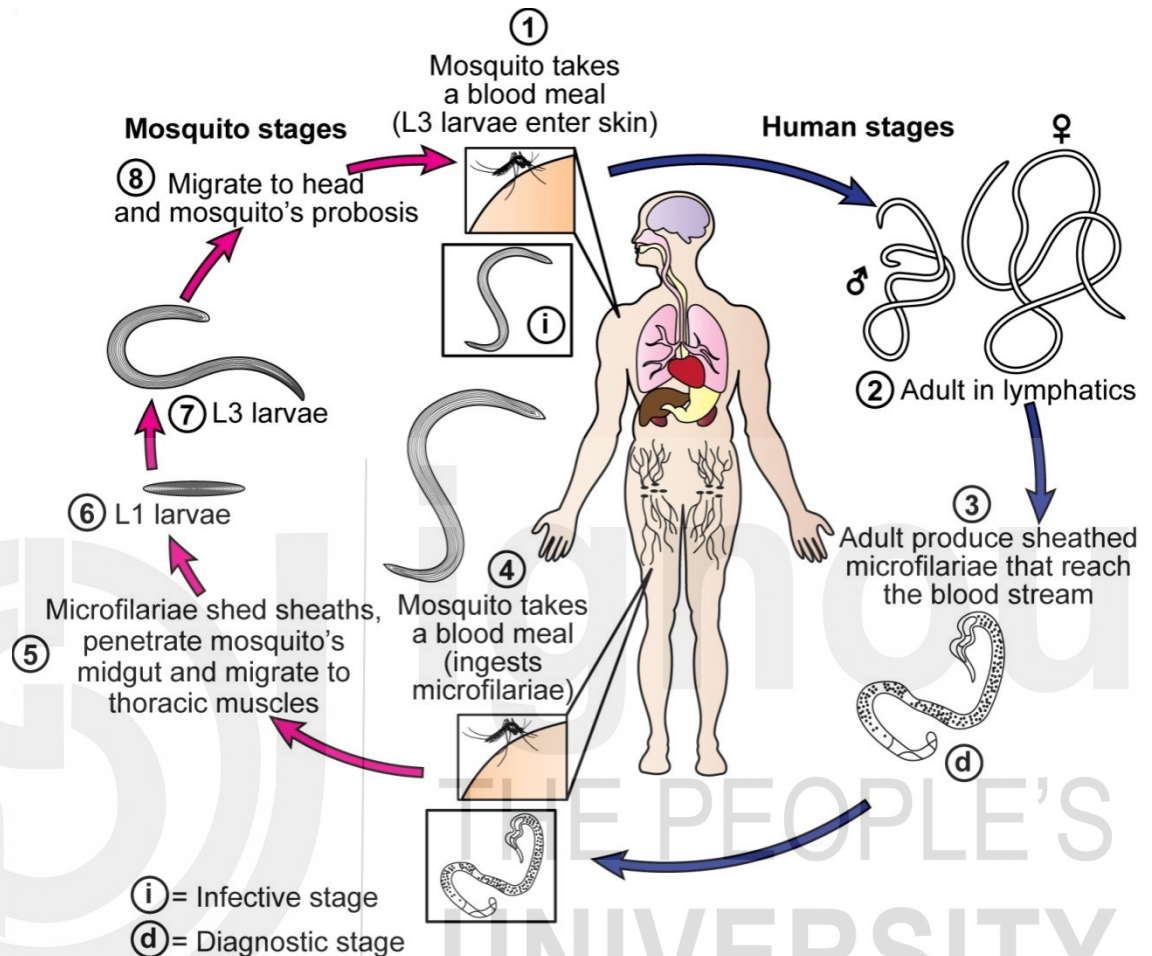


Fig. 11.7: Life cycle of Filarial worm, *Wuchereria bancrofti*

11.5.4 Pathogenicity

The pathogenic effects in the human beings are caused by mainly the adults of *Wuchereria bancrofti*. The disease is commonly called **Wuchereriosis, Bancroft's Filariasis or Classical Filariasis**. The disease is generally asymptomatic showing no external signs of infection. However, these cause the damage to the lymphatic system, kidneys and the immune system of the body. In case the infection is severe, the host shows various pathological symptoms. The microfilariae though circulate in the blood are not known to cause any pathogenic effects, except in severe cases. The disease caused by microfilariae is known as **Occult Filariasis**.

Various pathogenic effects caused by filarial worm are:

- Inflammation of the lymphatic system – **lymphangitis**. Inflamed areas are extremely painful and appear as cord-like swellings.
- Inflammation of the lymph nodes – **lymphadenitis**. It is accompanied by high fever for 3-5 days and increase in neutrophils

- c) Accumulation of fluid in the serous membrane surrounding the testes – **Hydrocoele**. It is less common in India.
- d) Excretion of milky-white urine due to the presence of chyle (milky fluid of small intestine) – **Chyluria**. It contains fat particles, albumin and fibrinogen along with microfilariae and RBCs.
- e) Blockage of the lymph flows in the body due to their accumulation in the lymph nodes – **Elephantiasis**. The affected part of the body becomes edematous and enlarge enormously becoming solid like a tumour. The surface of the skin becomes rough, dry, thick and papillomatus. *Wuchereria bancrofti* can affect the legs, arms, vulva, breasts, and scrotum.

11.5.5 Diagnosis

Individuals infected by filarial worms may be described as either “microfilaraemic” or “amicrofilaraemic”, depending on whether microfilariae can be found in their peripheral blood. Filariasis is diagnosed in microfilaraemic cases primarily through direct observation of microfilariae in the peripheral blood. Occult filariasis is diagnosed in amicrofilaraemic cases based on clinical observations and, in some cases, by finding a circulating antigen in the blood.

Those who develop the chronic stages of elephantiasis are usually free from microfilariae (amicrofilaraemic), and often have adverse immunological reactions to the microfilariae, as well as the adult worms (Source NVBDCP).

11.5.6 Treatment

- Administration of single dose of anti-filarial drugs to the entire community (mass drug administration), yearly once for 5-6years.
- Diethylcarbamazine (DEC) and albendazole for mass drug administration.
- Other drugs used –Ivermectin, Benzimidazole, Levamisole, Piperazine, Mebendazole, Imidazole.

11.5.7 National Filaria Control Programme (NFCP)

The National Filaria Control Programme (NFCP) was launched in the country in 1955 after pilot project conducted in Orissa from 1949 to 1954. The objective of NFCP are to delimit the problem, to undertake control measures in endemic areas and to train personnel to man the programme. The main control measures were mass administration of Diethylcarbamazine (DEC), antilarval measures in urban areas and indoor residual spray in rural areas.

The NFCP strategy was based on use of recurrent anti-measures at weekly intervals. These included:

- (a) Environmental methods; such as source reduction by filling ditches, pits, low lying areas, de-weeding, desilting, etc.
- (b) Biological control of mosquito breeding through larvivorous fish.
- (c) Anti-parasitic measures through 'detection' and 'treatment' of microfilaria carriers and diseased person with DEC, by Filaria Clinics in towns covered under the programme (source NVBDCP).

11.5.8 Elimination of Lymphatic Filariasis (ELF)

In 1997, The World Health Assembly adopted resolution, (WHA 50.29), for the Elimination of Lymphatic Filariasis as a global public health problem by 2020. In 2002, National Health Policy set a goal for ELF in India by 2015, which was extended to 2017. In 2004, ELF covered 202 endemic districts in 20 States/UTs. Subsequently it was scaled up to cover all the 256 endemic districts (21 States/UTs) targeting a population of about 650 million.

The twin pillars of LF elimination strategy include:

- Transmission control: To prevent occurrence of new infections by annual MDA with DEC and Albendazole for 5 years or more to population at risk except children below 2 yrs, pregnant women and seriously ill persons.
- Disability prevention and morbidity management for those who already have the disease: Home based management –limb hygiene for lymphoedema and Hospital based management – surgical correction for hydrocele.

JE virus is transmitted between mosquitoes, in particular, *Culex tritaeniorhynchus* and animals such as pigs. Humans are incidental or 'dead-end' hosts, because they usually do not develop high enough concentrations of the virus in their bloodstreams to infect feeding mosquitoes.

11.6 JAPANESE ENCEPHALITIS (JE)

Japanese Encephalitis is a viral disease (Fig. 11.8) transmitted by infective bites of female *Culex tritaeniorhynchus*, *Culex vishnui* and *Culex pseudovishnui*. However, some other mosquito species also play a role in transmission under specific conditions. It is primarily zoonotic in its natural cycle and man is an accidental host.

Natural hosts of JE virus include water birds of Ardeidae family (mainly pond herons and cattle egrets). Pigs play an important role in the natural cycle and serve as an amplifier host since they allow manifold virus multiplication without suffering from disease and maintain prolonged viraemia. Due to prolonged viraemia, mosquitoes get opportunity to pick up infection from pigs easily and transmit to human beings. Man is a dead end in transmission cycle due to low and short-lived viraemia. Mosquitoes do not get infection from JE patient.

