

MANUAL ON INTEGRATED VECTOR MANAGEMENT INDIA



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Preface

Integrated vector management (IVM) is a rational decision-making process to optimize the use of resources for vector control. This manual has been attempted as operational framework to guide implementers of vector-borne disease control programmes in planning more efficiently. Though IVM is being implemented and its various components and strategies are available in disease specific documents, it was felt necessary that one comprehensive document will facilitate all for reference. IVM offers an opportunity to find solutions and implement programmes in an efficient, cost effective, ecologically sound and sustainable manner.

The intention of this manual on integrated vector management (IVM), therefore, is to provide guidance to the state and district level programme officers of vector-borne disease control programme along with other stake holders including NGOs, Civil society etc. The target audience is officials at central, district and grassroot levels. The manual provides background concept of IVM, relevant information about entomological surveillance, techniques, analysis, interpretation and decision making process at local level to use available tools and adopt feasible methods to combat the vectors. Emphasis on IVM may involve both reorientation of vector borne disease control programme and involvement of local health authority to implement IVM. Capacity-building, in particular human resource development has always been a challenge but because the IVM strategy requires skilled staff and adequate infrastructure for its implementation, the available human resource need to be mobilized and trained. The Inter-sectoral collaboration will result in cost savings and benefits to other health services also.

(Dr. P. K.Srivastava)

Address

Vector control experts are one of the important asset in vector control units in a health department or programme. The entomologists are guiding force for vector control and such an effort of compiling the scattered documents on various aspects of vector control under IVM. Many places where malaria and lymphatic filariasis are coendemic already have vector control programmes that target *Anopheles* mosquitoes for malaria control. Effective coordination between the two programmes can ensure optimal use of resources to benefit both. Similarly vector control operations for arboviral diseases are also being undertaken through source reduction, anti-larval programme and indoor and out-door fogging. Entomological surveillance, including monitoring of insecticide resistance and management, is essential for vector control of all vector borne diseases. When there is no vector control programme or expert, a decision must be made about whether investment in vector control will pay off in terms of removing diseases as a public health problem in the long term. This requires careful analysis of the costs of training and infrastructure for vector control that is not limited to one disease but can be adapted for any other vector-borne disease in the context of an integrated vector management strategy and plan.

This effort of bringing a manual on IVM is appreciable and I expect that it will be used as reference for many years to come,

A.C.Dhariwal

Address

Vector management has been a priority in one or the other ways among society. The Government has been supporting its different components in different ways through different departments. The scattered way of implementation has not been able to satisfy the community to its desired level. The visibility has also been lost. The timely and synchronized efforts within available resources can be productive provided doers and service providers own the responsibility and avoid/control vectors in their vicinity.

The Integrated vector Management is to provide variety of options to be used in isolation or in combination depending on situation. The consolidation of all the tools, methodology and precautions has been a great effort to facilitate the generations to come.

This can be one of the important referral Manual and will be useful to all the stakeholders of health and non-health sectors.

(Dr Jagdish Prasad)

1

Introduction

Integrated vector management (IVM) is a rational decision-making process to optimize the use of resources for vector control. It requires a management approach that improves the efficacy, cost effectiveness, ecological soundness and sustainability of vector control interventions with the available tools and resources. Integrated approach is vital in successfully combating vector-borne diseases. Various key elements of IVM are advocacy, social mobilization, strengthening of regulatory and legislative controls for public health, empowerment of communities, collaboration within health and other sectors in planning and decision-making, use of available resources for vector control, implementation of evidence-based strategies and capacity-building.

Though different integrals of IVM have been under implementation in isolation or in combination in different situations, there is a felt need that a comprehensive document describing IVM concept, its components and strategy for different diseases are compiled. The NVBDCP aims to achieve effective vector control by the appropriate biological, chemical and environmental interventions of proven efficacy, separately or in combination as appropriate to the area through the optimal use of resources. Efforts are made for collaboration with various public and private agencies and community participation for vector control. IVM is done by using similar vector control methods to control vector borne diseases malaria, kala azar, Japanese encephalitis, dengue, chikungunya and Lymphatic filariasis. The IVM includes implementation of all feasible strategies safely with or without insecticides to manage vector population in such a way so that disease transmission is kept under check. It also includes management of insecticide resistance. The

measures of vector control and protection include mainly IRS to control adult mosquitoes , source reduction, treatment of breeding sites with chemical, biological agents and personal protection using bed nets (ITNs/LLINs)

At central level, Directorate, NVBDCP facilitate in framing policy and strategic plan for implementation by states/UTs. The infrastructure for implementation is provided by states/UTs and financial resources are shared by both central and state governments as per policy.

The existing disease-specific vector control programmes and surveillance services need emphasis on integration within the decentralized health system. This approach requires new skills and capacities for analysis and decision-making. The availability of medical entomologist is crucial for each district but often it is not the reality. In such situation, health or public health staff in districts, PHC, sub-centre and villages needs to be trained in the technical, operational and managerial aspects of IVM in making them more capable and less dependent on centralized expertise. However, there has to be a linkage of vector control and vector surveillance activities under integrated vector management (IVM) at central and local levels. This brings health services closer to the community and will increase the motivation of health staff. Vector control becomes more sustainable in this process as local decision-makers are then more accountable.

Even within decentralized health systems, vertical programmes or its activities with effective coordination at district and local level is essential for establishing and maintaining an IVM strategy. For example, the personnel of indoor residual spraying programmes at district and subdistrict levels can work together with local authority or partners in implementation strategy and an appropriate division of tasks. The vertical programmes must allow such flexibility in planning based on local circumstances, with accountability to local leaders and representatives.

IVM strategy also requires collaboration between the health and other sectors and civil society with roles and responsibilities and terms of reference for all stake holders. Health impact assessment of ongoing or new projects is very crucial to identify any risks for vector-borne disease and tackle it. Various departments with construction activities need to be sensitized and so as to prevent vector breeding by adopting appropriate strategy or technology. Partnerships at state, district levels with their active participation require intensive capacity building and advocacy. This does not mean that responsibility of vector control unit will be shifted. They will have overall responsibility and should continue to acquire the skills to facilitate the partnership and guide its activities. Other stake holders like civil society organizations and communities would also play roles in implementing the activities. Technical support on IVM strategy should be with central and state Governments. The inclusion or exclusion of tools or technologies are guided by research with documentary evidence. Operational research conducted for programmes supports in ensuring amendments in guidelines and managing resources. The support from ICMR and NCDC in operational research is taken and deliberations through expert group are considered by TAC before a final decision taken under implementation of strategy or new tools under programme.

Most of diseases under programme are being targeted for elimination but during elimination of a disease or in a post-elimination phase, reduced financial support can not be ruled out which will affect the success gained through IVM. Though a disease may no longer be a public health problem but the management of vector populations must be sustained to avoid any outbreak or upsurge due to weak surveillance and vector control. There are many examples where such outbreaks or upsurge are noticed and health officials responsible for this are either non-existent or looking after other jobs resulting in loss of skill to tackle the problems. IVM, therefore should be considered as investment and not expenditure especially in public health programme.

2

Arthropods

Arthropods are the most abundant and diverse phylum of animals. Arthropods live in all of the major habitats. The smallest arthropods can only be seen with an electron microscope and the largest were prehistoric dragonflies with wing spans over a foot in length. Arthropods were likely the first animals to become terrestrial. Arthropods were the first organisms to develop flight. Millions of years before birds and bats, insects dominated the air. Today, flight is so common in arthropods that the major classification characteristic for insects is the structure of their wings.

Phylum Arthropoda (**arthro** = joint; **poda** = foot) derived from the Greek, and literally means "joint legged"). This is the most numerous phylum of all living organisms, both in number of species and in number of individuals. Arthropods include the insects, spiders, mites, ticks, ostracods, copepods, scorpions, centipedes, shrimps, and crayfishes. Of these, insects make up > 50% of all the nominal species of organisms in the world.

There are five main characteristics that all arthropods share. These include:

- **bilateral symmetry** - the left and right sides of the arthropod body are mirror images of one another
- **segmented body** - the arthropod body is made-up of repeating units (pairs of legs, claws, or breathing structures)
- **exoskeleton** - provides protection, prevents water loss, and provides support
- **jointed appendages** - enable the arthropods to move their legs, mouthparts, and claws despite the fact that their body is covered by a rigid exoskeleton

- **numerous pairs of limbs** - arthropods have many pairs of legs, some arthropods have fewer or smaller limbs, others have larger, specialized limbs such as claws limbs such as claws

The exoskeleton of an arthropod is a hard external structure made of chitin that protects the arthropod, prevents desiccation and provides structural support. Since the exoskeleton is rigid, it cannot grow with the arthropod and must be molted periodically to allow for increases in size. After molting, a new exoskeleton is secreted by the epidermis. Muscles connect to the exoskeleton and enable the animal to control the movement of its joints.

3

General Classification of Arthropods

To understand the placement of vectors especially insects in animal kingdom, the broad classification is described. Under invertebrate of animal kingdom, Arthropoda is one of the biggest Phylum which is divided into ten or more classes but more significant classes are these five:

Insecta: Mosquitoes, sand flies, fleas, house flies etc.

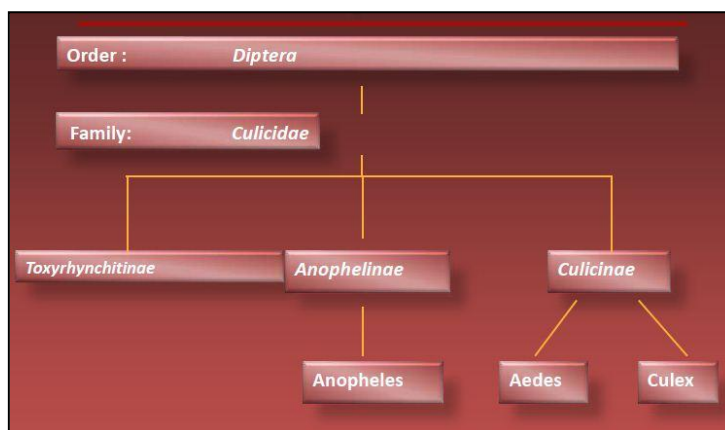
Arachnida: Ticks, mites, spiders, and scorpions

Maxillopoda: Copepods

Chilopoda: Centipedes

Diplopoda: Millipedes

Mosquitoes and sand flies are grouped into class Insecta and further mosquitoes are placed in order Diptera with different families, genera and species. Genus is a category for a taxon including one species or a group of species, presumably of common phylogenetic origin, which is separated from related similar units (genera) by a decided gap, the size of the gap being in inverse ratio to the size of the unit (genus). Species is a group of actually potentially interbreeding natural populations, which are reproductively isolated from such groups.



Family culicidae (mosquitoes) and its classification:

The family culicidae comprises of 3300 species belonging to 34 genera.

The salient feature of family Culicidae

- a) Elongated mouthparts of the female mosquitoes are adopted for piercing and sucking except few species.
- b) The antenna are long, plumose in male and pilose in the female and
- c) The characteristic wing venation with flat striated scales on the longitudinal vein and posterior borders.

Family culicidae Stephen 1829

- (Mosquitoes) these are small flies with slender abdomen, long proboscis, and antennae plumose in males, pilose in females.
- Wings are long and narrow with one or more cross veins at or beyond the middle of the wing without extra seventh vein and a marginal fringe of scales as well as a patterned distribution on veins.
- Ocelli absent.
- Mesonotium divided into scutum, scutellum and post scutellum. E.g. Mosquitoes
- Every species is different from each other genetically.

The family culicidae sub families

The family Culicidae is divided into three sub families:

- a) Toxirhynchitinae
- b) Anophelinae
- c) Culicinae

Sub family Toxyrhyngitinae (Megarhim)

The characters of sub family Toxyrhyngitinae

1. Adults are large and colourful and ornamented mosquitoes being metallic bluish or greenish with orange and red tufts of hairs on some abdominal segments.
2. Proboscis is recurved in both sexes and incapable of piercing the skin to take blood meals
3. The larvae are large and stout and often dark reddish in color like culicinae.
4. They are mainly found in natural container habitats i.e. tree holes; pitcher plants, bamboo (stumps) and other containers i.e. tins, cans, and pots. This sub family contains only one Genus comprising of about 65 species.

These genera are not medically important. The species exist in tropics of Asia, Africa, Central and South America and also at eastern part of United States of America and coastal area of Russia and Japan. The species exist in East coast.

Sub family Anophelinae

The characters of Anophelinae:

- a) Scales attached to the wing vein
- b) Long tubular mouthparts adopted for piercing and sucking
- c) The palpi of anopheles are as long as proboscis.
- d) The abdomen never entirely covered with scales.

The ventral surface covered with scales but dorsal surface the scales are absent.

The scutellum is crescent shaped with evenly distributed marginal hairs.

The larva has its head in distinct capsules; the fused thoracic segments are wider than abdomen. The antennae are not equipped with stout apical spines and there are no elongated air tube siphons. The palmate hairs are present on the abdominal segments.

Genus anopheles contains 30 genera and most are important vectors of malaria.

The sub family Culicinae

Genera having the following characters which differentiate culex from other sps.

1. The coloration and pattern of the thoracic and
2. abdominal scales and bristles.
3. The scales covering and veins of wings
4. Presence of pulvilli
5. Structure of the male hypogeum and
6. Color and ornamentation of legs.

This sub family culicinae is most medically important, there are 30 genera but five are medically important.

- 1) Culex
- 2) Aedes
- 3) Mansonia
- 4) Anopheles
- 5) Armigeres

4

Vectors of Public Health Importance

There are many vectors of public health importance across the world. Some are of importance in India but with changing climate and reemergence of diseases, it is important to have an overview of these vectors.

Arthropod	Disease	Agent
Mosquito		
<i>Anopheles</i> spp.	Malaria	<i>Plasmodium</i> spp.
	Lymphatic Filariasis	<i>Brugia timori</i>
<i>Culex</i> spp.	Lymphatic Filariasis	<i>Wuchereria bancrofti</i>
	West Nile	West Nile virus
<i>Culex vishnui</i> Sub Group	Japanese Encephalitis	Flavivirus
<i>Mansonioides</i> spp.	Lymphatic Filariasis	<i>Brugia malayi</i>
	Yellow Fever	Yellow fever virus
	Allergic Dermatitis	Allergens
Sand fly	Kala-azar	<i>Leishmania donovani</i>
	Cutaneous Leishmaniasis (Oriental Sore)	<i>L. tropica</i>
	Muco-Cutaneous Leishmaniasis (Espundia)	<i>L. braziliensis</i>
	PKDL	<i>Leishmania donovani</i>
	Papataasi Fever	Papataasi virus

Fleas	Bubonic Plague	<i>Yersinia pestis</i>
	Murine Typhus	<i>Rickettsia typhi</i>
	Tungiasis	<i>Tunga penetrans</i>
Housefly		
	Q Fever	<i>Coxiella burneti</i>
	Epidemic Diarrhoea	
	Amoebic Dysentery	Entamoeba, Giardia
	Bacillary Dysentery	Shigella
	Cholera	<i>Vibrio cholerae</i>
	Enteric Fever (Typhoid)	<i>Salmonella typhi</i> <i>Salmonella paratyphi</i>
	Helminthic Diseases	Egg and Cysts
Blow Flies spp.	Myiasis	Dipterous Larvae
Stable or Dog Fly (Biting House Fly)	Vicious Biter and Blood-Sucker Painful Bites to Man Cattle, Dog	-
Tabanids (Horseflies)	Painful Bites Loiasis or "Eye Worm infection"	<i>Chrysops dimidiatus</i>
	Tularemia	<i>Chrysops discalis</i>
Black flies	Onchocerciasis	<i>Onchocerca volvulus</i>
Tsetse-flies	African Trypanosomiasis	<i>Trypanosoma gambiense</i> <i>Trypanosoma rhodesiense</i>
Triatomine or Reduvid Bugs or Kissing Bugs	American Trypanosomiasis or Chagas Disease	<i>Trypanosoma cruzi</i>
Biting Midge (Culicoides)	Filarial Infections (Non - Pathogenic)	<i>Mansonella perstans</i> <i>Mansonella streptoserca</i> <i>Mansonella ozzardi</i>

Bed Bugs	Bites cause Swelling and Irritation, Disrupt Sleep	
Head Lice	Heavy Infestations lead to Swelling, Hardening and Darkening of the Skin	
Body Lice	Epidemic Typhus	Rickettsia prowazekki
	Trench Fever	Rochalimaea quintana
	Epidemic Relapsing Fever	Borrelia recurrentis
Cockroaches	Mechanical Transmission of Food and Water borne Pathogens. Also produce Strong Allergens	
Hard Tick <i>Haemophysalis spinigera</i>	Kyasanur Forest Disease	Flavivirus
Dermacentor spp.	Tick Paralysis	Rickettsia
Dermacentor marginatus	Tick Borne Encephalitis	Virus
Soft Tick Ornithodoros spp.	Endemic Relapsing Fever	<i>Borrelia duttoni</i>
Mites Trombiculid Mite	Scrub Typhus	Rickettsia
	Rickettsial pox	
<i>Sarcoptes Scabiei</i>	Scabies	-
House Dust Mite (<i>Dermatophagoides Sp.</i>)	Asthma-like Reaction	Allergens
Tropical Rat Mite (<i>Ornithonysscus bacoti</i>)	Dermatitis	-
Cyclops	Diphyllobothriasis	Diphyllobothrium latum
	Dracontiasis	Dracunculus medinensis
Crabs	Paragonimiasis	Paragonimus westermanni

5

Identification of Vector Species

Mosquito is an insect with two wings which belongs to :

Order : Diptera
Family : Culicidae
Sub Family : A) Culicinae
B) Anophelinae
C) Toxorhynchitinae

These are characterized by distinct needle shaped mouthparts consisting of proboscis. The proboscis is used by the female mosquito for sucking blood. Anopheles mosquitoes most frequently occur in tropical and subtropical regions and are also found in temperate climates and even in the Arctic zone during the summer. As a rule *Anopheles* are not usually found at an altitude above 2,000-2,500 meters.

Systematic position of mosquito in animal kingdom

PHYLUM : ARTHROPODA

- Paired jointed Appendages & Chitinous exoskeleton

CLASS : INSECTA (with 6 legs)

- With 3 pairs of legs & more than 750,000 species
- Characterized by distinct head, thorax & abdomen
- Thorax is divided into 3 segments & each segment contains one pair of appendages

DIVISION: PTETYGOTA (Winged Insects)

SUBDIVISION: ENDOPTERYGOTA

- Mouth parts adapted for biting
- Development of wings is internal
- Metamorphosis is holometabolous

ORDER : DIPTERA (Two winged real flies)

- Species more than 85,000
- Insects with a single pair of membranous wings, the hind pair is modified into halteres
- Mouth parts are suctorial
- Prothorax & metathorax small and fused with the large mesothorax, tarsi commonly 5 segmented
- Metamorphosis complete

SUBORDER : NEMATOCERA

- Diptera possess antennae longer than the head and thorax and usually many segmented
- Arista is wanting
- The palpi are usually 4 or 5 segmented and pendulous
- Larvae possess a well developed exerted head & horizontally biting mandibles

FAMILY : CUCULICIDAE (34 genus & 3,000 species)

- Generally with an elongated piercing proboscis & no ocelli
- Palpi stiff & non-pendulous
- Legs long, Antennae densely plumose in males & pilose in females
- Larvae and pupae aquatic and very active

SUBFAMILY

1. CULICINAE

- (2446 species under 30 genus)
- Scutellum is trilobed
- Eggs lack floating characteristic
- Abdomen is completely clothed with broad scales which nearly always lie flat

2. ANOPHELINAE - (375 species under 3 genus)

- 60 sp. are important vectors
- 51 species found in India & only 10 are important vectors
- Scutellum rounded or strap shaped

- Antennae are about as long as proboscis in both the sexes
- Legs long & slender with no distinct tibial bristles & pulvilli
- Wings with distinct markings
- Larvae lacking air-tube and dorsal surface of body with palmate hairs

2. **TOXORHYNCHITINAE** - (63 species under 1 genus)

- Sucks the juice from fruits & does not feed on blood

SUB FAMILY CULICINAE

TRIBES

- | | |
|----------------|--------------------|
| 1. Aedeomyiini | 2. Aedini |
| 3. Culicini | 4. Culisetini |
| 5. Ficallrini | 6. Hodgesini |
| 7. Mansoiini | 8. Orthopodomyiivi |
| 9. Saliethini | 10. Uranotaeniini |

SUBFAMILY ANOPHELINAE

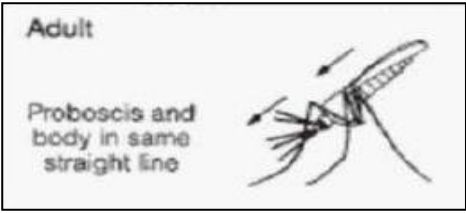
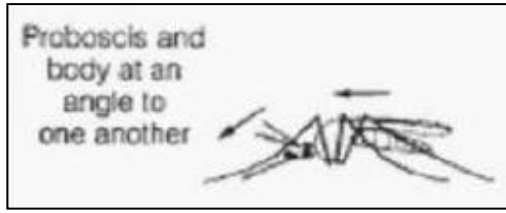
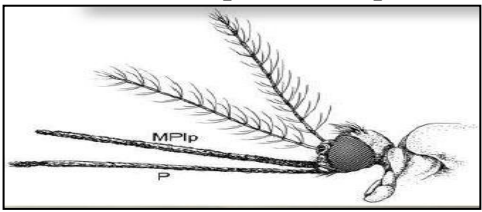
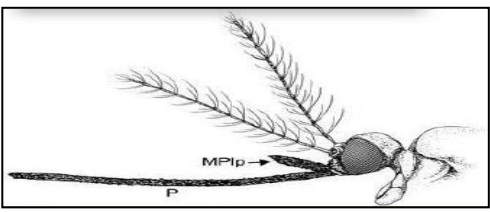
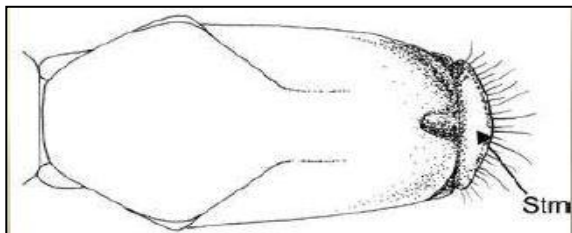
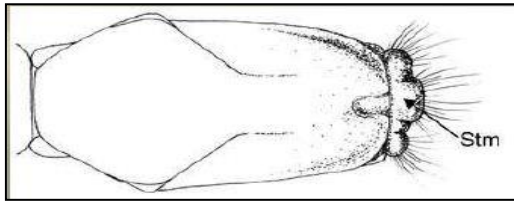
GENUS

1. Bironella
2. Chagasia
3. Anopheles

Subgenus of Genus Anopheles

- 1) Anopheles (21 species in India)
- 2) Cella (30 species in India)
- 3) Kerteszia
- 4) Lophopodomyia
- 5) Nyssorthynchus
- 6) Stethomyia

MOSQUITO BIONOMICS : Differences between adult Anopheles and Culex

Anopheles	Culex
<p>1. Resting posture The proboscis, head and abdomen are in a straight line with the abdomen pointing away from the resting surface</p> 	<p>The proboscis and body are not in a straight line, the abdomen being inclined towards the resting surface</p> 
<p>2. Wings Generally spotted with white and dark scales</p>	<p>Generally unspotted with only dark scales</p>
<p>3. Palpi (female) Palpi slender and equal to the proboscis</p> 	<p>Palpi stub like reduced</p> 
<p>Palpi (male) Club-shaped at the distal ends and nearly equal to proboscis</p>	<p>Pointed, bent and usually longer than the proboscis</p>
<p>4. Scutellum Half moon shaped with a uniform row of hairs along the margin</p> 	<p>Trilobed, with three bunches of hairs on the lobes</p> 
<p>5. Abdomen Without scales or with a few scattered scales</p>	<p>With uniform rows of over-lapping flat white and dark scales</p>

Medically important genera & species of mosquito

Genus	Number of species	
	In world	In India
Anopheles	364	51
Aedes	888	111
Culex	715	57
Mansonia	23	4
Other genus	12	26
Total	16 genus & 249 species in India 34 genus & 2968 species in the world	

MORPHOLOGY OF ADULT MOSQUITOES AND LARVAE

Mosquitoes belong to family Culicidae. Mosquitoes can be differentiated from other insects by the following characters.

1. They are slender flies, generally with an elongated piercing proboscis.
2. The Palpi are stiff and not pendulous
3. Legs are long.
4. Antennae densely plumose in the males and pilose in the females.

Generalized adult Mosquito

1. Wings fringed with scales along the posterior margin and the second longitudinal vein is forked only once.
2. Larvae and pupae aquatic and very active

Family Culicidae is divided into three sub families:

1. Anophelinae
2. Culicinae
3. Toxorhynchitinae.

The mosquitoes of subfamily anophelinae are generally referred to as anophelines, which include all the malaria vectors and of culicinae as culicines, which include all the vectors of filariasis, JE, dengue and yellow fever. Mosquitoes of these 3 sub families differ basically in many characteristics. The anophelines can be differentiated from culicines by the following features.

Toxorhynchitinae

This sub family includes only one genus Toxorhynchites. The adults of the genus can easily be identified by their large size and hook shaped proboscis. The larvae are also big in size and are predacious in nature and hence used as a biological control agent against the other species.



Culex

The mosquitoes of this genus can be differentiated from others by the following characters.

1. Margin of squama (S) fringed (Fig. 3.2)
2. Vein 6 ending well beyond the level of fork of vein 5 (Fig3.2)
3. Pulvilli (p) present (Fig.3. 8)
4. Spiracular (sp) and post spiracular bristles (psp) absent (Figs. 3.9).
5. Number of species are involved in the transmission of filariasis and JE.

The important species are

- I. Cx. quinquefasciatus (Filariasis)
- II. Cx. tritaeniorhynchus (JE)
- III. Cx. vishnui (JE)

Aedes

The following characters can differentiate the mosquitoes of this genus.

1. Margin of squama fringed
2. Vein 6 ending well beyond the level of fork of vein 5
4. Ornamentation and scaling very various.
5. Claws of female usually toothed.
6. Pulvilli absent

Mansonia

The mosquitoes of this genus can be differentiated by following characters.

1. Margin of squama fringed
2. Vein 6 ending well beyond the level of fork of vein 5
3. Tergite 8 of female with a row or patch of short tooth like spines
4. Yellowish brown, moderate sized and robustly built mosquito
5. Larvae get attached themselves to the roots of the water plants like Pistia and Eichornia.

I. MORPHOLOGY OF ANOPHELES - IMMATURE STAGES

The immature and adult stages of mosquitoes are passed in two completely different environments. The immature stages (i.e., eggs, larvae and pupae) require an aquatic environment, and the adult mosquito an aerial and terrestrial one.

Egg

Anophelines lay their eggs separately over the surface of water each egg having lateral air floats to keep it afloat. Culicines of the genus *Culex* and *Mansonia* lay their eggs on the water, in a boat-shaped mass referred to as an egg raft; whereas those to the genus *Aedes* are laid separately, often in dry hollows or containers which become flooded after rain. These “dry-laid” eggs are able to retain their viability without water for very long periods.

Larva

Eggs of mosquitoes generally hatch after two or three days in contact with water. The larva is about 1.5 mm long when newly hatched and about 10 mm long when fully grown. During growth the larva casts its skin four times, the stages between successive moults being known as instars. The larva of a mosquito is made up of head, thorax and abdomen – the last being composed of nine distinct segments. A mosquito larva breathes through two orifices, called spiracles; those of the Anophelines being situated on the eighth abdominal segment so that, in order to breathe, the larva rests in a horizontal position at the surface of the water. In Culicines larvae, the spiracles are situated at the end of a tubular organ, called the siphon, which extends from the eighth abdominal segment. Since the spiracles must lie in the plane of the water surface, the culicine larva must hang down from the water surface by the tip of its siphon in order to breathe. An exception is the genus *Mansonia*, in which the siphon is highly modified for piercing and adhering to stems of aquatic plants from which air is drawn for breathing purposes.

Pupa

The pupa is a non-feeding stage, of several days duration, providing for the morphological and physiological changes required for transformation of the larva to the adult. The general appearance of the pupa is of a comma with an exaggerated “dot” and small “tail”. The “dot” is occupied by the head and thorax while the “tail” encases the abdomen which terminates in a pair of paddles. The pupa is mobile and able to dive rapidly when disturbed. When quiescent, the pupa rests at the surface of the water, suspended by a large air cavity within its body. Breathing is carried out, at the surface of the water, by a pair of respiratory trumpets extending from the thoracic area. In general, culicine pupae can be distinguished from Anophelines pupae by their considerably longer respiratory trumpets.

Adult

The adult mosquito emerges, thorax first, from the pupal skin, by swallowing air to increase the internal pressure within the pupal skin and then to enable the mosquito to extend its soft limbs into the adult form. After emergence, the adult mosquito rests for a few minutes on the discarded pupal skin for its wings to expand and harden prior to flight. The proboscis takes longer to harden and is too soft, during the first day after emergence, for the female to take a blood meal. The adults of both sexes feed on plant juices but only the female feeds on blood. Egg development is dependent on a blood meal for almost all Anophelines and most Culicines. In some species the first batch of eggs can be laid without a blood meal (autogeny). While there are precise morphological differences between Anophelines and Culicines, which are outside the scope of this manual, the former may generally be distinguished from the latter by the appearance of the wings. With the exception of species of the subgenus Anopheles, the Anophelines wing is generally patterned with

dark and pale areas whereas the culicine wing is unpatterned and has a uniformly plain appearance. Another visual distinction is that, at rest, the body of an anophelines mosquito forms an angle nearly vertical with the surface while that of a culicine mosquito lies almost parallel to the surface.

Mosquito bionomics

Bionomics deals with the relationship between a given species and its environment. An understanding of mosquito bionomics is therefore of key importance in the Epidemiology of mosquito-borne diseases and in planning methods of mosquito control. Climatic factors play an important part in species distribution, behaviour, survival and vectorial status. Water is an essential component of the mosquito environment and whether it is running, standing, clean or polluted, sweet or brackish, shaded or sunlit, frequently determines which species of mosquito breeds in it. The environments of the immature stages and adult mosquito are interdependent since the adult mosquito must have access to water for egg laying. The adult mosquito environment is, however, largely aerial and terrestrial, the former environment being necessary for mating and dispersal and the latter providing habitats for feeding, resting and completion of the cycle of ovarian development from blood meal to egg laying. The environments of the immature stages of the adult mosquito are considered in more detail below.

The environment of the immature stages of the mosquito

The environment of the immature stages of the mosquito is aquatic, with its mobile stages (i.e., larva and pupa) dependent on atmospheric air for breathing and therefore typically spending much of their lives suspended from the surface film of the aquatic environment.

There is an optimal range of water temperatures for growth of the immature stages of the mosquito. The range is lower for species living in

temperate than in tropical zones and varies somewhat between different species living in the same geographical zone: thus temperature is one of the factors that limits the geographical distribution of a species. Within these optimal ranges, however, there is a largely direct relationship between temperature and growth. For example, mosquitoes breeding in the tropical zone, in water at 23^o-27^o C, usually complete their aquatic growth within two weeks. Moderately frequent rainfall usually increases the opportunities for prolific breeding, but repeated and heavy rainfall causes severe flooding resulting in a temporary flushing out of breeding places and reduction in mosquito population. The depth to which light penetrates the water in which the mosquito is breeding is generally not an important factor since the immature stages live largely at the water surface, but the extent to which the breeding place is shaded or exposed to sun determines which species of mosquito inhabit a particular water body. Hedges, planted to give shade over breeding places, or clearing of forests to allow sun to penetrate have been successfully used for environmental control of several malaria vectors (*Anopheles minimus* and *An. balabacensis*). Unless islands of vegetation are present to provide local breeding sites, mosquito larvae are not found on open surfaces of large bodies of deep fresh water (e.g., lakes ponds, rivers or reservoirs) but are confined to their sheltered shallow edges. The immature stages of some species (*An. gambiae*) are found throughout the entire surface of swamps and of shallow temporary rainwater pools. Some species (*An. funestus*) breed in clear fresh water with vertical vegetation whereas others are adapted to breeding in brackish water (*An. sudaicus*) or highly polluted water (*Culex quinquefasciatus* = *Cx pipiens fatigans*). The aquatic environment of some species is associated with particular plants. For example, *Mansonia* larvae are linked with the presence of fresh water lettuce (*Pistia*) and *Aedes simpsoni* with axillary breeding in banana plants. Other species (*Ae. Aegypti*,

Eretmapodites chrysogaster) breed in great numbers in small containers such as old tins, tyres and coconut husks.

Thus while mosquitoes, as a group, are found breeding in an almost infinite variety of sizes and types of water body, each species is generally associated with certain types of breeding places. In some species, however, breeding is restricted to a narrow range of habitats while others breed readily in a wide range of waste - types. The classifications given in Annex 1 attempts to identify the major and most common mosquito vectors, their biology and breeding habitats, and to indicate, for each type, the most suitable environmental management measures for control.

The environment and habits of the adult mosquito

Mating

Mating usually occurs within 24-48 hours after emergence. In some species the males form a swarm, frequently located over contrasting or sharply defined points, e.g., the top of a tree, stake or rock, or over the corner to of a building. Swarming usually occurs at dawn or in the evening, but may be seen in shaded areas in the middle of the day. Females entering the swarm are seized and the pair drops out of the swarm. After insemination, the spermatozoa are stored in the female mosquito, in an organ called the spermatheca, and drawn on for fertilization of all the eggs produced throughout the remainder of its life (monogamy).

Dispersal

The adult mosquito of most species does not fly great distances and the male is a much weaker flyer than the female. Thus the presence of a large number of adult male mosquitoes indicates that breeding places of that particular species are close by. In normal atmospheric circumstances, most individual mosquitoes of tropical species apparently fly within a range of 1-3

km, although there are records of a few species or occasional individual mosquitoes traveling much further. Certain temperate-zone species travel 4-5 km and there are records of those that traveled up to 10 km. Dispersal is largely downwind and strong winds can carry mosquitoes very much greater distance than normal. For e.g there is a report *Anopheles pharoensis*, in Egypt, being windborne, up to 280 km from the nearest breeding places. Dispersal of mosquitoes through human agency has occurred since the earliest times but with the increasing number of vehicles (boats, buses, trains and aircraft), the threat of passive dispersion of vector species is much greater today, and effective countermeasures such as vehicle disinfections are required.

Baiting habits

Many of the habits of adult mosquitoes are linked to their being both cold-blooded and physiologically ill-fitted to withstand very dry environments. Flight, host seeking, and feeding generally take place in a warm humid environment. Species that are associated with open terrain and sunlit habitats fly and feed between the hours of dusk and dawn when the air is more humid. Many of these species have a peak of biting activity in the latter half of the night when relative humidities are at their highest. For example, in *An. gambiae* s.l. and *An. funestus*, the principal vectors of malaria in Africa, the peak of biting occurs about an hour before dawn . Many species associated with dense vegetational habitats such as forests or plantations, where daytime humidities are generally higher than in open terrain, fly and feed during daylight hours. Their peaks of biting activity vary widely between different species and may occur in daylight hours (*Aedes simpsoni*) or shortly after dusk (*Ae. africanus*). Mosquitoes, which feed inside houses, are described as Endophagic and those that feed outdoors as Exophagic. The exact feeding pattern of mosquitoes indoors varies with different species and circumstances but usually mosquitoes enter houses to feed in the early hours of the night.

Host preference

The environment of the adult female mosquito includes a host. If the preferred host is man, the mosquito is referred to as anthropophilic; as zoophilic; and if there is no fixed preference, as an indiscriminate biter. In the absence of the preferred host, some species (e.g., *An. gambiae*) are facultative feeders and will feed readily on other animals.

Seasonal prevalence

In certain areas, mosquitoes are seasonally exposed to a hostile environment created by extremes of climate. In temperate zones, winter temperatures are survived by some form of hibernation. In the colder parts of the temperate zone, hibernation in *Aedes* spp. is in the egg stage and there may be only one generation a year. In the less cold parts of the temperate zone, most Culicines spend the midwinter months in the larval stage. The female adults of some, e.g., *Culex pipiens*, hibernate in sheltered places such as cellars or outbuildings, surviving on body fat. Adults of other species such as *Anopheles atroparvus* hibernate partially, taking an occasional blood meal off man or domestic animal, indoors, during a warm spell, but in these circumstances ovarian development does not follow after the blood meal. This physiological condition is described as gonotrophic dissociation.

Extrinsic incubation period and mosquito longevity

Since the mosquito is cold-blooded, the climate in which it lives greatly affects its capability for disease transmission by influencing the rate of development of the parasite within the vector and the longevity of the mosquito.

Malarial and filarial parasites undergo a developmental cycle within the mosquito host during which the former, but not the latter, parasites, multiply. Viruses multiply within the mosquito host but do not appear to undergo any

cyclical change. For all three groups there is a period between the host mosquito's first infectious blood meal and its first feed transmitting the infection. This interval is known as the extrinsic incubation period and varies in length in response to the temperature of the host mosquito's environment. For example development of the malaria parasites, *Plasmodium falciparum* and *P. vivax*. Is indefinitely retarded at 19^o C and 15^o C respectively and below; and in *P.falciparum* it is completed in 10 days at 30^o C and in 27 days at 20^o C (Macdonald 1957 a Roa and Iyengar found that mean temperatures below 24^o C and 34^o C inhibited growth of the filarial parasite *Wuchereria bancrofti* in *Culex quinquefasciatus* Say et al. c found that the filarial larvae matured in 20 days at 23-24^o C and in 14 days at 29-31^o C. Further details on the extrinsic incubation period of *W. bancrofti* are given by Sasa.d Davise found that the extrinsic incubation period of the African Asibi strain of yellow fever virus in *Aedes aegypti* was 4 days 37^o C, and 18 days at 21^o C. The mosquitoes were not infective after a period of 30 days at 18^o C.

While some species of mosquito live longer than others, temperature and humidity affect their survival. Except at extremely high or low humidities, when mosquitoes are unable to regulate their water loss, longevity is in general greater at the higher ranges of humidity and lower ranges of temperature. *Anopheles culicifacies* was found to survive about 10 days at a 60-65% relative humidity and at 30-35^o C, compared with 30 days at 80-90% relative humidity and 27-30^o C.

