Chapter 3: MATERIAL AND METHODS
The present study was undertaken to understand the epidemiological intricacies in the transmission dynamics of malaria and to identify the risk factors responsible for the enhanced receptivity and vulnerability to malaria in Goa during the past about two decades. This is a detailed epidemiological study carried out in both rural and urban areas of Goa from 2002 to 2007. A comparative analysis particularly of epidemiological data has also been done with the prior period from 1990 to 2001. Prior to the present study, small scale epidemiological studies have been carried out. The interrelationship of the different factors and parameters pertaining to the risk of malaria has been studied during the present investigation. The epidemiological and entomological data have been collated and new data generated so as to identify the key factors responsible for the enhanced receptivity and vulnerability and therefore sustained malaria transmission in the last decade in Goa with the aim to recommend suitable intervention strategies for control malaria. For this study, material used and methodology were as follows.

**Literature Search:**

The information and literature on malaria in Goa for the last one Century, of other malaria endemic States of India and other affected countries was collected from various sources listed below.

1. Archives of India, Goa.
2. Central Library, Goa.

5. Xavier Centre of Historical Research, Goa.


7. Goa University.

8. World Health Organisation reports.


10. Electronic data bases.

11. Print media/Local newspapers, etc.

The publications in Portuguese language were translated in English with the help of expert translators. Besides published work, reports on malaria situation in Goa and other states of India and malaria endemic countries of the world was collected from books, technical reports and other documents related to malaria. The scientific journals accessed were Indian Journal of Malariology, Journal of Communicable Diseases, Bulletin of Indian Society of Malaria and Communicable Diseases, Bulletin of Vector borne Diseases and Journal of Vector borne Diseases available in NIMR library and NVBDCP collection. Large numbers of foreign journals were also accessed from the internet and print outs of the relevant publications in free access journals were taken and used in the study. The published literature in both Indian and International journals were also collected from the National Institute of Malaria Library and collections.

Study area:

1. Physical Features
The vital information on Goa with respect to the physical maps (physiography), terrain features and soil type distribution was collected from the Directorate of Agriculture, Government of Goa. A series of maps of Goa were collected showing following features.

1. Goa Administrative map showing state and 'Taluka' Boundaries, Rail & Railway lines, Rivers and water bodies.
2. The physiographical features of different areas.
4. Rainfall zones in Goa.
5. The different soil types in Goa.
6. Soil suitability for various crops.
7. Present land use pattern with respect to different type of crops, forest, barren areas and built areas.
8. The soil available water capacity from very low to medium type.
9. Soil drainage from poorly drained to excessively drained soils.
10. Surface soil texture ranging from sandy to silt clay.
11. Land irritability in different areas of Goa.

These coloured maps were photocopied and computerised. A correlation between malaria prone areas and the different geographical features, soil types which are conducive for malarialogic conditions created and also due to the various developmental activities was studied.

2. Meteorological data

The Meteorological data for the state of Goa from 1980 to 2007 was collected from the Meteorological Observatory, Government of India, at Panaji.
The month wise data collected was on following meteorological parameters.

1. Temperature: maximum and minimum.
2. The percentage of relative humidity observed at 5.30 and 23.30 hours.
3. Month wise rainfall (mm) pattern for all years and number of rainy days/ month.

This data was computerized month and year wise. The climate data was analysed to study relationship of each weather parameter viz, Rainfall, Humidity and Temperature with epidemiological and entomological data to identify the key climatic factors influencing transmission of malaria.

3. Developmental Activities

A detailed survey of construction projects in the state of Goa, particularly in the capital city, Panaji was carried out. Panaji was chosen for the study being one of the major high risk areas and also because of the fact that it contributes to 50% or more to the malaria problem in Goa and here the transformation from traditional houses to multi storey complexes has completely changed the sky line of the city (Fig. 3.1 to 3.3).

