



Anti-Malaria Campaign
Ministry of Health
Sri Lanka



Guidelines for Entomological Surveillance of Leishmaniasis in Sri Lanka

2024

Foreword

Leishmaniasis is an emerging public health concern in Sri Lanka, necessitating comprehensive investigations and targeted control measures. The vector of leishmaniasis, sand fly, is abundant in many parts of the country. Conducting entomological surveillance to identify vector species, study their bionomics, and monitor their spatial and temporal variations is crucial for developing effective control strategies against the disease.

The Anti Malaria Campaign (AMC) has been entrusted with the responsibility of controlling leishmaniasis in line with the National Strategic Plan for its prevention and control. Recognizing the importance of vector surveillance, AMC identified it as a key component of the program to develop suitable control measures for the disease. To address this need, AMC took the initiative to develop a comprehensive guideline for sandfly surveillance with the active participation of representatives from relevant staff categories.

I am delighted to announce the completion of the “Guideline for Entomological Surveillance of Leishmaniasis in Sri Lanka.” I trust that all relevant staff will utilize this guideline effectively, contributing significantly to the control of leishmaniasis in the country.

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Acknowledgements

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Abbreviations

AMC	Anti Malaria Campaign
AMCHQ	Anti Malaria Campaign Headquarters
CL	Cutaneous leishmaniasis
RMO	Regional Malaria Officer
IRC	Indoor Resting Collection
ORC	Outdoor Resting Collection
CBT	Cattle Baited Net Traps
HDNT	Human Double Net Traps
LT	Light Traps
ST	Sticky Traps
CBHC	Cattle Baited Hut Collections
HEO	Health Entomology Officer
PHFO	Public Health Field Officer
SMO	Spray Machine Operator
SKS	Saukya Karya Sahayaka
PBS	Phosphate Buffered Saline

Guidelines for Entomological Surveillance of Leishmaniasis in Sri Lanka

1.0 Background

Cutaneous leishmaniasis (CL) is an emerging disease in Sri Lanka. Hematophagous females of sand flies are vectors of CL and *Phlebotomous argentipes* is the principal vector of *L. donovani* in Sri Lanka (Karunaweera *et al.*, 2003, Gajapathy *et al.*, 2011, Jayathilake and Robinson, 2020). However, the exact number of species capable of transmitting Leishmaniasis in Sri Lanka remains to be explored. Studies on the prevalence and abundance of sand fly fauna are confined to limited areas (Ozbel *et al.*, 2016, Gajapathy *et al.*, 2012, Wijerathna *et al.*, 2022) and a clear picture of population dynamics and distribution of sand flies is yet to be established in the country.

Entomological surveillance provides information for vector control to reduce or interrupt disease transmission by controlling sand flies. Monitoring insecticide resistance in sand fly vectors is essential for effective and sustainable disease control. This document describes a comprehensive entomological surveillance programme for sand flies in Sri Lanka.

2.0 Objectives

General objective

To assess the abundance, population dynamics, bionomics, temporal and spatial distribution of the sand flies for effective vector control for prevention and control of Leishmaniasis in Sri Lanka.

Specific objectives

1. To monitor the abundance and temporal and spatial distribution of sand fly populations
2. To study bionomics and ecology of sand flies
3. To incriminate vectors of Leishmaniasis in Sri Lanka
4. To monitor insecticide resistance of sand flies
5. To monitor the effectiveness of vector control interventions

3.0 Types of Entomology Surveys

1. Sentinel Surveys

Long-term observations in fixed locations to follow trends in vector density, species distribution and bionomics of the vectors over time.

2. Foci investigations

Short-term investigation in established foci of transmission.

3. Spot checks

Rapid survey to detect vector resurgence or transmission potential as needed.

