OPERATIONAL MANUAL
ON LEISHMANIASIS VECTOR CONTROL, SURVEILLANCE AND MONITORING AND EVALUATION

World Health Organization

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on leishmaniasis vector control, surveillance, monitoring and evaluation

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Abbreviations and acronyms

CDC	United States Centers for Disease Control and Prevention
CL	cutaneous leishmaniasis
DEET	*N,N*-diethyl-*m*-toluamide
IRM	insecticide resistance management
IRS	indoor residual spraying
ITN	insecticide-treated net
LLIN	long-lasting insecticidal net
NGO	nongovernmental organization
PCR	polymerase chain reaction
PKDL	post-kala-azar dermal leishmaniasis
VL	visceral leishmaniasis
WHO	World Health Organization

Glossary

The definitions given below apply to the terms as used in this document; they may have different meanings in other contexts.

Anthroponotic

Leishmaniasis that is transmissible from human to human via the bite of infected female phlebotomine sand flies without the involvement of an animal reservoir host.

Anthropophilic

Sand flies that are attracted to humans as a source of a blood-meal even when non-human hosts are available.

Note: A relative term requiring quantification to indicate the extent of preference for feeding on humans versus animals; usually expressed as the human blood index (proportion of sand flies that have fed on humans out of total fed). Sand flies that prefer to blood-feed on humans rather than animals are described as anthropophagic.

Biting rate

Average number of sand fly bites received by a host in a unit of time, specified by host and sand fly species.

Note: For ethical reasons, traditional human bait collection is not recommended under operational programme conditions. The biting rate is estimated by proxy methods such as the number of sand flies caught in a light trap hung near an untreated bed net with a volunteer human bait or by animal-baited traps or by an indirect method such as molecular analyses of blood-meals.

Case classification of cutaneous leishmaniasis (CL) by WHO operational definition

A case of CL comprises clinical signs (skin or mucosal lesions) with parasitological confirmation of the diagnosis (positive smear or culture) and/or, for mucocutaneous leishmaniasis only, serological diagnosis.

Case classification of post-kala-azar dermal leishmaniasis (PKDL)

Probable-PKDL case: A case in an area endemic for kala-azar with multiple hypopigmented macules, papules or plaques or nodules with no sensitivity loss.

Confirmed PKDL: A case in an area endemic for kala-azar with multiple hypopigmented macules, papules, plaques or nodules positive for the parasite or a positive slit skin smear or biopsy in a polymerase chain reaction (PCR) test.

Case classification of visceral leishmaniasis (VL) by WHO operational definition

A case of VL (kala-azar) comprises clinical signs (mainly prolonged irregular fever, splenomegaly and/or weight loss) with serological and/or parasitological confirmation.

Case detection

Identification and confirmation of a case of leishmaniasis in a community according to diagnostics and clinical definitions.

Controlled before-and-after trial

A trial in which pre- and post-intervention measurements are made of the outcome of interest (entomological infection or clinical parameters) in both the intervention and the control arm.

Density of sand flies

Number of female sand flies in relation to the number of specified shelters or hosts (e.g., per room, per trap or per person) or to a given period (e.g., overnight or per hour), with specification of the method of collection.

Note: This term refers strictly to the population density or abundance of adult female sand flies, which is a highly insensitive measure of leishmaniasis transmission.

Disease surveillance

Continuous, systematic collection, analysis and interpretation of disease-specific data and use in planning, implementing and evaluating public health practice.

Elimination threshold of VL in South Asia

Elimination of visceral leishmaniasis as a "public health problem" is defined as a reduction in disease incidence < 1 case/10 000 population in a geographical unit, which may be a district or a sub-district.

Endemic area

An area in which the full cycle of transmission has been demonstrated at any given time (i.e., where the population of a competent vector is maintained + parasite reservoir) *and* at least one locally acquired case was reported in the previous 10 years

Endophagic

Sand fly females that blood-feed indoors.