Resistance to insecticides

During the last 5-6 decades, control of mosquitoes and other insects of public health importance have been largely achieved by means of synthetic chemical insecticides. Their use on a vast and increasing scale has led to the widespread development of insecticide resistance, which has been defined as

“the development of an ability in a strain of insects to tolerate doses of toxicants which would prove lethal to the majority of individuals in a normal population of the same species b. The number of insecticide-resistant arthropods of public health importance has raised form 2 in 1946 to 155 in 1980, and insecticide-resistant mosquitoes from 7 in 1957 to 98 in 1980. Resistance has appeared to chlorinated hydrocarbon, organophosphate and Carbamate insecticides. As there are few alternative groups of chemical insecticides to fall back on, it has become urgent to develop alternative means of mosquito control.

Insecticide resistance is inherited and is induced through selection of individual insects that survive dosages of insecticides, which kill the susceptible individuals.

The mechanism of inheritance varies with different insect groups and different insecticides. It can be of a simple Mendelian character attributable to a single gene allele, or of a complex nature involving oligogenes or multiple-gene interaction. The physiological basis of resistance also varies with species and insecticides, and may arise through enhanced metabolism of the insecticide, reduce penetration of the insecticide into the insect, or reduced nerve sensitivity.

Entomological Surveillance

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

Collection of adult mosquitoes

The collection of adult mosquitoes is made for::

- (i) Qualitative studies - To study the prevalence, distribution, behaviour of different mosquito species in different macro and micro environmental conditions.
- (ii) Qualitative studies - To study the vector relative density and abundance, longevity, infectivity, impact of anti -vector measures on the vector population, impact on the transmission.

Several methods for sampling of mosquitoes are available which are undertaken alone or in combination with others depending on objective of survey.

Hand collection of mosquitoes

Principles and objective of the method

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

Collection of mosquitoes

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

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

- (II) For outdoor collection, mosquitoes sheltering under overhanging banks of streambeds, in cracks, in banks or walls, under bridges, culverts and in tree holes should be thoroughly searched in the area.
- (III) **By test tube:** Test tube without rim and having a length of about 100 mm (20 mm diameter) are used for the collection of mosquitoes. After locating a mosquito with torch light, hold a test tube in the middle and bring its mouth slowly over the insect; then move the tube slightly to dislodge the mosquito, slide the hand up the tube and quickly place a finger over the open end and plug it with cotton.
- (IV) **Catches off baits:** Mosquitoes are collected directly off the human or animal baits using sucking tube while they land on the host to bite or while in the process of biting a human or an animal host. This method is one of the most important for collecting partially or entirely exophilic mosquitoes. Mosquitoes may also be collected while resting in the vicinity of the bait, either before or after feeding.
- (V) **Hand net catches:** Small hand net about 15 cms in diameter, made of fine mosquito netting and provided with long handle is being used to catch adult mosquitoes resting in human and animal habitations in large number. The usual procedure is to gently spray the hut with a non-toxic oil (Risella or citronella oil) paying attention to cracks and crevices. The disturbed mosquitoes are collected by sweeping the net.
- (VI) **Spray sheet collection:** The method is applied during the daytime, usually early in the morning between 06.30 and 10.00 hours, depending on the situation and objective. All occupants, animals and easily removable objects like foodstuff, drinking water, furniture, etc. are first removed from the structures where collection is to be made. All doors and windows should be

closed and the floor of the hut should be covered with white sheet. The hut space then sprayed with ordinary hand-pump containing 0.1 per cent pyrethrum in kerosene oil @ 15-30 ml/1000 cu.ft. After filling the room with insecticides mist, the collector leaves the hut and closes the doors. Ten minutes after the spray, the doors are opened and the sheets is lifted with four corners and brought outside in daylight. The mosquitoes are collected with entomological forceps and transported to the laboratory. The mosquito thus collected can be used for dissection of malaria / filarial parasites, ovarian age grading and precipitin test, etc.

(VII) **Trap collection**-Traps are used extensively for collecting mosquitoes which are flying in search of food, shelter or egg laying sites or due to some external unfavourable influences, whether natural (wind, change of humidity, temperature and light) or produced by human being like smoke, insecticides, ventilation, etc. Some of the important traps used for the collection of adult mosquitoes are window trap, magoon trap, malaise, light trap, etc.

(VIII) Window trap - These are the most widely used for mosquito collection in malaria programmed. They are placed in the path of incoming or outgoing flying mosquitoes and are used without any attractant. The window trap consists of a wooden frame, a cube of six sides of one foot each, five sides of which are closed with mosquito nettings while to the sixth side a deep conical funnel of netting or provided. The frame of the trap should fit exactly into the window frame of the house so that no space is left to escape from it or the open areas around window trap should be plugged with cotton or cloth etc. (Fig.=====). Window trap collections give information on the circulation of mosquito in different physiological conditions from outside to inside and vice versa.

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

(X) **Light trap** - The basic principle of the light trap is that the mosquito attracted at night to the bright electric light enters under the hood of the trap where they are exposed to a strong downward air current produced by a fan operated by an electric motor. The mosquitoes are collected in a holding cage attached to it . Light trap have mostly been used for collecting outdoor flying mosquitoes. This trap is good for routine sampling of culicine mosquito population and for the study of culicine vectors of virus diseases. Fig.5.3 shows construction of CDC type of light trap.

Collection of adult sandfly

Hand collection

This is the most common method wherein sandfly sitting on a surface are caught with the help of an aspirator or test tube and a torch light. This method is particularly useful for longitudinal monitoring of man-hour densities. However, sin

sandfly collection the ordinary mosquito barrier netting between glass tube and rubber tubing of the aspirator must be replaced by a muslin cloth as the smaller size of sandflies enable them to escape through ordinary mosquito net.

Trap collection: Usually 4 types of traps are used:

- a. **Sticky trap:** This is the most extensively used trapping device wherein sandflies are trapped in a layer of castor oil. Suspended arched sticky papers/foils of standard size (20 x 30 cms) are placed at a height of about 4-5 cms from ground with convex sticky side towards ground. Traps are usually laid in the evening and collected on following morning. Sandfly density per trap is calculated for comparisons. Sticky traps are particularly useful in collecting sandflies from hidden shelters like burrows, cracks, tree holes, etc. For some species showing repellency to castor oil, other vegetable oils are required to be used. However, in Indian these can be safely used against *Ph. argentipes*.
- b. **Illuminated Sticky trap:** Box shaped batteries are hung on the walls facing sticky traps to make them illuminated. In some studies, these traps have provided higher catch as compared to ordinary traps.
- c. **Light traps:** CDC miniature light traps are often used for sandfly collections. However, nylon mesh cage suspended in a rigid frame are better than the collapsible cages provided with the traps. Further, for sandflies they are modified to give UV light or white light.
- d. **Funnel traps:** These are particularly useful in collecting flies from rodent burrows. Traps are placed just at the mouth of the burrow to catch the flies emerging out of burrows. The inner side is provided with sticky paper or foil. Other traps used in mosquito collection like double bed net, stable net,

malaise trap, magoon trap, etc. can also be used but the effectiveness is not yet well demonstrated.

Bait collections

Both human and animal baits can be used. However, the fact that sandflies are well known for their patchy distribution must be kept in mind while designing bait sampling. Due to clustering habit of sand flies, bait sampling must be extended to cover all parts of a village.

Larval collections

Objectives:

- I. To establish the breeding habits of different species.
- II. To establish the geographical distribution of the vectors.
- III. To establish the active breeding places.
- IV. To study the development of aquatic stages.
- V. To evaluate the impact of anti-larval measures on the larval density.
- VI. To collect samples of larvae for rearing adults for taxonomic studies or biological observation (bioassay/susceptibility tests.)

Larval collection method

- (a) Dipping
- (b) Netting
- (c) Pipetting.

- A. Dipping:** The dipping method is the most frequently used for the collection of mosquito larvae. The collecting equipments viz. Enamel bowl, flying pan or ladle (Fig. ===) should be immersed in the breeding places (edges of swamps, ditches, streams, rice fields other bodies of waters) at an angle of 45° . The surface water will flow into the larval container automatically along with the larvae, if any. If the dipper is immersed too slowly the larvae are disturbed and go to the bottom. There should be an interval of 2-3 minutes between each dip to allow stage III IV larvae and pupae to come to the surface again. In case surface should be agitated to cause the larvae to sink, clear away the vegetation and then wait for 3-4 minutes for larva to come to the surface and collect them with dipper. The larval density is assessed in terms of average larval density per dip.
- B. Netting:** Larvae may be collection from large stretches of water along the edge of streams, ponds, wells, and other large water bodies. A larval net (Fig.===) consists of a ring of iron frame, so-25 cm in diameter, to which a nylon / muslin cloth net is attached, measuring about 10 cm long. A long wooden handle is attached to the ring. For collecting larvae, the net is held at an angle of 30° and skimmed rapidly through the surface water near emerging or floating vegetation. The net is inverted and washed out in a bowl of water and the larvae collected with a pipette. The density is measured in terms of density per larval net. The usual pond net devoid of handle and provided with nylon string attached to four points on the iron ring at equal distances is used for collecting larvae from wells. Join the four pieces of string are such a way that the ring forms an angle of 30° and attach this to a rope tied with it. While collecting larvae from a well, put a small weight in the net to keep its bottom under the water surface. The net is moved around the border of the well two three times, it is then withdrawn,

inverted in a white enamel bowl containing water. The larvae are collected by pipette. The density is measured in terms of larvae per well net.

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

Collection of larvae from tree holes or axils of leaves

The larvae can be collected from the small, narrow tree holes or from the axils of leaves using a wide pipette or a siphon. The water can be siphoned off with a piece of rubber tubing and the holes may be washed two or three with extra water to retrieve left over larvae. Dry tree holes can be investigated for eggs, either by scrapping the deposits from the bottom or by filing them with water, agitating with a stick for 10-15 minutes and then siphoning the contents.

Collection of *Mansonia aquatic stages:* For collection of *Mansonia* larvae, a one-foot square bottom tin/wooden tray is kept over floating vegetation and the number of plants is counted. The plants are then removed to an enamel tray with water and the plants are then well shaken to disentangle the *Mansonia* larvae from the roots. Then the number of larvae and number of plants are counted and the average number of larvae and pupae per plant estimated.

Collection of immature stages of Sand fly: Sand flies breed in cracks, crevices and other places with soils rich in organic contents. The resemblance in soil and larval coloration makes it difficult to detect larvae visually in their habitat. The soil is collected, kept in a Petri dish and then examined under microscope (40 x magnification). To facilitate screening of larger soil samples, a floatation technique is often practiced. The soil samples are immersed in a saturated sugar

solution i.e. 3 parts sugar + 5 parts water. Larvae and pupae float in this solution. These are then passed through a series of sieves and finally the residues are examined under the microscope.

Xenomonitoring or xenosurveillance

Entomological techniques are also useful for lymphatic filariasis programmes in a more indirect way. Direct assessment of worms in vector mosquitoes with polymerase chain reaction (PCR) techniques is increasingly used to detect recurrence of new infections during post-MDA surveillance. This tool is called xenomonitoring or xenosurveillance. As the threshold for lymphatic filariasis elimination is so low (1-2%) antigenaemia prevalence in the human population, large numbers of mosquitoes must be collected and processed for testing with this method. The samples are usually examined in pools, the pool size being determined by the estimated prevalence of infection. The collection sites must be representative and widespread because of the heterogeneity in infection rates in humans. A standard protocol for sampling and testing need to be made available available with more diagnostic centres with facility need to be established.

Adult surveys for Aedes

Adult vector surveillance provides data on seasonal trends in population, transmission dynamics, and evaluation of adulticiding interventions.

Resting collections

During periods of inactivity, adult mosquitoes typically rest indoors, especially in bedrooms, and mostly in dark places, such as clothes closets and other sheltered sites. Resting collections require systematic searching of these sites for adult mosquitoes with the aid of a flashlight. A labour-intensive method is to capture the adults using mouth or battery-powered aspirators and hand-held nets with the aid of flashlights. Recently, a much more productive, standardized and less labour-intensive method using battery-operated back-pack aspirators has been developed. Following a standardized, timed collection routine in selected rooms of each house, densities are recorded as the number of adults per house (females, males or both) or the number of adults per human-hour of effort. When the mosquito population density is low, the percentage of houses positive for adults is sometimes used.

Landing/biting collections

Landing/biting collections on humans are a sensitive means of detecting low-level infestations, but are very labour-intensive. Both male and female *Ae. aegypti* are attracted to humans. Because adult males have low dispersal rates, their presence can be a reliable indicator of close proximity to hidden larval habitats. The rates of capture, typically using hand nets or aspirators as mosquitoes approach or land on the collector, are usually expressed in terms of landing/biting counts per man hour.

As there is no prophylaxis for dengue or other viruses transmitted by *Aedes* mosquitoes, it is highly desirable, for ethical reasons, that adult captures of *Aedes*

vectors should be based on “landing collections” only with the instruction to avoid being bitten by mosquitoes.

Oviposition traps : “Ovitrap” are devices used to detect the presence of *Ae. aegypti* and *Ae. Albopictus* where the population density is low and larval surveys are largely unproductive (e.g. when the Breteau index is less than 5), as well as under normal conditions. They are particularly useful for the early detection of new infestations in areas from which the mosquitoes have been previously eliminated. For this reason, they are used for surveillance at international ports of entry, particularly airports, which comply with international sanitary regulations and which should be maintained free of vector breeding. An ovitrap enhanced with hay infusion has been shown to be a very reproducible and efficient method for *Ae. aegypti* surveillance in urban areas and has also been shown to be useful to evaluate control programmes, such as the impact of adulticidal space spraying on adult female populations.

The standard ovitrap is a wide-mouthed, pint-sized glass jar, painted black on the outside. It is equipped with a hardboard or wooden paddle clipped vertically to the inside with its rough side facing inwards. The jar is partially filled with water and is placed appropriately in a suspected habitat, generally in or around homes in the environment. The “enhanced CDC ovitrap” has yielded eight times more *Ae. aegypti* eggs than the original version. In this method, double ovitraps are placed. One jar contains an olfactory attractant made from a “standardized” seven-day-old infusion, while the other contains a 10 percent dilution of the same infusion. Ovitrap are usually serviced on a weekly basis, but in the case of enhanced ovitraps, they are serviced every 24 hours. The paddles are examined under a dissecting microscope for the presence of *Ae. aegypti* eggs, which are then counted and stored. In areas where both *Ae. aegypti* and *Ae. albopictus* occur, eggs should be hatched and larvae or adults identified, since the eggs of those species cannot be

reliably distinguished from each other. The percentage of positive ovitraps provides a simple index of infestation levels, or if the eggs are counted, it can provide an estimate of the adult female population.

Larval surveillance: The commonly-used larval indices (house, container and Breteau) are useful for determining general distribution, seasonal changes and principal larval habitats, as well as for evaluating environmental sanitation programmes. However, they generally have no relevance to the dynamics of disease transmission. The precise levels of vector infestation that constitute a “risk” level for dengue transmission are influenced by many factors, including mosquito longevity and immunological status of the human population. Therefore, the limitations of these indices must be recognized and studied more carefully to determine how they correlate with adult female population densities, and how all indices correlate with the disease-transmission risk. The development of alternative, practical and more sensitive entomological surveillance methodologies is an urgent need by researchers.

Frequency of sampling: Ongoing Control programmes using integrated strategies do not require sampling at frequent intervals to assess the impact of control measures. However, feedback on monthly basis as monitoring may guide intensification if required.

7

Malaria Vectors

Vector bionomics

The term bionomics is defined as the inter relationship of an organism to its biotic and abiotic environment. Climatic factors play an important role in species distribution, behavior, survival and vectorial status. The relative number of malaria vector species in an area determines the transmission of pathogens to the human populations. An understanding of vector bionomics is of key importance in epidemiology of vector borne diseases and planning methods of vector control. The environment of immature species and adult vectors are interdependent since the adult vector must have access to water for egg laying. The adult vector environment is however largely aerial and terrestrial, the former environment being necessary for mating and dispersal and the later providing habitats for feeding, resting, and completion of the life cycle of ovarian development from blood meal to egg laying. Malaria transmission dynamics and its prevalence are governed by stable and unstable environmental factors affecting vector, man and parasite. As a consequence of these, malaria disease entity presents a very complex phenomenon.

Distribution

There are about 424 species of anopheles mosquitoes throughout the world. Of these some 70 species are of major importance. Anopheline mosquitoes are found in all parts of India from the sea level upto an altitude of 2000 to 2500 meters. There are records of mosquitoes found in deep mines particularly culicine mosquitoes. They have been found at depths of over 1000 meters in the Kolar gold mines in Karnataka. However, anophelines have been found only at depths of 90 to 180 meters in the coal mines of Bihar. Anophelines are found in all over the Indian

subcontinent, from Ladakh in the north to Kanyakumari in the south; and Andaman and Nicobar islands in the Bay of Bengal to Lakshadweep in the Arabian sea.

Malaria Vectors

Out of 58 species of anopheline mosquitoes in India, 9 species are vectors of malaria.

1. *Anopheles culicifacies*
2. *Anopheles fluviatilis*
3. *Anopheles minimus*
4. *Anopheles philippinensis*
5. *Anopheles dirus*
6. *Anopheles stephensi*
7. *Anopheles annularis*
8. *Anopheles varuna*
9. *Anopheles sundaicus*

An. culicifacies, *An. fluviatilis*, *An. minimus*, *An. philippinensis*, *An. dirus* and *An. stephensi* have been considered as principal vectors of malaria whereas three species viz. *An. annularis*, *An. varuna* and *An. sundaicus* have been considered to be local importance in the transmission of the disease. *An. stephensi* is mainly involved in the transmission in urban areas.

Vector bionomics

The transmission potential, endemicity levels, vectors of malaria and other factors of malaria differ from area to area. The vectors require different intervention strategies for malaria control.

Vector control strategy in North Eastern states

In tropical rain forests where both *An. dirus* and *An. minimus* are vectors, malaria transmission becomes stable leading to hyperendemicity. *An. dirus*, and *An. minimus*, are susceptible to commonly used insecticide - DDT. If indoor residual insecticide is properly sprayed during the transmission period, the malaria endemicity can be controlled and interruption of transmission can be achieved. It also appears that transmission control in villages which are close to the forest fringes where *An. dirus* transmits malaria, may not be possible by IRS. *An. dirus* is an exophilic vector although a poor flier, but after taking the blood meal, it leaves the human dwelling to rest outside. This vector has least contact with insecticide sprayed indoors in human dwellings. It avoids such a contact on account of its habit of leaving houses immediately after blood meal. Why a poor flier like *An. dirus* leaves the house immediately in spite of its abdomen full of blood meal, while *An. minimus*, a strong flier rests indoor on insecticide sprayed surface after blood meal is partially explained by the blood meal which is one third in quantity as compared with the blood meal taken by *An. minimus*. Even after a full blood meal *An. dirus* is lighter in weight than *An. minimus*. After blood meal it is aerodynamically more stable than *An. minimus* and therefore it goes out of human dwelling.

It is therefore, mooted that malaria transmission cannot be controlled by IRS in areas under the vectorial influence of *An. dirus*. In many places it has been observed that in *An. dirus* areas wherever insecticidal spray coverage with insecticides was of a very high order and also the quality of spray was very good, transmission interruption or reduction was observed. *An. minimus* and *An. fluviatilis* are endophagic and endophilic. They rest inside the house to partially digest the meal before they fly out to rest outside. Thus they are exposed to insecticide for a much longer periods resulting in interruption of transmission by these two vectors.

Areas under the influence of *An. fluviatilis* and *An. Culicifacies*

An. culicifacies transmits malaria in epidemic form in unstable states like Haryana, Punjab, Western UP, Rajasthan, MP and a few pockets in other states. *An. fluviatilis* supported by *An. culicifacies* also transmits disease in deciduous forest in peninsular hills at forest fringes. In such areas control can be achieved by IRS where *An. fluviatilis* is a vector. If *An. culicifacies* is resistant to DDT, then IRS is not likely to achieve results. In such areas both methods combined, i.e. IRS and ITN/LLIN will give better results. The binomics of vectors including their distribution, sphere of influence, endemicity, transmission, larval habitats, resting places, biting time, feeding habits, flight range and insecticide resistance are prerequisites for effective vector control measures in different eco-epidemiological settings.

Areas under the influence of *An. stephensi*

An. stephensi, the main vector in urban areas and in Rajasthan is responsible for the transmission of malaria in these areas. It breeds in clean water and in various containers where the water is being stored. In rural areas of Rajasthan this is the main vector which breeds in underground water storage tanks called Tankas. For the control of this vector various anti-larval methods are being used.

Malaria vectors

An.culicifacies

Distribution: Widely distributed in India. Not reported in Andaman and Nicobar Islands and Lakshadweep. Occurs sporadically in North Eastern India.

Breeding places: Breeds in rainwater



pools and puddles, borrowpits, river bed pools, irrigation channels, seepages, rice fields, wells, pond margins, sluggish streams with sandy margins. Extensive breeding of *An.culicifacies* is generally encountered following monsoon rains.

Resting habits: Rests during daytime in human dwellings and cattlesheds.

Biting time: Biting goes on throughout the night but peak biting occurs from 19.00 to 04.00 hrs.

Feeding habits: A zoophilic species but when high densities build up relatively larger numbers feed on men.

Flight range: About 1-3 kms.

An. fluviatilis

Distribution: Widely distributed in the foothill areas including both peninsular and north-east India.



Breeding places: Breeds typically in slow running streams, seepages and irrigation channels; also recorded from rice fields and shallow wells.

During heavy rains the breeding of *An.fluviatilis* is often flushed out.

Resting habits: Rests indoors in human dwellings and cattlesheds.

Biting time: Generally enters houses at dusk and completes feeding before midnight with peak from 09.00 to 11.00 hrs.

Feeding preferences: This species is in general highly anthropophilic; may be mainly zoophagic in northern India.

Flight range: Limited flight range.

An. minimus

Distribution: Distribution is restricted to the north-eastern states. This species was thought to have been eliminated as a result of insecticidal spraying in 1950ís and 1960ís but reappeared in late 1970ís.



Breeding places: *An.minimus* breeds in shaded slow flowing streams with grassy margins, swamps, ditches, channels, shallow earth wells; occasionally found to breed in borrowpits, rice fields and seepage from flowing water.

Resting habits: Rests in houses and cattlesheds, preferring to rest on the lower portions of walls.

Biting time: Peak biting activity occurs from 18.00 to 19.00 hrs outdoors and 24.00 to 02.00 hrs indoors. Biting time may vary from locality to locality and seasonally.

Feeding habits: A highly anthropophilic species, and as a consequence a very efficient vector of malaria. Flight range. Normally 0.5 km but can disperse upto 2 kms from the original locality.

An. philippinesis

Distribution: Distributed in West Bengal, North Eastern states and Andaman and Nicobar Islands. Breeding places. Breeds in tanks, swamps, ditches, rice fields, pools, leaf axils, shaded lake margins, inundated drains and water bodies with generally good growth of vegetation.

Resting habits :During daytime adults rest in cattlesheds and human dwellings.

Biting time: Biting outdoors and indoors throughout night with two biting peaks from 20.00 to 22.00 and 02.00 to 04.00hrs.

Feeding habits: Predominantly zoophagic but also bites man. Flight range. Normally upto 0.8 km.



An. dirus

Distribution: Distribution restricted to the forested areas of the seven north eastern states.

Breeding places: Breeds in pools and rain water collections in deep forest and forest fringes, stream margins with decaying organic matter, and animal foot prints during high monsoon.



Resting habits: Enters human dwellings to bite and rest but has a tendency to leave houses soon after blood meal.

Biting time: The peak biting activity is from 22.00 to 02.00 hrs.

Feeding habits: High preference for human blood but also bites monkey, other primates and cattle.

Flight range: Flight range varies from 1 to 2.5 km in forests.

An. stephensi

Distribution: Distributed throughout India except at higher altitudes; found sporadically in the north-east.

Breeding places: Breeds in wells, overhead and ground level water tanks, cisterns, rain water collections in roof

gutters, peridomestic containers, and underground water storage tanks. In Rajasthan desert it breeds and rests in the water storage tanks called Tankasí in the rural areas.

Resting habits: Rests in human dwellings and cattlesheds. Inside human dwellings it may rest on hanging objects, behind curtains etc. Outdoor resting has been observed in wells and underground cement tanks.

Biting time: Biting varies from area to area and seasonally, but peak biting activity is generally from 22.00 to 24.00 hrs.

Feeding habits: An indiscriminate feeder and bites both man and animals.

Flight range: Limited flight range in the urban areas but in rural areas the flight range may be upto 3 kms.



An. annularis

Distribution: Occurs all over the country. Not found in the Andaman and Nicobar and Lakshadweep islands.

Breeding places: Breeds in still waters with abundant vegetation in a variety of



water bodies; also breeds in wells, moats, tanks, borrowpits, rice fields and other water bodies such as lakes and stream margins with vegetation.

Resting habits: During day time rests in houses, cattlesheds and mixed dwellings, and also rests outdoors in small numbers.

Biting time: Peak biting activity takes place from 22.00 to 24.00 hrs.

Feeding habits: A zoophilic mosquito; biting on man is infrequent.

Flight range: Normally upto 1 km.

An. varuna

Distribution: Distributed widely in the country from north east plains, peninsular India, and the Lakshadweep islands.

Breeding places: Breeds in rain water pools, tanks, ponds, rice fields, drains, irrigation channels, wells and slow moving streams with plenty of shade provided by overhanging vegetation.

Resting habits: Rests indoors during daytime in human dwellings, cattlesheds and mixed dwellings. Rests outdoors near stream banks.

Biting time: Biting goes on throughout night, but the peak biting activity is from 24.00 to 02.00 hrs.

Feeding habits: Resting habits may differ from area to area.

Flight range: About 1 km.

An. sundaicus

Distribution: Reported from coastal Orissa, Andhra Pradesh and West Bengal in 1950s. At present it is restricted to Andaman and Nicobar Islands.

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



Resting habits: Rests indoors in human dwellings, cattlesheds and mixed dwellings.

Biting time: Biting goes on throughout the night but peak biting is from 20.00 to 02.00 hrs.

Feeding habits: An opportunistic feeder, prefers to bite man.

Flight range: About 1-3 kms.

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Filaria Vectors

ulex quinquefasciatus breeds in association with human habitations. It is the most common house frequenting mosquito. Breeds in any type of habitat ranging from fresh and clear to brackish, turbid and polluted water with decayed organic matter from



garbage and human waste accumulations in ground pools, ditches, drains, sewage, septic tanks etc. and in various kinds of artificial containers (bottles, cans, flower pots, vases, bowls, jars, cement tanks etc., (Indoor / outdoor) in general, uncommon in small containers but common in larger ones. They are the dominant species in order of prevalence found more or less fluctuations. Maximum breeding is found in water collections with high organic pollution with pH range 6.0 - 7.0. Eggs are laid in rafts containing 150 - 400 eggs depending on quality & quantity of blood meal. At optimum temperature of 25-30°C, eggs hatch within 24-48 hrs. All the 4 instars are voracious feeders. The larvae feed on diatoms, algae and other microorganisms. There is no morphological difference between I & IV instar except an egg breaker on dorsal side of head of I instar larva. The larvae hang head downward in water with siphon tube projecting out of surface for respiration. Any minute disturbance makes them to swiftly go to bottom for considerable period (1-2 Mts.). IV instars give rise to comma shaped pupae lasting up to 24-48 hrs. Pupae do not feed but very active; respire through a pair of respiratory trumpets. Adults emerge through a slit formed between 2 trumpets. After emergence, either sex sits

for sometime over pupal case or nearby vegetation for stretching wings and legs. Entire life cycle will be completed in 10-14 days.

Environment of Adult mosquito

Mating: Females generally do not take blood meal before mating (12-24 hrs from emergence). Mating takes place in dusk after a nuptial dance.

Dispersal: Average flight range of *Cx. quinquefasciatus* is about 2-3 km. Males are weak fliers.

Biting Habits: Blood feeding takes place within 24-48 hrs. after mating from sun set until dawn. Highest peak : Late night or third - quarter of night. It is Endophagous as well as exophagous.

Host Preference : Highly anthropophilic; also frequently attacks birds such as fowls and a lesser extent, other domestic animals including dogs, cats and pigs.

Resting Habits: Enter houses for feeding at night and for resting during daytime.i.e. Endophilic and endophagous. Rest in dark corners of walls, on hanging objects, cobwebs, inside shoes, cupboards, under cots, tables and chairs, bathrooms etc. with less human activity.

Gonotrophic Cycle : Under optimal atmospheric condition (25-30⁰ C & > 70% RH) each gonotrophic cycle is completed in 3-4 days. Before each cycle, females require a blood meal.

Seasonal Prevalence: They are fairly abundant throughout the year with two annual peaks in northern parts of the country (February to March & September to October).

Extrinsic Incubation Period and Longevity: Rao and Iyyengar found mean temperature below 24⁰ C and above 34⁰ C, inhibited growth of *W.bancrofti* in *Cx.quinquefasciatus*. Khalil et al., found filarial larvae matured in 20 days at 23-24⁰ C and in 14 days at 29-39⁰ C.

Average longevity varies form 15-45 days (females): Longevity reduced during adverse climatic conditions. During rainy seasons, average longevity will be comparatively more.



Bionomics of *Mansonia* Speceies:

Important vectors

Mn.annulifera, *Mn.uniformis* and *Mn.indiana*

The environment of Immature

The immatures need presence of floating vegetation like Pistia, Salvinia, Eichhornia etc. for survival. *Mansonia* spp. lay eggs (clusters) on under surface of

floating water plants. 80 - 120 eggs/clusters. Eggs are elliptical in shape with a spike at distal end. Within 48 hrs. 1 instar emerges. On emergence, larvae



immediately seek out a rootlet and get attached by way of modified siphon tube (Highly chitinised & serrated along the margin with 4 hooks at tip to facilitate uninterrupted attachment). Attachment continues during pupal stage as well; moultings occur in attached conditions. Tips of pupal breathing trumpet is also modified for attachment. Larvae & pupae get available oxygen from air spaces of the floating vegetation. Just before emergence of adults pupae get detached and come to surface; adult escape through slit on the pupal skin. Life cycle from egg to adult takes about 21-24 days depending upon climatic factors.

Breeding of *Mansonia* spp. is restricted to fresh water ponds, lakes, swamps & channels with floating vegetation and high organic pollution with a pH range of 6.2-6.8.

Pistia stratiotes, the most preferred plant for breeding of *M. annulifera* wherever replaced completely by *Salvinia*, *M. uniformis*, on the increase.

Environment and habits of adult mosquito

Dispersal: Not strong fliers, easy to catch since hop about like sandflies. Silent in flight with limited flight range.

Biting Habits: Active throughout night with peak biting activity in I & II quarters of night. Endophagous in nature.

Host Preference: *Mn.annulifera*, highly anthropophilic; *M.uniformis*, more zoophilic.

Resting Habits: *Mn.annulifera*, endophilic & endophagous, rest in dark corners of house, *Mn.indiana* mostly confined to cattle sheds & other outdoor situations.

Seasonal prevalence: *Mn.annulifera*: Peak density: Between January & May
M.uniformis: September to December. However, infected & infective mosquitoes (both species) found throughout year but slightly higher rates during June - August. *M.uniformis* density is higher where *Salvinia* host plants are completely replaced by *Pistia*.



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JE Vectors

Culex vishnui subgroup mosquitoes, comprising *Cx. tritaeniorhynchus*, *Cx. vishnui* and *Cx. pseudovishnui*, have been implicated as major vectors of JE in India as well as in many countries of south-east Asia. These mosquitoes are usually found in rural rice growing and pig-farming regions of Asia, but can also be found at the outskirts of cities in close proximity to human populations. They prefer to breed in rice fields, and outbreaks of JE are commonly associated with intensive rice cultivation.



JE virus has been recovered from 19 mosquito species in different parts of India and the prominent vectors are *Culex tritaeniorhynchus*. Majority of the isolations were from *Cx. tritaeniorhynchus* and this mosquito has been considered as the primary vector based on relative abundance, widespread distribution, and frequent virus infection. JE virus has also been isolated from *Culex gelidus*, which prefers to breed in marshy depressions containing abundant aquatic vegetation. JE virus has also been isolated from *Mansonioides* species of mosquitoes. The aquatic plants such as *Pistia*, *Salvinia* and *Eichhornia*, are essential for the larval development of *Mansonia*. In India, it was observed that *Mansonia* is probably a secondary vector which maintains JE virus during inter-epidemic period, with *Cx. tritaeniorhynchus* as the primary vector. *Cx. pseudovishnui*, *Cx. whitmorei*, *Cx. gelidus*, *Cx. epidesmus*, *Anopheles*

subpictus, *An. peditaeniatus* and *Mansonia uniformis* are suspected to play some role in the epidemiology of JE in Gorakhpur. Recently, JE virus was also detected from both male and female adult mosquitoes of *Cx. tritaeniorhynchus*, *Cx. gelidus*, *Cx. pseudovishnui*, *Cx. bitaeniorhynchus*, *Cx. vishnui*, *Cx. infula*, *Cx. fuscocephala* and *Ma. indiana* reared from wild caught immatures from Gorakhpur region showing vertical transmission.^{3,10} Vertical transmission of JEV occurred in both hot and cool seasons. Thus the JE virus is regularly maintained in nature during the non-transmission seasons also.

JE Vector bionomics

Life cycle consists of egg, four instars of larvae, pupa and adult. The whole cycle takes more than a month; however, duration depends on temperature and other ecological conditions.

JE vectors are mainly exophilic and endophagic in nature. The risk of transmission increases when the human dwellings and animal sheds particularly piggeries are situated very close to each other. When they are situated far from each other, the risk of transmission is reduced. Because of outdoor resting habits and crepuscular nature, the vector control using indoor residual spray is technically not feasible. In addition to this, due to vast and enormous breeding habitats like perennial ponds, paddy fields and other water bodies, larval control using various anti larval measures is also not feasible as it is resource intensive.

A conducive ecosystem comprising of irrigation canals, rice fields, ponds, ditches and lakes favour JE vector breeding particularly in rural areas. However in semi urban areas, breeding of *Culex vishnui* group of mosquitoes found in small ponds and ditches with water hyacinth and other aquatic plants. Surface water bodies such as ponds and canals are perennial breeding sources for JE vector breeding and provide a wintering and staging ground for a number of migratory waterfowls and

a breeding ground for resident birds. They also act as mother foci for vector mosquitoes. After the monsoon, vectors spread to other water stagnation areas and rice fields. Thus breeding control with appropriate larvicides or using larvivorous fish in all permanent water bodies, before the start of monsoon and paddy irrigation, may check proliferation of breeding of JE vectors and may even contain the vector population during JE transmission season. The epidemic form of the disease and mortality due to JE can be minimized by applying the effective prevention or control measures in order to reduce the mortality.

Dengue viruses are transmitted from person to person by *Aedes* (*Ae.*) mosquitoes of the subgenus *Stegomyia*. *Ae. aegypti* is the most important epidemic vector, but other species such as *Ae. albopictus*, *Ae. polynesiensis*, members of *Ae. scutellaris* complex, and *Ae. (Finlaya) niveus* have also been incriminated as secondary vectors. All except *Ae. aegypti* have their own restricted geographical distribution and, although they may be excellent hosts for dengue viruses, they are generally less efficient epidemic vectors than *Ae. aegypti*.

***Aedes* mosquito**

The *Aedes* mosquito can spread dengue fever, chikungunya, and yellow fever, West Nile viruses, and other diseases. *Aedes* is now found in all continents throughout the globe (except Antarctica), was originated in Africa. It ranges from sea level to 2200 meters (approx.) above Mean Sea Level (MSL).

Only the female *Aedes* mosquito bites for blood which is needed for development of eggs, whereas, males feed on nectar. These mosquitoes are attracted to different chemical compounds emitted by mammals (including human beings) and use these compounds to find the host. This is a day biting mosquito and prefers to rest in hard to find dark areas inside the houses.

Life-cycle

The life cycle of *Aedes* mosquitoes consist of four stages i.e. Egg, Larva, Pupa and Adult. The cycle can be divided in aquatic and terrestrial phases. The Egg, Larva and Pupa are aquatic stages, whereas, adults are terrestrial. Under favorable conditions, the life-cycle of *Aedes* mosquito completes in an average of one week (egg to adult). In unfavorable conditions, the eggs can be viable for over a year in a

dry state, which allows the mosquito to re-emerge after a cold winter or dry spell.

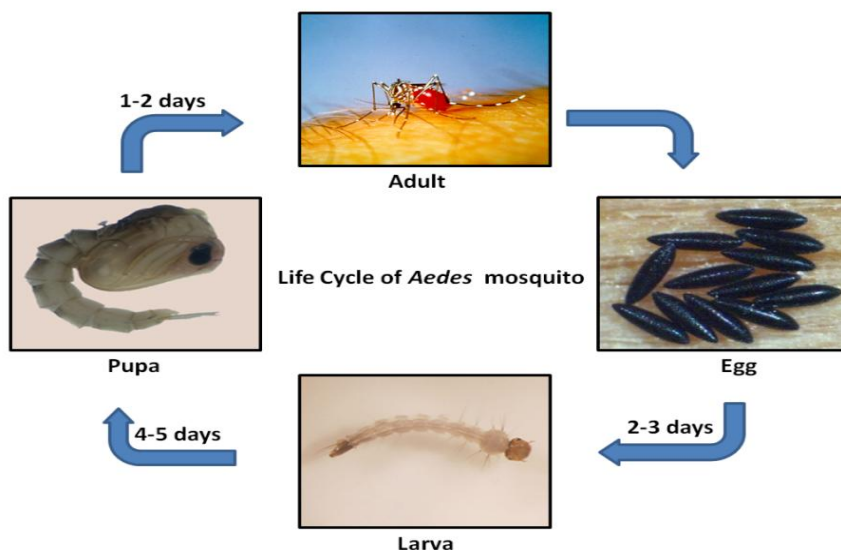


Fig- The life-cycle of Aedes mosquito

Seasonality

The density of Aedes mosquito is more during monsoon and post-monsoon season. At the onset of the rains, there is a population spurt due to the creation of multiple breeding foci. In dry area or water scarcity areas, the vector density is linked to water storage practices. Therefore, in such situation, the prevalence of the species may be independent of the rainy season. The species has been observed to be at its lowest ebb during winter months.

Aedes (Stegomyia) aegypti (L.)

Ae. aegypti is the main vector in urban, semi-urban and rural areas. The species has perfectly adapted to domestic habitats and breeds in man-made receptacles. Ae. aegypti selects obscure places for resting. More than 90% individual do not rest on sprayable surfaces. The species is strongly anthropophilic having highly preference for human blood. It is day-biter and important vector of Dengue, Chikungunya, Yellow Fever, West Nile virus (in India- only Dengue and Chikungunya).