Table 1: Entomological surveillance activities required for different Surveys

	Sentinel surveys	Foci investigations	Spot checks
Objectives	To establish baseline information on the role of vectors in transmission, geographical and seasonal distribution, feeding and resting behaviour and susceptibility to insecticides	To take appropriate and timely reactive measures as part of epidemiological investigation	Proactively identify the existence and the density of vectors in receptive and vulnerable areas.
Site selection	<p>Fixed sentinel sites should be identified based on the leishmania case incidence/number of confirmed cases in a particular region.</p> <p>Sentinel sites should be comprised of adjoining 1-3 GN areas based on case distribution with the highest number of confirmed Leishmaniasis cases.</p>	Sites of foci investigations are locations identified with persistent or recurrent transmission.	Spot checks should be carried out in selected locations with suspected high transmission potential

	Sentinel surveys	Foci investigations	Spot checks
When to be implemented	On monthly basis	As soon as the epidemiological investigation indicates the presence of active foci of transmission or persistence or recurrence of disease transmission.	During the period of high vector prevalence and the period of influx of sources of infection into areas
Indicators to be monitored	<ul style="list-style-type: none"> ➤ Sandfly density (seasonal) ➤ Vector feeding and resting behaviour ➤ Vector habitats ➤ Infection of vectors ➤ Susceptibility to insecticides 	<ul style="list-style-type: none"> ➤ Vector density ➤ Vector feeding and resting behaviour ➤ Vector infection rate ➤ Susceptibility to insecticides 	<ul style="list-style-type: none"> ➤ Vector presence and density or absence ➤ Vector geographical distribution

4. 0 Selection of sites for entomological surveillance

Stratification of the districts is done based on the number of Leishmaniasis cases. Districts are categorized as high, moderate and low levels of transmission. The entomology surveys conducted are sentinel surveys and foci investigations depending on the risk (Table 2).

Table 2: selection of the type of survey based on the level of transmission

	Level of transmission		
Type of survey	High	Moderate	Low
Sentinel survey	✓	x	x
Focus investigation	✓	✓	✓
Spot checks	✓	✓	✓

Based on the reported Leishmaniasis cases in 2023, districts were classified into three transmission levels to determine the type of entomological surveillance.

High transmission (>300 cases): Kurunegala (including Maho), Anuradhapura, Hambanthota, Polonnaruwa, Matara, Moneragala, Mathale, Rathnapura

Moderate transmission (10-300 cases): Ampara, Gampaha, Kandy, Kegalle, Badulla

Low transmission (<10 cases): Puttalam, Vavuniya, Mullaithivu, Trincomalee, Batticaloa, Kalmunai, Killinochchi, Jaffna, Mannar, Galle, Colombo Kalutara, Nuwara Eliya

In low-risk regions, sand fly collections can be done during malaria entomological surveys in potential sites to study sand fly abundance and bionomics.

5.0 Entomological techniques

1. Resting collection (Hand/mechanical indoors and outdoors)

Live female and male adult sand flies can be collected preferably with an aspirator and a torchlight while resting on indoor surfaces in human dwellings (Indoor Resting Collection-IRC), animal shelters buildings and outdoor surfaces (Outdoor Resting Collection-ORC). This method is suitable for collecting live specimens of wild sand flies for species identification and testing for insecticide susceptibility. It is, however, relatively labour-intensive. Mechanical aspirators are more productive but can damage specimens. Collected sand flies can be released into paper cups lined with fine mesh netting for further study. The peak collection times range but can be timed to determine where and when sand flies rest. The height of the walls to be searched for sand flies would be approximately 2m.

2. Cattle Baited Net Traps (CBT)

Where sand flies are highly zoophilic, this method can be useful for collecting sand flies that rest on the walls of the traps after feeding on cattle used as bait. This method is useful for collecting specimens for entomological studies such as host preference studies and insecticide resistance testing. The mesh size of the net should be smaller to trap sand flies.

3. Human double net traps (HDNT)

This method can be useful for measuring human biting rates for vectorial capacity assessment. Female sand flies seeking human blood meals are collected with aspirators as they land on the outer cover of the inner net and the inner cover of the outer net. A human should sleep inside the inner net to attract sand flies towards the net trap. The mesh size of the net trap should be smaller to prevent sand fly escape. Male sand flies can be collected from this technique as well.

