

Malaria entomology and vector control

Learner's Guide



**World Health Organization
HIV/AIDS, Tuberculosis and Malaria
Roll Back Malaria**

July 2003

Trial Edition

© **World Health Organization 2003**

All rights reserved.

This health information product is intended for a restricted audience only. It may not be reviewed, abstracted, quoted, reproduced, transmitted, distributed, translated or adapted, in part or in whole, in any form or by any means.

The designations employed and the presentation of the material in this health information product do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions except the names of proprietary products are distinguished by initial capital letters.

The World Health Organization does not warrant that the information contained in this health information product is complete and correct and shall not be liable for any damages incurred as a result of its use.

Table of Contents

Foreword.....	3
Learning Units	
1. Introduction to malaria entomology.....	5
2. Identification of malaria vectors	11
3. Sampling malaria vectors.....	21
4. Susceptibility and bioassay tests	35
5. Vector incrimination and malaria control	41
6. Malaria vector control.....	61
7. Malaria stratification and vector control.....	91
8. Management of malaria vector control programmes	97

Foreword

This module covers essential aspects of malaria entomology and vector control. It is multipurpose as the depth and selection of learning units depends on the background of the audience and learning objectives. It can be used to train field vector control workers, laboratory technicians, or health workers working in malaria vector control programmes at different levels. The latter audience may not need details of the field and laboratory techniques but rather focus should be given to the units that deal with epidemiological application of selective vector control options, strategies and management of vector control. The first two categories of audience may need additional resource materials if a course is entirely for laboratory and field techniques. It can be used to train those operating at national and district malaria (vector) control programmes with responsibility for planning, implementation, monitoring and evaluation of vector control activities.

The course is designed for a 7 day period. Learning units 1-5 introduce malaria entomology and role of entomology in malaria control including identification of malaria vectors both adult and larval stages, collection techniques, laboratory skills to determine vector stages and sporozoite infection rates, and techniques for insecticide resistance and residual efficacy. You will examine the biology of vectors and their incrimination as vectors using real-life examples where you calculate the most important entomological indicators of malaria transmission. Learning Unit 6 gives the basic principles for the selection and implementation of vector control methods. The advantages and limitations of each method are discussed. You will examine the role of integrated vector control in a malaria programme.

Learning Unit 7 includes the epidemiological stratification of malaria and the role of vector control in different epidemiologic strata. Finally, Learning Unit 8 brings together the fundamentals of malaria entomology and the management of vector control as part of a malaria control programme, including the importance of monitoring and evaluating vector control implementation.

The module was originally prepared by Dr Tarekegn Abose Abeku and Dr Pushpa Herath, with technical inputs from Drs Maru Aregawi, Elil Renganathan and M.C. Thuriaux. Dr Yemane Ye-ebiyo contributed to the development of the unit that deals with malaria stratification. Two publications previously produced by WHO, namely, *Entomological Field Techniques for Malaria Control* and *Entomological Laboratory Techniques* have been used as background documents in developing Learning Units 1-5, although most of the material has been extensively re-written and adapted to the needs of malaria control programme managers. We are grateful to Dr M. Zaim for his valuable inputs and providing a background (unpublished) WHO document on judicious use of insecticides, which proved useful in writing Learning Unit 8. Other background documents used in the rest of the learning units have been acknowledged in the text. Finally, we would like to acknowledge comments provided by several experts in WHO Headquarters and WHO Regional Office for Africa, in particular Drs K. Cham, P. Guillet, L. Manga, M. Nathan and B. Ameneshewa. The last version of the module was updated by Dr Robert H. Zimmerman.

Timetable – Malaria Entomology and Vector Control

Day	Topic	Teaching method*	Hrs
1	Introduction of tutor, facilitators and participants		1
	Introduction of the course goal and objectives		½
	UNIT 1 Introduction to malaria entomology	PRS/DEM	1 ½
	UNIT 2 Identification of malaria vectors	PRS/DEM/PRC	3
	Film (Malaria Entomology)	FLM	1
2	UNIT 3 Sampling malaria vectors	PRS/DEM	2
	UNIT 4 Susceptibility and bioassay tests	PRS/DEM/PRC	3
	UNIT 5 Vector incrimination and malaria control	PRS/PRC	2
3	UNIT 5 Vector incrimination and malaria control (continued)	PRC	1
	Field work (collection of adult mosquitoes and larvae)	PRC	6
	Organization and preservation field-collected specimens	PRC	1
	UNIT 4 Calculating mortality rates of susceptibility and bioassay tests		½
4	UNIT 4 Discussion of susceptibility and bioassay results	PRC	1
	UNIT 5 Identification and dissection of field-collected specimens (continued)	PRC	3
	UNIT 6 Malaria vector control - introduction	PRS/GRP	3
5	UNIT 6 Malaria vector control - demonstration of vector control methods	DEM	4
	Malaria vector control - implementation plan and integrated vector control	PRS/GRP	3
6	UNIT 7 Stratification and malaria vector control	PRS/GRP	3
	UNIT 8 Management of malaria vector control	PRS/GRP	4
	Closure	GRP/PRS	1

*PRS = Presentation by tutor

DEM = Demonstration

PRC = Laboratory practical

FLD = Field work

FLM = Film show

GRP = Group exercises or discussions followed by plenary discussion

Learning Unit 1

Introduction to malaria entomology

Learning objectives

By the end of this Unit you should be able to:

- describe how malaria is transmitted
- describe the life cycle of the mosquito and relate this to the transmission of malaria
- understand the purpose and role of entomological studies in malaria control

Malaria entomology is the study of biology and ecology of the mosquitoes that transmit malaria. The aim is to understand the relationship between the vector, its ecology and behaviour, the parasite and the host in order to develop and implement effective vector control strategies. In this Unit, a brief introduction will be given about the transmission of malaria and the life cycle of mosquitoes that transmit it. The importance and purpose of entomological studies in malaria control programmes will also be discussed in detail.

1.1 Malaria transmission

Malaria is caused by the *Plasmodium* parasite which spends its life in both humans and certain species of mosquitoes. Four species of *Plasmodium* cause malaria in humans: *Plasmodium falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. Of these, *Plasmodium falciparum* is the most important in most parts of the tropics and is responsible for most severe illnesses and deaths.

Malaria parasites are transmitted by female mosquitoes belonging to the genus *Anopheles*. Male *Anopheles* mosquitoes only feed on plant juices and nectar and cannot transmit malaria. The life cycle of the malaria parasite is divided into three different phases - one in the mosquito (the **sporogonic cycle**) and two in the human host. The **erythrocytic cycle** (in human blood cells) and the **exo-erythrocytic cycle** (outside the blood cells) (Fig.1.1)

If the right stages of the parasite (the male and female **gametocytes**) are ingested by the mosquito when she takes a blood meal, they will form male and female **gametes** within the mosquito's stomach (midgut). The gametes unite to form the **zygote**, which can move and is called the **ookinete**. The ookinete penetrates the wall of the midgut and becomes a round **oocyst**. Inside the oocyst, the nucleus divides repeatedly, with the formation of a large number of **sporozoites** and enlargement of the oocyst. When the sporozoites are fully formed, the oocyst bursts, releasing the sporozoites into the mosquito's body cavity (haemocoel). The sporozoites migrate to the salivary glands. The time necessary for the development of the sporozoites varies with temperature and to a smaller extent with the species of the malaria parasite and with humidity, but generally it is about 8-15 days.

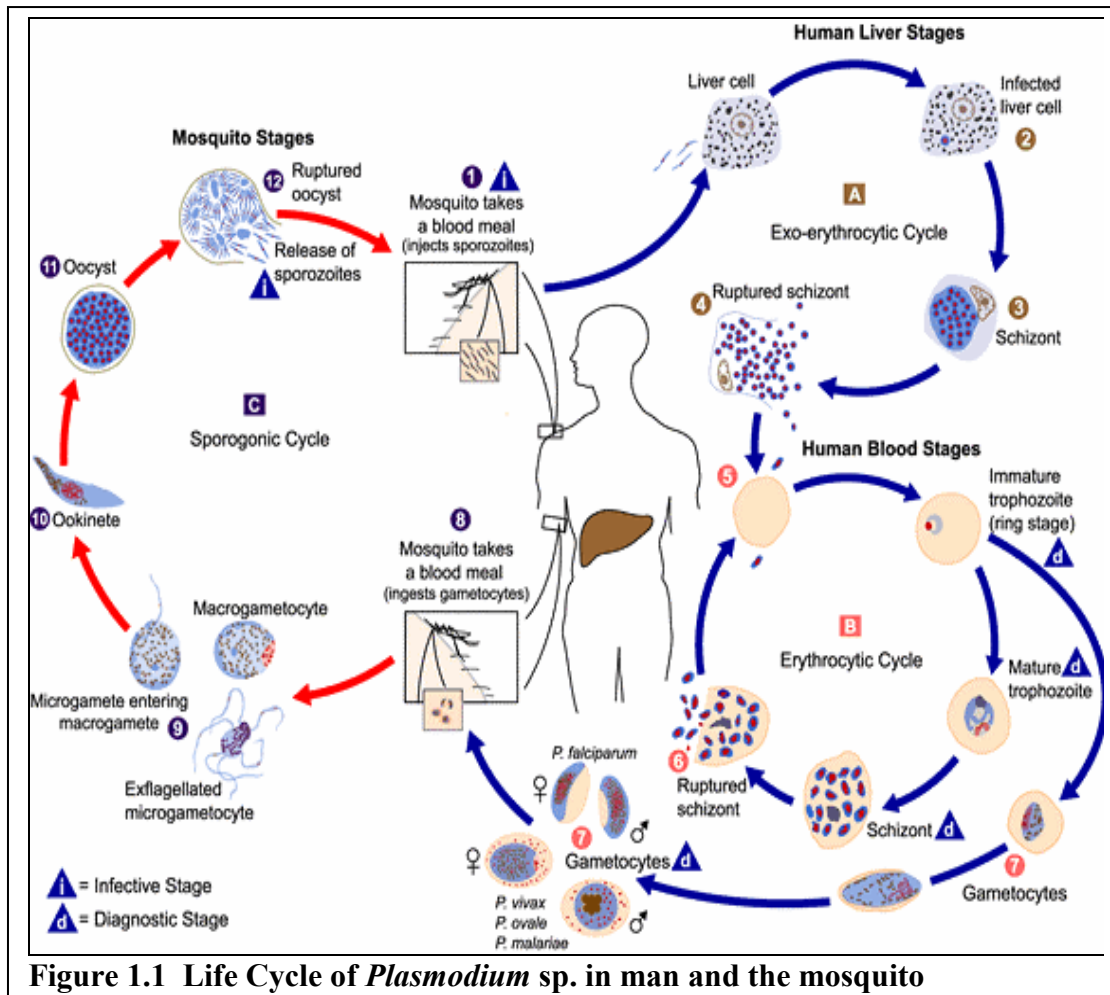


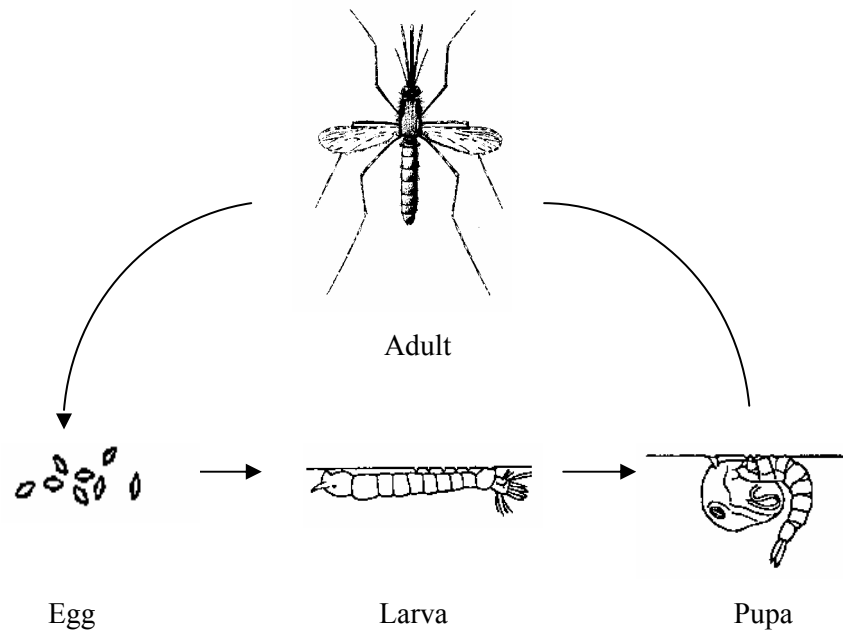
Figure 1.1 Life Cycle of *Plasmodium* sp. in man and the mosquito

The sporozoites (the infective stage of *Plasmodium*) are injected with saliva when the mosquito next feeds. The parasites enter the person's blood system and migrate to the liver cells where they multiply. Over a period of 7-12 days, the parasite multiplies until the infected liver cell bursts. Then the parasites (**merozoites**) are released into the bloodstream and invade the red blood cell where they multiply again. The infected red cells are destroyed, the parasites invade fresh red blood cells and the cycle is repeated. A female mosquito takes a blood meal so that her eggs mature; since she lays several batches of eggs during her lifetime, she will have several opportunities to transmit malaria.

1.2 Life cycle of anopheline mosquitoes

There are about 400 species of *Anopheles* mosquitoes. Approximately forty species worldwide can transmit malaria and of these only 15 are vectors of major importance. Some anophelines prefer to bite animals and transmit malaria parasites to humans rarely. Others do not live long enough for the parasite to develop in the mosquito or the parasite does not seem to be able to develop.

Mosquitoes have four different stages in their life cycle: the **egg**, **larva**, **pupa** and **adult** (Fig. 1.2). The time taken for the various stages to develop depends on temperature and nutritional factors in their environment. Development is shorter at higher temperatures.

Figure 1.2 Life cycle of an *Anopheles* mosquito

Eggs

A female anopheline mosquito normally mates only once in her lifetime. She usually requires a blood meal after mating before the eggs can develop. Blood meals are generally taken every two to three days; before the next batch of eggs is laid. About 100 to 150 eggs are laid on the water surface during oviposition. Oviposition sites vary from small hoof prints and rain pools to streams, swamps, canals, rivers, ponds, lakes and rice fields. Each species of mosquito prefers different types of habitats to lay eggs.

Under the best conditions in the tropics, the average lifespan of female anopheline mosquitoes is about three to four weeks. A female mosquito continues to lay eggs throughout her lifetime. Most females will lay between one and three batches of eggs during their life, though some may lay as many as seven batches.

Larva

A larva hatches from the egg after about one or two days and generally floats parallel under the water surface, since it needs to breathe air. It feeds by taking up food from the water. When disturbed, the larva quickly swims towards the bottom but soon needs to return to the surface to breathe.

There are four larval stages or **instars**. The small larva emerging from the egg is called the **first instar**. After one or two days it sheds its skin and becomes the **second instar**, followed by the **third** and **fourth instars** at further intervals of about two days each. The larva remains in the fourth instar stage for three or four more days before changing to a pupa. The total time spent in the larval stage is generally eight to ten days at normal tropical water temperatures. At lower temperatures, the aquatic stages take longer to develop.

Pupa

The pupa is the stage during which a major transformation takes place, from living in water to becoming a flying adult mosquito. The pupa is shaped like a comma. It stays under the surface and swims down when disturbed but does not feed. The pupal stage lasts for two to three days after which the skin of the pupa splits. Then the adult mosquito emerges and rests temporarily on the water's surface until it is able to fly.

Adult

Mating takes place soon after the adult emerges from the pupa. The female usually mates only once because she receives sufficient sperm from a single mating for all subsequent egg batches. Normally the female takes her first blood meal only after mating, but sometimes the first blood meal can be taken by young virgin females. The first batch of eggs develops after one or two blood meals (depending on the species), while successive batches usually require only one blood meal.

The feeding and resting habits of mosquitoes are of great importance in control programmes and for this reason they must be well understood. Most anopheline mosquitoes bite at night. Some bite shortly after sunset while others bite later, around midnight or the early morning. Some mosquitoes enter houses to bite and are described as being **endophagic**; others bite mostly outside and are called **exophagic**.

After the mosquito takes a blood meal she usually rests for a short period. Mosquitoes that enter a house usually rest on a wall, under furniture or on clothes hanging in the house after they bite and are said to be **endophilic**. Mosquitoes that bite outside usually rest on plants, in holes, in trees or on the ground or in other cool dark places and are called **exophilic**.

Host preferences are different for different species of mosquitoes. Some mosquitoes prefer to take blood from humans rather than animals and are described as being **anthropophagic** while others only take animal blood and are known as **zoophagic**. Clearly, those who prefer to take human blood are the most dangerous as they are more likely to transmit diseases from man to man.

1.3 Malaria control

Malaria control involves the diagnosis and treatment of malaria cases, preventing mosquito bites, and killing mosquitoes. The following methods of control can prevent mosquito bites: insecticide-treated bed nets, mosquito repellents, and screening houses to prevent mosquitoes from entering.

Eliminating breeding sites and killing larvae, pupae and adult mosquitoes will help reduce the number and, in the case of adults, the longevity of vectors. Breeding sites can be eliminated by draining or filling areas where water collects or by modifying the preferred habitats of particular vector species, for example by clearing streams so that the water flows faster. Larval breeding can be reduced or prevented by:

- spreading a thin film of oil on the water surface to prevent larvae from breathing
- covering the water surface with floating materials that deter the mosquitoes from laying eggs
- treating the water with **larvicides** to kill larvae
- putting fish or other predators that eat mosquito larvae in the breeding sites

In some areas, malaria transmitted by vectors that rest indoors can be prevented or controlled by spraying the insides of houses with a **residual insecticide**. Before and more usually after biting, an endophilic mosquito rests on a wall, ceiling or in other dark areas inside the house. If the surfaces it rests on have been sprayed with residual insecticide, the mosquito may eventually pick up a lethal dose and be prevented from transmitting the parasite. The aim of residual spraying is to reduce the longevity of mosquitoes below the time it takes for the malaria sporozoites to develop and to reduce mosquito density.

Mosquitoes can develop resistance to a wide range of insecticides. It is important to know when a vector species develops resistance in order to decide whether on the most appropriate resistance management measure such as interruption of spraying, change of insecticide, or by other means.

1.4 Role of entomological studies in malaria control

Information on the epidemiology of malaria is essential if the disease is to be controlled. Entomological, parasitological and clinical studies provide useful information on the characteristics of malaria transmission in an area as well as the habits and habitats of the specific vector species.

Entomological studies have several important roles to play in malaria control, including the following:

- identification of the **vectors** responsible for transmission of the disease
- provision of basic information on the **habits and habitats** of vector species for purposes of **planning** effective control measures
- monitoring the **impact of control measures** (for example, by determining changes in vector population density, rates of infection, susceptibility of vectors to insecticides, and residual effects of insecticides on treated surfaces)
- contributing to the **investigation of problem areas** where control measures prove unsuccessful

Vector control programmes should be planned on the basis of entomological studies. Entomological and other epidemiological studies can provide answers to several questions, some of which are listed below. In subsequent Units, you will learn important skills that will enable you to answer these questions.

- Is there malaria **transmission** in the area? If so, in which specific situation and what are the geographical limits of the disease?
- Are there any important mosquito borne diseases other than malaria, if so which ones?
- Which anopheline species are **present** in the area? Which of them are important as **vectors** of malaria?

- What proportion of the vector species **feed on humans**? Among the vectors that feed on humans, what proportion **rest indoors**?
- Where do most of the vector mosquitoes prefer to bite humans, and where does most **man-vector contact** take place, indoors or outdoors? What is the peak biting time of the vector?
- How many **infective bites** are received on average per night per person?
- Which **type of water bodies** is preferred for breeding by a particular vector species in the area?
- During which epidemiological and economic conditions should a vector control strategy to **reduce** transmission be recommended or not recommended?
- What proportions of the vector population are **susceptible or resistant** to insecticides?
- How can we determine the duration of efficacy of an insecticide deposited on a surface (e.g. a sprayed wall or an insecticide-treated bed net)?
- How do different vector **control options** affect malaria transmission, malaria morbidity and mortality? Which vector control options are appropriate against the specific habits and habitats of the vector species? How can we evaluate the **short and long-term effectiveness** of a vector control strategy?

The cost of undertaking comprehensive studies should always be weighed against the benefits to a malaria control programme. Entomological studies must only be carried out to provide a practical answer to clearly defined control-oriented research questions when data is unavailable or inadequate.

Malaria entomology is **not** limited to vector control. Any malaria control strategy should be based on a thorough understanding of the **transmission characteristics** of the disease. Understanding the characteristics of malaria transmission will involve both theoretical studies (e.g. using mathematical models) and empirical observations. Entomological parameters form the basis of such studies.

Entomological studies are also important in the estimation the expected impact of the various control measures. This helps to decide whether some measures are more useful than others and whether some control measures are **dangerous** to implement. We will touch on some of these issues in the final learning unit, but the use of malaria entomology in advanced theoretical studies of malaria is beyond the scope of this course.

Exercise 1.1

Your tutor will now demonstrate the life cycle of *Anopheles* mosquitoes in an insectary. You will visit the insectary in groups of 10. Observe the demonstrated live specimens of each of the stages of the *Anopheles* life cycle.

Learning Unit 2

Identification of malaria vectors

Learning objectives

By the end of this Unit you should be able to:

- Distinguish mosquitoes from other insects
- Tell male and female mosquitoes apart
- Distinguish female anopheline mosquitoes from female culicine mosquitoes
- Differentiate between anopheline and culicine eggs, larvae and pupae
- Describe major external morphological features of adult and larval anophelines used in species identification
- Use a species identification key

2.1 Distinguishing mosquitoes from other insects

Mosquitoes belong to the **phylum Arthropoda**. Arthropods include (among many others) spiders, beetles, ticks, butterflies, houseflies and mosquitoes. They can be recognized by the following characteristics:

- the body is composed of several parts or segments, some of which may be jointed
- the body is covered with a tough skin called exoskeleton
- the body normally has paired, jointed legs and antennae

Within Arthropoda, there are several **classes**, including the **class Insecta** – mosquitoes are members of this group. Insects have the following characteristics:

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

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

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

Fig.2.1 shows the main parts of the adult mosquito. The body, as in all insects, is divided into head, thorax and abdomen. Four characteristics can be used to describe adult mosquitoes: only one pair of wings; a long proboscis; the body is covered with scales; and wings have **veins** that show a defined pattern (as shown in Fig. 2.4.)

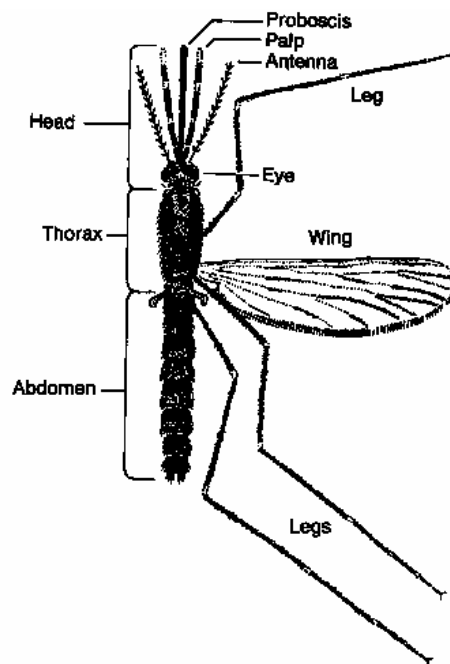


Figure 2.1 Main parts of the adult mosquito

2.2 Distinguishing anophelines from culicines

Distinguishing characteristics of anophelines and culicines are illustrated in Figs. 2.2 and 2.3.

Eggs

Culicine eggs clump together in a "**raft**" (*Culex*) or float separately (*Aedes*); anopheline eggs float separately and each of them has "**floats**".

Larvae

The **culicine** larva has a breathing tube (**siphon**) which it also uses to hang down from the water surface, whereas the **anopheline** larva has **no siphon** and rests parallel to and immediately below the surface.

Pupae

Pupae of both anophelines and culicines are comma-shaped and hang just below the water surface. They swim when disturbed. The breathing trumpet of the anopheline pupa is short and has a wide opening, whereas that of the culicine pupa is long and slender with a narrow opening. However, it is difficult to distinguish anopheline from culicine pupae in the field.