4. Migrant Population

The district and ‘taluka’ wise distribution of the population of construction workers for North Goa and South Goa was collected from the Census Department at Panaji. The information on the migratory population i.e., persons engaged in construction/developmental activities in Goa was collected through KAP studies carried out in Panaji. The data was analysed according to state of origin and the age and sex of migrant population engaged in construction activities (Fig. 3.4).
Fig. 3.1 Showing traditional houses of Goa in the foreground and newly built multi storey structures in the background.

Fig. 3.2 Showing changed sky line of Panaji where extensive development has taken place since 1980s. Multi Storey buildings have replaced traditional houses.
Fig. 3.3 A typical Construction complex where water stagnations are responsible for breeding of Anopheles stephensi.

Fig. 3.4 Showing masonry tank under construction. Such water filled tanks are source for vector breeding.
Mosquito Population Surveys:

1. Geographical Reconnaissance of Breeding Habitats

A survey of the potential mosquito breeding habitats was carried out in Panaji area. Panaji has 15 wards. The habitats were surveyed in each ward and the information was recorded.

Various types of potential breeding habitats such as ground tanks, overhead tanks, underground tanks, sumps, wells, swimming pools, curing/stagnant waters, rain water collections, bottles, tyres, coconut shells, grinding stones, drums, barrels, containers, drains, chambers and other miscellaneous habitats were surveyed in each ward and the information was recorded. The survey of the number of wells present in each ward of Panaji was carried out.

Depending on the nature and type of breeding habitats recorded, the breeding habitats were categorized into permanent and temporary breeding sites. The temporary breeding habitats were further separated into project associated potential breeding habitats and rain associated intra/extradomestic temporary habitats. The preferential breeding places of the vector species were also noted and recorded, on the basis of which the data was segregated into Anophelines, Culicines and Aedine breeding habitats. The information on geographical reconnaissance of the breeding habitats carried out was recorded in designed proforma-3.1.
<table>
<thead>
<tr>
<th>SR. NO.</th>
<th>BUILDING NAME AND ADDRESS</th>
<th>BREEDING SITE</th>
<th>SURFACE AREA / DIAMETER</th>
<th>NUMBER ASSIGNED</th>
<th>CURRENT BREEDING STATUS</th>
<th>MEAN DENSITY/DIP</th>
<th>REMARKS</th>
</tr>
</thead>
</table>

**BREEDING SITE CODE:**
- WL = WELL
- OHT = OVERHEAD TANK
- UGT = UNDERGROUND TANK
- CON = CONSTRUCTION SITE
- CW = CURING WATER
- FT = FOUNTAIN
- SUM = SUMP
- COC = COCONUT SHELL
- BOT = BOTTLE
- BRL = BARREL
- POT = POT
- PON = POND
- GT = GROUND TANK
- CHM = CHAMBER
- SCR = SCRAP
- TYR = TYRE
- ACP = AC PLANT
- SPT = SEPTIC TANK
- FBK = FIRE BUCKET
- SLC = SLUICE VALVE CHAMBER
- DRN = DRAIN
- CTM = CONCRETE MIXER
- CNT = CONTAINER
- TWC = TERRACE WATER
- LC = LIFT COLUMN WATER
- DRN = DRAIN
- SWP = SWIMMING POOL
- POL = POLYTHENE SHEET
- STW = STAGNANT WATER
2. Larval sampling

Larval sampling was done weekly in Panaji, Margao, Candolim/Calangute, Bicholim, Ponda, Vasco/Cortalim, & Sanguem areas covering various types of breeding habitats in different ecotypes in both urban and rural areas. The breeding habitats surveyed for immature stages of mosquitoes were both permanent and temporary habitats (Fig. 3.4 to 3.12). Under the temporary habitats both rain associated and those not associated with rains were covered. Among permanent sites covered were sumps, cement tanks, plastic/iron/asbestos tanks, overhead tanks, swimming pools, fountains, wells, while the temporary sites covered were, curing waters, stagnant rain waters, iron/plastic barrels, tyres, buckets, bottles, grinding stones, coconut shells, containers and drains. Dippers and plastic containers/bowls of 300 ml capacity, pasteur pipettes, galvanized iron buckets of 5 litre capacity, plastic/enamel trays, netted cloth, cotton, rubber bands, labels, pen were used during larval sampling and for the labelling of containers.

