Module 6

Japanese Encephalitis

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

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

Disease Profile

Japanese encephalitis virus (JEV) is a flavivirus related to dengue, yellow fever and West Nile viruses, and is spread by mosquitoes. JEV is the main cause of viral encephalitis in many countries of Asia with an estimated 68 000 clinical cases every year. Although symptomatic Japanese encephalitis (JE) is rare, the case-fatality rate among those with encephalitis can be as high as 30%. Permanent neurologic or psychiatric sequelae can occur in 30%–50% of those with encephalitis. 24 countries in the WHO South-East Asia and Western Pacific regions have endemic JEV transmission, exposing more than 3 billion people to risks of infection. There is no cure for the disease. Treatment is focused on relieving severe clinical signs and supporting the patient to overcome the infection. Safe and effective vaccines are available to prevent JE. WHO recommends that JE vaccination be integrated into national immunisation schedules in all areas where JE disease is recognised as a public health problem.

The first case of JE viral disease was documented in 1871 in Japan. However, the virus was first isolated in Japan in 1935. Japanese encephalitis virus JEV is the most important cause of viral encephalitis in Asia. It is a mosquito-borne flavivirus and belongs to the same genus as dengue, yellow fever and West Nile viruses. The main vector of JE is *Culex tritaeniorhynchus*, spread across India. The first case of Japanese encephalitis viral disease (JE) was documented in 1871 in Japan. The annual incidence of the clinical disease varies both across and within endemic countries, ranging from <1 to >10 per 100,000 population or higher during outbreaks. A literature review estimates nearly 68,000 clinical cases of JE globally each year, with approximately 13,600 to 20,400 deaths. JE primarily affects children. Most adults in endemic countries have natural immunity after childhood infection, but individuals of any age may be affected.

JE in India

The most serious outbreak of Japanese encephalitis (JE) in three decades has left nearly 800 dead and over 3500 infected in 30 districts across the state of Uttar Pradesh since July. More deaths have been reported in the adjoining state of Bihar. In Nepal—which shares an open border with Uttar Pradesh—259 people have died as a result of the mosquito-borne disease endemic in much of the paddy belt in Asia.

Vector

Culex tritaeniorhynchus is a species of mosquito and is the main vector of the disease Japanese encephalitis. Females target large animals for blood extraction, including cattle and swine, and are strongly anthropophilic. In India, the JE virus

has been isolated from 17 mosquito species in wild-caught specimens from different parts of the country. Maximum isolations have been recorded from the *Culex vishnui* group consisting of *Cx. tritaeniorhynchus, Cx. vishnui and Cx. pseudovishnui.* Female mosquitoes get infected after feeding on a vertebrate host harbouring the JE virus and after 9–12 days of extrinsic incubation period, they can transmit the virus to other hosts.[12]

Culex vishnui
Culex pseudovishnui
Culex tritaeniorhynchus
Culex fuscocephala
Culex quinquefasciatus
Culex gelidus
Culex whitmorei
Culex bitaeniorhynchus
Culex infula
Culex epidesmus
Anopheles barbirostris
Anopheles peditaeniatus
Anopheles subpictus
Mansonia annulifera
Mansonia indiana
Mansonia uniformis

Culex vishnui subgroup of mosquitoes are very common and widespread, and breed in water with luxuriant vegetation, mainly in paddy fields, and their abundance may be related to their breeding in rice fields, shallow ditches, pools, fish ponds, etc. Preference for breeding places varies with location. Paddy fields are favourable breeding places during the rainy season and irrigation channels bordering the paddy fields support breeding during the non-monsoon season. Rainwater collections in low-lying areas with aquatic vegetation / submerged grasses support the breeding during post-monsoon months. However permanent water collection in ponds, ditches etc. with aquatic vegetation such as water hyacinth, elephant grass, etc. provide favourable breeding places during all months. In view of the breeding habitats of the vector mosquitoes, JE is usually associated with rural areas with paddy cultivation. *Cx. tritaeniorhynchus*, the principal vector of JE has been reported to be an outdoor rester (exophilic) but may rest indoors during some part of the year. Vectors of JE are zoophilic and feed outdoors as well as indoors. They prefer to feed on cattle and also feed on pigs. Cattle such as cows may reduce risk by diverting vector mosquitoes (zooprophylaxis).

