Material and Methods
3.1. Study areas

3.1.1 Study area for spatio-temporal distribution of malaria

(i) Udalguri District

Udalguri district is situated between 91°42’-92°22’ E Longitude and 26°28’-26°56’ N Latitude and has 1852.16 km² of area comprising 3 primary health centres (PHCs) namely Khairabari, Udalguri and Orang. The district consists of 144 sub-centres (SCs) covering a population of about 0.8 million and has international border with Bhutan and state border with Arunachal Pradesh in the north (Fig. 3.1). The inhabitants are socio-economically backward and have mixed population of Bodos, Nepalese, Adivasis and Rabhas. The low literacy rate, poverty, reluctance to accept medical treatment, migratory mode of living, etc. are some important features of the villagers. The climate is sub-tropically humid with semi-dry hot summer and cold winter season. During summer season (May to Early September), heavy rainfall occurs and the district experiences flood every year. The annual rainfall, temperature and humidity of the area ranges from 1500- 2,000 mm, 13.5- 34.5 °C and 82-88% respectively which make the district conducive for mosquito proliferation.

(ii) Sonitpur District

Sonitpur district is situated in the northern part of the central Brahmaputra valley at 92° 16’ E to 93°43’ E longitude and 26°30’ N to 27°01’ N latitude (Fig. 3.1). The northern and southern boundaries of the district are covered by the foothills of Himalayas and river Brahmaputra. The district has approximately 5,324 km² of area and average altitude of about 70-75 meter mean sea level. The human population is 1.7 million (2001 census) with population density of 315 persons/ sq km. Different types of ethnic groups, like, Bodo, Nepali, Adivasi and Assamese, with very low socio economic condition, are living in the region. The district has a total of 1851 registered villages and a few non-registered forest
fringed villages formed due to resettlement activity (Source: Economic survey Assam, 2007-08). Average temperature during summer ranges from 32° C to 35° C and 15° C to 20° C in winter. Mean annual rainfall ranges from 1700 to 2200 mm and plays a major role in determining the climate of the region. Monsoon period starts in June and ends in September, however the rainfall starts from the early part of April. Many rivers coming from eastern Himalayas in the north flow over the plains of Sonitpur before ending in river Brahmaputra. Several forest reserves are located in the foothill region of the district, covering about 1417 km² of area (Source: Economic survey Assam, 2007-2008). The prevailing climatic conditions of Sonitpur district are conducive for breeding and proliferation of vector mosquitoes. The health infrastructure includes 6 Govt. hospitals, 8 PHCs, 11 dispensaries and 288 health SCs in order to extend health service to the people.

Fig. 3.1: Study area for spatio-temporal distribution of malaria.
3.1.2. Study area for mosquito distribution and insecticide resistance

(i) Tezpur: Four villages Udmari, Balitika, Paruwa and Rupkuria of Tezpur area around Solmara cantonment in Sonitpur district were selected for vector distribution and insecticide susceptibility survey (Fig. 3.2). The base map of the study area was prepared by digitization of Google earth images and Tezpur toposheet (1:50,000 scale).

![Fig. 3.2: Mosquito survey sites in Tezpur area.](image)

Table 3.1: Global Positioning System (GPS) locations of the study villages in Tezpur area.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Village</th>
<th>GPS location</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Udmari</td>
<td>92°46'43.03&quot; E 26°41'18.6&quot; N</td>
</tr>
<tr>
<td>2.</td>
<td>Balitika</td>
<td>92°47'36.2&quot; E 26°42'2.3&quot; N</td>
</tr>
<tr>
<td>3.</td>
<td>Paruwa</td>
<td>92°47'39.6&quot; E 26°40'35.3&quot; N</td>
</tr>
<tr>
<td>4.</td>
<td>Rupkuria</td>
<td>92°48'9.7&quot; E 26°41'31.8&quot; N</td>
</tr>
</tbody>
</table>
(ii) **Orang:** Mosquito survey and insecticide susceptibility studies were carried out in four villages in Orang area near the Orang National Park in Udalguri district (Fig. 3.3).

