

Module 7

Visceral Leishmaniasis (Kala-azar)

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

- Disease Transmission
- Vectors of VL
- Biology
- Life Cycle
- Vector Surveillance
- Entomological Indicators
- Adult Vector Control
- Larval Vector Control

Introduction

Leishmaniasis is caused by a protozoa parasite from over 20 *Leishmania* species. Over 90 sandfly species are known to transmit *Leishmania* parasites. There are 3 main forms of the disease:

Visceral leishmaniasis (VL), also known as kala-azar is fatal if left untreated in over 95% of cases. It is characterized by irregular bouts of fever, weight loss, enlargement of the spleen and liver, and anaemia. Most cases occur in Brazil, East Africa and India. An estimated 50 000 to 90 000 new cases of VL occur worldwide annually, with only between 25 to 45% reported to WHO. It remains one of the top parasitic diseases with outbreak and mortality potential. In 2020, more than 90% of new cases reported to WHO occurred in 10 countries: Brazil, China, Ethiopia, Eritrea, India, Kenya, Somalia, South Sudan, Sudan and Yemen.

Cutaneous leishmaniasis (CL) is the most common form of leishmaniasis and causes skin lesions, mainly ulcers, on exposed parts of the body, leaving life-long scars and serious disability or stigma. About 95% of CL cases occur in the Americas, the Mediterranean basin, the Middle East and Central Asia. In 2020 over 85% of new CL cases occurred in 10 countries: Afghanistan, Algeria, Brazil, Colombia, Iraq, Libya, Pakistan, Peru, the Syrian Arab Republic and Tunisia. It is estimated that between 6,00,000 to 1 million new cases occur worldwide annually. In India, cases of Cutaneous leishmaniasis are frequently reported in the states of Himachal Pradesh, Assam, Rajasthan, and Kerala. However, these are not being captured in the programme. The diagnosis and treatment protocols remain the same as for V. leishmaniasis.

Blood-sucking arthropods transmit a variety of human pathogens acting as disseminators of the so-called vector-borne diseases. Leishmaniasis is a spectrum of diseases caused by different *leishmania* species, transmitted word wide by sand flies.

Kala-azar or Visceral leishmaniasis is the most common endemic form of the disease in 33 districts of Bihar, some districts of eastern UP, West Bengal and 4 districts of northern Jharkhand. It is transmitted by the biting of an infected sandfly. *Phlebotomus argentipes* is the only known vector of kala-azar in India. Phlebotomines are the sole or principal vectors of Leishmaniasis. Sand flies are small, hairy insects of 2–4 mm in length.

The causative agent (parasite) is a protozoa named *Leishmania donovani* in Indian sub – continent.

Classification

Phylum: Arthropoda
Class: Insecta (Hexapoda)
Order: Diptera (Two winged Insect)
Sub Order: Nematocera
Family: Phlebotomidae / Psychodidae
Sub Family: Phlebotominae
Genus : Phlebotomine
Species : argentipes

Details of Phlebotominae Family

- Contains more than 600 species.
- 30 species have been reported in India.
- Important species are-
- *P. argentipes*
- *P. papatasi*
- *P. sergenti*
- *Sergentomya punjabensis*
- *Sergentomya babu*

Medical importance of sandfly

P. argentipes transmit protozoal disease Visceral leishmaniasis, Cutaneous leishmaniasis, and Mucocutaneous leishmaniasis.

Visceral leishmaniasis is the main public health issue in India. Post Kala-azar Dermal leishmaniasis (PKDL) is also a major health issue worldwide.

Reproduction and Oviposition

Adult sand flies mate soon after emergence. Female sandflies usually lay 30–70 eggs during a single gonotrophic cycle, which are deposited in cracks and holes in the ground or animal burrows and tree holes. The eggs require a microhabitat with high humidity to survive. Generally, one blood meal results in the production of a single batch of eggs.

Development and Life Cycle

In contrast to mosquitoes and other Diptera, sandflies do not have an aquatic stage in their life cycle. Still, humidity is an important factor that together with temperature is detrimental to and influences sand fly development. The sandfly lays eggs and breeds moist soil, cow dung debris and muddy situations with high humidity (Figure 26).