The larval sampling of mosquito population was carried out in overhead tanks, sumps, fountains, masonry tanks etc with the help of 300 ml dippers. Plastic containers/bowls were used to draw samples from iron/plastic barrels. Pasteur pipettes were used to collect samples of immature from stagnant rain waters and curing water collections, narrow tyres and intradomestic containers. Galvanized iron bucket was used to sample larval stages of mosquitoes from the wells. The method used for collecting the larvae and pupae was as follows. The dipper was gently lowered into the water at an angle of about 45° until one side was just below
Fig. 3.5 Sampling of curing water in progress for mosquito immature stages which are brought to the laboratory and identified after the adults have emerged.

Fig. 3.6 Bore wells are a common site at the construction sites. They support breeding of mosquitoes especially the vector *An* *stephensi*. 
Fig. 3.7 Larval samples are being collected from an Over Head tank.

Fig. 3.8 Wells are perennially suitable for vector breeding if devoid of larvivorous fishes.
Fig. 3.9 Sampling of immature stages in progress in masonry tank retained after construction work has been completed.

Fig. 3.10 Masonry tank being sampled at an active construction site.
Fig. 3.11 Swimming pool samples being carefully examined for smaller instars of mosquitoes.

Fig. 3.12 Ornamental tank being sampled for mosquito immatures.
the surface so that water was drawn in along with the larvae and pupae. Care was exercised not to disturb water surface much so that immature stages of mosquitoes were least disturbed during the sampling. In this manner, five samples were drawn from each well, fountains, overhead tanks, masonary tanks, etc. 4 samples were drawn from the corners/sides and one from the centre.

The immature stages of mosquitoes i.e. larvae and pupae found in the various habitats were transferred in the plastic containers along with water. The containers were individually labelled showing date, place and type of breeding habitat. The containers were placed in trays and brought to the laboratory. The mouths of the containers were covered with a netted cloth to provide atmospheric oxygen to the immature stages during transportation. Care was taken to avoid jerks to the water in the containers to prevent injury and mortality to the immatures while transportation especially of the 1st and 2nd instar larvae.

In the laboratory, the mosquito immature stages were transferred in to the enamel or plastic trays of 5" width x 10" Length x 3" height containing tap water. The immatures were reared in the insectary at room temperature (25-30°C). A pinch of food (Cerelac™ powder) was provided to the growing larvae once daily till the development of pupal stage. Dead larvae were pipetted out and discarded on daily basis. Larvae and pupae were shifted to fresh water every 3rd day.
### PROFORMA - 3.2

**LARVAL SURVEYS OF MOSQUITOES IN GOA**

**DATE:**

**PHC/UHC:**

**VILLAGE/WARD:**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Breeding site</th>
<th>Dips</th>
<th>Anopheles</th>
<th>Culex</th>
<th>Aedes</th>
<th>Sample No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>I+II</td>
<td>III+IV</td>
<td>P</td>
<td>T</td>
</tr>
<tr>
<td>D1</td>
<td></td>
<td></td>
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<td></td>
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<td>D2</td>
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<td>D3</td>
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<td>D4</td>
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<td>D5</td>
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<tr>
<td><strong>Total</strong></td>
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<td>D1</td>
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<td>D2</td>
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<td>D3</td>
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<td>D5</td>
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</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

D1=Dip one, P=Pupa, T=Total number of larvae, I+II (1st & 2nd Instar larvae), III+IV (3rd & 4th Instar larvae)
The pupae were transferred into plastic bowls containing tap water and covered with a piece of nylon net having a hole in the centre and plugged with cotton until the emergence of adults. This hole was kept plugged with cotton to prevent the emerged adult mosquitoes from escaping. The adult mosquitoes after emergence were aspirated and identified using the standard keys of Christophers (1933); Barraud (1934) and Puri (1954).

After every collection, the containers, bowls, dippers, pipettes and trays used for larval collections were washed and cleaned thoroughly with soap solution. The results of the emerged adults from various samples were recorded in a designed proforma 3.2.