The *Ae. aegypti* can be easily separated from *Ae. albopictus* on the basis of presence of silvery white sickle-shaped pattern of scale on scutum.



Adult *Ae. aegypti*



Ae. aegypti: Silvery white 'sickle-shaped pattern of scale on its scutum

Breeding places

Ae. aegypti mosquitoes prefer to breed in manmade containers, viz., , i.e water storage containers, uncovered/partially covered water tanks (cement tanks, overhead tanks, underground tanks), exterior extensions of building, evaporation coolers in arid/semi arid regions of tropics, pitchers and trash outdoors viz. discarded buckets, bottles, tyres, and coconuts shells etc. in which water stagnates for more than a week.

Resting places

The *Ae. aegypti* mosquito rests generally in indoor situations like inside the houses, office premises, business establishments, tyres, dumping places etc. It prefers to rest in dark corners of the houses, on dark clothes, umbrellas, under furniture & beds, shelves, coolers, behind hangings, shoes, besides house hold articles, curtains etc. and other hard to find dark places, but rarely on walls.

Lifespan and Survival

The lifespan of an adult *Ae. aegypti* is upto four weeks depending on certain biotic and abiotic factors (like temperature, relative humidity etc.). The developmental time of *Aedes* taken from hatching to pupation is negatively related with

temperature. The lower temperature is a limiting factor in mosquito development. The female longevity, fecundity and haematophagic activity are also directly affected by increased temperatures. However, it is also believed that females lived longer than males (1.5 times longer than males under constant temperatures).

Feeding and biting habits

The feeding behaviour of *Ae. aegypti* is a complex phenomenon. The females mosquitoes feed on the blood for maturation of ova and its survival.. The frequency of taking meal is related to the oviposition. Often feeds on several persons during a single blood meal in a short period of time.

Gonotrophic Cycle and Egg laying behavior

Gonotrophic cycle is the time lapse between blood sucking and egg laying. It is normally averages from 3 to 4 days which depends on fluctuation of temperature. During the cool-dry months, the delay in taking a blood meal reduces the degree of man-mosquito contact and the vectorial capacity of the mosquito.

The eggs (upto 100-120) are laid singly on damp surfaces just above the water line. During one gonotrophic cycle, female lay eggs in different containers. The egg-laying activity of female *Aedes* mosquitoes peaks in the late afternoon or early evening, and there is little oviposition during midday.

Flight range and dispersal

The flight range for *Aedes* is generally 100 meters but it can fly upto 400 meters. Through passive transportation, different stages of *Aedes* (especially eggs) may disperse to newer areas.

Aedes (Stegomyia) albopictus (Skuse)

The *Ae. (Stegomyia) albopictus* is considered to play a secondary role in transmission of dengue in the peripheral areas. *Aedes albopictus* is the secondary vector in sylvatic areas. It is an Asian species but has spread globally through transportation of dormant eggs with tyre export. It is a feral species & spread disease in built-up areas, particularly in parks and gardens. It also co-breeds in

peri-domestic locations in Trash. The species feeds on man & also on other animals. The *Ae. albopictus* can be easily separated from *Ae. aegypti* on the basis of presence of white strips down the center beginning at the dorsal surface of the head & continuing along the thorax of *Aedes albopictus*.



Adult *Ae. albopictus*



Ae. albopictus: White strips down the center beginning at the dorsal surface of the head & continuing along the thorax

Breeding places

Aedes albopictus mosquitoes prefer to breed in natural habitats like tree holes, latex collection cups of rubber plantations, leaf axils of pine apple plants, coconut shells etc. However, where population increases significantly, the species breeds in domestic habitats as well.

Resting places

The *Ae. albopictus* mosquito rests outside in bushes, shrubs, long grasses in and around per-domestic situations. But sometimes found in domestic conditions as well.

Lifespan and Survival

The lifespan of an adult *Ae. albopictus* is also upto four weeks depending on certain biotic and abiotic factors same as *Ae. aegypti*.

Feeding and biting habits

The *Ae. albopictus* feeds on different vertebrate hosts including human being. It is also a day-biter.

Gonotrophic Cycle and Egg laying behavior

The Gonotrophic cycle and egg laying behavior of *Ae. albopictus* and *Ae. aegypti* is almost same. The eggs (upto 100-120) are laid singly and during one gonotrophic cycle, female lay eggs in different habitats.

Flight range and dispersal

The flight range for *Ae. albopictus* is upto 400 meters. It may also disperse to newer areas through passive transportation (especially eggs).

Outbreak investigations in many geographical locations also reveal the role of *Ae. albopictus* as it was either predominant or the only species detected. *Ae. albopictus* is known to outcompete and even replace other species occupying the same niche.

Kala azar Vector Sand Fly (*Phlebotomus argentipes*)

- *Phlebotomus argentipes* (sand fly) only vector of VL in India. Adult sand fly small, fuzzy, delicately proportioned, 1/4th the size of mosquito. Length ranges from 1.5 to 3.5 mm.
- Favorable ecological conditions proliferating the density of sand flies include (i) alluvial soil (ii) high sub-soil water, (iii) monthly mean maximum temperature below 37° C (iv) Annual rainfall of 1250 mm or more (v) mean annual RH of 70% or more with 80% for at least 3 months (vi) altitude below 600 meters.
- In India distribution mostly on the eastern half of the country though reports of its prevalence have also emerged from other parts as well.
- *Ph. argentipes* found throughout the year in majority of areas of prevalence with complete absence in winter months. In Bihar a minor peak observed in March/April and major peak in August/September.
- Opportunistic feeder, mostly zoophilic, poor flies, hops covering a distance of less than than ½ metre. Resting sites include cracks and crevices, burrows, tree holes, termite hills, earthen mounds, under stone and foliage etc.
- Longevity under lab conditions ranges from 23-27 days but in field conditions from 16-20 days.
- Life cycle in four stages, egg, four instars of larva, pupa and adults, total time taken from egg to adult reported to be 20-36 days with average 26.75 days in laboratory.
- Sampling Techniques include (i) Hand collection, (ii) light trap collection and (iii) sticky traps.

Leishmaniasis are the vector borne diseases which exist either as zoonosis (in most of the endemic areas of the world) or anthroponosis (Indian sub-continent i.e. India, Bangladesh, Nepal). The vectors of various Leishmaniasis worldwide over belong to Order Diptera of class Insecta (Phylum Anthropoda).

Sand flies are grouped in two sub-families namely Psychodinae and Phlebotominae. Only the members belonging to family Phlebotominae are transmitting agents for different types of Leishmaniasis.

In India, so far three sand flies species have been incriminated as vectors of leishmaniasis, they are:

Phlebotomus argentipes as only known vector of Visceral Leishmaniasis.

Phlebotomus papatasi as the vector of anthroponotic or urban Cutaneous leishmaniasis.

Phlebotomus salehi as the vector of rural (zoonotic) Cutaneous leishmaniasis.

The adult sand fly is a small, fuzzy, delicately proportioned fly, usually 1/4th of the size of the mosquito. The length of sand fly body ranges from 1.5 to 3.5 mm. The males and unfed females can pass through mosquito net easily. The elongated wings are hairy, held erect on the abdomen and are bigger than the size of the body. The body, wings and legs are heavily covered with long hairs. The sand fly could be spotted easily because of the posture of the wings which are always held vertically erect when at rest.

5.1 Biology and Bionomics

The sand flies are associated with warm climate and could be grouped into two categories namely species associated with wet zone and the species associated with

arid zone and this association further delimits the distribution of different types of Leishmaniasis. Napier (1926) suggested ecological factors favorable for transmission of Visceral Leishmaniasis or Kala-azar as:

1. Alluvial soil
2. High sub-soil water
3. Monthly mean maximum temperature below 37°C
4. Monthly mean minimum temperature about 7.2°C
5. Annual rainfall 1250mm or more
6. Mean Annual Relative Humidity of 70% or more with more than 80% for at least 3 months
7. Abundant vegetation
8. Altitude below 600 meter

These factors inter-alia favour *Phlebotomus argentipes*, the only known vector of visceral leishmaniasis in India, to survive with high prevalence through greater part of the year facilitating transmission.

Adults

Distribution

P. argentipes is one of the widely distributed sand flies and is essentially the species of wet zone. It is widely distributed along side of the equator and in India, its distribution could be seen on the eastern half of the country and western limits could be marked by joining a line from Bombay to Delhi. Though commonly the sand flies are not found at the altitude above 600 meters, sporadic occurrence in India has been recorded in Kasauli at a height of 1200 meters and at 1300 meters in Pauri Garhwal in Himalayas. The species is predominantly distributed all along the eastern coast from West Bengal to Kanyakumari.

Seasonal prevalence

As already mentioned, high relative humidity, warm temperature, high sub-soil water and abundance of vegetation favors proliferation of *P. argentipes* and accordingly, depending on this condition seasonal prevalence varies from area to area. In India the species is found throughout the year in majority of areas of prevalence with complete absence in winter months in areas with extremes of temperatures like Assam. With the onset of warm weather coupled with humidity, the density increases till June with sudden decline due to high temperature. It is again followed by increasing trend reaching the maximum during and just after the monsoon rain. A similar trend has been reported in Bihar with a minor peak in March/ April and a major peak in August/ September in densities of *P. argentipes*.

Feeding Behavior

All species of sand flies feed on plant sugar and the females often feed on vertebrate host including man. The females of genus *Phlebotomus* feed on mammals. Though *P. argentipes* are commonly known as zoophilic, it has been observed by various workers that the anthropophilic index largely depends on the sampling. For example, the samples collected from human habitation showed 69.6% anthropophilic index whereas the samples collected from cowshed in the same area showed only 21.6% anthropophily. The same was true in the case of bovid blood index which was 96% in the cowshed and 44% in the human dwellings. This indicates clearly that *P. argentipes* are primarily indiscriminate (opportunistic) feeder and the type of blood meal largely depends on availability of host in its immediate vicinity. However, studies undertaken in Nilgiri hills indicate a high level of zoophily to the extent of even 100%.

Flight Range

Sand flies are not capable of flying very long distances. Their usual mode of flight pattern is a series of short erratic hopping in which the fly usually covers a

distance less than ½ meter. Further, the dependence of the most of the vectors of humidity for their survival limits their movements.

Resting Sites

The most favored resting sites for sand flies include soil cracks and crevices, burrows (rodent burrows), tree holes, termite hills, caves, bird tunnel, in earthen mounds, under stone and foliage, etc. In eastern parts of India like Bihar & West Bengal, *P. argentipes* prefers to rest indoors, about 9-10 times higher in cattle dwellings than the human dwellings. In western India sand flies rest outdoor also in considerable numbers. As could be seen, all these resting places provide them dark and damp shelter where the microclimatic humidity is very high. They usually leave these shelters at dusk and are active in open in the evening and night. Usually sand flies remain active throughout the night but they are sensitive to decreasing temperature and air currents. Even a gently breeze of 1.5-2 metre/second may greatly reduce their activity.

Longevity

In India, *P. argentipes* have been reported to undergo 5 gonotrophic cycles under laboratory conditions, duration each cycle being 4 to 5 days at 26+ 2°C i.e a minimum longevity up to 23-27 days under laboratory conditions. Field studies conducted on parity have also shown presence of triparous and “four parous” females in nature indicating probability of longevity under field conditions to be upto 16-20 days in a proportion of natural population. However, longevity in field is directly dependent on ecological factors.

Immature Stages

Egg

The freshly laid eggs are creamy white in colour which later becomes dark. The eggs are usually deposited in cracks and crevices with high organic content, humidity and darkness. Sometimes eggs are also found in loose soil. The eggs are [glued to the surface through flattened while the convex side faces upwards. The egg shell has sculptors and their size varies from 0.336 – 0.432 mmx0.096-0.160 mm. A wide range has been observed for total number of eggs laid per female (5-68). The eggs hatch in 3-4 days at 26+ 2oC in laboratory.

Larva

The creamy white larva with distinct head, thorax and abdomen has numerous hairs on its body. The larva feeds on organic matter available in the soil. There are four larval stages:

I instar: The delicate larva is whitish with a brown head capsule lacking eyes. A pair of black caudal bristles and presence of egg breaker on the posterior portion of head are the characteristic features of I instar. The average life is 2-4 days.

II instar: Presence of 2 pairs of caudal bristles, round 3rd antennal segment and absence of egg breaker are the features of II instar which lives for about 2-5 days.

III instar: Presence of 2 caudal bristles on completely dark last abdominal segment, partially elongated 3rd antennal segment and yellowish body hairs on a well developed larva help in identifying III instar which lasts for about 3-4 days.

IV instar: It is a well developed brown larva with dark brown head capsule, elongated, oval 3rd antennal segment with a pointed seta. Two pairs of spiracles and two pairs of well developed caudal bristles are conspicuous. The stage lasts for 4-7 days and transforms into pupa. The total larval period may vary from 11-29 days.

Pupa

The elongated comma shaped pupa is milky white in the beginning and turns brown. It is a non feeding stage lasting for about 6-10 days. The sexes are differentiated in this stage. The total life cycle from egg to adult is reported to take about 20-36 days with average 26.75 days in *Ph. argentipes* in laboratory.

5.4 Processing & Mounting

Each field caught specimen of sandfly is to be kept in 5% KOH solution or soap solution for 24 hours. Alternatively, the specimen may be kept in 5% KOH and gently heated for about 4-5 minutes. This helps in removing the hairs, cuticular wax layer and viscera. The alkali/ soap is then removed by thorough washing with ordinary water at least thrice. The specimen is then dissected in mounting media, the head is oriented ventral side up to expose buccal cavity. The 8th abdominal pleura are stretched gently to expose spermethecae in females. In males, terminalia is properly oriented for examination.

Mounting Media

Hoyer's media is the most commonly used medium for sandfly mounting. It consists of following constituents:

Distilled water	- 100cc
Gum Arabic	- 60gm
Chloral hydrate	- 400gm
Glycerine	- 20cc

After mounting the specimen, it is left for 48-72 hours in an incubator/room temperature for making the cuticular structures clearer.

Malaria transmission:

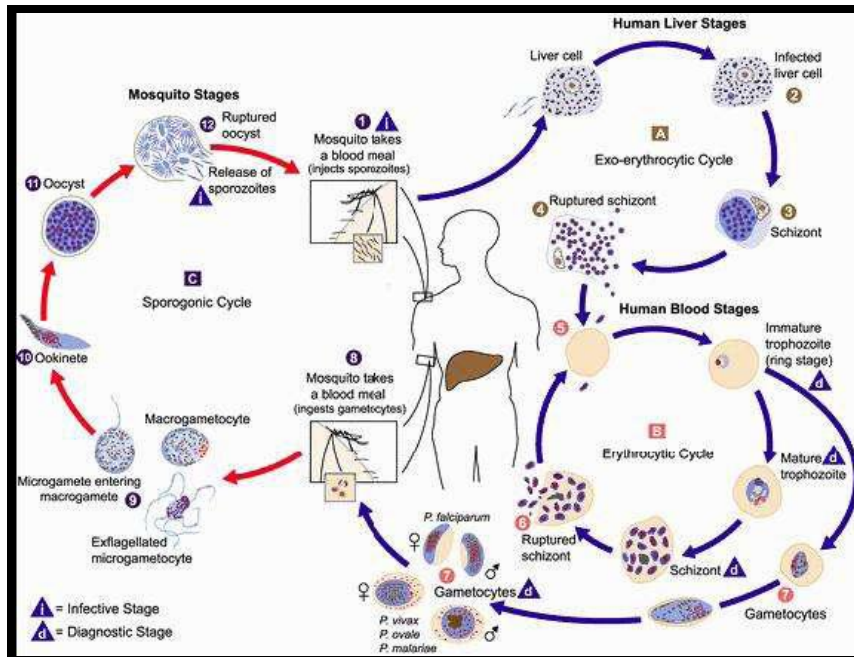
Malaria is transmitted by a female Anopheles mosquito bite which has been infected through a blood meal taken from an infected person. A single infected vector, during her lifetime, may infect several persons. After about a week of taking infected blood meal, mosquito is able to transmit malaria. This is known as vector transmission.

Because the malaria parasite is found in red blood cells of an infected person, rarely malaria can also be transmitted through blood transfusion (parasite can stay alive for nearly two weeks at 4° C in bottled blood), organ transplant, or the shared use of needles or syringes contaminated with blood. This is known as direct transmission. Malaria may also be transmitted rarely from a mother to her unborn infant before or during delivery known as "congenital" malaria.

In India *Plasmodium vivax* (*P. vivax*, PV) and *Plasmodium falciparum* (*P. falciparum*, PF) are responsible for most human malaria. Their proportions are nearly equal. There are two other plasmodia (*Plasmodium malariae* and *Plasmodium ovale*) that cause malaria in humans, but they are rare in India. *P. falciparum* is the variety which is responsible for almost all the deaths due to malaria. *P. vivax* causes debilitating illness, but in recent past deaths due to vivax malaria is also being reported.

The malarial parasite undergoes 2 cycles of development - the human cycle (asexual cycle) and the mosquito cycle (sexual cycle). Man is the intermediate host and mosquito the definitive host.

*Diagram depicting Life Cycle of Plasmodium species
in man and the mosquito*



1) Asexual cycle in human being

The asexual cycle begins when an infected anopheles mosquito bites a person and injects sporozoites. There are 3 phases in the human cycle.

a. Hepatic Phase

After entry into blood circulation, the sporozoites disappear within 60 minutes from the peripheral circulation. Many of them are destroyed by phagocytes, but some reach the liver cells. After 1-2 weeks of development (depending upon the species), they become hepatic schizonts, which eventually burst releasing a shower of merozoites. The number of merozoites produced from a single sporozoite varies – as many as 40,000 in *P. falciparum*, whereas only 200 – 15,000 in other species. In *P. falciparum*, the intrahepatic schizonts rupture almost simultaneously and there is no persistent tissue phase (exo-erythrocytic phase). In other species, the hepatic

forms may remain dormant (hypnozoites) for long periods, liberating merozoites at various intervals, causing relapses of malaria.

b. Erythrocytic Phase

Many of the merozoites released from the liver cells are quickly destroyed, but a significant number attach themselves to specific receptor sites on the RBCs, penetrate them and pass through various stages of trophozoite and schizont. The erythrocytic phase ends with the liberation of merozoites, which infect fresh RBCs. The clinical feature of fever with chills coincides generally with the rupture of RBCs. The cycle is repeated over and over again until the condition worsens or when it may be slowed down by the immune response of the host. The duration of each erythrocytic cycle varies between species – 48 hours for *P.falciparum*, *P.vivax* and *P. ovale*; and 72 hours for *P. malariae*.

c. Gametogony

Some of the erythrocytic forms of plasmodia do not divide further but develop into male and female gametocytes. Not all **infected** persons are **infectious** (can infect anopheline mosquitoes). The blood of the person has to have mature male and female gametocytes and the density should be minimum 12/ cumm of blood to be infective. These gametocytes take over a week to appear in the blood. Gametocytes do not cause any symptoms in humans. Most drugs like chloroquine kill the asexual forms that cause the fever but leave intact the sexual forms that are infective especially in case of *P falciparum*. Thus an apparently normal person may harbour the disease and contribute to its spread.

2) Sexual Cycle in Mosquito

The mosquito cycle (sporogony) begins when gametocytes are ingested by the vector mosquito while feeding on an infected person. The male gametocytes, after

reaching the stomach of the mosquito and develop into 4-8 filaments called “microgametes”. The female gametocyte undergoes maturation to become a “macrogamete”. The microgametes get attracted to the macrogamete, and one of the microgametes fertilizes the macrogamete. The resulting zygote is at first motionless, but within 18-24 hours, becomes a motile ookinete, which penetrates the stomach wall of the mosquito and develops into an oocyst on the outer surface of the stomach. The oocyst further develops into numerous sporozoites, when the oocyst ruptures and releases the sporozoites into the body cavity of the mosquito. Many of the sporozoites migrate to the salivary glands and the mosquito becomes infective to man. The period required for the development of the parasite from the gametocyte stage to sporozoite stage is about 10-20 days depending on atmospheric temperature and humidity. This period is known as the “extrinsic incubation period”. The sporozoites (the infective stage of *Plasmodium*) are injected with saliva when the mosquito next feeds.

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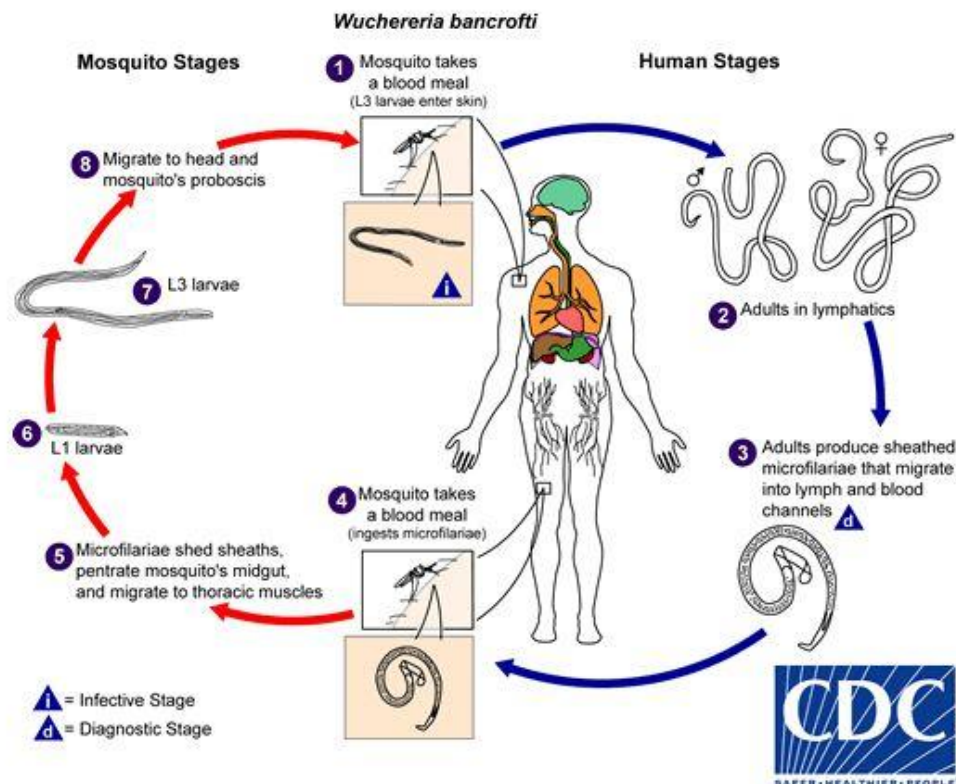
Filaria (Transmission)

To define the role of entomology in GPELF, the dynamics of transmission of lymphatic filariasis parasites between their human host and their mosquito vector, the geographical distribution of filarial parasites and vector competence must be understood.

3.1 Filarial parasites

Lymphatic filariasis is caused by three species of parasitic worm, *Wuchereria bancrofti*, *Brugia malayi* and *B. timori*, which have generally similar life cycles

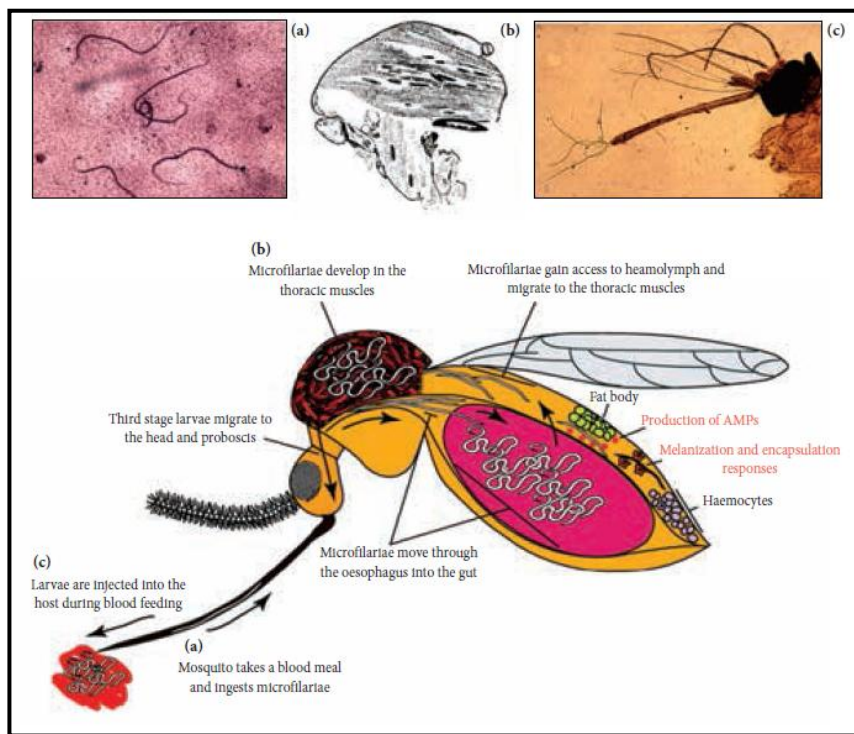
Biology - Life Cycle of *Wuchereria bancrofti*



In the human body, adult worms (male and female) live in nodules in the lymphatic system and, after mating, produce numerous microfilariae, which circulate in the bloodstream. The lifespan of adult worms is 4–6 years. Microfilariae migrate between the lymph system and blood channels to reach the peripheral blood vessels, often at times of the day that coincide with the peak biting activity of local vectors.

When female mosquitoes ingest a blood meal, they consume microfilariae with the blood. In the mosquitoes' stomachs, they lose their sheath, and some of the parasites migrate through the stomach wall to reach the thoracic flight muscles, where they develop into first-stage larvae (L1). The larvae grow and moult into second-stage larvae (L2) and moult again to produce highly active infective third-stage larvae (L3), a process that takes 10–12 days from the L1 stage to the L3 stage.

The infective larvae migrate to the mosquito's proboscis, where they can infect another human host when the mosquito takes a blood meal. The L3 are deposited on the skin and find their way through a bite wound. The L3 develop to fourth-



stage larvae (L4) as they migrate through the human body to the lymphatic vessels and lymph nodes, where they develop into adult worms. See *Figure 8*.

Transmission in the vector

The transmission dynamics of lymphatic filariasis are complex, involving two genera of parasite (*Wuchereria* and *Brugia*) and a number of genera of mosquito carriers. The four main genera are *Anopheles*, *Culex*, *Aedes* and *Mansonia*.¹ The biological features of the vector–parasite relationship should be understood in order to define the entomological variables critical to lymphatic filariasis transmission and the threshold for interrupting transmission. Unlike the transmission of malaria and arboviruses, that of lymphatic filariasis is inefficient, and a large number of bites from infectious mosquitoes is required to initiate a new infection with microfilaraemia (33).

Many factors contribute to the inefficient transmission of lymphatic filariasis (*Figure 9*). Firstly, microfilariae do not multiply in the mosquito body; hence, the number of L3 is limited by the number of microfilariae ingested. Second, only those mosquitoes that survive more than 10 days will contribute to transmission of the parasites (*Figure 9* and *Box 6*). Those mosquitoes that die before the L3 develop cannot play a role in the transmission cycle. Third, the L3 are deposited on the skin and have to find their way into the bite wound (rather than being injected with the mosquito saliva like malaria sporozoites). In view of all these factors, the transmission of lymphatic filariasis parasites is considered to be less efficient than that of other vector-borne parasites, such as malaria and dengue.

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Dengue (Transmission)

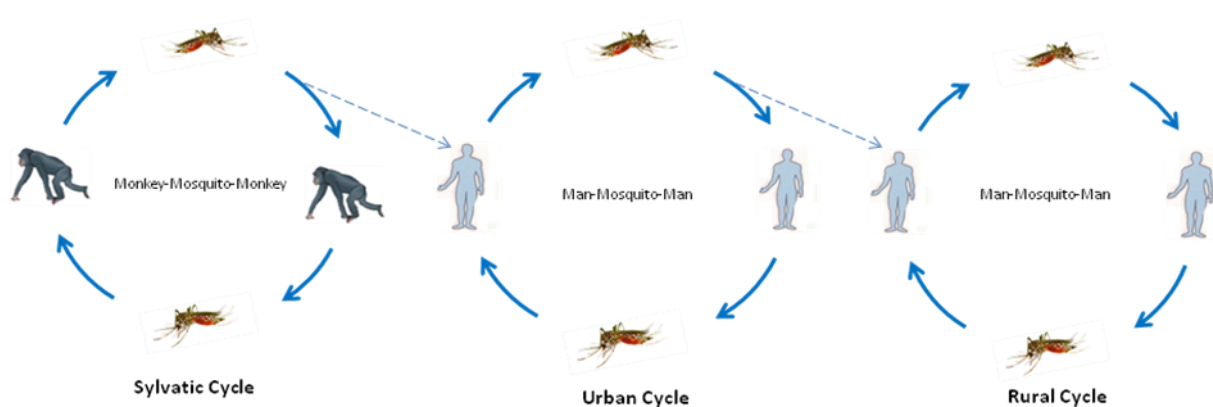
Transmission of Dengue depends upon a complex relationship between epidemiological factors viz., agent (virus), host (man and mosquito), and the environment (abiotic and biotic factors). The complexity of relationship among these factors eventually determines the level of endemicity in an area.

The causative agent of Dengue i.e. Dengue virus is categorized under the genus *Flavivirus*. The Dengue virus has four serotypes which are designated as DENV-1, DENV 2, DENV-3 and DENV-4. These serotypes may be in circulation either singly or more than one in any area at the same time. Infection with any one serotype confers lifelong immunity to that virus serotype. The secondary infections are associated with elevated risks of severe disease outcomes. However, the primary and secondary infections are distinguishable based on their antibody responses. All four serotypes are reported from various parts of India.

Dengue virus infects humans and several species of lower primates. People of all ages and both genders are at risk. Secondary Dengue infection is a risk factor for DHF including passively acquired antibodies in infants. Travel to Dengue endemic area is most important risk factor, if the patient develops fever more than two weeks after travel, Dengue is unlikely, Migration of patients during viraemia to a non endemic area may introduce the disease into that area if the vector is already available in that area. The geographical spread of Dengue has been reported to be occurring mainly by the traveling of people from endemic area to non endemic areas.

Dengue viruses are transmitted from infected person to others by the bite of female *Aedes* (*Ae.*) mosquitoes. In India, *Ae. aegypti* is the main vector in most urban areas; however, *Ae. albopictus* is also incriminated in many states. *Ae. albopictus* has posed serious threats of dengue transmission in southern states and NE states.

Dengue viruses are highly adapted to their mosquito-hosts and their origins are believed to be as mosquito-viruses in sylvatic life cycle between forest mosquitoes and non-human primates. From the sylvatic cycles the human to human cycle possibly evolved through mosquitoes feeding on humans. This evolutionary adaptation not only enabled the co-circulation of the four serotypes but also had a great influence to their pathogenicity for humans. The chain of dengue transmission is shown in the figure:



Under optimal conditions, the adult is emerged in seven days (after the aquatic stages in the life cycle of *Ae. aegypti*). The climatic conditions particularly temperature and rainfall play key role on the life cycle, breeding and longevity of vectors and thus transmission of these diseases. At low temperature, it may take several weeks to hatch. Average survival of *Ae. aegypti* is 4 weeks and *Ae. albopictus*

is about eight weeks. During the rainy season, when survival is longer, the risk of virus transmission is greater. There is also evidence of vertical transmission of Dengue virus from infected female mosquitoes to the next generation.

The female *Ae. aegypti* usually becomes infected with Dengue virus when it takes blood meal from a person during the acute febrile (viraemia) phase of Dengue illness. After an Extrinsic Incubation Period (EIP) of 8 to 10 days, the mosquito becomes infected and virus is transmitted when the infective female mosquito bites and injects its saliva into the wound of the person and through this the cycle of Dengue continues.

Transmission dynamics of dengue is correlated to the abundance of vector density. During Monsoon and post-monsoon the preponderance of vector increases due to abundance of breeding habitats in rain fed containers due to which every year during the period of July-Nov, an upsurge in the cases of Dengue has been observed. The disease has a seasonal pattern i.e., the cases peak after monsoon and it is not uniformly distributed throughout the country. However, the states in southern and western parts of the country report perennial transmission.

A person infected with dengue usually manifests symptoms after 4-10 day's Intrinsic Incubation Period (Average 5-7 days). The viraemic period ranges from 2 to 12 days with an average of 4-5 days. During this period, a dengue-infected person is capable of transmitting dengue viruses to *Aedes* mosquitoes.

Mosquito density increases rapidly during warmer periods as a result of shorter life cycle development. Simultaneously, increase in temperatures shortens the duration of the EIP of dengue viruses, prolongs the infective days of a mosquito, and therefore increases the dengue transmission rate. Prolonged rainfall creates numerous temporary breeding habitats for *Aedes* mosquitoes, which in turn increases dengue transmission in time and space.

The *Ae. aegypti* mosquito prefers to bite humans, and is easily disturbed by the movement of host during feeding. Thus, an *Aedes* mosquito has to bite several persons to complete a blood meal. During the process *Aedes* infects several persons in the same household or in close proximity resulting in clustering of cases. The horizontal and vertical dispersions of *Aedes* in a geographical area influences dengue transmission. Studies indicate flight range of *Aedes* mosquitoes normally less than 100m however in search of blood meal it can fly upto 400m and upto the 21st floor (height = 60 meter). This behavior allows *Aedes* mosquitoes to lay eggs and transmit dengue rapidly, not only in the same apartment building, but also in the neighborhood.

Climate may also influence the distribution of dengue incidence. The impacts of temperature and rainfall on dengue transmission are partly translated through the effects of temperature and rain on the rates of biological development, feeding, reproduction, population density, and survival of *Aedes* mosquitoes. At higher temperatures, *Aedes* mosquitoes emerge from eggs to adults in a shorter period and also the incubation period for dengue viruses shortens. A smaller size mosquito tends to have increased feeding frequency, which increases the rate of dengue transmission. Increasing temperatures can also shorten gonotrophic development, or the cycle from blood-feeding to egg maturation and breeding. Studies have shown that dengue viruses may reduce incubation time in mosquitoes from approximately two weeks to one week at temperatures of 32°C and above.

The monsoon season provides ample breeding habitats for *Aedes* mosquitoes, although heavy rainfall can potentially flush away larvae or pupae or the immature stage of *Aedes*. Rainfall converts numerous artificial and natural sources into breeding habitats for *Aedes* mosquitoes. *Aedes* mosquitoes adapt to harsh environmental conditions, which are sometimes produced by vector control

programs, by laying their eggs in unusual outdoor habitats, or even on dry surfaces to wait up to several months for the appropriate amount of rain water to hatch. Therefore, the population density of *Aedes* mosquitoes can increase rapidly after rainfall. In general, any object or container that holds or traps 10 ml of water can be a potential breeding site; thus making vector control an extremely challenging task. Scarcity of water during dry season also increases storage which in turn creates breeding sources for *Aedes* mosquitoes if containers are improperly covered or attended.

In summary, mosquito population increase rapidly during warmer periods as a result of shorter life cycle development. Simultaneously, increase in temperatures shortens the duration of the extrinsic incubation period of dengue viruses, prolongs the infective days of a mosquito, and therefore increases the dengue transmission rate. Though heavy rainfall could shorten the life span of outdoor *Aedes* mosquitoes, and potentially destroy immature stage, it also creates numerous temporary breeding habitats for *Aedes* mosquitoes, which in turn impacts dengue transmission.

Seasonal and geographic differences in temperature and anticipated climate change undoubtedly influence mosquito population dynamics, individuals' traits related to vector biology (lifespan and vector competence for arboviruses), and disease transmission patterns. Temperature is regarded as one of the most important abiotic environmental factors affecting biological processes of mosquitoes, including interactions with arboviruses.

In absence of availability of any commercial vaccine against dengue, the strategies for prevention and control of dengue is mainly dependent on the vector control measures particularly source reduction activities in minimizing the risk of transmission.

Chikungunya fever is viral disease, caused by an arbovirus and transmitted by *Aedes* mosquito. Chikungunya virus (CHIKV) is a single-stranded RNA Alphavirus from the family *Togaviridae*. It is a debilitating but non-fatal viral illness. Chikungunya usually starts with sudden fever, chills, headache, nausea, vomiting, joint pain, rash and arthralgia. The arthralgia is predominantly affects the small joints of the hands and feet, ankles, wrist and elbow joints with lesser involvement of the larger joints. Swelling of the joints may be noticed one or two days after the onset of pain and may persist for two to few weeks. Stiffness and pain on movement is worse in the morning. In few cases the arthralgia may persist for years especially in the elderly, although joint deformities are not noted.

The word Chikungunya is derived from east African dialect - Swahili, means which contorts or bends up. This refers to the contorted (or stooped) posture of patients who are afflicted with the severe joint pain (arthritis) which is the most common feature of the disease.

It is spread by the bite of *Aedes* mosquitoes, primarily *Aedes (Ae.) aegypti*. Humans are the major source, or reservoir of Chikungunya virus for mosquitoes. The infected person cannot spread the infection directly to other person (i.e. it is not contagious disease). The Chikungunya virus is transmitted from human to human by the bites of infected female mosquitoes. Most commonly, the mosquitoes involved are *Ae. aegypti* and *Ae. albopictus*, two species which also transmit dengue. These mosquitoes bite throughout daylight hours, although peaks of activity are in the early morning and late afternoon. The *Ae. aegypti* mosquitoes breed in a wide

variety of manmade containers which are common around human dwellings, whereas *Ae. albopictus* prefer to breed in natural habitats.

The *Aedes* mosquitoes generally acquire the virus while feeding on the blood of an infected person. After Extrinsic Incubation Period for 8 to 10 days, the mosquito becomes infected. The infected mosquitoes while probing for blood feeding pass on the virus alongwith saliva to a healthy person. A mosquito once infected is capable of transmitting the virus for the rest of its life. Transovarial transmission is not documented in case of Chikungunya virus.

The Intrinsic Incubation Period of Chikungunya virus is 5-7 days. The virus circulates in the blood of infected humans for several days, at approximately the same time that they have Chikungunya fever. *Aedes* mosquitoes may acquire the virus when they feed on an individual during this period. Chikungunya cases start appearing in post-monsoon period i.e. May onwards with a peak between the months of July and August, as during this period vector density is very high.

Chikungunya virus likely originated in Central/East Africa, where the virus has been found to circulate in a sylvatic cycle between forest-dwelling mosquitoes and nonhuman primates. In these areas, sporadic human cases occur, but large human outbreaks were not common. However, in urban centers of Africa as well as throughout Asia, the virus can circulate between mosquitoes and naive human hosts in a cycle similar to that of dengue viruses.

