



Review Article

Entomological Sampling Strategy, Sampling Tools and Analytical Techniques with regard to Vector-Borne Diseases: Indian Perspective

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A B S T R A C T

Entomological surveillance is an integral component of surveillance activities for any vector-borne disease. The vector surveillance in a given area shall depend on the laid down objective and keeping in view of the expected outcome. The sampling strategy for vector surveillance shall depend on routine entomological surveillance, outbreak situation and insecticide resistance monitoring. The selection of any sampling strategy and technique should always be kept in mind for its appropriate application depending on the target species. The sampling strategy shall imply that vector surveillance teams are supposed to select different options depending on the short or long term study at hand. The present ongoing operational vector sampling methods usually lack standardisation, quantitative comparisons across different situations in case of outbreak situations and one time measurements, and it makes it difficult to assess the real situation. The sampling strategy should always meet the requirement of the laid objectives to fulfil the desired outcome. The present document is an attempt to act as a guiding principle for use as appropriate sampling strategy for any vector-borne diseases in the country.

Keywords: VBDs, Sampling, Sampling Size, CCHF, KFD, CDC Light Trap

Introduction

In India, malaria, filaria, dengue, chikungunya, zika, japanese encephalitis, and visceral leishmaniasis are posing a major public health problem, while Kaynasur forest disease (KFD), crimean congo haemorrhagic Fever (CCHF) and Scrub Typhus are new emerging public health threats in different geographical settings. Though, entomological surveillance has become very extinct due to the poor infrastructure of entomologists in the country, there is a need to standardise various tools for vector surveillance (adult and larval) for vector-borne diseases (VBDs) based on the area-specific

needs and endemicity of VBDs. The surveillance tools to be selected and applied in the field situations are always based on whether one is going for larval or adult vector sampling. One important aspect of preparedness for vector sampling for vector-borne diseases is to understand the pre-requisite of surveillance tools, methodology, and entomological indicators.

Any effective/ efficient vector surveillance plan or strategy shall always depend on the development of a well-considered sampling strategy for a particular vector species. The selection of the sampling strategy and technique should



always be kept in mind depending on the target species. This implies that vector surveillance teams select different options depending on the short or long term study at hand. The operational vector sampling methods usually lack standardisation, quantitative comparisons across different situations in case of outbreak situations and one time measurements, and it makes it difficult to assess the real situation. The sampling strategy should always meet the requirement of the laid objectives to fulfil the desired outcome.

Some documents are available on the subject with regard to the global perspective for the sampling tools and techniques.¹⁻⁴ However, little reference is given with regard to appropriate sampling procedure and technique during outbreaks/ epidemics for the vectors transmitting VBDs in a given situation. Entomological surveillance may be applied in field situations to collect the spot prevalence and distribution of vector species. The outcome would help to understand different compositions of vector species over time and space in different eco-settings. The WHO Global Vector Control Response further emphasises the need for effective, locally adapted, and sustainable vector control based on increased capacity and enhanced entomological surveillance.^{5,6} Further guidance on entomological surveillance is provided in the WHO Malaria Surveillance, Monitoring and Evaluation: a reference manual that includes entomological surveillance requirements at different levels of malaria transmission.^{7,8}

There is sporadic material available in the literature searched on specific vector sampling strategies with regard to the recent prevalence and distribution of vector species in the Indian context. The data on vector surveillance, its distribution and biology is quite old and is limited to only short terms and periodic vector collections without following any standardised sampling techniques.

An attempt has been made in this manuscript to develop a standard vector sampling strategy for each vector species for an expected outcome. This sampling strategy shall be a way forward to describe a uniform sampling surveillance method to collect vector species. This protocol may help field entomologists to detail the point prevalence and distribution of vector species in different ecological niches, identification, and quality control. The sampling strategy may be planned based on the biology of the vector species and targeted for surveillance for its adult or larval sampling (surveillance).

A sampling strategy should be made as per area-specific objectives in mind and should be planned in such a way that the sample to be used on vector species is representative of the population/ area. It would be more appropriate to select the entomological tool as the laid objectives of the study planned if it is short term or long term keeping aligned with the sampling strategy.

Sampling is simply selecting a portion of the vector population in a refined area, which will be a representation of the whole problem. The sampling strategy may be the planning, one needs to set forth to be sure that the sample encountered represents the vector population from which the samples are drawn.