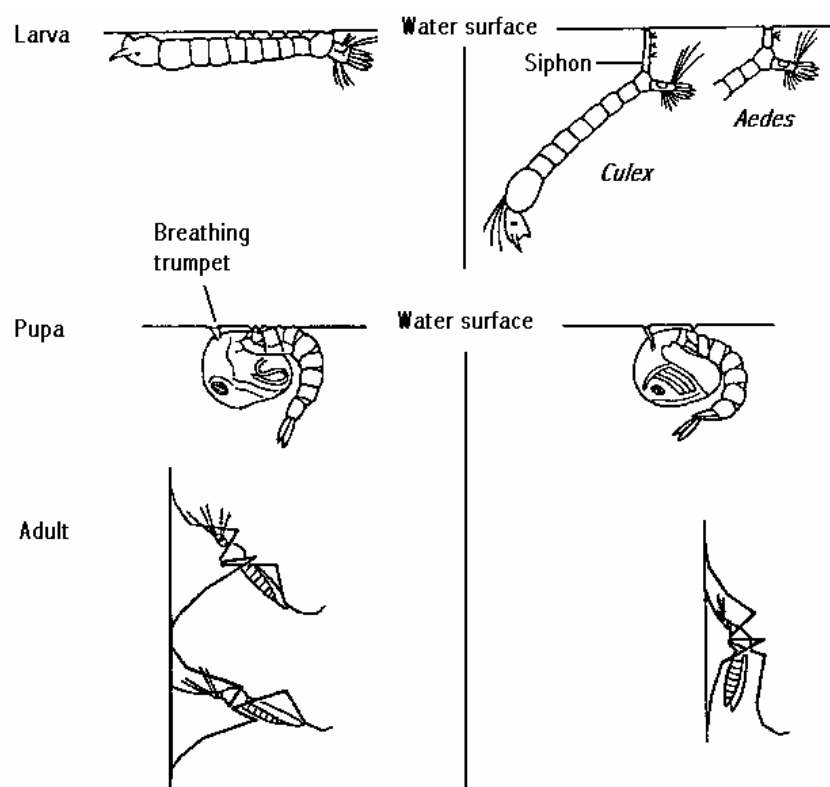


Figure 2.2 Comparison between anopheline and culicine mosquitoes

Adults

With live mosquitoes, you can distinguish between adult anopheline and culicine mosquitoes by observing their resting postures. Anophelines rest at an angle between 50° and 90° to the surface whereas culicines rest more or less parallel to the surface (Fig. 2.2).

Anopheline mosquitoes can also be distinguished from culicines by the length and shape of the palps. The differences (Fig. 2.3) are:

- In female anophelines, palps are as long as proboscis; in female culicines, palps are very much shorter than proboscis.
- In male anophelines, palps are as long as proboscis and club-shaped at tip; in male culicines, palps are longer than proboscis, with tapered tips.

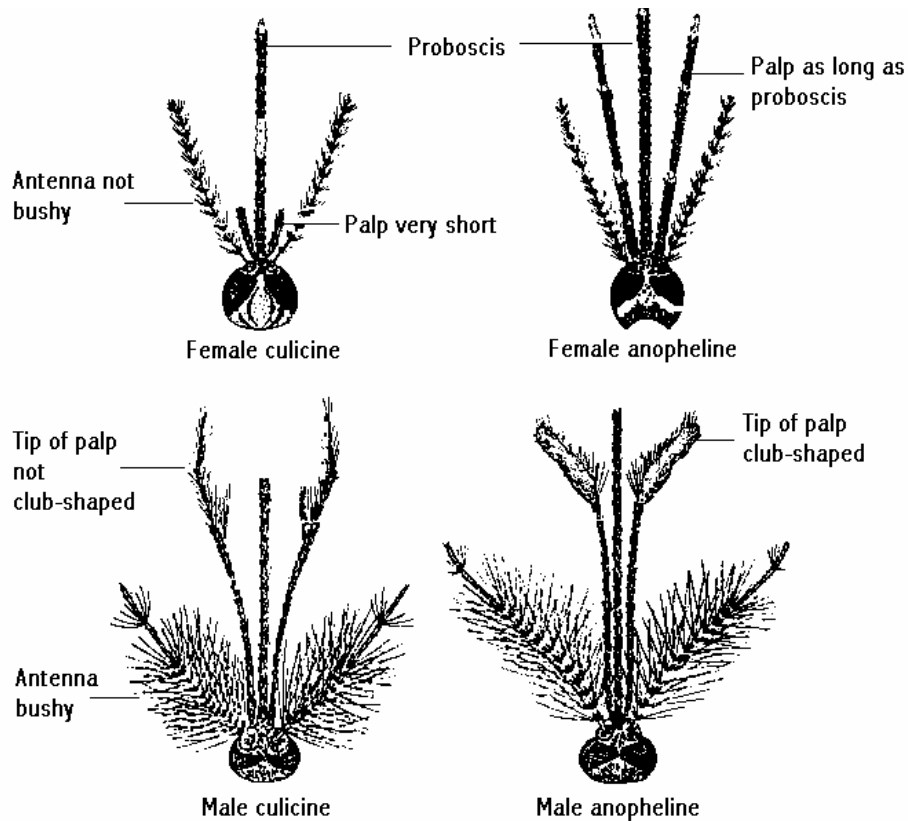


Figure 2.3 Heads of male and female anopheline and culicine mosquitoes

2.3 Distinguishing female *Anopheles* from males

It is important to be able to distinguish females from males because only the female *Anopheles* takes blood meals and transmits malaria,

On the antennae of the female the hairs are few in number and short (Fig. 2.3). The male has very long hairs on the antennae, which consequently have a bushy moustache-like appearance.

2.4 Identifying anopheline species

You will now learn how to classify common vector species in your area using **identification keys**. Information collected during mosquito surveys is only useful if the mosquitoes are accurately identified. It is therefore essential that you be able to identify the species of adults and larvae. Identification of pupae is very difficult and when pupae are obtained in the field they should be kept alive and allowed to emerge into adults because the adults can be identified more easily. We will describe some external characteristics of adult and larval anophelines that are useful in species identification.

a. The external anatomy of adult *Anopheles*

Head

The head has a pair of large compound eyes. A pair of antennae is joined to the head between the eyes (Fig. 2.3). A pair of palps below the antennae is composed of five parts in anopheline mosquitoes. The palps are covered with scales which may be of different colours and used in species identification. A proboscis protrudes from the ventral part of the head and extends forward.

Thorax

The thorax has a pair of wings and a pair of halteres on the upper surface and three pairs of legs on the lower or ventral surface.

The wings have several veins on them; each vein is given a number and/or a name (Fig. 2.4). The **vein** along the front edge of the wing is called the **costa** and the short vein behind it is called the **subcosta**. There are six other veins numbered 1-6 of which veins 2, 4 and 5 are forked. These veins are covered with scales. The scales are usually brown, black, white or cream in colour. The back edge of the wing has fine scales. Many anophelines have wings spotted with dark and pale areas which together with other characteristics are used for species determination.

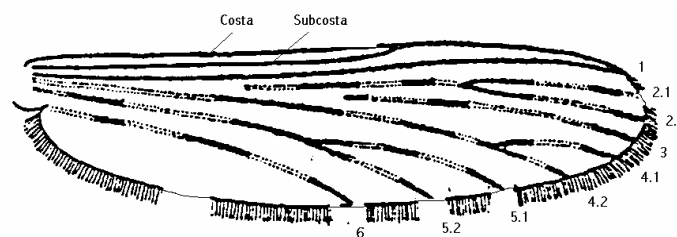


Figure 2.4 *Anophele's* wing

The legs are long and made up of a short **coxa** joined to the body, followed by a short **trochanter**, then a long **femur**, a long **tibia**, and long **tarsus** which are made up of five parts (figure 2.5). The five parts are numbered 1-5 with segment 1 being closest to the body. At the end of the leg is a pair of claws. The legs are also covered with scales which may be of different colours and used in species identification.

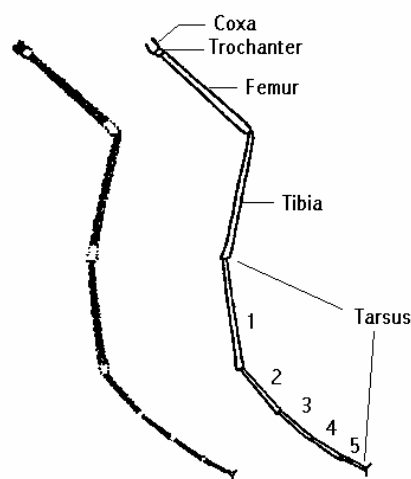


Figure 2.5 *Anopheles* leg

Abdomen

The abdomen has eight visible segments. The upper plates are called **tergites**, and the lower plates are called **sternites**. They are joined by a membrane which allows the distension of the abdomen when the female takes the blood meal.

b. External anatomy of *Anopheles* larva

The body of the larva is divided into three segments; the head, thorax and abdomen. All parts of the body have hairs attached to them.

Head

The head has a pair of antennae, one on each side. The shafts of the antennae have hairs at the end and on the sides (Fig. 2.6). A pair of **mouth brushes** lies at the front of the head. The upper surface of the head has several hairs; the position and shape of these hairs are important as a means of identification.

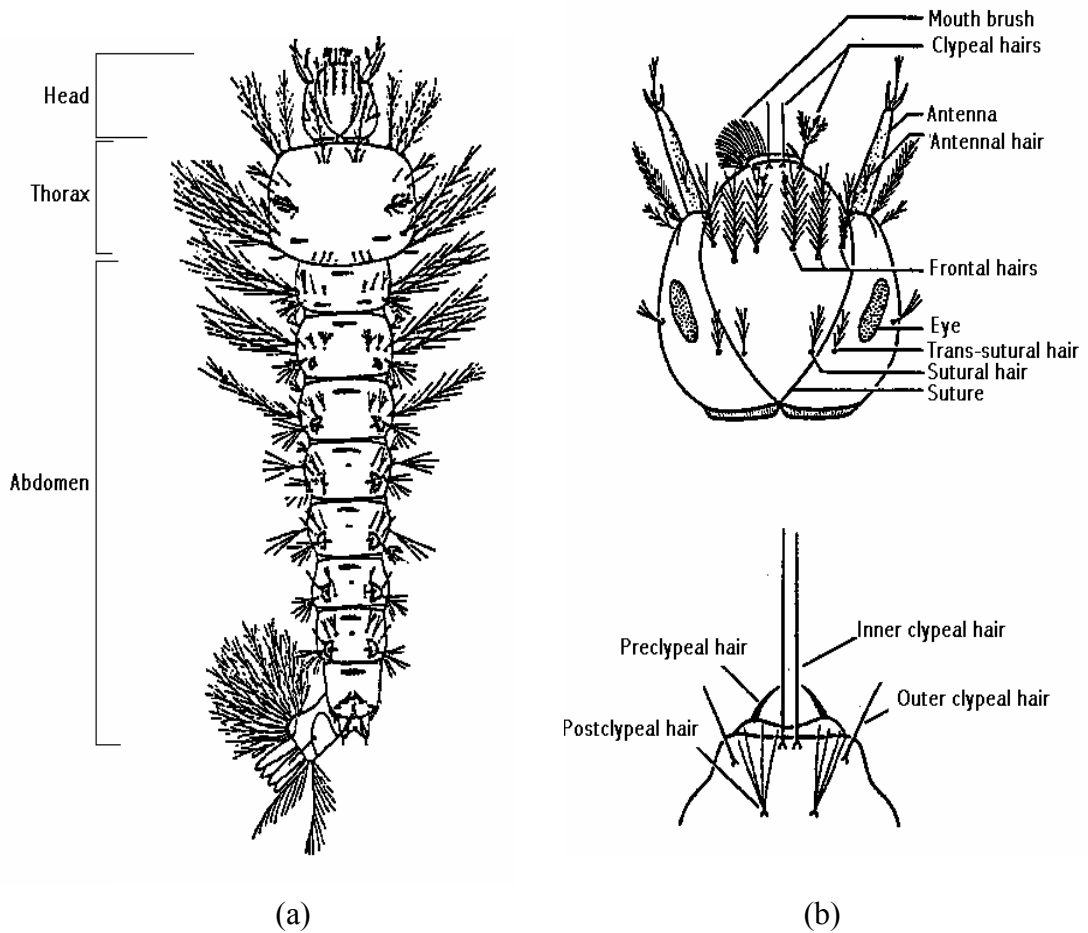


Figure 2.6 (a) Body parts and (b) head of an anopheline larva

Thorax

The thorax is formed of three parts: the **prothorax**, the **mesothorax** and the **metathorax** (Fig. 2.7). The hairs on these parts of the thorax are called prothoracic, mesothoracic and metathoracic hairs. Both the upper and lower surfaces have hairs. On the lower surface of the ventral part of the thorax there are several hairs including three groups on each side with four hairs in each group. These groups are the **prothoracic pleural** group, the **mesothoracic pleural** group and the **metathoracic pleural** group. These hairs are also important in species identification.

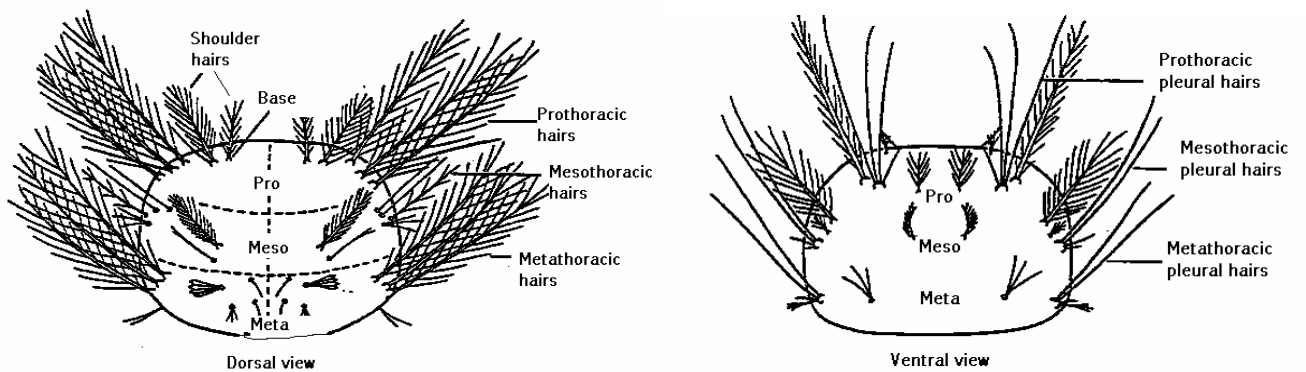


Figure 2.7 Thorax of an anopheline larva (dorsal and ventral views)

Abdomen

The abdomen has eight similar segments and two modified segments: the 9th segment has a pair of spiracles and the 10th is the anal part (Fig. 2.8). Well-developed fan-shaped hairs, called **palmate hairs**, are present on segments IV-VI and sometimes also on segments I-III. Each segment has up to four **tergal plates** on its dorsal side. There is usually a pair at the anterior and a second pair at the posterior of each segment, and there are also two accessory plates. The 9th abdominal segment is joined with the 8th segment and carries the spiracles through which the larva breathes. On each side of the 9th segment is a **pecten**, which is a triangular plate with comb-like teeth. Most of the upper surface of the anal segment is occupied by a large tergal plate called the **saddle**. Hairs may arise from the saddle or from the anal segment. On the lower surface of the anal segment is a series of hairs called the **ventral brush**. Four anal gills extend from the anal segment.

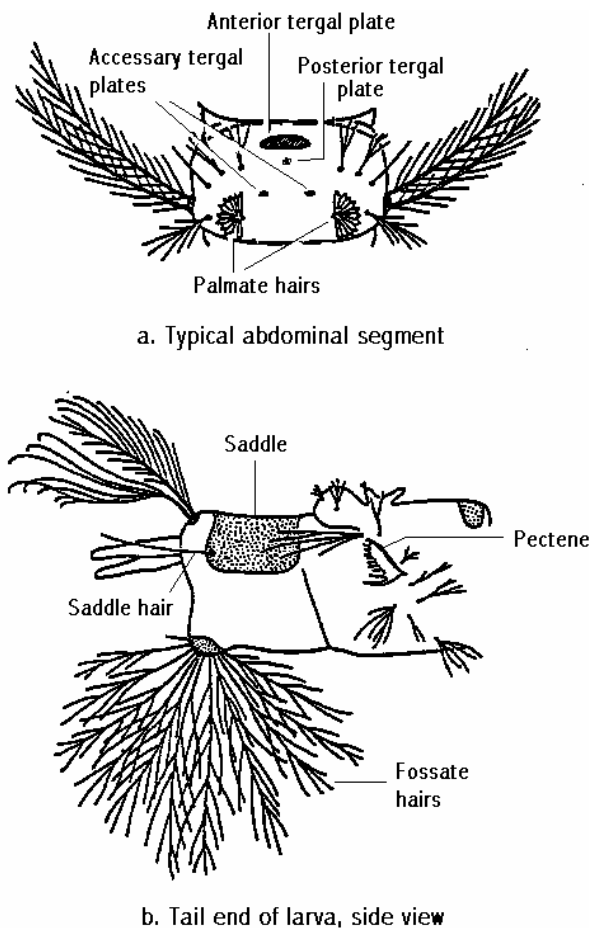


Fig. 2.8 Abdominal segments of an anopheline larva

Identification keys and how to use them

Keys for the identification of anopheline adults and larvae have been developed for most parts of the world. You must first be sure to select a taxonomic key which has been developed for the geographic area concerned or as near to it as possible.

The type of identification key that is most commonly used has pairs of statements grouped together and is called a **dichotomous** or **couplet** key. In this type of key only one of each pair of statements correctly describes your specimen. You must decide which statement is correct for your specimen. At the end of the statement will be either a number indicating which couplet to use next or the correct name of your specimen. If you go to another couplet, choose the correct answer in that couplet and continue working through the key until you have identified the name of your specimen.

If you have a specimen in which the wings have pale and dark scales, the legs are speckled and half of the proboscis is pale, in the following key your specimen would key out to species E.

- | | | |
|----|--|-----------|
| 1. | Wing scales are dark..... | 2 |
| | Wings with pale and dark scales..... | 3 |
| 2. | Legs with dark scales only..... | Species A |
| | Legs with pale and dark scales..... | Species B |
| 3. | Legs with dark scales only..... | Species C |
| | Legs with pale and dark scales (speckled)..... | 4 |
| 4. | Proboscis all dark..... | Species D |
| | Proboscis with pale scales on apical half..... | Species E |

Other techniques of species identification

Some anopheline species are similar in external morphology, while they are actually different species. These species are genetically related and are known as **sibling species**, and are morphologically grouped under the same **complex**. For example, in the *Anopheles gambiae* complex (also known as *Anopheles gambiae* sensu lato or s.l.), there are seven different species: *A. gambiae* sensu stricto (s.s.), *A. arabiensis*, *A. quadriannulatus species A*, *A. quadriannulatus species B*, *A. bwambae*, *A. merus*, and *A. melas*. It is not possible to differentiate between these species by using an identification key that is based on external morphology. If you cannot identify the particular species by external morphology, you should record the name of the complex, for example, *A. gambiae* s.l.

The techniques generally used for identification of sibling species include: cytogenetic identification, molecular techniques, enzyme electrophoresis, and use of cuticular hydrocarbons and crossing experiments. These techniques require advanced skills or sophisticated laboratory equipment, and are beyond the scope of this learning unit.

Exercise 2.1

In the laboratory, live and preserved specimens of anopheline and culicine mosquitoes at the various stages of their life cycle will be demonstrated. Take your time and observe the preserved or pinned specimens to see the differences among the different stages of the life cycle.

Exercise 2.2

You will be provided with a compound and dissecting microscope, forceps, dissecting needles and freshly pinned adult female anophelines and larval specimens on slides. Identify the specimens to the species (or species complex) level.

Learning Unit 3

Sampling malaria vectors

Learning objectives

By the end of this Unit you should be able to:

- understand the importance and use of different mosquito surveys
- use different methods to collect mosquitoes and describe their purposes
- describe the methods of handling and transportation of live mosquitoes
- transport live larvae and pupae collected in the field to the laboratory and preserve them
- describe breeding sites of malaria vectors

Introduction

Surveys are an essential component of malaria vector control programmes, operational activities and research. There are four primary types of surveys that are used in vector studies. They are preliminary surveys, regular or trend observations, spot checks, and focal investigations

1. Preliminary surveys

Preliminary surveys are original, basic, short-term surveys used to gather baseline data for **planning** vector control measures. They provide information on the identity of specific vector species; their resting and feeding habits, seasonal densities, and longevity; the types of water bodies used as breeding sites; and their sensitivity to available insecticides in order to facilitate the selection of the most cost-effective insecticide.

2. Regular or trend observations

These are long-term observations carried out regularly, e.g. monthly or half-yearly, for the purpose of monitoring and **evaluating** the impact of control measures. They provide information on changes in vector density, infection rates, behaviour, and susceptibility of vectors to insecticides.

3. Spot checks

Spot checks are carried out in localities that are chosen at random. Since the fixed stations often used to monitor mosquito populations may not be representative of all areas, spot checks may be conducted randomly in selected areas to **supplement routine observations** or obtain a clearer indication of the effects of control measures.

4. Focal investigations

Focal investigations are undertaken in areas of **new or persistent malaria transmission** to determine why there is transmission or why the disease is not responding to the measures being applied, and to identify the best approaches to control.

3.1 Hand collection of indoor-resting mosquitoes

Many of the anopheline species that are malaria vectors rest indoors. Hand collection provides information about usual resting places, resting density, and seasonal changes in density. It also provides live specimens for susceptibility and bioassay tests and for observations on mortality among mosquitoes from insecticide-treated houses or houses with insecticide-treated bed nets.

Equipment - Sucking tube, flashlight, paper cups with covering net, cotton wool, rubber bands, mosquito cages, a card box container or insulated picnic box, chloroform, and towels (Fig. 3.1).

How to use the sucking tube:

- With the mouthpiece in your mouth, hold the sucking tube with its opening 1-2 cm away from the mosquito
- Move the end of the sucking tube closer to the mosquito and, at the same time, suck gently but quickly so as to draw the mosquito into the tube
- Place your finger over the tube to prevent the mosquito from escaping
- Place the end of the tube, with your finger still in position, near the hole in the mesh covering the paper cup. Remove your finger and quickly put the tube into the hole
- Blow gently into the mouthpiece so as to transfer the mosquito to the paper cup; at the same time, tap the tube with your index finger to disturb resting mosquitoes.

Do not collect more than five mosquitoes in one sucking tube before transferring them to the paper cup.

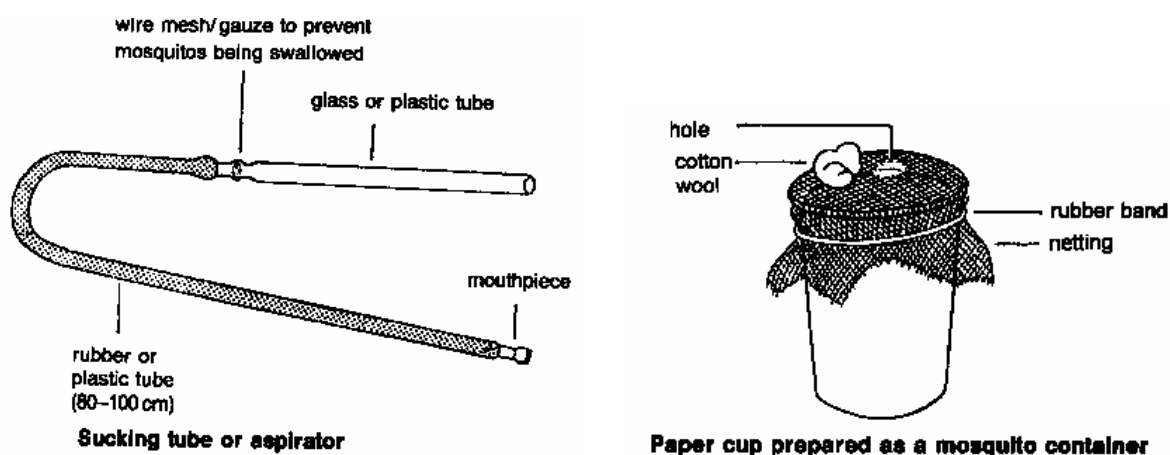


Figure 3.1 Sucking tube (or aspirator) and paper cup for hand collection of adult mosquitoes.

Hand collection of indoor-resting mosquitoes

You should normally collect mosquitoes early in the morning after the house occupants are up and dressed. In any village you should search at least 10 houses in order to provide a representative sample.

Mosquitoes caught alive in houses may be kept for 24 hours. This will allow you to check the 24-hour mortality rate among mosquitoes collected from sprayed houses or from houses with insecticide-treated bed nets.

Examine the whole house or, if it is too large, spend up to 15 minutes searching room by room. Pay special attention to rooms in which people slept the previous night. With the aid of the flashlight, look for mosquitoes on walls, on the ceiling, behind and under furniture, inside large pots and jars, and under beds. Conduct a systematic search of the house starting at the main door and searching to the left moving clockwise around the inside of the house.

Use a separate cup for each house. The cups must be clearly labelled in pencil with at least the following information: locality, date and time of collection, time spent on collecting in minutes, house number or householder's name, type of structure (house, animal shelter, store, etc.), whether sprayed and if so when, number of people and/or animals in the room during the previous night, and your name. Alternatively, you may include on the label only the locality, date, house number and your name, and use a collection form (to accompany the paper cup) to fill in the complete information.

Keeping mosquitoes alive in the field

If mosquitoes are to be kept for some time in the field and during transport, take precautions to keep them in good condition:

- Soak pieces of cotton wool in 5-8% sugar solution, squeeze out any excess sugar solution and place the cotton wool over the tops of the cups
- Place cups holding mosquitoes upright in a cardboard box or, preferably, an insulated picnic box
- Cover the cups with a damp towel and keep the towel damp until the mosquitoes reach the laboratory
- Make sure that you keep mosquitoes in places that are free from insecticide contamination and away from ants.
- Before transport, pack newspaper or other material between the cups to minimize movement.

Killing mosquitoes

Add a few drops of chloroform (or ethyl acetate) to a pad of cotton wool and place it on top of the netting of the paper cup. Cover the cup with a glass Petri dish to prevent the chloroform from evaporating. Do not use a plastic Petri dish as this will be dissolved by the chloroform. Take standard safety precautions when handling chloroform and any other chemical.