4. Light traps (LT)

Battery-powered light traps are commonly used to collect host-seeking, moving sand flies or gravid females and males overnight (dusk to dawn). The method can be used to collect sand flies both indoors and outdoors overnight. Sand flies are attracted by a light source in a trap usually placed about 1 m above the ground with the bottom of the collecting device about 15 cm from the ground, in the room most inhabitants sleep, thus attracting more vertically resting or active sand flies. Light trap collections capture mainly blood-searching females live and damaged.

Sticky traps (ST)

Sticky traps with paper or cardboard soaked in mineral or vegetable oil (eg. Castor oil) near sand fly breeding or resting habitats are placed vertically off the ground or horizontally at ground level overnight. This is an inexpensive, quantitative method.

5.1. Work plan for Sentinel surveys/ Foci investigations

Sentinel surveys are conducted monthly in selected sites. Foci investigations are carried out as required (Table 1). Each survey is planned for 4 days during which a minimum of 02 CBT, 01 HDNT, IRC (30 rooms), ORC (6 mhrs), LT (15), ST (60), Cattle baited hut collections (CBHC) (If possible and available) and insecticide susceptibility tests are conducted. Details are given in Table 3.

Table 3: Work plan for Sentinel survey/Focus investigation

	Day 1	Day 2	Day 3	Day 4	Total Effort
Cattle Baited Trap Collection (CBTC)		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		02
Light Trap collection (LT)		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	15
Sticky trap collection (ST)		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	60
Resting Collections IRC ORC	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		30 rooms 6 hrs
HDNT (full night)			<input checked="" type="checkbox"/>		01
Insecticide Susceptibility test		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	02 per year
Cattle Trap setting	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>			
Light Trap setting	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		
Sticky trap setting	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		

6.0 Processing for identification and preservation of adult sand flies

Correct identification of phlebotomine sand flies is a critical first step in processing samples for studies collected in the field during vector surveillance. Collected sand flies are killed using Chloroform/Ether, sorted by collection method and sex and preserved for studies in 70% -80% Ethanol in separate labeled vials and stored at room temperature.

Specimens should be processed and mounted for identification on glass slides. Specimens should be dehydrated in 70% ethanol, 90% ethanol, absolute ethanol, and xylene for 5 minutes each. Then they are cleared in 10% lactophenol for 1–2 hours. Specimens are dissected on a glass slide separating the terminal part of the abdomen, wings and entire head with a fine needle and mounted temporarily or permanently.

6.1. Preparation of temporary and permanent mounts

The dissected specimens are mounted temporarily on slides with cover slips in lactophenol and identified under the microscope. Permanent mounts are prepared in Hoyer's medium for later identification of genus and species. The sand flies are identified based on morphometric characters described in the standard identification keys published in Sri Lanka and internationally.

7.0 Guide to separate male and female

Terminalia of sand flies should be observed under a stereo microscope for gender identification: Females are characterized by terminal ends lacking clasping structures and males are characterized by terminal ends with clasping structures (Fig 1).