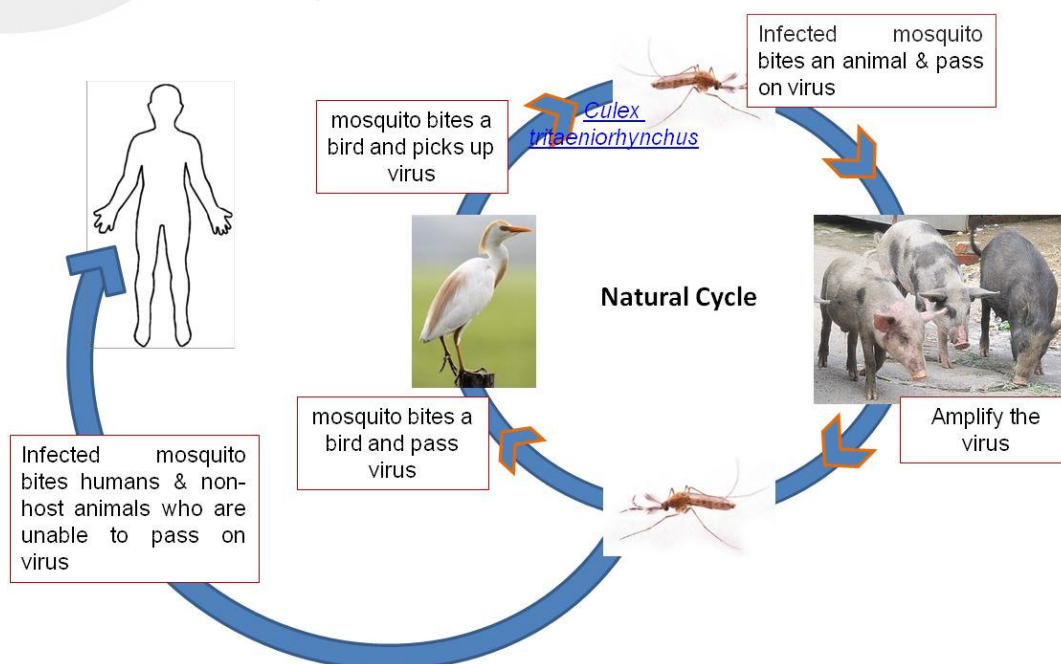


Fig. 11.8: JE cycle in human as well as zoonotic.

JE virus is neurotropic arbovirus and primarily affects central nervous system. The infection leads to classical symptoms similar to any other virus causing encephalitis.

Infections of JE virus are widespread in India. First evidence of the presence of JE virus dates back to 1955 when first case was confirmed. In India, annual incidence of JE ranges between 1714 and 6594 causing fatalities between 367 and 1665 (NVBDPC).

11.6.1 Pathogenicity

JE virus infection may result in febrile illness of variable severity. In children, gastrointestinal pain and vomiting may be the dominant initial symptoms. Severe disease is characterized by rapid onset of high fever, stupor, disorientation, coma, tremors, spastic paralysis, hypertonia, loss of coordination, focal neurological deficit etc. Prodromal stage (the period after incubation and before the characteristic symptoms of infection occur) may be abrupt (1-6 hours), acute (6-24 hours) or more commonly subacute (2-5 days). The disease usually lasts for a week but may prolong due to complications.

Amongst patients who survive, some are fully recovered through steady improvement, however some suffer with permanent intellectual, behavioral or neurological sequelae such as paralysis, recurrent seizures or the inability to speak.

11.6.2 Diagnosis

Clinically it is difficult to differentiate between JE and other viral encephalitis as JE virus infection presents classical symptoms similar to any other virus causing encephalitis. A laboratory test is required to confirm JE infection and to rule out other causes of encephalitis. Testing for JE-specific IgM antibody in a single sample of cerebrospinal fluid (CSF) or serum by Enzyme Linked Immuno-Sorbant Assay (ELISA) has been recommended by WHO.

Other laboratory tests available for detection of JE virus include; Haemagglutination Inhibition Test (HI); Complement Fixation Test (CF); Antigen Detection - RPHA, Indirect Fluorescence Antibody test, Immuno-peroxidase etc.; Genome Detection - RTPCR, etc.

11.6.3 Treatment

JE vaccine is produced in limited quantities at the Central Research Institute, Kasauli (HP). Three doses of the vaccine provide immunity lasting for a few years. The vaccine is procured directly by the state health authorities. Vaccination is not recommended as an outbreak control measure as it takes at least one month after second dose to develop antibodies at protective levels and the outbreaks are usually short-lived.

Ironically, there is no specific treatment of JE. Clinical management is supportive and in the acute phase. The treatment is directed at maintaining fluid and electrolyte balance in the body, and control of convulsions, if present. Maintenance of airway is crucial for the patient to recover. The state

governments have been advised to make anticipatory preparations in the endemic districts for timely availability of medicines, equipment and accessories as well as sufficient number of trained medical, nursing and paramedical personnel. The Government of India supports training programmes (source NVBDCP).

SAQ 2

Read the following question and tick mark (✓) the correct answer.

- i) Filaria is caused by a
 - a) Bacterium
 - b) Nematode
 - c) Protozoan
 - d) Virus
- ii) Japanese Encephalitis is a
 - a) Muscular disease
 - b) Bacterial diseases
 - c) *Aedes*-borne disease
 - d) Neurotropic disease
- iii) What is NFCP?
 - a. National Fertilizer Control Programme
 - b. National Filarial Control Programme
 - c. National Food Corporation of India
 - d. National Fund for Control of Population
- iv) Filariasis is caused by
 - a. *Entamoeba histolytica*
 - b. *Plasmodium vivax*
 - c. *Wuchereria bancrofti*
 - d. *Ascaris lumbricoides*

11.7 PREVENTION AND CONTROL

Culex-borne diseases can be controlled or prevented by keeping the vector population under control. The use of excessive insecticides to control mosquito population has not only caused development of resistance in the mosquitoes but has also increased the environmental pollution to a great extent. Integrated vector management approach is, thus recommended for the

control of mosquito population which involves use of more than one method with a view to obtain maximum benefit. The various methods to control the *Culex* vector are as below.

11.7.1 Anti-Larval Measures

This method deals with reducing mosquito population by removing the breeding ground and killing larva. A few measures are as follows:

- (a) Stagnant water bodies act as breeding ground for mosquito. By not allowing water to remain accumulated for long, breeding of mosquitoes can be prevented.
- (b) Application of kerosene, fuel oil, burnout diesel, mosquito larvicidal oil etc., in stagnant water once a week, prevents aerial respiration by mosquito larva without which they cannot survive.
- (c) In larger water bodies, like ponds, application of Paris green is recommended to prevent mosquito breeding. Paris green contains copper aceto arsenite which causes stomach poisoning in the larva.
- (d) Introduction of larvicidal fish such as *Gambusia* and *Lebistes* (Guppy) in water bodies acting as mosquito breeding ground is recommended as a measure to control mosquito population. These fishes feed upon mosquito larva voraciously and kill them.
- (e) Introduction of naiads(nymphs) of dragonfly and mayfly; and cyclopoid copepods in water bodies can also control mosquito larvae by feeding upon them.

11.7.2 Anti-Adult Measures

This measure deals with killing of adult mosquitoes. It includes:

- (a) **Use of mosquitocidal chemicals:** Spraying of DDT@ 1+2 gm/Sq meter applied 1 to 3 times in a year or spraying of lindane, malathion etc. in a lesser dose can kill adult mosquitoes.
- (b) **Application of space sprays and fumigants:** Spraying or fumigation of the houses and neighbouring areas with insecticides, such as pyethroids, malathion, etc. can kill the mosquito adults.
- (c) **Genetic control:** Mosquitoes can also be controlled by genetic methods, such as sterile male technique, chromosomal translocation, sex distortion and gene replacement methods. However, these methods have limitations and are expensive.

11.7.3 Protection Against Mosquito Bites

Infection occurs due to biting of parasite carrying mosquitoes. Protection against mosquito bite is the most effective measure to keep the disease under control. It can be achieved through:

- (a) Use of mosquito net with not less than 150 holes/sq inch.
- (b) Screening of building with copper and bronze gauze.
- (c) Use of mosquito coils, mosquito mats and repellent cream to keep the mosquito away.

SAQ 3

Fill in the blanks :

- i) *Culex*-borne diseases can be controlled by killing in their breeding grounds and using space
- ii) Stagnant water bodies act as, for mosquito.
- iii) Mosquitoes can be controlled genetically bymale technique and replacement.
- iv) Mosquito nets used against *Culex* mosquitoes should not have holes per square inch.