![Fig. 3.3: Mosquito survey sites in Orang area.](image)

**Table 3.2: GPS location of study sites in Orang area.**

<table>
<thead>
<tr>
<th>Survey site</th>
<th>GPS points</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>92°16'15.84&quot;E 26°38'23.75''N</td>
</tr>
<tr>
<td>2.</td>
<td>92°15'49.89&quot;E 26°41'45.38''N</td>
</tr>
<tr>
<td>3.</td>
<td>92°17'32.43&quot;E 26°41'51.99''N</td>
</tr>
<tr>
<td>4.</td>
<td>92°19'38.30&quot;E 26°41'55.29''N</td>
</tr>
</tbody>
</table>
3.1.3 Study area for socio-economic factors and malaria risk

Orang PHC (Fig. 3.4), situated between 92° 7’-92° 22'E Longitude to 26° 33’- 26° 56’N Latitude on the north bank of river Brahmaputra at 345 ft (MSL), is dominated by ethnic tribes of Bodo, Adivasi and Rabha. Low literacy rate, poverty and reluctance to accept medical treatment are the important features of the villagers. The climate is humid with semi-dry hot summer and cold winter. The annual rainfall, temperature and humidity of the area range from 1500 to 2000 mm, 13.5- 34.5°C and 82- 88% respectively. Study area has many small streams and covered with tea gardens. Rajiv Gandhi National Park with an area of 78.80 km² is located in the study area. From a climatic point of view, the study region is conducive for malaria epidemics.

Fig. 3.4: Socio-economic survey sites.
3.2. Methods of study

3.2.1. General methodology

3.2.2. Collection of data and application of geographical information system (GIS)

3.2.2.1. Pilot survey

Pilot survey was carried out to collect preliminary data and information about the study area needed for fieldwork. In the pilot survey the local health workers were contacted and the villages were visited to obtain the necessary information and pin-pointing the study areas.

3.2.2.2. Field work and data collection

Collection and screening of useful data for extracting communicable information from field is the important component in methodology. Comprehensive fieldwork has been done in many field visits during the study period to collect all related and necessary data to extract information and gather experience from the observation of ground reality. The steps followed for data collection and analysis:

(i) Secondary data collection

Secondary data of malaria epidemiology were collected from the state health offices located in the study area, Defence Research Laboratory (DRL), Tezpur and districts health offices located at district headquarter. References collected from the literature of previous work related to study were thoroughly consulted. All these collected information was used to create a good secondary data base.

(ii) Primary data collection

The accuracy and relevance of secondary data was verified by primary data which is essential for drawing right conclusion and communicating the right information. The primary data was collected through intensive field survey and questionnaire properly designed for the purpose.
(iii) Analysis of primary and secondary data

Based on the collected data, the high and low risk areas for malaria prevalence were identified and different features and factors favorable for spread of malaria were recognized and analyzed. All types of data were computed and analyzed with the computer operated software programmes.

3.2.2.3. Applications of GIS

GIS application has provided an excellent means for analyzing and visualizing data, revealing trends, dependencies and interrelationships existent among the complex variables which are not possible otherwise. We have used GIS in mapping the malaria epidemiology data, malaria vector distribution and their resistance status in selected areas. The steps carried out by GIS application are as follows:

(i) Digitization of data layers

Different data layers of the study area, like district boundaries, rivers, transport connectivity, location of health centres, distribution of malaria vectors etc. were digitization by using ArcGIS software, version 9.3.

(ii) Adding attributes

Different attributes of the layers were added to database for further analysis and thematic mapping.