Figure 26. Suitable Habitats for Sandfly breeding

Sand flies have a 4 stage life cycle – egg larva pupa and adult. Normally, oviposition occurs between 5–8 days after blood feeding. Eggs hatch 4–20 days, although, this is likely to be delayed in cooler weather (Figure 27). Larval development involves 4 instars and is completed in 20 to 30 days depending on species, temperature and nutrient availability.

Environmental extremes for example heat; cold or drought can cause larvae to diapause, prolonging development time to months. Larvae are mainly scavengers, feeding on organic matter for example fungi, decaying leaves, animal faeces and decomposing matters.

The pupal stage is a non-feeding and sessile stage which lasts 6–13 days before the adult sandfly emerges.

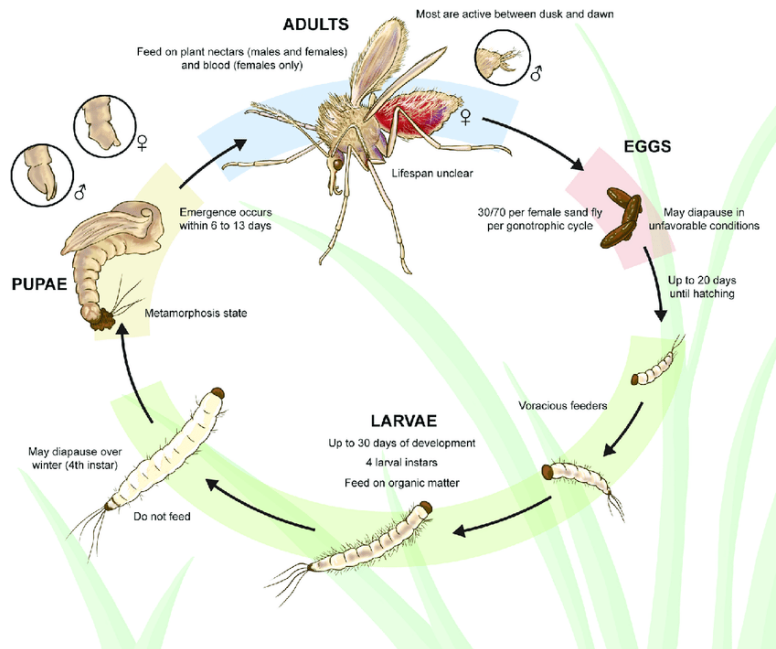


Figure 27. Life Cycle of Sand Fly

Habitat Preferences

Sandflies occur predominantly in warm, humid, tropical climates and semi-desert vegetation habitats, although a few species occur in temperate zones. They can colonize rural, semi-urban and urban areas.

Sand flies require a humid climate for their eggs to develop and larvae need a cool, moist habitat with decaying debris to feed upon. Adult sand flies often inhabit rock crevices, caves and rodent burrows and in semi-domestic settings rest in cool, dark and humid corners of animal shelters or human dwellings. Both rodent burrows and semi-urban areas provide ready access to blood meals in addition to shelters.

Adult Feeding Behavior

Both male and female sandflies feed on plant juices and sugary secretions. Females feed on blood to produce eggs. Feeding activity is influenced by temperature, humidity and air movement. Sand flies are weak fliers so even a light wind can inhibit flight and reduce biting.

Sandflies feed at dusk and during the night when the temperature falls and humidity rises.

Sand flies naturally feed on blood from a wide range of mammalian and avian host species and are found in large numbers in rural environments close to animals and humans. Female sandflies feed on a wide variety of vertebrate hosts, including humans, livestock, dogs, urban and wild rodents, reptiles, amphibians and birds. Feeding probability is also positively associated with increasing host population and decreasing movement. As a result, it is probable that in urban and semi-urban settings, humans and domestic dogs are the main targets of sand flies.

As ectothermic insects, the large-scale territorial boundaries of sand flies are constrained by climate, particularly minimum winter temperature affecting larval survival that reduces adult biting activity.

Activity

The extent to which sandfly population densities vary throughout the year depends on the local climate, with significant climate changes in temperature and humidity resulting in fluctuations in sandfly numbers.

Various species of sandflies have different seasonal activity periods and daily peaks of biting activity. Adult sand flies are weak fliers travelling with characteristic short hopping flights and they usually disperse no more than a few hundred meters from their breeding sites. Most species fly horizontally near ground level.