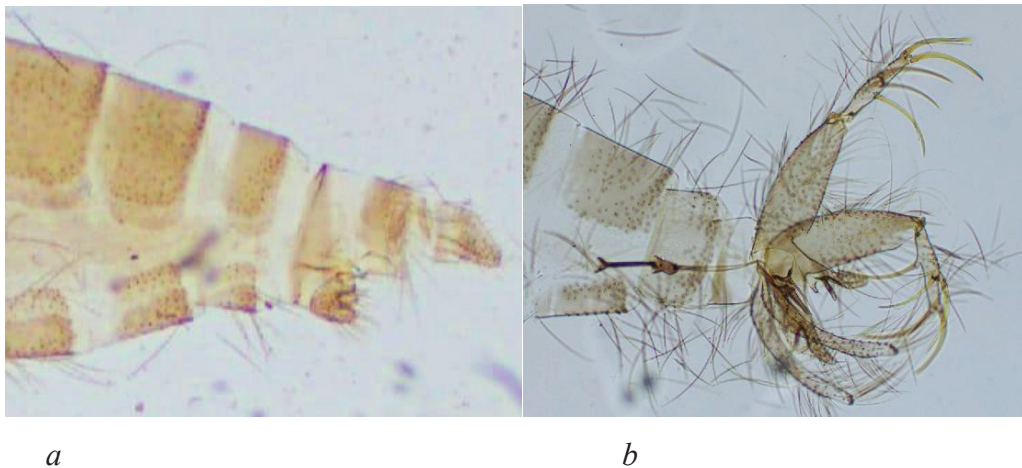


Fig 1. a) Terminalia of a Female sand fly b) Terminalia of a Male sand fly observed under a dissecting microscope

8.0 Guide to identify up to the Genus level

8.1. Key to sand fly Genera in Sri Lanka based on male morphology

-Style with 4 or 5 spines; not all spines terminal (Fig 2c)- Genus *Phlebotomus*

-Style with 4–5 spines, usually terminal; if not all spines terminal, 2 spines terminal and 2 sub-terminal, often in pairs (Fig. 2d) -Genus *Sergentomyia*

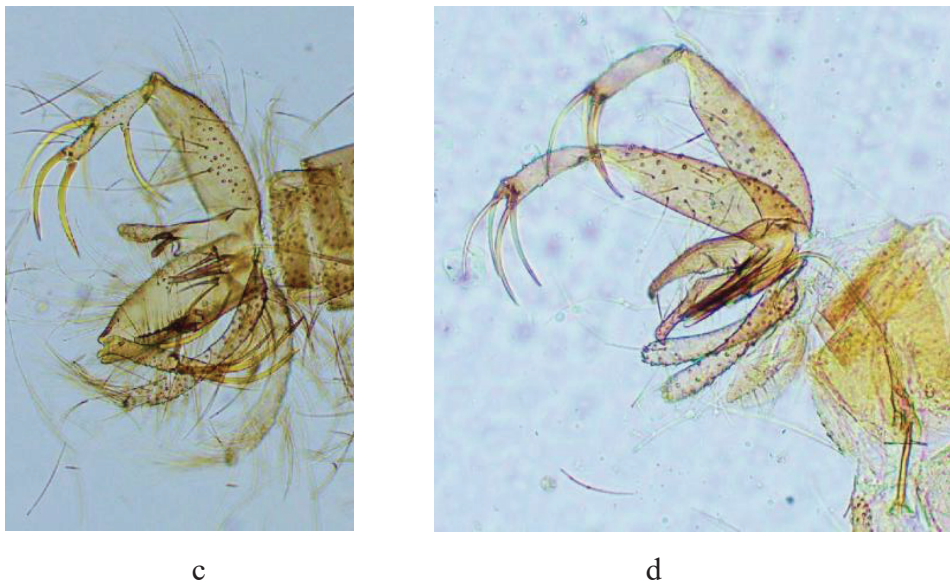


Fig 2. Morphological features of male sand fly used for Genus identification
 c) Gonostyle of *Phlebotomus* d) Gonostyle of *Sergentomyia*

8.2. Key to sand fly Genera in Sri Lanka based on female morphology



Fig. 3. Female sandfly. left: Genus *Sergentomyia* Right; Genus *Phlebotomus*

-Wings broader, and asymmetrical along the length (Fig. e). Cibarium unarmed or with scattered spicules without a pigment patch (Fig. f) - Genus *Phlebotomus*

-Wings narrow, lanceolate, symmetrical along the length (Fig. g). Cibarium with one or more rows of teeth, a pigment patch is usually present (Fig. h) - Genus *Sergentomyia*



Fig4. Morphological features of female sand fly used for Genus identification
 e) Wing of *Phlebotomus* f) Cibarium of *Phlebotomus* g) Wing of *Sergentomyia*
 h) Cibarium of *Sergentomyia*.