(iii) Analysis and mapping

Different thematic representations in form of maps are made for meaningful analysis and interpretation. Thematic representations like sub-centre wise spatio-temporal mapping of malaria and extraction of high risk areas based on epidemiological data, vector distribution and insecticide resistance have been developed and presented in the form of visual maps for easy and quick retrieval.
3.3. Methods for spatio-temporal distribution of malaria in Udalguri and Sonitpur districts

3.3.1. Geographical Information System database

The mapping of sub-centres in Udalguri and Sonitpur districts was started with preparing a base map using ESRI® ArcMap™ 9.2 software (Redland, CA). Topological maps (1:50,000 scale) obtained from the Survey of India (Govt. of India) were used to extract the layers like district boundary, rivers and health centres. Location of sub-centres, mini primary health centres, community health centres and hospitals were recorded with the help of Garmin iQue® M5 GPS. Later the registered sub-centres were imported into ArcGIS environment. A team consisting of surveillance worker, malaria inspector, assistance malaria officer and district malaria officer helped in preparing jurisdiction of each sub-centre.

Epidemiological data of the district was collected from the Joint Director of Health office of Udalguri district which comprised information on population, blood slide examined, malaria positive cases, number of positive cases for *P. falciparum* etc. from 2006-2010. These data were later attached with the jurisdiction data of each concerned sub-centre and different maps were prepared with the help of GIS. Maps of parasitological indices such as Annual Parasite Incidence (API) which is number of blood slides positive per 1000 population in a year and *P. falciparum* (*Pf*) proportion (per cent positive slides for *P. falciparum*) were prepared using GIS database for each year. Based on the conditions *Pf >30%* and *API >5* consistently during 2006-08/09-10 sub-centres were depicted as malaria hotspots.

3.3.2. Conditions for sub-centres depicted as malaria hotspots

The following conditions were laid down for sub-centres to qualify as hotspots. Only the sub-centres which followed all the set conditions were depicted as malaria hotspots.

1. *Pf >30%* and *API >5* consistently during 2006-10.
Steps followed for above process were such as:

(i) Sub-centre wise layers for API (L₁, L₂, L₃, L₄, L₅) and Pf % (L₆, L₇, L₈, L₉, L₁₀) were created for 2006-10.

(ii) Sub-centres with >5 API cases were extracted from three layers L₁ to L₅ were integrated using Boolean operator “Intersection” to get layer L₁₁:

\[ \text{Layer } L_{11} = \bigcap \text{B}_5 \in \text{all } L_i \]
\[ i = 1, 2, 3, 4, 5 \]

Where B₅ stands for sub-centres having >5 API belonging to each layer L₁ to L₅

Thus layer L₁₁ = \{B₅ : B₅ ∈ each layer Lᵢ, i = 1, 2, 3, 4, 5 \}

(iii) Sub-centres with >30% Pf cases were extracted from three layers L₆ to L₁₀ were integrated using Boolean operator “Intersection” to get layer L₁₂:

\[ \text{Layer } L_{12} = \bigcap \text{B}_{30} \in \text{all } L_j \]
\[ j = 6, 7, 8, 9, 10 \]

Where B₃₀ stands for sub-centres having >30% Pf belonging to each layer L₆ to L₁₀

Thus layer L₁₂ = \{B₃₀ : B₃₀ ∈ each layer Lⱼ, j = 6, 7, 8, 9, 10 \}

(iv) Malaria hot spots were obtained by integrating layers L₁₁ and L₁₂ using Boolean operator “Intersection” to get layer L₁₃:

\[ \text{Layer } L_{13} = \bigcap \text{B}_{5}, \text{B}_{30} \in \text{all } L_k \]
\[ k = 11, 12 \]

Where B₅ stands for sub-centres having >5 API and B₃₀ for sub-centres having >30% Pf

Thus layer L₁₃ = \{B₅ : B₃₀ ∈ each layer, Lₖ, k = 11, 12 \}

3.4. Methods for mosquito distribution and insecticide resistance

3.4.1. Mosquito collections

Adult mosquitoes were collected using 6 volts battery operated centre for disease control (CDC) miniature light trap (John Hock, USA) hung at two meters from the ground level in human dwellings overnight and by aspiration using hand held aspirators (John Hock, USA) from the walls inside human dwellings from selected areas of the Udalguri and
Sonitpur districts. The CDC light traps were operated overnight (1800-0600 h) daily in the human huts, whereas using aspirators based hand collection, the mosquitoes were collected during the early morning hours (0400h-0700) from various human hut indoor resting places, such as walls, roofs, clothing and adjoining cattle sheds. The adults were identified following the standard mosquito identification keys and nomenclature (Nagpal et al., 2005; Reinert, 2001; Wattal and Kalra, 1961). The collected females were assayed for diagnostic doses of DDT (4%), deltamethrin (0.05%), malathion (5%) and lambda cyhalothrin (0.05%) using the standard WHO method.