3. Adult mosquito collections

1. Adult collection with Mosquito Trap (Mosquito Magnet™)

The collection of adult mosquitoes was carried out in the problematic and non-problematic wards in Panaji city. A Mosquito trapping machine, LPG cylinder (15 kg), Octenol cartridge, collection bag, plastic vials, silica gel, zipper bags, marker pens were used. PRO model of Mosquito Magnet™ trap (MM™-Trap) was used for the sampling of adult mosquitoes. This trap has been developed by the American Biophysics Corporation, North Kingstown, Rhode Island, USA and is commercially available in India under the trade name Mosquito Magnet™ (Fig. 3.13a). The mosquito magnet was run on 15 kg liquefied petroleum gas (LPG) cylinder.

Functionally, the MM™-Trap mimics human breath and uses a counter flow technology, which enables it to emit a plume of carbon dioxide, heat and moisture, while the Octenol cartridge acts as a mosquito attractant. A small fan in the machine blows down the mosquitoes into a collecting bag and traps
them. In each locality, this trap was operated up to 9 times during the period of collection. The device was run round the clock to trap both nocturnal and diurnal adult mosquitoes in a removable bag.

![Mosquito Magnet Trap (Pro Model)](image)

**Fig. 3.13a Mosquito Magnet Trap (Pro Model) used for the Mosquito Sampling**

The 24 hours collection of adult mosquitoes was carried out in four localities (wards) of Panaji viz., Market area, Boca-de-Vaca, Miramar and Caranzalem. On each day of collection, the insects trapped were removed and put into labelled zipper bags, once at 10:00 hrs and then at 17:00 hrs and transported to the insectary. The mosquitoes were segregated from other insects and identified using the keys of Christopher's (1933); Barraud
(1934) and Puri (1954). The data was recorded locality and species wise in
designed proforma 3.3.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Species</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>An. stephensi</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>2</td>
<td>An. subpictus</td>
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</tr>
<tr>
<td>3</td>
<td>An. vagus</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>4</td>
<td>An. jamesi</td>
<td></td>
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<tr>
<td>5</td>
<td>An. tesellatus</td>
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<tr>
<td>6</td>
<td>An. peditaeniatus</td>
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<tr>
<td>7</td>
<td>An. pseudojamesi</td>
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<tr>
<td>8</td>
<td>An. subpictus</td>
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<tr>
<td>9</td>
<td>An. barbirostris</td>
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<tr>
<td>10</td>
<td>An. nigerrimus</td>
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<tr>
<td>11</td>
<td>Ae. aegypti</td>
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<tr>
<td>12</td>
<td>Ae. albopictus</td>
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<tr>
<td>13</td>
<td>Ae. vittatus</td>
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<tr>
<td>14</td>
<td>Cx. quinquefaciatus</td>
<td></td>
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</tr>
<tr>
<td>15</td>
<td>Cx. vishnui</td>
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<tr>
<td>16</td>
<td>Cx. tritaeniorynchus</td>
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<tr>
<td>17</td>
<td>Cx. bitaeniorynchus</td>
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<tr>
<td>18</td>
<td>Cx. gelidus</td>
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<tr>
<td>19</td>
<td>Cx. lutzia fuscanus</td>
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<tr>
<td>20</td>
<td>Cx. pseudovishnui</td>
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</tr>
<tr>
<td>21</td>
<td>Mansonia uniformis</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>22</td>
<td>Armegeres subalbatus</td>
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</tr>
</tbody>
</table>
2. **Collection of Landing Mosquitoes**

Biting rhythm of mosquitoes was studied by capturing landing of mosquitoes on human baits from 18.00 to 06.00 hours. The study was conducted in 3 ecotypes viz., coastal, sub-coastal and hilly terrains covering both urban and rural areas of Goa (Fig. 3.14 to 3.17).

A preliminary survey was conducted to identify and select the villages/construction site areas for all night bait collections in both rural and urban areas of Goa. The inhabitants of the selected houses/ and construction sites were informed about the purpose of mosquito collections.