3.2 Spray sheet collection of indoor-resting mosquitoes

Spray sheet collection involves using a pyrethrin space spray to knock down mosquitoes resting inside a house and collecting them on white sheets spread on the floor and other flat surfaces in the house.

It is unlikely that you would obtain all the mosquitoes resting in a house using the hand collection method. Using the spray sheet collection method, it should be possible to collect practically all the mosquitoes from a well-closed room sprayed with a fine mist of pyrethrin solution. This method of collection allows quantitative studies to be undertaken, including measurement of,

- indoor resting density (the number of mosquitoes resting indoors during the day)
- man-biting density (indirectly)
- seasonal changes in indoor resting density
- number of mosquitoes remaining in a given room after a hand collection

Equipment - White cotton sheets (sizes 2m x 1m, 2m x 2m and 2m x 3m); hand sprayers; pyrethrin solution; kerosene; small Petri dishes; paper cups; hand lens; forceps; a container (or preferably a picnic box) for transporting mosquitoes; cotton wool; filter paper; a torch.

The hand sprayers should be of the double-action type with an air valve (Fig.3.2). The pyrethrin solution should be prepared at a concentration of 0.2%-0.3% in kerosene. Take the necessary safety precautions when handling pyrethrin, and always keep away from the reach of children.

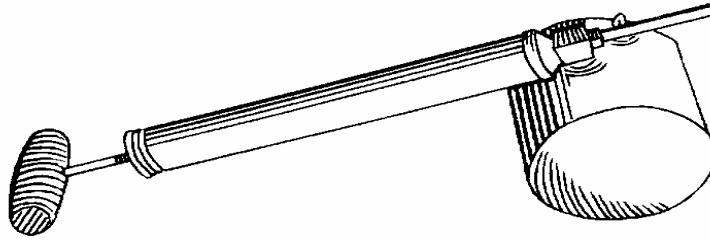


Figure 3.2 Hand sprayer

Preparation of rooms for spray sheet collection

It is normal for the work to be performed by a team of three or four people so that collections can be made in eight to ten rooms in each locality.

Ensuring that you disturb any resting mosquitoes as little as possible, prepare a room for spraying as follows:

- Remove all animals
- Remove or cover all food
- Remove all small items of furniture
- Cover all openings and eaves with cloth or mosquito netting
- Spread the white sheets so that they completely cover the floor and all flat surfaces of the remaining furniture; sheets should also be spread under tables, beds and other places where mosquitoes may hide
- Close all windows and doors.

Carrying out space spraying and collection of mosquitoes

One of the team members should walk round the outside of the room and spray in open spaces or holes in the walls and eaves. The same person or another member of the team should then enter the room, close the door and, moving in a clockwise direction, apply spray towards the ceiling until the room is filled with a fine mist. The operator should leave the room quickly and make sure that the door remains closed for at least 10 minutes.

Starting from the doorway, pick up the sheets one at a time by their corners. Carry the sheets outside. Collect the knocked down mosquitoes outside in daylight using forceps. Place collected mosquitoes in a labelled Petri dish with a layer of damp cotton wool and filter paper on top of the cotton wool. Use separate Petri dishes for each house, and label the dishes with all the essential information.

3.3 Outdoor collection of adult mosquitoes

Some mosquito species enter houses at night to bite and rest indoors during the day. Some species bite indoors, but leave the house soon after biting. Other species do not enter houses but bite outside and then rest on vegetation or on solid surfaces in sheltered places such as the banks of streams and ditches, holes in rocks, culverts, cracks in stone walls, caves, animal burrows, on the trunks or stems of larger trees, and in old termite mounds.

Data from outdoor collections is important in evaluating the impact of vector control measures. It provides information about the species that habitually rest outdoors and any alterations in the relative numbers of mosquitoes resting outdoors following the application of insecticides and use of insecticide-treated bed nets in houses.

Outdoor collection is performed in either the natural resting places described above or in shelters specially constructed for that purpose. Artificial shelters have the advantage of providing concentrated sites for collections and more representative samples that can be used for quantitative work.

Equipment - The equipment required for outdoor collection is the same as that listed under Hand collection of indoor-resting mosquitoes. In addition, a hand net and a drop net may be used. Since the preparation or construction of artificial shelters will be undertaken during field practice, you also require: a barrel, two spades, a pickaxe, and an axe.

Outdoor collection methods

The common methods used to collect mosquitoes resting on vegetation involve the use of a sucking tube, a hand net, or a drop net. Anopheline species that normally rest on solid surfaces are collected with the aid of a sucking tube from natural or artificial shelters. Artificial shelters may consist of large barrels or boxes, perhaps set into riverbanks, or they may be pits dug in the ground (Fig. 3.3). Well-placed shelters normally yield more mosquitoes than natural environments.

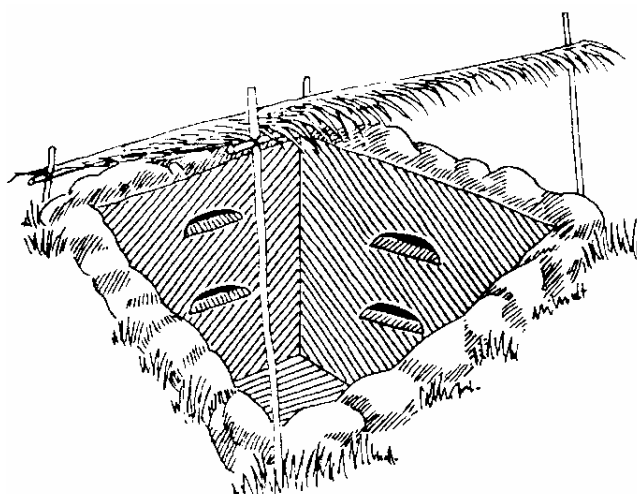


Figure 3.3 Pit shelter with roof

A hand net or sweep net is used to collect mosquitoes resting on vegetation (Fig. 3.4). The correct method of use is to move the hand net swiftly over the tops of tall grasses or close to the ground around bushes.



Figure 3.4 A hand net

Make sure that you record the type of shelter, the number of collections, and the total time spent collecting.

3.4 Direct catches of mosquitoes from baits

Female mosquitoes are attracted to humans and/or animals to obtain blood meals. The number of vectors biting humans is therefore a major determinant of malaria transmission, and it is important to know:

- which anopheline species bite humans and which prefer to bite animals
- which of those that bite humans are vectors of malaria
- how often a person is bitten by a vector
- whether the vectors bite indoors or outdoors
- their peak biting time
- the seasonal variations in the numbers of mosquitoes biting humans

Equipment - Sucking tube, flashlight, paper cups with net covers, alarm clock, wooden pegs and a rope (to tether the animal bait), wooden pegs (for the tether), hammer, cotton wool, towels, card box container or an insulated picnic box.

Human baits

Human baits should take an appropriate and effective antimalarial prophylaxis to avoid contracting malaria during collection of biting mosquitoes. Furthermore, it is not necessary to permit mosquitoes to feed; they should be collected as soon as they settle on the skin, since it can be safely assumed that biting would normally follow. Landing rates should therefore be measured instead of biting rates. Although collection of mosquitoes off human baits is useful as a direct measurement of human biting rates, there are ethical concerns because the baits are at risk of infection. You will need to consider this concern and acquire ethical clearance before using this technique. We recommend that you avoid using this technique unless it is absolutely essential, especially if other safer techniques are available that can provide proxy estimates of human biting rates. These techniques will be described in subsequent sections.

If possible, select a house in the area of the village with the greatest number of cases of malaria. A human collector is seated indoor and another is seated outdoor. Collectors should switch sites every hour. The collections are often made during the entire night (if necessary) or during part of the night. Collectors may also work in shifts during the night.

Adjust your clothing so that your legs are exposed as far as your knees and sit quietly. When you feel a bite, quickly turn on your torch collect the mosquito¹ with your sucking tube and transfer it to a paper cup. Use one cup for each hour of collecting. Do not smoke while collecting.

Alternatively, one person can serve as bait and another as collector. The person acting as bait sits or lies in a quiet place, inside or outside the house as appropriate, with his or her clothing adjusted to expose as much skin as is acceptable. The collector checks for and collects biting anophelines every two or three minutes.

Note the habitual sleeping time of the local people; you will use this information to study whether most man-vector contact occurs indoors or outdoors, and the number of bites that average villagers receive at each site during the night.

Animal baits

Collecting from animal bait is normally carried out in the same location and at the same time as collecting from human bait. Before sunset, select a tame animal from the village, usually a cow. The collecting site should be near the place where the animal usually passes the night. Tie the animal securely and examine the animal every two to three minutes and collect all the anopheline mosquitoes you find. Keep each hour's collection in a separate paper cup.

3.5 Collecting mosquitoes in baited trap nets

In this section, you will learn how to use trap nets; the purposes of collection by this method are the same as those of direct collecting.

Animal-baited trap nets generally produce more mosquitoes than can be collected by direct capture from animals; the opposite, however, is true for human-baited trap nets. For this reason, standard night collecting from bait usually involves direct collection from humans indoors and outdoors, and collection from animal-baited trap nets outdoors.

Equipment - Sucking tube, torch, paper cups with net covers, cotton wool, towels, an insulated picnic box, an alarm clock, two camp beds, two small mosquito nets with frames to fit the camp beds, two trap nets for human bait, one trap net for animal bait, wooden pegs and a rope (for tethering the animal), hammer, pegs and string (for securing trap nets), and a needle and thread (for repairing trap nets).

¹ Unless you are an *extremely experienced* entomologist, collect all mosquitoes and sort out anophelines afterwards rather than trying to select anophelines during bait-catching.

Collecting by means of human-baited trap nets

The direct catch of mosquitoes using human baits is often not recommended because of ethical concerns; the collectors might be exposed to malaria infection. If it is an ethical problem to use human collectors, you must find an alternative method of collection that gives a representative sample of the vector population that would bite humans.

One technique uses two trap nets set up so one is a sleeping room and the other is the trap net. Put up the inner net around a camp bed to protect the person acting as bait (Fig. 3.5). Stretch the bottom of the outer net tightly and tie it to pegs in the ground, leaving 15-20 cm between the ground and the lower edge of the net. At sunset, get into the trap and lie on the bed. Set the alarm clock to ring after one hour. When the alarm rings, collect all the anophelines in the trap net. The collecting period should not exceed ten minutes. Get back onto the bed and set the alarm as before. Repeat the procedure throughout the night.

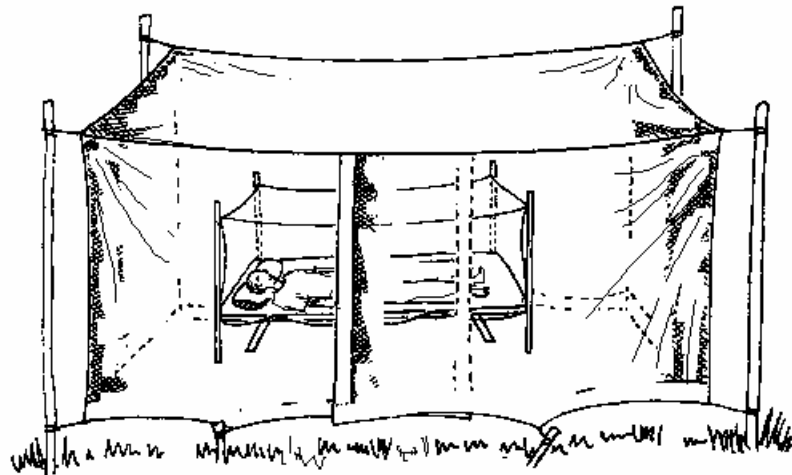


Figure 3.5 Trap net with human as bait

Collecting by means of human-baited CDC light traps

A CDC (Centre for Disease Control) light trap is installed in the bedroom beside a bed with a mosquito net. The human bait will sleep under the net, while the light trap will attract female anophelines that have entered the room to bite the person under the net, and thus can be used as a proxy to study the biting rate. In the house, only one person (known as a sleeper) sleeps alone during the night. One CDC light trap is positioned indoor fitted with incandescent bulbs and laid close to the human volunteer sleeping under an untreated bed net in his/her usual sleeping place. The light trap is installed at about 1.5 m above the floor next to the foot of the bed of the person. Trapped mosquitoes are removed the next morning.

Collecting by means of animal-baited trap nets

An animal-baited trap net is situated close to where the animal is customarily kept overnight. Animal-baited traps are normally used outdoors. The trap net (Fig. 3.6) is similar to that used for collecting mosquitoes attracted to human bait. The animal must be securely tethered so that it cannot break free and damage the trap or harm itself.

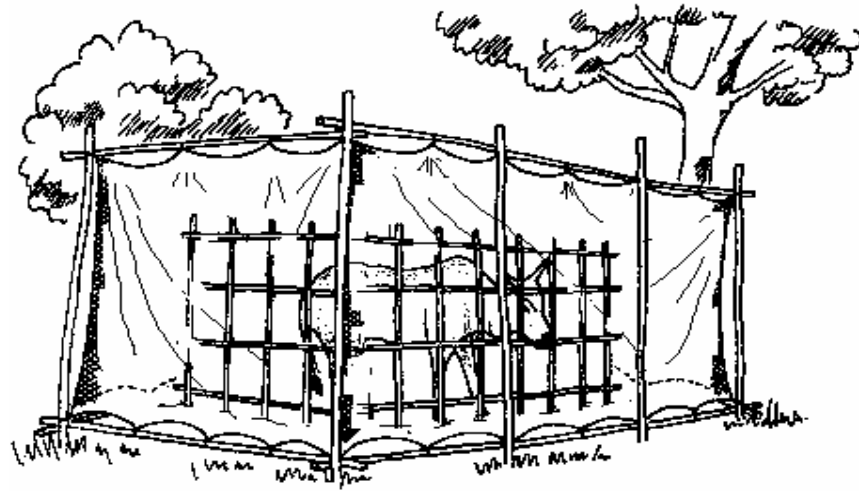


Figure 3.6. Animal-baited trap net

If there is to be repeated collection at the site, a small enclosure may be built to confine the animal. Place the animal in the trap at sunset and collect mosquitoes every three hours.

3.6 Methods for collecting larvae and pupae

Where to look for anopheline larvae and pupae

Each type of mosquito prefers to lay its eggs in a particular kind of water. Some will lay only in fresh, clear, water with some shade, others only in brackish water; some may even lay eggs in very small quantities of water, such as a hoof-print.

It is most important for you to know the preferred breeding sites of the anopheline mosquitoes that transmit malaria in your area, and the densities of larvae and pupae at these sites. Collecting from different types of breeding site in an area will allow you to:

- determine the species present
- ascertain the preferred breeding sites of each vector species
- make an assessment of the effectiveness of a vector control programme

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

Potential breeding sites include:

- small rain pools, hoof-prints, drains and ditches, where the entire surface of the water should be examined
- brackish water (where fresh water and salt water mix)
- streams, which should be searched at the edges where there is vegetation and the water moves slowly
- ponds, lakes, swamps and marshes where larvae usually occur in vegetation around the edges, but can sometimes be found far from the shore among floating vegetation
- special sites, such as wells and water containers made of cement, where the entire surface of the water should be examined

Whatever the collecting method used, you must always approach the breeding place cautiously, facing the sun: if the larvae are disturbed by shadows and movement many of them will swim downwards and disappear from view. You will then have to wait quietly for several minutes until they return to the surface of the water.

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

Use of the dipper

A white enamelled dipper is preferable, because this allows you to see the larvae most easily (Fig. 3.7).

- Lower the dipper gently into the water at an angle of about 45° , until one side is just below the surface
- While dipping, care should be taken not to disturb the larvae and thus cause them to swim downwards – if they are disturbed, wait for a minute or two until they come up to the surface again, and then continue dipping
- Move along the breeding site, skimming the surface of the water with the dipper
- Lift the dipper out of the water, making sure that you do not spill the water containing the larvae and pupae
- Hold the dipper steady until larvae and pupae rise to the surface of the water
- Collect the larvae and pupae by means of a pipette and transfer them to a bottle or vial
- Do not throw the residual water back into the breeding place as this may further disturb the larvae and pupae
- Count number of dips in each type of breeding place –this helps in calculating **larval density** in each type of water body. The larval density in each breeding site can be calculated by instar or as the number of 3rd and 4th instar larvae of each species collected per dip (or per 100 dips if the density is too low). Also note the time spent in minutes while collecting in each type of breeding place.

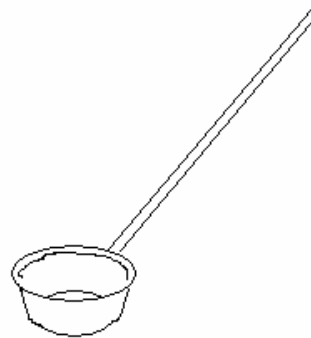


Figure 3.7 Dipper

Use of the larval net

A larval net for collecting larvae and pupae in ponds and lakes consists of a fine mesh net mounted on a wooden handle and a plastic bottle or tube tied to one end (Fig. 3.8). To collect larvae and pupae, sweep the water surface by holding the net at an angle and moving it through the water. Larvae and pupae on the water surface will be swept into the net and will collect in the plastic bottle or tube.

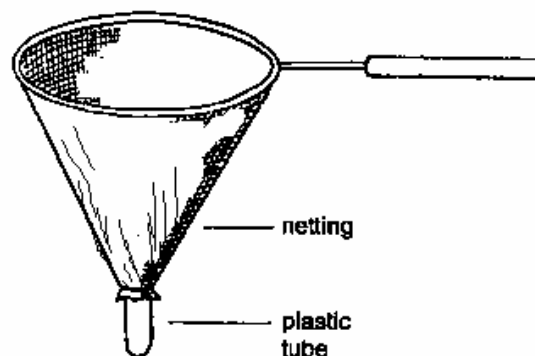


Figure 3.8 Larval net

A simple net with no attached bottle or tube can be used. After sweeping, the net should be inverted into a bowl of water and its contents dislodged. The water in the bowl is then searched for larvae and pupae, which are picked up and transferred to a bottle or vial by means of a pipette.

The net used for sampling from wells is similar to the larval net but lacks a wooden handle; instead, it is held at an angle by four strings and controlled by a long string or rope.

3.7 Transporting live larvae and pupae

Place all the specimens from a particular breeding place in one bottle or vial and label it. The label must be written in pencil on a piece of paper and dropped into the specimen bottle. Do not use a ballpoint pen as the ink dissolves in water.

The larvae and pupae collected must arrive alive and undamaged at the laboratory. Cap each bottle or vial tightly so that water cannot spill. Make sure that there is air in the top 1-2 cm so that the larvae and pupae can breathe for a few hours. If a larger air space is left, the water will become agitated during transportation and the specimens will suffer damage, particularly loss of hairs.

If the journey to the laboratory takes more than two to three hours, remove the stoppers every two hours to provide the specimens with fresh air. Pack bottles and vials carefully so that they are not jolted during transport. If the larvae are to be used in insecticide susceptibility tests they should be transported in water in a large vacuum flask or other large container.

3.8 Killing and preserving larvae and pupae

Hold the vial containing the larvae over a flame of a burning cotton wool soaked with alcohol (placed on a piece of rock) for about 30-60 seconds. Alternatively, you may transfer larvae to hot water (50°-70°C) using a pipette.

- Carefully pour the water until as much water as possible has been removed from the vial while keeping the killed larvae in the vial.
- Add 70% alcohol (ethanol) to the vial.
- Add a plug of loose cotton wool to the vial.
- Prepare a label with all of the following information written in *pencil* (do *not* use a pen): locality, type of breeding site, number of dips taken, time spent in minutes, date of collection, and name of collector.
- Put the label inside the vial above the cotton wool.
- Close the vial tightly (Fig. 3.9).

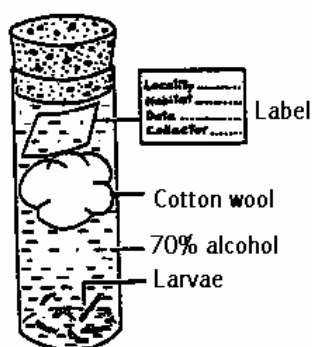


Figure 3.9 Preserving larvae in vials

Exercise 3.1

In the laboratory, you should practise the following:

- Using a sucking tube, pick adult mosquitoes from a cage and put them in paper cups
- Pick live larvae and pupae using droppers and put them in vials
- Killing and preserving adults, larvae, and pupae.

Learning Unit 4

Susceptibility and bioassay tests

Learning objectives

By the end of this Unit you should be able to:

- measure the level of insecticide resistance in a vector population
- determine the residual efficacy of an insecticide deposit on a sprayed surface or material at a specified time after the spraying

One of the most important reasons to sample vector populations is to determine their susceptibility to insecticides. When insecticides are used in malaria control, it is important that you monitor changes in the susceptibility level of the target vectors from time to time. The residual efficacy of the insecticide used should also be determined at intervals after its application or use. In this Unit, you will learn the skills required to carry out these activities.

4.1 Susceptibility tests

Physiological resistance to insecticides

Susceptibility tests are carried out to determine the proportion of the vector population that is physiologically resistant to a particular insecticide.

Physiological resistance to insecticides has been defined as the 'ability of a population of insects to tolerate doses of an insecticide which would prove lethal to the majority of individuals in a normal population of the same species.

The efficacy of indoor residual spraying (IRS) and insecticide impregnated nets (ITNs) depends, among other things, on the proportion of vectors resting on the sprayed surface and on the susceptibility of the vectors to the insecticide used. It is therefore important to monitor the development and extent of insecticide resistance in a given vector population.

Equipment

Susceptibility test kit (including exposure/holding tubes, copper and silver rings, insecticide-impregnated filter papers, oil-impregnated control papers, sucking tubes), thermometer, wooden box with large holes, towels, cotton wool, paper cups with cover nets, rubber bands, markers or wax pencils, mosquito cage.

Method of determining the susceptibility of adult mosquitoes

The standardized WHO method involves checking the mortality of several female *Anopheles* of a known species exposed in special tubes to filter papers impregnated with a lethal concentration (known as **discriminating dose**) of a given insecticide dissolved in mineral oil.

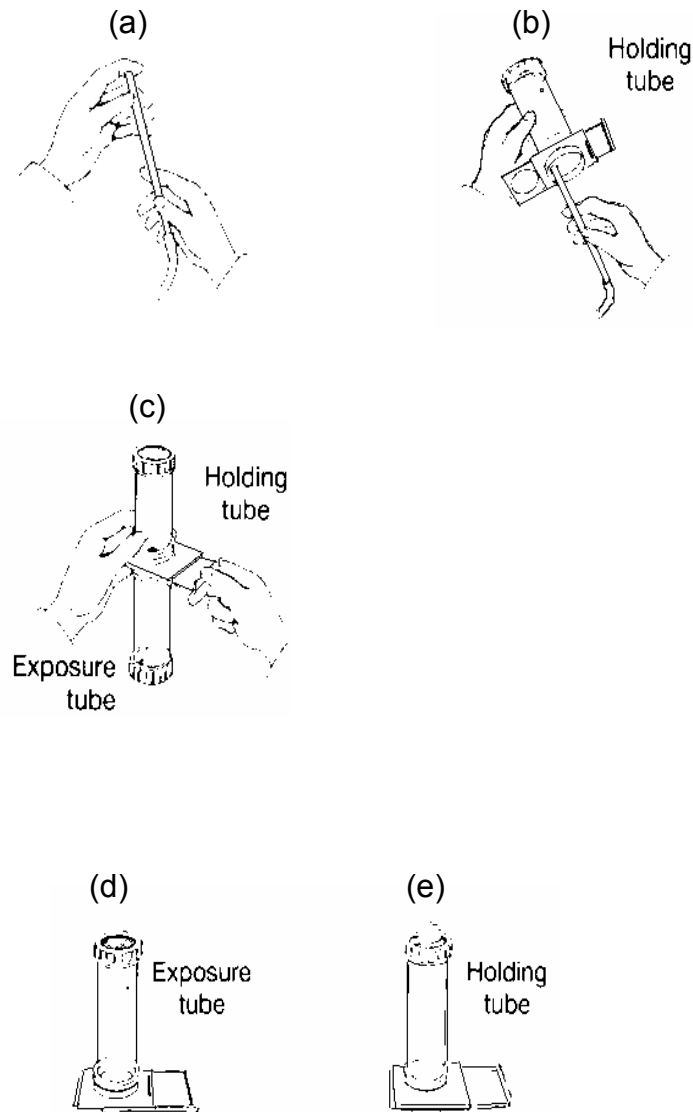


Figure 4.1 Method for determining the susceptibility of adult mosquitoes

Collect as many mosquitoes by means of an aspirator (Fig. 4.1a).

- Transfer 15-25 fed mosquitoes to a special plastic holding tube lined with insecticide free paper (Fig. 4.1b).
- Connect a plastic tube lined with filter paper impregnated with mineral oil (used as control) with the holding tube and transfer 15-25 mosquitoes to the tube through a hole in the slide between the 2 tubes (Fig. 4.1c); also transfer the same number of mosquitoes to plastic tube with insecticide-impregnated filter paper. (The filter papers are held in place by special rings). Use separate sucking tubes to transfer mosquitoes to the exposure and control tubes to avoid contamination.
- Close the slide and allow the exposure and control tubes to stand upright for the determined period, which is usually one hour (Fig. 4.1d).
- After the exposure period transfer the mosquitoes back to the holding tube, which should stand upright for 24 hours, with a piece of moist cotton wool on the gauze end and in a wooden box with large holes for ventilation and covered with a damp towel (Fig. 4.1e); you should monitor temperature and humidity in the box.
- Count dead mosquitoes killed by the contact with the insecticide and those killed in the control tube at the end of this recovery period.
- You will need four replicates of the experiment to calculate % mortality in the exposure and control tubes.
- The rates of mortality are:
 - Control mortality where $C = (\text{number of dead mosquitoes})/(\text{total number of mosquitoes})$ in control tube
 - Exposure mortality where $E = (\text{number of dead mosquitoes})/(\text{total number of mosquitoes})$ in tube with insecticide
- If control mortality is greater than or equal to 5% and less than or equal to 20%, the value for exposure mortality E should be corrected by using the following formula (Abbott's formula):

$$\text{Corrected exposure mortality (\%)} = \left(\frac{E - C}{100 - C} \right) \cdot 100$$

Where, E is the (uncorrected) exposure mortality expressed in % and C is the control mortality expressed in %.