In India a major epidemic of Chikungunya fever was reported during the last millennium (during 60s & 70s). After quiescence of three decades in 2006, Chikungunya outbreak occurred again in India. Total 1.39 million clinically suspected cases have been reported by 16 states/UTs out of 35 in the country. As on date, Chikungunya cases were reported from 28 States. Both the urban and rural areas were affected. All age groups were affected and no sex differentiation

reported from any of the states. Multiple cases in a family were observed and clustering of cases was common in majority of affected areas. The areas reporting Chikungunya was mostly overlapping with Dengue affected areas.

The clinico-epidemiological features during 2006 outbreak was that the onset of disease with moderate to high grade fever, chills associated with moderate to severe arthralgia and rash. The rash was most intense on trunk and limbs. Residual arthritis, swelling and pain on movement were reported by maximum patients, which persisted from few weeks to several months after recovery.

Fatalities related to Chikungunya virus are rare. Chikungunya virus infection (whether clinically apparent or silent) is thought to confer life-long immunity (herd immunity). Chikungunya fever displays interesting epidemiological profiles: major epidemics appear and disappear cyclically, usually with an inter-epidemic period of 7-8 years and sometimes as long as 20 years. A distinctive feature of Chikungunya virus is that it causes explosive outbreaks, before apparently disappearing for a period of several years to decades.

Japanese encephalitis (JE) is a disease of major public health importance in India because of its epidemic potential, high case fatality rate and presence of life-long complications in survivors. Japanese encephalitis virus belonging to the mostly vector-borne Flaviviridae family. Japanese It is a single-stranded RNA virus that belongs to the genus *Flavivirus* and is closely related to West Nile and Saint Louis encephalitis viruses. In areas at risk, Japanese encephalitis is primarily a disease of children, but it can occur in a person of any age. JE virus is transmitted to humans through the bite of infected *Culex vishnui* group of mosquitoes, particularly *Culex tritaeniorhynchus*.

The main reservoirs of the JE virus are pigs and water birds, and in its natural cycle, virus is maintained in these animals. Man is an accidental host and does not play a role in JE transmission. About 20-30% cases die, and amongst survivors few develop physical and mental disabilities.

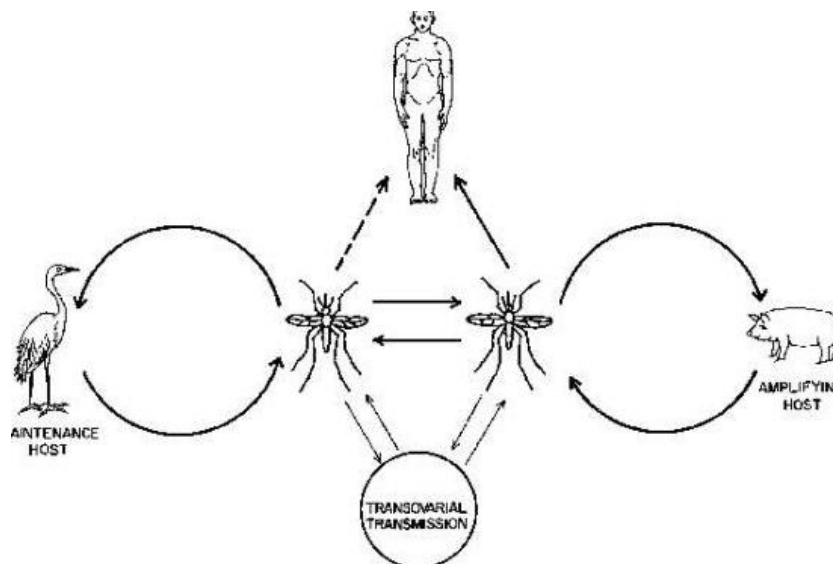
The first case of Japanese Encephalitis (JE) was reported in India in 1955 from Vellore, Tamilnadu. The first major outbreak was reported in 1973 from Burdwan district of West Bengal. Since then, JE has been reported from 21 states in the country till date. JE was reported for the first time in UP in 1978 when crop pattern in Eastern UP changed from sugarcane to paddy cultivation. In temperate areas of Asia, JE virus transmission is seasonal. Human disease usually peaks in the summer and fall. In the subtropics and tropics, transmission can occur year-round, often with a peak during the rainy season.

India is the world's second largest producer of rice after China. Of the 414 rice growing districts of India, more than 50% are rain fed and vast area of land under paddy cultivation contributes significantly in the rapid building up of vector population. Besides, promotion of piggeries through Integrated Rural Development Schemes (IRDS) has accelerated the spread of the disease in various parts of the country.

Man is an accidental host and during specific ecological conditions the man get the infection through the bite of an infective mosquito belonging to Culex vishnui group. In human beings, the virus circulates for a short period and moves to central nervous system mainly affect the brain resulting the inflammation of brain tissues, hence the name encephalitis. Infection in man is the dead end of transmission.

Mosquitoes are zoophilic (feed on animals – vertebrate hosts) – female mosquitoes get infected after feeding on viremia hosts. After 9–12 days, incubation period – mosquitoes transmit disease to other vertebrate hosts. The average life of Culex mosquito is 21 days. Culex can fly over long distances of 4–5 kms. Epidemics occur during monsoon and post monsoon period because the vector density is high. However, in endemic areas, sporadic cases may occur throughout the year. These mosquitoes breed profusely in a variety of ground clean oxygenated waters having luxuriant growth of water hyacinth and other emerging and free floating vegetarian in paddy fields, irrigation channels, shallow ditches, pools, etc. Culex vishnui group of mosquitoes are mainly zoophilic but may feed on human blood at high vector density level.

Vectors of JE generally start their biting activity at dusk and continue to be active till the early hours of the morning. JE is primarily a disease of rural areas however; epidemics have also been reported in peri-urban areas where similar conditions exist.



Outbreaks of JE usually coincide with the monsoon and post monsoon months when the vector density is high. However, in endemic areas, supporting the breeding of vector species and availability of reservoir hosts, sporadic cases may occur round the year.

17

Kala Azar (Transmission)

Visceral Leishmaniasis or Kala-azar is transmitted by the bite of infected sandflies. *Phlebotomus argentipes* is the only known vector of Kala-azar in India. The seasonal prevalence of this species varies from area to area depending upon the ecological conditions. Disease transmission is highest in the rainy season. The vector breeds in humid soil rich in organic matter and near cattle sheds and mud-houses. It rests most commonly in cracks & crevices of thatched mud-houses. The peak biting time of the vector is around midnight.



Adult Sand Flies



Sand flies feeding on the host



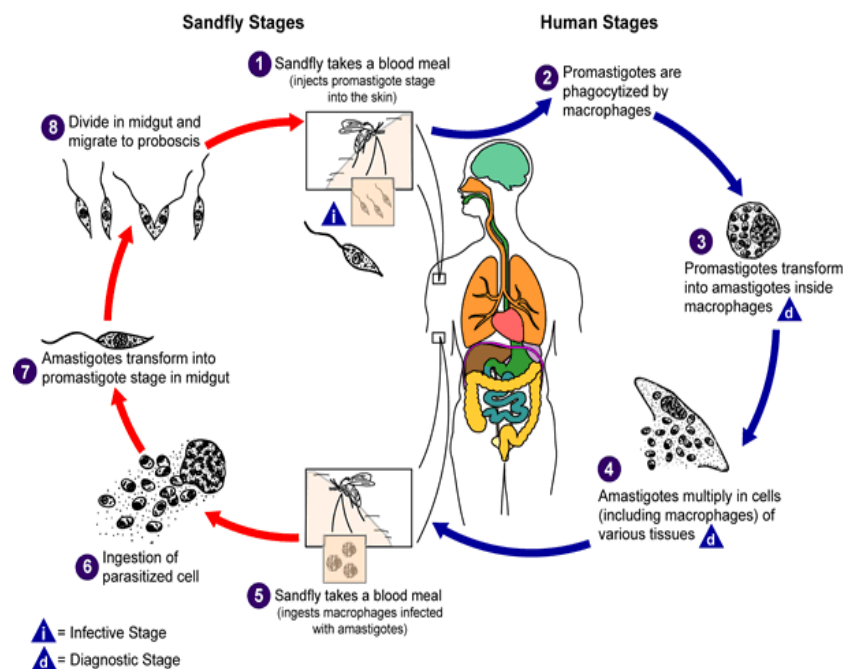
Kala-azar in India has a unique epidemiological feature of being anthroponotic, i.e. human beings are the only known reservoirs of infection. The female sandflies pick up the amastigote stage (LD bodies) of the parasite while feeding on an infected human host. The parasites undergo development and multiplication in the gut of sand flies to become numerous flagellates (Promastigote or Leptomonad stage) which migrate to their mouthparts. The cycle in the sand flies is completed in about 8 days. Infection is transmitted to healthy human beings when such infective sand flies bite them.

- **Incubation period:** Kala-azar being a chronic disease, incubation period significantly varies. Generally it varies from 1-4 months but in reality the range

is from 10 days to 2 years, however in India the range varies from 4 months to 1 year.

- **Extrinsic incubation period:** The extrinsic incubation period in the vector sand flies vary from 4-25 days which is the time required for the vector to become infective after an infective blood meal.
- **Age & Sex:** Kala-azar is reported amongst all age groups, however in earlier surveys carried out for the purpose of research children in the age group of 5-9 were most afflicted with male to female ratio of 2:1.
- **Environmental and Ecological factors:** The prevalence of vector is dependent upon the environmental factors which include humidity, temperature and rainfall. The ecological factors like alluvial soil, kuccha mud-houses and large scale vegetation also influence build up of vector density.

The parasite primarily infects reticulo-endothelial system and may be found in abundance in bone marrow, spleen and liver. Post Kala-azar Dermal Leishmaniasis (PKDL) is a condition in which the *Leishmania donovani* invades cells of skin and develops lesions. This results in skin manifestations of PKDL. Some of the Kala-azar cases manifest PKDL after a few years of treatment. Recently it has been claimed that PKDL may appear without passing through visceral stage.



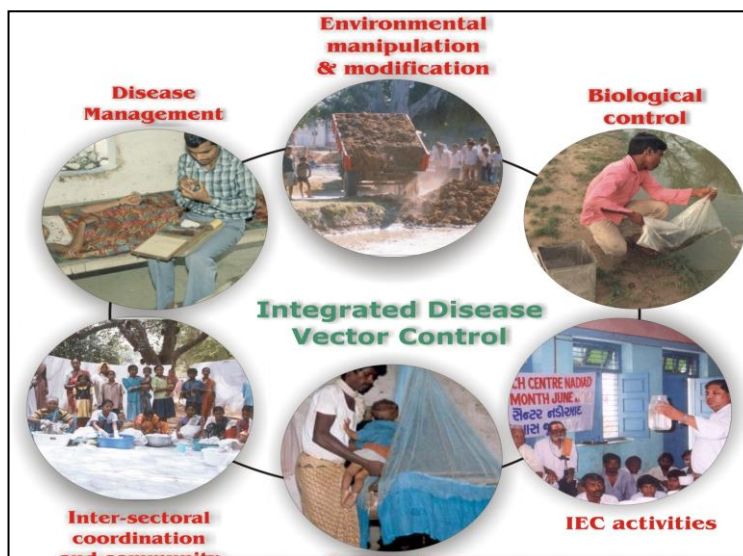
Source: CDC. Life cycle of Kala-azar

Integrated vector management (IVM) is a rational decision-making process to optimize the use of resources for vector control. It requires a management approach that improves the efficacy, cost effectiveness, ecological soundness and sustainability of vector control interventions with the available tools and resources. Integrated approach is vital in successfully combating vector-borne diseases. Various key elements of IVM are Advocacy, social mobilization, strengthening of regulatory and legislative controls for public health, empowerment of communities, collaboration within health and other sectors in planning and decision-making, use of available resources for vector control, implementation of evidence-based strategies and capacity-building. The basic concepts for IVM implementation are:

- Which mosquito species are locally important as vectors of human diseases?
- Which mosquito species are important as the primary source of annoyance?
- What are the important breeding sites of different mosquito species?
- What is the seasonal pattern of mosquito breeding?
- What are the resting places of adult mosquitoes?
- What are the feeding preferences of vector mosquitoes?
- To map out and locate all potential larval development habitats
- To identify the mosquito species present
- To predict the time and location of effective control strategies.

Though different integrals of IVM have been under implementation in isolation or in combination in different situation, there is a felt need that a comprehensive document describing IVM concept, its components and strategy for different diseases are compiled. The NVBDCP aims to achieve effective vector control by the

appropriate biological, chemical and environmental interventions of proven efficacy, separately or in combination as appropriate to the area through the optimal use of resources. Efforts are made for collaboration with various public and private agencies and community participation for vector control. Integration of IVM is done by using identical vector control methods to control vector borne diseases malaria, kala azar, Japanese encephalitis, dengue, chikungunya and Lymphatic filariasis.



STRATEGY: The IVM includes implementation of all feasible strategies safely with or without insecticides to manage vector population in such a way so that disease transmission is kept under check. It also includes management of insecticide resistance either by rotation within the same group or different group

TOOLS: There are many tools available and recommended for vector control. Some are used for personal protection and some are their combination are used as public health measures

SOURCE REDUCTION

1. BIOLOGICAL
2. CHEMICAL
3. ADULTICISDE
4. LARVICIDE

IVM will be implemented involving

- entomological surveillance in sentinel and random sites at monthly intervals;
- appropriate use of insecticides for supervised IRS with full support (including spray wages) from NVBDCP;
- scaling-up use of LLIN;
- treatment of community owned bed-nets;
- intensified anti-larval operations in urban and peri-urban areas within the states / districts;

scaling up use of larvivorous fish with exploring outsourcing to NGOs under PPP model; and

- promotion of source-reduction, minor engineering etc. by involvement of panchayati raj institutions at village level.

Supportive interventions including IEC and BCC activities through village health and sanitation committee meetings on monthly basis, inter-sectoral collaboration meetings in district and blocks with API more than 1 and involvement of other sectors for social mobilization towards prevention and control with coordinated efforts of district programme managers. Training, Monitoring and supervision for the activities will be undertaken as well as monitoring towards timely performance of the activities.

Environmental management involves any change that prevents or minimizes vector breeding and hence reduces human-vector contact. Environmental methods to control *Ae. aegypti* and *Ae. albopictus* and to reduce man-vector contact are source reduction, solid waste management, modification of man- made breeding sites, and improved house design. The major environmental management methods used for the control of the immature stages of dengue vectors are summarized as below.

- ***Improved water supply:*** Whenever piped water supply is inadequate and available only at restricted hours or at low pressure, the storage of water in varied types of containers is encouraged, thus leading to increased *Aedes* breeding. The majority of such containers are large and heavy (e.g storage

jars) and can neither be easily disposed of nor cleaned. In rural areas, unpolluted, disused wells become breeding grounds for *Ae. aegypti*. It is essential that potable water supplies be delivered in sufficient quantity, quality and consistency to reduce the necessity and use of water storage containers that serve as the most productive larval habitats.

- ***Mosquito-proofing of overhead tanks/ cisterns or underground reservoirs:*** Where *Ae. aegypti* larval habitats include overhead tanks/cisterns and masonry chambers of piped waterlines, these structures should be mosquito-proofed⁽⁴²⁾. Similarly, mosquito-proofing of domestic wells and undergroundwater storage tanks should be undertaken.
- ***Flower pots/vases and ant traps:*** Flower pots, flower vases and ant traps are common sources of *Ae. aegypti* breeding. They should be punctured to produce a drain hole. Alternatively, live flowers can be placed in a mixture of sand and water. Flowers should be removed and discarded weekly and vases scrubbed and cleaned before reuse. Brass flower pots, which make poor larval habitats, can be used in cemeteries in place of traditional glass containers. Ant traps to protect food storage cabinets can be treated with common salt or oil.

Aedes breeding in incidental water collections

Desert (evaporation) water coolers, condensation collection pans under refrigerators, and air conditioners should be regularly inspected, drained and cleaned. Desert water coolers generally employed in arid/semi-arid regions⁽⁴⁴⁾ of South-East Asia to cool houses during summer contain two manufacturing defects. These are as follows:

The exit pipe at the bottom of the water-holding tray is generally fixed a few centimetres above the bottom. This exit pipe should be fitted at such a level that

while emptying the tray, all the water should get drained off without any retention at the bottom.

Desert coolers are normally fitted to windows with the exit pipe located on the exterior portion of the tray. These sites are usually difficult to access, and, therefore, there is a need to change the design so that both the filling and emptying of the water-holding trays can be manipulated from the room, thus eliminating the need of climbing to approach the exit pipe at the exterior of the building.

It is recommended that each country should develop regulatory mechanisms to ensure the design specifications as outlined above for manufacturing desert coolers.

Building exteriors

The design of buildings is important to prevent *Aedes* breeding. Drainage pipes of rooftops sunshades/porticos often get blocked and become breeding sites for *Aedes* mosquitoes. There is a need for periodic inspection of buildings during the rainy season to locate potential breeding sites.

- ***Mandatory water storage for fire fighting: Fire prevention regulations*** may require mandatory water storage. Such storage tanks need to be kept mosquito-proofed. In some municipalities in India⁽⁴⁵⁾, timber merchants are required to maintain two metal drums (50 gallons) full of water for fire fighting. These drums should be kept covered with tight lids. Also, metal drums used for water storage at construction sites should be mosquito-proofed.
- ***Solid waste disposal:*** Solid wastes, namely tins, bottles, buckets or any other waste material scattered around houses, should be removed and buried in land fills. Scrap material in factories and warehouses should be stored appropriately until disposal. Household and garden utensils (buckets, bowls and watering devices) should be turned upside down to prevent the

accumulation of rain water. Similarly, canoes and small boats should be emptied of water and turned upside down when not in use. Plant waste (coconut shells, cocoa husks) should be disposed of properly and without delay.

- **Tyre management:** Used automobile tyres are of major importance as breeding sites for urban *Aedes*, and are therefore a significant public health problem. Imported used tyres are believed responsible for the introduction of *Ae. albopictus* into the United States, Europe and Africa⁽⁴⁶⁾. Tyre depots should always be kept under cover to prevent the collection of rain water.
- **Filling of cavities of fences:** Fences and fence posts made from hollow trees such as bamboo should be cut down to the node, and concrete blocks should be filled with packed sand, crushed glass, or concrete to eliminate potential *Aedes* larval habitats.

Personal Protection

Protective clothing: Clothing reduces the risk of mosquito biting if the cloth is sufficiently thick or loosely fitting. Long sleeves and trousers with stockings may protect the arms and legs, the preferred sites for mosquito bites. Schoolchildren should adhere to these practices whenever possible. Impregnating clothing with chemicals such as permethrin can be especially effective in preventing mosquito bites.

Mats, coils and aerosols

Household insecticidal products, namely mosquito coils, pyrethrum space spray and aerosols have been used extensively for personal protection against mosquitoes. Electric vaporizer mats and liquid vaporizers are more recent additions which are marketed in practically all urban areas.

Repellents

Repellents are a common means of personal protection against mosquitoes and other biting insects. These are broadly classified into two categories, natural repellents and chemical repellents. Essential oils from plant extracts are the main natural repellent ingredients, i.e. citronella oil, lemongrass oil and neem oil. Chemical repellents such as DEET (N, N-Diethyl-m-Toluamide) can provide protection against *Ae. albopictus*, *Ae. aegypti* and anopheline species for several hours. Permethrin can be used as effective repellent when impregnated in cloth

Insecticide-treated mosquito nets and curtains

Insecticide-treated mosquito nets (ITMN)/LLINs have limited utility in dengue control programmes, since the vector species bites during the day. However, treated nets can be effectively utilized to protect infants and night workers who sleep by day. They can also be effective for people who generally have an afternoon sleep. LLIN has two advantages over traditional nets in that the wide mesh permits better ventilation and light, and the treated thread enables a slow release of insecticides (Synthetic Pyrethroids) to the fibre surface, ensuring a long residual effect (over a year).

Biological Control

The application of biological control agents which are directed against the larval stages of dengue vectors in South-East Asia has been somewhat restricted to small-scale field operations.

Fish : Larvivorous fish (*Gambusia affinis* and *Poecilia reticulata*) have been extensively used for the control of *An. stephensi* and/or *Ae. aegypti* in large water bodies or large water containers in many countries in South-East Asia. The applicability and efficiency of this control measure depend on the type of containers.

Bacteria: Two species of endotoxin-producing bacteria, *Bacillus thuringiensis* serotype H-14 (*Bt.H-14*) and *Bacillus sphaericus* (*Bs*) are effective mosquito control agents. They do not affect non-target species. *Bt.H-14* has been found to be most effective against *An. stephensi* and *Ae. aegypti*, while *Bs* is the most effective against *Culex quinquefasciatus* which breeds in polluted waters. There is a whole range of formulated *Bti* products produced by several major companies for control of vector mosquitoes. Such products include wettable powders and various slow-release formulations including briquettes, tablets and pellets. Further developments are expected in slow-release formulations. *Bt.H-14* has an extremely low-level mammalian toxicity and has been accepted for the control of mosquitoes in containers storing water for household use.

Chemical Control

Chemicals have been used to control *Ae. aegypti* since the turn of the century. In the first campaigns against the yellow fever vector in Cuba and Panama, in conjunction with widespread clean-up campaigns, *Aedes* larval habitats were treated with oil and houses were fumigated with pyrethrins. When the insecticidal properties of DDT were discovered in the 1940s, this compound became a principal method of *Ae. aegypti* eradication programmes in the Americas. When resistance to DDT emerged in the early 1960s, organophosphate insecticides, including fenthion, malathion and fenitrothion were used for *Ae. aegypti* adult control and temephos as a larvicide. Current methods for applying insecticides include larvicide application and space spraying⁽⁵¹⁾.

Chemical larviciding

Larviciding or “focal” control of *Ae. aegypti* is usually limited to domestic-use containers that cannot be destroyed, eliminated, or otherwise managed. It is

difficult and expensive to apply chemical larvicides on a long-term basis. Therefore chemical larvicides are best used in situations where the disease and vector surveillance indicate the existence of certain periods of high risk and in localities where outbreaks might occur. Establishing the precise timing and location are essential for maximum effectiveness. Control personnel distributing the larvicide should always encourage house occupants to control larvae by environmental sanitation. There are three insecticides that can be used for treating containers that hold drinking water.

Temephos 1% sand granules

One per cent temephos sand granules are applied to containers using a calibrated plastic spoon to administer a dosage of 1 ppm. This dosage has been found to be effective for 8-12 weeks, especially in porous earthen jars, under normal water use patterns. The quantity of sand granules required to treat various size water containers is shown in Annex VIII. Although resistance to temephos in *Ae. aegypti* and *Ae. albopictus* populations has not been reported from the South-East Asia Region, the susceptibility level of *Aedes* mosquitoes should be monitored regularly in order to ensure the effective use of the insecticide.

Insect growth regulators

Insect growth regulators (IGRs) interfere with the development of the immature stages of the mosquito by interference of chitin synthesis during the molting process in larvae or disruption of pupal and adult transformation processes. Most IGRs have extremely low mammalian toxicity (LD50 value of acute oral toxicity for methoprene (Altosid) is 34 600 mg/kg). In general, IGRs may provide long-term residual effects (three to six months) at relatively low dosages when used in porous earthen jars. Because IGRs do not cause immediate mortality of the immature mosquitoes, countries with legislation stipulating that the breeding

of *Aedes* larvae is an offense, will require some alteration of the law, so as not to penalize home owners who use these compounds.

Bacillus thuringiensis H-14 (Bt.H-14)

Bt.H-14, which is commercially available under a number of trade names, is a proven, environmentally-nonintrusive mosquito larvicide. It is entirely safe for humans when the larvicide is used in drinking water in normal dosages⁽⁵²⁾. Slow-release formulations of *Bt.H-14* are being developed. Briquette formulations that appear to have greater residual activity are commercially available and can be used with confidence in drinking water. The use of *Bt.H-14* is described in the section on biological control. The large parabasal body that forms in this agent contains a toxin that degranulates solely in the alkaline environment of the mosquito midgut. The advantage of *Bt. H-14* is that an application destroys larval mosquitoes but spares any entomophagus predators and other non-target species that may be present. *Bt.H-14* formulations tend to rapidly settle at the bottom of water containers, and frequent applications are therefore required. The toxin is also photolabile and is destroyed by sunlight.

Space sprays

Space spraying involves the application of small droplets of insecticide into the air in an attempt to kill adult mosquitoes. It has been the principal method of DF/DHF control used by most countries in the Region for 25 years. Unfortunately, it has not been effective, as illustrated by the dramatic increase in DHF incidence in these countries during the same period of time . Recent studies have demonstrated that the method has little effect on the mosquito population, and thus on dengue transmission ^(53,54,55). Moreover, when space spraying is conducted in a community, it creates a false sense of security among residents, which has a detrimental effect on community-based source reduction programmes. From a political point of view, however, it is a desirable approach because it is highly visible and conveys the

message that the government is doing something about the disease. This, however, is poor justification for using space sprays. The current recommendations are that space spraying of insecticides (fogging) should not be used except in the most extreme conditions during a major DHF epidemic. However, the operations should be carried out at the right time, at the right place, and according to the prescribed instructions with maximum coverage, so that the fog penetration effect is complete enough to achieve the desired results.

When space sprays are employed, it is important to follow the instructions on both the application equipment and the insecticide label and to make sure the application equipment is well maintained and properly calibrated. Droplets that are too small tend to drift beyond the target area, while large droplets fall out rapidly. Nozzles for ultra-low volume ground equipment should be capable of producing droplets in the 5 to 27 micron range and the mass median diameter should not exceed the droplet size recommended by the manufacturer. Desirable spray characteristics include a sufficient period of suspension in the air with suitable drift and penetration into target areas with the ultimate aim of impacting adult mosquitoes. Generally, there are two forms of space-spray that have been used for *Ae. aegypti* control, namely “thermal fogs” and “cold fogs”. Both can be dispensed by vehicle-mounted or hand-operated machines.

Thermal fogs

Thermal fogs containing insecticides are normally produced when a suitable formulation condenses after being vaporized at a high temperature. Generally, a thermal fogging machine employs the resonant pulse principle to generate hot gas (over 200°C) at high velocity. These gases atomize the insecticide formulation instantly so that it is vaporized and condensed rapidly with only negligible formulation breakdown. Thermal fogging formulations can be oil-based or water -

based . The oil(diesel) - based formulations produce dense clouds of white smoke, whereas water-based formulations produce a colorless 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.

Ultra-low volume (ULV), aerosols (cold fogs) and mists

ULV 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, droplet size is important and the equipment used should be capable of producing droplets in the 10 to 15 micron range, although the effectiveness changes little when the droplet size range is extended to 5-25 microns. The droplet size should be monitored by exposure on teflon or silocone-coated slides and examined under a microscope. Aerosols, mists and fogs may be applied by portable machines, vehicle-mounted generators or aircraft equipment.

House-to-house application using portable equipment

Portable spray units can be used when the area to be treated is not very large or in areas where vehicle-mounted equipment cannot be used effectively. This equipment is meant for restricted outdoor use and for enclosed spaces (buildings) of not less than 14m³. Portable application can be made in congested low-income housing areas, multistoried buildings, godowns and warehouses, covered drains, sewer tanks and residential or commercial premises. Operators can treat an average of 80 houses per day, but the weight of the machine and the vibrations caused by the engine make it necessary to allow the operators to rest, so that two or three operators are required per machine.

Vehicle-mounted fogging

Vehicle-mounted aerosol generators can be used in urban or suburban areas with a good road system. One machine can cover up to 1500-2000 houses (or approximately 80 ha) per day. It is necessary to calibrate the equipment, vehicle speed, and swath width (60-90m) to determine the coverage obtained by a single pass. A good map of the area showing all roads is of great help in undertaking the application. An educational effort may be required to persuade the residents to cooperate by opening doors and windows.

The speed of the vehicle and the time of day of application are important factors to consider when insecticides are applied by ground vehicles. The vehicle should not travel faster than 16 kph (10 mph). When the wind speed is greater than 16 kph or when the ambient air temperature is greater than 28°C, the insecticide should not be applied. The best time for application is in the early morning (approximately 0600-0830 hours) or late afternoon (approximately 1700-1930 hours).

Performance of fogging machines

Estimates have been made of the average coverage per day with certain aerosol and thermal fog procedures .

Insecticide formulations for space sprays

Organophosphate insecticides, such as malathion, fenitrothion and pirimiphos methyl have been used for the control of adult *Aedes* vectors. Undiluted technical grade malathion (active ingredient 95%+) or one part technical grade diluted with 24 parts of diesel have been used for ULV spraying and thermal fogging respectively. For undiluted technical grade ULV malathion applications from

vehicles, the dosage on an area basis is 0.5 liters per hectare.

Apart from the above-mentioned formulations, a number of companies produce pyrethroid formulations containing either permethrin, deltamethrin, lambda-cyhalothin or other compounds which can be used for space spray applications. It is important not to under-dose during operational conditions. Low dosages of pyrethroid insecticides are usually more effective indoors than outdoors.

Also, low dosages are usually more effective when applied with portable equipment (close to or inside houses) than with vehicle-mounted equipment, even if wind and climatic conditions are favourable for outdoor applications. Outdoor permethrin applications without a synergist should be applied at concentrations ranging from 0.5% to 1.0%, particularly in countries with limited resources and a lack of staff experienced in routine spraying operations.

Insecticidal Residual Spray (IRS)

Insecticidal Residual Spray is one of the most cost-effective control measures for Malaria and Kala-azar in India. To maximize the impact of IRS, it should be synchronized with case detection. The objective of IRS is to interrupt the transmission by reducing numbers of infective vectors. This can be achieved by ensuring safe and correct application of the insecticide to indoor surfaces of houses and animal shelters. For malaria only human dwelling and for kala azar both human dwelling and animal shelters are covered.

The success of IRS operations depends on the planning and implementation. IRS plans should be developed before end of the year so that there is no last minute rush during implementation.

IRS planning should be made, based on the capacity for achieving complete and uniform coverage. When there is resource constraints it is preferable to limit the size of the operation and achieve quality coverage.

IRS for Malaria:

IRS is at present carried out in high risk areas ($API \geq 2$) with coverage of about 80 million population. DDT is the insecticide of choice; in areas where the vector has shown resistance to DDT, the alternatives are malathion and synthetic pyrethroids. Two rounds of spraying are done for DDT and synthetic pyrethroids to provide protection during the entire transmission season; in the case of malathion, three rounds of spraying are required.

About 60% of the high risk areas targeted under IRS are under coverage with DDT. The real coverage by IRS is however limited by the low community acceptance due to the white marks left on plastered surfaces, acrid smell associated with malathion, re-plastering of wall after completion of IRS, etc.

As the programme intends to expand the use of LLINs in high risk areas targeted for vector control, it would not expand the use of IRS further. The focus would be on improving the quality of IRS with meticulous microplanning and intensive monitoring and supervision. With quality IRS, there is every chance that disease control would be possible in these areas in the coming 2-3 years and areas previously qualifying as high risk would shift to low risk. This would bring about a decline in the requirement of insecticides for spray in the following years. The first round of spray in an area is usually done to coincide with the time of build-up of vector populations which precede the malaria transmission season.

During the strategic plan period (2012-17), IRS coverage will be targeted primarily at achieving a minimum of 80% coverage of IRS eligible population living in high

endemic areas. These are the areas not targeted for community-wide coverage with LLINs or conventional ITNs. It is possible that as LLINs are scaled up, the IRS eligible population will become smaller, but the rate at which this will happen cannot be determined in advance.

Surveillance on insecticide resistance will form a critical component for taking decision on the choice of insecticide to be used. Therefore, the surveillance of resistance by NIMR and Zonal entomologists will be strengthened.

DDT will continue to be used but efforts will be made to progressively scale down its use. Research for alternative insecticides will be intensified in adherence to Stockholm Convention. The state health services will be responsible for safe disposal of DDT and other insecticides. Environment management plan will be implemented to minimize the damage to the environment due to insecticides. The prioritization made in India for implementation is as below:

For areas having API less than 1

- Vector control- By minor engineering processes like desilting, deweeding and cleaning of canals and irrigation channels, biological control, by use of larvicides and environmental management
- Involvement of PRIs in rural areas and municipal bodies in urban areas by sensitizing them
- Cooperation from VHSCs and nodal officers for MNREGA

For areas having API between 1-2

- Vector control by source reduction and biological control

For areas having API between 2-5

- (b) Vector control by distribution of LLIN if acceptability of IRS is low.
- (c) For areas which can be supervised and accessible –Quality IRS for selective vector control based on epidemiological impact of earlier vector control measures, if needed; these areas can also be provided with LLINs

For areas having API above 5

For areas having perennial transmission (more than 5 months in a year)

- (c) 2 rounds of IRS with DDT or 3 rounds with Malathion
- (d) Priority distribution of LLINs as per the guidelines
- (e) Vector bionomics studies for future change of strategy

For areas having seasonal transmission (less than 5 months in a year)

- 1 round of IRS with DDT before start of transmission
- Focal spray whenever and wherever needed
- Priority distribution of LLINs as per the guidelines

IRS for Kala azar: The entire village needs to be covered if selected for IRS. Once micro planning is well developed for endemic areas, efforts should be made to utilize Geographic Information System (GIS) and Remote Sensing (RS) for improving the plan. Following criteria are applied while selecting areas for IRS:

- All villages within a Block PHC which reported Kala-azar cases in the past three years;
- New villages which reported cases during year of spray;
- Villages free of Kala-azar, but on search were found to have cases conforming to the case definition.

It is important that while making micro action plan for IRS by district add 10% enhanced budget in action plan to cover any new village(s) reported KA case during the spray round.

Two rounds of IRS with DDT 50 % at a dose of 1gm/sq meter are carried out in a year. Spraying should be started before onset of Kala-azar transmission season which coincides with time of build-up of vector populations. The build-up in vector population starts in March and peak Kala-azar transmission season is from June to October. The effectiveness of DDT lasts for about 10 weeks. Therefore, two rounds of DDT are done, the first in February - March and the second in May - June, to control the vector population and for providing protection during entire transmission season. This schedule may be modified in consultation with meteorological department based on local weather conditions. As it is difficult to conduct spray operations during monsoon, it may sometimes be necessary to delay the 2nd round till the monsoon subsides.

For Kala-azar elimination, the insecticidal spray is done up to a height of 6 feet only as the sand fly vector cannot hop above this height. Cattle sheds are also to be covered for interrupting transmission of Kala-azar. The varanda and areas with full sun light should be avoided for spray. Kala-azar vector prefers dark humid areas, ill ventilated rooms for resting. Therefore, special attention with good quality spray needs to be undertaken to cover these dark humid areas. The average requirement of DDT is 150 grams per house in the rural areas and the average

surface area for spray per house is 75 square meters. For one million population the requirement of DDT is 75 metric ton (MT) for both the round ie 37.5 MT for each round of spray.

Stirrup pumps are at present used mostly for IRS in India. Hand compression pumps can also be used after proper training. Hand compression pumps have advantage over stirrup pumps as in stirrup pumps, two workers are required to cover area with DDT where as in the case of compression pump only one person required to cover the area. State can choose any other pumps at their discretion.

The requirement of equipment per squad is as follows:

- Stirrup pumps (3) OR Hand Compression pumps (2)
- Spray nozzle tips for spray pumps (2)
- Bucket 10 liters (4)
- Asbestos thread (3 meters)
- Measuring mug (1)
- Straining cloth (1 meter)
- Pump washers (2)
- Plastic sheet 3X3 meters (1)
- Register for records (1)
- Writing material to identify households covered by IRS
- Tools for minor repairs
- Personal protection equipment (PPE) for each member of the squad including a pair of gloves

Use of Synthetic Pyrethroid (SP)

Though *P. argentipes* is still susceptible to DDT spray in majority of the areas, however, RMRI, NCDC and CARE India have reported resistance of the vector to DDT spray in districts Vaishali, Muzaffarpur, Sitamadhi, Samastipur and Patna. In

view of this and considering high endemicity and insecticide (SP) availability, seven high endemic districts in Bihar were identified to be brought under IRS with Alphacypermethrin 5% during 2015. It has been proposed to extend Synthetic Pyrethroid spray in 21 districts (Bihar-15, Jharkhand-4 and West Bengal-2) during 2016.

Manpower requirements

The spray operations of one round should be completed in 45 – 60 days. This requires thorough planning and proper deployment of staff. Prolonging the duration of 1st round will create problems in carrying out the 2nd round in time, particularly with monsoon period closely following the originally planned schedule. The spray squads should be supervised well to ensure quality (correct dose, uniformity and completeness of application) of the IRS. The training of spray personnel should also be good. The supervisor of the spray teams should be a regular staff member of spray squad team. A spray squad comprises 5 field workers and one superior field worker. The number of houses to be sprayed is determined by terrain in which the team is operating. The population to be covered should be divided by 5 since each household has an average of about 5 members. Calculate the number of spray teams that would be required in each district based on the number of houses to be sprayed. Each spray squad should be supervised by a suitably trained health worker/supervisor. This individual is different from the spray squad supervisor. The task of supervision of the spray squads should not be assigned to health workers who are expected to provide general health services. This is because the quality of services will suffer if the health workers are taken away from their work to supervise IRS. One supervisor would be required to be responsible for adequacy of work of no more than 5 teams.

Training of spraying squads and supervisors

The training of IRS team comprises of training of the health workers who are responsible for supervising the spray operations, and training of the spray teams. District focal point for Kala-azar is responsible for organizing the training. The training components are:

- Importance of uniform and complete spraying
- Obtaining cooperation from the community
- Safe storage of the insecticide
- Preparation of insecticide suspension
- Correct use of the equipment
- Maintenance of the equipment
- Safety precautions and personal protection measures to be observed during the spraying operations
- Safe disposal of insecticide waste.

The training should be at least 3 days duration and include hands on training on the correct use of spray equipment and the observance of all the steps needed from the preparation of the suspension to safe disposal of left over insecticide suspension.

The training of the supervisors should include community involvement for ensuring community acceptance and participation, which in turn is expected to achieve the completeness of coverage in all the targeted dwellings and the cattle sheds. The training of the supervisors should also include supportive supervision, which includes the use of a standard checklist and problem solving. The person responsible for solving the day to day problems of spray men is the spray squad supervisor. Each district proposed to be covered by IRS should develop a training plan. The training should be completed one week before the first spraying operations. Avoid a long interval between the spray operations and the training. It

should be an integral part of the district work plan for elimination of Kala-azar. Each district should prepare a report on training of the supervisors and the spraying teams in the following format.