Endophilic

Sand flies that rest indoors.

Environmental management

Changing aspects of the environment in order to adversely affect sand fly populations and/or exposure to *Leishmania*-infected sand flies.

Exophagic

Sand fly females that blood-feed outdoors.

Exophilic

Sand flies that rest outdoors.

Focus; new focus

A focus is defined as any circumscribed geographical endemic area.

Note: People can be infected in a given focus but the case may be reported in another location because of travel or lack of access to health care. A new focus is a circumscribed geographical area from which new cases are reported but in which leishmaniasis transmission had not been reported in the previous 10 years.

F1 sand fly progeny

The first generation of sand flies reared in a laboratory from the eggs of wild-caught females; one of the two generations of sand flies that can be used in standard resistance monitoring bioassays.

Indoor residual spraying (IRS)

Operational procedure and strategy for leishmaniasis vector control involving spraying of interior surfaces of human dwellings and animal shelters in their vicinity with a residual insecticide to kill endophilic sand flies.

Insecticide discriminating concentration

A standard concentration of an insecticide active ingredient to which a sample of sand flies are exposed for a standard time that reliably kills susceptible sand flies so that any survivors may be assumed to be resistant to the insecticide.

Insecticide resistance

A heritable trait that enables sand flies to survive exposure to a standard concentration of an insecticide, reducing the performance of the intervention. Resistance may be due to physiological or behavioural adaptation.

Infected sand flies

Female sand flies with any stage of *Leishmania* parasite forms in their gut.

Infective sand flies

Female sand flies with *Leishmania* metacyclic parasite forms in the anterior midgut or stomodeal valve.

Insecticide-treated net (ITN)

A net treated with insecticide to repel or kill vectors that come into contact with the treated material.

Intra-sectoral

Working in a (health) sector.

Long-lasting insecticidal net (LLIN)

A factory-treated mosquito net made of material into which insecticide is incorporated or bound onto the fibres. The net must retain its effective biological activity without re-treatment for at least 20 WHO standard washes under laboratory conditions and 3 consecutive years of recommended use under field conditions.

New World

The Americas.

Old World

The world other than the Americas.

Outbreak (of leishmaniasis)

Occurrence of leishmaniasis cases in excess of the expected number in a defined community, geographical area or season. An outbreak may occur in a restricted geographical area or may extend to several countries. It may last for a few days or weeks or for several years.

Protective efficacy

Percentage reduction in a clinical or entomological outcome in a population that has received an intervention. Calculated as (1 – risk ratio) x 100, where the risk ratio is the risk of exposure of the intervention group divided by that of the control group.

Public health intervention

An activity (e.g., tool or policy) implemented to improve the physical or mental health of a population.

Randomized controlled trial

A field trial in which the unit of interest (e.g., individuals or villages) is randomly assigned to receive either the intervention or the control arm.

Sentinel surveillance

Sentinel surveillance is surveillance based on the collection of data from a sample (random or non-random) of collecting sites as indicator data for the rest of the population, in order to identify cases of a disease early or to obtain indicative data about trends of a disease or health event. Examples are the use of a few hospitals to monitor the composition of influenza virus and check that the vaccine includes the right components, or the use of a network of general practitioners to monitor diseases or health events (e.g. attempted suicide, requests for HIV testing). One instance of sentinel surveillance is the use of a particular population group (e.g., monitoring the serology of syphilis among pregnant women as an indicator of syphilis trends in the general population). Sentinel surveillance is inappropriate for those situations where every case requires public health action, e.g., poliomyelitis.

(This definition is based on WHO recommended surveillance standards, 2nd edition, 1999. World Health Organization).

Note: this standard definition needs to be adapted to the context of entomological surveillance.

Spray round

Spraying of all sprayable structures in an area designated for coverage in an indoor residual spraying programme during a discrete period

Note: Depending on the residual activity of the insecticide and the dynamics of leishmaniasis transmission, one or more spray rounds may be required each year in the same area.