For instance, if control mortality C is 10% and crude exposure mortality E is 40%, the corrected exposure mortality is $[(40 - 10)/100 - 10] \cdot 100 = 33\%$. If control mortality is greater than 20%, the experiment should be discarded.

4.2 Bioassay tests

The residual efficacy of an insecticide on a sprayed surface is determined by **bioassay** tests. This is done by checking mortality of the target mosquito vector exposed to the sprayed surface at intervals of weeks or months after the spraying. This technique can be also used to evaluate the quality of a residual spraying operation. It is also used to determine residual efficacy of an insecticide on bed nets. This can be used to decide when to re-treat bed nets and also to assess the quality of treatment.

Equipment

Bioassay kit (including plastic cones, adhesive sponge tape, bent sucking tube, normal sucking tubes), cardboard paper, small nails, hammer, cotton wool, paper cups with cover nets, rubber bands, markers, mosquito cage, wooden box with large holes, towels. The plastic cone and the special (bent) sucking tube are shown in Fig. 4.2.

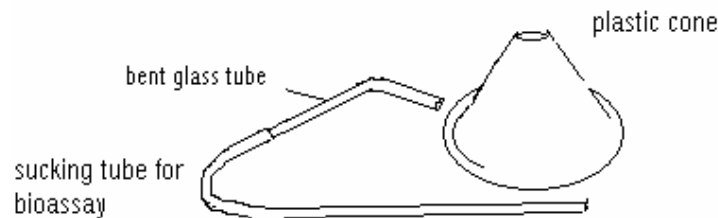


Figure 4.2 Plastic cone and sucking tube for bioassay test

Residual efficacy of insecticide on sprayed surfaces

- Line the edges of the plastic cone with adhesive sponge tape
- Using nails or tape, fix the cone to the sprayed surface. (Fix different cones at three heights: low, middle and high)
- Nail insecticide-free cardboard paper to the wall and fix plastic cones to the paper (to be used as a control)
- Transfer 10 mosquitoes (preferably susceptible strains from an insectary) into each cone and put a piece of cotton wool in the opening of the cone (use separate sucking tube to transfer mosquitoes to control cones)
- After a specified exposure period (usually 30 minutes), carefully take out the mosquitoes from the cones and transfer them to separate (and labeled) paper cups
- Count the number of mosquitoes dead (or 'knocked down') at the end of the exposure period, but do not remove mosquitoes that appear dead, as some of them might later recover
- Place damp cotton wool on top of the paper cups, put them in the wooden box and cover with damp towel
- After 24 hours, count the number of mosquitoes dead and calculate percent mortality in both the exposure and control paper cups
- Repeat the experiment on different walls (in the same house) and in different houses to have a representative sample. In each experiment, use the same number of mosquitoes for exposure and control
- If control mortality is between 5% and 20%, the exposure mortality should be corrected by using Abbott's formula described under susceptibility tests. If control mortality is greater than 20%, the experiment should be discarded.

Residual efficacy of insecticide on bed nets

The bioassay procedure for insecticide-treated bed nets is similar to the procedure used for sprayed walls, except that the cone is attached to the fabric with a rubber band, and the mosquitoes are usually exposed only for three minutes.

Exercise 4.1

Susceptibility tests: You will be provided with live, fed female mosquitoes in the laboratory. In pairs, prepare holding tubes and introduce 15 fed females to the holding tubes. When all groups have introduced the mosquitoes, half the groups will prepare exposure tubes and the other half control tubes. After all tubes have been prepared, all groups will be asked to transfer the mosquitoes to the exposure and control tubes and to label each tube with the group number indicating whether it is an exposure or a control tube, and the time of the day. After one hour of exposure, you should transfer the mosquitoes back to the holding tubes for 24 hours of observation. After 24 hours, all results will be combined to calculate mortality rates.

Exercise 4.2

Bioassay tests: Again, as in Exercise 4.1, you will form pairs and half the groups will install cones on treated bed nets and half on untreated bed nets. Each pair should transfer 10 fed mosquitoes to the cones, and after three minutes the mosquitoes should be taken out and placed in paper cups for 24 hours of observation. Again, the results will be combined for calculation of mortality rates.

Learning Unit 5

Vector incrimination and malaria control

Learning objectives

By the end of this Unit you should be able to:

- Describe the methods used to incriminate malaria vectors
- Identify the entomological indicators of malaria transmission
- Calculate the entomological indicators associated with the resting habits, feeding habits, human-vector contact and entomological inoculation rates for malaria vectors
- Measure the components of the vectorial capacity model and understand its value for malaria control
- Interpret the entomological measurements and their implications for malaria vector control

In Learning Unit 1 you briefly looked at the transmission dynamics of malaria and the role of entomology in the study and control of malaria. In Learning Unit 3 you were introduced to the sampling methods used in entomology. In this unit you will see how the information in these two learning units come together to incriminate a vector and determine potential control approaches.

5.1 Vector Incrimination

The entomological components used to incriminate a vector include:

- Presence, abundance and percent of mosquitoes of a given species infected with sporozoites
- Age or parity of the vector
- Feeding behavior of the vector:
 1. where a mosquito bites,
 2. when a mosquito bites, and
 3. what host is preferred

From this data you can calculate and compare several entomological indicators.

- The man-biting rate
- resting habits
- longevity
- infectivity
- human blood index
- entomological inoculation rate and,
- vectorial capacity

5.2 Vector Incrimination Techniques

Determining the **abdominal condition or blood digestion stages** of vectors is important component in vector incrimination studies. Many times you need to know when a mosquito takes blood and how long it takes to digest the blood, develop eggs and lay eggs and return to take a blood meal again. It is one of the important components needed to calculate a vectors capacity to transmit malaria (see below). Dissection and examination of **ovaries** is required in order to study longevity and the age of a vector population.

In addition, to determine the proportion of infective vectors (infection rate in vectors) you need to dissect the **salivary glands** and examine them for the presence of sporozoites or use a biochemical method.

In this learning unit, you will learn to implement these techniques.

Important structures within a female mosquito

Before dissecting an adult mosquito, it is essential to know the position of the different organs within its body. Fig. 5.1 shows the structures inside a female mosquito as if the mosquito was cut in half vertically along the middle of the body. The positions of the various structures are

- The salivary glands lie inside the thorax, but are joined to the head by salivary ducts.
- The stomach or midgut lies in the abdomen, and the Malpighian 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 and join at the ampulla to form a common oviduct.
- A single spermatheca where the male sperm is stored is attached to the common oviduct.

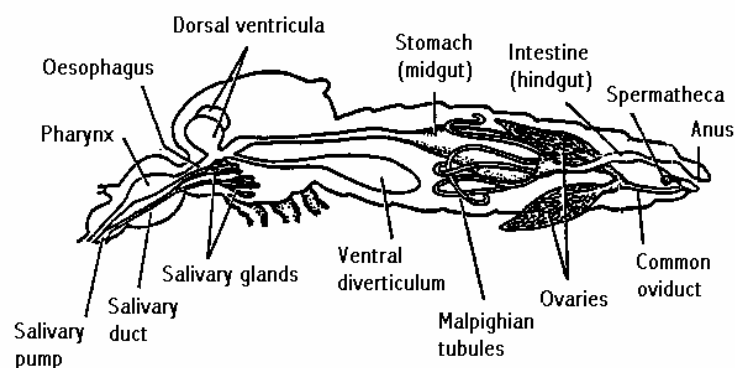


Figure 5.1 Internal anatomy of a female mosquito

Recognizing blood digestion stages

Blood digestion stage refers to the appearance of the abdomen of the female *Anopheles* as the result of the blood digestion and ovarian development. In anophelines, ovary maturation (egg development) occurs at the same time as blood digestion. Based on their blood digestion stage or abdominal condition, anophelines can be grouped as **unfed**, **freshly fed**, **half-gravid**, and **gravid** (Fig. 5.2).

1. Unfed - The abdomen is flattened.
2. Freshly fed - The abdomen appears bright or dark 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 two segments on the ventral surface and at most five segments on the dorsal surface.
3. Half-gravid - The blood is dark in colour—almost black—and occupies three to four segments on the ventral surface and six to seven on the dorsal surface of the abdomen. Ovaries occupy most of the abdomen.
4. Gravid - The blood is reduced to a small black patch on the ventral surface or may be completely digested. The ovaries occupy all the rest of the abdomen.

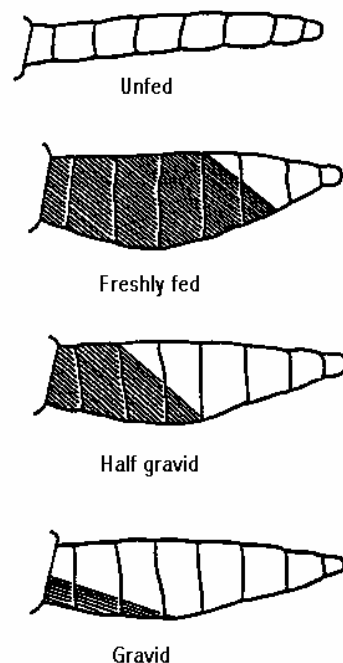


Figure 5.2 Abdominal conditions of a female

Dissecting ovaries and determining parity

Equipment needed to dissect ovaries - dissecting (or stereoscopic) microscope, compound microscope, dissecting needles, fine forceps, slides, dropper and distilled water.

Dissection of the female mosquito to obtain ovaries for parity determination

Parity determination is done by dissecting out the ovaries and examining them to see if they are **parous** (those that have taken a blood meal at least once and laid eggs at least once) or **nulliparous** (mosquitoes that have not taken a blood meal yet and have not laid eggs).

Only females which are unfed or freshly fed are suitable for this method of parity determination. To dissect out ovaries, proceed as follows:

- Kill the female and remove legs and wings.
- Place the mosquito on a slide and add a drop of distilled water (Fig. 5.3).
- 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. The ovaries will come out of the abdomen.
- Cut through the common oviduct and separate the ovaries from the rest of the specimen.
- Transfer the ovaries to a drop of distilled water on another slide and allow them to dry.

Differentiating between nulliparous and parous ovaries

- Examine the dried ovaries under a compound microscope using the 10x objective, and if necessary, confirm using the 40x objective.
- Females in which the ovaries have coiled tracheolar skeins are nulliparous (Fig. 5.4).
- 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.

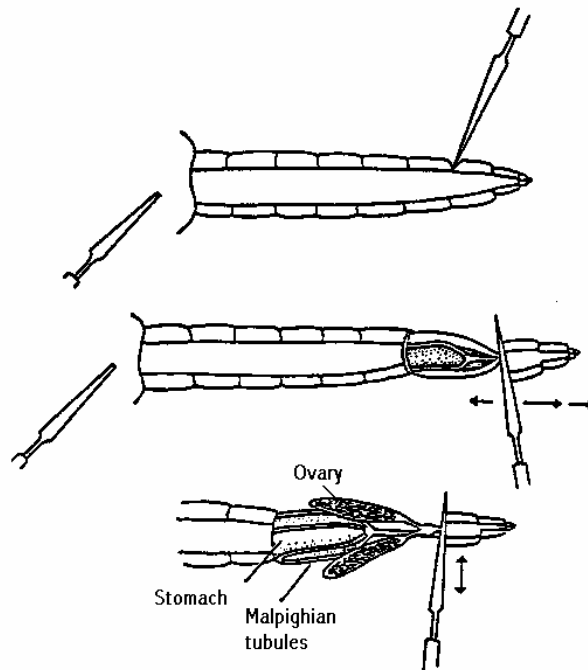


Figure 5.3 Dissecting ovaries

By measuring the proportion of parous mosquitoes in a vector population one can monitor changes in vector populations and evaluate the impact of an intervention. For example, if a population is increasing it usually is because more nulliparous adults are emerging, therefore the parous rate decreases. Conversely, as a population gets older, with fewer mosquitoes emerging, the parous rate increases.

The aim of residual insecticide spraying is to reduce malaria transmission by killing mosquitoes that enter dwellings to rest before or after feeding and hence reduce their longevity and their ability to transmit malaria. If residual spraying is effective there will be less parous mosquitoes compared to nulliparous mosquitoes after spraying than before spraying; or if compared to areas that were not sprayed. Parity is an entomological indicator used to determine if malaria transmission has been reduced.

A nulliparous mosquito can not transmit malaria because it has not obtained the *Plasmodium* parasite yet. Even a female that has laid eggs once (or twice) it may not yet be old enough to transmit malaria parasites because the gonotrophic cycle – the time from the first blood seeking to the second blood seeking – averages only three days and sporozoite development takes 10-12 days. Therefore, a mosquito may need three gonotrophic cycles before it is able to transmit malaria. The dissection of ovaries and their examination are therefore essential tools in entomological analysis and assessment of impact of vector control interventions.

Note: In some anopheline species it is possible to observe the scars that form on the common oviduct after each oviposition. Therefore, you can estimate the age of the mosquito by counting the number of scars and multiply this number by the gonotrophic cycle. This method is hard to do and is usually only done in special research projects.

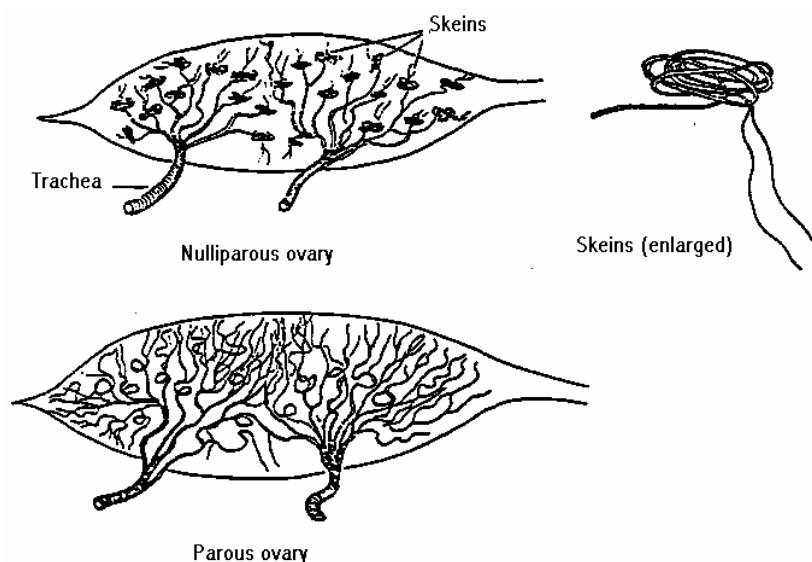


Figure 5.4 Appearance of nulliparous and parous ovaries

Dissecting and examining salivary glands for sporozoites

The salivary glands are examined for sporozoites in order to determine which mosquito species carry malaria parasites and the percentage of each species that is infected. Determination of sporozoite rates is necessary to confirm the role of a particular mosquito species as a vector to determine intensity of malaria transmission (inoculation rate) and assess impact of malaria control interventions. The dissection technique indicates whether or not the mosquito is infected with *Plasmodium*, but does not distinguish the species of parasite.

Equipment needed to dissect the salivary glands: dissecting microscope; compound microscope; dissecting needles; fine forceps; slides; dropper; 0.65% saline solution.

Procedure for dissecting salivary glands:

- Kill the mosquito, identify the species and remove legs and wings. You do not need to dissect the salivary glands of nulliparous females because they are not infected.
- Place the mosquito on a slide, lying on its side with the head pointing to the right (Fig. 5.1).
- Place a small drop of saline solution close to the front of the thorax.
- Hold the thorax firmly with a blunt dissecting needle in your left hand.
- Place the needle held in your right hand on the neck of the mosquito without cutting the neck.
- Gently pull the head away from the thorax – 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.
- Separate the glands with the other needle, and place them in a drop of saline solution.
- Cover the salivary gland with a standard 18 x 18 mm cover slip.

Examining freshly dissected salivary glands for sporozoites

If the glands have not been crushed by the cover slip, gently press the cover slip with a dissecting needle so that the glands break and sporozoites are released. The glands should be examined under a high-power 40x 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.

Staining sporozoites

- Place a drop of adhesive on the top side of the cover slip and take it off carefully, by using the tip of a dissecting needle, and turn it over with the wet side up; fix it temporarily to one end of the slide. In this way the sporozoites that stick to the cover slip can be saved and stained.
- Draw a circle around the salivary glands and sporozoites with a grease pencil on the reverse side of the slide (this makes it easy to locate the specimen later).
- Allow the preparation to dry and protect it from ants and flies.
- Fix it by immersing the whole slide for a few seconds in methanol.
- Stain for 30 minutes with 5% Giemsa stain in buffer solution. The slide may be left face up and the stain applied with a dropper to flood the specimen and cover slip.
- Wash well with buffer solution and examine under the high power of a compound microscope.

Note on the ELISA technique

There are other techniques for determining infection rates in mosquitoes. The most often used is the enzyme-linked immunosorbent assay (ELISA). In this technique, the thorax and head parts of dried mosquitoes of known species are ground in specific solution. Many samples can then be placed in a special apparatus with several wells coated with *Plasmodium* species-specific antibodies. If the corresponding antigen from a particular species is present in the sample, then they will bind to the wells while the negative samples are washed away. Enzymes and substrates which form colour reactions are then used to recognize positive wells. This technique saves time and permits the identification of the particular *Plasmodium* species that caused the infection in the mosquito.

A similar ELISA technique is used to identify the source of blood meals of mosquitoes. In this case, blood samples collected on filter papers by squashing freshly fed mosquitoes will be tested using antibodies prepared from several known animal hosts.

Exercise 5.1

Your tutor will demonstrate an assortment of abdominal conditions under a dissecting microscope. Then (in pairs) you will be asked to determine the blood digestion stages of female anopheline mosquito specimens.

Exercise 5.2

In pairs, you will dissect ovaries of unfed and freshly fed females. Allow the ovaries to dry, and then under the compound determine if they are parous or nulliparous. Practice until you distinguish parous mosquitoes from nulliparous mosquitoes.

Exercise 5.3

Your tutor will demonstrate how to dissect out the salivary glands. Then you should practice the technique yourself. Examine the glands under the compound microscope.

Exercise 5.4

There will be a field trip to allow you to practice the various mosquito collection techniques that you learned in Learning Unit 3 and the dissection techniques demonstrated in the current learning unit. In the field, you will work individually and in groups to carry out the following activities:

- Using sucking tubes, flashlights and paper cups, search for indoor-resting mosquitoes in three houses
- Using sucking tubes, torches and paper cups, spend at least 20 minutes searching for outdoor-resting mosquitoes
- In groups of four, carry out spray-sheet collections in one house per group
- Using dippers, vials and pipettes, collect larvae and pupae from natural breeding sites for at least 30 minutes
- Practise sitting with bare legs indoors and outdoor during night-landing collections (because time is short, this will be done during the daytime for the sake of practice and demonstration).
- Transport live specimens to the laboratory

Exercise 5.5

In groups of two, kill the mosquitoes you collected during the field trip and identify the abdominal conditions and species. Practice dissecting ovaries and salivary glands of the field-collected mosquitoes.

5.3 Entomological indicators of transmission

In this section you will learn how to use the techniques discussed so far to study the incriminate vectors in relation to the control of malaria. You will learn skills required to correctly interpret entomological information.

In order to explain most of the important concepts, we will take an actual example of an entomological study carried out in 1964-65 in an upland valley in Ethiopia to gather base-line data on local anophelines². The study was designed to understand the characteristics of malaria transmission and the habits and habitats of the local vector species in order to plan an effective control programme.

²Rishikesh N (1966) *Observations on anopheline vectors of malaria in an upland valley in Ethiopia*. Unpublished document of the World Health Organization, WHO/Mal/66.554.

Some of the results of the study have been re-analyzed in the light of current knowledge and new control tools. The objective is to illustrate how entomological information is used for vector control.

Study design and sampling techniques

Selection of study villages and description of the area

The area lies in central Ethiopia within the Great Rift Valley. The terrain is relatively flat and the altitude is between 1600 and 1800 metres. The population (about 420 000), is largely rural. The people engage in agriculture and stock herding, living in scattered clusters of "tukuls", which are the prevalent type of rural dwellings. Cattle are herded in open enclosures close to human habitations or herded for the night into a section partitioned off from the rest of the house by a loose framework of posts and twigs.

The main rainy season extends from June to the end of October while a short rainy season occurs in March and April. The hottest months are March, April and May. The coldest months are November and December.

Six villages were selected as observation posts, three in Awasa sector and three in Adamitulu sector (now Zway sector). A **sector** is an area delineated for the purpose of malaria control; the then Ethiopian Malaria Eradication Service had been established some four years prior to the study. These were chosen primarily on entomological grounds, but attention was also paid to their malaria endemicity and accessibility throughout the year. The area had never been sprayed with insecticides when the study was conducted. Table 5.1 shows parasite and spleen rates in the selected villages. Of the infections recorded, 6.6% were mixed infections of both vivax and falciparum malaria, 61.8% *P. falciparum*, 25.0% *P. vivax*, 6.6% *P. malariae*.

Table 5.1 Parasite and spleen rates in selected villages.

Village	Month & Year	Blood films examined	Parasite rate (%)	Spleen exams	Spleen rate (%)
Abella Wondo	Jun 64	59	0	55	13.0
Galle	May 64	49	4.1	37	35.1
	Oct 64	194	13.4	-	-
	May 65	92	5.4	-	-
Awasa Tabor	May 64	52	13.5	45	26.7
	Nov 64	37	8.1	-	-
Bulbula	May 64	40	15.0	30	50.0
Woldia	Nov 64	181	2.6	-	-
	Dec 64	206	2.4	-	-
Ajiti Washgula	Nov 64	47	31.9	-	-
	May 65	75	4.0	-	-

Entomological sampling techniques

Indoor resting collections -Indoor resting mosquitoes were sampled in the six villages once a month by the spray sheet collection method. The collections were analysed according to species and abdominal condition (see Table 5.2). The salivary glands were dissected to establish infection rates.

Night landing collections -Night landing catches were made normally twice a month (at Abella Wondo). Human baits were employed to catch the anophelines landing on their bare legs. Indoor catches were carried out throughout the night from 6pm to 6am, while outdoor catches were limited to the period 6 to 10pm (Table 5.4), beyond which hour none of the inhabitants are normally to be found outdoors (except for one set of concurrent indoor and outdoor catches that were carried out in order to understand the feeding habits of the vectors if given equal opportunity throughout the night at both sites (Table 5.3). Two collectors each were stationed indoors and outdoors and worked in four-hour shifts. The indoor capture stations also contained their normal occupants at the relevant times. The collected samples were identified in the morning and they were dissected to examine the salivary glands for sporozoites. The ovaries were also dissected to determine the parous rates.

Artificial outdoor shelters were installed and were inspected once a month.

RESULTS

Indoor resting densities

Table 5.2 shows the indoor resting collections for each anopheline species per house per day.

Exercise 5.6a

Calculate the indoor resting density per house per day for each species for the month of October 1964. It is calculated by dividing the total number of females of a particular species by the total number of houses inspected.

Table 5.2 Results of indoor resting collections in Awasa Sector (1964-65)

Month & year	No. houses	No. occupants*	A. gambiae s.l.**				A. pharoensis				A. funestus			
			Unfed	Fed	Half-gravid	Gravid	Unfed	Fed	Half-gravid	Gravid	Unfed	Fed	Half gravid	Gravid
Jun 64	8	35	11	135	59	96	0	36	21	21	1	0	0	0
Jul 64	17	75	91	904	378	141	4	102	46	43	7	18	12	11
Aug 64	15	66	458	1041	459	678	11	60	19	14	2	26	7	14
Sep 64	18	79	149	586	270	236	8	101	45	45	1	25	8	5
Oct 64	18	79	185	802	438	340	14	46	16	15	3	80	9	9
Nov 64	23	101	8	65	51	38	0	10	9	7	3	47	13	34
Dec 64	24	106	2	25	13	9	2	13	4	4	3	43	2	4
Jan 65	24	106	1	9	6	4	1	3	3	4	0	4	1	2
Feb 65	23	101	0	0	0	0	0	3	2	1	0	0	0	2
Mar 65	23	101	0	1	0	0	0	5	6	3	1	0	0	0
Apr 65	23	101	1	5	3	6	5	28	8	12	0	17	2	2
May 65	23	101	2	34	19	22	2	29	13	15	1	12	1	0

*This column was not reported by the investigator but was estimated from average household size taken from a later study.

** From later studies it has established that the particular species referred to here as *A. gambiae* s.l. is *A. arabiensis*.

Feeding habits

Feeding habit refers to whether the vectors prefer to feed indoors (**endophagy**) or outdoors (**exophagy**) and the times of feeding during the night (**nocturnal biting cycle**).