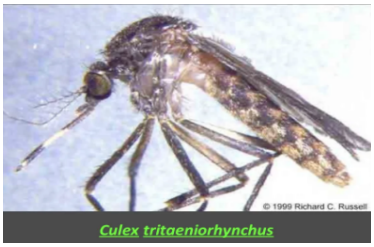
Host

Sources of Infection

- Arthropod-borne viruses (Arboviruses)
- Enzootic or zoonotic disease
- Amplifying hosts

  – Pigs (the main reservoir)
  – Wading birds (egrets, herons), bats

- Incidental host
- Horses, humans (dead-end host)



Culex tritaeniorhynchus

Vector Bionomics

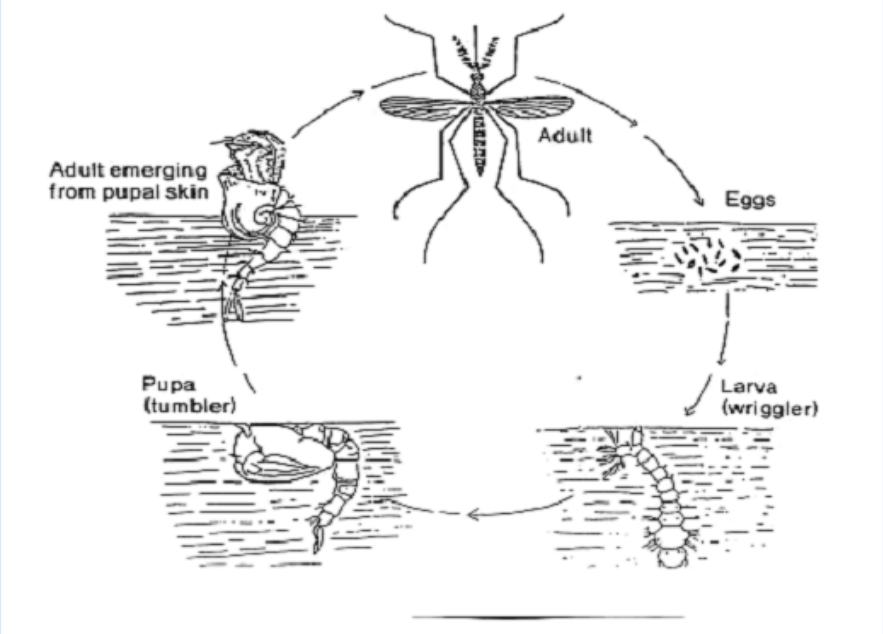
Breeding Habitat

The larval habitat of *Cx. tritaeniorhynchus* primarily consists of low-lying waterlogged areas such as grasses and fallow rice fields, but this species can also be found in wells, ponds, and ditches and has been reported in urban environments in close proximity to human populations, such as water storage containers in houses. It can be found in locations where the annual mean temperature ranges from 8.2–28.9 °C, with elevations of a maximum of 838 m above sea level.

Resting and Biting Behaviour

Primarily outdoor resting in vegetation and other shaded places, but in summer may also rest indoors. They are principally zoophagic/ cattle feeders, though human and pig feeding is also recorded.

Life Cycle of *Culex:* The stages of life cycles for JE vectors include egg, larva, pupa and adult as for other culicine mosquitoes and depicted below (Figure 23).



Egg (2 days) Larva (6-10 days), Pupa (2 days) Adult (3-5 weeks)

Figure 23.Life Cycle of Culex

Prevention and Control

- Safe and effective JE vaccines are available to prevent disease. There are 4 main types of JE vaccines currently in use: inactivated mouse brain-derived vaccines, inactivated Vero cell-derived vaccines, live attenuated vaccines, and live recombinant (chimeric) vaccines.
- Personal preventive measures include the use of mosquito repellents, long-sleeved clothes, coils and vaporisers.

JE is a disease reported primarily from rural agricultural areas, particularly in rice cultivation areas, where vector mosquitoes proliferate in close association with pigs, wading birds and ducks, the principal amplifying hosts. Vector mosquito is able to transmit the JE virus to a new host with an incubation period of 14 days.

For planning vector control measures, the bionomics of vector mosquitoes in an area needs to be studied.

Objectives of Entomological Surveillance

1. To identify the JE vector mosquitoes in an area
2. To monitor JE vector abundance in JE endemic areas
3. To detect the JE virus in vector mosquitoes
4. To suggest appropriate vector control measure

Procedure

Entomologist and insect collectors or biologists in the zones/ districts is responsible for entomological surveillance in JE endemic areas. An entomological team may also conduct these studies. The index villages may be identified in the district for entomological surveillance.

Choice of index villages

- At least 3 villages in which JE has occurred in the recent past (past five years)
- At least 2 villages which remained unaffected to date would be monitored in each affected block.
- Sampling would be carried out on a fortnightly basis
- Surveillance would be carried out round the year to know the JE vector density, their resting behaviour, feeding behaviour and detection/ isolation of JE virus from vector mosquitoes.