Synergist

A substance that does not itself have insecticidal properties but that, when mixed and applied with insecticides of a particular class, enhances their potency against sand flies. The mode of action of most synergists is to block the insects' metabolic systems that usually detoxify the insecticide in their system.

Transmission season

Period of the year during which most sand fly-borne transmission of leishmaniasis infection occurs.

Vector, proven or suspected

Proven vector: anthropophilic sand flies that also pick up parasites from animal reservoirs which are naturally infected with parasites that are indistinguishable from those found in human reservoirs.

Suspected vector: anthropophilic sand flies with a geographical distribution compatible with that of endemic foci; may be suspected on the basis of epidemiological evidence, naturally infected but parasite not identified or is a proven vector elsewhere.

Vector or entomological surveillance

Regular, systematic collection, analysis and interpretation of entomological data for risk assessment, planning, implementation, monitoring and evaluation of vector control interventions.

Vector control

Measures of any kind against leishmaniasis-transmitting sand flies, intended to limit their ability to transmit the disease

Note: Ideally, vector control reduces leishmaniasis transmission rates by reducing the vectorial capacity to a point at which transmission is interrupted.

Vectorial capacity

Number of new infections that the population of a given vector would induce per case per day at a given place and time, assuming that the human population is and remains fully susceptible to *Leishmania* parasites.

Note: Not to be confused with "vector competence", which is the ability of sand flies to complete *Leishmania* parasite development and migration of metacyclic forms to the anterior midgut, allowing their transmission when the infective female sand fly feeds again.

Vector susceptibility

The extent to which a sand fly population is susceptible (i.e., not resistant) to insecticides.

Zoonotic

Refers to a disease that can be transmitted from living vertebrate animals to humans via the bites of infective sand flies.

Zoophilic

Refers to sand flies that are attracted to animals. Sand flies that prefer to blood-feed on animals rather than humans are described as anthropophagic.

1. Introduction

1.1 Leishmaniases

Leishmaniases are usually poverty-related vector-borne neglected parasitic diseases caused by infection with more than 20 species and subspecies of *Leishmania*, a protozoan parasite *(1, 2)*. Secondary, uncommon transmission routes are also reported *(3–7)*. The main clinical forms of leishmaniases are summarized in **Box 1**.

Box 1. Main clinical forms and symptoms of leishmaniases:

- **Visceral leishmaniasis (VL)**, also known as kala-azar in the Old World, is the most serious form of the disease as it is usually fatal if untreated. Common clinical features include persistent fever (> 14 days), enlargement of the spleen and liver, weight loss and anaemia.
- **Post-kala-azar dermal leishmaniasis (PKDL)** is a skin condition that usually occurs after treatment of VL and is prevalent mainly in areas endemic for *Leishmania donovani* in East Africa and South Asia. It may appear as macular, papular or nodular rashes, usually on the face, upper arms and trunk and also on other parts of the body. Typically, it appears 6 months to 1 or more years after VL has been treated but may occur earlier. It has also been reported in patients without a history of VL. Up to 50% of VL cases in East Africa (mainly in Sudan) develop PKDL, usually as a papular or maculopapular rash, whereas on the Indian subcontinent, 10–15% of treated kala-azar patients develop the condition.
- **Cutaneous leishmaniasis (CL)** is the most common form of leishmaniasis and causes skin lesions, mainly ulcers, on exposed parts of the body. Life-long scarring and disfigurement may occur at the sites of lesions *(1)*. It occurs throughout the Americas, the Mediterranean basin, East Africa, the Middle East and Central and South-East Asia.
- **Mucocutaneous leishmaniasis** is a highly disfiguring disease and can even be life-threatening. It destroys mucous membranes and the cartilage of the nose, mouth and throat and can lead to aspiration pneumonia. Mucocutaneous leishmaniasis occurs mainly in Brazil, Ethiopia, Peru and the Plurinational State of Bolivia.