Degree of endophagy/exophagy and nocturnal biting cycle were estimated by concurrent whole night indoor and outdoor landing collections (Table 5.3).

Table 5.3 Concurrent indoor and outdoor night-landing collections

Time		A gambiae s.l.		A pharoensis	
		Indoor	Outdoor	Indoor	Outdoor
pm	6-7	1	6	0	2
	7-8	2	4	0	3
	8-9	1	7	0	8
	9-10	3	13	1	2
	10-11	4	8	0	10
	11-12	5	9	0	1
am	12-1	6	9	0	0
	1-2	3	9	0	2
	2-3	2	13	1	1
	3-4	2	16	0	1
	4-5	3	4	0	0
	5-6	18	38	0	0
Total		50	136	2	30

Exercise 5.6b

Calculate the ratio of indoor vs. outdoor biting for each species. Which species is exophilic? Which is endophilic?

Man-biting rates

The **man-biting rate** refers to the average number of bites per person per night by a vector species, and its estimation involves both the feeding habits of the vector and the night-time habits of the local people. Man-biting rates can be calculated directly from man-landing collections and indirectly from spray sheet collections.

a. Direct calculation of the man-biting rate

To calculate the man-biting rates, the night-time habits of the local people need to be taken into consideration. In the present survey, observations show that an average villager spends about one hour outdoors between 6 pm and 10 pm and the remaining hours of the night indoors. Practically all the villagers are indoors by 10 pm. Night landing catches were undertaken from 6 pm to 6 am indoors whereas outdoor catches were restricted to the period 6pm to 10 pm (Table 5.4).

Table 5.4 Results of the night-landing collections in Awasa Sector (Abella Wondo station), 1964-65.(a) *A. gambiae s.l.*

Month And Year	No. of Nights of catch	No. of baits		Total catches indoors		Total catch outdoors 6 - 10pm	Man-biting rate		
		Indoors	Outdoors	6pm-10pm	10pm – 6am		Indoors (3+8 hrs)	Outdoors (1 hr)	Total (12 hrs)
Jul 64	2	2	2	12	84	16	23.3	1.0	24.3
Aug 64	2	2	2	81	340	76			
Sep 64	1	2	2	5	7	12			
Oct 64	2	2	2	4	21	34			
Nov 64	2	2	2	2	1	9	0.6	0.6	1.2
Dec 64	2	2	2	0	0	4	0.0	0.3	0.3
Jan 65	2	2	2	0	0	2	0.0	0.1	0.1
Feb 65	2	2	2	0	0	0	0.0	0.0	0.0
Mar 65	2	2	2	0	0	0	0.0	0.0	0.0
Apr 65	1	2	2	0	0	0	0.0	0.0	0.0
May 65	2	2	2	0	0	0	0.0	0.0	0.0

(b) *A. pharoensis*

Month & Year	No. nights of catch	No. of baits		Total catch indoors		Total Catch outdoors 6 - 10pm	Man-biting rate		
		Indoors	Outdoors	6pm-10pm	10pm - 6am		Indoors (3+8 hrs)	Outdoors (1 hr)	Total (12 hrs)
Jul 64	2	2	2	19	17	92	7.8	5.8	13.6
Aug 64	2	2	2	37	83	74	27.7	4.6	32.3
Sep 64	1	2	2	23	31	143	24.1	17.9	42.0
Oct 64	2	2	2	12	12	105	5.3	6.6	11.9
Nov 64	2	2	2	2	1	33	0.6	2.1	2.7
Dec 64	2	2	2	0	1	42	0.3	2.6	2.9
Jan 65	2	2	2	3	7	44	2.3	2.8	5.1
Feb 65	2	2	2	7	0	9	0.3	0.6	1.9
Mar 65	2	2	2	0	1	8	0.0	0.5	0.8
Apr 65	1	2	2	0	0	11	2.6	1.4	1.4
May 65	2	2	2	11	2	19	2.6	1.2	3.8

Table 5.4 also shows some of the calculated values of the man-biting rates. The indoor and outdoor components of the man-biting rates are calculated separately and the total man-biting rate is obtained by adding the two rates together.

The outdoor component M_y is the average number of bites per bait per hour during the period 6pm to 10 pm.

$$M_y = \frac{ty}{uTc_y}$$

with,

$T =$	number of hours between 6 pm. and the latest time after which all villagers stay indoors (here $T = 4$)
$t =$	average number of hours spent by each villager outdoors after 6 pm. (here $t = 1$)
$y =$	number collected outdoors during period T
$c_y =$	number of collectors outdoors
$u =$	number of nights of collection

For July 1964, for instance, the denominator of the outdoor man-biting rate M_y is 16 (two nights for two collectors * T); the numerator is $(t)(y)$, with $t=1$ and y number of outdoor captures = 16; M_y is therefore $16/16 = 1$

The indoor component (M_x) is the average number of bites per indoor bait in four hours between 6 and 10 pm, plus the average number of bites per indoor bait in eight hours between 10 pm and 6 am.

$$M_x = \frac{\left(1 - \frac{t}{T}\right)x_1 + x_2}{uc_x}$$

with,

$T =$	number of hours between 6 pm and the latest time after which all villagers stay indoors (here $T = 4$)
$t =$	average number of hours spent by each villager outdoors after 6 .m. (here $t = 1$)
$x_1 =$	number collected indoors during period T (6 pm -10 pm)
$x_2 =$	number collected indoors after period T (10 pm - 6 am)
$c_x =$	number of collectors indoors
$u =$	number of nights of collection

For July 1964, the denominator of the indoor man-biting rate (M_x) is 4 (2 nights for two collectors); the numerator = $[1 - (t/T)]x_1 + x_2$ with $t/T = 0.75$, x_1 the number of indoor captures during the period 6pm to10 pm. (12) and x_2 the number of indoor captures during the period during the period 10 pm to 6 am. (84), thus the numerator of (M_x) = $[(1-0.25) 12] + 84 = 93$ and (M_x) = 23.3.

The total man-biting rate (M) is given as:

$$M = M_x + M_y = 23.3 + 1 = 24.3$$

The results for July 1964 indicate that an average villager would be bitten by 24.3 *A. gambiae* s.l. per night during that particular month. Of these bites, 23.3 were received indoors while only 1 was received outdoors; even though the vector has an exophagic habit. These results demonstrating that the sleeping habits of the people influences where human-vector contact occurs - indoors.

Exercise 5.6c

Calculate the man-biting rates for *A. gambiae* s.l. and *A. pharoensis* for the months of August and September 1964. From the results of your calculation, where does most human-vector contact take place, indoors or outdoors? Which species is endophilic? Do these results differ from the ones calculated in Exercise 5.6a?

b. Indirect calculation of the man-biting rate from spray sheet collections

This method uses spray sheet collections to estimate the man biting rate. The man-biting rate (per night) is obtained by dividing the total number of fed mosquitoes by the total number of human occupants who spent the night in the houses used for collection:

$$M = \frac{F}{w}$$

where,

F = total number of freshly fed mosquitoes of the particular species and w = total number of human occupants in houses used for collection.

The above estimation of the man-biting rate makes two implicit assumptions:

- (1) All fed mosquitoes found in the houses used for collection took their blood meals from the occupants of the same houses; and
- (2) No fed mosquitoes left the houses after taking their blood meals until the time of collection. If these assumptions are more or less true, this method is more efficient and less labour intensive for estimation of the man-biting rate.

Nevertheless, some vector species such as *An. arabiensis* can significantly (up to 30%) feed on animals and can be found resting in human dwellings. This might mean that you have to adjusting the results accordingly by multiplying “M” by the proportion of females found fed on human blood.

Host preference

Host preference is usually determined by analyzing the sources of the mosquito blood meals. The proportion of mosquitoes with human blood (referred to as the **human blood index, HBI**) in a vector species can be then used as an indication of the degree of **anthropophily** of that particular species. The HBI is calculated by

$$\text{HBI} = \frac{\text{Number of Mosquitoes with Human Blood}}{\text{Total Number of Mosquitoes with blood}}$$

In the current study, the HBI was not determined so we will use an estimate of 0.6 for both *A. gambiae* s.l. and *A. pharoensis* for this exercise.

Resting habit

An important index is the **proportion of blood meals taken on man followed by resting indoors**. One element of the success of Indoor residual spraying (IRS) in interrupting transmission is the proportion of the vectors that rest on the sprayed surface before and after feeding on humans. The aim of residual spraying is to reduce the chance of infected vectors reaching an infective age.

The proportion of blood meals taken on humans and followed by indoors resting is calculated as:

$$f = \frac{kHD}{NPM}$$

where,

- $k =$ a correction value of 1.16
- $H =$ **Human-blood index**, not calculated during the Ethiopian survey, but for which we use the arbitrary value 0.6
- $D =$ **indoor resting density** (total number of females collected divided by number of houses used for the spray-sheet collection)
- $M =$ **man-biting rate for October** (see exercise 5.6)
- $P =$ duration of **resting indoors after feeding**, in days; $P = 1 + G/F$, where G is the total number of half-gravid and gravid females (spray-sheet collections) and F is the number of freshly fed females (spray-sheet collections)
- $N =$ average number of persons per house (**household size**)

For October 1964 the values that are the same for both *A. pharoensis* and *A. gambiae* s.l. are:

$$k = 1.16$$

$$\text{Inhabitants: } 79; \text{ houses: } 18 \quad N = 79/18 = 4.4$$

and separately

	<i>A. gambiae</i> s.l.	<i>A. pharoensis</i>
H (human blood index)	0.6	0.6
Total number of females	1765	91
D (indoor-resting density)	$1765/18 = 98.06$	$91/18 = 5.06$
Fed females	802	46
Half-gravid females	438	15
Gravid females	340	16
P (indoor resting post feeding)	$1 + [(340 + 438)/802] = 1.97$	$1 + [(16 + 15)/46] = 1.67$
M (man-biting rate)	8.1*	11.9*

(* see your results exercise 5.6 and Table 5.4, respectively)

$$f = 1.16 (D) H/(N)(M)(P)$$

thus,

$$\begin{aligned} \text{for } A. \text{ gambiae s.l.} \quad f &= (1.16) (98.06)(0.6)/(4.4) (8.1) (1.97) = 0.972 \\ \text{for } A. \text{ pharoensis} \quad f &= (1.16) (5.06) (0.6)/(4.4) (11.9) (1.67) = 0.040 \end{aligned}$$

Exercise 5.7

Write a brief description of the results shown above. Compare your results with those of the facilitator.

Longevity and infectivity

Two other factors affect the likelihood of being bitten by an infective mosquito:

- 1. The survival of a female mosquito after a blood meal (probability of surviving one day after blood meal, denoted as p and expectation of life for n days (n being the number of days for a sporogonic cycle to be completed))**

The result of ovarian dissections between July and December 1964 in Awasa Sector was as follows:

$$\begin{aligned} A. \text{ gambiae s.l.} \quad 72/108 &= 0.667 \\ A. \text{ pharoensis} \quad 107/276 &= 0.388 \end{aligned}$$

Given an interval of two days between blood meals, the **probability of surviving one day** (denoted as p) can be estimated as:

$$p = \sqrt{\text{Proportion parous}}$$

Thus, $p = \sqrt{0.667} = 0.817$ for *A. gambiae* s.l. and $\sqrt{0.388} = 0.623$ for *A. pharoensis*. If a 3-day interval is assumed, we have $p = \sqrt[3]{0.667} = 0.874$ for *A. gambiae* s.l. and $\sqrt[3]{0.388} = 0.729$ for *A. pharoensis*.

The above formula for p assumes that the mosquito population has a stable size and age structure, and the death rate is independent of age. For this reason, the proportion parous is usually averaged over the whole population cycle, to eliminate the effect of seasonal fluctuation in population size and age structure.

It is also possible to calculate the probability of surviving through n days. As p is the probability of surviving one day, p^n is the probability of surviving n days. For example, at an average daily temperature of 27°C, it would take about 10 days for *P. falciparum* to complete the sporogonic cycle in the vector³. The probability that

³ The duration of sporogony as a function of temperature can be calculated by the formula $n = T/(t - t_{\min})$, where n = duration of sporogony; $T = 111, 105$ and 144 for *P. falciparum*, *P. vivax* and *P. malariae*, respectively; t = actual average temperature in degrees centigrade and $t_{\min} = 16$ for *P. falciparum* and *P. malariae* and 14.5 for *P. vivax*.

this parasite can be transmitted by *A. gambiae* s.l. and *A. pharoensis* is 0.874^{10} ($=0.26$) and 0.729^{10} ($= 0.042$), respectively.

We can also calculate the **expectation of life** for each species as:

$$\frac{l}{-\ln p}$$

Using that formula and the data of the previous paragraphs, expectation for *A. gambiae* s.l. is 7.4 days and 3.2 days for *A. pharoensis*.

At Abella Wondo (Awasa Sector), the average daily temperature during the months of July-December is usually 20°C. At this temperature, it takes approximately 28 days to complete the sporogonic cycle for *P. falciparum*. The probability of transmission of falciparum malaria by *A. gambiae* s.l. is therefore 0.874^{28} ($=0.023$ or 2.3%). During the same period, out of 2434 females of this species, 3 were found infected, i.e. 0.1%. The low sporozoite rate (or the low probability of transmission) in the study area could be the result of both the survival probability of the vectors and the ambient temperature.

2. The sporozoite rate of female mosquitoes and the number of infective bites per night.

The sporozoite rates for one of the Sectors (Awasa) are given in Table 5.5.

Table 5.5 Salivary gland dissection, *A. gambiae* s.l., Awasa Sector (1964-65)

Month & Year	No. dissected	No. sporozoite positive	Sporozoite rate (%)
Jun 64	128	0	0.00
Jul 64	212	0	0.00
Aug 64	580	0	0.00
Sep 64	630	0	0.00
Oct 64	803	2	0.25
Nov 64	162	1	0.62
Dec 64	47	0	0.00
Jan 65	20	0	0.00
Feb 65	0	-	-
Mar 65	0	-	-
Apr 65	0	-	-
May 65	38	0	0.00
Total	2620	3	0.11

In Awasa sector, 3/2620 (0.11%) *A. gambiae* s.l. were found positive and during the same period, 6/1918 *A. gambiae* s.l. (0.31%) were sporozoite positive in Adamitulu Sector. In both sectors, out of a total of 2577 *A. pharoensis* collected none were found positive.

Suppose you were an inhabitant of Abella Wondo (in Awasa Sector) in 1964. How many infective bites of *A. gambiae* s.l. would you expect during the month of October 1964 if you had no protection against mosquito bites?

This question can be re-phrased as: “From all *A. gambiae* s.l. females that could have taken their blood meals on you during October 1964, how many of them would have been infective?” To answer this question, you will need two quantities:

1. the sporozoite rate, and
2. the man-biting rate

The sporozoite rate was 0.25% (Table 5.5). On average, you would be bitten by 8.1 *A. gambiae* s.l. per night (your calculation). The number of infective bites per person per night, known as the **Entomological Inoculation Rate (EIR)** and is calculated as:

$$\text{EIR} = \text{man-biting rate} \times \text{sporozoite rate (\%)} / 100$$

The EIR is therefore, $8.1 \times 0.0025 = 0.0203$ per person per night. Assuming that you would be bitten by same number of females every night in October 1964, you would expect $0.0203 \times 31 \text{ days} = 0.63$ infective bites during the whole month.

Alternatively, you can arrive at the same solution as follows. If 8.1 *A. gambiae* s.l. bite you every night, $8.1 \times 31 = 251.1$ could have bitten you during the whole month. From the sporozoite rate, you expect that 0.25% of these mosquitoes are infective; thus the expected infective bites would be $0.0025 \times 251.1 = 0.63$ infective bites per person per month (which is less than 1 infective bite). When expressing the EIR, always remember to indicate the time dimension (whether it is per night, per month or per year). Incidentally, note the low number of infective bites in this particular area. In some parts of Africa that are highly endemic, a person might receive one infective bite *every night!*

A similar calculation for *A. gambiae* in November 1964 (Table 5.4 and Table 5.5) shows a man-biting rate of 1.2 per person per night (lower than in October) and a sporozoite rate of 0.62% or 0.0062 (higher than in October); the EIR is $1.2 * 0.0062 = 0.00744$ infective bites per person per night or $0.00744 * 30 = 0.22$ infective bites per person per month. It is likely that in November the remaining vectors were older mosquitoes (likely to be infected) but the lower biting rate diminished the EIR.

Exercise 5.8

Form into working groups and answer the following questions

- a) From the results of earlier observations and of calculations on life expectancy and on human-vector contact, which of the two anopheline species do you think is the most important vector of malaria in the area? Why? (Give three reasons).
- b) If you decide to use residual spraying to control *A. gambiae* s.l., how do you time the application of an insecticide with six months residual efficacy? Refer to indoor resting densities and man-biting rates.

Present your results in plenary.

Vectorial capacity

Vector capacity is an index (or model) that is defined as the capacity of a vector population to transmit malaria in terms of the potential number of secondary inoculations originating per day from an infective person. The formula of the vectorial capacity (C) is given as:

$$C = \frac{ma^2 p^n}{-\ln p}$$

where,

m = density of vectors in relation to man

a = number of blood meals taken on man per vector per day (= human blood index multiplied by 0.5, if a gonotrophic cycle of two days is assumed)

p = daily survival probability (or proportion of vectors surviving per day)

n = incubation period in the vector (days)

The formula can be derived as follows: a person is bitten by ma vectors in one day; a fraction p^n of these vectors survive the incubation period; they survive $(1/-\ln p)$ days, during which time they feed on a persons per day; multiplying ma by a , and then by p^n and $(1/-\ln p)$ yields the above formula. It is difficult to measure all these parameters correctly and there exists several assumptions that need to be met.

Nevertheless, the vectorial capacity is one of the most important concepts in the theoretical studies of the epidemiology and control of malaria. For example, using this concept, it can be shown that halving the survival p (by using residual spraying) produces a much greater reduction in vectorial capacity than halving a , which is itself more effective than halving the density m .

Closure Discussion

As a class you will now review the key concepts of the vector biology and how they relate to malaria transmission and vector incrimination. Your tutor will raise the following question and you will develop a list of the components of a vector's biology that increases the risk of malaria. Place the class results on a flip chart.

- 1) What characteristics of the vector aquatic habitats contribute to the risk of malaria?
- 2) What life history characteristics of the adult vector increase its chances of transmitting malaria?
- 3) What biting activity behaviors increase the potential of malaria transmission?
- 4) What human activities and behaviors put them at risk to malaria?
- 5) Do you need to measure all the components of the vectorial capacity model in order to monitor the entomological component of malaria transmission? Explain.
- 6) The EIR has become an important entomological indicator of malaria transmission for comparing regional differences? Why do you think this has happened?

Learning Unit 6

Malaria vector control

Learning objectives

By the end of this Unit, you should be able to:

- know the role and objectives of vector control in malaria prevention and control
- describe vector control options, their expected impacts, their advantages and their limitations
- identify operational issues likely to influence vector control planning and implementation
- demonstrate a technical understanding of the main vector control measures including indoor residual spraying and insecticide-treated nets
- include integrated vector control approaches at your place of work

Introduction

The role of vector control is to augment the impact of early diagnosis and prompt treatment of malaria cases. Vector control should be implemented:

- to reduce malaria incidence where urgent malaria problems exist such as situations where previously malaria-free individuals, populations or communities are at high malaria risk
- to curtail the spread of malaria in areas where the parasite is resistance to anti-malarial drugs
- to prevent epidemics

to reduce the environmental risks of transmission by:

- maintaining a low level of malaria in endemic areas
- preventing the reintroduction of malaria
- contributing to health, development and improvement in general living conditions

Effective selection and implementation of selective vector control should also adhere to the principles and strategies of Roll-Back Malaria (RBM), which are:

- evidence-based actions
- harmonious partnerships
- sustained national and local capacities for vector control
- appropriate supporting mechanisms and environments

6.2 Vector Control Methods

Interventions using vector control methods are related to three major control measures (Figure 6.1).

- 1. Larval control**
 - Source reduction
 - Larvivorous fish
 - Larviciding
- 2. Reducing man-vector contact**
 - Insecticide-treated mosquito nets (ITN)
 - Improved housing
 - Repellents and mosquito coils
- 3. Adult mosquito control**
 - Insecticide treated nets
 - Indoor residual spraying (IRS)
 - Space spraying

Not all of these methods are applicable to the diverse malaria epidemiological and operational situations that can occur. For example, IRS had a dramatic impact on malaria during the so-called malaria eradication era, but in many situations it might not be sustained because of financial and operational constraints. Figure 6.1 gives a schematic representation of the states into which the human and vector populations are distributed, and the points of impact with the malaria control interventions.

Exercise 6.1

You will again work in small groups. The tutor will give you a theoretical life cycle diagram of a malaria vector. Your goal is to put on the diagram the potential vector intervention methods for each life stage of the vector. Classify them as reducing man-vector contact, adult mosquito control and larval control. Refer to the reading about vector control methods at the end of this learning unit when developing this exercise. Present this information in plenary and discuss your group's conclusions.

Vector control methods vary in efficacy, resource requirements, and potential delivery systems and personnel implementing the method. Some methods are highly specific and others broad ranging. Figure 6.2 demonstrates the complexity of the vector control selection process. The central point revolves around a particular epidemiological situation. This will be discussed in Learning Unit 8. In this learning unit you should think about the impact of the vector control method on the vector population.

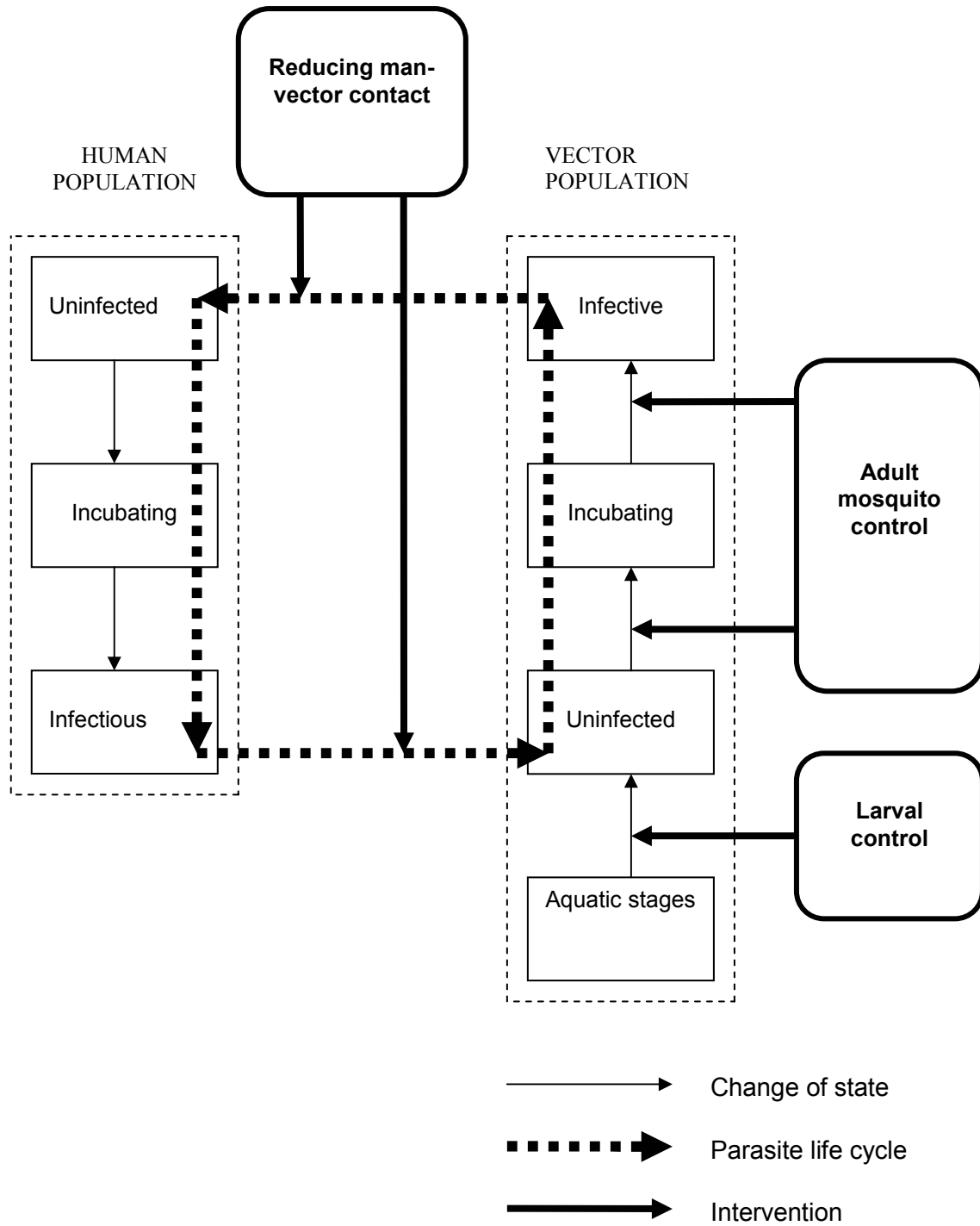
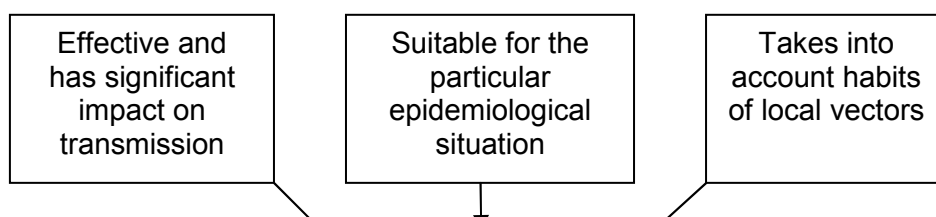


Figure 6.2 shows the most important criteria for selection of appropriate use of vector control options for a given epidemiological situation.