11.8 SUMMARY

Let us summarise what we have learnt so far:

- *Culex* adults are usually unicolour mosquitoes. Some species of the subgenus *Culex* have wings without scales, markings on the legs and pale spots on the wings similar to *Anopheles*. The *Culex* species is different from that of *Anopheles* and *Aedes* in appearance and habits. It is known to contribute to the spread of diseases; West Nile Virus, filariasis and encephalitis.
- *Culex* is a common mosquito found in natural as well as manmade environments. *Culex* breeds in wide variety of habitats; natural ponds, dirty water, sewage water, rice fields wells, etc. filled with water containing high biological content.
- *Culex* mosquito life cycle has four major stages, *i.e.* Eggs, larvae, Pupae & Adults .The length of life cycle can vary from 7-14 days in optimal conditions of water temperature at 25-27°C.
- Adult females lay 150-300 eggs per oviposition. Eggs are held together in the form of a raft which traps air bubbles to keep eggs floating on surface of water. At rest, larva lies at an angle to the surface of water. Abdomen lacks palmate hairs. It has long tubular respiratory siphon at the tip of which lies a spiracle for aerial respiration while four tracheal gills are located in the 9th segment for aquatic respiration.. The pupa is comma-shaped. The head and thorax are merged into a cephalothorax with the abdomen curving around underneath.
- In India, *Culex* species transmits mainly two diseases; Filariasis and Japanese Encephalitis.
- Lymphatic filariasis,spread by *Culex quinquefasciatus*,is caused by the worms *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*. These worms occupy the lymphatic system, including the lymph nodes causing various forms of diseases, lymphangitis, lymphadenitis and hydrocoele. In chronic cases, these worms lead to the swelling of limbs, called elephantiasis.

- Filariasis is seen mainly in developing countries. Lymphatic filariasis is often associated with urbanization, industrialization, illiteracy, poverty and poor sanitation. Migration of people favors the spread of filariasis.
- The programmes running at the national level for control and elimination of *Filaria* include National Filaria Control Programme (NFCP) & Elimination of Lymphatic Filariasis (ELF).
- Japanese Encephalitis (JE) is a viral disease, transmitted by infective bites of female mosquitoes mainly belonging to *Culex tritaeniorhynchus*, *Culex vishnui* and *Culex pseudovishnui* group. It is primarily zoonotic in its natural cycle and man is an accidental host.
- In children, gastrointestinal pain and vomiting may be the dominant initial symptoms of JE. Severe disease is characterized by rapid onset of high fever, stupor, disorientation, coma, tremors, spastic paralysis, hypertonia, loss of coordination, focal neurological deficit etc.
- The prevention and control measures of *Culex* are similar to other mosquitoes. The mosquito can be controlled at larval as well as adult stage using chemical measures, biological agents, genetic techniques and mosquito nets, etc.

11.9 TERMINAL QUESTIONS

1. Briefly discuss about the causative agent, transmission, symptoms and diagnosis of JE.
2. Describe life cycle of *Culex* mosquito. Support your answer with a well labelled diagram.
3. Write a short note on National Control Programme of Filariasis.
4. Describe the transmission cycle of Filariasis in brief. Add a note on the nocturnal periodicity of filarial worm.
5. Write various methods of *Culex* control.
6. Draw a labeled diagram of:
 - a) Life cycle of JE
 - b) Filarial worm transmission Cycle

11.10 ANSWERS

Self Assessment Questions

1. i) b, ii) c.
2. i) b, ii) c, iii) b, iv) c.
3. i) larvae, adults, sprays, ii) breeding ground, iii) sterile, gene, iv) 150.

Terminal Questions

1. Refer to Section 11.6.
2. Refer to Section 11.2.
3. Refer to Sub-Section 11.5.7.
4. Refer to Sub-Section 11.5.3.
5. Refer to Section 11.7.
6.
 - a) Refer to Figure 11.8.
 - b) Refer to Figure 11.7.



UNIT 12

DIPTERANS AS DISEASE VECTORS–III (*Aedes* MOSQUITOES)

Structure

12.1	Introduction	12.7	Chikungunya
	Objectives		Pathogen
12.2	Life Cycle of <i>Aedes</i>		Transmission Cycle
	Adults	12.8	Prevention and Control
	Eggs		Environmental Management
	Larvae		Methods
	Pupae		Biological Control
12.3	Behavior		Chemical Control
	Flying		Insect Growth Regulators (IGRs)
	Biting		Thermal Fog
	Resting		Ultra-low Volume (ULV)
	Oviposition		Aerosols (cold fogs) and Mists
12.4	<i>Aedes</i> -borne Diseases	12.9	Summary
12.5	Dengue	12.10	Terminal Questions
	Pathogen	12.11	Answers
	Transmission Cycle		
12.6	Zika		
	Pathogen		
	Transmission Cycle		

12.1 INTRODUCTION

In the previous two units you have studied about disease spread by *Anopheles* and *Culex* species of mosquitoes as vectors. In the present unit you will study the diseases transmitted by *Aedes* mosquitoes.

It is a culicine mosquito closer to *Culex* in appearance. *Aedes* is a genus of invasive mosquitoes found mostly in tropical and subtropical countries. About 125 countries of the world are affected by the menace of *Aedes*. There are about 888 species of *Aedes* worldwide, out of which only 25 species are disease vectors. In India, about 111 species of *Aedes* genera are found, of which mainly two species are involved in transmitting diseases such as Dengue, Chikungunya and Zika. The two species prevalent in India are *Aedes aegypti* (Asian Tiger mosquito) and *Aedes albopictus*. In other countries, *Aedes* is also responsible for the transmission of yellow fever, Eastern Equine Encephalitis and West Nile virus. In Polynesia, the species *Aedes polynesiensis* is even responsible for the transmission of human lymphatic filariasis.

Objectives

After you have read this unit you should be able to:

- ❖ explain the life cycle of *Aedes* mosquito,
- ❖ know about the diseases transmitted by *Aedes* species, recognize their symptoms and discuss the remedial measures,
- ❖ discuss the disease epidemiology and affecting factors, and
- ❖ describe the various preventive and control measures of *Aedes* mosquitoes.

12.2 LIFE CYCLE OF AEDES

Aedes mosquitoes are holometabolous insects. Their life cycle, thus comprise four stages; adult, egg, larva, and pupa.

12.2.1 Adults

Aedes are small and dark-coloured mosquitoes. They have unique patterns of light and dark scales on the abdomen and thorax, and alternating light and dark bands on the legs. In India, *Aedes aegypti* and *Aedes albopictus* are the main vectors that transmit the viruses causing dengue and Chikungunya. *Ae. aegypti* can be distinguished from the other species by presence of a white lyre-shaped mark on its thorax whereas, *Ae. albopictus* has a single line marking on thorax extending up to head (Fig. 12.1).

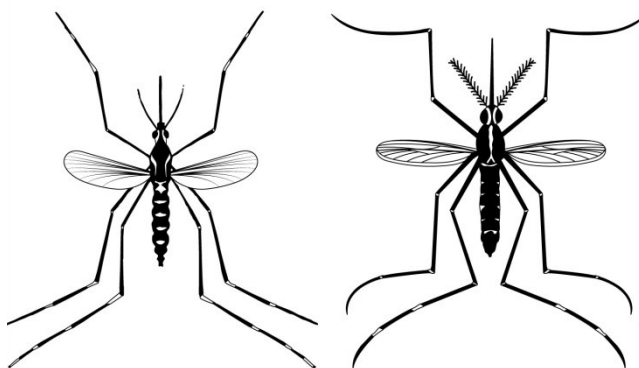


Fig. 12.1: *Aedes aegypti* (left); *Aedes albopictus* (Right).

Male *Aedes* can survive for 4-7 days while females can survive from 4 to 6 weeks. These are mostly active and bite during the daytime; the peak biting periods are early morning and in the evening before dusk. Male and female adults feed on nectar of plants; however, females also feed on the blood in order to produce eggs.

12.2.2 Eggs

After taking a blood meal, female *Ae. aegypti* mosquitoes lay eggs on the damp surfaces in areas likely to be temporarily flooded, such as tree holes and walls of man-made containers like barrels, drums, jars, pots, buckets, flower vases, plant saucers, tanks, discarded bottles, tins, tyres, water cooler, etc. and places where rain-water gets collected or is stored. The eggs are laid singly and spread out over hours or days, depending on the availability of suitable substrates. A female can produce up to five batches of eggs during her lifetime, each batch containing on an average 100-150 eggs. The number of eggs laid is generally dependent on the size of the blood meal.

The eggs of *Ae. aegypti* are smooth, long, ovoid, cigar-shaped and roughly one millimeter long. Freshly laid eggs appear white but within minutes they turn shiny black (Fig. 12.2). In warm climates, eggs may hatch in as little as two days, whereas in cooler temperate climates, hatching can take up to a week. Unlike eggs of other mosquitoes, ***Aedes* eggs can survive for very long periods in a dry state, often for more than a year. However, they hatch immediately once submerged in water. This makes the control of dengue vector very difficult.**



Fig. 12.2: Cigar-shaped *Aedes* eggs singly laid on the wall of containers.

12.2.3 Larvae

The hatched larvae feed on organic particulate matter in the water, such as algae and other microscopic organisms. Larvae are very active and move in the water rapidly. They have a respiratory siphon at the abdominal end through which they breathe in atmospheric oxygen (Fig. 12.3). Thus, they are often found hanging from the water surface at an angle.