3.4.2. Insecticide tested

(i) Pyrethroids: Pyrethroids are used for both IRS and treatment of LLINs in the form of alpha cypermethrin, bifenthrin (not recommended for use in LLINs), cyfluthrin, deltamethrin, permethrin, lambda cyhalothrin and etofenprox (WHO, 2006). In the present study lambda cyhalothrin and deltamethrin were used as the test compound. These have been the chemicals of choice in public health for the past few decades because of their relatively low toxicity to human, rapid knockdown effect and relative longevity (3-6 months when used for IRS). They are the only insecticides used currently in WHO recommended LLINs (WHO, 2006). Pyrethroids open sodium channels, leading to continuous nerve excitation, paralysis and death of the vector (Brown, 2006); they also have an irritant effect, causing an excito-repellency response, resulting in hyperactivity, immediate knockdown, feeding inhibition, shorter landing times and undirected flight, all of which are associated with reducing the biting ability of vectors.

(ii) Organochlorines: Organochlorines are used in IRS in the form of DDT, which was the insecticide used predominantly in the eradication campaigns of the 1950s (Curtis, 1996). At the Stockholm Convention on Persistent Organic Pollutants in 2001, use of DDT was banned for all applications except disease control, because of its environmental effects when used in
large volumes in agriculture. As the number of equally effective, efficient, alternative insecticides for public health is limited, continued use of DDT was permitted until "locally safe, effective, and affordable alternatives are available for a sustainable transition from DDT". Like pyrethroids, DDT has been popular because of its rapid knockdown effect, relative longevity (6–12 months when used for IRS) and low cost. Despite chemical structural differences, DDT and pyrethroids have similar modes of action (Brown, 2006).

(iii) Organophosphates: Organophosphates comprise a vast range of chemicals, but those recommended for use for IRS vector control are fenitrothion, malathion and pirimiphos-methyl (WHO, 2009). The insecticides in this class are highly effective but do not induce an excitorepellency response from the vector, and in their current formulations have shorter residual activity (2-3 months when used for IRS) than pyrethroids and DDT. Organophosphate insecticides prevent breakdown of the neurotransmitter acetylcholine, resulting in neuromuscular overstimulation and death of the vector. In the present study malathion was used as test insecticide.

3.4.3. Procedure for testing insecticide susceptibility in adult mosquitoes

The WHO tube test kit consists of two plastic tubes (125 mm in length, 44 mm in diameter), with each tube fitted at one end with a 16-mesh screen (Fig. 3.4). One tube (exposure tube) is marked with a red dot, the other (holding tube) with a green dot. The holding tube is screwed to a slide unit with a 20-mm hole into which an aspirator will fit for introducing mosquitoes into the holding tube. The exposure tube is then screwed to the other side of the slide unit. Sliding the partition in this unit opens an aperture between the tubes so that the mosquitoes can be gently blown into the exposure tube for starting treatment, in which the tube is vertical, and then blown back to the holding tube generally after one hour exposure time.
Fig. 3.5: WHO tubes and procedures for testing the susceptibility of adult mosquitoes to insecticides.