Report on training for IRS- District				
	Total number	Training courses (No.)	Dates	Venue
Supervisors and malaria workers				
Spray team members				
Spray supervisors				

Spray Programme

The district plan should include a plan for IRS developed on the basis of endemic areas identified for spraying. The plan should include identification of dates when the selected villages are proposed to be sprayed. Each supervisor should then develop a plan for each spray team. This plan should be used to calculate the requirement of the insecticide, which should be supplied and safely stored at least one week before the spray. A prototype framework is summarized below.

PHC*	Sub centers	Village (for sub center 1)	Date of spray	Spray team members
Name 1	Name 1	Village 1		
	Name 2	Village 2		
	Name 3	Village 3		

Spray operations

Spray operations comprises of estimation of needs for each squad, correct use of spraying technique, full coverage of all the targeted households, proper maintenance of the spraying equipment and preparing daily reports with stock checking. DDT for spraying is provided in sacks by the national programme. The estimated amounts of insecticide based on the requirements should be given to each spray squad. This should be distributed to the spray team and the team supervisor should be asked to maintain an account. Based on the plan for IRS, a village wise programme should be developed by the supervisors with indication of the number of households to be covered in each village. A copy of the spray schedule should be kept by the supervisor. This would facilitate the supervision of the operations.

Spraying Technique

It is extremely important that the technique of preparation of the suspension and of spraying meets the recommended procedures.

Preparation and Spraying Methods

The suspension of DDT for spraying or other insecticide should be prepared when the team is ready to start the spraying. The suspension should be prepared correctly so that sufficient quantities of the insecticide are sprayed to be effective. Prepare 10 liters of the suspension at a time. This is enough for covering 500 meters square surface area. Therefore this will be sufficient for 6-8 households for Kala-azar.

The following steps need to be taken;

- Place the required quantities of DDT wettable powder or other insecticide in a 15-liter bucket
- Add volume of water with a mug that is considered adequate to make a paste of DDT or other insecticide as appropriate.

- Do not put too much water at this stage.
- Once the paste is made then pour water on the paste and keep mixing vigorously to make a uniform suspension and add measured volume of water i.e. 10 liters.
- After this procedure filter the solution through a clean cloth to remove any particulate matter. The barrel of the spray pump is placed in the bucket containing the spray suspension.
- One person operates the pump and the other is responsible for the spray. If a compression pump is used it can be operated by one person.
- The spray lance should be kept 45 cms away from the surface to be sprayed. The swath should be parallel.
- It is applied in a vertical swath about 53 cms wide.
- There should be an overlap of about 7.5 cms between two swaths.
- Spraying should be done from the top downwards.
- The top should be about 6 feet from the ground. The spray should not drip on to the floor.
- The discharge rate should be 740-850 ml per minute.
- The person who is responsible for pumping the material should give 20-26 strokes per minute with 10-15cms plunger movement at a pressure of 10 pounds per square inch.
- Spraying into a bucket for one minute and measuring the discharge rate per minute helps to ensure that the discharge rate is satisfactory.

If the discharge rate exceeds 850 ml minute then the nozzle should be rejected. A blockage in the nozzle is a frequent problem. The nozzle cap should be removed by unscrewing it and replaced by a new nozzle. The blocked nozzle should be kept

immersed in water for a few hours and then cleaned off blockage. Do not use fine wire etc to remove blockage as this will widen the hole size of nozzle and the discharge rate will more. The unused insecticide should be disposed off safely as per the guidance of ECoP (environmental code of Practices) developed by NVBDCP. The buckets that were used should be cleaned properly ensuring safe disposal of the waste to ensure that it does not contaminate the environment. The deposits on the wall should be uniform and no areas should be skipped. This is an indication of good spray. The supervisor should check the quality of the spray. This is easy to do in the case of DDT since DDT leaves white deposits after the spraying has been done. It takes about 3 minutes to spray about 150 sq meters area. This is the average size of a dwelling in rural areas of India. The size of the house to be sprayed can vary from country to country. There are always some households that are not covered in the first round. These should be covered under subsequent mopping up round on the same day or on a pre-decided different day.

Routine maintenance of the equipment

The spray equipment is subject to normal wear and tear since the insecticides are corrosive. To reduce the deterioration the following actions should be undertaken at the end of each day:

- The discharge line should be disconnected at the delivery outlet at the end of spraying.
- The bucket and the discharge line should be emptied.
- The spray pump should be thoroughly rinsed with clean water
- The filter assembly should be rinsed and cleaned. Filter should be removed from the valve by grasping it at its screen and slightly twisted on pulling it out.
- Reassemble all the clean parts except the nozzle. Put clean water in the tank, seal the tank and pump air into it. Open the control valve and let the water

flow from the lance to flush the hose, filters, control valve and lance. Remove the tank cover and dry the inside of the tank.

- Clean the nozzle tip by washing thoroughly with water. Remove any dirt from the orifice with a fine bristle/a brush. Never use a wire or nails to clean the nozzle.

Minor Repair of the spraying equipment

Minor repairs can be done in the field. Some examples are as follows:

- Cleaning the nozzle
- Cleaning of the discharge line
- Tightening of the hose clamp
- Tightening of the gasket
- Tightening of the nut and compression of the cut off valve
- Replacement of the nozzle.

Instructions for the spray squad members

- A simple flyer should be provided to each member of the spray squad. This should be in simple local language with appropriate illustrations.
- Wash your hands thoroughly with soap and water after preparing the insecticide spray. This is to be repeated every time the spray operation is stopped. Washing of hands thoroughly with soap and water is advised when the team takes a lunch or tea break.
- The personal protection comprising of apron, gloves, mask and goggles should be worn during the insecticide spray.
- Avoid direct contact of the insecticides with eyes or skin. If this happens wash the skin coming in contact and adjacent skin thoroughly with soap and water. Eyes should be flushed repeatedly with clean water for a period of at least 5 minutes or 10 times to protect you against any harmful effects of the insecticides.

- If irritation persists even after thorough washing, seek medical advice.
- If any member of spray team suffers from any symptoms while spraying operations, medical attention should be sought without any delay.

Information about use of hand compression pump

A hand-compression sprayer consists of a tank for holding a liquid insecticide formulation, which can be pressurized by means of a hand pump attached to it. The compressed air forces the liquid from the tank via a hose with a cut-off valve, a lance and a nozzle. barrel of the sprayer should be capable of withstanding an internal pressure of 14 kg/cm² and for this purpose the metal walls should not be less than 0.63 mm thick. The diameter of the plunger shaft should not be less than 12 mm. The plunger bucket of the pump should be made from nitrile rubber or chrome-tanned leather. The plunger assembly should be easily removable for cleaning and repair in the field. The handle may be shaped D or T. The handle grip should be about 30 mm in diameter. Further, the length in the case of T-type handle should not be less than 20 cm.

Actions ensured by the operators/supervisors

- The compression sprayer is pressurized before commencing spraying, and not continuously pumped. The pump is filled to levels usually at about $\frac{3}{4}$ liquid to $\frac{1}{4}$ air. A smaller air volume in relation to liquid volume would not retain sufficient pressure for long periods.
- When the tank is not in use, the spray lance is held in a bracket and nozzle cup, which protects the nozzle from damage.
- The nozzle tip is the most important part of the sprayer. It should deliver a precise amount of spray suspension per minute (740-850 ml) at a certain pressure (40 PSI or 2.8 kg/cm²) in the tank, and maintain a uniform spray pattern and swath width (53 cm or 21”).

Intervention measures to restrict the transmission of VBDs by controlling the vector population form the main part of the vector control. Effective vector control strategies are based on the following facts.

- Knowledge and understanding of vector biology
- Surveillance of vector species
- Incrimination of vector species
- Public education and implementation of effective control measures.

Vector control programme in India, as in the case with many anti-malaria programme elsewhere, in the world, mostly rely on usage of natural and synthetic chemical molecules, which have potential to kill the target insects. Presently different formulations of synthetic chemical insecticides are in use for vector control. Wettable powder (WP) formulations are used for indoor residual sprays while emulsion concentrate (EC) formulations are used for larval control. For IRS, insecticides in use are DDT 50% WP, Malathion 25% WP and synthetic pyrethroids. Synthetic pyrethroids include Deltamethrin 2.5% WP, Cyfluthrin 10% WP, Lambdacyhalothrin 10% WP and Alphacypermethrin 5% WP. The synthetic pyrethroids are also used for impregnation of bednets. Most of the insecticides having residual effect are sprayed indoors, so that mosquitoes after taking the blood meal from an infective person will rest in the house, pick up sufficient insecticide particles sprayed on the walls and other indoor surfaces of the house and their longevity will be reduced so much that they do not survive long enough to become infective. In areas where the vectors are strongly endophilic, i.e. they

tend to rest indoor, IRS of human dwellings can give very effective control. Vectors that are exophilic i.e. they tend to rest outdoors but tend to feed or rest indoors briefly, can be effectively controlled by IRS with insecticides that have good airborne effect. In areas where vectors are strongly exophilic and/or exophagic, i.e. they rest and bite outdoors, other control methods, such as use of ITNs or outdoor space spraying (for emergency control), should be considered. In practice, the effectiveness of house spraying for malaria control depends on adherence to the specified criteria of the insecticide and application procedure, public acceptance of spraying, the availability of well maintained equipment and adequately trained spray personnel, depends on local circumstances and is influenced by the distribution of malaria and malaria vectors, distance from the active breeding sites, the flight range of the vectors and demographic features.

Selection of insecticides

Several factors need to be considered in the selection of an insecticide for spraying, including availability, cost, residual effectiveness, safety, vector susceptibility and excito-repellency. The insecticides used as adulticides for IRS are DDT, Malathion and different synthetic pyrethroids.

Insecticides used under NVBDCP

The following formulations/compounds are used under the NVBDCP for control of malaria:

DDT (Dichloro-diphenyl-trichloroethane)

In India, DDT has been in use for malaria control since 1946. Recently there has been a tendency to curb the use of DDT due to its persistence in the environment. It is a fact that if DDT is applied in agriculture, it contaminates water resources, enters the

biochain and at each step of the biochain, it gets more concentrate (bio-magnification) till it reaches human beings. In the human body, it is stored in the body fat and is excreted in milk. Since DDT persists for a long time in the environment, there has been apprehension that it will produce adverse effects on human health. A study group of WHO has recommended that at this stage there is no justification on toxicological or epidemiological grounds for changing current policy towards IRS with DDT for VBD control.

DDT may therefore be used for vector control, provided that all the following conditions are met:

- It is used only for indoor spraying
- It is effective
- The material is manufactured to the specifications issued by WHO
- The necessary safety precautions are taken in its use and disposal.

The GoI has constituted a mandate Committee on DDT which reviews the use of DDT in public health and decides its quantity to be released for the NVBDCP every year. DDT has also an added advantage. It is comparatively cheaper than the other insecticides and even in those areas where resistance to DDT has been recorded with WHO test kits the epidemiological impact of good spray operations is seen because of its excito-repellent action.

Requirement of DDT

The requirement of DDT is 150 MT per population of a million for two rounds of spray. In areas where a third round is proposed in selected villages, the additional requirement is estimated to be 75 MT per population of a million.

Malathion

Malathion 25% WP is used under the programme in areas with DDT resistance. The disadvantage of organophosphorous compounds is that unlike their use in

agriculture where a farmer uses the organophosphorous compound for crop protection only once or twice a year, the spray squads engaged in spraying residual insecticide in human dwellings work with these compounds for periods extending upto 6 or 7 months. This long exposure results in acute toxic symptoms and if not controlled properly may lead to mortality. Therefore, the spraymen engaged in spraying of organophosphorous compounds is to be provided with more elaborate protective garments and their blood cholinesterase level is to be checked periodically to assess the toxic impact of the compounds. These compounds are also toxic to domestic pets. Under Indian conditions, three rounds of spray with organophosphorous compounds are done as against two rounds of spray with DDT. In the case of Organophosphate poisoning, the patient should be transported as soon as possible to a doctor to receive an antidote. 2-4 mg of atropine should be given intravenously (for children 0.5 to 2 mg according to weight). Depending on symptoms, further doses of 2 mg. should be given every 15 minutes for 2-12 hours in severe cases. Auto-injections are also available for administration of atropine.

In case of OP poisoning, the patient should be transported as soon as possible to a doctor to receive an antidote. Organophosphate poisoning, 2-4 mg of atropine should be given intravenously (for children 0.5 to 2 mg according to weight). Depending on symptoms, further doses of 2 mg should be given every 15 minutes for 2-12 hours in severe cases. Automatic injections are available for administration of atropine.

Requirement of Malathion 25%

The requirement of Malathion 25% is 900 MT per population of a million for three rounds of spray. In areas where an additional round is proposed in selected villages, the additional requirement is estimated to be 300 MT per population of a million.

Synthetic pyrethroids

These are new insecticides introduced for control of VBDs in India. The cost of these insecticides is higher than the cost of DDT and Malathion. Currently the insecticides of this group registered with Central Insecticide Board for use in the programme are Deltamethrin 2.5%WP, Cyfluthrin 10%WP, Alphacypermethrin 5% WP and Lambdacyhalothrin 10% WP. In treating synthetic pyrethroid poisoning, vitamin E oil preparations are given for prolonged paraesthesia. Only in cases of definite allergic symptoms should corticosteroids be administered. On occurrence of convulsions after severe intoxication, intravenous injection of 5-10 mg Diazepam (or any other benzodiazepine derivatives) should be given.

Requirement of synthetic pyrethroids

The requirement of Deltamethrin 2.5% WP is 60 MT per population of a million for two rounds of spray. In areas where an additional round is proposed in selected villages, the additional requirement is estimated to be 30 MT per population of a million.

The requirement of Cyfluthrin 10% WP is 18.75 MT per population of a million for two rounds of spray. In areas where an additional round is proposed in selected villages, the additional requirement is estimated to be 9.375 MT per population of a million.

The requirement of Lambdacyhalothrin 10% WP is 18.75 MT per population of a million for two rounds of spray. In areas where an additional round is proposed in selected villages, the additional requirement is estimated to be 9.375 MT per population of a million. The requirement of Alphacypermethrin 5% WP is 37.5 MT per population of a million for two rounds of spray. In areas where an additional

round is proposed in selected villages, the additional requirement is estimated to be 18.75 MT per population of a million.

IRS Formulation and doses

S.No.	Name of Insecticide	Amount of Insecticide to prepare 10 litres of suspension	Dosage per sq.metre of active ingredient	Residual effect in weeks	Area (in sq.m) to be covered by 10 litres of suspension	Requirement of insecticide per million population (in MT)
1	DDT 50% WP	1.000 Kg	1 gm.	10-12	500	150.00
2	Malathion 25% WP	2.000 Kg	2 gm.	6-8	500	900.00
3	Deltamethrin 2.5% WP	0.400 Kg	20 mg.	10-12	500	60.00
4	Cyfluthrin 10% WP	0.125 Kg	25 mg.	10-12	500	18.75
5	Lambdacyhalothrin 10% WP	0.125 Kg	25 mg.	10-12	500	18.75
6	Alphacypermethrin 5% WP	0.250 Kg	25 mg.	10-12	500	37.50
7	Bifenthrin 10% WP	0.125 Kg	25 mg.	10-12	500	18.75

□ In the case of Malathion, the requirement shown above, is for the three rounds

Larvicide formulations and dosages

S.No.	Name of larvicide	Commercial formulation	Preparation of ready to spray formulation	Dosage			Frequency of application	Equipment required	Remarks
				1 square meter	50 Linear meter	Per Hectare			
1	MLO	100% petroleum project product	As it is	20 c.c.	1 Litre	200 Litres	Weekly	Knapsack/Hand compression Sprayer	To be applied along the shore of water body
2	Temephos (Abate)	50% EC	2.5 c.c. in 10 Litres of potable water	20 c.c.	1 Litre	200 Litres	-do-	Knapsack/Hand compression Sprayer	Can be applied in clean water
3	Bacillus thuringiensis var israelensis WP	Wettable Powder	5 Kg in 200 litres of Water	-	-	5 Kg.	Fortnightly	Knapsack/Hand compression Sprayer	For both clean and non-potable polluted water
4	Bacillus thuringiensis var israelensis 12 Aqueous Suspension (12AS)	Aqueous Suspension	1 litre in 200 Litres of water	-	-	1 Litres	Weekly	Knapsack/Hand compression Sprayer	Clean Water
			2 litre in 200 Litres of water			2 Litres			Polluted Water
5	Diflubenzuron 25% WP	25% Wettable Powder	100 gms in 100 Litres of water	-	-	25 gm a.i	Weekly	Knapsack/Hand compression Sprayer	Clean Water
			200 gms in 100 Litres of water			50 gm a.i			Polluted Water
6	Pyriproxyfen	0.5% Granular	Ready-to-use	-	-	2 kg.	3 Weekly	Granular Applicator / Hand Broadcast	Clean Water
						4 kg.			Polluted Water

Change of insecticide

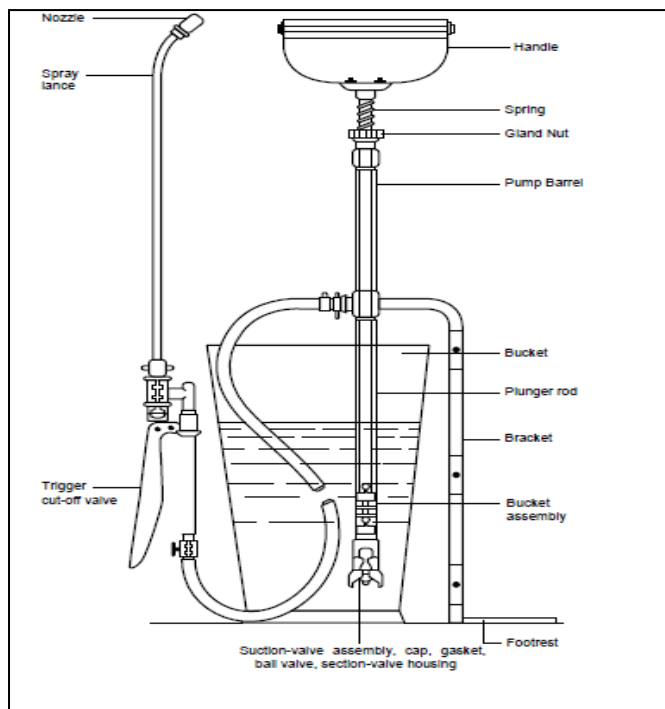
If a change of insecticide is warranted, the state government should support their choice of alternative insecticide by documentation of data on vector resistance studies and field observations on epidemiological impact of spray in respect of insecticide in use. The change of insecticide will always be decided in mutual consultation between SPO, ROH&FW and the Directorate of NVBDCP with concurrence of state and central governments. The proposal for any such change of insecticide should follow the following steps:

- The state government submits the proposal for change of insecticide to Directorate of NVBDCP in the month of January-February. All technical data on vector resistance, epidemiological impact of the current insecticide in use, along with financial outlay, quantity of alternative insecticide chosen, with comparative cost difference for spray operations should be included in the proposal. The proposal should be discussed in the annual action plan meeting in Directorate of NVBDCP.
- Mutual consultations between the SPO, ROH&FW and Directorate of NVBDCP in the month of March-April and report prepared in this regard for submission to Technical Advisory Committee (TAC) for approval under the chairpersonship of the DGHS, GoI.
- Approval of MOH&FW should be obtained in the month of April-May.
- Insecticide should be procured for next year's spray operations and fixing of delivery schedule should be ensured so that the insecticide reaches the periphery by March-April next year i.e. well before starting the first round of spray operations.

Manpower Requirement

The Expert Committee 1995 recommended that 52 squads are required for 5 months spray period to cover a population of one million with DDT / synthetic

pyrethroids. 87 squads are required for 4½ months for 3 rounds of Malathion spraying. Each spray squad consists of 5 field workers working with two stirrup pumps and one Superior Field Worker. It is expected that a spray squad can on an average, cover 60 to 80 houses per day. One squad will take 12 to 17 days to cover a subcentre area with an average population of 5,000.



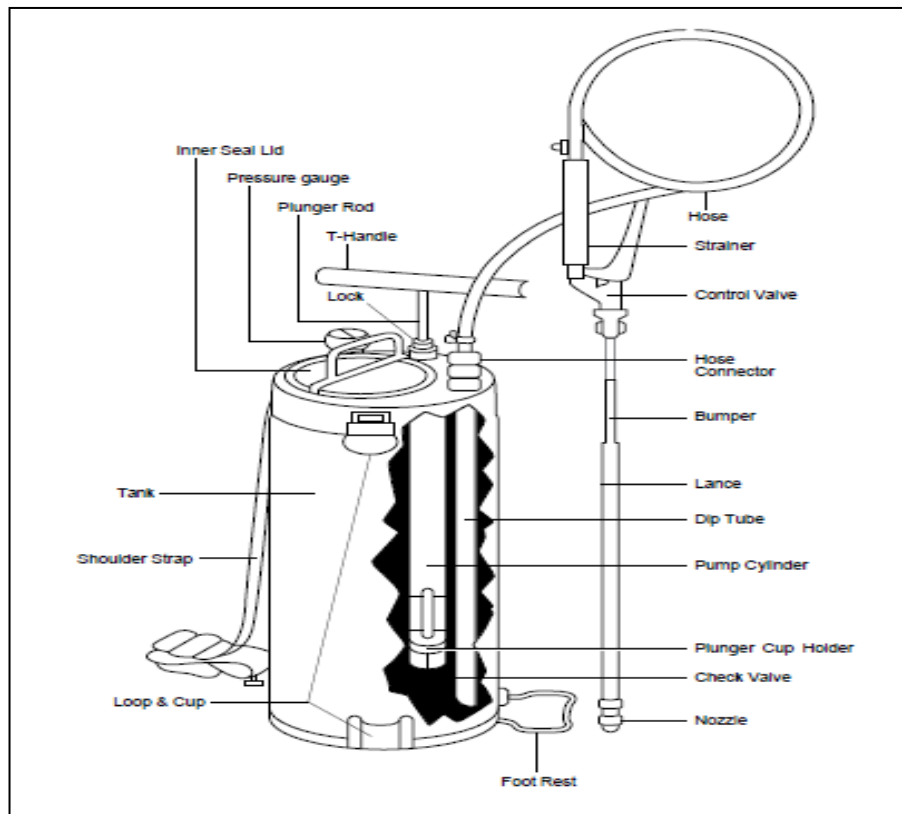
Equipments

Each squad will require the following equipment which must be available well in time before spray operations:

- Stirrup pumps - 2
- Spray nozzle tips for spray pumps - 2
- Bucket 15 litres - 1
- Bucket 5 litres - 1
- Bucket 10 litres - 1
- Asbestos thread - 3 metres
- Measuring mug - 1
- Straining cloth - 1 metre
- Pump washers - 2
- Plastic sheet (3x3 metres) - 1

Hand Compression Pumps requirement is one per person so either with same numbers of spray workers, spraying can be completed quickly or number of spray workers can be reduced.(Details after deliberations-----)

The squad supervisor must have extra spray pumps, nozzle tips, washers, asbestos threads. A set of tools for minor repairs should also be available which should include a pipe wrench, pliers, screwdrivers and



a set of spanners. A good quality nozzle should be used.

Each squad must also be provided with personal protection gear including masks and soap to wash.

Sprayers equipments under NVBDCP -

Knap spray is used for larviciding in urban areas under UMS .

Hand compression and stirrup pumps are in use for Indoor Residual spraying.

The fogging is only recommended during outbreak/epidemic situations for interruption of transmission.

The preparation of the spray suspension is made just before the start of the spray operations every day. It is important that the suspension is made correctly so that the correct dosage is applied on the sprayed surfaces. The procedure for the preparation of the suspension is the same irrespective of the insecticide. However, the quantity of the insecticide used per 10 litres of water will depend on the insecticide used.

The required quantity of the insecticide is measured with a plastic mug and poured into a 15 litre bucket. A paste is made with a small quantity of water. The remainder of water is then poured slowly into the bucket and the insecticide water mixture is stirred vigorously to obtain a uniform suspension. The suspension is then poured into another bucket through a cloth sieve to remove any particulate matter that might clog the nozzle of the spray pump. The insecticide suspension should be stirred vigorously at least every hour.

All food, cooking utensils, bedding and clothes must be protected from the insecticide by taking them outside the house before spraying starts.

The barrel of the stirrup pump is put in the bucket containing the spray suspension. One man operates the pump and the other man sprays. The spray lance should be kept 45 cms (18 inches) away from the wall surface. The swaths should be parallel. Spray is applied in vertical swaths 53 cm (21 inches) wide. Successive swaths should overlap by 7.5 cm (3 inches). Spray is done from roof to floor, using downward motion, to complete one swath; then stepping sideways and spraying upwards from floor to roof. Do not let the spray drip to the floor. Spraying is done only on inner surfaces, including eaves and roofs. The discharge rate should be 740 to 850 ml per minute. To obtain the above discharge rate, the pump man should give 20 to 26

strokes per minute with 10-15 cms plunger movement at a pressure of 10 PSI (0.7 kg/sq.cm) at the nozzle tip. Spraying into a bucket for one minute and measuring the quantity of the suspension in a graduated mug will check the correct discharge rate (740 to 850ml / minute). The nozzle tip should be discarded if the discharge rate exceeds 850 ml per minute.

If the spray stops due to a blockage in the nozzle, the nozzle cap should be unscrewed to remove the blockage and replaced with a new one. The blocked nozzle should be put in a container with water for a few hours before the blockage is removed with a finer wire.

A good quality spray should lead to uniform deposit on walls and other sprayable surfaces. This is easy to verify for DDT and malathion sprays as the insecticide deposits are clearly visible. Deposits of synthetic pyrethroids are visible on wooden structures. The supervisor through physical verification should verify the quality and coverage of spray randomly.



It takes about 5 minutes to spray a house with an average surface area of 150 sq. metres. A summary of spray operations in each village should be maintained by the SFW and verified by the health worker showing the areas covered and room coverage (Form VC-1).

Space spray - Space sprays are transient, short lived treatment with insecticide fogs, aerosols and mists delivered from portable or vehicle mounted generators . This method of treatment is not designed to give a residual effect , but rather to produce high densities of small insecticide droplets Which, while drifting down wind ,will saturate the airspace,penetrating vegetation and buildings to reach both flying and resting mosquitoes. Success also depend upon producing droplets repeated which are too small to settle readily and which while airborne are available for impaction on all flying mosquitoes or are trapped by filter settlement on their hairs.

SPACE SPRAY -

Space sprays are transient , short lived treatments with pesticidal fogs, aerosols, mists delivered from portable or vehicle-mounted generators or from aircraft. This method of treatment is not designed to give a residual pesticide effect, but rather to produce high densities of small pesticide droplets, which, while drifting down-wind, will saturate the airspace, penetrating vegetation and buildings to reach both flying and resting mosquitoes. Success depends upon producing droplets which are too small to settle readily, and which, while airborne, are available for impaction on all flying mosquitoes or are trapped by filter settlement on their hairs. Success all dependent upon repeated treatments to control reinfestation from hatching from pupa to adult or by immigration into the treated area.

Space treatment operations require a higher level of training and coordination than do residual treatment operations. Monitoring of the vector population should occur throughout the activities to changes in vector development, distribution and density. This requires training and discipline of field staff as well as the ability to utilise the information obtained from monitoring to change the control plans as needed. Since pesticides, especially those applied through space sprays, have some

degree of toxicity to non-target organisms, environmental monitoring and safety strategies should be applied. Persons operating and maintaining the equipment should have training in safe handling of it as well as in procedures to ensure that the equipment is effective and efficient. The latter requires understanding of dosage and calibration, droplet size, application timing, application speed and swath, personal and public safety.

It is of a paramount importance that treatment should coincide with vector density, the daily period of vector activity, and the daily local meteorological conditions as well as with human activity which might disperse the spray otherwise impede its applications. For space spraying against *A. aegypti*, the optimum times for outdoor applications are at sunrise (between one hour before and three hours after) and at sunset (from two hours before). Unfortunately, these times correspond to urban traffic rush hours, which can make spraying operations difficult. Ground winds should not exceed 13 km/h or be less than 3 km/h. Treatments should be geared to the incubation of the virus in the mosquito, and treatment intervals planned such that treatments occur before each brood of mosquitoes becomes ineffective.

The volume median diameter (VMD) is widely used to measure droplet size for space spraying. There are a number of methods in use which determine droplet size, but determination of droplet size is not essential in control programmes as operational experience and programme monitoring determines the success of the work and indicates failure. The distinction between aerosols and mists is based on the distribution of droplet size as follows.

Sprays	Droplet size in microns (0.0001cm) (Volume median diameter)
Aerosol	Less than 50

Mists	50-100
Fine Sprays	100-400
Coarse sprays	More than 400

Space spraying equipment should generate a spray near the optimum diameter range. Droplet size varies with nozzle air pressure and condition, pesticide viscosity and flow rate, and operational factors such as type of equipment. Space spraying equipment may require special formulations. For instance, thermal foggers usually require the pesticides to be diluted in an oil, whereas cold mists and aerosol sprays require liquid technical grade or ULV formulations for ULV application and diluted formulation for low and high volume application.

Space spraying may be used to apply dusts, thermal fogs and ULV cold fogs by portable or vehicle mounted ground equipment or by air. For *A. aegypti* control, either thermal foggers or ULV equipments is used. WHO specifications should be consulted when ordering equipment.

Thermal Fogs

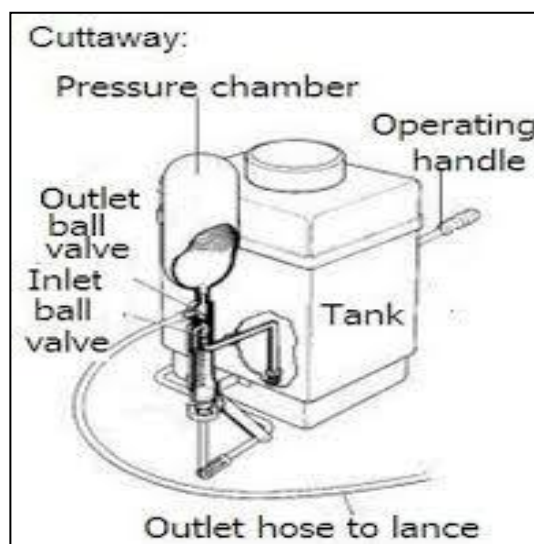
The concept of thermal fogging of pesticides originate from the smoke machines which were used during World War II by the US Navy for making ships invisible to the enemy. Thermal fogs are produced by aerosol generators, using heat to volatilize oil solutions of pesticides. When injected into the cooler temperature of the atmosphere, the hot oil carrying the insecticide condenses in the form of fog. Droplet size is generally proportional to the flow rate. The VMD for thermal fog should range from 23µm to 30 µm.

Thermal fog generators are available in several sizes and are made by a variety of manufacturers. Rates of application range from about 20 to 450 litres per hour. The

most common equipment in use in DHF endemic countries of South-East Asia for emergency control of *A. aegypti* is the hand carried thermal fogger, such as the Swig Fog. Indonesia has a total of nearly 100 fog machines and 17 vehicle-mounted cold aerosols (LECO). Some factors to be considered in the selection of fog generators for space treatment are: (i) terrain, (ii) local climatic conditions, (iii) initial and operating costs, (iv) availability of repair services, (v) size of area to be protected and (vi) location of the area to be treated, as fog reduces visibility of traffic.

Cold Aerosols

The space spraying of liquid pesticides at a minimum volume of liquid pesticide, usually less than 500ml/ha, is known as ULV. ULV is generally considered to be the application of aerosols in the 1 to 50 μ m VMD range. ULV is directly related to the application volume not to the droplet size. Nevertheless, droplet size is important and the equipment used should be capable of producing droplets in the 10 to 15 μ m range, although the effectiveness ranges little when droplet size is extended to 5 to 25 μ m. ULV is known as an economic space spray method when compared to economic space spray method when compared to high volume (HV) application of water diluted pesticides. The main savings result from: (i) the elimination of diluents; (ii) the elimination of formulation costs; and (iii) the speed of application. As with HV spraying, uniform coverage of the target area by the ULV depends on: (i) accurate liquid flow rate; (ii) accurate operation speed; (iii) proper application techniques and (iv) climatic conditions. Types of ULV application equipment available at present time are: (i) portable and vehicle mounted motorized aerosol generators; (ii) portable and vehicle-mounted motorised mist blowers; (iii) portable and vehicle-mounted thermal foggers; and (iv) aircraft-mounted ULV systems. The type of application equipment needed will depend on: (i) the size of the target area; (ii) the accessibility of target areas; (iii) the vector species to be controlled; (iv) the cost of the equipment and maintenance services; and (v) the safety of operation.



MAINTENANCE OF SPRAY EQUIPMENTS

Spray Equipments

Insecticides will be effective only if applied precisely by the most efficient applicators. Equipment selection should be based on the type of pesticide to be applied and the size and scope of the spraying job involved. Five key factors should be considered when selecting application equipment.

- 1) Will it do the job? (Effectiveness)
- 2) Is it safe? (Safety)
- 3) Is it Offensive? (Public relations aspects)
- 4) Is it expensive? (Cost)
- 5) Is it durable? (Durability)

An important consideration in the selection of spraying equipment is the size of droplets produced by the equipment during normal use. The type of the spray depends on the droplet size, which is generally described by volume median diameter (VMD) expressed in micrometers (μm). This is the number which divides the aerosol or spray into

two equal parts by volume, one half containing droplets smaller than this diameter and the other half containing largest droplets.

Sprays may be classified according to droplet VMD as follows:

Aerosols	50um
Mists	50 - 100 um
Fine sprays	100 - 250 um
Medium sprays	250 - 400 um
Coarse sprays	400 um

The equipment for the production of fine and coarse sprays generally comprise of nozzles and pumps. The knapsack sprayer, compression sprayers and stirrup pumps are most commonly used in vector control programmes.

Equipments for the application of larvicides and residual insecticides

Knapsack sprayer

This is carried on the back and a shield is provided so that it does not come into actual contact with the back. A skirt is usually fitted to the bottom of the container to prevent the direct contact with the ground. Knapsack sprayer is a continuous type of Sprayer (Fig 6.1) and the discharge rate is fairly constant. Unskilled operators with little education can use the sprayer. Maintenance is usually simple. The operator has not only to bear the weight of the sprayer but also simultaneously to operate the pump lever with one hand and direct the spray with the other. Lighter the equipment and lesser the effort needed for operation, the better will be the spray application.

Compression sprayer

This equipment is simple to use and versatile for vector control purposes. However as the liquid is discharged from the container, the air space increases in volume and the pressure falls. It is therefore necessary to pump air to maintain a steady working pressure. (Fig 6.2).

Stirrup pumps

These sprayers are widely used in vector control programmes because they are less costly than compression sprayers. They can be used with any type of hydraulic nozzle (Fig. 6.3). These sprayers consist of pump, attached discharge hose and spray lance, the pump being provided with a bracket and foot-rest or stirrup. The discharge outlet is usually placed at the top-end of the pump and should be preferably sloped downwards to prevent the delivery hose from bending or collapsing. The spray discharge is continuous because an air chamber maintains spraying pressure. This type of sprayers needs two operators, one for pumping while the other can direct spray.

Equipments suitable for space spray

These are either operated electrically or through internal combustion engines. Different type of equipment necessary for thermal fogging and cold aerosol spraying.

Thermal fogging equipment

For indoor fogging the hand-carried thermal fogger generally called as Swing-fog can be used. In this machine, oil is injected into the exhaust gas of a pulsejet internal combustion engine at a point where it will be completely vaporized and then immediately discharged. Devices employing this method can be hand or shoulder-carried (Fig. 6.4). But in view of the fire hazard and the possibility of misuse of diesel, which is generally used, the cold aerosol sprays are preferred.

Microsol

This is electrically operated for producing cold aerosol spray. In this applicator (Fig. 6.5), vortical nozzles are employed wherein the air stream is given a rotary motion. This considerably increases its shearing action on the liquid so producing droplets in the size range of mists and aerosols.

Because of the large orifices in the vortical nozzles, the problem of blockage is minimal. The popularity as a means of producing aerosols has grown to such an extent that they are replacing the thermal devices that have hitherto been used for this purpose.

Mist Blower

Power-operated gaseous energy sprayers, or mist blowers consist of four main parts: a power source which can be either operated electrically or by internal combustion engine, a fan or blower, a pesticide container and a nozzle (Fig 6.6). The use of restrictors with small bores in the liquid feed permits the mist blower to be used for ultra-low-volume applications requiring a high air-to-liquid ratio in order to achieve the most effective atomization of the liquid.

LECO-ULV. Generator

These generators overcome many of the shortcomings with thermal fogging machines. The equipment consists of a power-driven high-performance blower to supply the air, a container and a pump for the spray liquid and a directional head in which several smaller nozzles are mounted on a T-shaped head to give a wide swath discharge (Fig 6.7). These can be mounted on a trolley or vehicle. They are less noisy in operation than the thermal fogger and there is less likelihood of creating a traffic hazard.

Care and maintenance of spray equipments

All applicator equipments require diligent care if they are to be kept operating properly. Several basic rules should be followed in the care of a sprayer.

1. Handle it carefully.
2. Keep it clean.
3. Strain the formulations through proper filters.
4. Rinse it out thoroughly with water after use and pump 1 litre of water through it.
5. Every 3 months, disassemble it completely, put small metal parts into kerosene, allow to set clean with a small bottle brush, soak nozzles, spray lance and tank with trisodium phosphate solution (washing soda), and clean with a scrubbing brush, then rinse thoroughly. Replace worn gaskets, broken parts etc. Reassemble it. Pump clean water through it.

Maintenance of power operated equipments:

1. Power equipments should be covered when not in use.
2. Have regular preventive maintenance on all motors.
3. Replace damaged parts immediately.
4. Allow only experienced personnel to operated power equipment.

Spray equipments, use, handling and maintenance including safety measures

The discovery of organic pesticides provided man with new and powerful weapons for his incessant war against insect pests, diseases and needs, since the introduction of DDT in 1940's pesticides have played a major role in the control of vector borne diseases. Insecticides will be effective only when applied precisely by the most suitable applicators. Hence the equipment selection should be based on the type of insecticide to be applied.

The commonly used spray equipments varies according to the type of spray. In the case of mosquito control measure depending with the type of spray, different spray equipments are used as shown below.

Type of Spray	Spray equipments
Indoor residual spraying	Hand compression sprayers, stirrup pumps.
Indoor space spraying	Mists blowers, power operated mist blowers Aerosol generators
Exterior space spraying	ULV equipment, Power operated mist blowers, Thermal, fogging equipment, Aerosol generators.
Larviciding	Hand compression sprayers, Knapsack sprayers, Granule applicators, Dusters.