Leishmaniases are a major health problem in the Region of the Americas, east and north Africa and western and south-east Asian regions, and their global burden varies temporally. According to Global burden of diseases study in 2019, 498 000–862 000 new cases of all forms of leishmaniasis were estimated to occur each year, resulting in up to 1.6 million disability-adjusted life years lost *(8)*. In order for accurate reporting of the leishmaniasis burden, the WHO Global leishmaniasis programme requests all Member States to submit data on six indicators annually to the Global Health Observatory. In addition, detailed regional and national data were published in 2021 in the *WHO Weekly Epidemiological Record (9)*.

1.2 Vectors of leishmaniasis

Leishmania parasites are transmitted to humans through the bites of infected phlebotomine sand flies. More information on the life-cycles of leishmaniasis and sand flies is given in **Annex 1**. An estimated 31 species of *Phlebotomus* and 47 species of *Lutzomyia* are proven vectors of human leishmaniases *(10)*. The main vectors responsible for transmitting human leishmaniasis in the WHO regions and settings are discussed in **Annex 2**. The distribution and behaviour of different sand fly species depend on their setting. Vector, human and parasite dynamics can be highly complex in different nosogeographical entities, particularly for zoonotic CL in the Americas. These are discussed further in section 2.

1.3 Scope of the manual

Despite the significant role of vector control in national leishmaniasis control programmes *(11)*, the programmatic community perceives vector control as the weakest component of leishmaniasis control strategies in terms of resources, scientific evidence of the usefulness of interventions and capacity for quality-assured implementation. Therefore, the main objective of this manual is to provide practical tools, techniques and procedures to strengthen sand fly control and surveillance in order to improve implementation of leishmaniasis control programmes. The manual provides a rationale for programme managers in different geographical regions on the types of vector control interventions to be used in different epidemiological and environmental settings and also how to measure their impact.

The manual covers both CL and VL (**Box 1**). Wherever possible, equal emphasis is placed on CL and VL, despite the imbalance of relevant evidence and material on CL in the literature.

To develop the manual, all six WHO regional offices were asked to nominate experts in sand flies and the leishmaniases. From the list prepared, experts with experience in operational surveillance and control as well as in research on sand fly biology and ecology in different WHO regions were selected. A group of these experts was invited to first develop a table of contents and then draft different sections of the manual. The draft manual was then peer reviewed by a larger group involving the WHO regional and country offices. Finally, it was peer reviewed at a WHO consultation and finalized.

1.4 Subject areas covered

- Section 2 presents the distinct scenarios and dynamics of leishmaniasis transmission and vector control in different eco-epidemiological settings.
- Section 3 describes how to organize vector surveillance activities to be used in a leishmaniasis control programme; including types of surveillance systems, vector parameters to be measured, insecticide resistance monitoring and management. Capacity requirements in vector surveillance and control are considered; including human resources, financial resources and infrastructure.
- Section 4 summarizes the main methods used for leishmaniasis vector control worldwide, such as indoor residual spraying (IRS), insecticide-treated nets (ITNs),

environmental management and new methods, including when, where and how to use them.

- Section 5 covers monitoring and evaluation, including quality assurance, choice of insecticide, quality of IRS and ITNs, coverage of interventions, staff training and core entomological indicators.
- Section 6 addresses data management, repository and reporting, including data collection and data dissemination.
- Section 7 describes operational research requirements. Gaps in evidence identified during the preparation of this manual will indicate the operational research to be considered by programme managers and researchers. Evidence on sustainability and cost–effectiveness is considered for use in decision-making.
- The annexes provide additional resources and materials for training and operational purposes.