The filter papers are held in position against the walls of the tubes by four spring wire clips, two steel clips for attaching the plain paper to the walls of the holding tube and two copper clips for attaching the insecticidal paper inside the exposure tube. The only time that the exposure and holding tubes are connected is during transfer of the mosquitoes.
3.4.5 Bioassay

The bioassay was performed following standard WHO protocol and involved tarsal contact exposure to insecticide impregnated papers in the standard susceptibility test kit (WHO, 1963, 1992, 1998). DDT (4%), deltamethrin (0.05%), malathion (5%) and lambdacyhalothrin (0.05%) and silicone oil pre-impregnated papers were obtained from vector control research unit, Universiti Sains Malaysia, Malaysia. Mosquitoes were introduced into the exposure tubes containing insecticide impregnated papers for 1 h. The number of knockdown mosquitoes was monitored at every 10 minutes intervals to record the knockdown times (KDT). The tests were accompanied by control test where the mosquitoes were exposed to the papers treated with silicone oil only. After completion of the exposure time, mosquitoes were transformed into holding tubes and fed upon 5% sucrose solution. Mortalities were recorded 24 h post exposure and sensitivity status was graded as per the recommended criteria (Fig. 3.5; 3.6).
3.4.6 Data analysis and interpretation

Mortality in control between 5 and 20% was corrected with Abbott’s formula (Abbott, 1925) and expressed as corrected percent mortality.

\[
\text{Corrected } \% \text{ mortality} = \frac{\% \text{ Observed mortality} - \% \text{ Control mortality}}{100 - \% \text{ Control mortality}} \times 100
\]

Insecticide sensitivity status of mosquitoes was determined as follow (WHO, 1992; 2013b)
- Corrected mortality rates < 80% indicates resistant
- Corrected mortality rates > 98% indicates fully sensitive
- Corrected mortality ranging between > 80% to < 98% indicates suspected resistance that needs to be verified

Fifty and 99% knockdown times (KDT\textsubscript{50} and KDT\textsubscript{99}) were estimated using the Ldp line software.

3.5. Methods for socio-economic factors and malaria risk

3.5.1. Study design

A cross-sectional study was carried out during March to September 2012 in four randomly selected health sub-centres (SCs), namely Kadabil, Dhalanibasti, Gelabil and Bhogdol in Orang PHC, while ignoring the actual situation of malaria and assuming that all the SCs were homogen. During the surveys about 900 houses were visited to ascertain their willingness to participate in the study, thereafter a total of 71 house heads representing 385 individuals of households were interviewed in the present study. In the remaining houses the people were not ready to participate in the study due to one or other reasons. The head of households was the person who was perceived to be the primary decision maker in the family, whereas the households were the individuals living together in a house.

3.5.2. Data collection

Detail about the personal attributes, knowledge of malaria and socio-economic parameters were recorded in a well structured questionnaire, printed in English and Assamese
A local health worker, in the presence of community head recorded the responses of each individual interviewed. The specific variables recorded included; age, gender, education level, knowledge about malaria, house type, monthly income, other disease along with malaria, mosquito net use, type of mosquito net used (LLIN/ITN), frequency of bed net use and source of obtaining bed net, proximity of health centre and approximate number of mosquito bites per day. The knowledge about malaria was ascertained by taking verbal response to the following questions;

1. What is malaria?
2. How malaria is caused?
3. How malaria spreads?
4. How malaria can be avoided?
5. What is the treatment?
6. What government is doing about malaria?
7. Do you know that government provides bed net free of cost and antimalarial treatment is available free of cost in the government health centres?

The participants who responded well to all the questions were recorded having good knowledge, those who responded to at least any 5 questions were recorded as adequate, while those who could not answer more than 3 questions were recorded as having poor knowledge of malaria. In addition to recording the data, in-depth interviews and group discussions were done with the local people, who aggregate during the study to take an idea about the findings recorded in questionnaire sheet.

3.5.3. Statistical analysis

Association of the malaria with the gender, poverty, house type, knowledge, education, proximity to the health centre, use of bed nets, mosquito bites per day and other
awareness parameters were analyzed using chi-square test. Similarly, trend of malaria prevalence for poverty, proximity to the health centre and mosquito bites per day was determined using chi-square test. Relative risk (RR) for gender, house type, knowledge and use of bed nets was determined using Katz approximation. Whereas, odds ratios (OR) for the type of bed net use, knowledge about LLIN and supply of bed nets were determined using Wolf approximation.