The salient features of the different spray equipments are given in nutshell below:

1	Knapsack sprayer/ Hand compression sprayer	A continuous type of sprayer with lightweight. Discharge rate is fairly constant can be used by unskilled operators. Maintenance usually simple.
2	Stirrup pump	Less costly than Hand compression Sprayers. Any type of hydraulic nozzle can be used. Discharge rate is constant when constant pressure is maintained. Two spray men are required for each sprayer for its operation.
3	Mist blowers	Simple manual and power operated sprayers are available. Use of restrictors with small bores permits proper mist formation
4	ULV equipment	It is usually a two-stroke engine. The oil and petrol ratio is usually 1:24. The most milable oil is 30 SAE oil. Multigrade oil should never be used. The droplet size (VMD) needs to be 20 - 50 um. Useful and economical method of temporary control of mosquitoes.
5	Thermal fogging equipment	Can be used both the indoor space spray and exterior space spray. Operating on a pulse jet system. The droplet size may be 10 - 50 um. Useful in urban and semi urban area. There is a fire hazard with some type of machines, hence greater care is needed.

The important factors that are to be considered while selecting the spray equipments are

- 1) Effectiveness
- 2) Safety
- 3) Acceptability
- 4) Cost
- 5) Durability.

While all attempts must be made to select most effective equipment available in the country, safety should be the prime consideration to eliminate health hazard to the operators as well as to general public. A defective non-standard equipment shall result in safety hazard and environmental pollution

Having selected spray equipment the maintenance of the equipment both during spray and after spray are also important. For proper maintenance the following aspects are to be looked for, to tide over the operational difficulties in field.

- a. One pair of pliers, screw driver (of different sizes) one small adjustable wrench, a knife and a string or greased string
- b. In the case of power sprayers, a spare spark plug, a plug spanner, suitable tachometer
- c. A toolbox
- d. Spare equipments (one or two for each team if available)

Precautions

- 1 Handle the spray equipment carefully
- 2 Keep the equipments clean
- 3 Strain the formulation through proper filters
- 4 Revise the equipment thoroughly with water after use and pump water through it
5. Keep (a) Extra nozzles, washers and spare parts
- 6 Keep the equipments under lock and key when not needed for use with due care to the various parts during storage.

Safety Precautions

Before Spraying

- 1 Choose only recommended insecticide, which is the least toxic
- 2 Read the instructions issued by the Authorities concerned now and then on the insecticides under use and on the spray equipment supplied.

- 3 Check the spraying equipment
- 4 Ascertain that all components are clean
- 5 Replace worn-out parts
- 6 Check the nozzle spray pattern and discharge rate
- 7 Make sure that appropriate protective clothing available for use
- 8 Train all concerned with the application method
- 9 Check that the pesticide/insecticide are kept in dry, locked store
- 10 Do not transfer insecticide into other containers especially into containers used to hold soft drinks
- 11 Notify the area about your spray programme
- 12 Use the pesticide/insecticide only when really needed

During spraying

- 1 Take only sufficient insecticide for the days' application from the store to the site.
- 2 Recheck the insecticide under use and the equipment
- 3 Make sure correct formulations are made
- 4 Wear appropriate clothing
- 5 Avoid contamination of the skin (avoid splashing)
- 6 Avoid drifting of the insecticide while spraying
- 7 Never eat, drink or smoke when mixing or applying the insecticide
- 8 Never blow out clogged nozzles with your mouth
- 9 Follow correct spray technique
- 10 Never allow children or unauthorized persons to be nearby during mixing.

After spraying

- 1 The left out insecticide in tank should be emptied and disposed off in pits dug on the wasteland.
- 2 Never leave unused insecticide in sprayers
- 3 Never empty the tank into irrigation canals or ponds
- 4 Always clean equipments properly
- 5 After use, oil it and then keep away in storeroom
- 6 Do not use empty containers for any purpose. Crush and bury the containers preferably in a land filled dump.
- 7 Clean buckets, sticks, etc. used in preparing the spray solution
- 8 Remove and wash protective clothing and footwear
- 9 Wash yourself well and put on clean clothing
- 10 Keep an accurate record of insecticide usage.

Procedure for Treating Mosquito Nets and Curtains

The steps described below mainly refer to treatment of mosquito nets with permethrin. The net treatment technique can be easily used for curtains.

(a) Calculate the area to be treated

Measure the height, length and width of the net. Assuming a rectangular mosquito net is 150 cm high, 200 cm long and 107 cm wide, the calculations are as follows:

Area of one end	$107 \times 150 = 16,050 \text{ cm}^2$
Area of one side	$200 \times 150 = 30,000 \text{ cm}^2$
Area of top	$107 \times 200 = 21,400 \text{ cm}^2$

The sides and ends need to be multiplied by 2:

(d) Determine how much insecticide is needed

Assume that a permethrin emulsifiable concentrate will be used, and the dosage desired is 0.5 grams per square metre. To determine the total grams required, multiply the net size by the dosage:

(f) Determine the amount of liquid required to saturate a net

In order to determine the percentage solution to be used for dipping, it is first necessary to determine the approximate amount of water retained by a net. Another term for dipping is soaking.

Pour five litres of water, but preferably a dilute solution of the insecticide to be used, into a plastic pan or other suitable container. For cotton, a 0.3% solution can be tried; for polyethylene or other synthetic fiber, a 1.5% solution can be tried. Add the net to the solution till it is thoroughly wet and then remove it. Allow the drips to fall into a bucket for 15 to 30 seconds. Set the net aside. Repeat the process with two other nets. Cotton nets can be lightly squeezed but not the synthetic ones. Measure the water or solution remaining in the dripping/soaking container and in the bucket to calculate the amount of liquid used per net.

Quantities of 1% Temephos (Abate) Sand Granules

Size of water jar, drum or other container in litres	Grams of 1% granules required	Number of teaspoons required, assuming one teaspoon holds 5 grams
Less than 25	Less than 5	Pinch: small amount held between thumb and finger
50	5	1
100	10	2
200	20	4
250	25	5
500	50	10
1000	100	20

Procedure, Timing and Frequency of Thermal Fogging and ULV Space Spray Operations

Basic steps: The steps listed below are to be followed in carrying out the space spraying of a designated area:

1. The street maps of the area to be sprayed must be studied carefully before the spraying operation begins.
2. The area covered should be at least 300 metres within the radius of the house where the dengue case was located.
3. Residents should be warned before the operation so that food is covered, fires extinguished, and pets are moved out together with the occupants.

4. Ensure proper traffic control when conducting outdoor thermal fogging since it can pose a traffic hazard to motorists and pedestrians.
5. The most essential information about the operation area is the **wind direction**.
6. Spraying should always be done from downwind to upwind, i.e. going against the direction of the wind.

Vehicle-mounted spraying

1. Doors and windows of houses and buildings in the area to be sprayed should be opened.
2. The vehicle is driven at a steady speed of 6-8 km/hr (3.5-4.5 mile/hr) along the streets. Spray production should be turned off when the vehicle is stationary.
3. When possible, spraying should be carried out along streets that are at right angles to the wind direction. Spraying should commence on the downwind side of the target area and progressively move upwind.
4. In areas where streets run parallel as well as perpendicular to the wind direction, spraying is only done when the vehicle travels upwind on the road parallel to the wind direction.
5. In areas with wide streets with houses and buildings far from the roadside, the spray head should point at an angle to the left side of the vehicle (in countries where driving is on the left side of the road). The vehicle should be driven close to the edge of the road.
6. In areas where the roads are narrow, and houses are close to the roadside, the spray head should be pointed directly towards the back of the vehicle.
7. In dead-end roads, the spraying is done only when the vehicle is coming out of the dead-end, not while going in. The spray head should be pointed at a 45° angle to the horizontal to achieve maximum throw of droplets.

8. Vector mortality increases downwind as more streets are sprayed upwind in relation to the target area.

Portable thermal fogging

1. Thermal fogging with portable thermal foggers is done from house to house, always fogging from downwind to upwind.
2. All windows and doors should be shut for half an hour after the fogging to ensure good penetration of the fog and maximum destruction of the target mosquitoes.
3. In single-storey houses, fogging can be done from the front door or through an open window without having to enter every room of the house. All bedroom doors should be left open to allow dispersal of the fog throughout the house.
4. In multi-storey buildings, fogging is carried out from upper floors to the ground floor, and from the back of the building to the front. This ensures that the operator has good visibility along his spraying path.
5. When fogging outdoors, it is important to direct the fog at all possible mosquito resting sites, including hedges, covered drains, bushes, and tree-shaded areas.
6. The most effective type of thermal fog for mosquito control is a medium/dry fog, i.e. it should just moisten the hand when the hand is passed quickly through the fog at a distance of about 2.5-3.0 metres in front of the fog tube. Adjust the fog setting so that oily deposits on the floor and furniture are reduced.

Back-pack aerosol spraying with ULV attachments

Basic points

1. Each spray squad consists of four spraymen and one supervisor.
2. Each sprayman sprays for 15-30 minutes and then is relieved by the next sprayman. For reasons of safety, he must not spray when tired.
3. The supervisor must keep each sprayman in his sight during actual spraying in case he falls or needs help for any reason.
4. Do not directly spray humans, birds or animals that are in front of spray nozzles and less than five metres away.
5. Spray at full throttle. For example, a ULV
6. Fontan nozzle tip 0.4 can deliver 25 ml of malathion per minute, and a 0.5 tip, 65 ml. The smaller tip is usually preferred unless spraymen move quickly from house to house. Some machines can run for about one hour on a full tank of petrol.

House spraying technique

1. Do not enter the house. House spraying means spraying in the vicinity of the house.
2. Stand 3-5 metres in front of the house and spray for 10 to 15 seconds, directing the nozzle towards all open doors, windows and eaves. If appropriate, turn away from the house and, standing in the same place, spray the surrounding vegetation for 10 to 15 seconds.
3. If it is not possible to stand three metres from the house due to the closeness of houses and lack of space, the spray nozzle should be directed towards house openings, narrow spaces and upwards.
4. While walking from house to house, hold the nozzle upwards so that particles

can drift through the area. Do not point the nozzle towards the ground.

5. Spray particles drift through the area and into houses to kill mosquitoes which become irritated and fly into the particles. The settled deposits can be residual for several days to kill mosquitoes resting inside houses and on vegetation not exposed to the rain.
6. This technique permits treatment of a house with an insecticide ranging from 1 to 25 grams in one minute. The dosage depends on the discharge rate, concentration of insecticide applied, and time it takes to spray the house. For comparison, an indoor residual house spray may require 30 minutes of spraying to deposit 300 grams of insecticide. This assumes a dosage of two grams per square metre to 150 square metres of sprayable surface.

Information to be given to inhabitants

1. Time of spraying, for example 0630 to 1000 hours.
2. All doors and windows should be open.
3. Dishes, food, fish tanks, and bird cages should be covered.
4. Stay away from open doors and windows during spraying, or temporarily leave the house and/or the sprayed area until the spraying is completed.
5. Children or adults should not follow the spray squad from house to house.

Timing of application

Spraying is carried out only when the right weather conditions are present and usually only at the prescribed time. These conditions are summarized below:

For optimum spraying conditions, please note

1. In the early morning and late evening hours, the temperature is usually cool. Cool weather is more comfortable for workers wearing protective clothing. Also, adult *Aedes* mosquitoes are most active at these hours.

2. In the middle of the day, when the temperature is high, convection currents from the ground will prevent concentration of the spray close to the ground where adult mosquitoes are flying or resting, thus rendering the spray ineffective.
3. An optimum wind speed of between 3 and 13 km/hr enables the spray to move slowly and steadily over the ground, allowing for maximum exposure of mosquitoes to the spray. Air movements of less than 3 km/hr may result in vertical mixing, while winds greater than 13 km/hr disperse the spray too quickly.
4. In heavy rain, the spray generated loses its consistency and effectiveness. When the rain is heavy, spraying should stop and the spray head of the ULV machine should be turned down to prevent water from entering the blower.
5. Spraying is permissible during light showers. Also, mosquito activity increases when the relative humidity reaches 90, especially during light showers.

Frequency of application

The commencement and frequency of spraying generally recommended is as follows:

1. Spraying is started in an area (residential houses, offices, factories, schools) as soon as possible after a DF/DHF case from that area is suspected. At least one treatment should be carried out within each breeding cycle of the mosquitoes (seven to ten days for *Aedes*). Therefore, a repeat spraying is carried out within seven to ten days after the first spraying. Also, the extrinsic incubation period of dengue virus in the mosquito is 8 to 10 days.

Introduction

The entomological specimens like adults and immature collected during Entomological Surveys should be preserved for Taxonomic studies, demonstration and Dissection analysis or for other diagnostic procedures. An important work in an entomological laboratory is preparing, mounting and storing of Entomological specimens. The methodology adopted for the preservation, storage and transportation of Entomological specimens (Adults & larvae with reference to mosquito are dealt herewith.

I. Mounting of adults

Adult mosquitoes have to be preserved for Taxonomic study and for demonstration. Mosquitoes must be pinned so that they are examined under a microscope.

a. For storing specimens in Insect Box**Materials required**

Long stainless steel Insect Pins
Small minuten Entomological Pins
Cork sheets
Strip cutter blade
Labels
Forceps
Mounting Block

Procedure

- Kill specimens with Ether or Chloroform and not by Tapping which will damage the scales and legs and make the specimen difficult to identify
- Cut a 1.5 cm X 0.25 cm strip from the cork sheet and pierce a alpin through one side of the cork strip
- Pierce a large pin through the other end of the cork strip and push it so that the height of the minute pin is above the large pin.
- Place the killed mosquito on its back facing the ventral side up on a white sheet of paper
- By holding the cork assembly towards the ventral side of the mosquito insert the minute pin into the thorax at right angle between the 2nd and 3rd pair of legs.
- Press the minute pin so that it goes through the Thorax
- Adjust the wings and legs of the mosquito so as to spread them evenly on either side taking care of the legs or wings without damage.
- Write the name of village, block, taluk, state, Country, name of collector, habitat, date & time of collection on a label and pierce it with the large pin facing the information up



- Likewise in a second label write the name of specimen and pierce it with the large pin and adjust the gap between the two labels about 1 cm and 1cm from the base of the cork assembly
- Mount the pinned mosquitoes in rows in an insect storage box.

The insect storage boxes are treated with Preservatives. Melted Napthalene are poured to the sides of the box or beech wood creosote soaked cotton can be placed in a small vial and secured inside the box. Powdered Napthalene can also be placed inside the box. The pinned mosquitoes are mounted in the box in rows using entomological forceps

b. For storing single or more mosquitoes in a single tube

Materials required

Minute Entomological pins
 Corks sheets
 Strip cutter blade
 Napthalene
 Entomological Forceps
 Glass specimen tube (8cm x2.5 cm) with cork stopper
 Wax

Procedure

- Cut a piece of long cork to fit lengthwise in the middle of a large glass specimen tube
- Cover the Cork with white paper to give a contrasting background
- Place the killed mosquito on its back facing the ventral side on a white sheet
- Insert the minute pin into the thorax at right angle between the 2nd and 3rd pair of legs on the ventral side of the mosquito.
- Write label on one side of the cork
- Mount the pinned specimens on the other side of the cork with help of a forceps

- The bottom of the cork is dipped in Melted Napthalene
- Insert the cork with the pinned mosquitoes in the tube and close it tightly with the stopper until the cork is gripped.
- Seal the tube with wax

Mosquitoe (s) mounted in this way can be kept for several years and can be examined under a microscope without being taken out of the tube.

Softening of dried specimens

If specimens have been dead for some time and become dry and brittle, they must be softened and relaxed before pinning.

Materials required

Dried specimens

Air-tight container

Cotton wool

Phenol or Chloro-cresol

Procedure

- A cotton wool soaked in water or wet sand is placed on the bottom of airtight container
- Add a few drops of phenol or Chloro-cresol to the cotton wool to prevent the growth of fungus
- Place a fine wire mesh over the cotton wool
- Place the dried mosquitoes on the surface of the fine wire mesh and Close the container
- Leave the specimen as such for several days till the specimens softens

II. Preservation of mosquito larvae

For the identification of the mosquito species, the larvae collected during the entomological surveys can be preserved and mounted

Materials required

Wide mouth pipette

70% Ethanol

95% Ethanol

Hot water

Beakers

Glass vials

Cotton wool

Microslides & Coverslip

Lactophenol, Nail varnish & Euparal

Procedure

- Pickup the 4th Instar with the pipette and transfer it to the clear water in a container
- If the larvae is of early instars keep them until they become 4th Instar
- Transfer the larvae to hot water (70^o C) to kill the them without any deformity
- Transfer the killed larvae to 70% alcohol in a container and leave them for 24 Hours
- Then transfer the larvae to small containing 95% alcohol and Plug the vial with cotton wool
- Label the tube with Locality, Type of Habitat, Date of collection, Name of collector

Mounting of Mosquito Larvae

Mounting media such as Lacto phenol (Lactic acid 10 ml, Phenol 10gm, Glycerol 20 ml & Distilled water 10 ml and store in a dark bottle) and Polyvinyl lacto phenol can be used for quick mounting and can be kept of several years. For a more permanent mount Euparal is used.

Procedure

- At the center of a clean-labeled slide put one drop of clean water and draw a circle for the placement of the larva
- Transfer one larva using a dropper over the water drop and adjust the specimen facing dorsal side up
- Remove the water with a filter paper strip
- Cover the specimen with a small drop of mounting medium
- And a small drop of Mounting medium to the center of the cover slip
- Invert the cover slip and gently lower onto the specimen so that the mounting medium join together
- Leave the side to dry and while drying if any space appears between the slide and cover slip fill it with more mounting medium
- Apply clear nail varnish to the edge of the cover slip to form a seal
 - a) Label the slide with Locality, Type of Habitat, Date of collection, Name of collector , Species
- Examine the specimen under the microscope
- For Euparal mounting place the larvae in 70% alcohol for 24 Hrs
- Transfer the larvae to 95% Ethanol and keep for 24 Hrs
- Transfer the larvae to clove oil the larvae sink on to the bottom
- Place the larvae in a drop of Euparal at the center of a clean-labeled slide and mount it as before
- Allow the slide to dry (This will take several days and require no sealing
- Ready for microscopical examination and can be store for any number of years

III. Preparing the adult specimens for transportation

a. Preparing the unpinned Adult specimens for transportation

Whenever the mosquitoes collected from a field laboratory has to be sent to a central laboratory or Research Institute of Diagnostic laboratory it is necessary to adopt certain methodology. The packing must be done very carefully so that the specimens do not get damaged.

Materials required

Pill boxes

Tissue paper

Self Adhesive tape

Procedure

- Place a soft tissue paper in the bottom of a small screw capped container
- Place a single layer of specimens on the tissue paper without touching each other
- Cover the specimens with another layer of one or two of tissue paper
- Place a single layer of specimen on the top layer of tissue paper
- Likewise gently pile up more layer of specimens
- Fill the remaining space with tissue paper
- Seal the container with adhesive tape
- Place the container in a large container and pack it
- Wrap and write the address

b. Preparing individual pinned Adults specimens for transportation

Materials required

Pinned specimens

Vials with cork stoppers

Packing materials

Packing container

Procedure

- Place the pinned specimen on the inner side of the cork stopper using forceps
- Carefully insert the cork stopper with the pinned specimen on to the specimen vial
- Pack the vials in a strong box with plenty of packing material
- Wrap and write address on the box

c. Preparing pinned Adult specimens in a box for transportation

Materials required

Pinned specimens

Entomological Box

Cardboard

Cotton wool

Wrapping paper & packing material

Procedure

- Insert the pinned specimens very firmly into the Entomological Box
- Keep apart the specimens from one another so as to avoid knocking one another
- Make sure that the pinned specimens are lower than the top pin
- Cut and place a piece of cardboard across the top pins so as to cover the top
- Fill in the space between the cardboard and top of the box with cotton wool

- Fasten the lid firmly
- Pack the Entomology box inside a larger, stronger box with plenty of packing material
- Wrap the outer box and write address

Iv. Preparing the larval specimens for transportation

Materials required

Vials with rubber stopper

Hypodermic Needles

A strong Box

Packing Material

Procedure

- Preserve the larvae in 70% Alcohol in small vials with rubber stoppers
- Fill each vial to the top with alcohol
- Insert the rubber stopper
- Pierce the rubber stopper with a Hypodermic needle to release my trapped air
- Pack the vials into a strong container
- Fill spaces between the vials with packing material
- Wrap the container and write the address

V. Preparing eggs for despatch

Sometimes it will be necessary to collect and send the mosquito eggs of research studies and maintenance of progeny in other institutes. The following methodology is adapted to preserve and transport of mosquito eggs.

Procedure

- Line a small funnel with filter paper
- Pout the suspension containing the mosquito eggs on to the filter paper
- Let the water completely let out from the filter paper
- Remove the filter paper and wrap in another piece of filter paper. The paper should be damp and no excess water
- Place the filter paper in a Zip lock cover and put the cover inside container
- Wrap the container and write the address

Dispatch the eggs as soon as possible after they are laid

MOSQUITO DISSECTION

Recognition Of Abdominal Conditions

Unfed: The abdomen is flattened

Fully fed: The abdomen appears bright red from the blood in the midgut. The ovaries occupy only a small area at the tip of the abdomen and this part is not red; it includes only 2-3 segments on the ventral surface, and at most, 4 segments on the dorsal surface.

Fed late stage: The blood is now dark red and the ovaries occupy 4-5 ventral segments and 6 dorsal segments.

Half gravid: The blood is dark in colour - almost black - and occupies 3 or 4 segments on the ventral surface (sternites) and 1 or 2 on the dorsal surface (tergites) of the abdomen nearest to the thorax. Ovaries occupy most of the abdomen.

Gravid: The blood is reduced to a small black patch on the ventral surface. The ovaries occupy all the rest of the abdomen. In some mosquitoes the floats on the side of the eggs are visible through the abdominal wall.

The ovarian stages and the abdominal conditions are generally related in the following way:

Abdominal condition	Ovarian stage
Unfed	Ovaries at stage 1 (newly emerged females)
	Ovaries at early stage 2 (females which have already laid eggs)
Freshly fed	Ovaries at stage 2 (newly emerged females)
	Ovaries at stage 3 (females which have already laid eggs)
Half gravid	Ovaries at stage 3 or 4
Gravid	Ovaries at stage 5

Dissection of the female mosquito to examine the Midgut for Oocysts

In some malaria surveys it is necessary to know the number of mosquitoes that are infected with the Oocyst stage of the parasites (examination for sporozoites is dealt with later). This can be determined by an examination of the midgut of mosquitoes. Before dissecting an adult mosquito, it is essential to know the position of the different organs within its body.

Equipment needed

Killing tube, dissecting needles, forceps, slides, cover-slips, mercurochrome stain, bottle to store stain, measuring cylinder, labels, dropper, formal - glycerine solution, nail varnish.

Important structures within a mosquito

ows the structures inside a female mosquito as they would appear if the mosquito was cut in half vertically along the middle of the body and lying on its lower surface.

- The salivary glands lie inside the thorax, but are joined to the head by salivary ducts.
- The midgut lies in the abdomen, and the Malphigian tubules are at the bottom end of the midgut.
- The ovaries lie on either side of the gut in the posterior part of the abdomen, oviducts from each ovary join at the ampulla to form a common oviduct.
- A single spermatheca is attached to the common oviduct.

Dissection of the female mosquito to examine the midgut for Oocysts

By determining the proportion of mosquitoes, which are infected with oocysts, one can get a rough measure of the rate of infection in the mosquito population and the possibility of finding sporozoites by further dissection. Examination for oocysts can also be used to follow in the laboratory the time required for the development of the infection in the mosquito and for the production of mature sporozoites.

Stages suitable for dissection

It is best to dissect unfed females caught in a biting catch, or those in which the blood has already been digested.

Dissecting out the midgut for examining of oocysts

- Identify the specimen and Remove legs and wings

- Place specimen on slide in a drop of saline solution. The specimen could be also placed near to a drop of saline solution and the midgut transferred in the solution once extracted from the abdomen.
- Arrange the specimen so that the thorax points to the left and the end of the abdomen to the right.
- Make small cuts in the abdomen between the sixth and seventh abdominal segments, cutting both the upper and lower surfaces.
- Hold the thorax firmly with the blunt needle or forceps.
- Pull the end of the abdomen with the other needle so that the abdomen breaks in the area of the cuts. The midgut, Malphigian tubules and ovaries should then come away with the tip of the abdomen.
- Cut the foregut in the region of the first abdominal segment by cutting through the middle of it.
- Pull the midgut free from the abdomen
- Hold the foregut with a needle and cut off the hindgut behind its junction with the midgut.
- Remove the Malphigian tubules and ovaries.
- Remove all parts of the mosquito except the midgut.
- Make sure you have the right amount of saline solution. If there is too little, the midgut will break when you add a coverslip and it will be difficult to see the oocysts. If you have too much the midgut will not flatten enough when you put on the coverslip and carefully cover the stomach with a coverslip.
- If you have too much saline solution, carefully remove some by using a small piece of filter-paper.
- Examine the midgut for oocysts under a compound microscope using the X10 objective. If you do not see oocysts with this magnification, use the X40 objective.

- Gently push the edge of the coverslip in a forward direction to rotate the midgut. A human hair can be placed between the slide and the coverslip. This makes it easier to rotate the midgut without crushing it. Examine it until the presence of oocysts has been confirmed. It is necessary to examine every part of the surface of the midgut and as soon as an Oocyst is identified no further examinations are necessary unless you intend to count the number of oocysts.

Dissection of the female mosquito to examine salivary glands for sporozoites

The salivary glands are examined for sporozoites to determine which mosquito species carry the malaria parasite and to determine the percentage of each species that is infected. Determination of sporozoites rates is necessary to confirm the role of a particular mosquito species as a transmitter of malaria. The proportion of mosquitoes with sporozoites gives an indication of the magnitude of malaria transmission which may be occurring which is important information for evaluating the impact of malaria control measures.

Equipment needed

A killing tube, 0.65% saline solution, dissecting needles – one blunt and one fine pointed, fine forceps, slides, dropper, balance, measuring cylinder, bottles for stain and buffer, labels, methanol, Giemsa stain, methanol; coverslips, modeling clay (or plasticine), Euparal, diamond pencil and a grease pencil.

Dissecting salivary glands

For dissection, mosquitoes should be anaesthetized or stunned and their wings and legs removed. There are two different ways to dissect salivary glands.

Method 1 (Fig. ====)

- Confirm the species of the mosquito (it will not be possible to do this after the dissection for obvious reasons).
- Place the mosquito on a slide, lying on its side with the head pointing to the right.
- Cut off the head with a needle and discard the head
- Place a small drop of saline solution close to the front of the thorax.
- Hold the thorax firmly with the blunt needle held in your left hand.
- Gently squeeze the thorax with the needle held in your right hand and as you squeeze, the salivary glands will be forced out of the thorax.
- Separate the glands with the other needle and place into the drop of saline solution.
- Remove the body of the mosquito.
- Cover the salivary gland with a standard 18 x 18 mm coverslip (if a large coverslip is used you will need to use more saline solution).

Method 2 (Fig. =====

- Confirm the species of the mosquito
- Prepare a slide with a drop of saline solution
- Place the mosquito on its side on the slide with the head pointing to the right.
- Hold the thorax firmly with the blunt dissecting needle in your left hand.
- Place the needle held in your right hand on the neck of the mosquito but do not cut the neck.
- Gently pull the head away from the thorax and the glands will come out of the thorax, attached to the head.

- If the glands do not come out with the head, they may be obtained by gently squeezing the thorax in the same way as described in method I.
- Cover the glands with a coverslip.

Examining salivary glands from fresh dissections (Fig.===)

- If the glands have not been crushed by the coverslip, gently press the coverslip with a dissecting needle so that the glands break and sporozoites are released.
- The glands should be examined under a high power X40 objective so that the unstained sporozoites can be seen moving. Reduce the illumination either by lowering the condenser or by partially closing the iris diaphragm to get better contrast for an easier detection of sporozoites.

Determination of parity (Fig. ====)

The aim of residual insecticide spraying is to reduce malarial transmission by kill mosquitoes that enter dwellings to feed. Older mosquitoes are more likely to have been killed, as they would have entered houses more often. If residual spraying is effective, there will be fewer old (parous) mosquitoes and less likelihood of malaria transmission. Therefore, by measuring the proportion that is parous in a vector population, one can evaluate the impact of spraying.

There are less old females because they have no time to become aged because they are killed by insecticide. But as breeding sites are not concerned there is still production of nulliparous females and since the parous rate is a proportion of the parous to nulliparous females, this rate will be lower.

By examining the ovaries, one can tell if a mosquito is parous (has laid at least one batch of eggs) or nulliparous (has not laid any eggs). For sporozoites

development a mosquito must live 10-12 days or more and therefore nulliparous mosquitoes are not old enough to transmit malaria parasites.

Dissection of ovaries and their examination are essential tools in entomological analysis and assessment. This unit is, therefore an extremely important part of the course.

Equipment needed

A killing tube; dissecting needles; forceps; dropper; distilled water; slides (slide box for storage).

Only females which are unfed or freshly fed (ovary stages 1-2) are suitable for this method of determining parity.

1. Nulliparous females are those which have never laid eggs.
2. Parous females are those which have laid eggs at least once.

Dissecting out the ovaries (Fig. ====)

1. Kill the female
2. Remove legs and wings
3. Place the mosquito on a slide
4. Add a drop of distilled water
5. Cut the abdomen on each side of the body between the sixth and seventh segment.
6. While holding one needle on the thorax, pull the tip of the abdomen away from the rest of the body with another needle held in the right hand.
7. The ovaries will then come out of the abdomen approximately where the Malphigian tubules or the midgut are situated.
8. Cut through the common oviduct and separate the ovaries from the rest of the specimen.

9. Transfer ovaries to a drop of distilled water on another slide and allow it to dry.
10. Six ovaries can be thus arranged on one slide unless correlations are made with ovarian stage, in which case one slide will be used for each mosquito.
11. Protect the specimens from ants and houseflies while they are drying by placing them in an ant-proof cupboard.

Differentiating between nulliparous and parous ovaries (Fig. ====)

- Examine the dried ovaries under a compound microscope using the X10 objective, and if necessary, confirm using the X40 objective.
- Females in which the ovaries have coiled tracheolar skeins are nulliparous.
- Ovaries in which the tracheoles have become stretched out are parous.
- In some females not all developed eggs are laid; if some eggs (usually less than five) are retained in the ovaries, the female is parous.

Storing ovaries

Dried ovaries may be stored for months, but must be protected from ants. They should also be stored in boxes, which contain naphthalene or camphor to prevent the growth of fungus.

Introduction

The purpose of the susceptibility test is to detect the presence of resistant individuals in an insect population as soon as possible so that alternative control plan can be made in time to deal with the situation when the insecticide in question is no longer having the desired effect. When originally investigating insect population two approaches are necessary:

- A. **establishment of base-line susceptibility of normal population** : By “normal” is meant a population never subjected to insecticidal pressure and in which resistant individuals are rare. Exposure of such a population to serial concentrations of insecticide or serial time exposures to a single insecticide concentration should yield a straight-line relationship between the logarithm of the concentration or time and probit mortalities. From such data it is possible to predict by extrapolation that concentration or time will normally kill all the individuals of a susceptible population. This is the discriminating or diagnostic concentration or time.
- B. The frequent exposure of a population under insecticide selection pressure to this diagnostic concentration or time should be done to detect the appearance of abnormally tolerant individuals and to monitor changes in their frequency.

Condition of mosquitoes

Although there is seldom a large difference in susceptibility between the sexes, female mosquitoes (preferably blood -fed) should be used exclusively in field tests. This is because they survive better and show lower control mortalities.

If mosquitoes are scarce, it is permissible to use a mixture of fed and unfed females provided the proportion of each is recorded. Mosquitoes may be collected from sprayed and unsprayed premises in the zone, but their source should be reported on the form provided. In instances where it is not possible to collect a sufficient number of adult mosquitoes for testing, specimens may sometimes be provided by collecting the immature stages and rearing them to adults, In some circumstances females without a blood meal may used exclusively e.g. those recently emerged a collection of larvae.

Conditions of test

The experiments should be carried out indoors, if possible, in buildings free from insecticidal contamination and extremes of temperature, humidity, illumination and wind. Where possible, subsequent comparison tests should be made under similar conditions of temperature and humidity. Transport of insects to a base laboratory often results in mortality from causes other than the insecticide; this will show up as high mortality in the controls.

Composition of the test fit

Equipment and\or insecticide may be ordered separately. For insecticide the order should specify both the insecticide and number of boxes for OCand of boxes for op and Carbamate papers

Equipment

- (a) 8 plastic tubes, 125 mm in length and 44 mm in diameter; 2 of which (with red dot) are used to expose the mosquitoes to the insecticides, 2 (with green dot) are used for the control exposure without insecticide and 4 (with green dot) are used as holding tubes for the pre-test sorting and post exposure observation. Each tube is fitted at one end with 16-mesh screen.
- (b) 4 slide units, each with a screw cap on either side and provided with 20 mm filling hole.
- (c) 16 sheets of clean paper (12x15 cm) for lining the holding tubes.
- (d) 8 spring wire clips to hold the papers in position against in the wall of the tubes. The 6 steel clip should be used only for the holding and the control exposure tubes; the 2 copper clips should be used for the insecticide exposure tubes.
- (e) 2 glass aspirator tubes 12 mm internal diameter, together with 60 cm of tubing and mouthpieces.
- (f) 1 roll of self-adhesives plastic tape.
- (g) Instruction sheets and 20 report forms, plus 3 sheets of log probability paper for plotting regression lines using variable times with one concentration.

Insecticides

- a. 1 box papers impregnated with DDT (p,p'-isomer) 4.0%² concentration I box papers impregnated with dieldrin concentration 0.4%²
- b. 1 box papers impregnated with malathion concentration 5.0%²
- c. 1 box papers impregnated with fenitrothion concentration 1.0%³
- d. 1 box papers impregnated with propoxur concentration 0.1%³

Procedure

In to each of the holding tubes, insert a piece of clean white paper rolled into a cylinder to line the wall and fasten it in position with a spring-wire clip (silver). Attach the slides to the tubes.

- (a) Collect up to 100 female mosquitoes with the aspirator provided (Fig. 1.1). Damage resulting from careless handling of mosquitoes collecting may produce very high mortalities. Mosquitoes should be collected in lots of not more than 10 (Fig. 1.1) and gently transferred to the holding tubes through the filling-hole in each side (Fig. 1.1) to give 15 to 25 per tube. Any departure from these figures may impair the reliability of the results.
- (b) A pre-test holding period may necessary to guard against including damaged specimens it the end of this time the damaged insects are removed.
- (c) Into each of the exposure tubes introduce a sheet of impregnated paper, rolled into a cylinder to line the wall and fastened into position with an appropriate spring-wire clip.
- (d) Introduce the mosquitoes into the exposure tube by attaching it to the vacant screw top in the slide (Fig. 1.1). The slide should be pulled out to a point beyond the filling -hole so that no part of it occludes the tube openings; the mosquitoes are slide may be filed down into the exposure tube. (If necessary, the small safety knob on the slide may be filed down to facilitate this operation. Close the slide. Detach the holding tube and set it aside.
- (e) Leave the exposure tubes standing upright with screen end up for the required exposure period (Fig. 1.1) (under conditions of moderate, diffuse illumination, and adequate humidity).
- (f) At the end of the required exposure period, transfer the mosquitoes to the holding tubes by reversing procedure (e). When some mosquitoes have been knocked down in the course of an exposure, the exposure, tubes should be held horizontally and tapped to dislodge the insects from the slide before the latter is

withdrawn. Attach the holding tube, open the slide and gently blow the mosquitoes into the holding tube; close the slide and remove the exposure tube. Then set the holding tube so that it stands on the slide and place a pad of moist cotton wool on the screen (Fig. ===.). Cardboard cartons or cups or other suitable containers may be used instead of the holding tubes, provided that they are used consistently.

- (g) Keep the holding tubes for 24 h in a secluded, shaded place, where the temperature does not exceed 30°C. Wherever feasible, the maximum and minimum temperature of the site of the holding tubes should be recorded. If necessary, the tubes should be protected from ants by placing them on a platform standing in a pan of water. If conditions are very hot and dry, a moist chamber may be prepared by suspending damp toweling in a container, and measuring the maximum and minimum temperature within.
- (h) Mortality counts are made after 24 h. Remove the dead mosquitoes by gently detaching the slide and cautiously moving the tube aside. Affected specimens that are unable to walk should be counted as dead. As an aid to counting the living specimens, they are stunned by a sharp jerk of the tube or stupefied by chloroform or ether. The anesthetics should not be allowed to come into direct contact with the plastic tube and screw cap, which are soluble in these compounds. The results should be recorded on the forms provided.
- (i) Four replicate tests should be done with each of the diagnostic concentrations and preferably, 4 controls with oil-treated papers.
- (j) Tests with control mortality in excess of 20%, though unsatisfactory, should be recorded. An investigation into the causes of control mortality should be made and steps taken to avoid it. A possible cause may be the collection of mosquitoes from sprayed dwellings. In this case, it may be necessary to collect specimens from unsprayed ones, or to test adults reared from aquatic stages.

4. General Remarks

Each impregnated paper may be used up to 20 times, and up to 3 weeks after removal from the package, provided all possible precautions are taken against evaporation of the insecticide solution. Organochlorine papers can be left in the tubes, with the open end well wrapped, and placed in the kit box, which in turn should be kept in a cool place. No paper should be used more than 3 weeks after removal from the package

For organophosphates and Carbamates, impregnated papers should not be left for more than 3 weeks in exposure tubes and the same precautions for wrapping and storing should followed.

After an impregnated paper has been removed, the package should be resealed carefully with the plastic tape provided. The packages should be kept in a cool place, but not in a refrigerator, as too low a temperature may cause crystallization in the higher insecticidal concentrations. Prolonged storage at high temperatures should be avoided and papers should not be used after the expiry date shown on the box. The expiry date is valid only if the packages are kept sealed at all times.

Results

(a) Percentage mortality should be recorded on the report form. If the control mortality is between 5% and 20% the percentage mortalities should be corrected by Abbott's formula

$$= \frac{\% \text{ Test Mortality} - \% \text{ Control Mortality}}{100 - \% \text{ Control Mortality}} \times 100$$

(b) Results obtained where control mortalities exceed 20% should be recorded but not corrected. The accuracy of the interpretation of results depends on the reliability of the data obtained. Utilizing the maximum number of specimens per tube (25) decreases the effect of individual differences in response.