2. Sand fly vector bionomics and transmission dynamics in various geographical settings

Sand fly species are found in a wide variety of arid, semi-arid, temperate, semi-tropical and tropical areas, including forest, desert and savannah areas, where they may occupy sylvatic, peridomestic or domestic habitats. Like other haematophagous (blood-feeding) insects, sand flies may bite indoors (endophagic) or outdoors (exophagic), rest indoors (endophilic) or rest outdoors (exophilic) or exhibit any combination of these behaviours. They may show an attraction and preference for biting human (anthropophilic) or animal hosts (zoophilic) or be opportunistic, feeding on the closest accessible host or those of greatest biomass, or may take mixed blood. These behavioural characteristics play a significant role in shaping the epidemiology of CL and VL and therefore the methods that can be used to control leishmaniasis in a given setting. Vector-based control and early detection and treatment of cases are necessary to successfully manage leishmaniasis and prevent onwards transmission. The appropriateness of these control methods varies by endemic region, depending on the local ecology, epidemiology of leishmaniasis and dominant vector species.

2.1 Factors to consider in planning vector control

Planning for vector control requires understanding of the dynamics of leishmaniasis transmission. The main questions for a programme manager to consider before implementing a control programme are common to all transmission settings and regions. They concern the etiology of leishmaniasis and are most pertinent to vector bionomics and vector control and surveillance.

Other major considerations, relating to resources and capacity, are addressed in section 3.

- Is transmission zoonotic or anthroponotic? If it is zoonotic, the control programme might have to identify and then target interventions against a given reservoir(s), in addition to treating human cases. If it is zoonotic, is there more than one main reservoir? If more than one reservoir host is involved or not all reservoirs are identified, the control strategies will be more complex.
- What is or are the vector/s? The control programme can use tools suited to the behaviour of a particular vector species but might have to be more adaptable if more than one vector is involved.
- Is the endemic zone widespread or clustered? Endemic zones may be widespread but, within a zone, the distribution of CL, VL and PKDL cases can be focal.
- The sand fly species compositions trapped in domestic, peridomestic and adjacent sylvatic habitats may differ in their behavioural ecology, adaptability and

habitat integrity. These are important considerations for surveillance, as many houses must be monitored to reduce the effects of heterogeneity.

- Is non-domestic transmission significant? Theoretically, it is much easier to focus vector control strategies when transmission is predominantly domestic. The place where most people are infected depends on the location and feeding behaviour of the vector and the behaviour of humans (e.g., forest workers may be infected predominantly in sylvatic habitats). Therefore, the demographic distributions (age, sex, occupation) of cases and the bionomics of vectors must be known in order to effectively target the control programme. Also, cases may be infected at one place and manifest and reported at another place.

Vector bionomics

Programme managers require recent evidence on the bionomics of adult sand fly vectors in their setting in order to select appropriate control tools and the optimal timing of their use. The focus is on the behaviour of adult sand flies, because very little is known about the breeding sites and behaviour of immature stages of sand flies in nature.

- What is the seasonality of vectors?

  The seasonality of vectors is subject to change according to environmental factors and should therefore be monitored periodically. The time at which female flies acquire infections, and hence the risk of infection of humans, is not clear. Paradoxically, maximum transmission does not necessarily occur at the peak of vector abundance, as this is due to a sudden increase in the emergence of adult sand flies which are still uninfected, having never taken a blood meal, and are thus not infectious to humans.

- Is or are the vector(s) endophilic or exophilic?

  Knowledge of resting behaviour can guide programme managers in selecting control tools. For example, IRS is a useful tool for controlling vectors that rest predominantly indoors; however, long-term use may divert sand flies to resting outdoors. Therefore, resting behaviour must be monitored throughout the programme.

- Is or are the vector(s) endophagic or exophagic, what do they bite, and when do they bite?

  Sand flies bite determines when people are at greatest risk and therefore determines avoidance measures and whether interventions such as ITNs are appropriate. Because of the high risk of infection for VL, it is ethically unacceptable to use unprotected humans as baits for determining peaks in biting times. Indirect measurements of diurnal sand fly activity could be monitored with United States Centers for Disease Control and Prevention (CDC) light traps modified to separate hourly collections. Biting rates are more commonly estimated by placing standard CDC light traps in houses and analysing the blood-meals of captured sand flies with molecular techniques; however, such studies

are prone to bias due, e.g., to trap placement and host availability. Another alternative is to use a human-baited trap, i.e., to use human volunteers from the same area who sleep under an untreated net beside a CDC light trap.