Entomological sampling methods take advantage of specific vector behaviours, and each method has its own biases, advantages, and disadvantages. Selecting the appropriate sampling method and its placement (location and time) is critical to collecting relevant and accurate data. For example, a human-baited trap (e.g., CDC light trap hung near a human) placed inside houses may function very well with only indoor biting (endophagic) and anthropophagic (human-biting) mosquitoes, and sampling will thus not be representative of vectors that are more exophagic (outdoor-biting) or prefer feeding on animals (zoophagic). In other words, that sampling will be biased toward indoor biting, human host-seeking vectors. Also, each method functions differently with local vector species, their bionomics, and the local environment, hence validating sampling methods locally prior to widespread use is critical. For example, a method that works in one vector for an area may not work in another area for other vector species due to local vector behavioural differences. Once vectors have been sampled in the field, they are usually brought to the laboratory for analysis. Indicators including vector occurrence; sporozoite rate; frequency, intensity, and mechanism of insecticide resistance; human blood index; and bio-efficacy of insecticide, among other indicators, all require analysis with standardised entomological techniques.

Entomological Sampling Strategy

The entomological sampling strategy is comprised of the following steps for getting the desired outcome based on the objective in place.

Step 1: Situational Analysis to define Sample and Target Population

The epidemiological information for 3-5 years would be required to identify the hot spots to establish the endemicity of the vector-borne disease. Analysis may be done village wise/ PHC wise for the district to understand the clustering of old/ new cases. Epidemiological indices may also be taken into consideration at the micro-levels. At least 50 households would be required for each index case reported recently.

Step 2: Mapping of Breeding Potential Areas and defining the Sample Size

It is essential to enlist the potential breeding habitats of vector species in the defined area to be taken as the sample size. The number and area of sampling need to be based on the objective and necessary degree of precision for the

expected outcome. The degree of accuracy to achieve the desired goal would depend on the appropriate sample size with right sampling intervals in the study areas having confidence in the representation of the vector species in that area.

Step 3: Fixed and Random Site Selection

After deciding the sample size for undertaking vector surveys, the sampling tools and technique are to be selected keeping in view of the objective of the study. The sampling tools may be chosen based on the need for fixed and random sites.

Fixed Sampling: At least four representative areas of the population may be selected as fixed sampling stations based on the past and recent endemicity of the disease. Fixed sampling is usually done for undertaking a longitudinal study on the seasonal vector prevalence, distribution, and biology in specific ecological niches.

Random Sampling: Random sampling is basically the selection of the sample sites which is done in and around the fixed sites on a random basis so that the left area may also be a part of the representative area planned for sampling. The only important point to be kept in mind is that the random sampling spots should be in the vicinity or periphery of the fixed stations. Changing the sampling sites should be done from time to time. Usually, four random stations are to be taken up during each vector surveillance and are not to be repeated.

Step 4: Selection of appropriate Sampling Tool and its Application (Technique)

The selection of the appropriate sampling tool is quite important keeping in view the objective. The sampling strategy is always planned based on the sampling of larval stages or adult vectors and the success of the yield shall depend on the application of proper sampling tools and techniques in the field conditions. There may be insufficient information on vectors if the sampling strategy is not proper with proper selection of representative areas, sampling tools, and procedures.

An attempt has been made to highlight the guiding principle for planning a proper sampling strategy in a given area for the specific vector surveillance. It is also important to mention that sampling strategies may differ during outbreak situations, routine surveillance, and longitudinal study on the vector biology and entomological indicators.

During outbreaks of any VBDs, the sampling strategy needs to be focused on the area/ population affected by the diseases based on vector habitat, prevalence, and transmission. Sampling is short term and indicates the presence of a vector and its probable role in the transmission of a particular disease under surveillance.

During routine vector surveillance, the sampling technique is based on the frequency and time interval based on multiple sites. The outcome of such routine vector surveillance may help in planning a proper vector control strategy.

Sampling of Mosquito Vectors

Objective

- Prevalence and distribution of mosquito vectors
- Vector biology and bionomics
- Parasite/ virus detection among vectors
- Preference for different vector species in eco-settings
- Understanding the level of susceptibility status against insecticides among mosquito vectors
- Evaluation of the vector control activities in the area

The above objectives can be achieved with the selection of an appropriate sampling technique and in view of the endemicity of the area and the presence of evidence for the vector mosquito species. However, entomological sampling may be undertaken in the sentinel sites, potential hotspots, and susceptible populations.

