

Chapter 2

Mosquito Surveillance Tools Used and Methodology Followed in Ecological Study on JE Vectors in Northern India

Abstract A simple cost-effective, operationally feasible sampling method was developed for *Culex tritaeniorhynchus* predominantly exophilic JE vector mosquito species primarily for two reasons, viz: (i) to have an in-depth knowledge on the ecology of Mosquito vectors of Japanese encephalitis virus from Northern India, (ii) to overcome the problem regarding inadequate entomological evidence faced during repeated JE/AES outbreak investigation in different parts of India. The aim of this chapter is to describe the new mosquito surveillance tool used and methodology followed in ecological study on Mosquito vectors of Japanese encephalitis virus from endemic and non endemic areas of Northern India.

2.1 Introduction

Northern India comprising of National capital Delhi, Haryana and north western part of Uttar Pradesh (UP) constitute a transition semiarid zone between the arid Western India (Rajasthan) and humid belt of Eastern India (Bihar and West Bengal). Semiarid areas are characterised by high temperature (35–45 °C) during summer, hottest months (April–June) with precipitation less than potential evaporation. Almost all precipitation occurs as rains between July and September in these areas. Vegetation in semiarid zone is composed of stunted and scattered trees, shrubs, bushes and grasses.

2.2 Study Area

This study is based primarily on enormous field data accumulated by the author of this book (Bina Pani Das) on Japanese encephalitis (JE) vector bionomics from Northern India (Delhi, Haryana and Uttar Pradesh) at the national institute of communicable diseases (NICD), now known as national centre for disease control (NCDC), over a period of 9 years 1998–2006. Studies on JE vectors at NICD were broadly divided into three phases, viz.

2.2.1 Phase 1: Non-Endemic Area—In and Around Delhi (1998–2002)

As the existing sampling tools were not adequate for predominantly exophilic JE vector (*Culex tritaeniorhynchus*) in the area, a need-based simple technique “Bina Pani Das (BPD) hop cage Method” was developed (Das 2000, 2009) to monitor vector abundance round the year. The tool developed and standardised at NICD was later found to be useful in studying nearly every aspect of adult JE vector bionomics (Chaps. 4, 5).

Studies on larval bionomics of JE vector in study areas of Haryana, both at field as well as laboratory, resulted into the discovery of a new bio-control agent (*Chilodonella uncinata*, a ciliate parasite) for mosquito vectors of human diseases (Malaria, Dengue and Chikungunya, JE)—(Das 2003, 2008). In view of potential biolarvicidal activity of *Ch. uncinata*, the Inventor and the Author of this book (Bina Pani Das) was allowed to file National and International patent applications during in 2001, for which entire financial assistance was provided by Department of Biotechnology, Ministry of Science and Technology (Das 2004). International search report was of “A” category and so far patent has been granted by six countries including USA (Patent # 7141245, date. 28.11.2006).

Initially *Ch. uncinata*, ciliate parasite was discovered in wild caught JE vector larvae (Das 2003). Thereafter, these were isolated, purified, colonised and when formulation was prepared it was found to be more effective against larvae of urban malaria vector (Das 2008).

2.2.2 Phase 2: Endemic Area I—Karnal District, Haryana State of India (2002–2004)

BPD hop cage Method was field tested and its efficacy was compared with those of conventional sampling tools (Drop net and Hand catch Method) for sampling outdoor resting population of vectors of Japanese encephalitis virus (JEV) in selected study villages of district Karnal (JE endemic), Haryana state of India during 2002–2004 (Chap. 6 of this book).

2.2.3 Phase 3: JE Endemic Area II—Saharanpur District, Uttar Pradesh State of India (2005–2006)

A longitudinal study, on ecology of vectors of JEV, was carried out in Saharanpur District, a JE endemic area of Uttar Pradesh, northern India to develop a spacio-temporal strategy for the control/management of JE vectors (Chap. 7).

2.3 Methodology

2.3.1 *Metrological Data*

Data on climatic conditions of Delhi during study period was collected from Mousam Bhawan, New Delhi and that of Karnal District (Haryana) and Saharanpur District (Uttar Pradesh) were collected from the Soil Research Institute, Karnal and Army establishment, Saharanpur, respectively.

2.3.2 *Epidemiological Data*

Data on suspected cases and deaths due to Acute Encephalitis Syndrom (AES)/JE during the study period and that of recent past of Karnal and Saharanpur District were collected from District Heath Officer, Karnal and Saharanpur District, respectively. While, data related to country as a whole from 1978 onwards were obtained from national vector-borne disease programme (NVBDCP).