Distribution of Reports

It is of considerable importance that WHO should receive copies of results obtained from the use of this test kit. It is therefore requested that copies be sent to the following addresses

For Anopheline species;

1. World Health Organization, Malaria Action Programme, 1211 Geneva, Switzerland
2. The appropriate WHO Regional office; and
3. Project Headquarters.

The fourth-copy should be retained by the investigator.

For non-Anopheline species;

1. World Health Organization, Division of Vector Biology and Control 1211 Geneva, Switzerland ; and
2. The appropriate WHO Regional office.

The third and fourth copies should be retained by the investigator.

Tentative diagnostic concentrations and exposure times for adult mosquitoes

	Anophelines		C. quinquefasciatus	
DDT	4%	1 hour	4%	4 hours
Dieldrin	0.4%	1 hour	4%	1 hour
Malathion	5%	1 hour	5%	1 hour
Fenitrothion	1%	2 hours	1%	2 hours
Propoxur	0.1%	1 hour	0.1%	2 hours
Chlorphoxim	4%	1 hour	-	-
Permethrin	0.25%	1 hour	0.25%	3 hours **
Deltametrin	0.025%	1 hour	0.025%	1 hour**

*Except for An. Sacharovi.

**Exposure tubes held flat so that mosquitoes that are knocked down remain in contact with the paper during the entire time specified.

LARVAL SUSCEPTIBILITY TEST

Introduction

Purpose and limitations of the test

The purpose of the susceptibility test is to detect the presence of resistant individuals in a mosquito larval population as soon as possible so that alternative control plans can be made in time to deal with the situation when the insecticide in question is no longer having the desired effect.

When originally investigating larval population two approaches are necessary

- a. The establishment of the base-line susceptibility of a normal population. By 'normal' is meant a population never subjected to insecticidal pressure and in which resistant individuals are rare. Exposure of such a population to serial concentrations of insecticide should yield a straight-line relationship between the logarithm of the concentration and probit mortalities. From such data it is possible to predict by extrapolation that concentration which will normally kill all the individuals of a susceptible population. Double this concentration is taken to be the discriminating or populations lacking resistant genes.
- b. The frequent exposure of a population under insecticide selection pressure to this diagnostic concentration should serve to detect the appearance of abnormally tolerant individuals and to monitor changes in their frequency.

Establishing the base-line

Batches of mosquito larvae exposed to different concentrations of insecticides and the mortality at each level is determined. It is suggested that a

preliminary test be made on a wide range of concentrations using the standard exposure of 24 hours. This will indicate the general level of susceptibility; further tests should then be made with at least 4 concentrations, some of which will give partial mortality (i.e. at least one of them should give 100% mortality and two from 5 to 50% mortality.). Four replicate tests at each concentration should then be made and from the results a log-probit regression line constructed on the logarithmic-probability paper provided, The line should be straight if the population is homogeneous and if so, the concentration expected to produce 99.9% mortality can be extrapolated from it

Subsequent routine check by diagnostic concentrations

In routine monitoring for resistance, it is not necessary to employ the full range of concentration used to establish the baseline of susceptibility. Only the diagnostic concentration should be used. Tentative diagnostic concentrations for mosquito larvae are shown in Table 1.

These data were obtained with IV instar at 23°C. It is possible that they will not correspond to those for wild-caught larvae under field conditions and are only given for guidance; they should be checked under various field conditions before use.

Tests at the diagnostic concentration should be repeated periodically with at least four replicates of 25 larvae. The occurrence of survivors at this concentration will rarely be due to normal variability provided that the physiological age and condition of the larvae and the experimental conditions are the same as when establishing the base-line data. If survivors are repeatedly found resistance is confirmed.

Composition of the Kit

Equipment and insecticide should be ordered separately. For insecticide the order should specify the insecticide and the number of each standard solution.

Equipment

- (a) 4 1-ml pipettes for insecticide and 1 for ethanol and 5 rubber suction bulbs.
- (b) 3 droppers with rubber suction bulbs.
- (c) The following materials for use in making a strainer; 2 wire loops, 1 piece of nylon netting (30cm²) and 1 tube of cement. It is suggested that 2 pieces of netting be cut and cemented to opposite sides of the large end of the wire loop, More cement should then be applied around the outside of the loops to join the 2 pieces of netting when dry, the netting may be trimmed with scissors. The kit contains sufficient netting for replacement purposes.
- (d) Instruction sheets, 20 report forms and 3 log-probability papers for plotting regression lines.

The user is expected to provide his\her own collecting and test vessels. Disposable plastic cups which hold 250 ml to a rim may be used when available.

Insecticides

Standard solutions

Malathion	781.25mg\l,	156.25mg\l,	31.25mg\l,	6.25mg\l
Temephos	156.25.mg\l,	31.25mg\l,	6.25mg\l,	1.25mg\l
Bromophos	31.25mg\l.	6.25mg\l,	1.25mg\l,	0.25mg\l
Fenitrothion	“	“	“	“
Febthio	“	“	“	“
Chlorpyrifos	6.25mg\l,	1.25mg\l,	0.25mg\l,	0,05mg\l

All these alcoholic solutions are supplied in 50 ml bottles. One 50 ml bottle alcohol for control is supplied for solutions and of 4 standard solutions or less.

Caution

Alcohol used for solutions and control has been denatured by addition of 2% butanone.

Procedure

Base-line

- (a) For a complete test with one insecticide, sufficient larvae should be collected from the field in order that about 300 individuals of the same species may be selected; they should be in their third or early fourth instar and should be retained in the water in which they were collected until selected for testing. Any larvae showing abnormalities, for example a fuzzy appearance due to the presence of parasites on the body surface, should be discarded. Lots of 20-25 larvae are distributed in each of 12 ml. beakers, each containing 25 ml of water. Their transfer is effected either by means of the strainer, or by means of a dropper during the process they should be rinsed lightly in clean water.
- (b) Into each of 12 glass vessels, approximately 7.5. - 10 cm in diameter, (Jars, bowls or 500 ml beakers) place 225 ml of water. The vessels should be such that the depth of Jar is between 2.5 and 7.5cm. Distilled water or tap water may be used or even water obtained from a well or stream, but it should be as free as possible from chlorine or organic contaminants. It should be noted that distilled water obtained commercially may contain traces of poisonous heavy metals and this will give high mortalities in the controls. Certain species such as salt-marsh or tree-hole mosquitoes may suffer upon transfer to relatively pure water, an effect that will also be reflected in high control

mortalities; in this case water from the breeding site should be used provided that it is free from insecticides and filtered to exclude most of the organic matter. The average temperature of the water should be approximately 25°C, it must not be below 20°C or above 30°C .

- (c) Prepare the test concentrations by pipetting 1 ml of the appropriate standard insecticide solution just above the surface of the water in each of the glass vessels and stirring vigorously for 30 seconds with a glass rod. In preparing a series of concentrations, the most diluted should be prepared first. There should be 2 replicates at each concentration, and 2 control-replicates. The 2 controls should be prepared by the addition of 1 ml of the alcohol to the water in each container.
- (d) Within 15-30 minutes of the preparation of the test concentrations, add the mosquito larvae to them by tipping the contents of the small beakers into the vessels.
- (e) After a period of 24 hours, make mortality counts. In recording the percentage mortalities for each concentration, the moribund and dead in both replicates should be combined. Dead larvae are those that cannot be induced to move when they are probed with a needle in the siphon or the cervical region. Moribund larvae are those incapable of rising to the surface (within a reasonable period of time) or of showing the characteristic diving reaction when the water is disturbed; they may also show tremors incoordination or rigor.
- (f) Discard the larvae that have pupated during the test. If more than 10% of the control larvae pupate in the course of the experiment, the test should be discarded. Tests with a control mortality of 20% or more are unsatisfactory and should be repeated.

- (g) When 4 replicates have been performed with the same population of mosquito larvae, adequate data should be available for constructing a base-line susceptibility. The results should be recorded on the forms provided.

Diagnostic concentrations

For routine checks, the same procedure is applicable except that larvae are exposed to only one concentration, established from the base-line data or given for guidance in Table .

General remarks

- (a) The accuracy of the concentrations provided will be affected if the alcohol is allowed to evaporate from the standard solutions. The bottles should therefore be tightly stoppered after use. The contents should no longer be used when they have decreased below 5ml; fresh standard solutions should then be prepared from the stock solutions.
- (b) Test vessels should be carefully cleaned after use to remove traces of insecticide. They should be thoroughly rinsed, scrubbed with detergent and water (or cleaned with potassium dichromate and sulfuric acid), and rinsed again. Pipettes should thoroughly cleaned with acetone or alcohol.

Results

To construct the dosage-mortality regression line the results obtained should be plotted on the logarithmic-probability paper provided. The regression line may be fitted by eye and the concentrations expected to kill various percentages can be read from it. The concentration to kill 50% is known as LC50%; that 95% kill as LC95, etc. The curve can be extended to estimate the LC99.9 (though it must be realized that this is very approximate).

If the control mortality is between 5% and 20% the percentage mortalities should be corrected by Abbott's formula

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

The accuracy of the interpretation of results depends on the reliability of the data obtained. Utilizing the maximum number of specimens per test (25) decreases the effect of individual differences in response. Regression lines based on similar numbers of specimens offer greater reliability.

Table: Tentative diagnostic dosages for larval mosquitoes (mg / litre)

	Anopheles	Culex quinquefasciatus	Aedes aegypti
Malathion	3.125	1.00	1.0
Fenitrothion	0.125	0.125	0.06
Fenthion	0.05	0.05	0.05
Temephos	0.25	0.02	0.02
Chlorpyrifos	0.025	0.01	0.01

BIO-ASSAY

Introduction

Bioassays are methods for estimating the potency of a material by means of the response of living matter to it. The main objective of this bio- assay test is to assess the potency of an insecticide deposit for adult mosquito at a various times after application on different surfaces and thus to detect the onset of a definite decline in the toxic effect of the deposit due to ageing sorption or other factors. The test is designed to provide information that may assist in (a) comparing the residual action of different insecticides or insecticide formulations, and (b) ascertaining whether or not the spraying has been carried out satisfactorily. The method will not measure the amount of insecticide remaining on the wall nor will it by itself measure the overall rate of mortality of vectors being achieved during the campaign the campaign, since can be assessed only by other entomological measurements in the area.

It is recommended that where possible such elementary methods as window -trap collections and survival tests be used in conjunction with bioassay test. This is particularly advisable where the bioassay method is used to determine the time at which an insecticidal deposit on a given surface has lost its potency or to decide on the appropriate insecticide dosage and spacing of spraying cycles for effective vector control. The method is not suitable for measuring the susceptibility or resistance of a population.

Conditions of the test

Rigid rules cannot be laid down as to the number and frequency of bioassay tests should be conducted in various parts of the world. It must be recognized that each geographic area has its own set of conditions with respect to rainfall, temperature, humidity surfaces, and vector species. Therefore, the use to which

the test is put in a given area and the interpretation of the results will necessarily depend on local conditions. With these factors in mind the following guiding principles are suggested

- (a) The first tests for the evaluation of any given insecticide should be undertaken within a few days after its application or as soon as the deposit is completely dry. Where a previously sprayed area is due for bioassays should be performed beforehand to ascertain the potency of the deposits present.
- (b) Using this method, it is of great importance to carry out the tests on an adequate scale and at regular intervals, It is necessary to and to evaluate separately the potency of the insecticide deposit on each main type of resting surface. On a given type of surface, not less than 10 points, variously situated, should be chosen for more than 3 points being in any one house. At least 2 controls should be used for every 10 bioassay tests. The control runs are carried out in a suitable situation away from the sprayed premises and on an unsprayed surface. For this purpose, it is suggested that the investigator carry with him a stock of index cards or similar unsprayed material to be discarded after each test.
- (c) When the main objective of the investigator is to determine the rate of loss of potency, there will be advantages in using the same points throughout the series of tests. He should therefore mark the points carefully at the time of the first tests and should take particular care to avoid rubbing or in any way impairing the deposit at these points during the performance of the tests. If, on the other hand, different points are selected for the subsequent tests, more information will be gained on the overall potency of the insecticide deposit on the type of surface examined.
- (d) Subsequent routine checks. Following the initial bioassay. Subsequent tests should be made at intervals not exceeding 1 month. If successive tests are

carried out at the same time of day the comparability of the date will be improved.

- (e) Choice of test insect. Wherever possible, the local vector species should be used in the bioassay. If there is more than one vector species, each should be used separately, and it is preferable to take wild-caught specimen because of their greater hardiness. If a species of mosquito other than the local vector is used, it should preferably be from a laboratory colony. It is recommended that the mosquitoes selected for test be females, all of which have recently been fed and show the presence of a blood meal. The susceptibility of the mosquito population to the insecticide being bio-assayed must be established before the first bio-assay (using the standard adult mosquito test kit) and re-checked each time a cycle of testing is carried out in order to ensure the validity of the bio-assay results. A marked change in susceptibility during the course of the bioassay testing would invalidate the results.

Composition of test kit

- (a) 24 conical chambers of transparent plastic, 8.5 cm in diameter at the base and 5.5 cm high.
- (b) 4 hard-glass aspirator tubes, 1 cm in outside diameter (with one end bent so as to facilitate removal of mosquitoes from the exposure chamber), together with 60cm of flexible rubber or plastic tubing. These tubes are suitable for handling mosquitoes of ordinary size. For very large species, tubes with an inside diameter of 12mm should be used. These tubes can be of glass or transparent plastic.

The exposure chambers are best loaded with a straight glass or plastic tube of appropriate diameter and this is not supplied in the test kit.

- (a) 2 rolls of adhesive plastics tape

- (b) 1 box of upholstery tacks with large heads
- (c) 2 rolls of adhesive plaster
- (d) Instruction sheets

Procedure

The exposure chamber is fastened to the selected spot on the surface to be tested with the upholstery tacks, or with some appropriate device that will hold the chamber tight against the surface. It is often advantageous to fasten a strip of the plastic sponge tape to the flange of each test chamber and leave it there permanently. Particular care should be taken flange of each test chamber on the surface while it is being attached or removed.

At least 10 but not more than 15 mosquitoes are collected with a straight 'loading' tube and introduced into the chamber by blowing gently, great care being taken that the end of the tube does not touch the test surface. There should be no backpressure; this can be avoided by boring a dozen small holes at the outermost tip of the exposure chamber.

The cage containing the stock of mosquitoes to be used in the bioassay test should never be taken inside a house that has been sprayed with insecticide but should be left on insecticide-free surfaces outside the houses. The cages should be handled with care so as not to contaminate them with insecticide from the hands of the operator.

- (a) The chamber is left undisturbed for a standard period of time, long enough to cause 100% mortality of the test mosquitoes. It has been found that 30 minutes is usually suitable and it is recommended that this period be chosen when bioassays are first stated in a given area. However, a longer period may be used if necessary.

- (b) At the end of the exposure period, the mosquitoes are collected carefully by means of the bent transfer tube exposure period, the aperture of the chamber (without touching the test surface) and are transferred immediately to the holding containers. Paper cups of about 250-ml capacity are cheap and convenient but other type of container may be used.
- (c) The room temperature and relative humidity are recorded at the beginning and end of each day's testing, and at hourly intervals during the work period.
- (d) The recovery cages are kept for 24 hours in a secluded shaded place where the temperature does not exceed 30°C if feasible. Maximum and minimum temperatures during the recovery period should be recorded. The humidity may be kept high by use of damp toweling where necessary.
- (e) Exposure chambers and transfer tubes are carefully washed in detergent after each use, rinsed, and allowed to drain dry. No attempt should be made to reserve certain chambers and tubes for the control mosquito, and others for the test mosquitoes.
- (f) **Warning.** It is of vital importance; it is essential to use the same transfer tube, the course of the day's work. To prevent the undetected occurrence of such contamination, it is essential to use the same transfer tube first for the test mosquitoes and then for the control mosquitoes. If a single transfer observed in the control mosquitoes and then for the control mosquitoes is used for 4 or 5 test chambers and then for a control chamber, and a high mortality is invalid, and the tested should be repeated using a separate transfer tube should be considered and for the control. Mosquitoes, all results with that transfer tube should be considered and for the control. If the transfer tubes should become contaminated with insecticide removed by suction or in any other way from the wall surfaces being tested, it becomes impossible to ascertain how much of the observed mortality is due to the exposure of the

mosquitoes on the wall surface and how much to insecticide in the transfer tube or holding containers. Even when a separate transfer tube is used for each chamber, there is some risk that insecticide will be departed transfer from the wall surface to the holding container inadvertently increased.

Results and interpretation

After 24 hours, the dead and live mosquitoes are counted. It is essential that observed mortalities (in%) be recoded for each individual test. If it is found that the control mortality is above 10%, it is recommended that the number of controls in the subsequent series of tests be increased to 4 for each series of 10 tests. Where control mortalities exceed 20%, the series of tests should be considered unsatisfactory and repeat possible.

The mortalities on the different points (on one type of surface only) are average where the control mortalities between 5% and 20%, the average observed mortality is corrected by Abbott's formula

$$= \frac{\% \text{ Test Mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

Immediately after the average value, the lowest and highest values should be written in parentheses, thus; '68% (20%-90%)', to indicate the range of variation in the results. Wide differences in mortality rates from one point to another may reflect either the unevenness of spraying or a differential in the rate of loss of potency, due by the particular composition of the surface, soot that may be deposited on the surface by domestic fires, the microclimate, or other localized variables. Even if all such factors

could be eliminated, there would still remain as a cause of variability the inherent differences in susceptibility of the individual mosquitoes used in the tests.

A marked drop in the bioassay mortality in a spray, area. While not in itself proving that a fresh application of insecticide is necessary, can underline the need for other investigations on the vector species designed to evaluate the continued effectiveness of vector control.

ENTOMOLOGICAL PARAMETERS

An understanding of entomological parameters is important in the study of dynamics of vector borne diseases in a given terrain both in presence or absence of anti-vector measures. These parameters are useful in assessment of intensity of transmission in an area as well as assessment of anti vector measures, the proper understanding of which depends in integration of values obtained under individual parameter. The important entomological parameters used in various vector control programmes may be arbitrarily grouped into two important aspects as follows:

1. Vector Control

The following important parameters are collected:

- II. Mosquito density - Per Man Hour Density, Per Room Density etc.,
- III. Out-door mosquito population sampling
- IV. Larval density in breeding places
- V. 24 hours survival of vectors collected from sprayed houses
- VI. Bio-assay - contact bio-assay and susceptibility test Aerial bio-assay
- VII. Abdominal condition of female mosquitoes
- VIII. Parity Rate

2. Disease Transmission - for this following parameters are usually collected:-

- I. Man-biting rate - man mosquito contact
- II. trap collections - light/bait
- III. Seasonal ineffectivity of vector and incrimination of vectors
- IV. Anthropophilic index

V. Duration of gonotrophic cycle

The important entomological parameters used in various vector control programmes are in brief as under:

1. Adult Mosquito Density: PMD (Per Man hour Density) PRD (Per Room Density)

A. Per Man Hour Density

Method

This index is calculated for each vector species and total anophelines. Index is calculated from the daytime hand collection made by the Insect Collectors. Aspirators tube flash light technique is commonly used for collecting the mosquitoes.

$$\text{Per Man Density} = \frac{\text{No. Mosquitoes (male \& female) collected}}{\text{No. of Insect Collector} \times \text{Time spent in search in hour}}$$

Significance

This parameter is useful to know:

- a) Mosquito fauna of the area
- b) Seasonal prevalence of mosquitoes and vectors
- c) Resting habits, both in-doors and out-doors
- d) Impact of vector control measures.

B. Per Room Density (from Pyrethrum Space Spray Catch)

Method

The pyrethrum solution is prepared by mixing one part of 2% Pyrethrum Extract with 19 parts of kerosene oil and sprayed in suitable rooms whose openings (windows and doors) can be temporarily closed. The above pyrethrum solution is

sprayed at the rate of ½ oz. Per 1000 sq.feet room space. Before spraying white cotton sheets are stretched at the floor and after spray the room is closed for sometime. The knock down mosquitoes are collected, identified and recorded. After making such collections 3-4 rooms, the indoor resting mosquito per room on an average may be calculated.

ii) Significance

- a) Determination of mosquito/vector prevalence in an area of low indoor resting densities
- b) Specially useful in places where indoor hiding mosquitoes are not easily detected through hand collection method
- c) When large number of mosquitoes is required for dissection.

C. Out-Door Collection (Per Man Hour Density) through trapping devices

- i) Method - Hand collections of mosquitoes in outdoors are made through trapping devices like pit-shelters, box shelters etc.
- ii) Signification - This parameter indicates dispersal of mosquito population in space and time.

D. Mosquito collection on animals

- I. Method - Hand collection with the help of aspirator tube and flashlight, from the animals in the proximity of human dwelling is done during the usual hours.
- II. Significance
 - (a) This parameter indicates preference of mosquitoes for animals, or human for blood meals.

(b) If mosquito has predilection for animals, the latter may be used as zooprophyllactic.

2 Larval density

(i) Method

Larval collection is done with the help of standard ladle, net well net etc. The common approach is to work out per dip density of larvae. Commonly a minimum of four dips is applied in each breeding places at different points and number of larvae an average per dip is determined.

(ii) Significance

- a) An auxiliary method of detection of vector prevalence in time and space.
- b) This parameter is used in understanding mosquito breeding habits.
- c) This indicator is used in assessing the antilarval measures in urban areas.

3. Man Mosquito Contact

A. Man biting rate

(i) Method

Mosquitoes are collected from the human baits in the night during usual sleeping hours. Index is determined either indoors or outdoors usually from 6.00 P.M. to 6.00 A.M. Hourly collections are recorded. No. of mosquitoes collected per night on each bait becomes the parameter.

(ii) Significance

- a. This parameter helps in understanding the vectorial potency and quantum of man mosquito contact in space and time.
- b. This indicates differential man feeding propensity.
- c. Helps in understanding the site of vector-man contact.

- d. Helps in deciding whether indoor residual insecticidal spraying is advisable. To understand animal biting rate, collection may be done on animal baits.
- e. Helps in understanding Endophagy or Exophagy

B. Human / Animal bait traps

(i) Method

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

(ii) Significance

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

4. 24 Hours Survival Rate

(i) Method

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

(ii) Significance

- a) This indicator gives an idea about the impact of residual insecticide spray.

b) This gives indication of inherent population characteristic of daily mortality.

5. Seasonal Infectivity of Vectors / Sporozoites Rate

(i) Method

Mosquitoes collected from known positive houses and nearby houses are dissected in 0.68% Saline for Salivary glands dissection. The glands so obtained are to be examined under Compound Microscope. The sporozoite rate (infectivity rate) may be calculated as follows

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

(ii) Significance

The significance of this parameter is

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

6. Oocyst Rate

(i) Method

Mosquitoes are dissected for gut, which may be examined under low power for presence of Oocyst on the gut wall. The rate may be calculated as follows.

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

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

(ii) Significance

The above rate is useful in;

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

7. Vector infection rate (VIR)

Proportion of vector females containing developing (I & II) and developed (III) forms of filarial parasite.

$$\text{VIR} = \frac{\text{No. of vector females with I, II and III stages of parasite}}{\text{Total No. Vector females dissected}} \times 100$$

Significance

Crude Index for the extent of sources of human infection

8. Vector infectivity rate

Proportion of vector females with developed stage (III) only.

$$\text{VIR} = \frac{\text{No. of vector females with III stage alone}}{\text{Total No. Vector females dissected}} \times 100$$

Significance

Indicates Transmission potential in community.

9. Average No. of Infective larvae per infective mosquito

10. Parity rate

1) Method

a) Detinova technique

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

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

b) Polovodova technique

Here also ovary of the mosquito is dissected as above and the follicular dilatations in the ovariole stalk are observed under microscope and the number of dilatations on knots counted. Each dilatations represents the happening of one egg-laying. The rate is calculated as under:

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

(ii) Significance

This parameter is useful in;

- a) Determining the physiological age of the mosquito
- b) Evaluating the impact of anti-vector measures
- c) Determining the structure of vector population

11. Bio-assay tests

A. Contact Bio-assay

(i) Method

Mosquitoes are exposed to a sprayed wall surface for a standard period of time (usual 30 minutes as per standard WHO method and mortality is recorded at the end of 24 hours holding period.

(iii) Significance

This parameter helps in

- a. Determining the residual efficacy of insecticide on different surface and in different duration of time.
- b. Evaluating the equality of insecticidal spraying.

B. Aerial Bio-Assay

i) Method

- a. Mosquitoes from unsprayed areas/lab bred are kept in a small mosquito cage and later is suspended in the middle of a sprayed room. The mortality in the mosquito is observed after 24 hours and the cage is taken out of the room.
- b. The caged mosquitoes may be placed outside the house at suitable places for assessing the efficacy of fogging.

ii) Significance

This parameter helps in

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

12) Mosquito/vector susceptibility to insecticides

A. Determining susceptibility status of adult mosquitoes

i) Method

Blood feed female adult mosquitoes are exposed to diagnostic doses of insecticides for standard time i.e. DDT 4% X 1 hr; Dieldrien 0.4 x1 Hr. Malathion 5% X 1 Hr. Fenitothion 1% X 2 Hrs.; and propoxure 0.1 X 1 Hr. The mosquito mortality is recorded at the end of 24 hours holding period. The control mortality if 5 to 20% the test mortality may be corrected by following Abbot's formula.

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

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

Significance

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

B. Susceptibility test of mosquito larvae

Method

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

Significance

- a. To establish baseline susceptibility level of vectors to different insecticides before these insecticides are put to usage and subsequently monitor any change in susceptibility level in course of time.

- b. To change the existing control measures or to suggested alternative control methods.

Anthropophilic index

Method

Bloodmeal samples from freshly fed females are collected on filter papers and the human blood index is determined either by precipitin test or gel-defusion test or any other method and the index is calculated as under.

$$\text{HBI} = \frac{\text{No. of mosquitoes showing source of human blood}}{\text{Total No. of mosquitoes whose blood meals have been identified.}}$$

Significance

This index is important in understanding;

- a) The feeding preference and biting frequency of mosquito on men
- b) Change in feeding behaviour of mosquitoes.

Abdominal conditions of female mosquito

Method

The mosquitoes collected during early morning hours are examined for abdominal conditions, like, fullfed, unfed, semi-gravid & gravid and recorded. The percentage of each type may be worked out.

$$\% \text{ Full fed} = \frac{\text{No. of mosquitoes with FF abdominal}}{\text{Total nos. of mosquitoes examined}} \times 100$$

Significance

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

Duration of Gonotrophic cycle

Method

- a. Direct observations on known fed mosquitoes in cage
- b. Estimate ratio of bloodfed and gravid and semigravid in morning pyrethrum space spray collection.

Significance

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

Determining the proportion of parous females

$$\text{The proportion of parous females} = \frac{\text{No.of parous females}}{\text{Total No.of females examined}}$$

$$\text{Parous rate} = \frac{\text{No.of parous females}}{\text{No.of females examined}} \times 100$$

For container breeders

House index % Houses & their premises positive for Immatures (More than 10 % High less than 1% Low Risk).

Container Index % Water Holding Containers positive for Imatures

Breteau Index No. of Positive containers per 100 houses (100 houses ideal; More than 50% High Risk; Less than 5% Low Risk).

Adult Biting / Landing Rate: No of mosquitoes collected at bait per unit of time. Collections at selected dimly lit rooms; 15 - 20 mts. / room; Timings: 9:00 -11:30 & 5:30 16:00 hrs. Collecting PMHD (More than 2 High Risk ; 0 - 2 Low Risk).

Significance

1. Vector prevalence / potential in time & space
2. Breeding Habits
3. Impact of control measures
4. Variations in their relative abundance

Man Biting Rate

Collecting mosquitoes directly while landing or during the process of biting a human / animal host throughout night (18:00 -6:00 hrs) for night biting species.

Hourly collections

1. Average No. of mosquitoes / Bait / Night is estimated.
2. Date to be monitored at least once a month for a year.
3. All females, to be processed for Age, infection and Infectivity.

Significance

1. Vectorial potency / Quantum of Man-Mosquito contact
2. Indicates differential man feeding propensity
3. Site of Vector - Man contact
4. Man - Animal Biting Ratio
5. Feeding Behaviour (Endophagy / Exophagy)

Sand fly

Age Determination: Usual method of age determination of sand flies is the examination of ovariole relics. The ovaries are dissected in sterile saline and the ovarian follicles are examined for dilatations. Each relic represents one gonotrophic

cycle. The examination of accessory glands for secretory granules also provides criteria for determination of age (parity).

Host Preference: The blood meal of a freshly fed sand fly is sampled on a filter paper which may be subjected to precipitin test, Gel-diffusion technique or ELISA to determine the source of blood meal.

Vector incrimination: After dissecting a sand fly in sterile saline, midgut is examined for presence of flagellates. If found positive, head should also be dissected for examination of cibarium, pharynx and proboscis. The promastigotes must be spread on a slide, fixed with methanol and stained with Giemsa or Leishman stain. The presence and promastigote, however does not confirm the species of the parasite as all promastigotes are morphologically indistinguishable. For confirmation, samples should either be subjected to xenodiagnosis or to biochemical characterization of parasite.

Determination of susceptibility to insecticide: The conventional WHO susceptibility test kit must be used. Freshly fed *Ph. argentipes* can be subjected to preliminary screening on the basis of silvery white legs. However, after recording the data, all sandflies, subjected to test must be examined under microscope after mounting and due corrections be made in the observations before interpreting the results.

Introduction

Each year thousands of public health insecticides containers are emptied and become waste items that require disposal. . All these insecticides are registered by Central Insecticide Board for public health use in the country with safety levels . The Insecticides Rules, 1971 has a provision (Rule 44) that sets clear cut guidelines for disposal of used packages, surplus materials and washings of insecticides. FAO/WHO recommends that the practice of disposal of insecticide packaging at the place of use by burying or burning be prohibited. For many years, the laws and regulations governing the safe use of insecticides also have placed some restrictions upon their disposal. Label instructions include warnings about container and rinse water disposal, and caution against the contamination of foods, feeds and water supplies. Disposal inconsistent with label instructions is a violation. Newer product labels show more extensive disposal instructions.

The NVBDCP aims to achieve effective vector control by the appropriate biological, chemical and environmental interventions of proven efficacy, separately or in combination as appropriate to the area through the optimal use of resources. Integration of Integrated Vector Management (IVM) is done by using identical vector control methods to control malaria and Kala-azar and other vector borne diseases . Measures of vector control and protection include:

1. Measures to control adult mosquitoes in rural areas: Indoor Residual Spray (IRS)

2. Anti-larval measures in urban areas : chemical, biological and environmental
3. Personal protection: use of bed nets, including insecticide treated nets (ITNs) and Long Lasting Treated Nets (LLINs).
4. Integrated Vector Management (IVM).

The NVBDCP is currently using IRS as the primarily method of vector control in rural settings, and anti-larval measures in the urban areas. Insecticides like DDT, Malathion and Synthetic Pyrethroids are recommended in the programme for IRS. The state health departments will be responsible for safe disposal of DDT and other insecticides. General safety precautions while handling insecticides and guidelines for proper storage, transportation and safe disposal of insecticides and insecticide containers are mentioned below for further reference.

General safety precautions while handling insecticides

Exposure to insecticides may occur when handling and spraying insecticides. The exposures to insecticides may occur in following situations:

- When handling the insecticide product during opening of the package, mixing and preparation of the spray.
- When spraying the insecticide.
- When disposing the insecticide solution and containers

General precautions:

1. The operator should also wear a protective hat and face shield or goggles.
2. Do not eat, drink or smoke while working.
3. Wash hands and face with soap and water after spraying and before eating,

smoking or drinking.

4. Shower or bath at the end of every day.s work and wear new clean clothes.
5. Wash overalls and other protective clothing at the end of every working day in soap and water and keep them separate from the rest of the family.s clothes.
6. If the insecticide touches the skin, wash off immediately with soap and water.
7. Change clothes immediately if they become contaminated with insecticides.
8. Inform the supervisor immediately if one feels unwell.

Protective clothing and equipment

Absorption of insecticide occurs mainly through the skin, lungs and mouth. Specific protective clothing and equipment given below must be worn in accordance with the safety instructions on the product label

- Broad-rimmed hat (protects head, face and neck from spray droplets).
- Face-shield or goggles (protects face and eyes against spray fall-out).
- Face mask (protects nose and mouth from airborne particles).
- Long-sleeved overalls (worn outside of boots).
- Rubber gloves.
- Boots.

Storage

1. Insecticide storehouses must be located away from areas where people or animals are housed and away from water sources, wells, and canals.
2. They should be located on high ground and fenced, with access only for authorized persons. However, there should be easy access for insecticide delivery vehicles and, ideally access on at least three sides of the building for fire-fighting vehicles and equipment in case of emergency.

3. Insecticides must NOT be kept where they would be exposed to sunlight, water, or moisture which could affect their stability.
4. Storehouses should be secure and well ventilated.
5. Containers, bags or boxes should be well stacked to avoid possibility of spillage. The principle of **.first expiry first out.** should be followed.
6. Stock and issue registers should be kept upto date. Access to the insecticides should be limited to authorized personnel only.
7. The store room should have a prominently displayed mark of caution used for poisonous or hazardous substances. It should be kept locked.
8. Containers should be arranged to minimize handling and thus avoid mechanical damage which could give rise to leaks. Containers and cartons should be stacked safely, with the height of stacks limited to ensure stability.

Transportation

1. Insecticides should be transported in well sealed and labeled containers, boxes or bags.
2. Insecticides should be transported separately. It should NOT be transported in the same vehicle as items such as agricultural produce, food, clothing, drugs, toys, and cosmetics that could become hazardous if contaminated.
3. Pesticide containers should be loaded in such a way that they will not be damaged during transport, their labels will not be rubbed off and they will not shift and fall off the transport vehicle onto rough road surfaces.
4. Vehicles transporting pesticides should carry prominently displayed warning notices.
5. The pesticide load should be checked at intervals during transportation, and any leaks, spills, or other contamination should be cleaned up immediately using accepted standard procedures. In the event of leakage while the transport vehicle is moving, the vehicle should be brought to a halt

immediately so that the leak can be stopped and the leaked product cleaned up. Containers should be inspected upon arrival at the receiving station. There should be official reports to the national level and follow-up enquiries in the event of fires, spills, poisonings, and other hazardous events.

Disposal of remains of insecticides and empty packaging

1. At the end of the days work during IRS activities, the inside of the spray pump should be washed and any residual insecticide should be flushed from the lance and nozzle.
2. The rinsing water should be collected and carefully contained in clearly marked drums with a tightly fitted lid. This should be used to dilute the next days tank loads or disposed properly by the supervisor at disposal sites like pits or digs.
3. Never pour the remaining insecticide into rivers, pools or drinking-water sources.
4. Decontaminate containers where possible. For glass, plastic or metal containers this can be achieved by triple rinsing, i.e. part-filling the empty container with water three times and emptying into a bucket or sprayer for the next application.
6. All empty packaging should be returned to the supervisor for safe disposal according to national guidelines.
7. Never re-use empty insecticide containers.
8. It shall be the duty of manufacturers, formulators of insecticides and operators to dispose packages or surplus materials and washing in a safe manner so as to prevent environmental or water pollution.
9. The used packages shall not be left outside to prevent their re-use.
10. The packages shall be broken and buried away from habitation.

Disposal of Expired Insecticides

1. Adequate measures should be undertaken to avoid expiry of stocks in storehouses.
2. First Expiry First Out. principle should be strictly followed during stock movements.
3. Information about near expiry stock, should be provided to Dte. of NVBDCP, Delhi well in time so that the stock can be re-allocated to other locations.
4. The expired stock should be returned to manufacturer for disposal as per guidelines preferably through incineration process.
5. The chemical efficacy should be tested before disposal of expired insecticide to find out possibility of usage. The efficacy and active ingredient percentage of insecticide is tested and certified by the authorized testing laboratory.

DESIGN FOR THE SOAK PITS

A soak pit is a specially designed hole in the ground for disposing of insecticide remnant after the day's IRS activities. A properly sited and constructed soak pit protects the environment from getting contaminated with insecticides.

Siting of the Soak Pit

The soak pit should preferably be located within the unused areas of Sub-

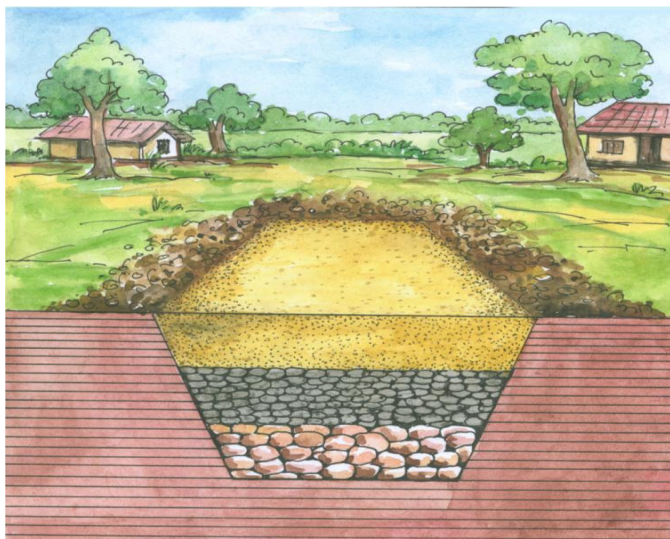


Centre/ Panchayat/ government offices of a village. However, such pits should not be within 100m of any water body or drinking water source. The soak pit should be

constructed only in areas where ground water table is at a depth of more than 5m below ground level.

Construction of the Soak Pit

A soak pit measuring 1m by 1m by 1m is usually sufficient absorb the effluent produced from one round of spraying operation. The bottom of the pit is lined with a layer of coarse gravel followed by a layer of stone aggregate. It is then filled with 1.5 to 2 bags



charcoal (where feasible) and 1.0 to 1.5 bags of sawdust/sand/ morrum/coarse soil. This would create a filter one meter in depth. As the effluent percolates through this filter medium, the insecticides filter out.