9.0 Identification up to Species level

Please refer to sand fly species identification keys listed below to identify the species. However, it is recommended to identify *Phlebotomus* up to the species level and *Sergentomyia* up to the genus level.

- a) Wijerathna, T., Gunathilaka, N. Morphological identification keys for adults of sand flies (Diptera: Psychodidae) in Sri Lanka. *Parasites Vectors* **13**, 450 (2020).

- b) Lewis DJ. The phlebotomine sandflies (Diptera: Psychodidae) of the Oriental Region. Bull Br Museum Nat Hist. 1978;37:217–343.
- c) Kalra NL, Bang YH. Manual on entomology in visceral leishmaniasis. New Delhi: World Health Organization; 1988

10.0 Dissections for identification of the Leishmania parasite in sand flies and identification of blood meal

Sand fly specimens collected in HDNT, IRC, ORC in sentinel sites and foci are dissected in PBS to identify *Leishmania* parasite in the fore gut. If parasites are observed, the slide should be washed into a vial and frozen. The head and the genitalia should be mounted for species identification. The dissected samples should be sent to AMCHQ for molecular confirmation of parasite and sand fly species.

Identification of the source of blood meal is done using molecular (PCR) and immunological methods. Sand fly specimens from resting and light trap collections from sentinel sites and foci should be sent to AMC HQ.

Above studies will be carried out by collaborating with a research unit/ university.

11.0 Insecticide resistance monitoring

Testing for insecticide susceptibility is important for potential insecticides which can be used in the future for Leishmaniasis vector control and the insecticides that are used for the control of other vectors. Testing at least one compound in each insecticide class for non-blood-fed wild-caught females of known vector *Phlebotomus argentipes* is recommended in entomological sentinel sites (Table 4).

The baseline susceptibility of new classes of insecticides with unrelated modes of action that have not previously been applied or used in the target area may also, be tested as possible alternative products. This testing will help to establish the phenotypic resistance profile of the vector at the field level. If resistance is found samples need to be sent to AMCHQ for biochemical and Molecular analysis of

insecticide resistance. Susceptibility testing should be conducted at least annually in sentinel sites/ Foci.

Table 4: Discriminating Concentrations for *Phlebotomus argentipes* (WHO, 2023)

Insecticide class	Insecticide	DC for 1-hour exposure	Control oil/solvent
Pyrethroids	Alphacypermethrin	0.1%	Silicon oil
	Deltamethrin	0.05%	
	Permethrin (40:60 cis:trans isomer ratio)	1%	
Carbamates	Bendiocarb	0.1%	Olive oil
Organophosphates	Malathion	5%	Olive oil
	Pirimiphosmethyl	100 mg/m ²	Acetone only

Note: Susceptibility test for Lamdacyhalothrin 0.05% is also recommended

12.0 Work Force involved in entomological surveillance

The Entomology team at the Regional Malaria Office carry out the Leishmaniasis entomology surveys. The team comprises Health Entomology Officer (HEO) and supportive staff (PHFO, SMO, SKS). Depending on the requirement, technical support from district Entomologist /other entomology teams shall be obtained.

13.0 Data Reporting

Data recording forms are filled for each survey by HEO and submitted to RMO. A preliminary report is submitted just after the entomology survey and the full report is submitted after completing the identification within one week. One copy should be sent to AMCHQ by RMO. A digital database should be developed to be updated one week after the completion of the survey at the district level and the real-time data can be seen in a dashboard at the national level.

14.0 Quality control (cross-checking/Supervision)

Morphological identification of sand fly species will be verified at the national level by AMCHQ. A proportion of permanently mounted slides labeled properly from different species of collected sand flies should be submitted to AMCHQ for cross-checking to ensure the quality of species identification.