- Is or are the vector(s) resistant to insecticides in various classes?

  Insecticide-based vector control tools are effective only if the vector is susceptible to the insecticide to be used. Currently, there is no centralized database of insecticide resistance in sand fly species worldwide; however, target-site pyrethroid resistance mutations (knockdown resistance (*kdr*) L1014F/S) have been detected in several populations of sand flies *(12)*. The susceptibility of the sand fly population should therefore be determined at baseline and at regular intervals during the programme with standard WHO susceptibility bioassays to ensure that the target vector has not developed resistance to the selected insecticide.

Other considerations

- type of targeted area, such as urban or rural, that may require different approaches, e.g., ITNs and sanitation may be more acceptable than IRS in urban areas;
- transmission patterns, e.g., perennial, seasonal incidence peaks or occasional focal outbreaks;
- programmatic capacity for systematic vector surveillance, and delivery, monitoring and evaluation of the impact of vector control interventions;
- human and financial resources and capacity;
- availability of adequate supplies such as of insecticides, ITNs, spray equipment, transport; and
- the cost–effectiveness of the selected intervention, and community acceptance and participation.

2.2 Transmission settings and vector control

The following sections summarize the evidence available to programme managers on VL and CL control in various scenarios of transmission and WHO regions (see also **Annexes 3–5**) and provide further details and sources of information. Each case study takes into account the questions in section 2.1 on etiology and vector bionomics. In practice, not all the fundamental questions are addressed before the start of a programme, and, as indicated by the sources provided, the available data may be scanty, restricted to a small area or no longer valid because of shifts in vector behaviour over time. Gaps in evidence may indicate the direction of operational research (section 7).

2.2.1 Peridomestic and anthroponotic transmission of VL in the South-East Asia Region

Transmission of VL in the South-East Asia Region is relatively simple, involving only one vector, *Ph. argentipes*, one parasite, *Le. donovani*, and humans as the reservoir hosts. This and other factors, such as confinement of the disease to limited geographical areas,

effective vector tools, effective diagnosis and free treatment, ensured high political commitment, allowing programme managers to target VL for elimination as a "public health problem", defined as a reduction in incidence below 1 case/10 000 population at district or sub-district level. Because of the endophilic behaviour of *Ph. argentipes*, two rounds of IRS with a synthetic pyrethroid, ideally conducted before observed peaks in sand fly density, are currently the only vector control method used routinely. As a large proportion of sand flies feed on both cattle and humans, routine IRS includes spraying the internal walls of all houses and cattle sheds in targeted areas, from the ground up to 2 m, as it was observed that *Ph. argentipes* are most commonly found at low heights. Thus, roofs are not sprayed, except in areas co-endemic for malaria. The quality of the insecticides selected should be tested pre- and post-shipment, and the susceptibility of *Ph. argentipes* must be tested at least once each year. As it is currently unclear whether ITNs reduce VL incidence, their distribution to at-risk populations (unless in areas co-endemic for malaria) is not supported financially in any national VL control or elimination programme. Systematic longitudinal sampling of *Ph. argentipes* populations to monitor the impact of IRS on their density and the presence of *Le. donovani* infection is not currently routinely performed within programmes but may be done with partners when possible.