Exercise 6.2

Working in small groups complete Table 6.1 regarding the expected effects of the various vector control methods on the different aspects of the vector population. You may use the following signs in the blank cells of the table: (+) reduction expected; (-) no effect and (+/-) effect doubtful or conditional on other factors. Present your groups results in plenary. Again, refer to the reading on vector control methods at the end of this learning unit when developing this exercise.

Table 6.1 Aspects of the vector (and components of vectorial capacity) that are likely to be affected by various vector control methods.

Method	Larval density (m)	Adult density (m)	Adult survival (p)	Human biting habit (a)
Larval control				
Source reduction				
Larvivorous fish				
Larviciding				
Reducing man-vector contact				
Insecticide-treated mosquito nets and other materials				
Improved housing				
Repellents and Mosquito coils				
Adult mosquito control				
Insecticide-treated mosquito nets and other materials				
Indoor residual spraying				
Space spraying				

+ reduction expected

- no effect

+/- effect doubtful or conditional on other factors

Demonstration

At this time, you will proceed to an area where the commonly used vector control methods, equipment and chemicals will be demonstrated. You will practise the methods and discuss their use, maintenance, operations and safety.

6.3 Selection of vector control methods – their advantages and limitations

In a **class setting** the tutor will lead a discussion on the criteria that are used to select vector control methods. One important question is how do you select your control methods in your place of work? Do you have any experience that you can remember when these criteria were not used? If so, discuss these experiences.

Exercise 6.3

An important component of the selection process for is to know the advantages and limitations of each method. In your working group, develop a list of advantages and limitation for the methods listed in Table 6.1. You can refer to the information provided in the reading at the end of this learning unit as you develop your list.

Exercise 6.4

In this exercise the tutor will assign your group one of two interventions to be used in the case study for Ethiopia. Imagine that the local government has been given conflicting opinions on whether to use INTs or IRS. Your group will have to justify your selection, develop the intervention plan, and promote it in plenary. Use the reading at the end of this module as a guide in the development of each programme.

Integrated vector control

The principles of vector control have changed during the past fifty years. In fact, public health officials in the mid-20th century aimed their efforts at vector eradication, not control. Today's emphasis on **integrated vector control (IVC)** enhances control and makes more efficient use of scarce resources.

Up until this point in the course you have developed the ability to understand and use vector control methods. The question now arises “what do you do if your vector control method does not satisfy your criteria for success? For example, if you have set your health outcome to be the reduction of malaria by 35% in 5 years and you see in year three that you will only be able to achieve a 15% reduction, you will either have to accept the lower outcome or improve your control programme.

If after a thorough evaluation of the vector control programme, you find that the human-vector contact and adult densities are not decreasing as expected you would first suspect a decrease in susceptibility of the vector to the insecticide. However if the vector is still susceptible to chemical control - what do you do? You are forced to think of using another vector control method or a complement to the original method selected. In other words you have to consider integrating your vector control programme and managing it in a different way.

Integrated vector control has been defined as “the rational use of all appropriate means of control in a mutually compatible, safe and cost-effective manner in order to achieve vector suppression and control of disease transmission (WHO, 1983)”.

A more ecological definition of integrated control based on the vectors biology was suggested by Zimmerman (1992) and states that:

Integrated vector control is a unified plan of control that selects the most appropriate methods of control, based on the environmental conditions and the population dynamics of the vector, which maintains the vector population at a level that does not cause a health problem.

Vector control is a component of **Integrated Vector Management (IVM)** and the philosophy of IVM influences how vector control is carried out. IVM will be discussed further in Learning Unit 8.

Recently, it has been proposed that we study the entire system in order to have efficient and effective health prevention and control programme. The concept of health has changed from the health of an individual and population of a single species to one that considered multiple populations of species in a biological community or ecosystem, including people (Nielsen 1998).

This concept can be illustrated by the **human improvement system** and the interrelationship of the various components (Fig. 6.3).

Exercise 6.5

Form your working groups and answer the following questions.

1. Are any of any of these definitions in use at your place of work? If so, which one?
2. Describe an integrated vector control programme that is in place where you work.
3. Which definition would you like to see incorporated into the malaria vector control programme where you work? Is your selection realistic?
4. What are the advantages and limitations associated with each concept?

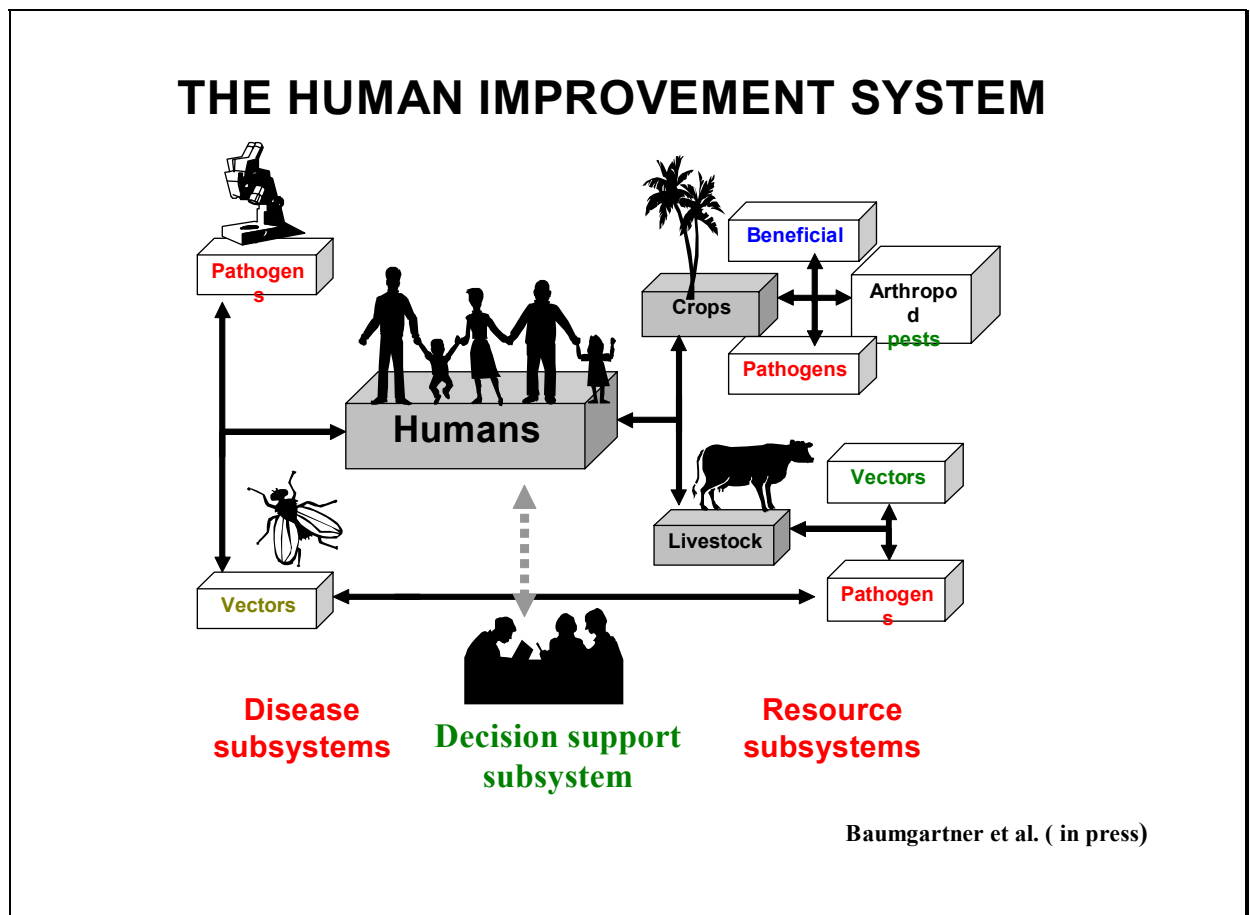
Develop an integrated vector control programme for one to three of the following case scenarios. The tutor will decide how many you should do. Justify your selections of vector control methods.

1. An irrigated rice agroecosystem with low EIR, a high density exophagic, partially endophilic and 50% HBI. Both *P. falciparum* and *P. vivax* are prevalent.

2. A coastal malaria situation in a series of villages adjacent to a national marine reserve. The reserve is protected against modification and contamination by national law. The vector breeds in salt and freshwater, it is enophagic, but exophilic.
3. A village in a tropical rainforest where 90% of the reported cases are *P. falciparum* and high EIR. There are two vectors present. Both are endophagic and endophilic. The HBI's are 80% and 40%, respectively. The first species breeds in the seasonal rivers and marshes and the other is a generalist and breeds in all types of habitats both natural and manmade.

Present your results in plenary. The tutor will start a class discussion on vector control and the questions presented in this exercise.

Figure 6.3 Human Improvement System



Reading - vector control methods

1. Larval control

Larval control is indicated as the sole method of vector control only if a high proportion of the breeding sites within the vector's flight range of the community to be protected can be located, accessed and managed. Larval control may be also undertaken to supplement or synergize the effects of other vector control interventions. Larval control affects only the vector density and requires a high coverage to be effective. Only a proportional reduction in vectorial capacity through reduction in m is expected from larval control, whereas reducing adult survival p and m (by using residual spraying) produces a much greater reduction in vectorial capacity. Effective larval control is most feasible where breeding places are limited in number, easily recognized, and easily accessible.

Larval control may be useful:

- in densely populated areas with relatively few breeding places
- during very dry periods in endemic areas, when the breeding sites are very limited, definable and manageable
- in refugee camps
- development areas such as irrigation scheme and construction sites

Source reduction

The term **source reduction** refers to any measure that prevents the breeding of mosquitoes or eliminates their breeding sites. Source reduction is a component of **environment management** which aims to modify the environment in order to deprive the vector population of its requirements for survival (mainly breeding, resting and feeding), thus reducing human-vector contact and transmission risks.

If such measures bring about long lasting or permanent changes on land, water or vegetation, they are referred to as **environmental modification** (e.g. filling, drainage, planting water-loving trees such as eucalyptus trees in swampy areas and closing or covering breeding sites). When such measures have a temporary effect and need to be repeated, they are known as **environmental manipulation** (e.g. water-level fluctuation, intermittent irrigation, flushing, changing water salinity, clearing vegetation in streams, and irrigation canals).

Many development-linked activities (e.g. irrigation) lead to environmental changes and often inadvertently increase the risk of malaria transmission. Appropriate safeguards and mitigation actions are required in the planning, construction, and maintenance phases of development projects. Irrigation canals should be lined and the vegetation cleared to discourage breeding in the canal edges and allow free flow of water. Periodicity of water release can also be adjusted to allow flushing of larvae from the pooling canal beds.

Larvivorous fish

Larvivorous fish feed on mosquito larvae. Some of the most successful species introduced in different countries are the top minnow or mosquito fish (*Gambusia affinis*) and the guppy (*Poecilia reticulata*). *Gambusia* is most efficient in clean water, while *Poecilia* can be used successfully in organically polluted water. *Poecilia* tolerates higher temperature than *Gambusia* and may therefore be more effective in rice fields in hot areas. However, unlike *Gambusia*, it cannot survive temperatures below 10°C. The annual killifishes, *Cynolebias*, *Nothobranchius* and *Aphyosemion*, have drought-resistant eggs and can be used in breeding sites that temporarily dry out, such as borrow-pits and irrigated rice fields. In addition, locally collected fish have been evaluated for their efficacy in controlling mosquitoes and a number of species have proven useful. The use of local larvivorous fish is particularly important to avoid the risk of disturbing the ecological balance by introducing “exotic” fish species.

Larviciding

Larviciding includes the use of chemicals or biological agents or toxins to kill larvae and pupae. Larvicides are used in breeding sites that cannot be drained, filled or where other larval control methods are too expensive or impossible to use. Larviciding is indicated only for vectors which tend to breed in permanent or semi-permanent water bodies that can be identified, and where the density of the human population to be protected is sufficiently high to justify the treatment. These prerequisites reduce the indications for larviciding to urban areas, labour or refugee camps and development projects. In these situations, it is possible that larviciding programmes may be complementary to environmental measures aimed at controlling malaria and other mosquito-borne diseases or nuisance mosquitoes in integrated control programmes. Because of limited indications for larviciding for malaria control and the high degree of coverage needed if it is to be effective, it is very important to define precisely the area and the points where treatment should be applied.

The residual effect of larvicides varies considerably depending on the water quality and type of the breeding place, but is relatively short for most larvicides. Most treatments must be repeated at fairly short cycles which may vary from 2-10 weeks. A variety of larvicides are or have been used for malaria control (see Table 1), including chemicals and insecticides of biological origin; these vary in their modes of action, efficacy, safety, formulations, cost and availability. Larvicides of potential use are discussed below.

Petroleum oils

These are used for stagnant water bodies which are unsuitable for animal drinking and irrigation. Oils act mainly by forming a film on the water surface, thereby preventing larvae from breathing. The heavier the oil the less dispersible it will be and the more easily blocked by vegetation.

Common chemical insecticides

Organophosphate insecticides (Table 1) are widely used despite increasing levels of resistance in some areas. Temephos, which has a very low mammalian toxicity, has been the most widely used mosquito larvicide worldwide. It may be applied to water used for the irrigation of food crops, and has also been used for treating drinking-water. It is, however, toxic to fish. Fenthion is also commonly used when there is no risk of contamination of drinking water and food.

Insect growth regulators

These are chemical compounds that are highly toxic to mosquito larvae by preventing their development into adults. Their use has generally been limited by their high cost. Insect growth regulators can be divided into: (a) juvenile hormone analogues, which prevent the development of larvae into viable pupae or of pupae into adults (they do not kill larvae); and (b) chitin synthesis inhibitors, which interfere with the moulting process by killing the larvae when they moult.

Table 1. WHOPES recommended compounds and formulations for control of mosquito larvae.

Insecticide	Formulation ^a	Dosage of active ingredient
Oils		
Fuel oil	Solution	142-190 L/ha
Fuel oil + spreading agent	Solution	19-47 L/ha
Organophosphates		
Temephos	EC, GR	56-112 g/ha
Fenthion	EC	22-112 g/ha
Pirimiphos-methyl	EC	50-500 g/ha
Chlorpyrifos	EC	11-25 g/ha
Insect growth regulators		
Diflubenzuron	GR	25-100 g/ha
Methoprene	EC	20-40 g/ha
Pyriproxyfen	GR	5-10 g/ha
Microbial insecticides:		
<i>B. thurigiensis</i> <i>israelensis</i>	Slow-release formulations	(b)
<i>B. sphaericus</i>	Slow-release formulations	(b)

^a EC = emulsifiable concentrate; GR = granule

^b The dosage will depend on the formulation used.

Larvicides of biological origin

Bacillus thuringiensis israelensis (Bti) produces toxins which are very effective in killing mosquito larvae after ingestion. It is harmless to other insects, fish, higher animals and humans at normal dosages and, at appropriate doses, may be suitable for use in water used for drinking (with due attention to potential microbial contaminants in the formulated product) or for the irrigation of food crops. It has the disadvantage that it is active by ingestion and is rather heavy, sinking into the water, while anophelines are surface feeders. It breaks down quickly in the environment and must be reapplied periodically. Another bacterium, *B. sphaericus*, also produces a toxin. It has characteristics similar to those of *Bti* but is more effective in polluted water while *Bti* is more effective in clean water.

2. Reducing man-vector contact

Insecticide-treated mosquito nets (ITN) and other materials

One could categorize ITNs either as an adult mosquito control or reducing human-vector contact due to their combined effect.

Large-scale implementation of ITN programmes is part of an integrated approach to malaria control in many countries. As with the other vector control methods, this intervention also needs evidence-based actions, adaptation to local conditions, monitoring and evaluation, operational research, appropriate resources and capacities, and partnerships with community and inter-sector actions. As a malaria prevention and control intervention, ITN programmes follow some basic concepts:

- used as a method of personal protection for high risk groups
- used for transmission control with a target of high coverage exceeding 80% of the entire population

Nets treated with pyrethroids give greater personal protection than untreated nets by irritating, repelling or killing mosquitoes before they can find a place to bite through the net. In villages where impregnated nets are widely used, a reduction in vector density and longevity has often been observed. However, such a "mass effect" has sometimes not been found. Some of the most remarkable impacts on malaria using ITN's have been attributed to improved personal protection. Pyrethroids have an important, but compound-specific, excito-repellent effect on most vector species and so far only this group of compounds has been proved to be both safe and effective in treating mosquito nets. The presence of one insecticide treated mosquito net in a room may also partially protect individuals sleeping outside the net.

The present lack of an alternative class of insecticide for ITN's is a cause for concern because of the potential for insecticide resistance; although it is not currently a major problem. Moreover, at least in the case of *A. gambiae* in West Africa, effective protection can be obtained even in the presence of a high frequency of *kdr*-resistance gene (knock-down resistance gene) in the vector population.

Technical and operational issues to be considered in planning ITN programmes

Some of the questions in relation to the technical, socio-cultural, economic and operational issues likely to influence the efficacy and implementation of the ITN strategy that need to be considered when planning are:

- What are the behavioural patterns of the vectors? Are they mostly exophagic or endophagic and what are the peak biting periods, especially in relation to peoples' sleeping patterns? Are people outdoors (outside ITNs) at times when mosquitoes bite most?
- What are the night time movements and habits of people likely to affect exposure to vectors, including the time they go to bed? (This will vary with age, gender, and occupation).
- What are the attitudes of the people towards net use?
- Is there any preference for size, shape and colour of the bed nets?
- Who uses nets already? From where do they get the nets and at what costs?
- Are there seasonal variations in net use patterns?
- How do people react to insecticide used?
- What is the economic status of most people – this will affect net ownership, the ability to pay for insecticides and net (re)treatments?

Sustainability is more likely when:

- communities pay for the nets and ideally for net retreatments, also
- financial management is handled through community-based revolving funds and cost-recovery systems, even though initial support (seed money, logistics, technical guidance, training and capacity building) is essential

ITN service delivery (i.e. ensuring that people needing ITN have access to them) may be more promising and realistic where potential delivery systems and services already exist or when the potential exists to access these, for example,

- Properly functioning district and sub-district health management systems including primary health care
- Other formal, non-formal, structured systems and networks, which (within and outside of the health sector) may be reaching the communities – whether these are already involved in ITN activities or have the potential to do so
- Where the private sector and social marketing services reach the peripheral communities
- Well-structured, operationally functional NGOs and local associations are already engaged in ITN activities or can be motivated to do so
- Where the community leaders have confidence of the people
- When women play lead roles in health care in the community, make decisions in the household, and potential exists for women's associations to be involved in ITMN activities (e.g. sewing and selling nets)

Netting material and mosquito net models

The following terms are used to characterize netting materials:

Material: Nets are made of cotton or synthetic fibres (nylon, polyester or polyethylene). In general, polyester and nylon nets are preferred because they are cheaper and more durable than cotton materials, easier to impregnate and insecticides are more effective on them than on cotton.

Mesh: the number of holes per square inch. For example, a mesh size of 156 has 12x13 holes per square inch. The 156 mesh is considered a standard for bed nets.

Denier: an indication of the weight (and therefore the strength) of the thread. It is defined as the weight in grams of 9,000 meters of a single thread. A denier of 100 is strong and often recommended. Nets with 70 denier are also used, but are fragile.

Colour: Blue, green or pink are commonly used because they do not show dirt and they avoid cultural problems associated with white. In some areas however white nets are preferred.

Shape: Nets are made usually in two shapes: rectangular and conical (or circular). Large scale programmes often use rectangular nets.

Size: Four sizes of rectangular nets are commercially available (Table 2).

Table 2. The most commonly used mosquito nets

Size	Width (cm)	Length (cm)	Height (cm)
Single	70	180	150
Double	100	180	150
Family	130	180	150
X- family	190	180	150

The conical nets in use are approximately 8.76 m² for the single nets, 10.20 m² for double nets, 11.64 m² for the family size, and 14.52 m² for X-family size.

Treating procedures for mosquito nets

Treatment procedure - Nets are treated by dipping them in basins or plastic bags containing insecticide mixed with water. To simplify the treatment of nets, one dose of insecticide is added to 0.5 or 2 L of clean water for polyester and cotton nets, respectively, regardless of their size. These doses have been based on the highest recommended concentrations and for a family size net of 15 m². They are expected to give longer persistence, (i.e. to tolerate more washes), and will have a more visible impact on nuisance mosquitoes. This impact is important in achieving greater compliance, since it is the main motivating factor for most people to use insecticide treated nets.

Mosquito nets may be treated at the household (home treatment) or community (mass treatment) levels. Dip-it-yourself kits for home treatment may be available through shops, health centres and special community programmes. Mass treatment by trained personnel may be provided by dipping centres and mobile teams. After dipping, take the wet net out of the treatment container and gently wring out any excess liquid to allow it to drip back into the container. To dry, place the fabric horizontally on a plastic sheet or other clean, non-absorbent surface, under a shade away from sunshine.

Insecticides and formulations for treatment - Table 3a and 3b list the insecticide products recommended by WHOPES (WHO Pesticide Evaluation Scheme) for the treatment of mosquito nets.

Table 3a. Amount of insecticides recommended for net treatment

Insecticide product ^a	Dosage per mosquito net
Alpha-cypermethrin 10%SC	6 ml
Cyfluthrin 5%EW	15 ml
Deltamethrin 1%SC	40 ml
Deltamethrin WT	One tablet
Etofenprox 10%EW	30 ml
Lambda-cyhalothrin 2.5%CS	10 ml
Permethrin 10%EC	75 ml

^a SC = aqueous suspension concentrate; EW = emulsion, oil in water; WT = water dispersible tablet; CS = capsule suspension (microencapsulated); EC = emulsifiable concentrate.

Table 3b. WHOPES-recommended insecticide products for treatment of mosquito nets⁴

Insecticide product ^a	Dosage - Active ingredient (a.i.) mg/m ² of netting
Alpha-cypermethrin 10%SC	20-40
Cyfluthrin 5%EW	50
Deltamethrin 1%SC (also WT 25%)	15-25
Etofenprox 10%EW	200
Lambda-cyhalothrin 2.5%CS	10-20
Permethrin 10%EC	200-500

^a SC = aqueous suspension concentrate; EW = emulsion, oil in water; WT = water dispersible tablet; CS = capsule suspension (microencapsulated); EC = emulsifiable concentrate.

Safety issues - While field use of pyrethroids for the treatment of mosquito nets at the recommended dose poses little or no hazard to those treating the nets, the supply of insecticide “over the counter” (OTC) for the treatment of nets by householders has particular safety concerns. It is

⁴ WHO specifications for public health pesticides are available on the WHOPES homepage on the Internet at www.who.int/ctd/whopes.

strongly recommended, therefore, that insecticides for the home treatment of mosquito nets be marketed only in single-unit doses. Moreover, if presented as a liquid formulation in bottles, the use of child-proof caps is mandatory. The OTC supply of high-concentration permethrin (e.g. 50% EC) should be avoided. Only trained staff should use such high concentrations of permethrin.

Acute toxicity or irritation may occur as a result of the handling of insecticides when applying them to mosquito nets (see Annex). People directly involved in dipping large numbers of nets are at greater risk than people who occasionally treat their own nets. The use of rubber gloves is essential; mouth and nose masks should be worn when dipping large numbers of nets, especially with emulsifiable concentrate formulations.

Distribution of nets and re-treatment - Nets are most suitable for cost-recovery as they provide individual protection. In most current programmes distribution is usually channelled through the private sector, the public sector being limited to promotion, information and social marketing activities. There is not yet enough information as to how far these programmes will cover the most peripheral and impoverished sectors of the population, whom suffer most from the burden of malaria. Ways should be put into place to compensate for “market failures” and increase the methods of practises of accessibility to insecticide-treated mosquito nets in marginalized populations.

People often accept and use nets because they protect against nuisance mosquitoes including *Culex*, even though malaria control programmes promote insecticide-treated mosquito nets with the objective of controlling malaria. It is therefore essential to bring about behavioural changes in the communities through continuous education and advertising the fact that the use of ITN protect against malaria, and to promote a sustained use even during seasons of low vector density.

Social marketing of net - Social marketing of nets uses commercial marketing methods to create a demand for health products and services. Social marketing aims to meet a social need whereas traditional marketing aims to maximize profit. Social marketing could involve subsidy of products (nets and insecticides) and services. It should seek to achieve a balance between affordable prices and cost recovery. For example, a non-profit NGO known as Population Services International (PSI) has been social marketing nets in several African countries by combining education to motivate the use of nets with the provision of nets to low-income populations through the private sector. ITN and insecticides are branded, attractively packaged, widely marketed, effectively promoted to the poor and selected target groups, and sold at affordable prices.

When to apply - Special attention must be paid to net distribution systems and to periodic re-treatment of nets with insecticide. The activities required on the part of the malaria control programme will have to be adapted to the method of distribution when this vector control option is adopted.

A more serious problem is that of establishing functional periodic re-treatment cycles based on the epidemiological needs, the residual effect of the formulations on different materials, and the habits of the population in washing their nets.