For the collection of adult landing mosquitoes during all night collections, a canvas handbag, sucking tube or aspirator, test tubes, cotton, pocket stop watch, rubber bands, torch (with spare bulb and batteries), mosquito forceps, test tube stand, plastic vials, silica gel, zipper bags, marker pens, note book, pen, were used.

Aspirator/Sucking tube used for capturing adult mosquitoes consisted of a plastic tube (approximately 15 inches long) attached to a flexible rubber tubing (about 20 inches long) and a small plastic or glass mouth piece (Fig 3.18). A fine wire mesh / gauze separated the plastic and rubber tubes to prevent mosquitoes from entering into the mouth while collecting.

For the sampling of adult mosquitoes, two mosquito collectors and a human volunteer (bait) were involved. The human volunteer participating in the all night collections was explained about his role as bait for capturing landing mosquitoes on his body which was needed to study the biting
Fig. 3.13 Migrant construction workers live in the vicinity of the construction site where vector breeding takes place.

Fig. 3.14 All Night collections of mosquitoes on bait in progress at a construction site.
Fig. 3.15 All Night collections of mosquitoes on a bait is in progress in a rural area of Goa.

Fig. 3.16 All Night collections of mosquitoes on a bait is in progress in an urban area of Goa.
behaviour of mosquitoes from dusk to dawn. Informed consent was taken from the potential bait/volunteer (Form No. 3.4).

Fig. 3.18 An Aspirator (Suction tube) with the help of which hand catch of mosquitoes was done.

Only after voluntary consent was obtained, the human bait and mosquito collectors were made to participate in the collections. The necessary ethical approval was taken for conducting the adult mosquito collections on human volunteers from the ethics Committee of National Institute of Malaria Research, Delhi. The human volunteers and mosquito collectors were administered prophylactic doses of antimalarial, chloroquine @ 300mg stat (2 tablets of 150 mg ai) weekly until one week after the conclusion of the study. It may be mentioned that none of the volunteers and mosquito collectors suffered from malaria during the course of the study.
The person acting as a bait was made to expose his limbs i.e., hands up to the forearms and legs up to the knees. He was made to lie on a cot. The mosquitoes were collected by hand catch method using aspirator and torch following the standard procedures in the selected areas. With the mouth piece in the mouth, the suction tube was held by the collector with its opening about 1cm away from the mosquito. The open end of the sucking tube was moved closer to the mosquito and at the same time sucked quickly and gently so as to draw the mosquito into the tube. The captured mosquito was prevented from escaping by immediately covering the suction end with the thumb.

The mosquito was transferred into a clean test tube 150 mm long with 16 mm dia, by gently blowing it into the tube. The test tube was then plugged with cotton wool and placed in the test tube stand. Each test tube was labelled individually showing the hour, place and date of collection. Two to three test tubes were used for collecting mosquitoes during each hour of collection. On an average, 4 to 8 all night collections were done in each month.

Next day morning, the mosquitoes were transported to the laboratory. The live mosquitoes in each test tube were anaesthetized to facilitate identification of mosquitoes by putting 1-2 drops of ether on the cotton plug. WILD dissecting binocular microscope was used to magnify and identify the mosquitoes based on morphological features up to species level using the keys of Christopher's (1933), Barraud (1934) and Puri (1954).

Following precautions were taken during the mosquito collections.
1. Care was taken not to suck or blow too hard, as mosquitoes being fragile could easily lose legs or damaged and lose their characters which would hamper their identification.

2. Care was taken to prevent breakage of test tubes during transportation.

3. After every night collection, the test tubes were washed, cleaned and kept dry before re-use. This was essential to keep the test tubes free from dirt.

4. The oil or ointment that may act as mosquito repellent and smoking was strictly prohibited during the all night collections.