2.2.2 Domestic zoonotic transmission of VL in the Region of the Americas

In the Region of the Americas, VL is transmitted by one main vector species, *Lu. longipalpis*, and domestic dogs are the reservoirs of *Le. infantum*. The Brazilian Ministry of Health recommends that control activities focus on areas with autochthonous cases of VL classified as high, intense and very intense transmission on the basis of reported case numbers. Use of IRS to control domestic *Lu. longipalpis* populations had a limited impact, as there is little residue of the insecticides on the wall substrate in houses. Furthermore, a low or no significant effect was observed on peridomestic populations *(13, 14)* of *Lu. longipalpis* in Brazil due to behavioural resistance to pyrethroid treatment *(14)*. It has also been proposed that insecticide spraying of chicken sheds disrupts the lekking behaviour of *Lu. longipalpis*, causing new leks to be established in untreated household dining huts *(15, 16)*. Instead, the strategy of using deltamethrin-impregnated dog collars has been explored after laboratory and field trials on the efficacy of the collars and mathematical modelling that predicted their potential efficacy in VL control *(17–21)*. Collar loss was 50% between the first and last cycle, most of which occurred during the first and second cycle. Despite the losses, reductions of 50% in canine leishmaniasis incidence and prevalence were observed in intervention areas *(22, 23)*. In a municipality with highly endemic canine leishmaniasis in Brazil *(24)*, use of deltamethrin-treated dog collars contributed to a reduction in zoonotic VL with an effectiveness of 66%, and the dogs' survival rate at 50 months was > 90%. Analysis according to capture site showed a 21% decrease in peridomiciliary *Lu. longipalpis* (incidence rate ratio = 0.783; $P < 0.001$) in Montes Claros and 56% (incidence rate ratio = 0.44; $P < 0.001$) and 60% (incidence rate ratio = 0.40; $P < 0.001$) decreases in intra- and peridomiciliary *Lu. longipalpis* in Fortaleza, respectively. The reduction outside houses was expected, because *Lu. longipalpis* feed less on dogs with collars and resort to feeding on other domestic animals, livestock or humans *(23)*. The cost–effectiveness of this control strategy depends on the local transmission intensity, and it should always be used within the framework of integrated vector control, including environmental and dog population management.

A cluster-randomized trial of dog collars in a region of intense transmission in Brazil reduced the canine seroinfection incidence rate by 36% and the domestic/peridomiciliary female *Lu. longipalpis* abundance by 43% *(25)*.

2.2.3 Sylvatic and anthroponotic transmission of VL in Eastern Mediterranean and African regions

The transmission of VL in high-burden East African countries (Ethiopia, Kenya, Somalia, South Sudan, Sudan and Uganda) involves several vectors and occurs in two distinct ecological settings: the Acacia–Balanites savannah regions in the north, where *Ph. orientalis* is the major vector; and the savanna and forest areas in the south, where *Ph. martini* and *Ph. celiae* are found in association with *Macrotermes* termite mounds. The Sudanese states of Al Gedarif, Blue Nile and Sennar on the border with Ethiopia are reported to have high VL transmission, in part due to the acacia forests, as do the Ethiopian agricultural lowland states of Amhara and Tigray, where large families live in crowded, poor conditions favourable for sand flies. The potential non-human sylvatic reservoir hosts have not yet been fully identified. Unfortunately, effective control measures for sand fly vectors of *Le. donovani* in East Africa are lacking, and there has been limited evaluation of the available tools *(26–31)*. Although sand fly vectors are predominantly exophilic, the integrated vector management programme in Sudan principally targets malaria vectors that are endophilic: IRS with a bendiocarb in irrigated areas in the targeted states twice a year in June/July and December and distribution of long-lasting insecticidal nets (LLINs) to each household every 3 years in rotation. The timing of the two IRS rounds does not coincide with sand fly seasons. There is some evidence to suggest that pyrethroid-impregnated bednets provide effective personal protection against bites of *Ph. orientalis* *(27, 32)*, but residents use ITNs predominantly in the rainy season against nuisance malaria mosquitoes *(28)*. Importantly, householders tend not to use ITNs in the sand fly biting season (April–June) because of the intense heat *(30)*, when they usually sleep outside. Seasonal migrant agricultural workers and migrant populations in general do not use bed nets *(33)*. There is no VL vector control programme in Ethiopia, although ITN distribution and IRS applications are conducted in the context of malaria control *(34, 35)*. As IRS is not an appropriate intervention for exophilic species, use of targeted outdoor residual spraying of houses and village boundary fencing was explored in a pilot study in Sudan against *Ph. orientalis*, with some success *(36)*.