Following entomological investigations are to be carried out:

Larval Surveys

Larval Density & Mapping of Breeding Sites: Larval survey should be carried out by the entomological team periodically. All potential breeding sites will be surveyed and will be reported on the standard proforma. All permanent breeding sites of JE vectors would be identified (mapped) and provided to District officers for implementation of control measures.

Larvae collected in the field would be reared in the laboratory for the emergence of adult mosquitoes for identification of vector species. For this purpose, the standardised reporting format AESF-6 form for the breeding survey will be used by all entomological/ reporting units.

Adult Survey

Indoor/ outdoor resting collection and the Dusk collection should be carried out from fixed as well as random sites such as human dwellings/ cattle sheds/ mixed dwellings and outdoor situations such as bushes, plantations, standing crops, etc. by hand catch method using suction tubes. Per Man Hour Density (PMHD) is to be monitored and reported in a standard prescribed format. This collection would be carried out in the index villages only.

Figure 24.Hope Cage Method for JE Vector Surveillance

Cx. tritaeniorhynchus predominantly rests outdoors on agricultural crops and wild vegetation, depending on the local situation, where they can also be monitored by the BPD Hop Cage method; formerly known as the sweep cage method (NICD). The density of mosquitoes may be estimated as the average number of mosquitoes collected for 10 hop cages (Figure 24). The larger the area covered by hopping, the better representation of the mosquito density.

$$\text{Mosquito density (Per 10 HC)} = \frac{\text{Total number of mosquitoes collected x 10}}{\text{Total number of hops made on vegetation}}$$

Blood Meal Analysis

After identification of field-collected vector mosquitoes, stomach blood would be collected in filter paper and sent to the central laboratory for blood meal analysis by ELISA method to know the source of blood.

Guidelines for Collection and Transportation of Samples

- For preparing filter paper for blood meal squashes:
- Fold the filter paper in half, then fold again and fold twice more.
- Unfold the paper, which will now be marked by folds into 16 parts.
- Draw pencil lines along the folds from the edge of the filter paper towards the centre, but leave the central 5cm blank.
- On the inner edge of the filter paper, write a number in the centre of each paper the species of mosquitoes from which the blood was taken, where and when.

Making the Blood Meal Squashes

Blood from a freshly fed mosquito is best for the detection of blood meal sources. Older blood meals are not suitable.

- One filter paper must be used for only one species of mosquito that has been obtained from the same type of resting site, for instance inside houses, inside animal sheds and preferably outdoor natural resting sites. This reduces the chance of error in conducting the test as a circle of paper is punched out from each sector.
- Label the filter – paper with a number in the centre and enter the name of the mosquito species, the place of collection, the date and the time.
- Kill or anaesthetise the freshly-fed mosquitoes
- Place a female mosquito on the filter –paper approximately 1 cm from the edge and inside the area and level as number 1/
- Squash the abdomen using a blunt needle or the corner of a slide or a glass rod.
- Make sure that the squashed abdomen remains inside the area labelled 1. \
- Place a second female mosquito in the area labelled 2 and squash it.
- Each female must be squashed with the corner of a slide or a glass rod or the other side of a lead pencil which do not have exposed lead. If you use a slide; use each corner in turn and after four squashes, discard the slide; you have to make sure that blood from one specimen is not transferred to another (i.e. contamination) from the rod or slide.
- Continue in this way until all 16 areas of the filter paper have been used.
- Write the details that are asked for on the recording form and make two copies of it.
- Allow blood meal squashes to dry, making sure that the papers are protected from ants and humidity.
- Store filter papers by placing them on top of each other with a piece of plain paper between each set of papers on which there are squashes.
- Place filter papers in a desiccator or a refrigerator.
- When you have made all the squashes that you require, pack the filter papers into a self-sealing plastic envelope. If you do not have self-sealing envelopes, pack the filter paper(s) in a plastic bag and seal the end with a hot iron/ candle.
- Send the blood meals to the laboratory in which the identifications are to be made, including one copy of the record forms.
- Keep one copy of the record forms in your laboratory.

Recording Information

Record the following information for each filter-paper of blood meals:

- Name of the collector;
- Estimated ratio of people to pigs, cattle and other animals;
- Locality;
- The resting site from which the mosquitoes were obtained;
- Date and time on which the collections were made.

Susceptibility of JE Vector Mosquitoes and Larvae

Susceptibility status of JE vector mosquitoes to insecticides particularly Malathion in JE endemic areas should be carried out by entomological teams in the state/ ICMR/ any other institute. The map should be prepared in all JE endemic states about the status of resistance of vector mosquitoes to insecticides. Format JEF-9 will be used for reporting the susceptibility/ resistance status of vector mosquitoes.