5. Same bait was used throughout the collection duration.

6. The data was recorded locality and species wise in designed proforma 3.5.

3. Sporozoite ELISA Test:
The collected Anopheline female mosquitoes with the help of Mosquito Magnet™ and during all night collections and after their identification were dried and stored individually in plastic vials containing dried silica gel under cold conditions (0-4°C). The head and thoraces of An. stephensi females were tested by sporozoite ELISA method (Burkot et al., 1984) using antibodies to circumsporozoite proteins of *Plasmodium falciparum*, *Plasmodium vivax*-210 and *P. vivax*-247. End point results were read visually and confirmed at 450 nm using a Vmax kinetic microplate reader manufactured by Molecular Devices Corporation (Sunnyvale, CA, USA).
Project Title: An Epidemiological study on risk factors responsible for the enhanced receptivity and vulnerability to malaria in Goa- Ph.D. research work being pursued in NIMR (ICMR), Field station, Goa.

Information Sheet: Introduction
We are doing a research study on risk factors that help in the increase and spread of malaria in Goa. I am going to give you detailed information about this study and invite you to participate as a volunteer in this study. Before you decide about it, you can talk to any one you feel comfortable with. There may be some words that you may not understand. You may stop me and ask question if you desire as we go through the information and I will explain it to you. If you have questions later, you can ask me whenever you wish to.

You may be aware that Malaria is one of the common and dangerous diseases in Goa. Malaria is spread by a certain variety of mosquitoes. Generally such mosquitoes bite humans at different hours of the night. The purpose of this study is to find out when the mosquitoes that spread malaria in Goa attempt to bite us and in how many numbers. This information will be useful for finding out a solution for prevention of such bites and malaria.

In this study we need some volunteers and insect collectors who are trained in the collection of mosquitoes that may land and attempt to bite.

As a volunteer you will be first provided with a preventive (prophylactic dose) of chloroquine (300mg base) as per the guidelines of the National Vector borne Diseases Control Programme. This will be repeated every week till one week after the end of the study.

As a volunteer you will act as bait for mosquitoes and expose only the limbs i.e. hands and legs up to the knees to attract mosquitoes. Any mosquito that lands on these parts will be promptly sucked in the suction tube by the Insect Collector. This collection will be done from 18.00 to 0.00 hours on each collection night. This may cause some annoyance and irritation to you. However utmost care will be taken to promptly collected mosquitoes to avoid the biting. The collected mosquitoes will be kept separately hour wise in labelled test tubes and brought next morning to the laboratory for identification.

As a volunteer you will not be provided with any incentive to take part in the study. However for the transportation you will be reimbursed with not more than Rs. 120/- per night. During the course of the study we will follow you closely and keep track of any event during the study to prevent the risk of malaria to you.

The knowledge we get from this study will be shared with you at any time you desire. Confidential information will not be shared with any one and your name shall not be disclosed. Afterwards, we will use the results of the study in the Ph.D. thesis and publish them in the scientific journals in order that other people may learn and benefit from our study.

You do not have to take part to agree to take part in this epidemiological study if you do not wish to do so and may refuse to take part in the study. Even during the course of the study you may withdraw without assigning any reason why you have consented to take part in the study. This will not affect you in any way.

If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact any of us.

Mrs. Nandini S. Korgaonkar, NVBDCP Panaji Goa Contact Telephone No:0832-2225837
Dr. Ashwani Kumar, NIMR, FS, Panaji, Goa Contact Telephone No. 0832-2222444

Certificate of Consent
I have been invited to participate in a research study on risk factors that help in the increase and spread of malaria in Goa. I understand that it will involve me as a volunteer to attract mosquitoes. I have been informed about the risks and the protective dose of chloroquine medicine that will be given to me to prevent malaria at weekly interval and I undertake to consume the same without fail. I am aware that there may be no benefit to either myself and that I will not be compensated beyond travel expenses. I have been provided with the name of investigators who can be easily contacted using the numbers.

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I hereby consent voluntarily to participate in this study and understand that I have the right to withdraw from the study at any time without assigning reason for the same.