Selection of Sentinel Sites

- Sentinel sites are selected based on the recent hotspots or past endemicity of the disease at the village level in a district. The seasonal variability and the parasitic reservoir shall determine the onset of disease transmission in that area
- An area having a susceptible population near a high malaria transmission risk area and having potential breeding habitats for the vectors
- An area reporting frequent outbreaks in the past may be due to a migratory population or inaccessibility due to difficult terrain features

Entomological Sampling Tools

Entomological Surveillance

Scientists rely on the infestation level, available resources, and surveillance technique while choosing a suitable sampling technique. Vector control can be prioritised based on time and space, vector surveillance is critical in order to determine distribution, population density, larval habitats, and insecticide resistance. Such data is required while choosing the most suited vector control techniques.

Collecting Adult Mosquitoes

Adult mosquitoes are collected for both qualitative as well as quantitative studies. Qualitative studies investigate the relative density and abundance of vectors, longevity, infectivity, and impact of anti-venom treatments on vectors in different macro and micro-environments. Qualitative studies assess the vector relative density and abundance.

Collecting Mosquitoes by Hands

A test tube or sucking tube can be used to collect mosqui-

toes in which host species are being fed or resting (indoors and outdoors). This could be useful in determining diverse habits such as resting, feeding, ovipositing, as well as vectorial potential.

Collection of Mosquitoes

Using a torch light, adult mosquitoes can be found in dark places of rooms, around the ceiling, under shelves, among clothing, and other hangable articles. Large numbers of mosquitoes can be collected from sheds used for cattle.

Aspirator Tube or Sucking Tube: This is the most widely used and convenient method for mosquito collection.

Aspirator tubes are usually 30 to 45 cm long (8 to 12 mm inner diameter) and have plastic or glass tubing. A piece of mosquito netting is placed over the end of a shorter piece of rubber tubing that is inserted into the larger tube. This tool can be used to suck a resting mosquito while keeping the other end of the tube closed to check the movement of the mosquito.

Test Tube: Mosquitoes are collected in approximately 100 mm long test tubes with no rim. The test tube mouth is kept slowly over the resting mosquito and when it enters the tube, the mouth of the tube is tilted and closed with cotton.

Catch Off Baits: Mosquitoes can be sucked using a tube while they sit on the host to bite. This is one of the most important methods for catching partial or whole populations of mosquitoes.

Mosquitoes are collected directly off the human or animal baits using a sucking tube while they land on the host to bite or while they are in the process of biting a human or an animal host. Exophilic mosquitoes can be collected using this technique.

Hand Net Catches: An adult mosquito that is resting on human and animal habitations in high numbers is caught by a small hand net about 15 cm in diameter. The net is made of fine mosquito netting and comes with a long handle. First, a non-toxic oil (Risella or citronella oil) is sprayed in the place especially in crevices to perturb mosquitoes which can then be collected quickly in the net.

Spray Sheet Collection: Usually, from 06.30 to 10.00 in the morning, this method is applied during the day time, depending on the goals and conditions.

A white sheet should be placed on the floor and the structures must be empty of occupants, animals, food-stuffs, drinking water, and furniture before collection begins. 0.1 per cent pyrethrum in kerosene oil @ 15-30 ml/1000 cu ft is sprayed and doors and windows are closed. After 10 minutes, mosquitoes can be gathered from the sheet using entomological forceps.

Trap Collection: Flying mosquitoes can be collected using

traps. Window trap, magoon trap, malaise, and light trap are certain important types of traps. Ovitrap can be used to collect female mosquitoes.

Window Trap: These are primarily used to collect mosquitoes in various malaria programmes. These collections provide data regarding the movement of mosquitoes in diverse physiological conditions. This type of trap is used to capture mosquitoes flying into or out of a window or entrance. They do not contain any attractant, and the trap consists of a wooden frame shaped like a cube with each side of one foot and five sides covered with mosquito nets, while the sixth side has a deep cone (i.e., a hole).

Larval Collections

This helps in the raising of adults for stock, as well as establishing the breeding habits of different species, their geographical distribution, studying the larval stages and evaluating the impact of anti-larval measures on larval density. The larvae are also collected for determining the breeding habits of different species and their taxonomic studies.

Larval Collection Methods

Dipping: This technique is the most common one used to collect mosquito larvae. If the dipper is immersed too slowly, the larvae are disturbed and sink to the bottom, and that can lead to a loss of the collected larvae. This is why the bowls, frying pans, or ladles should be immersed at an angle of 45° in the breeding places (edges of swamps, ditches, streams, rice fields, and other bodies of water). A gap of 2 to 3 minutes between successive dips would allow larvae and pupae to come to the surface.

Netting: A larval net is a ring of iron frame measuring 25 cm in diameter surrounded by a nylon/ muslin cloth net measuring 10 cm long. A long wooden handle is attached to it. Larvae are collected along the edges of streams, ponds, and other large bodies of water.