Larvae can easily breed around the home in puddles, tyres, or within any object holding water. They pass through four instars, spending a short time in the first three, and up to three days in the fourth instar. The fourth instar larvae are approximately eight millimeters long. Male larvae develop faster and develop into pupae earlier than the females. The larval duration varies from 5-7 days, depending upon the temperature conditions. In colder environments, *Ae. aegypti* can remain in the larval stage even for months so long as the water supply is sufficient.



Fig. 12.3: (a) Larva of *Aedes aegypti* (b) hanging from the water surface through respiratory siphon.

12.2.4 Pupae



Fig. 12.4: Pupa of *Aedes aegypti*.

After the fourth instar, the larvae enter the pupal stage. Mosquito pupae do not feed but are mobile (called tumblers) and responsive to stimuli (Fig. 12.4). They breathe through respiratory trumpets present on their head and take approximately two days to develop. Adults emerge, the head out first, by ingesting air to expand the abdomen and split open the pupal case (Source: <http://www.denguevirusnet.com/life-cycle-of-Aedes-aegypti.html>)

The aquatic life cycle, from eggs to adults, of *Ae. aegypti* takes 8-10 days depending on temperature, which is inversely proportional to the duration (Fig. 12.5).

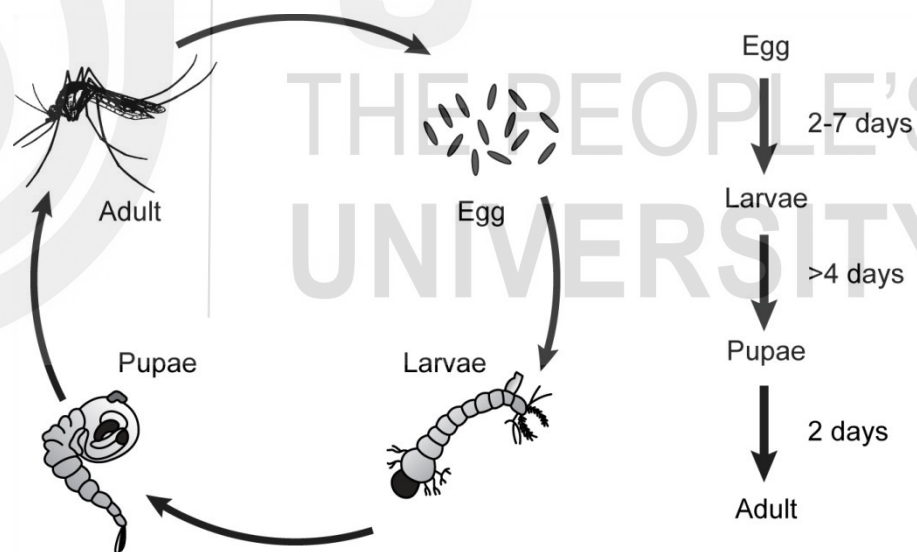


Fig. 12.5: Life cycle of *Aedes* Mosquito.

12.3 BEHAVIOR

Behavior of *Aedes* mosquitoes like flying, biting, resting, ovipositing etc. determines their vectorial capacity. Let's study about these.

12.3.1 Flying

Flight range of *Aedes* is very restricted. Studies suggest that most female *Ae. aegypti* may spend their lifetime in or around the houses where they emerge as adults. They have limited flight range of roughly 300 feet. If caught up in winds high above the ground, they can be carried great distances.

In general, mosquitoes that bite humans prefer to fly at heights of <25 ft.

Aedes mosquitoes have been found breeding in tree holes >40 ft. above ground. As per reports, they have been found breeding up to 8,000 feet in the Himalayas and 2000 feet underground in mines in India.

12.3.2 Biting

Male *Aedes*, like other species, does not suck blood while females need blood for the maturation of eggs. *Aedes* is *anthropophilic* (likes to bite humans) and is a day time biter with peak biting hours after sunrise and 2 hours before sunset.

In order to keep the bite painless during day time, *Aedes* has local numbness-causing components in its saliva. By the time host recognizes the mosquito bite, almost half of meal is taken after which they are normally disturbed due to alertness of the host. Therefore, they bite other hosts for completing their meal. As a result of such biting habit, an infected mosquito can spread infections to multiple persons till its blood meal is complete. One mosquito can normally bite 50-80 persons during its lifetime and therefore is more dangerous disease vector unlike *Anopheles* and *Culex*.

12.3.3 Resting

Aedes being a domesticated mosquito like to rest indoors in dark corners of houses; *i.e.* under the table, under beds, stairs, behind curtains, hanging cloths. etc.

12.3.4 Oviposition

Aedes is the only mosquito genera which lay eggs at waterline junction of container. The eggs are laid singly and without floats due to which they stick on to the container surface. A female lays 100-150 eggs in single oviposition distributing them among several containers (Figs. 12.6, 12.7 and 12.8).

Eggs can withstand long periods of desiccation, up to 1 year and can be transported to long distances in dry conditions. This residency period of eggs makes it very difficult to control as eggs remain dormant in any container.

Eggs can hatch when flooded and during rains.



Tender coconut shells



Buckets thrown in the open



Open box



Tyres thrown in the open



Open tank



Battery Boxes



Fountain



Solid Waste (Drums)



Tanks at Washing Place



Earthen Pots

Fig. 12.6: Different Breeding sites of *Aedes*.Fig. 12.7: Common Breeding sites for *Aedes aegypti*.Fig. 12.8: Common breeding sites of *Aedes albopictus*, top left Flower pot trays; top right Tyres; lower left leaf petioles; lower right tree holes.

SAQ 1

- i) What is the life span of *Aedes mosquito*?
- A. ♂ 4-7 days and ♀ 4 to 6 weeks
 - B. ♂ 15 days and ♀ 3 to 4 weeks
 - C. ♂ 1 month and ♀ 2 months
 - D. ♂ 2 days and ♀ 10 days
- ii) Eggs of *Aedes* are laid on the:
- A. water surface
 - B. dry surface
 - C. wall of container near water
 - D. wall of container inside water

12.4 AEDES-BORNE DISEASES

Several diseases are transmitted by different species of *Aedes* mosquitoes. These include dengue, Chikungunya, Zika, yellow fever, West Nile virus, Saint Louis Encephalitis virus, Eastern Equine Encephalitis virus. Among these, dengue and Chikungunya are the most prevalent diseases in India, while Zika has also been reported in a few states. All these diseases are transmitted by primarily *Ae. aegypti*. Other diseases have not been reported in India.

Let's study about the transmission and symptoms of *Aedes*-borne diseases found in India.

12.5 DENGUE

The origin of the word dengue is not clear. According to one theory, it is derived from the Swahili phrase "*Ka-dinga pepo*", meaning "cramp-like seizure caused by an evil spirit". It is believed that the Swahili word "dinga" may possibly have its origin from the Spanish word "dengue" meaning fastidious or careful. It describes the gait of a person suffering the bone pain of dengue fever. Dengue is also called as "Dandy Fever" because slaves in the West Indies who contracted dengue were said to have the posture and gait of a dandy.

As per the Chinese medical encyclopedia, the first probable dengue fever incidence, referred to as "water poison" associated with flying insects, was from the Jin Dynasty (265–420 AD). However, the first recognized dengue epidemics occurred almost simultaneously in Asia, Africa, and North America in the 1780s shortly after the identification and naming of the disease in 1779. The first confirmed case report dates back to 1789 and is by Benjamin Rush, who coined the term "breakbone fever" because of the symptoms of myalgia and arthralgia; extreme pain and stiffness of the muscles and bones.

The viral etiology and the transmission by mosquitoes were deciphered only in the 20th century. The socio-economic impact of World War II resulted in increased spread of disease at the global level. Nowadays, about 2.5 billion people, or 40% of the world's population, live in areas where there is a risk of dengue transmission. Dengue has already spread to more than 100 countries in Asia, the Pacific, the Americas, Africa, and the Caribbean. (Source: <http://www.denguevirusnet.com/life-cycle-of-Aedes-aegypti.html>)

12.5.1 Pathogen

Dengue is caused by Dengue virus (DENV), a mosquito-borne *Flavivirus*. DENV is a single positive-stranded RNA virus of the family Flaviviridae. The family also includes the West Nile virus, Zika virus, Yellow Fever Virus, and several other viruses which may cause encephalitis.

DENV causes a wide range of diseases in humans, from a self-limited Dengue Fever (DF) to a life-threatening syndrome called Dengue Hemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS). There are four antigenically different serotypes of the virus based on the antigens on their surface (although as per a 2013 report a fifth serotype has been found in Malaysia):

DENV-1

DENV-2

DENV-3

DENV-4

These serotypes are different strains of dengue virus that have 60-80% homology between each other. Infection by a particular serotype induces long-life protection against that, but only a short time cross-protective immunity against the other types. The first infection causes mostly minor disease, but secondary infection has been reported to cause severe diseases (DHF or DSS) in both children and adults.