Method for Collection and Transportation of Mosquitoes for Isolation of JE Virus

For entomological studies, virus isolation would be attempted from vector mosquitoes, which would be collected in a screw-capped clean test tube and sent to the laboratory at NIV/ CRME. Particularly, in epidemic situations, it becomes necessary to collect vector mosquitoes for isolation of the JE virus.

In an epidemic situation, it is desirable to collect mosquitoes from the affected areas, both indoors and outdoors, so that they may be processed for virus isolation. This may give an indication of the species acting as a vector of the area. Mosquitoes can be collected by standard methods such as aspirators, baited traps, biting collections and light traps.

The mosquitoes should be held alive in 'Barraud Cages' wrapped with moistened lint or cloth. If the collection locality is not far from the laboratory or transportation can be done within a day or two, they may be transported alive in Barraud cages. For such transportation, it is necessary to provide raisins soaked in water or a cotton pledget soaked in 10% glucose solution inside the Barraud cage.

If the collection locality is far from the laboratory and immediate transportation is not possible, mosquitoes may be identified, pooled species and stored in liquid nitrogen, refrigerators or on dry ice for subsequent transportation to the laboratory. If facilities for liquid nitrogen or dry ice storage are not available in the field, a transport medium may be used to store the mosquito pools. It is, however, necessary that such pools are constantly kept in the refrigerator or transported on wet ice. Since the Centre for Research in Medical Entomology (CRME), ICMR, Madurai, and Tamil Nadu have developed a technique whereby the JE antigen can be detected in even 28 days old desiccated mosquitoes and NICD has also detected JE virus antigen even after 20 months of mosquito collection from field. It would be possible to get the JE antigen detected from the mosquitoes regularly dispatched to CRME, Madurai post. However, care should be taken not to allow mosquitoes attached by fungus or affected by dilapidation before enveloping for dispatch.

Laboratory Support

The following virological investigation should be carried out in labs :

1. Screening/ isolation of JE virus from suspected JE vector mosquitoes.
2. Vector incrimination would be done in collaboration with NIV Pune, CRME Madurai and NICD, Delhi.

Integrated Vector Management

Adult Mosquito Control

Space Spray: ULV Fogging with Pyrethrum/ Malathion

Fogging is done for the containment of outbreaks.

Personal Protection: Insecticide Treated Bed Nets/ Curtains

Larval Control

Chemical larvicides, Bio-larvicides, Larvivorous fish, Environmental management.

JE Vector Control need to be chosen appropriately from the following IVM Options as to which will be effectively able to control the aquatic or adult population of JE Vectors.

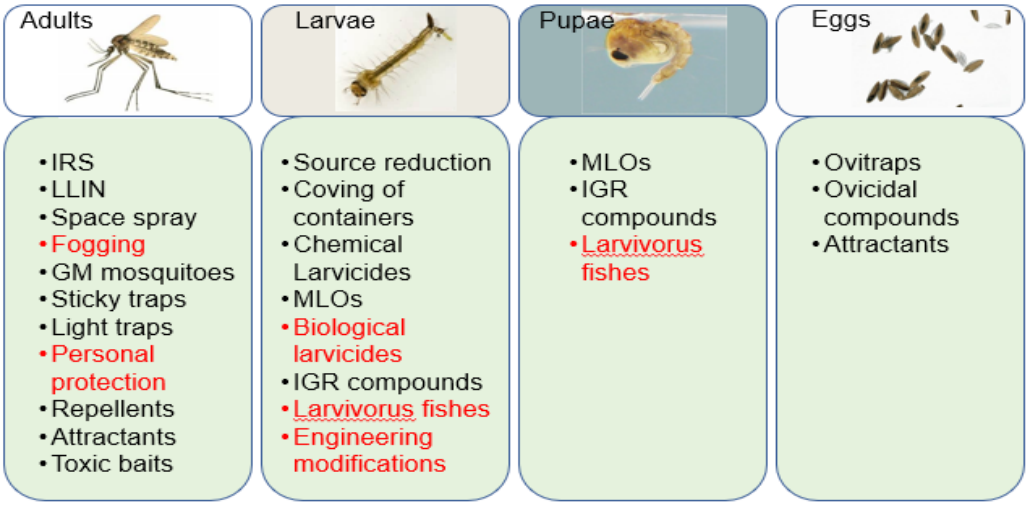


Figure 25.Vector Control Options for JE Vectors