Name of Participant: ___________________________
Signature of Participant/Thumb Impression: ____________
Date: ________ day/month/year

Name of witness: ___________________________
Signature of witness: ____________
Date: ________ day/month/year

Name of investigator: ___________________________
Signature of investigator: ____________
Date: ________ day/month/year

A copy of this Informed Consent Form has been provided to participant.
## PROFORMA-3.5
All night Landing Mosquito Collections on Human baits in Goa

<table>
<thead>
<tr>
<th>PHC/UHC:</th>
<th>VILLAGE/WARD:</th>
<th>COLLECTION LOCATION:</th>
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<table>
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<tr>
<th>Date</th>
<th>Species</th>
<th>♂-Male mosquito</th>
<th>♀-Female mosquito</th>
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</table>

♀-Male mosquito
♂-Female mosquito
Epidemiological Data Collection and Analysis:

Malariometric Indices

The various levels of endemicity of malaria were ascertained with the help of standard malariometric indices, surveillance indices and entomological indices. The following indices were worked out as a measure of malaria endemicity during the course of the present study.

i). Parasitological Indices

1. Annual Blood Examination Rate (ABER): It is expressed as the proportion of blood slides examined for malaria in a human population in a year.

It was calculated as follows.

\[
ABER = \frac{\text{No. of blood slides examined in a year}}{\text{Total Population covered}} \times 100
\]

2. Annual Parasite Incidence (API): It is expressed as the number of malaria positive cases in a particular year in a particular place per thousand populations and was calculated as follows.

\[
API = \frac{\text{Total No. of malaria positive cases in a year}}{\text{Population of the area}} \times 1000
\]

3. Monthly Parasite Incidence (MPI): It is expressed as the number of malaria positive cases in a particular month in a particular place per thousand populations.

\[
MPI = \frac{\text{No. of malaria positive cases in a month}}{\text{Total population of that area}} \times 1000
\]
4. **Annual Vivax Incidence (AVI):** It was expressed as the number of *Plasmodium vivax* positive cases in a particular year in a particular place per thousand populations and was calculated as follows.

\[
\text{AVI} = \frac{\text{Total No. of } P. \text{ vivax positive cases in a year}}{\text{Population of the area}} \times 1000
\]

5. **Annual Falciparum Incidence (AFI):** It was expressed as the number of *Plasmodium falciparum* positive cases in a particular year in a particular place per thousand population and was calculated as follows.

\[
\text{AFI} = \frac{\text{Total No. of } P. \text{ falciparum positive cases in a year}}{\text{Population of the area}} \times 1000
\]

6. **Slide Positivity Rate (SPR):** It was expressed as the proportion of positive slides out of those examined for malaria.

\[
\text{SPR} = \frac{\text{No. of slides positive for malaria}}{\text{Total No. of blood slides examined}} \times 100
\]

7. **Slide Vivax Rate (SVR):** It is the proportion of slides showing *Plasmodium vivax* infection out of the total slides examined for malaria.

\[
\text{SVR} = \frac{\text{No. of slides with } P. \text{ vivax infection}}{\text{Total No. of slides examined}} \times 100
\]

8. **Slide Falciparum Rate (SfR):** It is the proportion of slides showing *Plasmodium falciparum* infection out of the total blood slides examined for malaria.

\[
\text{SfR} = \frac{\text{No. of slides with } P. \text{ falciparum infection}}{\text{Total No. of slides examined}} \times 100
\]
9. **Plasmodium falciparum Proportion (Pf %)**: It is expressed as the proportion of slides showing *Plasmodium falciparum* infection out of the total positive slides with malaria infection.

\[
Pf\% = \frac{\text{Total No. of } P. \text{ falciparum cases}}{\text{Total No. of slides positive for malaria}} \times 100
\]

**ii). Entomological indices**

Similar to parasitological indices, maliariometric indices related to mosquitoes used were the following.

1. **Per Night Per Trap Density (PNPTD)**: It is expressed as the number of mosquitoes collected during the whole night (from sunset to sunrise) with the help of a trap. It can be expressed for the total mosquitoes collected or per species.

\[
\text{PNPTD} = \frac{\text{Total no. of mosquitoes collected during all the night}}{\text{Total no. of nights of collection}}
\]

2. **Larval Density**: It is expressed as the average number of larvae collected with the help of a dipper per dip.

\[
\text{Mean per dip density} = \frac{\text{Total Number of larvae collected in all the dips}}{\text{Number of dips}}
\]

**Malaria Data of Goa: 1963 to 2007**

The malaria incidence data of Goa state was collected from the State National Vector borne Disease Control Programme of the Directorate of Health Services, Government of Goa from the year 1963 till 2007. The year wise data for the above years collected was as follows:

1. Blood slides collected and examined.
2. Total number of blood slides found positive.
3. Total number of blood slides found positive for *P. vivax*.
4. Total number of blood slides found positive for *P. falciparum*.