DENV is a 50nm virus enveloped with a lipid membrane. There are 180 identical copies of the envelope (E) protein attached to the surface of the viral membrane by a short transmembrane segment. The virus has a genome of about 11000 bases that encodes a single large polyprotein that is subsequently cleaved into several structural and non-structural mature peptides. The polyprotein is divided into three **structural proteins**, capsid (C) protein, the envelope (E) glycoprotein and the membrane (M) protein; seven **nonstructural proteins**, NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5; and short **non-coding regions** on both the 5' and 3' ends (Fig. 12.9).

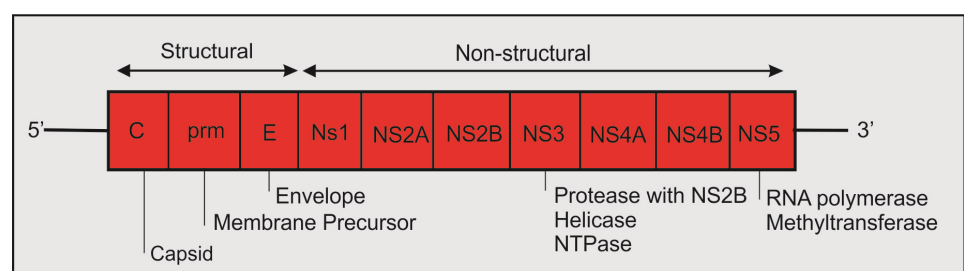


Fig. 12.9: Dengue virus genome structure with the structural and nonstructural genes.

The E glycoprotein is responsible for virion attachment to receptor and fusion of the virus envelope with the target cell membrane. In addition to the E glycoprotein, only one other viral protein, NS1, has been associated with a role in protective immunity. NS3 is a protease and a helicase, whereas NS5 is the RNA polymerase required for viral RNA replication.

12.5.2 Transmission Cycle

Aedes mosquito acquires dengue virus through three ways:

1. **Through infected patients:** Mosquito acquires virus while biting a dengue-infected person during viremic phase. This is called as **urban cycle**.
2. **From reservoir host:** Reservoir of any disease is said to be a non-human host that sustains the pathogen and becomes the potential source of human infection. Wild monkeys are reservoir hosts for the dengue fever virus and can spread the virus from the jungle to humans. This is called **sylvatic** or **peri-urban cycle**.

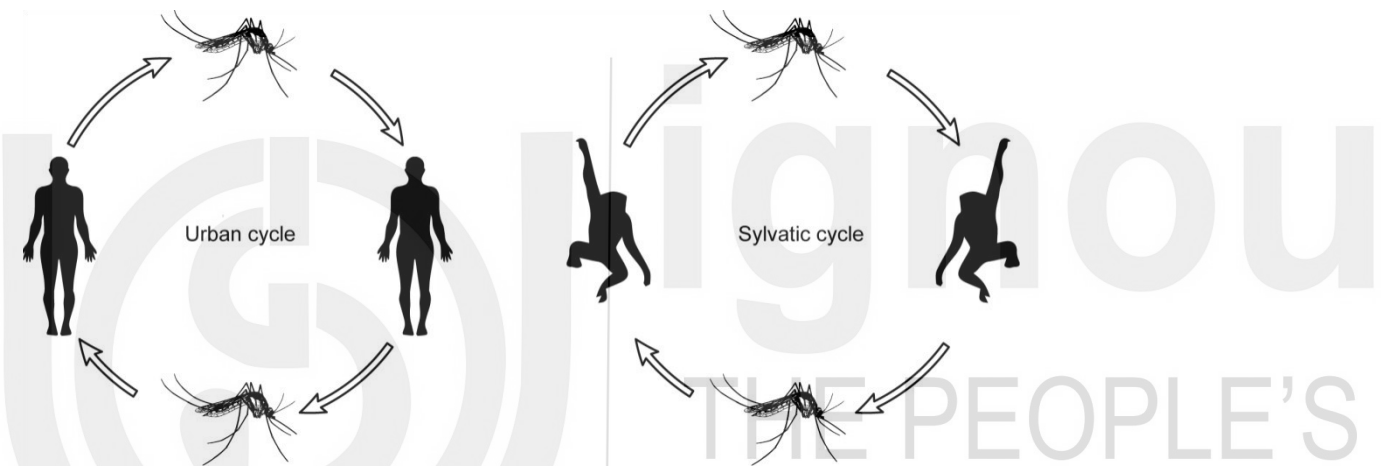


Fig. 12.10: Urban cycle and sylvatic cycle of *Aedes* mosquito.

3. **Transovarial Transmission (TOT):** The virus can be transmitted to the next generation of mosquitoes through eggs. The eggs are infected during multiplication of the virus in the body of mosquito and are passed to the next generation maintaining the virus in nature (Fig. 12.11).

The infection from one person to the other is known as **transverse transmission** while travelling of virus through generations of mosquito is known as **vertical transmission**.

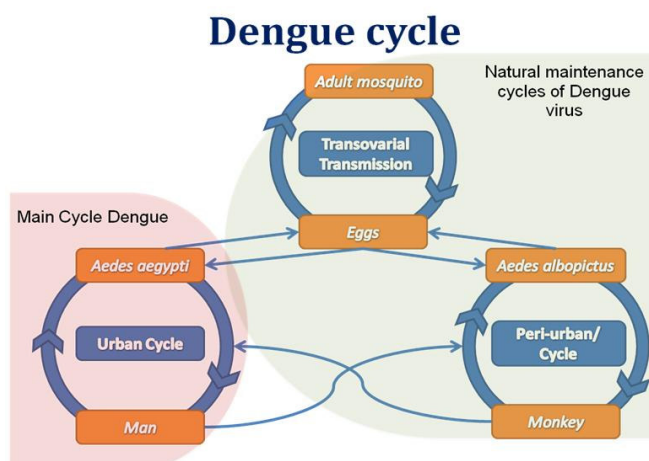


Fig. 12.11: Different cycles of Dengue occurring naturally.

Once virus reaches mosquito's body, it multiplies and reaches the salivary gland. The time period from the infected blood meal taken by mosquito to appearance of virus in salivary gland is known as **extrinsic incubation period** (or the duration between acquiring virus by the vector and when it becomes able to transmit the virus). This period is of approximately 8-12 days and temperature-dependent. Rise in temperature reduces the incubation period and enables quicker transmission.

After extrinsic incubation period, *Aedes* can transmit the virus to a healthy person while taking blood meal during which mosquito injects saliva in the human blood. The time duration from the bite of infective mosquito to appearance of 1st symptom of disease is known as **intrinsic incubation period** (Fig. 12.12).

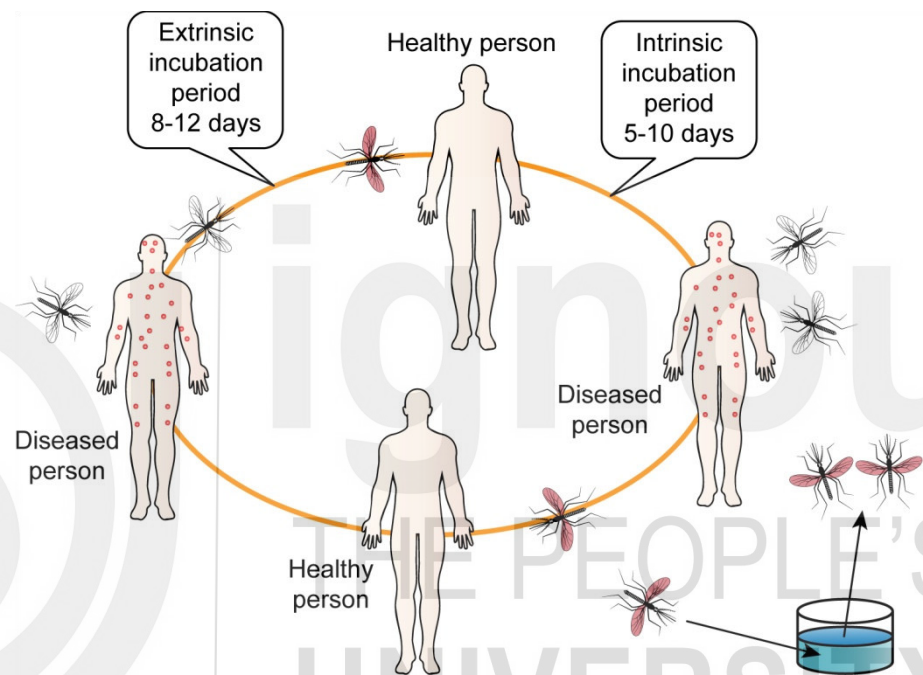


Fig. 12.12: Transmission Cycle of Dengue virus to healthy person through infected mosquitoes.

12.6 ZIKA

Zika fever is caused by the Zika virus (ZIKV), an arthropod-borne virus (arbovirus). The Zika virus is also a member of the *Flavivirus* genus in the family *Flaviviridae*.