From the epidemiological point of view, maximum protection is required during the transmission season or its peak, where transmission is perennial. When control programmes play an active role in the distribution of nets, whether free or subsidized, re-treatment is normally carried out at special events, such as National Anti Malaria Week (or day) or Health Day. These should be timed, if possible, to ensure the maximum coverage with freshly treated nets during the transmission season. Even when distribution is left to commercial undertakings, official events to promote and demonstrate the use of insecticide-treated mosquito nets should be organized just before the start of the transmission season.

The periodicity of re-treatment should be based on regional investigations that determine the actual residual effect of the insecticide under the conditions of use in the area concerned (climate, exposure to direct sun when used outdoors, washing habits, etc.) and on the seasonality of transmission. These studies should determine the best method of washing the nets, taking into account effects of local soaps, use of hot water, drying conditions, frequency of washing etc., which should be promoted by information, education and communication and during treatment or promotional events.

If nets are sold commercially and individuals are responsible for treatment, the users should be informed that if they wash their nets more often than recommended, they should also re-treat them more frequently. Wherever possible, insecticide treatment is better provided free of charge especially to the poor and vulnerable group.

When the risk of an epidemic is detected or even when an actual epidemic is detected at an early stage, it will be desirable to organize a re-treatment event in areas where coverage with treated nets is high, provided that this will not interfere with the implementation of emergency control measures that may be more effective.

Long lasting insecticidal mosquito nets

Long lasting insecticidal mosquito nets (LLINs) are ready-to-use pre-treated mosquito nets, which require no re-treatment during their expected life span (4-5 years). They have several important advantages over conventional mosquito nets. These include eliminating the need to retreat the nets (one of the main obstacles to the use of insecticide-treated mosquito nets in many endemic countries), avoiding problems associated with the storage and handling of insecticides by non-professionals, and in the community, reducing insecticide use, and minimizing the environmental hazards caused by the release of insecticide into natural water bodies. Olyset Net[®] is an example of LLIN material. It is a 100% high-density polyethylene net, blended with permethrin 2% (corresponding to about 1000 mg of active ingredient/m²) incorporated into the polyethylene polymer before yarn extrusion. The insecticide is slowly released from the polymer at the surface of the fibre. Residual efficacy is longer than that of conventionally treated nets. After washing, the biological efficacy is reduced initially, but diffusion of the insecticide from the inside of the yarn to the surface reinstalls it. Recent studies show that Olyset Net[®] washed 10 times recovered its efficacy in less than 15 days.

Other insecticide-treated materials

Curtains and hammocks can also be treated with pyrethroid insecticides and used to reduce man-vector contact. Curtains on doors and windows could be very important supplementary interventions to ITNs in areas with significant vector biting rate in the early evening, before people sleep.

Improved housing and location of settlements in relation to breeding sites

Household and community actions to improve the quality of housing (design, construction, alteration including screening/mosquito proofing) and to deter mosquito entry and indoor resting can have more permanent effects than insecticide related control methods. Improved housing also improves the living condition and general health of the population. These are also relevant in planned settlements including in development projects.

Poor housing is linked to higher risk. For example, incomplete houses with open walls, wide or unscreened eaves, houses with open windows and doors or without ceilings favour mosquito entry, houses with damp walls and floors favour resting, increase malaria risk. House protection with screening of windows, eaves and doors is an effective method of reducing human-vector contact if properly implemented and maintained. New settlements should be carefully planned, selecting the correct design, structure, construction material, and location in relation to breeding sites, to prevent malaria.

Repellents, mosquito coils and protective clothing

The use of repellents and protective clothing are useful for people who are outdoors during peak vector biting periods. Most repellents have a very short duration of effect (eight hours).

Repellents

Repellents are available as creams, lotions and aerosol soaps. These may be applied either directly on the skin or on clothes. The use of repellents is a measure of individual protection. They complement bed nets and house protection and can be used after dark before retiring under the mosquito net or by people who stay outdoors during part of the night. In epidemics, repellents have sometimes been distributed in for malaria control; although their cost-effectiveness is doubtful.

Mosquito coils

Some insecticides kill or repel mosquitoes at a distance when vapourized with a heating device. Mosquito coils are among the most popular and widely used insecticide vapourizers. Once lit, the coils smoulder, releasing the insecticide into the air at a steady rate for six to eight hours.

Protective clothing

Cloths that cover most of the body, i.e. long sleeve jackets and shirts, trousers and soaks can provide a certain level of personal protection from mosquito biting.

3. Adult mosquito control

Indoor residual spraying (IRS)

IRS significantly contributed to the success of malaria control in the 1950s and 1960s. Malaria was eradicated, or almost eradicated from many parts of the world. IRS remains a valuable option for malaria control, when applied in the right circumstances. However, large-scale and continued application of insecticides is not sustainable because of the high costs (insecticide purchasing and operational costs), vector resistance to insecticides, and environmental concerns.

Conditions for use and effectiveness of IRS

IRS is recommended only where:

- a majority of the vector population is endophilic
- the vector population is susceptible to the chosen insecticides
- a high percentage of the houses or structures in the operational area have adequate sprayable surfaces, and
- spraying is done correctly

Mosquitoes rest in various locations during the gonotrophic cycle. Resting takes place indoors in human habitations, in animal shelters and outdoors on vegetation. The preferred vector resting sites in houses are walls, eaves, under furniture, and cool, dark, humid places. Vectors resting on sprayed surfaces are more likely to encounter a lethal dose of the insecticide and die than those that do not rest on sprayed surfaces.

Criteria for selective IRS

The considerable resource requirements, import needs, environmental concerns in their use, and the potential for vector resistance development, compel highly selective targeting of IRS. As with any control intervention, the selection of IRS requires the definition of the population to be protected and the areas where the measure should be applied. The epidemiological situation determines which areas receive total coverage for a relatively long period of time and which areas are covered only after the detection of certain risk factors.

In the areas to be sprayed, IRS requires, in principle, the coverage of all potential places where the vector might rest, at least for the first few hours after feeding and while searching for a host within an epidemiological unit. An **epidemiological unit** is the area where the vector circulates freely between breeding places and blood sources. It may be as small as an isolated group of houses together with several breeding places. The extent and intensity of the malaria problem and the mobility of the population affected will determine the size of the unit of intervention. The unit can be as large as an entire valley or even vary between certain altitudes.

The main indications for IRS in malaria control are:

- the control of epidemics detected in the early stages of development, where spraying can be done early enough to cut off peak transmission
- the control of seasonal transmission in areas with high malaria mortality, morbidity, and disease severity in order to reduce peaks of incidence
- the prevention of epidemics following significant alarm signals of emerging risks in specific epidemic-prone areas (e.g. abnormally heavy rains or drought leading to an increase in vector-breeding sites, high humidity or high temperatures and the migration of large number of non-immunes into endemic areas)
- special risk situations, (e.g. non-immune population groups temporarily exposed to transmission risks) such as refugees camps, settlers in development project areas, labour camps, and army and police posts
- the reduction of transmission and the curtailment of the spread of drug-resistant parasites in areas with major drug resistance problems

Planning for IRS

Planning for IRS involves stratification and delineation of areas to be covered, with more precise definition of the operational boundaries and the frequencies and times of applications (i.e. macro-, micro-analysis of information to select targets).

Issues to be considered in planning IRS are:

- transmission and burden of malaria are often focal and may vary with malaria endemicity and vector density even within a small area
- aggregate indicators such as annual parasite incidence rates should not be the only criterion for undertaking IRS. Micro-analysis (micro-stratification) is necessary for IRS targeting
- the size of operational areas is influenced by vector distribution, distance from important breeding sites, vectors' flight range, demographic features, and distribution of malaria
- IRS may be limited to specific geographical areas, villages, and times of the year

The first decision to be made is whether IRS is a suitable intervention for the malaria problem in a particular area. The choice should be based on an evaluation of the results of previous vector control activities. To improve the interpretation of existing records it is necessary to collect information on local vector bionomics and behaviour.

For IRS to be effective, apart from the identification of an effective insecticide, a number of other conditions must be met:

- The vector should preferably be endophilic. However, spraying may be effective to some extent against vectors which are partially exophilic, i.e. they rest indoors only for a few hours after biting and then spend most of the time digesting blood and developing eggs outdoors.
- The human habitations should have walls that the insecticide can be applied to.
- Finally, the required coverage must be achieved before the onset of the transmission season and maintained for the length of the season. This is particularly important in the control of epidemics. When an epidemic is recognized following an alarming increase of malaria cases, it is essential to ascertain whether transmission is likely to continue; IRS is not advisable when the epidemic is subsiding and transmission is coming to its end.

Definition of targets of application

The targets to be sprayed should be clearly defined and a geographical reconnaissance of the area should be undertaken so maps and guidelines can be prepared for the spraymen as follows:

Areas to be sprayed. The intervention units must be mapped or clearly marked so that they can be easily recognized by the spraying squads. Maps and identifying criteria should be made available to guide those responsible for the spraying operations.

Structures. The types of structure to be sprayed should be defined and include all human habitations where vector-human contact is likely to occur. In many rural areas, for example, people may spend long periods of time in “farm huts” within their fields and these structures may be very important in maintaining transmission. Similarly, other structures, such as animal shelters, latrines, stores or outhouses, may be important resting places for blood-fed mosquitoes.

Sprayable surfaces - IRS requires a high degree of coverage of potential resting places, including all walls, ceilings and furniture. The spraying of window frames and both sides of doors is often necessary, since they may be temporary resting places for vectors entering or leaving a room.

Organizational and logistic requirements of IRS

IRS requires very high coverage in order to be effective. Spraying should be:

- *total* - all the dwellings are sprayed
- *complete* - cover all sprayable surfaces
- *sufficient* - uniform application of the required dose to all sprayable surfaces
- *regular* - repeated at regular intervals to ensure an effective residue is present during the transmission season

The need to cover all the houses means that a complete knowledge of the geography of the area is necessary and that the spraymen must cover all outlying houses and scattered populations. A geographical reconnaissance is generally required to update local maps and census data. Meeting these standards requires a

disciplined and competent organization with properly equipped and trained spraymen and efficient logistic support. Traditionally, IRS has been based on the operational model of the malaria eradication campaigns of the 1950s and 1960s, which called for a strong autonomous and centralized organization. This model no longer exists in most areas and the need for such centralization has been questioned, particularly where countries are embarking on a policy of decentralization. A successful IRS programme should pay special attention to:

- the logistics of operational support, supplies, supervision and monitoring
- planning required for the regular application of IRS and the technical guidance needed for decentralized operations
- the responsibility of individuals and the community. Decentralized operations will benefit from decisions and actions at the local level though sustained local capacity and participation of communities

Selection of insecticides for IRS

Residual insecticides for spray application are formulated as:

- **water-dispersible powder** – a dry powder insecticide mixed with a surface-active agent that allows it to dissolve in water. Made ready for mixing with water to form a spray suspensions normally containing 1% to 5% of active ingredient
- **emulsifiable concentrate** – a solvent + emulsifying agent in which the insecticide is dissolved. When mixed with water it forms an emulsion suitable for delicate surfaces and does not cause spots or stains, but this is more expensive
- **suspension concentrate** - particles of insecticide with wetting agent and water, providing a water-based suspension. They are non-flammable, long-lasting, but less effective than water-dispersible powder on porous surfaces

The choice of insecticide and its formulation should be based on the susceptibility of the local vectors, the characteristics of the various compounds; the type of wall/roof surface; and the formulations of the available products (e.g. residual effect), and their cost. Information on the insecticides approved by WHOPES for IRS is given in Table 4.

The choice of insecticide and its formulation should first consider its effectiveness against the local vector species and its safety. Susceptibility testing should therefore be done first. Even if an insecticide is effective elsewhere, it may be necessary to conduct small field trials to determine its effectiveness and residual efficacy under local conditions.

Once an insecticide and its formulation are selected, it is essential to choose a quality product. Products that appear to be similar may not contain the same concentration of active ingredient. Even if the concentration is accurate, the product may be poorly formulated, may not suspend well, block sprayers, and give uneven coverage. It may also deteriorate rapidly during storage and produce toxic derivatives.

WHOPES can assist national vector control programmes in strengthening and/or establishment of the capacity for quality control of pesticides. WHOPES can also provide the specifications, criteria and guidelines for this purpose⁵. When necessary the procedures can be carried out at WHO-designated collaborating centres on behalf of the programme. WHO country representatives can provide information on how to order insecticides using the WHO supply service.

Table 4. WHOPES recommended insecticides for IRS against malaria vectors

Insecticide compounds & formulations ^a	Class ^b	Dosage (g/m ²)	Mode of action	Duration of effective action (months)
Bendiocarb WP	C	0.1-0.4	Contact & airborne	2-6
Propoxur WP	C	1-2	Contact & airborne	3-6
DDT WP ^c	OC	1-2	Contact	>6
Fenitrothion WP	OP	2	Contact & airborne	3-6
Malathion WP	OP	2	Contact	2-3
Pirimiphos-methyl WP & EC	OP	1-2	Contact & airborne	2-3
Alpha-cypermethrin WP & SC	P	0.02-0.03	Contact	4-6
Bifenthrin WP	P	0.025-0.050	Contact	3-6
Cyfluthrin WP	P	0.02-0.05	Contact	3-6
Deltamethrin WP	P	0.01-0.025	Contact	2-3
Etofenprox WP	P	0.1-0.3	Contact	3-6
Lambda-cyhalothrin WP	P	0.02-0.03	Contact	3-6

^a EC = emulsifiable concentrate; WP = wettable powder; SC = suspension concentrate

^b OC = organochlorine; OP = organophosphate; C = carbamate; P = pyrethroid.

^c For the conditions for using DDT, see the Stockholm Convention on Persistent Organic Pollutants (POPs) (UNEP, 2001).

⁵ WHO (2000) Guidelines for the purchase of public health pesticides. Geneva, WHO, *document WHO/CDS/WHOPES/2000.1*.

Regulatory mechanisms, national policies and legislation for public health must be available in relation to the selection, importation, and use of insecticides. These will ensure the safety, quality, and efficacy of insecticides. In the long-term it will lead to vector resistance management. Insecticide registration must be based on adequate evaluation data (WHOPES, supplemented where possible with data from evaluations undertaken in the country itself).

Imported insecticides should conform to the WHO specifications for public health use. When procuring insecticides, reports of conformity of the selected insecticides to WHO specifications must be examined by an independent institution before the insecticides concerned leave the place or country of origin.

Resistance

The potential development of insecticide resistance is a common threat to any programme that relies on the continuous or repeated use of IRS. It is therefore important to monitor vector susceptibility periodically during programme operations (see Learning Units 4 and 8).

Resistance is often the result of using the same or related insecticide that is used in agriculture. The selection of the insecticide to be used in IRS should be based not only on the susceptibility of the vector population, but on the general use of insecticides in the area. It is also desirable to study the history of resistance in neighbouring areas and for the same vector species in other areas.

The same applies with respect to the avoidance of contact with the sprayed surfaces by the vector. It will be necessary to monitor possible changes in vector behaviour by means of exit traps and the observation of man-biting behaviour.

There is no reliable way of preventing the development of resistance however some procedures may be useful:

- Selective use of the insecticide by restricting coverage to areas and periods of recognized risk will allow, as far as possible, the dilution of resistant genes from non-sprayed areas. Such selective use should take into account all insecticide use in the area concerned, including use in controlling nuisance insects and in agriculture.
- Mixtures of unrelated insecticides may also be used provided that there is no resistance in the area concerned to the products used in the mixture. Such mixtures should be produced industrially to avoid problems of mixing incompatible products or formulations in the field. The safety of the mixtures should also be assessed in such applications.
- Mosaic spraying
- Insecticides may be rotated. Although this may present logistical and acceptance problems and has not been used systematically in indoor spraying, it may be simpler than the simultaneous use of two insecticides. Switching of insecticides has normally been done only after resistance has developed, but it is not clear whether programmed rotation will enable two insecticides to be used for longer periods than switching to another insecticide when resistance is detected and switching back if resistance levels decline.

Acceptability

House spraying requires the coordinated coverage of all sprayable surfaces at regular intervals (spraying cycle). The aim is to have a high coverage of all potential vector resting places with the effective dose of insecticide during the entire period when transmission is to be controlled.

When residual spraying is used, a plan must ensure that the required coverage will be achieved for the specified period and that sufficient human and material resources will be available for this purpose.

Whether spraying is done by a specialized organization or by the community itself, indoor spraying requires the continued collaboration of the population, which may easily be eroded if people are not made continuously aware of the need for vector control. This is particularly important if some of the early benefits of spraying, such as the control of nuisance insects, are lost with time. It is therefore essential to maintain active contact with the community through an effective information, education and communication mechanism.

Dosage

Dosage is the amount of insecticide applied per unit area. It is normally expressed as grams or milligrams of active ingredient per square metre (g/m^2 or mg/m^2) of sprayable surface. Doses vary considerably for the different insecticides. Most of the pyrethroids are effective at doses of 10 - 50 mg/m^2 , while DDT, organophosphate and carbamate insecticides generally require doses of 1 to 2 g/m^2 .

Preparation of houses before spraying

Correct spraying requires the careful preparation of the rooms to be sprayed. In particular: all food, cooking utensils, bedding and clothes must be protected from the insecticide by taking them outside the house before spraying starts; and all portable furniture and any pieces of furniture leaning against the walls should be removed so that the walls and all sides of all the pieces of furniture can be sprayed.

When to apply

The repetition of spraying operations at regular intervals is called the "spraying cycle". It is the interval between repetitions, e.g. a six-month cycle. Each spraying of all sprayable houses in an area over a period of time is called a "spraying round". The epidemiological requirements and the residual effect of the insecticide formulation on the main sprayable surfaces will determine the frequency of the spraying cycle.

In areas with seasonal transmission the insecticide selected for use should be effective during the period of time that transmission is likely to occur. Areas requiring continuous protection should be sprayed regularly. The requirement that effective coverage be maintained during the entire transmission season implies that spraying of the whole area to be protected be completed before the beginning of that season (often the rainy season). This requirement has operational implications that must be taken into account particularly when the operations are conducted by decentralized health services: in order to ensure the timely reception of supplies and the training or retraining of spraymen.

Residual spraying techniques

The application of IRS has been standardized throughout the world. It is always necessary to check the working practises of spraymen in order to ensure that neither humans nor the environment are endangered. This is particularly important when insecticides with greater acute toxicity are to be used.

IRS requires the application of a uniform dose of insecticide to all the sprayable surfaces. This can best be achieved by means of compression sprayers that meet WHO specifications. WHO-approved compression sprayers (Fig. 1) are sturdy enough to maintain the pressure needed to produce a flat fan swath and to resist rough handling in the field. WHO specification WHO/VBC/89.970 covers the quality requirements. These sprayers should be fitted with nozzle tips producing the required swath and discharge rate, and with pressure gauges or control flow valves (CFV) graduated to deliver the required rate of application. Nozzle tips erode fairly quickly when insecticide suspensions are used at high pressure and should therefore be made of highly resistant materials (hardened steel, ceramics, etc.) and be checked frequently to avoid waste of insecticide or irregular dosage.

The WHOPES manual on IRS⁶ describes procedures for the safe and effective use of insecticides for IRS and also covers the maintenance of equipment. The spray is applied in swaths 75 cm wide. Swaths should overlap by 5 cm. Spraying is done from the roof to the floor, using a downward motion, to complete one swath. Step sideways and spray upwards from floor to roof (Fig. 2).

⁶ WHO (2000) Manual for indoor residual spraying. Application of residual sprays for vector control. Geneva, WHO, document WHO/CDS/WHOPES/GCDPP/2000.3.

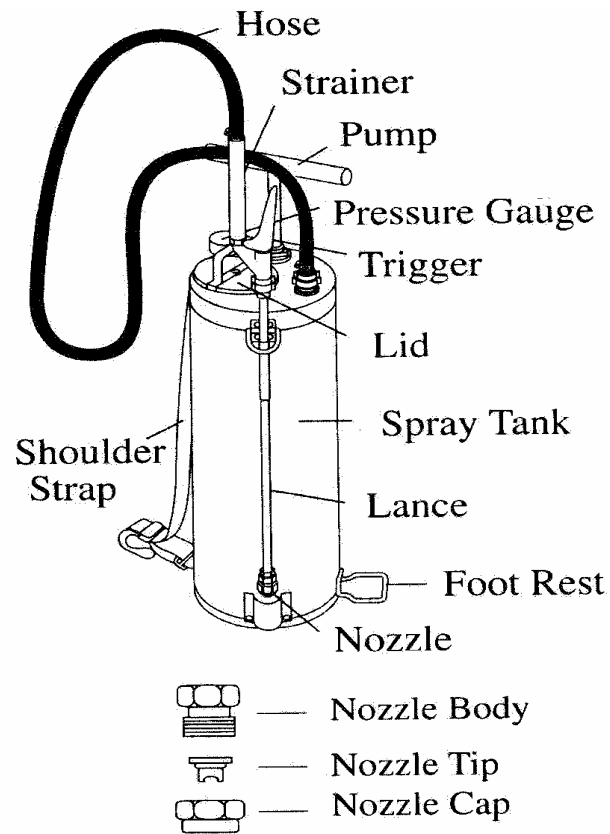


Figure 1 WHO-approved compression sprayer

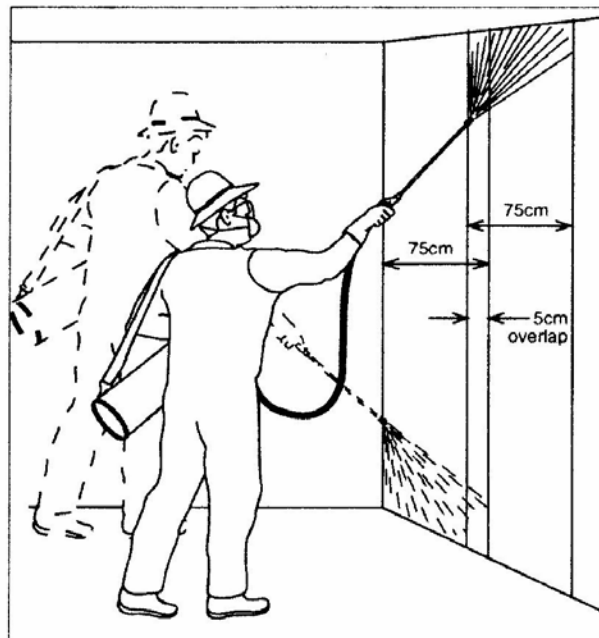


Figure 2 Spraying technique

Protective measures

The safe use of insecticides for IRS requires a number of precautions. The removal or physical protection of all foodstuffs and cooking or eating utensils is imperative. In addition, inhabitants should be advised not to enter a sprayed room until the spray is dry, and to sweep all floors before allowing free entry into the house. This is particularly important for families with small children or indoor domestic animals that may have greater contact with the floor.

The use of protective devices and safe working practises is essential to avoid or reduce the contamination of spraymen, packers and mixers with the insecticide. In most spraying programmes in which insecticides of low acute toxicity (such as DDT) have been used, it is sufficient to wear overalls, broad-brimmed hats to cover the neck of the overalls, gloves and shoes or boots (the openings of which should be covered by the long trousers of the overalls). More toxic or more irritating insecticides require more elaborate protective devices such as light masks, goggles, visors and respirators.

Packers and mixers have a higher risk of contamination and should therefore use rubber gloves, masks or respirators and protect their eyes with a visor made of transparent plastic attached to the hat. The current trend is for insecticides to be delivered from the manufacturer in pre-packed pump charges, preferably in water-soluble sachets, which can be dropped directly into the pump tank, so that packers and mixers are not needed. Suspension concentrate (SC) and emulsifiable concentrate (EC) formulations in dosage-regulating containers and water-dispersible granular formulations are also available and limit exposure during spray tank preparation.

Squad leaders must enforce safe practises and the appropriate use of protective devices. They must be familiar with early signs of intoxication and monitor members of their squad for any sign of poisoning.

Basic precautions to prevent unnecessary contamination include:

- Hands and face should be washed after filling each pump charge.
- Eating, drinking and smoking should be forbidden, except after washing and before starting to spray.
- Spraymen should not be exposed to insecticide for more than six hours each day.
- Overalls and hats should be washed daily, especially if they have been heavily contaminated.
- Spraymen must take a shower at the end of each day's work, particularly when they have been working with organophosphate insecticides.
- If respirators are used, they must fit well around the nose and mouth. They must be washed, dried and the cartridge must be changed daily or whenever it becomes obstructed.

Empty insecticide containers must be collected by the team supervisors and brought to the central storage area for proper disposal by qualified staff, in accordance with the FAO/WHO/UNEP guidelines⁷. It is also essential to follow the recommendations for the disposal of larger metal containers. Reuse of containers is always dangerous. If containers are to be reused they must be selected and cleaned by properly trained personnel.

Space spraying

Space spraying is defined as the destruction of flying mosquitoes by contact with insecticides in the air. The main objective is to reduce vector density and increase vector mortality as soon as possible. It has limited use in malaria control and is used as a complementary method of vector control. It is used mainly in conjunction with mass treatment of fevers. It has also been used with some reported success in the control of malaria epidemics or of highly exophilic vectors, such as *A. dirus* in refugee camps in Thailand and *A. nuneztovari* in Venezuela. It has been used in the emergency control of epidemics (in the developing phase) and where there is sufficient evidence that the main determinant factor is an abnormal vector density.