Disposal of the residue

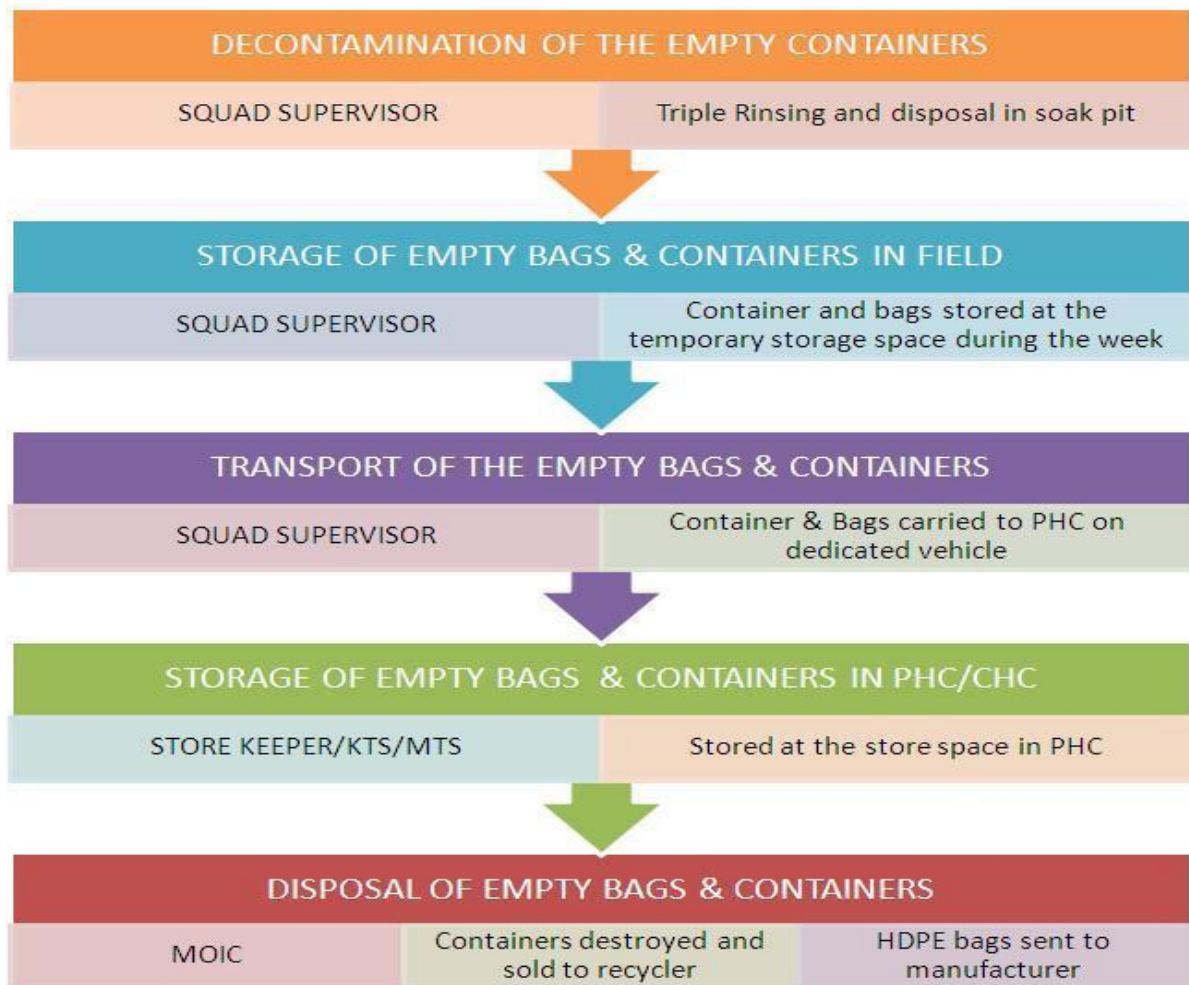
At the end of a spraying round, the residue of chemicals left on the surface of the pit should be scrapped by the spraying squad and disposed of into the waste pit of the PHC.

DISPOSAL OF BAGS/CONTAINERS

Empty bags or containers used for packing insecticide are contaminated with insecticides and have to be disposed properly. These wastes are generated in the field during IRS spray and, thus, needs to be collected and decontaminated before they are disposed. However, type and size of containers varies depending upon the insecticide used in a particular area and manufacturer of the insecticide.

The process of decontamination, collection and storage disposal is provided in Figure Below:

SCHEMATIC DIAGRAM OF HANDLING AND DISPOSAL OF EMPTY BAGS AND CONTAINERS



Collection and transportation to the PHCs

The Spray Supervisor would be responsible for collecting the empty bags and containers generated from the week's operations and would carry it back to the PHC. Empty containers should be rinsed (triple-rinsing) before they are transported to the PHC. The container wash water should be disposed of in the soak pit designed for disposal of waste water. A dedicated transport, e.g. cycle rickshaw, van rickshaw/ auto rickshaw, should be used for transporting the bags/containers

of insecticides. During transportation of the contaminated bags and containers, the load should be covered up with polythene sheets and tied up so that they are securely fixed. The vehicle should not be overloaded at the time of transportation

The empty bags and containers have to be deposited to the storekeeper. The storekeeper should verify the quantity and also maintain an account of the bags and containers returned. The empty bags and containers would be stored along with the insecticides before it is disposed. The following precautions should be adopted while handling empty bags/containers:

- The personnel handling the empty bags and containers should wear their PPEs (consisting of gloves, mask, apron and goggles and shoes).
- The empty containers and bags should be stacked properly and should not be strewn in the storage area.
- Any spill of the remaining insecticide should be contained and subsequently cleaned.

Disposal of the bags and containers at the PHCs

- The following guidelines should be followed for proper disposal of bags and containers from the PHC. Since jute bags, HDPE bags, HDPE containers and MS Containers are used for the packing of insecticides, the guidelines for disposal of each of these have been specified separately.
- **Gunny bags:** Gunny bags used for the packing of insecticides are usually double ply jute cloth lined with an impermeable liner. There is an LDPE bag inside the double ply jute bag in which the insecticides are packed. The LDPE bag is extracted and the jute bag can be allowed to decompose. The LDPE bags should be disposed in the deep burial pit at the PHC.
- **HDPE bags:** The bags should be returned to the PHC by the spray supervisor after being cut into two pieces. The store in-charge should maintain record of the bags which have been returned. The bags can then be

disposed of to a hazardous waste recycler. Alternatively, The HDPE can either be sent back to the district during cycle/year in the vehicle which supplies the insecticides to the PHC. These bags can subsequently be sent back to the manufacturer.

- **Containers:** The containers once rinsed can be used for as collection containers for hazardous wastes. Alternatively, PWD crushers (like JCB)/bulldozers could be used at each PHC to crush these containers. The crushed containers can be sold off to an authorised recycler.

Health Monitoring

- In case of accidental exposures or appearances of symptoms of poisoning, medical advice must be sought immediately.
- In case of organophosphorus (Malathion), regular monitoring of cholinesterase (CHE) level should be carried out and spraymen showing decline in CHE to 50% should be withdrawn and given rest and if needed medical aid

Long Lasting Insectidal Nets-

Both the bags for individual nets and the packaging used to wrap bales of nets are made of various materials⁴ including: *low density polyethylene (LDPE)*, *LDPE coated with polyethylene terephthalate (PET, polyester)*, *linear low density polyethylene (LLDPE)*, *biaxially oriented polypropylene (BOPP)*, *oxodegradable*⁵ (OXO) plastic bags, paper bags and various strapping bands.

Having been in direct contact with the pesticides present in the enclosed LLIN, an individual net bag is an "Empty Pesticide Container" as defined by the *FAO/WHO Guidelines on Management Options for Empty Pesticide Containers*.² The bags should therefore be handled in a manner consistent with that guidance. The Guidelines, which specify methods for the disposal of pesticide-contaminated packaging material, indicate that "unless empty pesticide containers are managed correctly, they are hazardous to both mankind and the environment". In particular, "burning

plastics and pesticides in an uncontrolled fire will not destroy the hazardous components completely and may generate dangerous persistent toxins".

WHO Recommendations for the Management of LLIN Packaging Material -

Options for the management of LLIN bags and baling material must be evaluated on a case by case basis. "Reuse" is currently not an option since no manufacturer produces reusable LLIN bags and baling material and it is unsafe to use them for any other purpose as such. The following recommendations apply only to the safe disposal and recycling of LLIN waste packaging (bags and baling material) and do not cover the LLINs themselves. Where possible, and with no reduction in the public health benefit, distribute LLINs without leaving any packaging with the intended LLIN user;

1. Recycle LLIN packaging: recyclers processing used LLIN bags and baling material should apply proper controls of their materials and processes to ensure the bags are only recycled into appropriate products which have "limited potential for human contact⁶ and are not likely to be recycled again;"
3. Ensure proper personal protective equipment (PPE) are used and measures strictly followed by workers involved in all stages of operations for collection, sorting, recycling and disposal of LLIN bags and baling material;⁸
4. Incinerate LLIN bags and baling material ONLY if specified high temperature incineration conditions for pesticide-tainted plastic can be assured⁹ following *Basel Convention Technical Guidelines* and in accordance with national regulations and requirements;
5. Store used LLIN packaging awaiting future safe recycling, disposal or other processing in dry, well ventilated and secure facilities;

6. If recycling or incineration is not possible, and if LLIN producers provide directions on methods for safe disposal, follow the manufacturer's recommendations. Alternatively, landfilling of bags and baling material in a properly engineered landfill is an option, as detailed in the *FAO/WHO Guidelines on Management Options for Empty Pesticide Containers*
7. National pesticide registration authority to make mandatory that manufacturers provide recommendations on the safe disposal and/or recycling of LLIN packaging. This will include information on labels of LLIN bags regarding the material used in the production of such bags;
8. Assure that disposal of LLIN packaging is included as a condition in the procurement of LLINs;
9. Develop national LLIN packaging management protocols for these wastes and assure that all stakeholders are aware of proper packaging disposal procedures that is aligned with national regulations and requirements;
10. Integrate good practice recommendations on the sound management of LLIN packaging into the existing national malaria strategy and related frameworks; and ensure that recommendations are aligned with national regulations concerning the safe handling and disposal of chemical waste (or pesticide-tainted waste).

Basel Convention Technical Guidelines for the Identification and Environmentally Sound Management of Plastic Wastes and for their Disposal specify that "The condition for the optimal incineration of material is: Temperature of 850°C-1100°C for hydrocarbon wastes and 1100°C-1200°C for halogenated wastes; sufficient (gas) residence time in the incinerator (EU legislation requires 2 seconds as a minimum): good turbulence; and excess of oxygen

Health Impact Assessment (HIA) is defined as "a combination of procedures, methods, and tools by which a policy, program, or project may be judged as to its potential effects on the health of a population, and the distribution of those effects within the population."

HIA is intended to produce a set of evidence-based recommendations to inform decision-making. HIA seeks to maximise the positive health impacts and minimise the negative health impacts of proposed policies, programs or projects.

HIA is a practical approach used to judge the potential health effects of a policy, programme or project on a population, particularly on vulnerable or disadvantaged groups. Recommendations are produced for decision-makers and stakeholders, with the aim of maximising the proposal's positive health effects and minimising its negative health effects.

The environmental factors such as terrain features (plain, desert, hilly and forests), ecology, climatic features, rainfall, humidity influence the presence of vector mosquitoes helping thereby in the transmission of a particular disease. The developmental project namely irrigation, dam, hydro-electrical projects, jhoom cultivation (deforestation) lead to direct health impact on the population in that area in terms of vector borne diseases.

Under the National Vector Borne Disease Control Programme, insecticides and larvicides are being used which are registered with Central Insecticide Board based on the toxicity data to ensure safe standard of human safety. However, Environmental Code of Practises (ECoP) envisaged under the orbit of the programme implementation. But, there is need for health impact assessment of the

spraymen handling insecticides and irrational use of fogging operations in the municipal corporations on the spraymen and community.

The procedures of HIA are similar to those used in other forms of impact assessment, such as environmental impact assessment or social impact assessment. HIA is usually described as following the steps listed, though many practitioners break these into sub-steps or label them differently:

1. *Screening* - determining if an HIA is warranted/required
2. *Scoping* - determining which impacts will be considered and the plan for the HIA
3. *Identification and assessment of impacts* - determining the magnitude, nature, extent and likelihood of potential health impacts, using a variety of different methods and types of information
4. *Decision-making and recommendations* - making explicit the trade-offs to be made in decision-making and formulating evidence-informed recommendations
5. *Evaluation, monitoring and follow-up* - process and impact **evaluation** of the HIA and the monitoring and management of health impacts

The main objective of HIA is to apply existing knowledge and evidence about health impacts, to specific social and community contexts, to develop evidence-based recommendations that inform decision-making in order to protect and improve community health and wellbeing. Because of financial and time constraints, HIAs do not generally involve new research or the generation of original scientific knowledge. However, the findings of HIAs, especially where these have been monitored and evaluated over time, can be used to inform other HIAs in contexts that are similar. An HIA's recommendations may focus on both design and operational aspects of a proposal.

HIA has also been identified as a mechanism by which potential health inequalities can be identified and redressed prior to the implementation of proposed policy, program or project .

A number of manuals and guidelines for HIA's use have been developed

Department of Health

The proposition that policies, programs and projects have the potential to change the determinants of health underpins HIA's use. Changes to health determinants then leads to changes in health outcomes or the health status of individuals and communities. The determinants of health are largely environmental and social, so that there are many overlaps with environmental impact assessment and social impact assessment.

Level of HIA

Three forms of HIA exist:

- Desk-based HIA, which takes 2–6 weeks for one assessor to complete and provides a broad overview of potential health impacts;
- Rapid HIA, which takes approximately 12 weeks for one assessor to complete and provides more detailed information on potential health impacts; and
- Comprehensive HIA, which takes approximately 6 months for one assessor and provides a in-depth assessment of potential health impacts.

It has been suggested that HIAs can be prospective (done before a proposal is implemented), concurrent (done while the proposal is being implemented) or retrospective (done after a proposal has been implemented) . This remains controversial, however, with a number of HIA practitioners suggesting that concurrent HIA is better regarded as a monitoring activity and that retrospective HIA is more akin to [evaluation](#) with a health focus, rather than being assessment

per se. Prospective HIA is preferred as it allows the maximum practical opportunity to influence decision-making and subsequent health impacts.

HIA practitioners can be found in the private and public sectors, but are relatively few in number. There are no universally accepted competency frameworks or certification processes. It is suggested that a lead practitioner should have extensive education and training in a health related field, experience of participating in HIAs, and have attended an HIA training course. It has been suggested and widely accepted that merely having a medical or health degree should not be regarded as an indication of competency.

Why use HIA?

Values

HIA is based on four values that link the HIA to the policy environment in which it is being undertaken.

1. **Democracy** - allowing people to participate in the development and implementation of policies, programmes or projects that may impact on their lives.
2. **Equity** - HIA assesses the distribution of impacts from a proposal on the whole population, with a particular reference to how the proposal will affect vulnerable people (in terms of age, gender, ethnic background and socio-economic status).
3. **Sustainable development** - that both short and long term impacts are considered, along with the obvious, and less obvious impacts.
4. **Ethical use of evidence** - the best available quantitative and qualitative evidence must be identified and used in the assessment. A wide variety of evidence should be collected using the best possible methods.

HIA and policy making

In this section we investigate how HIA contributes to policy making.

HIA can be a valuable tool for helping to develop policy and assisting decision-makers. The usefulness and need of HIA within policy and decision making is clear, HIA:

- is used in projects, programmes and policies
- assists policy development
- brings policies and people together
- involves the public
- provides information for decision makers
- addresses many policy making requirements
- recognises that other factors influence policy apart from HIA.
- is a proactive process that improves positive outcomes and decreases negative outcomes
- can provide what policy makers need

Suggestions for how an HIA practitioner might interact with the policy process and policy makers, a description of the different stages in policy making, plus some key steps for HIA practitioners, are also provided.

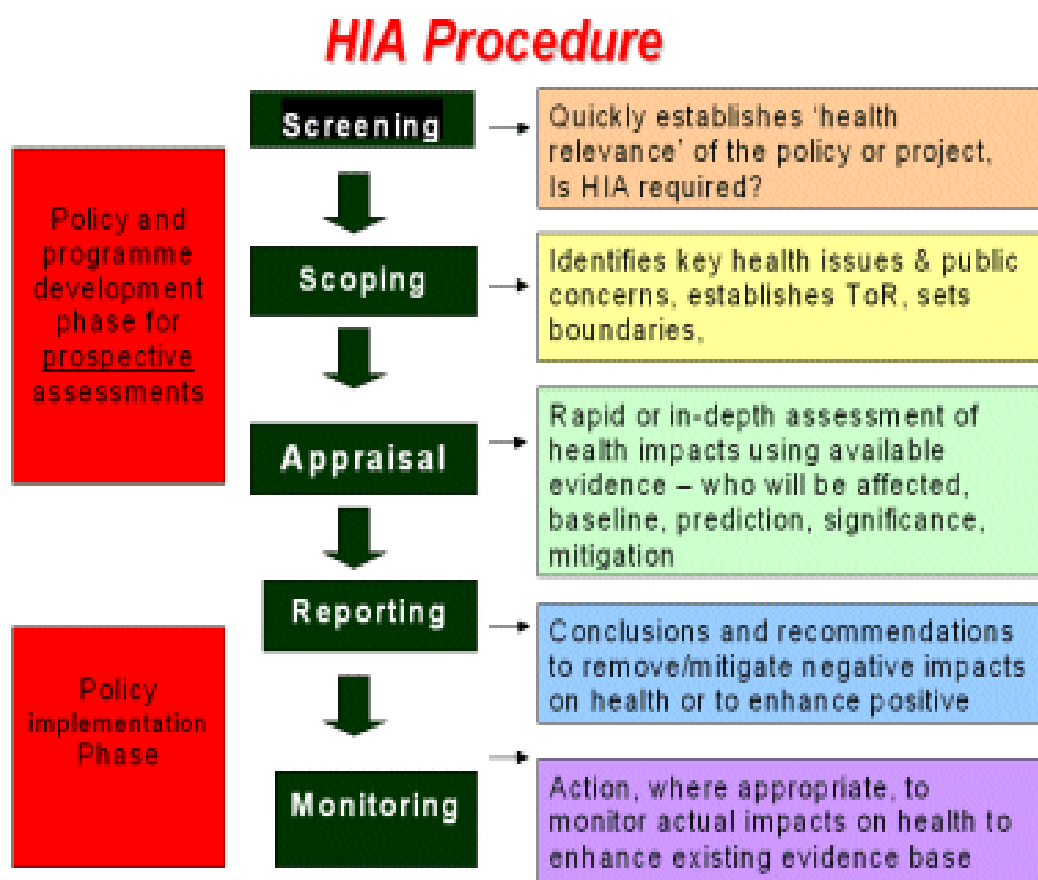
Tools and methods

How to undertake an HIA

This section will draw on a number of case studies to briefly describe the theory and practice of carrying out an HIA. Many [HIA guidance documents](#) have been produced, from all regions of the world and we encourage you to use these for detailed work. While there is no single agreed method for undertaking HIA, a

general pattern has emerged amongst methods and there is much overlap between them.

Guidance documents often break HIA into four, five or six stages. Despite the differing number of stages, it is important to note that there are no significant differences between the methods. Also, the theoretical stages often overlap and intermingle, and a clean separation is not often obvious in practice. The stages are:



Using evidence within HIA

One of the key values of HIA is the ethical use of evidence.

A wide variety of evidence should be collected and assessed, using appropriate and effective methods. This will provide the basis for evidence-based recommendations that can be provided to decision-makers, who can then make

decisions about accepting, rejecting, or amending the proposal in the knowledge that they have the best available evidence before them.

HIA considers several types of evidence. It goes beyond published reviews and grey literature to include the knowledge of stakeholders who are involved in or affected by a proposal. Where evidence of the quality and quantity demanded by decision-makers is not available, a note of this is made within the HIA.

For management of domestic and extra-domestic mosquito breeding places, adoption and enforcement of by-laws for use under Urban Malaria Scheme are framed as under:-

Control of malaria and other mosquito borne diseases.

Draft provisions suggested for adoption under appropriate section/rule prevailing in the State.

Application of this Provision

1. The State Govt./local authority constituted under any act may enforce the following provisions to the whole or any part of the State/local authority area.
2. (1) If the provisions have been extended, no person or local authority shall, after such extension:
 - (a) have, keep, or maintain within such area any collection of standing or flowing water in which mosquitoes breed or are likely to breed, or
 - (b) cause, permit, or suffer any water within such area to form a collection in which mosquitoes breed or are likely to breed, unless such collection has been so treated as effectively to prevent such breeding.

(2) The natural presence of mosquito larvae, in any standing or flowing water shall be an evidence that mosquitoes are breeding in such water.

Treatment of Mosquito Breeding Places

3. (1) The Health Officer may, by notice in writing, require the owner or the occupier of any place containing any collection of standing or flowing water in which mosquitoes breed or likely to breed, within such time as may be specified in the notice, not being less than 24 hours, to take such measures with respect to the same, or to treat the same by such physical, chemical or biological method, being measures or a method, as the Health Officer may consider suitable in the circumstances.

(2) If a notice under sub-section (1) is served on the occupier, he shall in the absence of a contract expressed or implied, to the contrary, be entitled to recover from the owner the reasonable expenses incurred by him in taking the measures or adopting the method of treatment, specified in the notice and may deduct the amount of such expenses from the rent which is then or which may thereafter be, due from him to the owner.

Health Officer's Power in Case of Default

4. If the person on whom a notice is served under provision 3 fails or refuses to take the measures, or adopt the method of treatment, specified in such notice within the time specified therein, the Health Officer may himself take such measures or adopt such treatment, specified in such notice within the time specified therein, and recover the cost of doing so from the owner or occupier of the property, as the case may be, in the same manner as if it were a property tax.

Protection of Anti-mosquito Works

5. Where, with the object of preventing breeding of mosquitoes in any land or building, the Govt. or any local authority or the occupier at the instance of the Govt. or local authority, (have constituted any works) in such land or building, the owner for the time being as well as the occupier for the time such land or building shall prevent its being used in any manner which causes or is likely to cause the deterioration of such works, or which impairs, or is likely to impair the efficiency.

Prohibition of Interference with such works

6. (1) No person shall, without the consent of the Health Officer, interfere with, injure, destroy, or render useless any work executed or any material or thing placed in under or upon any land or building, by the orders of the Health Officer with the object of preventing the breeding of mosquitoes therein.

(2) If the provisions of sub-section (1) are contravened by any person, the Health Officer may re-execute the work or replace the materials or things, as the case may be, and the cost of doing so shall be recovered from such person in the same manner as if it were a property tax.

Section in Respect of Household Cans and other Containers

7. The owner or occupier of any house, building, or shed or land shall not therein keep any bottle, vessel, can or any other container, broken or unbroken, in such manner that it is likely to collect and retain water which may breed mosquitoes.

8. All borrow pits required to be dug in the course of construction and repair of roads, railways, embankments, etc. shall be so cut as to ensure that water does not remain stagnant in them. Where possible and practicable the borrow pits shall be left clean and sharp edged and extra expenditure not exceeding 1 per cent of the cost of the earth work in any project may be incurred to achieve this. The bed level of borrow pits shall be so graded and profiled that water will drain off by drainage channels connecting one pit with the other till the nearest natural drainage nullah is met with. No person shall create any isolated borrow pit which is likely to cause accumulation of water which may breed mosquitoes.

9. In case of any dispute or difference of opinion in the execution of any antimosquito scheme or in its operation or any work under these provisions in which the jurisdiction of the Govt. of India, or Govt. of any other State is involved, the matter shall be referred to the Govt. of India for final say in the matter.

10. Powers of Health Staff to enter and inspect the premises

For the purpose of enforcing the provisions, the Health Officer or any of his subordinate not below the rank of Health or Sanitary Inspector may, at all reasonable times, after giving such notice in writing as may appear to him reasonable, enter and inspect any land or building within his jurisdiction and the occupier or the owner as the case may be, of such land or building, shall give all facilities necessary for such entry and inspection, and supply all such information as may be required of him for the purpose aforesaid.

International Health Regulations (IHR)

In today's connected world, health security is a global issue. The International Health Regulations (IHR) are legally binding regulations aiming to a) assist countries to work together to save lives and livelihoods endangered by the spread of diseases and other health risks, and b) avoid unnecessary interference with international trade and travel.

The Twenty-Second World Health Assembly (1969) adopted, revised and consolidated the International Sanitary Regulations, which were renamed the International Health Regulations (1969). During the Forty-Eighth World Health Assembly in 1995, WHO and Member States agreed on the need to revise the IHR (1969) against the backdrop of the increased travel and trade characteristic of the 20th century. The IHR (2005) entered into force on 15 June 2007 and are currently binding on 194 countries (States Parties) across the globe, including all 193 Member States of WHO. The purpose and scope of IHR 2005 are to prevent, protect against, control and provide a public health response to the international spread of disease in ways that are commensurate with and restricted to public health risks, and which avoid unnecessary interference with international traffic and trade. (Art. 2, IHR 2005). WHO plays the coordinating role, through the IHR, WHO keeps countries informed about public health risks, and works with partners to help countries to build capacity to detect, report and respond to public health events.

Core obligations for Member Countries

- Designate a National IHR Focal Point as the operational link for urgent communications concerning the implementation of the Regulations.
- Develop, strengthen and maintain the surveillance and response capacity to detect, assess, notify, report and respond to public health events, in accordance with the core capacity requirements under the IHR (2005).

- Notify WHO for all events that may lead to a Public Health Emergency of International Concern (PHEIC) within 24 hours of assessment by using the decision instrument [an algorithm].
- Respond to requests for verification of information regarding public health risks.
- Provide WHO with all relevant public health information, if a State Party has evidence of an unexpected or unusual public health event within its territory, which may constitute a PHEIC.
- Control urgent national public health risks that may threaten to transmit diseases to other Member Countries.
- Provide routine inspection and control activities at international airports, ports and some ground crossings to prevent international disease transmission.
- Make every effort to fully implement WHO-recommended temporary and standing measures and provide scientific justification for any additional measures.
- Collaborate with other Countries Parties and with WHO in implementing the IHR (2005), particularly in the area of assessment, provision of technical and logistical support, and mobilization of financial resources.

Core obligations for WHO

- Designate WHO IHR contact points as operational links for urgent communications concerning the implementation of the IHR (2005).
- Support Member Countries' efforts to develop, strengthen and maintain the core capacities for surveillance and response in accordance with the IHR (2005).
- Verify information and reports from sources other than official notifications or consultations, such as media reports and rumors, when necessary.
- Assess events notified by Member Countries (including on-site assessment, when necessary) and determine if they constitute a PHEIC.
- Provide technical assistance to Countries in their response to public health emergencies of international concern.
- Provide guidance to Countries to strengthen existing surveillance and response capacities to contain and control public health risks and emergencies.

- Provide all Member Countries with public health information to enable Member Countries to respond to a public health risk.
- Issue temporary and standing recommendations on control measures in accordance with the criteria and the procedures set out under the Regulations.
- Respond to the needs of Member Countries regarding the interpretation and implementation of the IHR (2005).
- Collaborate and coordinate its activities with other competent intergovernmental organizations or international bodies in the implementation of the IHR (2005).
- Update the Regulations and supporting guides as necessary to maintain scientific and regulatory validity.

Role of WHO in global system for alert and response

When a significant public health event takes place, WHO's comprehensive global alert and response system ensures that information is available and response operations are coordinated effectively

Country capacity building

To help countries review and, if necessary, strengthen their ability to detect, assess and respond to public health events, WHO develops guidelines, technical materials and training, and fosters networks for sharing expertise and best practice.

International Health Regulations in Ports, Airports and Ground Crossings

In case of arboviral / Vector Borne Diseases, all international air/sea ports and ground crossings with a perimeter of 500 meters should be kept free from vector of yellow fever/Dengue/Plague, if present, their density should be less than one to limit the spread of health risks to neighboring countries, and to prevent

unwarranted travel and trade restrictions so that traffic and trade disruption is kept to a minimum.

International transport, travel and trade contribute to economic development and welfare of populations, pose great public health risks. Today's high traffic at airports, ports and ground crossings – points of entry, can play a key role in the international spread of diseases through persons, conveyances and goods. The International Health Regulations (2005) provide a public health framework in the form of obligations and recommendations that enable countries to better prevent, prepare for and respond to public health events and emergencies.

Under the IHR, Member Countries are requested to maintain effective sustainable public health measures and response capacity at designated ports, airports and ground crossings, in order to protect the health of travelers and populations; keep ports, airports and ground crossings running as well as ships, aircrafts and ground transportation travelling in a sanitary condition; contain risks at source, respond to emergencies and implement public health recommendations, limiting unnecessary health-based restrictions on international traffic and trade.

In **India** an International Health Division under Directorate General of Health Services (Dte.GHS) monitors the IHR related activities in the country.

Activities undertaken for vector control in air/sea port and ground crossings:

- Strengthen regular epidemiological/entomological surveillance at international air/Sea ports and ground crossings. The National Vector Borne Disease Control Programme (NVBDCP) and National Centre for Disease Control (NCDC) regularly has to regularly monitor the *Aedes* breeding at International airports and sea ports to maintain *Aedes* breeding free status. If any breeding is detected actions need to be taken to immediately through port health authority to eliminate the source and prevention for future. The

observations/actions taken has to be shared with the IH division of Dte.GHS with suggestions, if any.

- Support the Ministry of Health and Family Welfare in the development of regular feedback reports and early warning systems for the diseases.
- Co-ordinate activities to review the legislative framework of the IHR implementation.
- NVBDCP and NCDC to organize and facilitate training on core capacity for entomological surveillance as identified in the field of VBDs.
- Support implementation of the IHR (2005) action plan.

GoI Policy for Yellow Fever

Yellow Fever (YF) does not occur in India. The conditions for transmission of YF are very conducive in India (presence of Aedes vector and susceptible population). GoI has been following a strict YF vaccination programme to prevent its entry to India. Strategy of GoI for prevention of entry of YF disease into India has been screening of all international passengers for vaccination against yellow fever disease at all points of entry in compliance of the IHR 1969 & 2005 and Aircraft Health Rules 1954 and Port Health Rules 1955. All passengers coming to India or passengers going from India to countries endemic for YF should have a valid International Vaccination Card for YF or they are quarantined for a period of 6 days or till the YF vaccination become valid (whichever is earlier).

YF disease will be treated as disease of Public health significance and all health measures being applied presently like disinsection of conveyance by spraying Aerosol (Aircrafts and Ships), vaccination requirements and quarantine of passengers and crew (as may be required) (as per Article 7, P.2(b), 42 and relevant annexure) will be continued as has been stipulated under Annex-II of IHR-1969.

Source: <http://www.who.int/ihr/ent>

For details of India's perspective on IHR refer : <http://www.mohfw.nic.in/index1>

Monitoring is very crucial and besides disease situation, there has to be provision of monitoring the entomological reports as well as output indicators. Some are indicated below but this chapter is under revision

- Percentage of targeted households / rooms sprayed
- Percentage of population in high-risk project areas protected with effective IRS
- Percentage of population in high-risk project areas protected with either effective IRS or LIN
- Percentage of population in high-risk project areas provided with effective ITNs/ LLINs
- Percentage of HH with LLIN
- Percentage of individual who slept under LLIN/ITN the previous night
- Number of LLINs distributed
- Number of ITNs retreated
- Number of LLINs replenished
- Number of Hatcheries established
- Number of Hatcheries with fishes

Vector surveillance : Vector surveillance is a critical component of the programme since it helps to plan IRS operations based on evidence. It is also necessary part of the elimination strategy because it is useful in determining the impact of IRS. There are no easy methods for estimating the size of sand fly population reliably.

- Number of breeding sites checked
- Number of Containers Checked
- Number of Houses Checked
- House index
- Container Index
- Breteau Index
- Pupal Index
- Adult vector per Man Hour density (PMHD)
- Susceptibility status

Supervision is an essential and integral part of IRS to ensure its efficacy and safety. This should be thorough to produce an impact and ensure that there are no ill effects. To be effective, supervision should be carried out at all levels. There should be a written plan for supervision and supervisory checklists are to be developed and used. Supervision will be effective if problems are identified and they are solved by the supervisors as soon as they are detected. Any unsolved problems should be referred to district authorities for resolution. All supervisory reports should be sent to the district to facilitate follow up action. The supervisory reports should be kept safely in the district and referred to whenever needed.

- Availability of plan with the spray squad. Review of the plan to ensure that the plan is being followed.
- Ensure that all members of the spray squad are present and are doing the job.
- Checking that the spraying is being done correctly according to the norms prescribed in the work manual of the spray squad.
- Examination of the spray equipment to ensure that it is in working condition and is being properly maintained as per the guidelines provided.
- Going with the squad to the households where there is refusal or reluctance for spraying
- Checking the records of the spray squads
- Discussions of plans for mopping up to cover the households where there was refusal or the houses were locked.

- Assessing the consumption of insecticides and making arrangements for additional supplies if required.
- Review of time schedule for the following week
- Visit randomly selected households and ask whether the house was sprayed or not.
- If the house was sprayed, then check for grey white deposits as evidence for spray.
- Check whether the deposits are uniform or not. Uniform deposits indicate that the spraying was satisfactory.
- Check to see if any portions of the dwelling or the cattle shed were skipped.
- Check whether the walls have been plastered with mud. If the walls have been plastered then determine when this was done to determine the time interval between the IRS and the plastering.
- Visit the households that were not covered and find out the reasons for non-coverage. Try to convince them to get their houses sprayed as a part of special mop up drive.
- Prepare a written report along with recommendations and share with the spray squads to ensure that the mistakes are corrected as soon as possible.

In case of Synthetic Pyrethroids, it is difficult to see the deposits on the wall during concurrent supervision. The droplets may be seen on the wooden structures in the rooms/ cattle sheds where insecticide has been sprayed.

Informing and involving the community

The supervisors should inform the community leaders and key persons in the villages about the plans for the spraying at least a week before the spraying is done. The spray team members should remind them at least one day before the operation.

During the first visit discuss the following issues with the community leaders and key persons in the community.

- Distribution of a simple flyer explaining the purpose of the spraying and including the common do's and don'ts developed in local language. Simple illustrations should be included to facilitate easy understanding of the people. This should be a part of BCC. The flyer should be left with several key persons in the village for distribution and briefing amongst the sections/segments they represent or influence. Tell the key persons to share the contents of the flyer to others in the community.
- What is proposed to be done and why. Explain that this is the most effective way of eliminating a dreaded disease Kala-azar. Their cooperation will be a key to success of the efforts.
- Inform the proposed date for spraying the village
- Discuss what specific role the community leaders and key persons can play to ensure that the spraying is complete and thorough. This would require that no household is missed and the spraying done must be complete.
- Explain that if all surfaces are not sprayed the sand fly would fly to the uncovered areas and the desired effect of spraying will not be obtained.
- The insecticide is harmful for the food items. Foods must not be exposed to the insecticides.
- The households must not do any mud plastering of the walls and the sprayed surfaces for 3 months after the spraying.
- One day prior to the spraying audio announcement for masses might be a useful way of informing and reminding the villagers. Other suitable options may be taken up in place of above source if that is not available.

Daily Summary, reporting of information

At the end of each day, the spray squads should prepare the summary of day's work. This includes information on the households targeted, households sprayed, insecticide consumed, insecticide left, and the problems encountered in the work. A daily summary of spray operations and daily consumption record of insecticide should be maintained in the perform annexed at **Annexure12**.

The daily report should be sent to the supervisor by all the spray squads for review and feedback by the supervisor in order to take corrective actions if required. The supervisors in turn should send the consolidated report to the focal point in the district once every week.

Monthly Reporting

Reporting of compiled data as per formats provided by NVBDCP needs to be ensured. The Vector control forms as VC1 to VC6 and Entomological surveillance forms are on websites and are annexed for reference.

These may be referred by State and Zonal entomological teams. IDSP and district level entomologists should also use this formats for reporting and analysis purposes.

Annual reporting

Compiled monthly data on Vector control and Entomological surveillance must be analysed and compared with previous year for onward submission to NVBDCP.

1. **What is Dengue and how Dengue spreads?**
 - a. Dengue is an outbreak prone seasonal viral disease, transmitted through bites of female adult *Aedes* mosquitoes
2. **How does the *Aedes mosquito* look like?**
 - a. These are small blackish mosquitoes with white stripes on abdomen & legs. These are also called tiger mosquitoes.
3. **Where *Aedes* mosquitoes breed?**
 - a. *Aedes* mosquitoes breed in stagnant water within and around houses. *Aedes* mosquitoes are container breeder viz., cement tanks, overhead tanks, underground tanks, tyres, desert coolers, pitchers, discarded containers, junk materials, potholes, rooftop, window parapet, ornamental fountain, lucky bamboo pot, money plant bottle etc, in which water stagnates for more than a week
4. **Does the *Aedes* breed in dirty polluted water & drains?**
 - a. No, they breed only in clean water.
5. **What is the average life span of an adult *Aedes*?**
 - a. The life span for adult mosquitoes is around three weeks.
6. **Where does Dengue vector rest?**
 - a. Dengue vector mosquitoes rest in indoor houses mainly in corners under furniture, beds, shelves, almirah and dark clothing hanging inside.

7. When does Dengue vector bite?

- a. Dengue vector mosquitoes bite during day time usually in morning (8-10 am) & afternoon (3-5 pm) hours.

8. What are the Vector control methods?

- a. Vector control/management includes:
 - i. Environmental management for Source Reduction Biological Control: Larvivorous fish viz., Gambusia and Guppy are recommended for control of *Ae. aegypti* in large water bodies or large water containers.
 - ii. Chemical control Larval control Temephos as chemical larvicide Diflubenzuron and Pyreproxifen as Insect Growth Regulator
 - iii. Adult control
 1. **Pyrethrum space spray** is used in indoor situations as space spray
 2. **Malathion fogging** is used outdoor using insecticide Malathion technical
 3. **Cyphenothrin 5% EC-** another molecule is also recommended in the programme for fogging which can be used both for indoor and outdoor fogging.
 4. Personal protection
 - Protective clothing
 - Repellents as household insecticide products, namely, mosquito coils, mats etc
 - Aerosols against mosquitoes
 - Insecticide treated mosquito nets or long lasting insecticidal nets (LLIN) to protect infants and night workers while sleeping in daytime.

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Photos



VC1. Primary record of IRS
(Superior Field Worker's Diary)

Village _____ Village Code _____
 Sub-Centre _____ PHC _____ Round _____
 Planned Date ____/____/____
 Date of Spray ____/____/____

	Number Targeted	Partial Covered	Complete Covered	%
Houses				
Rooms				
Population				

Code of Squad-----, Name of SFW/FW

1.-----, 2.-----
 3.----- 4.-----
 5.----- 6.----- Insectide:-----

S.No	Head	Total inmates	Total rooms	RCS	RPS	RR	RL	Remarks
1								
2								
3								
4								
5								
6								

RCS: Rooms Complete sprayed; RPS: Rooms Partially Sprayed; RR Rooms Refused

RL: Rooms Locked

(Signature)