In 1947, scientists researching yellow fever placed a rhesus macaque in a cage in the Zika Forest (zika meaning "overgrown" in the Luganda language), near the East African Virus Research Institute in Entebbe, Uganda. The monkey developed a fever, and researchers isolated from its serum a transmissible agent that was first described as Zika virus in 1952. It was subsequently isolated from a human in Nigeria in 1954. From its discovery till 2007, confirmed cases of Zika virus infection from Africa and Southeast Asia were rare.

The first outbreak of the disease outside of Africa and Asia was in April 2007, on the island of Yap in the Federated States of Micronesia. The condition was characterized by rash, conjunctivitis and arthralgia, and was initially thought to

be dengue. It was also suspected as Chikungunya and Ross River viruses. However, serum samples from patients in the acute phase of illness contained RNA of Zika virus. The Zika fever disease attack was relatively mild with 49 confirmed cases, 59 unconfirmed cases, no deaths and no hospitalizations.

A larger outbreak of Zika virus outside Africa and Asia was confirmed in April 2015, in Brazil. Around 500 patients had flu-like symptoms followed by rash and arthralgia. It was confirmed as Zika fever by RT-PCR technique. Local authorities linked the outbreak to increased flow of foreign visitors due to the 2014 FIFA World Cup, coupled with the large population of *Ae. aegypti* and *Ae. albopictus* in the region (Fig. 12.13).

Since April 2015, the Zika outbreak has been reported from South and Central America, and the Caribbean countries. According to the CDC (Centre for Disease Control and Prevention), Brazilian health authorities reported more than 3,500 microcephaly (children with smaller head due to birth defects) cases between October 2015 and January 2016 due to Zika infection in pregnant women. Some of the affected infants have had a severe microcephaly and some died due to underdeveloped brain (source : <http://www.zikavirusnet.com/history-of-zika.html>)

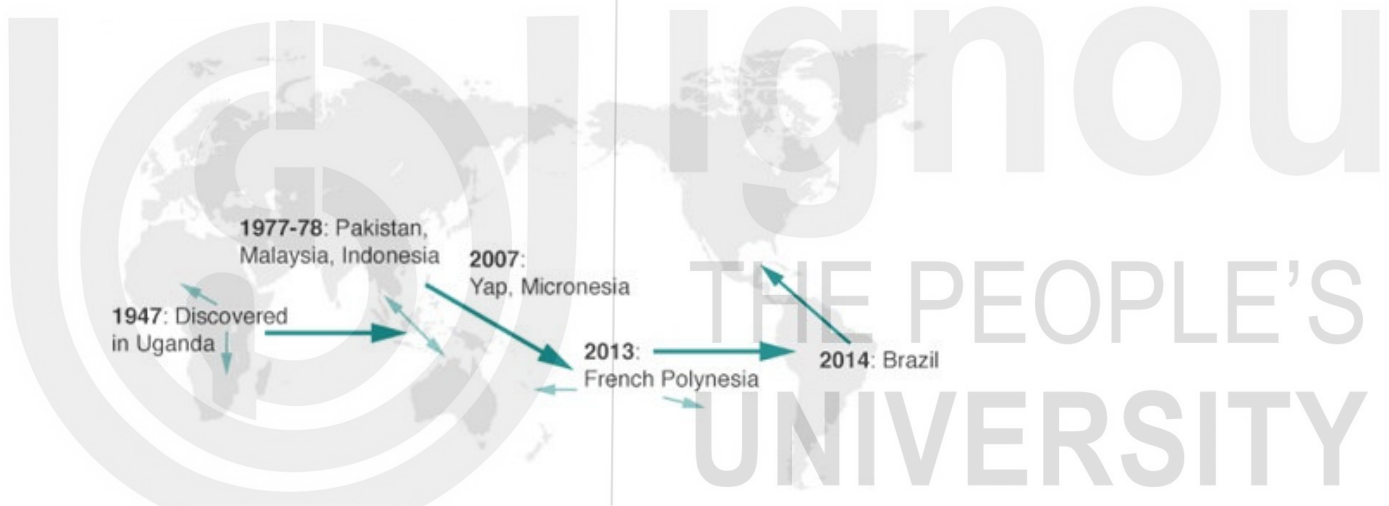


Fig. 12.13: How Zika virus spread from Uganda in Africa. Source: Lancaster University.

12.6.1 Pathogen

Like other viruses in the Flaviviridae family, Zika virus is enveloped and icosahedral with a non-segmented, single-stranded, positive sense RNA genome. Virus particles are 40 nm in diameter, with an outer envelope and a dense inner core. The genome of Zika virus is 10,617-nucleotide long and structurally similar to that of dengue virus with three structural proteins, capsid, premembrane/membrane, and envelope, and seven nonstructural proteins, NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 (Fig. 12.9).

12.6.2 Transmission Cycle

The transmission cycle of Zika through mosquitoes is similar to the dengue virus transmission. The extrinsic incubation period is about 3-14 days depending upon temperature and intrinsic incubation period is about 5-15 days.

Apart through mosquitoes, Zika virus can also be transmitted through body fluids during sexual intercourse and even from mother to the child inside her womb causing microcephaly (Fig. 12.14).

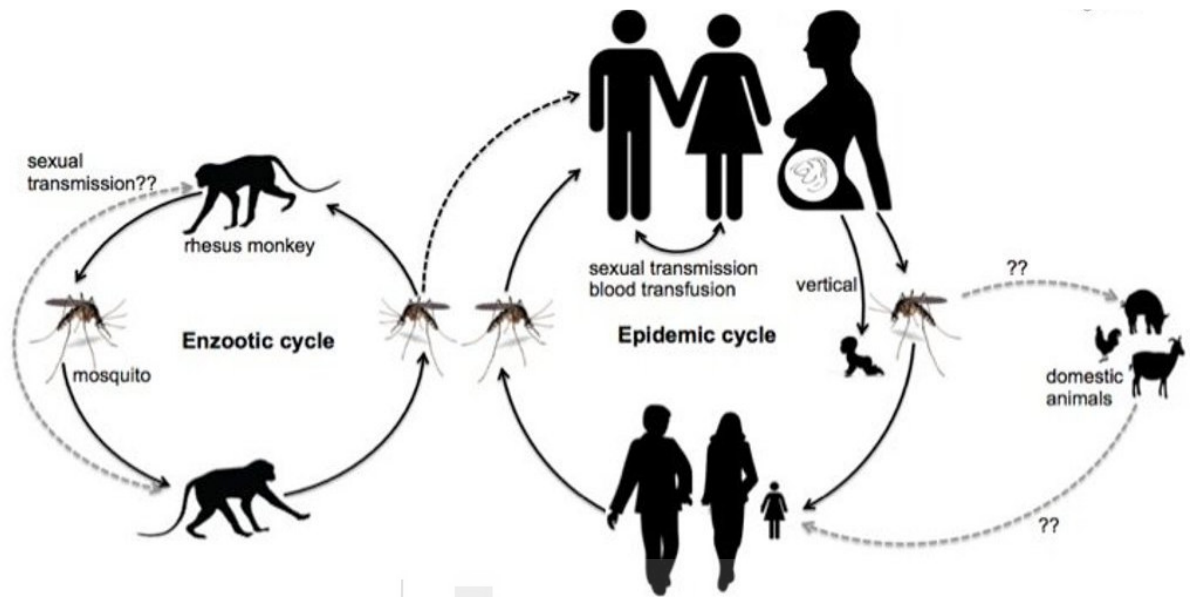


Fig. 12.14: The transmission of the Zika virus through mosquitoes, sexual contact or through blood transfusions.

12.7 CHIKUNGUNYA

Chikungunya is caused by the chikungunya arbovirus (CHIKV), a member of the *Alphavirus* genus in the family *Togaviridae*. A total of 29 different types of alphaviruses are known that cause diseases in humans and other mammals.

The disease was first detected in 1952 in Africa following an outbreak on the Makonde Plateau situated between Mozambique and Tanzania. The name chikungunya was then originated from the Makonde root verb *kungunyala* meaning “that which bends up” in reference to the stooped posture developed as a result of the arthritic symptoms of the disease. Subsequent authors assumed the derivation of the term Swahili, meaning “the illness of the bended walker”. Many other erroneous spellings and forms of the term are in common use including “Chicken guinea”, “Chicken gunaya” and “Chickengunya”.

Since its discovery in Africa, Chikungunya virus outbreaks occurred occasionally, but subsequent outbreaks spread the disease to other parts of the world. Numerous chikungunya re-emergence and outbreaks have been documented from Africa, Asia (India), and Europe, with irregular intervals of 2–20 years. In 2008, chikungunya was listed as a US National Institute of Allergy and Infectious Diseases (NIAID) category C priority pathogen. Currently, Chikungunya fever has been recorded from nearly 40 countries.