Space spraying has the following disadvantages:

- it may be wasteful (if it misses its target or is used against a widely dispersed target)
- it requires special and expensive equipment
- it has little residual activity
- its efficacy often depends on the meteorological conditions at the time of application, including wind direction, rain, and temperature

To have an impact on vector density the spray operation must be timed to coincide with peak activities of the vector. An important characteristic of space spraying is the size of the droplets dispersed, which governs the time they remain in suspension, and their ability to penetrate into spaces that are not fully open. Operational costs are high and the killing effect transient. Space spraying must be considered only exceptionally, for very limited periods of time.

Insecticides and formulations

Table 5 shows the insecticides suitable for use as thermal fogs or cold aerosols for mosquito control. Pyrethroids are becoming the predominant insecticides for use in space spraying, while organophosphates are becoming less acceptable because of their smell.

⁷ FAO (1999) Guidelines for the management of small quantities of unwanted and obsolete pesticides. Rome, FAO, *Field document GCP/INT/650/NET*.

Table 5. Selected insecticides suitable for use as aerosols and thermal fogs for mosquito control.

Compounds	Dosage of active ingredient (g/ha)
Organophosphates	
Fenitrothion	250 – 300
Malathion	112 – 600
Pirimiphos-methyl	250
Pyrethroids	
Cyfluthrin	1 – 6
Deltamethrin	0.5 – 1.0
Lambda-cyhalothrin	0.5 – 1.0
Permethrin	5 - 10
Resmethrin	2 – 4

Exercise 6.5

Some points need to be taken into account when considering the efficacy of IRS. First of all, this is a tool for interrupting or severely suppressing malaria transmission. IRS is not necessarily the best tool when other aims are targeted, e.g. restriction of morbidity and prevention of mortality. Therefore, the objectives of malaria control should be clearly specified. Secondly, the degree of perfection of operation is crucial. The coverage of the structures should be no less than about 90%, which may be a difficult task for cost and operational reasons. Work in small groups and answer the following.

- a) Partial spraying may be counterproductive: e.g. by spraying of cattle sheds and not fully covering residence dwellings. Why?
- b) Apart from the high refusal rate, what other problems diminish efficacy of IRS?
- c) The aim of IRS is to reduce numbers of mosquitoes. **True or False? Explain your answer.**
- d) IRS protects people living in sprayed structures from infection. True or False? **Explain your answer.**
- e) The number of people protected equals the number of inhabitants of sprayed houses, e.g. if 50% of houses have been sprayed, 50% of the population have been protected. **True or False? Explain your answer.**
- f) In cases of epidemics or emergency complexes, priority for IRS should be given to spraying of permanent houses. **True or False? Explain your answer.**

Learning Unit 7

Malaria stratification and vector control

Learning objectives

By the end of this Unit you should be able to:

- Describe the characteristics of the six major malaria epidemiological strata
- Select effective vector control options according to the local epidemiological characteristics and strata of malaria

In the last learning unit you looked at the components of vector control and the methods used to control vectors. Also, you described their advantages and limitations. In this learning unit, you will learn how to select appropriate vector control options according to the epidemiology of malaria.

7.1 Stratification of malaria by risk

Malaria risk is often stratified into three broad categories which depend upon the degree of transmission. Malarious strata can be classified as:

- malaria free
- unstable (epidemic), or
- stable (endemic)

Sometimes it is not easy to distinguish between stable or non-stable malaria. Also within each classification it is possible to further classify malaria. For example, in areas of unstable (epidemic) malaria it is possible to classify two distinct types of transmission:

- Highly seasonal but intensive transmission with more or less predictable pattern each year associated with explosive epidemics at five to ten years intervals
- Highly seasonal with very little or even no transmission for several years. These areas are also affected at times by dramatic and devastating epidemic, which often result from environmental or meteorological changes

The same is true with stable malaria. While some areas show a marked seasonal variation in intensity of transmission, others have a more uniform pattern of transmission throughout the year.

7.2 Stratification based on spleen and parasite rates

Endemicity of malaria can also be classified based on spleen and parasite rates among the population (Table 7.1).

Table 7.1 Classification of malaria endemicity based on spleen and parasite rates

Endemicity	Spleen rate	Parasite rate
Hypoendemic malaria	< 10% in children 2-9 years	<10%
Mesoendemic malaria	11-50% in children 2-9 years	11-50%
Hyperendemic malaria	50-75% in children 2-9 years and high (>25%) in adults	51-75%
Holoendemic malaria	>75% in children 2-9 years and low in adults	>75%

- **Hypoendemic** areas: very little transmission and low risk of infection to the population.
- **Mesoendemic** areas: typically rural villages mainly in subtropical regions with varying intensity of transmission and often prone to malaria epidemics.
- **Hyperendemic** areas: intense seasonal transmission but not sufficient enough for a very high proportion of the population to develop protective immunity.
- **Holoendemic** areas: year round transmission with a high degree of immunity among the population of all age groups, particularly adults.

It is important to note that **entomological inoculation rates** and **vectorial capacity** are also useful in expressing the risk of malaria infection and distinguishing between different eco-epidemiological types.

7.3 Stratification of malaria by epidemiological strata

Malaria can be classified into six major epidemiological strata for the purpose of defining malaria risk and potential control methods.

1. Unstable malaria (epidemic-prone) areas

The characteristics of epidemic-prone areas are:

- High seasonal transmission
- Highly variable risk of malaria from year to year
- Explosive seasonal proliferation of vectors
- Low survival rate of vectors due to mostly hostile outdoor environment
- Climatic conditions favourable for short periods of transmission
- High anopheline density required to sustain transmission
- *Plasmodium vivax* infections are common (In Africa, this is true only in the case of Ethiopia; parts of Kenya and Burundi but not in Southern Africa)

2. Stable malaria

The adult population usually shows a high level of immunity to malaria and therefore children are more often at risk of severe disease and death due to malaria than are adults.

3. Urban malaria

Malaria endemicity in urban settlements is often lower than rural communities and varies greatly in different parts of the towns or cities. Entomological studies have shown that the main factor for such variations is vector density.

By reducing of open space and potential breeding sites, and increasing domestic pollution, urbanization curtails anopheline breeding. The following are some of the consequences of urban development:

- The spread of the anopheline population over denser human population tends to reduce the amount of exposure of each person
- By limiting the dispersion of vectors from breeding sites, urbanization tends to localize malaria transmission. High population density at the edges of breeding sites potentially provide enough blood source for vectors thus diminishing the need for the mosquitoes to seek blood a greater distance from the breeding sites. This lowers the risk in the center of cities
- Urban housing conditions (e.g. mosquito-proofing) may limit vector- human contact

While urbanization is mainly characterized by reduction of malaria transmission and may create an island of non-immune people at its center, it is also associated with increased risks at the periphery. As urban areas expand the sanitation and good housing lags behind, particularly in the peri-urban area where poor people tend to congregate and immigrants settle. This exposes these high risk populations to areas of vector breeding and increases human-vector contact.

4. Economic development projects

Economic development projects attract a large labour force, many of whom are newcomers. This group is usually either non-immune to malaria infection or is susceptible to the local strains of *Plasmodium*, thus creating the potential for outbreaks. Also, poor housing and living conditions can expose them to higher malaria risks. Economic development projects also involve modification of land and water use that often favour vector proliferation and increases the risk of malaria.

5. Nomadic populations

Characteristics of nomadic populations

Nomads migrate with their herds to exploit scarce resources for their animals and themselves: thus exposing themselves and other populations to malaria. It is important be aware of these movements when planning the protection of nomadic people against malaria.

6. Complex emergencies (refugee and internally displaced populations)

Malaria is one of the major health problems that affect refugees and internally displaced populations. Exposure to transmission is often several times greater among refugees and displaced populations than among the local population.

Factors that can give rise to a high malaria transmission in complex emergencies include:

- a breakdown of health services
- the concentration of non-immune refugees in malaria risk areas
- malnourishment
- the placement of refugee camps on marginal land that is prone to flooding and vector breeding
- a lack of access of medicine to the displaced population

Complex emergencies usually evolve from an acute-emergency to post-emergency phases. The acute phase is characterized by sudden population displacement and high mortality, and may last for only a few months. To prevent morbidity and mortality, case management should be supplemented with vector control. Some methods are more suitable for the acute phase, others for the post-emergency phase.

Exercise 7.1

Form three working groups and answer the following questions related to the epidemiological strata of malaria.

1. What do you think are the impact of the entomological and environmental factors on the prevalence of parasite, immune response of the population, age distribution of infection, illness and death due to unstable malaria? Stable malaria? Compare and contrast the differences.
2. State the characteristics of stable malaria that can limit the impact of large-scale vector control measures such as residual spraying of houses
3. What do you think are the factors that affect vector density in urban settings?
4. Meet in plenary to discuss your conclusions with the rest of the class.

Exercise 7.2

Return to your working groups. The facilitator will select two to six of the epidemiological strata for your group to work with. You will determine which vector control options are most appropriate for each stratum. List your results and your justification for your selection on a flip chart. Present your results in plenary.

Discussion

The course coordinator will lead a final discussion on vector control options as they relate to epidemiological strata.

Learning Unit 8

Management of malaria vector control programmes

Learning Objectives

By the end of this Unit you should be able to:

- strengthen and direct surveillance and information management systems to provide support for decision-making on vector control
- develop the capacity to address the technical, operational, managerial, and policy aspects that are necessary to ensure efficient and effective functioning and cost-effectiveness of vector control
- establish a vector control monitoring system and select the indicators needed to monitor and evaluate vector and malaria control

Incorporate operational research in malaria vector control programmes

8.1 Deciding on suitability and feasibility of methods

In situations where a need for vector control has been identified, you must decide what methods are suitable and what methods give optimum cost effectiveness. From the technical point of view, your decision is guided by:

- The vector's breeding, resting, and feeding habits
- The peoples sleeping habits, occupations, net use patterns, and their attitude to insecticide and ITN programmes
- The environment of transmission: the types of places where people are at greatest risk of exposure to mosquito bites (house and housing environment, specific outdoor locations, types, extent, location and accessibility to important breeding sites)

Operational feasibility depends on:

- Presence of enabling environments
 - Provision of resources (human, material, logistic and financial)
 - Infrastructure for delivery of services, policies and legislatures
- Good planning and implementation schemes
 - Managerial (leadership, supervision, monitoring and evaluation),
 - Administrative (logistic and other support).

As already pointed out, the different vector control methods vary in efficacy, specificity, resource requirements, likely implementers, costs, appropriateness and delivery prospects in different situations. Methods with different levels of efficacy and resource requirements may be used in an area simultaneously at sub-unit levels, and/or at different times. The unit or size of operational areas depends on the method and the levels of stratification. Some methods may not produce an adequate impact on their own, but may complement or synergize the effects of other methods. The relative contribution of each method including its cost-effectiveness must be assessed to identify the methods that can contribute most. For instance, management must recognize that space spraying has only a minimal role except in the limited circumstances where its application is indicated.

8.2 Managing information

The effectiveness of vector control interventions depends on early detection, a timely and efficient response to the malaria problems and on changes in risks. This requires that correct information is received by those concerned with planning and implementing vector control through efficient decision-support systems (surveillance and information management) that can highlight such indications. The management must thus establish or strengthen the existing surveillance and information management systems in order to allow rapid collection, reporting, analysis, exchange, and use of the relevant information.

In terms of vector control, the surveillance and information management systems must be directed to:

- Pinpoint situations that demand vector control interventions
- Monitor ongoing interventions
- Recognize and act on emerging epidemics and epidemic risks
- Enable stratification at the macro- and micro-levels, to guide vector control implementations by defining major areas, specific ecotypes, localities and high risk population groups. Finer units of transmission such as high risk house units within villages, or individuals can be defined for micro-targeting vector control and thereby optimizing cost-effectiveness
- Provide the information necessary to guide decision makers, support agencies and vector control implementers
- Provide guidance for the overall planning, implementation, monitoring and evaluation of vector interventions and strategies
- Provide feedback to the localities where information is gathered for surveillance and for other purposes.

Managers should develop mechanisms and processes to ensure that the planning, implementation and evaluation of vector control measures is very closely linked to the relevant surveillance and information management systems.

An information base should contain:

- Broader aspects of the malaria problem or risks, e.g. major geographical areas, populations and seasonality of malaria risk
- The major determinants of transmission and risks in a given area, e.g. climatic factors, environmental changes, population movement, development projects
- The monitoring indicators likely to be most useful

- The available vector control methods, their advantages and limitations.
- The resources available and required (personnel, insecticides, nets, spray equipment, etc).
- Documentation on country and local experiences, achievements and failures in vector control.
- Specific and target-oriented information such as those related to the disease, parasite, vector, human host and demographic features of the areas targeted, and the environment of transmission.

Some information is indispensable while other information helps refine the information base to pinpoint houses or individuals at higher risk. It allows closer targeting of interventions, such as ITN distribution, and guides the improvement or modification of control strategies. Continued access to information on the disease status and trends is essential to assess the malaria problem and risk.

Not all of the information needed for vector control decision making needs to be collected on a regular basis. It can be collected to understand trends or for special purposes such as operational research.

Information is collected within the malaria control programme or the health sector on a regular basis. Other information is accessed from other sources within or outside the malaria and health services when needed. Be aware that other agencies collect data on other topics that are used in malaria vector control and one can get the information without duplicating effort (e.g. meteorological, environment, housing and construction). This data may be collected from researchers, non-governmental organizations (NGOs), communities, development projects, the private sector, and published documentation. Therefore, close collaboration and linkages with the different information bases is highly beneficial.

8.3 Quality control and standard setting

Management responsibilities include regular undertaking quality assurance checks on insecticides, mosquito nets, equipment and other supplies used and ensuring collaboration among malaria control personnel, WHOPEs, pesticide industry, national pesticide registration and regulatory bodies, researchers, and other relevant bodies for checks on insecticides and ITNs.

Other responsibilities include capacity building, training and operational research.

8.4 Role of entomological services

Entomological inputs are essential to guide the control of malaria. In general, the role of the entomological service in malaria vector control is to:

- Guide, support and participate in the planning, implementation, monitoring and evaluation of vector control, including epidemic prevention and control
- Link with the surveillance and information management systems within and outside the health sector, and capture the relevant information and data for the planning of vector control
- Undertake and participate in stratification process

- Contribute and participate in partnerships, including the development of information, education and communication materials
- Address the epidemiological aspects of malaria transmission and the broader environmental issues involved in transmission and risk
- Guide related vector control activities.

The entomological needs and inputs for malaria control depend on the,

- Objectives of the malaria control programme
- Vector control strategy, objectives and targets
- Vector control methods in use, and those likely to be used
- Institutional arrangements and resources available for vector control
- Available information
- Ongoing operational research, including future needs
- Ability to use entomological data for decision making and planning

Not all malaria control programmes have an entomological component or use it appropriately. Where it does not exist, it must be developed. In the short-term, arrangements must be made to get the minimum necessary information needed for vector control activities from relevant sources.

Experienced malaria control programmes often have an entomological component. Many countries are in the process of shifting from “eradication”, to “control” and towards an integrated and decentralized management of health services. In this process, the specific requirements and the issues including the managerial and other support for vector control and entomology are often overlooked or not addressed as required.

There is a need to:

- Redefine and reorient the role of the entomological service
- Strengthen the capacity of personnel, in keeping with the current demands and challenges of vector control, under the Global Malaria Control Strategy and the Roll Back Malaria Initiative
- Ensure that the managerial, resource, and support requirements for effective functioning are appropriately met, especially within the decentralized, integrated management of malaria

8.5 Partnerships in malaria vector control

Malaria is a national priority not just a local problem. It has a broad ownership. All stakeholders, irrespective of their level of involvement should be involved in the decision-making process for malaria control. Properly motivated and planned, a range of stakeholders can play a crucial role in malaria vector control. In addition to the malaria control programme personnel, the stakeholders and partners include:

- Macro-economic decision makers
- Sectors within and outside malaria and health system, including municipal governments
- Communities, households, and individuals

- NGOs, donors, bilateral and international agencies
- The private sector
- Academic and research institutes

The levels of implementation and involvement of the partners will depend on the vector control objectives, targets and methods. The roles of different stakeholders can be primary, supportive, collaborative or participatory. Some will have indirect roles. Clear guidelines must be given and the responsibilities assigned to each partner. Their responsibilities should be guided by their technical competence, resource provision, coordination, and social mobilization.

The primary responsibility of the partnership is to ensure action against mortality, morbidity, drug resistance and epidemics. Even if the main responsibility lies with malaria control and health services and municipalities, all partners should be part of the process. The primary responsibility for the insecticide-based interventions is most likely to lie within the malaria/health services. Macro-economic, policy and decision makers have crucial roles to play in dealing with environmental risks of transmission resulting inadvertently through development-linked activities, where high investments and specific policies and legislation are involved. Implementation responsibilities may vary from individuals/households, and communities, to the macro-economic level. Within the malaria/health services, entomologists have a major role in ensuring standards and specifications (insecticides, equipment, supplies, quality assurances), for operational research and evidence-based arguments, technical guidance and inputs, training, monitoring and evaluation, and promotion – including information, education and communication (IEC). Training needs (sensitization, orientation, knowledge, skills) for vector control and related activities must be identified and met for all categories of stakeholders and partners within and outside the health sector.

Comprehensive communication campaigns and IEC programmes are necessary for education, awareness raising behavioural changes and sensitization. IEC programmes must cover the current demands of malaria vector control, including selective vector control (target-oriented interventions), integrated vector management, partnerships and harmonized actions, community involvement in order to:

- obtain and sustain commitment and support at all levels from the highest political and macro-economic and decision-making levels to communities, individuals and the private sector
- (re)awaken people to the malaria problem, possible solutions, the potential role of different vector control interventions and their likely participation
- Incorporate epidemiological and socio-economic (cultural, religion, language literacy, etc) factors into the decision making for vector control

Examples of issues to be addressed through IEC programmes are to,

- develop and use target-group specific IEC messages
- enhance community awareness and sensitivity on the potentials and strengths of different vector control methods; where they could be used and where they will not work
- publicize relevant vector control related research findings, with appropriate messages to create a demand for their use or application
- provide models of locally suitable houses that can minimize human-vector contact, facilitate and support their use and monitor their impact
- help target vector control programmes related IEC in schools and other child organizations to improve the quality of homes and sustainability

These will entail development and use of suitable materials (posters, press releases, media messages for TV, radio and others) for national and local level advocacy. Other IEC materials and suitable approaches should be developed for district and regional levels, and for the local communities, households and individuals.

Political commitment for malaria vector control exists through the RBM initiative. However commitment needs to be reinforced to ensure that vector control is properly addressed.

8.6 Monitoring and evaluation of vector control

Vector control must be evidence-based, systematically monitored, and evaluated. The first step is to carryout a situation analysis on the vector and vector control. For example, an assessment of vector control measures, its achievement and success, and strengths and weaknesses will help identify the major areas, issues and problems affecting current implementation. Variables and indicators can be selected to monitor process and activities and evaluation of outcomes and impact. The situation analysis in particular guides the needs assessment for planning and for subsequent actions.

For vector control, a preliminary situation analysis must cover the following:

Malaria problem and risks

- What areas and populations are under malaria risk in relation to the country total?
- What malaria epidemiological situations and their characteristics are likely to influence (positively or negatively) the potential for vector control interventions in each epidemiological setting?
- What are the levels of malaria endemicity?
- What are the malaria incidence and mortality rates and trends?
- What are the predominant parasite species and their drug resistance status?
- What are the transmission patterns?
- Are there special situations or factors during the season of highest transmission that favour an increase in human-vector contact?

Management of vector control

- Existing national or sub-national vector control policy, strategies, objectives, and targets in relation to the country's needs.
- Organizational structures at different functional levels of the health system or management and the placement of malaria control, vector control and entomological components.
- Functions of vector control and entomological services within decentralized, integrated management of malaria control and in the primary health care system. What are their roles and responsibilities, and chain of command?
- Development-linked projects and their contributing risk to the malaria transmission; actions taken to prevent or avoid such risks; and availability of related policies. Presence of policy and legislation to enforce the incorporation of health issues in development-linked projects.
- Existence of system and capacity at all levels for proper documentation, analysis and feedback on vector control implementation
- Availability and relevance of policies and legislation on insecticides, including a national insecticide policy for public health. Procedures for the procurement of insecticides and quality assurance are needed.

Vectors

- Current knowledge on:
 - status of primary, secondary and potential vectors
 - breeding sites, resting preferences (indoor/outdoor) and feeding preference (humans vs. animals; indoors vs. outdoors)
 - peak biting periods
 - susceptibility and resistance status to insecticides in use and potential alternatives
 - date of latest investigation or update

On-going vector control interventions

- Total area and population under vector control in relation to the total area under malaria risk
- Vectors targeted
- Vector control methods in use, areas and populations targeted and coverage by each method and in what situations
- How and by whom vector control is planned, the processes in stratification and the level of stratification (macro and micro levels)
- Who other than the MOH are involved in vector control, what are they implementing, how are these consolidated, coordinated and monitored
- Decision-making process to undertake vector control; criteria used for IRS (e.g. when to start, stop, or continue ongoing IRS in an area; size of operational units/areas; the maximum and minimum)
- Type and amount of each insecticide used for IRS, larviciding and space spraying
- Criteria used for the selection of insecticides

- Likely efficacy and impact of each type of vector control on target vectors and on malaria
- Methods (and indicators) used to monitor and evaluate vector control and malaria
- Resources used (human, material, financial) and relative costs of each

Epidemics

- Areas and situations with epidemic potential and malaria risk
- Epidemic and epidemic risk indicators, if known, and their current use in the malaria programme (environmental, meteorological, entomological, population movements, use of drugs)
- Capacity to predict, detect and respond to epidemics
- Role of vector control and entomological services in epidemic prediction, detection and control

Quality assurance

- Methods and processes to ensure the quality, standards and specifications for insecticides, equipment and material used in vector control. Also a measure of the performances and function of these methods
- Issues, constraints and gaps in knowledge affecting cost-effective vector control; probable causes and processes for feedback to guide adjustments and improvements in the vector control programme
- Availability to maintain the programme and the equipment used
- Resources used and their cost

Safety

Ensure availability of guidelines on storage, handling and safe use of insecticides, including availability of protective measures for staff handling of insecticides

Indicators for monitoring and evaluation

Indicators must be defined for each aspect of vector control that you will monitor. Choice of indicators depends on the characteristics of the issues addressed. The indicators selected must be relevant. They must measure what is needed and be related to the programme objectives. They must be limited in number, easy to collect, reliable and sensitive to variation and change in vector control and malaria incidence. They must be simple, readily measurable and operationally useful. Indicators may be monitored regularly or selectively for trends depending on the variable and method used.

Exercise 8.1

Identify the operational and entomological indicators for (a) larval control (b) adult mosquito control and (c) control of human-vector contact for the vector control methods selected by your group for the epidemiological strata in Learning Unit 7. After your group discussion fill in Table 8.1, Indicate which indicators would need to be measured routinely, for trends or for special purposes. Present the results in plenary.

Table 8.1 Operational and entomological indicators to be monitored

Vector control	Operational	Entomological
Larval control		
Control of human-vector contact		
Adult mosquito control		

Return to your working groups and do the following exercise.

Exercise 8.2

Define the epidemiological indicators you would monitor for malaria in each of the epidemiological strata your group worked on in Learning Unit 7. Present your results in plenary.

Two additional management functions needed for a well designed and implemented malaria vector control programme are operational research and cost-effectiveness.

8.7 Operational research

Problem-solving operational research with direct relevance to vector control must be an integral component of a malaria vector control programme. The research aims are to identify control options and needs and enhancing the operational impact of control actions. It must cover the technical, operational and managerial aspects and implies that you need to review ongoing research and its relevance to short, medium and long term vector control needs. In addition the managers in charge of malaria and vector control programmes should,

- Identify knowledge and information gaps on vectors, transmission, vector control methods and insecticide resistance
- Identify priorities for research. Malaria control programme and vector control managers, entomologists, and field personnel may be able to pinpoint specific issues and problems likely to warrant investigation
- Prepare research agendas jointly with decision makers, entomologists, vector control planners and implementers and potential researchers
- Seek funding and resources for operational research
- Undertake the research jointly with the relevant partners
- Translate research findings into action against malaria and its vectors
- Use the findings of operational research to influence policies and subsequent planning and implementation

8.8 Cost-effectiveness studies

Cost-effectiveness analysis is an economic evaluation technique designed to aid decision-making. It involves assessing and comparing the effectiveness and the costs of alternative tactics of achieving a specific objective⁸. Its purpose is to identify how to run programme and arrive at a specified objective with minimal cost within a given budget. Cost-effectiveness analysis starts from a problem and a set of proposed solutions. The cost and effectiveness of each alternative tactic or approach is calculated and the benefits of each reviewed. The alternative with the lowest cost per unit of health effect is by and large preferred.

⁸ Phillips M, Mills A, Dye C (1993). *Guidelines for Cost-effectiveness Analysis of Vector Control*, World Health Organization document WHO/CWS/93.4.

A cost-effectiveness analysis has been conducted for malaria control in sub-Saharan Africa⁹. ITN and IRS were compared using the cost per disability-adjusted life year (DALY). In a very-low-income country, insecticide treatment of existing nets had a cost-effectiveness range of US\$4-10 per DALY averted; for provision of nets and insecticide treatment the range was \$19-85; and for residual spraying (two rounds per year) \$32-58. These results will be used by the health programmes of many countries to guide the economical needs of a malaria programme.

⁹ Goodman CA, Coleman PG, Mills AJ (1999). Cost-effectiveness of malaria control in sub-Saharan Africa. *Lancet*, **354**: 378-385.