Pipetting: If larvae are found in shallow breeding sites like hoof prints, they can be collected with a small pipette or a small spoon. They can also be gathered from tree holes or leaves with a large pipette or a siphon. The water can be removed with a straw.

Siphoning: It is done when the breeding is found in tree holes and small containers are present at limited access.

Sandfly Sampling

Sandflies are generally nocturnal; different species have different times of activity at night; however, they bite if disturbed during the daytime, for instance, in dark corners.⁹

Sampling from resting sites is usually done by the entomological teams by using different types of aspirators. It is important to distinguish between indoor and outdoor resting sites of sandflies. Light traps and sticky traps are also

used for the outdoor sampling of the sandfly populations.

Tick Surveillance

Surveillance of tick distribution, presence, and disease rates within a geographic area are targeted for tick surveillance. Depending on the population, dragging, flagging, and dry ice-baited traps can be used to collect ticks. Rodent surveys are used to collect ticks infecting live hosts. Leaf litter technique is used to collect ticks from throughout the garden.

Methodology

Sampling may be done during the forenoon period as the tick population (larval forms) is active and questing for host-seeking. Collection in the morning and evening is not recommended due to poor activity of the ticks. The dragging or flagging may be done at least 5-10 times at one site to cover the area completely. The clothes are thoroughly examined for the presence of ticks which are collected in vials. The details are to be noted with regard to the area, site and date, type of vegetation, and method used. Forceps may be used for removing adult ticks or nymphs.

A tick survey should be carried out by applying standard entomological techniques for all stages of vector species. Ticks should be sampled, collected, identified, and brought to the lab. They should be either live or stored in 70% ethanol to process them further for identification etc. Susceptibility test/ cone bio-assay shall also be carried out against insecticides as per WHO protocol.¹⁰

Hand Collection

Ticks can be gathered from six well-defined sites on each host animal to determine the total tick number. These sites are chosen as they have been shown to provide feeding areas for the different stages of common cattle ticks. All ticks can then be removed from these sites.

Collection of Tick Parasitising Live Host including Rodent Surveys

It is sometimes necessary to collect rodents and their ectoparasites in order to identify known vector-reservoir systems, or perhaps to discover new ones. Different kinds of rodent traps are available for rodent surveys and can be used keeping in view the objective of the rodent survey. Snap trap, live trap, Sherman trap, and multiple other traps are often used for rodent surveillance in the field situation as per the need and objective of the survey. Ticks are removed after processing the rodents in the lab. After thorough combing or brushing the rodent in a backward manner, the brushed out ectoparasites are collected in a white enamel tray and processed further for identification and labelling.

Mites

“Black plate” technique is really helpful in surveying mites.

Mites randomly crawl on the “black plates” (~12-inch square) composed of construction paper, paper plates, hard plastic, or similar materials kept directly on the earth where the rodent population concentrates.

A yellowish or orange mite called chigger lives in the ears or groin area of their rodent hosts. Trapping their rodent hosts is another method of efficiently sampling these mites.

Fleas

It is crucial to understand local flea species and their hosts in order to estimate risks of human plague infection and develop control measures that are appropriate for local conditions. Analysing relevant surveillance data can usually help determine the relative significance of local flea species as plague vectors. The rate of infection, number of fleas per host, and preferences data can be used to indicate future trends and provide a direction to control efforts.¹¹

Entomological Techniques

Morphological Identification

It is very important to know the taxonomic characteristics of a mosquito species encountered during entomological surveillance to understand and correlate the transmission dynamics. Appropriate identification keys shall help a trained technician to know and understand it by matching the morphological/ taxonomic characteristics of the vector mosquito sample to the genera and species level. Morphological characteristics mainly include banding on antennae and legs, among others. It would be good to make a collection of pinned specimens for reference in future.

Molecular Identification

Recently, polymerase chain reaction or PCR based diagnostics have become an important molecular biology technique. The same can be used to amplify DNA sequences that allow mosquito species to be identified based on species-specific differences in nucleotide sequence and, hence, amplicon length. PCR has a high rate of sensitivity and specificity and is thus a preferred technique for identifying mosquito biodiversity. Standard Operating Procedures (SOPs) are to be in place for the identified laboratories with BSL2 and ACL2 norms.

Salivary Gland Dissections

Salivary gland dissections allow for microscopic observation of sporozoites in freshly killed mosquitoes with regard to malarial parasites. Needles are used to dissect the salivary gland from the specimen, thus allowing the sporozoites to be observed under a microscope.

Ovary Dissections

Ovary dissections are done primarily to know and determine the age structure of the mosquito population differentiating

populations based on if they have had a blood meal or not. This technique is labour-intensive so training (and retraining) is required.