Biting and Disease Risk

Since adult females are the only haematophagous stage biting rates in a particular area would be strongly correlated to the abundance of adult females. Sandfly requires a minimum of 6 weeks to complete a life cycle with adult activity and, consequently, parasite transmission being mostly nocturnal and typically seasonal. The active season for adults is March to November.

Morphological identification of Phlebotomine sand flies to species level is difficult, usually requiring examination of internal structures.

Public Health Control Measures

The suitability of leishmaniasis control strategies depends on the epidemiology of infection and the ecological characteristic of the vector and reservoir hosts, deferring substantially for zoonotic and anthroponotic leishmaniasis,

Public health intervention to control leishmaniasis outbreak requires an integrated strategy, combining vector surveillance and action against vector and reservoir species. Surveillance aims to assess the risk of parasite transmission and guide vector control activity. Due to the difficulty in locating sandfly breeding sites, it centres on monitoring the distribution and abundance of the adult stages.

A variety of sampling methods are available. The most common are sticky traps to capture random selections of the sand flies flying in the immediate surroundings, light traps to catch host-seeking females attracted to light and aspirator collection to catch resting sand flies.

Entomological Parameters

1. Adult sampling techniques

Of the various methods available for adult sampling, the selection depends on the objective of the sampling, the biotope selected for sampling and the limitations of a particular technique. The most commonly used techniques are summarized below:

a) Hand Collection

This is the most common method wherein sandflies sitting on a surface are caught with the help of an aspirator or test tube and a torch light. This method is particularly useful for the longitudinal monitoring of manhour densities. However, in the sandfly collection, the ordinary mosquito-barrier netting between the glass tube and rubber tubing of the aspirator must be replaced by a muslin cloth as the smaller size of sandflies enable them to escape through ordinary. Mosquito net.

b) Trap Collections

Usually 4 types of traps are used :

i) Sticky trap

This is the most extensively used trapping device wherein sandflies are trapped in a layer of castor oil. Suspended arched sticky papers/foils of standard size (20x30 cm) are placed at a height of about 4-5cms from the ground with a

convex sticky side towards the ground. Traps are usually laid in the evening and collected the following morning. Sandfly density per trap is calculated for comparisons. Sticky traps are particularly useful in collecting sandflies from hidden shelters like burrows, Cracks, tree holes, etc. For some species showing repellency to castor oil, other vegetable oils are required to be used. However, in India, these can be safely used against *Ph. argentipes*.

ii) Illuminated Sticky traps

Box-shaped batteries are hung on the walls facing sticky traps to make them illuminated. In some studies, these traps have provided higher catch as compared to ordinary traps.

iii) Light traps

CDC miniature light traps are often used for sandfly collections. However, nylon mesh cages suspended in a rigid frame are better than the collapsible cages provided with the traps. Further, for sandflies, they are modified to give UV light or white light.

iv) Funnel traps

These are particularly useful in collecting flies from rodent burrows. Traps are placed just at the mouth of the burrow to catch the flies emerging out of burrows. The inner side is provided with sticky paper or foil.

Other traps used in mosquito collections like double bednet, stable net, malaise trap, magoon trap, etc. can also be used but the effectiveness is not yet well demonstrated.

c) Bait collections

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

Age Determination

Usual method of age determination of sand flies is the examination of ovariole relics. The ovaries are dissected in sterile saline and the ovarian follicles are examined for dilations. Each relic represents one gonotrophic cycle. The examination of accessory glands for secretory granules also provides criteria for determination of age (parity).

Host Preference

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

Vector Incrimination

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

Determination of Susceptibility of Insecticide

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

Sampling of Immature Stages

Sandflies breed in cracks, crevices and other places with soils rich in organic contents. The resemblance in soil and larval coloration makes it difficult to detect larvae visually in their habitat. The soil is collected, kept in a petri dish and then examined under microscope (40 x magnification). To facilitate screening of larger soil samples, a floatation technique is often practiced. The soil samples are immersed in saturated sugar solution i.e. 3 parts sugar+5parts water. Larvae and pupae float in this solution. These are then passed through a series of sieves and finally the residues are examined under the microscope.