12.7.1 Pathogen

Chikungunya virus is a small (about 60–70 nm-diameter), spherical, positive-strand RNA virus with an envelope and a nucleocapsid. The virus genome is 11,805 nucleotides long and encodes for two polyproteins – the non-structural polyprotein consisting of four proteins (nsP1, nsP2, nsP3 and nsP4) and the

structural polyprotein consisting of five proteins (Capsid, E3, E2, 6K and E1) (Fig.12.14). The 5' end of the RNA molecule is capped with a 7-methylguanosine while the 3' end is poly-adenylated. The E1 and E2 glycoproteins are expected to form heterodimers that appear as spikes on the viral surface covering the surface evenly. The envelope glycoproteins play a role in attachment to cells. (Source: <http://www.chikungunyavirusnet.com/chikungunya-virus.html>)

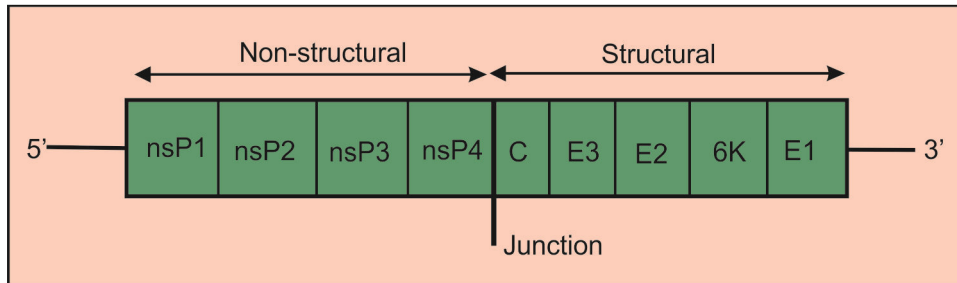


Fig. 12.14: Chikungunya virus genome structure.

12.7.2 Transmission Cycle

Transmission cycle of chikungunya is similar to that of dengue (Refer to Fig. 12.12).

SAQ 2

- i) Which of the following diseases is referred as water poison by Jin Dynasty of China?
 - A. Chikungunya
 - B. Zika
 - C. Yellow fever
 - D. Dengue
- ii) The disease which can be transmitted by pregnant mother to newborn baby is
 - A. Dengue
 - B. Zika
 - C. Chikungunya
 - D. Ross River
- iii) The duration from the bite of infective mosquito to appearance of its disease symptom is
 - A. Intrinsic incubation period
 - B. Host incubation period
 - C. Extrinsic incubation period
 - D. Disease period

The Alphavirus genus also includes O'nyong'nyong virus (ONNV) and Ross River virus (RRV). The O'nyong'nyong virus is also transmitted by mosquitoes and causes disease with symptoms similar to Chikungunya virus including arthritis, fever and rashes. This virus is so far limited to African countries mainly Uganda.

The Ross River virus is also transmitted by mosquitoes and causes rashes and arthritis. This virus is found in Australia and some of the surrounding islands.

12.8 PREVENTION AND CONTROL

Dengue fever/DHF control is primarily dependent on the control of *Ae. aegypti*, since no vaccine is yet available for the prevention of dengue infection and there are no specific drugs for its treatment. Earlier attempts relied almost exclusively on space spraying of insecticides for adult mosquito control. However, space spraying required specific operations that were often not adhered to, and most countries found its costs prohibitive as well. Subsequently, source reduction by clean-up campaigns and/or larviciding with insecticides has been promoted widely. However, their success has been limited on account of the variable degrees of compliance by communities and the non-acceptability of the larvicidal treatment either due to the bad odour of the insecticide used or inherent misgivings prevalent in some communities. Therefore, close cooperation with non-health sectors such as NGOs, civic organizations and community groups becomes essential to ensure community understanding and involvement, and to achieve a sustainable, successful DF/DHF vector control program.

The long-term and sustainable control of *Ae. aegypti* requires an integrated approach including all appropriate methods (environmental, biological and chemical) that are safe, cost-effective and environmentally acceptable.

12.8.1 Environmental Management Methods

Environmental management involves planning, organization, execution and monitoring of activities for the modification and/or manipulation of environmental factors or their interplay with human beings in order to prevent or minimize vector breeding and reduce human-vector-virus contact.

Three kinds of environmental management methods have been defined to control *Ae. aegypti* and *Ae. albopictus* and reduce man-vector contact. These are based on reduction in mosquito breeding through source reduction, solid waste management, modification of man-made breeding sites, and improved house design. These are:

- **Environmental modification:** This includes any long-lasting physical transformation of land, water and vegetation aimed at reducing vector habitats without causing unduly adverse effects on the quality of the human environment.
- **Environmental manipulation:** This incorporates planned recurrent activities aimed at producing temporary changes in vector habitats that involve the management of “essential” and “non-essential” containers, and the management or removal of “natural” breeding sites.
- **Changes in human habitation or behavior:** These feature the efforts made to reduce man-vector-virus contact.

Environmental modification

- Improved water supply
- Mosquito-proofing of overhead tanks/cisterns or underground reservoirs
- Filling, land levelling and transformation of impoundment margins

- Environmental manipulation
- Draining water supply installations
- Covering domestic water-storage containers
- Cleaning flowerpots/vases and ant-traps
- Cleaning incidental water collections
- Managing construction sites and building exteriors
- Managing mandatory water storage for fire-fighting
- Managing discarded receptacles
- Managing glass bottles and cans
- Tyre management
- Filling up of cavities of fences
- Managing public places

Personal protection

- Protective and long-sleeved clothing
- Use of mosquito mats, coils, aerosols and other insecticide vaporizers
- Use of mosquito Repellents, DEET, IR3535 or Icaridin
- Insecticide-treated materials, such as mosquito nets and curtains
- Screened windows and doors

12.8.2 Biological Control

Different biological agents are used to control *Aedes* sp. at the larval stage. A few are discussed below.

Fish: A few species of fish are predatory and feed on the *Aedes* larvae. These include, *Gambusia affinis* and *Poecilia reticulata*, etc.

Bacteria: Two species of endotoxin-producing bacteria, *Bacillus thuringiensis* (Bti) and *Bacillus sphaericus* (Bs), are effective mosquito control agents. They do not affect non-target organisms associated with mosquito larvae. Bti has an extremely low-level mammalian toxicity and has been accepted for the control of mosquito larvae breeding in water storage containers for household use. Bs is less effective against *Ae. aegypti* as compared to other mosquito species.

Copepods: The predatory role of copepod crustaceans was documented between 1930 and 1950. However, scientific evaluation was carried out only in 1980 in Tahiti, French Polynesia, where it was found that *Mesocyclops aspericornis* could cause a 99.3% mortality rate among *Aedes* larvae. The cyclopoids, *Mesocyclops* and *Macrocyclus* have been found most effective against first instar larvae.

Carnivorous plants: Recently, *Utricularia macrorhiza*, the common bladderwort, has been found to possess high predatory efficiency against larvae of *Ae. aegypti*.

12.8.3 Chemical Control

Chemicals have been used to control *Ae. aegypti* since the beginning of the 20th century. Insecticides are applied as larvicides or space sprays. *Aedes* larval habitats were treated with oil and homes were fumigated with pyrethrins. Organochlorines, like DDT, were used as adulticides till emergence of resistance against them (1960s) and accumulation of DDT in bird's egg shells, vegetables and fruits, etc. Organophosphate insecticides, such as fenthion, malathion and fenitrothion, were used as control agents against *Ae. aegypti* adults. Temephos is still used as mosquito larvicide. Nowadays, primarily pyrethroids, such as permethrin, allethrin, cypermethrin, etc. are used as mosquito control agents.

Chemical larviciding of *Ae. aegypti* is usually limited to domestic-use containers that cannot be destroyed, eliminated or otherwise managed. The WHO has issued guidelines on drinking water quality which provides guidance on the use of pesticides in drinking water. One of the approved insecticides in drinking water is 1% temephos and granules. These are applied to the containers @ dosage of 1 ppm which is effective for 8–12 weeks, especially in porous earthen jars under normal water use patterns.

12.8.4 Insect Growth Regulators

Insect growth regulators (IGRs) interfere with the development of the immature stages of the mosquito by interfering with the chitin synthesis during the moulting process in larvae or by disruption of the pupal and adult transformation processes. Pyriproxyfen is a juvenile hormone analogue that has been found extremely effective against *Ae. aegypti* at concentrations as low as 1 ppb or less. Very low doses of pyriproxyfen can also sub-lethally affect adults. Other IGRs used to control *Aedes* are methoprene, diflubenzuron, etc.

12.8.5 Thermal Fog

Thermal fogs are normally produced when a suitable insecticide formulation condenses after being vaporized at a high temperature. These are produced using thermal fogging machines. These generate hot gas (over 200 °C) at high velocity which atomize the insecticide formulation instantly. The insecticide is vaporized and condensed rapidly with negligible formulation breakdown.