ELISA for Pathogen/ Virus Detection

Enzyme-linked immunosorbent assay (ELISA) is a technique used to detect pathogen/ antigen virus infections in mosquito vectors. The head and thorax of the mosquito sample are used to test for the presence of the pathogen/ viruses using an ELISA assay. These can be identified as specific pathogens or viruses based on the monoclonal antibody used.

Blood Meal Analysis

Blood meal detection is done through ELISA or PCR and is used to know the source of the mosquito's blood meal. For this test, full-fed mosquitoes/ vector species are exposed to ELISA or PCR to identify host blood. The technique can be customised/ standardised to test for human, cow, and other animal sources (both domesticated and wild animals) based on the monoclonal antibody or host-specific PCR primers.

PCR for Pathogen/ Virus Detection

PCR can also be used to detect the presence of the parasite in the mosquito. Usually, the head and thorax are used to limit the DNA detection of infectious sporozoites that leave the abdomen and infect the salivary glands. Since this technique looks at DNA that is found in all stages of the parasite, care should be taken to mention this in any analyses as infection rates (presence of DNA) might not be reflective of infectious rates (presence of infectious sporozoites in the salivary glands). The absolute relationship between CS ELISA and Plasmodium PCR is not determined at present.

WHO Tube Bioassay

WHO tube bioassay procedures measure the susceptibility of local vectors to five classes of insecticides, including organochlorines, organophosphates, pyrethroids, carbamates, and neonicotinoids.¹² The test procedures may be used as outlined in the WHO Test Procedures for Insecticide Resistance Monitoring in Malaria Vector Mosquitoes. The intensity of resistance may also be measured. When presenting results on insecticide resistance, the sampling method used to capture the mosquitoes, as well as the mosquitoes used (F0 wild caught or F1 progeny), should be noted as these may bias results. Controls using colony-reared susceptible mosquitoes should be utilised when available. A parallel test with colony-reared, susceptible mosquitoes should be conducted to ensure that the test with the wild-type mosquitoes is being conducted correctly. When susceptible mosquitoes are not available, then only relying on survival controls of wild-caught mosquitoes is acceptable. When insecticide resistance data is presented, the nature of controls used

(susceptible mosquitoes, survival controls, or no controls) should be defined.

CDC Bottle Assay

The CDC bottle assay also looks at the frequency and intensity of insecticide resistance.¹³ When presenting results on insecticide resistance, the sampling method used to capture the mosquitoes, as well as the mosquitoes used (F0 wild caught or F1 progeny), should be noted as these may bias results. Controls using susceptible mosquitoes should be utilised when available.

PCR for Mechanisms of Insecticide Resistance

PCR can also be used to evaluate the presence of genes and alleles associated with insecticide resistance, including knockdown resistance (Kdr) target-site mutations as associated with resistance to pyrethroids and DDT (both Kdr-East and Kdr-West) and acetylcholinesterase (Ace-1) mutations, which are associated with carbamate and organophosphate resistance. Various species-specific and target site-specific tests are available and should be appropriately conducted based on the questions the tests aim to answer. Controls (resistant, susceptible and heterozygotes) should always be included in the tests, and understanding the interactions between each genotype (e.g., between Kdr-East and Kdr-West) is important when interpreting results.

Cone Bioassay

Cone bioassays evaluate the toxicity of insecticide-treated surfaces such as LLINs and walls treated with IRS. Here, susceptible mosquitoes are exposed to the treated surface for a certain amount of time to determine the effect.¹³ This method looks at the present and immediate effect of the intervention on susceptible mosquitoes and is usually used to assess the residual or temporal bio-efficacy of an intervention's active ingredient. Further guidance and protocols on laboratory and field entomology methodologies can be accessed through the WHO's Manual on Practical Entomology in Malaria.¹⁴

Conclusion

An appropriate sampling strategy should be in place for undertaking proper entomological surveillance in a given area and the selection of sampling tools may help, in turn, for the best outcome with regard to the information on vector prevalence, distribution, and its biology which would help in planning the prevention and control strategy against specific vector species. The use of insect surveillance should guide intervention selection, targeting, and success rates over time as it can provide insight into disease transmission.¹⁵

However, the selection of an appropriate entomological sampling strategy with the well-designed and standardised sampling tools shall help in timely and effective planning

and implementation of intervention measures. In India, analytical techniques with regard to vector-borne diseases are quite critical to understanding the role and dynamics of disease transmission through vectors in different geographical areas.

Conflict of Interest: None

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