From the above data, the following malarriometric indices were worked out.

1. *Plasmodium falciparum* percentage (Pf %).
2. *Plasmodium vivax* percentage (Pv %)
3. Slide Positivity rate (SPR)
4. Slide *falciparum* rate (SfR)
5. Slide *vivax* rate (SvR)
6. Annual Blood Examination Rate (ABER)
7. Annual *falciparum* Incidence (AFI)
8. Annual *vivax* Incidence (AVI)
9. Annual Parasite Incidence (API)

The year wise population data of the state was collected from the Health Intelligence Bureau (HIB) of the Directorate of Health Services. The mid year population figures were calculated based on Census of India, 1991 and 2001. The population figures were used for calculating ABER and API of different years.

**Seasonal Distribution of Malaria**

The month wise data of malaria morbidity of the state of Goa was tabulated and analysed from the year 1990 to 2001 and 2002 to 2007 and various malarriometric indices mentioned above were worked out. The data for all the above years was further analyzed according to different seasons of the year i.e. pre-monsoons, monsoons and the post monsoons.
Stratification of Goa based on malaria endemicity:

1. Malaria Data of Goa: District and PHC/UHC wise

The data of each district, Primary Health Centre (PHC)/Urban Health Centre (UHC) on malaria morbidity was tabulated from the year 1990 to 2001 and 2002 till 2007. Based on the population data of each PHC, the total blood slides collected and examined and the number of slides positive for *P. vivax* and *P. falciparum*, various maleriometric indices such as SPR, SFR, Pf% and API were worked out for each year for all the PHCs.

2. Stratification of Goa based on Malaria Endemicity

Based on the SPR, ABER and API calculated from 1990 to 2007 for each PHC/UHC and CHC of Goa, a colour scheme for stratification was developed and different colours were assigned to different levels of ABER, SPR and API for visual separation of PHCs according to their malaria endemicity during the above period.

3. Micro Stratification of Panaji based on Malaria endemicity

The area wise data of malaria was tabulated and analyzed from January to December 2004. For each month and all localities of the city, SPR, Pv% and Pf% was worked out. The data was also segregated according to the method of malaria surveillance i.e. Active Case Detection (ACD) and Passive Case Detection case (PCD). A coloured map of Panaji was prepared to denote municipal localities according to different malaria endemicity for prioritising malaria control.
4. Age and Sex Distribution of Malaria Cases in Panaji

Malaria cases were assigned to the following age groups of both sexes to study age and gender distribution of malaria incidence in Panaji in the year 2004. The age groups were < 1 year, 1 – 4 years, 4 – 8 years, 9 – 14 years, 15 – 20 years, 21 – 30 years, 31 – 40 years, 41 – 50 years, and > 50 years and malariometric indices were worked out for each of the above age groups for both the genders.

5. Demographic Distribution of Malaria in Panaji

The malaria incidence of Panaji for the year 2004 was analysed according to three demographic groups viz., local, labour and hotel/restaurant workers and the malariometric indices were worked out separately for each of these groups.

Identification of Malaria Risk Factors:

The malaria risk factors were identified based on relationship between malaria incidence and various parameters i.e. physical features, meteorological factors, developmental activities, migratory population, vector prevalence and the epidemic outbreak potential in the PHC/UHC and CHC responsible for receptivity and vulnerability to malaria transmission.

Malaria Control Strategy for Goa:

Based on the stratification of the PHC/UHC and CHCs into malaria endemic zones, seasonal trends of malaria, demographic distribution of malaria, vector prevalence, breeding ecology, previously conducted and published studies, a simple and specific malaria control strategy has been proposed for the state of Goa.