Thermal fogging formulations can be oil-based or water-based. The oil-based (diesel or kerosene) formulations produce dense clouds of white smoke, whereas water-based formulations produce a colourless fine mist. The droplet (particle) size of a thermal fog is usually less than 15 microns in diameter. The exact droplet size depends on the type of machine and operational conditions. However, uniform droplet size is difficult to achieve in normal fogging operations.

12.8.6 Ultra-low Volume (ULV) Aerosols (cold fogs) and Mists

Ultra-low volume (ULV) formulation involves the application of a small quantity of concentrated liquid insecticides. The use of less than 4.6 litres/ha of an insecticide concentrate is usually considered as an ULV application. ULV is directly related to the application volume and not to the droplet size.

Nevertheless, droplets in the 10–15 microns range provide higher efficacy than if the size range is extended to 5–25 microns. Aerosols, mists and fogs may be applied by portable machines, vehicle-mounted generators or aircraft equipment.

(Source: http://www.searo.who.int/entity/vector_borne_tropical_diseases/documents/SEAROTPS60/en/)

No effective antiviral medications exist to treat dengue infection. In cases of severe dengue, it is critical to maintain the patient's body fluid volume.

No commercial vaccine against dengue is available yet, although several candidate vaccines are currently in various trial phases.

SAQ 3

- i) Pyriproxyfen is an example of
 - A. Biological control
 - B. Chemical control
 - C. Environmental control
 - D. Insect growth regulator
- ii) Which of the following fish feeds on mosquito larvae?
 - A. *Heteropneustes*
 - B. *Clarias*
 - C. *Labeo*
 - D. *Gambusia*

12.9 SUMMARY

Let us summarise what we have learnt so far:

- *Aedes* is responsible for three major diseases in India; *i.e.* dengue, Chikungunya and Zika. *Aedes* is distributed in about 125 countries across the world. About 111 species are found only in India out of which 2 species; *Ae. aegypti* and *Ae. albopictus*; are the main vectors responsible for transmission of dengue and Chikungunya. The female *Aedes* mosquito acquires the virus while feeding on the blood of an infected person and transmits to other humans while sucking their blood.
- Male *Aedes* can survive for 5-7 days whereas females can survive from 4 to 6 weeks and produce on an average 100-150 eggs per batch. The number of eggs is dependent on the size of the blood meal. Eggs are laid singly on damp surfaces, such as tree holes and man-made containers like barrels, drums, jars, pots, buckets, flower vases, plant saucers, tanks, discarded bottles, tins, tyres, water cooler, etc.

- The larval stage (wiggler) is a feeding stage. These are often found hanging from the water surface at an angle. Larval development includes four instars and total duration lasts for 5-7 days. Larvae convert to a non-feeding but motile pupae which develop into flying adults after 2-3 days.
- The adult mosquito is a striped mosquito with prominent white markings on abdomen and legs. The adult behavior important for their vectorial capacity are feeding, resting, biting and egg laying.
- *Aedes*-borne diseases; dengue, Chikungunya and Zika are caused by viruses; DENV, CHIKV and ZIKV, respectively. The transmission cycle of virus is more or less similar in dengue and chikungunya, where virus is majorly transmitted through mosquito bite. However, Zika virus can also be transmitted through sexual intercourse and other body fluids. In addition, Zika can also travel vertically, from pregnant mother to the child, causing microcephaly in newborn babies.
- *Aedes* mosquitoes can be controlled by various methods. Environmental management methods involve environmental modification, sanitation and personal protection using repellents, screens, mosquito coils, etc. Biological agents used to control *Aedes* larvae include fishes, copepods, bacteria, insectivorous plants, etc. Several groups of chemicals in different formulations have been employed as control interventions, such as larvicides, space sprays, insect growth regulators, ULV, thermal fog etc.

12.10 TERMINAL QUESTIONS

1. Describe the life cycle of *Aedes aegypti*.
2. Write a short note on dengue prevention and control.
3. Describe the transmission of Zika virus with suitable diagram. Explain how is it different from the transmission of other *Aedes*-borne diseases.
4. What are the different methods of *Aedes* control?
5. Give difference between the following:
 - a) Extrinsic incubation period and intrinsic incubation period.
 - b) Transverse and vertical transmission.
 - c) Urban cycle and sylvatic cycle.
6. Draw a labeled diagram of:
 - a) Life cycle of *Aedes* mosquito.
 - b) Dengue transmission Cycle.
 - c) Zika Transmission Cycle.

12.11 ANSWERS

Self Assessment Questions

1. i) A, ii) C.
2. i) D, ii) B, iii) A.
3. i) D, ii) D.

Terminal Questions

1. Refer to Section 12.2.
2. Refer to Sub-Section 12.5.1 and 12.5.2
3. Refer to Sub-Section 12.6.2.
4. Refer to Section 12.8.
5. a) Refer to Sub-Section 12.5.2.
b) Refer to Sub-section 12.5.2.
c) Refer to Sub-section 12.5.2.
6. a) Refer to Fig. 12.5.
b) Refer to Fig. 12.11.
c) Refer to Fig. 12.14.

Acknowledgement of Figures

Fig. 12.14: Michigan Technological University

ANNEXURE I: MOSQUITO GROUPS AND THE DISEASES THEY TRANSMIT

Mosquito	Disease	Distribution
ANOPHELINES <i>Anopheles</i> (e.g. <i>A. gambiae</i> the main malaria vector in Africa)	Malaria	Endemic in most countries between latitude 30°N and the Tropic of Capricorn in the south with notherly extensions in Turkey, Syria, Iraq, Iran, Afghanistan and China
	Bancroftian filariasis	Endemic in tropical Africa, Middle East, South Asia, Far East and New Guinea, Tropical America
	Brugian filariasis	South-east Asia
	Arboviral disease	Africa
CULICINES	Bancroftian filariasis	Endemic in tropical Africa, Middle East, South Asia, Far East and New Guinea, Tropical America
<i>Culex</i> (e.g. <i>C. quinquefasciatus</i> the main vector of elephantiasis in urban areas)	Japanese Encephalitis	Siberia, Japan, Korea, China, Indonesia, Singapore, Malaysia. Thailand, Vietnam, Burma, Nepal, India and Sri Lanka. Epidemics in Japan and Korea
<i>Mansonia</i> Major biting nuisance and occasional vector	Brugian filariasis	South-east Asia and South-west India
	Arboviral diseases	Africa, Americas
<i>Aedes</i> (e.g. <i>A. aegypti</i> , the main vector of urban yellow fever and dengue)	Yellow fever	Endemic in West and Central Africa and in north and eastern parts of South America. Epidemics occur in central America.
	Arboviral diseases-Dengue Haemorrhagic Fever (DHF)	Endemic throughout the tropics, particularly Asia, the Pacific and Caribbean. While dengue occurs in Africa DHF has not be recorded
	Other arboviral diseases	Highly endemic in Africa and Asia and also found in the Americas
	Bancroftian filariasis	South Pacific, South Asia

ANNEXURE II: THE CHOICE OF CONTROL METHOD FOR DIFFERENT MOSQUITOES

Mosquito behavior	Control programme	Vector species	Control of transmission	Control schedule
Mosquito bites indoors	Screening of windows, doors, eaves	<i>Anopheles</i> spp., <i>Culex</i> spp. Few important <i>Aedes</i> spp., <i>Mansonia</i> spp.	Immediate none or partial effectiveness	When house is built. Repair annually.
Mosquito bites indoors at night	Bednets	<i>Anopheles</i> spp. (no <i>Aedes</i> spp.) <i>Mansonia</i> spp., <i>Culex</i> spp.	Immediate none or partial effectiveness	New bednet every 2-5 years
	Insecticide impregnated bednets		Partial or complete effectiveness	Net impregnation every 6-12 months
Mosquito rests indoors	Indoor residual spraying	<i>Anopheles</i> spp. <i>Aedes aegypti</i> (no other <i>Aedes</i> spp.) <i>Culex</i> spp., <i>Mansonia</i> spp.	2-3 weeks partial or complete effectiveness	Every 3-6 months, prior to transmission season
Mosquito larvae attach to roots of aquatic vegetation (especially the water lettuce <i>Pistia stratiotes</i>)	Removal of vegetation (especially water lettuce) from standing water	All <i>Mansonia</i> spp.	Partial or complete effectiveness	Check possible breeding sites weekly in the main growing season
All mosquitoes	Destruction of breeding sites Larviciding	Most vector species <i>Anopheles</i> , <i>Culex</i> , <i>Mansonia</i> , <i>Aedes</i> (except where breeding sites are unknown or inaccessible)	2-3 weeks total effectiveness 2-3 weeks partial/total effectiveness	Permanent Every 2-3 weeks for <i>Anopheles</i> , every 2-3 months for <i>Aedes</i>
	Space spraying	Standard control method for <i>Aedes simpsoni</i> ; effective against all other vector spp. But must be done at time when air conditions are right (early morning or in the evening)	Must be undertaken daily until situation controlled. Immediate, can be very effective	Weekly after an attack phase of 10 days
	Repellents	All mosquitoes	Immediate, lasts 1-6 hours; used during period of biting; very effective	Daily during hours of biting