2.3.3 *Sampling of Adult Mosquitoes*

Adult mosquitoes were collected and categorised as: (1) Outdoor collection; Indoor collections: (2) Hand catch, (3) Total catch by pyrethrum space spray and (4) Dusk collection.

2.3.3.1 **Outdoor Collection by BPD Hop Cage Method**

Outdoor resting mosquitoes were collected from 09:00 to 13:00 h from vegetations like jowar, mustard, and berseem at monthly interval from each study village using BPD hop cage method.

Construction hop cage The hop cage is a standard mosquito cage, measuring $30 \times 30 \times 30$ cm, each side made up of thin iron rod of 6 mm in diameter. The metal frame is covered with muslin cloth on its five sides, with a long cotton sleeve on the sixth side and a narrow cotton sleeve outlet on the opposite side. The sleeve of the cage is folded (Fig. 2.1) to allow entry of mosquitoes while hopping the cage on land and aquatic vegetation.

Mosquito collection method In order to collect resting mosquitoes from land vegetation, folded mosquito cage was allowed to hop through the shady vegetation near the ground (Fig. 2.2) and also low-level ground vegetation by a series of quick forward, backward, up and down movements through a distance of 5 ft (length wise) for about 2 min (Das 2000, 2009). This led to the trapping of mosquitoes

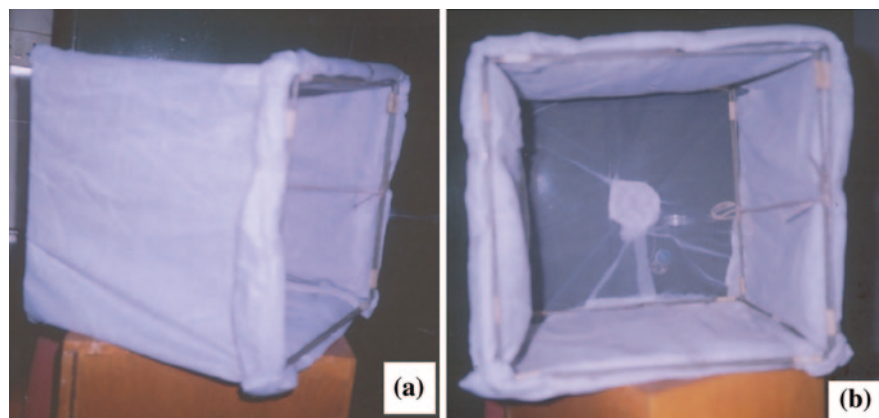


Fig. 2.1 Hop cage. **a** side view. **b** front view



Fig. 2.2 Collection of mosquitoes resting in secondary ground vegetation following BPD hop cage method [Adapted from Das (2009)]

in the cage. One side open sleeve of the cage is then immediately folded in order to prevent the trapped mosquitoes from escaping. The mosquitoes were retrieved from the narrow cotton sleeve outlet of the cage by a mouth aspirator tube. Such attempts are made at least ten times in one type of vegetation. For collecting mosquitoes from aquatic vegetation like hyacinth marshes same procedure was followed along the side of the water body (Fig. 2.3).

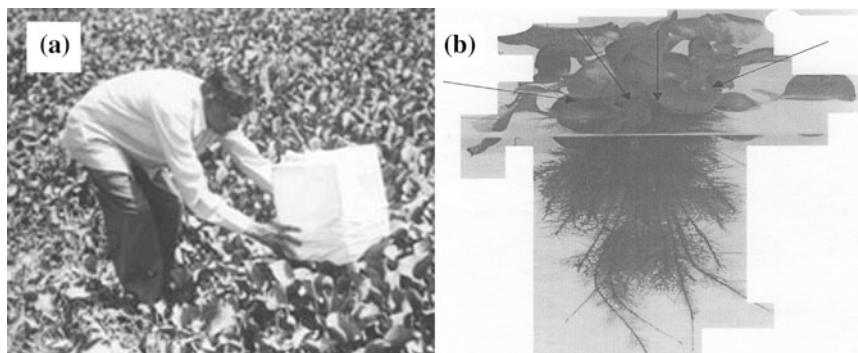


Fig. 2.3 **a** Collection of mosquitoes from aquatic vegetation (water hyacinth) using BPD hop cage method. **b** Arrows showing resting sites of mosquitoes among foliages of plant, just above the water level

Mosquito density measurement by hop cage Hop cage has to be used 10 times while collecting day-resting mosquitoes from land as well as aquatic vegetation and the mosquito density was measured as average number of female mosquitoes collected per ten hop cages (PTHC) by the following formula:

$$\text{Mosquito density (PTHC)} = \frac{\text{Total numbers of female mosquitoes collected}}{\text{Total numbers of hopping attempts made on vegetation}} \times 10$$

Each hop on vegetation covers an area of 1 sq. foot. The larger the area of the vegetation covered by hopping, the better representation of the mosquito density.