Bibliography

1. Operational manual on leishmaniasis vector control, surveillance, monitoring and evaluation. Geneva: World Health Organization; 2022. Licence: CC BY NC-SA 3.0 IGO.
2. Wijerathna, T., Gunathilaka, N. Morphological identification keys for adults of sand flies (Diptera: Psychodidae) in Sri Lanka. *Parasites Vectors* **13**, 450 (2020). <https://doi.org/10.1186/s13071-020-04305-w>
3. Standard operating procedure for testing the susceptibility of adult sand flies to insecticides in WHO tube tests. Geneva: World Health Organization; 2023. Licence: CC BY-NCSA 3.0 IGO.
4. Lewis DJ. The phlebotomine sandflies (Diptera: Psychodidae) of the Oriental Region. *Bull Br Museum Nat Hist.* 1978;37:217–343.
5. Kalra NL, Bang YH. Manual on entomology in visceral leishmaniasis. New Delhi: World Health Organization; 1988

Annex 01

Summary -Sand fly Vector Surveillance

H/AMC/LE 1

Summary -Sand fly Vector Surveillance

District / RDHS area
 MOH Area
 GN Division/s
 Localities
 GPS
 RMO Region/Entomological Team.....
 Type of Survey SS /FI/ SC
 Survey code
 Start Date
 End date

Technique	Work output	No. of <i>P. argentipes</i> sp. female	<i>Phlebotomus</i>		<i>Sergentomyia</i>		<i>Species</i>											
			Male	Female	Male	Female	<i>P. argentipes</i> S.I.											
							Male	Female	no. dissected	infection rate	Male	Female	no. dissected	infection rate	Male	Female	no. dissected	infection rate
I.R.C - Hand		per man hour																
I.R.C. - Prokopack	 per man hour																
C.B.T.C.	 per trap																
Sticky Trap Collection	 per trap																
Light Trap Collection	 per trap																
H.D.N.T.C. - Indoor	 per man hour																
O.R.C. - Hand	 per man hour																
O.R.C. - Prokopack	 per man hour																

Susceptibility Test

Collection technique	Insecticide & concentration (%)	No. of replicates in the test	No. tested	Test Mortality	No. of replicates in the control	No. in the control	Control Mortality	Corrected Mortality

Name of HEO.

Signature of HEO.

Comments:

RMO

Signature of RMO.

Outdoor Resting Collection of Sand flies (Hand /Prokopack)

Outdoor Resting Collections of sand flies (Hand / Prokopack)

RMO Region / Entomological team :
Survey code
Date of investigation

[illegible]

Signature of HEO

Annex 04

Cattle Baited Trap Collection for Sand fly

RMO Region / Entomological team :
Survey code
Total no. of trap
GPS

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[illegible]

Signature of HEO

Annex 05

H/AMC/LE 5

District / RDHS area
 MOH Area
 GN Division/s
 Locality

[illegible]

Signature of HEO

Annex 06

H/AMC/LE 6

District / RDHS area
 MOH Area
 GN Division/s
 Locality

[illegible]

*Type of collection site: Brick clin, Cattle shed, goat shed, firewood shed, Dog cage, poultry house

Name of HEO.....

Annex 07

Human Double Net Trap Collection (Outdoor/Indoor)

District / RDHS area

[illegible]

Note: P - Positive, N - Negative

Annex 08

Insecticide Susceptibility Test - Sand fly Adult

District / RDHS area
 MOH Area
 Details of collection site

GPS coordinates

Insecticide sprayed

[illegible]

*Stage -	A- F0 adult (wild collected);	B- F1 adults (lab reared)
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**** Type of paper - Insecticide only/ PBO only/ PBO and Insecticide**

Name of HEO

Signature of HEO

Annex : Types of Outdoor collection Sites

1. Brick pile
2. Cattle shed
3. Clay pots
4. Dog cage
5. Fire wood shed
6. Goat shed
7. Outer wall of house
8. Outer wall of toilet
9. Pile of roof tiles
10. Termite mound
11. Tree hole
12. Other