2.3.3.2 Indoor Collection

Indoor resting mosquitoes were collected from 06:00 to 08:00 h from human dwellings and cattle sheds in selected villages using Hand catch method spending 10 min per house. Mosquitoes were also collected during the day between 10:00 and 12:00 h from human dwelling (Total catch method) using pyrethrum space spray. Dusk collections were made around cattle shed and human dwelling by mouth aspirator tube and torchlight between 18:00 and 20:00 h. The abundance of mosquitoes in hand catch and in dusk collection was expressed as number of female mosquitoes collected per man hour (PMHD) and in Total catch as number of female mosquitoes collected per room density (PRD). Mosquito collections were transported to NICD/the respective field laboratories at Karnal and Saharanpur and sorted into sex, species using standard keys by Barraud (1934); Sirivanakarn (1976); Reuben et al. (1994), Das et al. (1990) and “Pictorial key to common species of *Culex* (*Culex*) mosquitoes associated with Japanese encephalitis virus in India”—Chap. 3 of this book. Abdominal condition of female mosquito species was noted and recorded.

2.3.4 Sampling of Mosquito Larvae

Larval collections were done by conventional dipping method. Fourth instar larvae were preserved using a simple standard method (Das 1986) and identified following Sirivanakarn (1976).

2.3.5 Mosquito Blood Meal Identification

Mosquitoes (*Culex tritaeniorhynchus*, *Cx. gelidus*, *Cx. bitaeniorhynchus*, *Cx. perplexus*) Leicester and *Aonopheles culicifacies* Giles collected from Saharanpur (July to October 2005) were processed at the National Institute of Malaria Research, Delhi for determining blood meal by enzyme linked immunosorbent assay (ELISA) following (Roy and Sharma 1987). The meal of fed mosquitoes was smeared on Whatman No. 3 filter paper by squeezing the stomach content of the mosquito, dried at room temperature and then kept in desiccator at 4 °C until use. For testing, the blood smears were eluted by cutting discs in microtitre plates containing 100 µl of 0.15 M Phosphate buffered saline (PBS), pH-7.2. The plates with eluates were kept at room temperature for 2 h followed by overnight at 4 °C. The elutes (~4 µl) were transferred by a clone-master template (Hyclone) from the wells onto a strip of nitrocellulose membrane (NCM) and dried at 37 °C for 10 min. After drying the NCM was washed thrice with 0.1 M Tris buffer (pH-7.5) containing 0.02 % Tween 20 (TBS-T). The NCM was then blocked with 1 % Bovine serum albumin (BSA) in TBS for 1 h at 37 °C. After blocking, the NCM was treated with anti-species globulin conjugated with peroxidase (Dakopatt, Denmark) at optimal dilution in PBS containing 1 % BSA. Finally, the reaction was visualised by enzyme specific substrate, 4-chloro-1-naphthol/H₂O₂ (SIGMA Aldrich). The reading of the coloured dots was denoted as +/++/+++ or ++++ comparing with reference positive and negative samples.

2.3.6 JE Virus Detection

Culicine mosquitoes were sorted into pools, each containing about 50 specimens according to species, sex, place and date of collection. These pools were kept as dry specimens at room temperature (25 ± 2 °C). Mosquito pools were transported without cold chain facility to Centre for Research in Medical Entomology, Madurai, Tamil Nadu for detection of JEV infection in vector mosquito species. These were processed using an antigen-capture ELISA (Gajanana et al. 1995, 1997). For this assay, monoclonal antibody (MAB) 6B4A-10 reactive against JEV and a polyclonal antibody broadly reactive, conjugated with peroxidase, SLE MAB, 6B6C-1 were used as capture antibody and detector antibody, respectively. The ELISA plate contained known positive (JEV infected suckling mouse brain homogenate) and

negative (homogenate of uninfected laboratory reared adult mosquito pools) controls. A mosquito pool was considered ELISA positive for virus antigen if its optical density (OD) value was greater than or equal to mean + standard deviation (SD) of the negative control (normal uninfected laboratory colony mosquitoes).

2.4 Pictorial Key for Identification of Common Species of *Culex* (*Culex*) Mosquitoes

A simplified key is prepared and incorporated in [Chap. 3](#) of this book for identification of four medically important genera and 17 common species of culicine mosquitoes, ten of which have been incriminated as vectors of JEV in India. This key is used to identify culicine mosquitoes collected in the present work from the study areas of Delhi, Haryana, Uttar Pradesh, Uttaranchal, Chhattisgarh and Andhra Pradesh.

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