2.2.4 Anthroponotic transmission of CL in the Eastern Mediterranean and European regions

In the Eastern Mediterranean and European regions, CL is transmitted by one main vector, *Ph. sergenti*. Although it is classified as anthroponotic, the contribution of non-human reservoirs to transmission should be explored further *(37)*. In most foci of *Le. tropica*, the vector is strongly endophilic *(38)*, and IRS and ITNs have been effective. In a cluster-randomized trial in Morocco of the relative efficacy and cost–effectiveness of IRS and LLINs relative to "standard of care environmental management", IRS was more effective and more cost–effective for the prevention of CL *(39)*. A study in the Syrian Arab Republic, however, showed that *Ph. sergenti* was more exophilic than previously reported, suggesting a possible change in behaviour in response to prolonged IRS *(40)*.

2.2.5 Peridomestic or sylvatic/zoonotic transmission of CL in the Region of the Americas

Transmission of CL in the Region of the Americas involves multiple vectors and multiple reservoir hosts, some not yet identified, which poses many challenges to programme managers. The lack of knowledge makes it difficult for vector control programmes to target interventions and so, in response to CL outbreaks in non-Amazonian Brazil, residual insecticides are sometimes sprayed on the inside and outside walls of at-risk houses by teams trained to target the specific resting places of the vectors of VL (*Lu. longipalpis*), arboviruses (mosquitoes) or Chagas disease (triatomine bugs). Such interventions may be better than no response in regions endemic for CL caused by *Le. braziliensis*, where there can be relatively high peridomestic densities of one or more of the incriminated vectors: *Lu. intermedia* (syn Nyssomyia *intermedia*) (northeast and southeast Brazil), *Lu. neivai* (syn Ny. *neivai*) (central-west, southeast and south Brazil, Argentina and Paraguay), *Lu. whitmani* (syn Ny. *whitmani*) (northeast, central-west and southeast Brazil) and *Lu. migonei* (syn *Migonemyia migonei*) (in all of those regions) *(41, 42)*.[1] The impact of these interventions, however, should be evaluated. In Peru, a trial of household vector control was conducted to assess how indoor spraying of walls and ceilings with lambda-cyhalothrin affected the risk of residents for contracting CL *(44)*. Spraying significantly reduced the indoor abundance of *Lu. peruensis* and *Lu. verrucarum*, by an average of 83% and 78%, respectively, the proportion of blood-fed sand flies (77%) collected in light traps and the proportion of susceptible householders who acquired leishmaniasis (54%) *(44)*. Similarly, in the Bolivarian Republic of Venezuela, catches of female, male and blood-fed female sand flies were significantly lower in sprayed than in control houses immediately after treatment, but the numbers of male and female flies recovered in sprayed houses to the levels in control houses after 7 and 11 weeks, respectively *(45)*.

2.2.6 Sylvatic or zoonotic transmission of CL (*Le. major*) in the Eastern Mediterranean and European regions

In the Eastern Mediterranean and European regions, the vector for transmission of CL is mainly *Ph. papatasi*, but other vectors are involved in transmission (**Annex 2**), with different reservoir hosts in different areas. The proximity of houses to sources of infected sand flies greatly increases the risk of infection. In new urban developments, houses on the periphery, close to "undeveloped" or undisturbed desert where rodent burrows are found have the greatest number of cases of zoonotic CL. As conurbations expand, the risk moves outwards with the moving edge of development. Because of the close association of sand flies with their rodent hosts and the propensity of sand flies to rest and breed in rodent burrows, destruction of rodent burrows has been attempted as a means of disease control *(46)*. It is generally accepted that most sand flies travel < 1 km from their breeding sites, which contributes to the geographically discontinuous nature of leishmaniasis foci, with characteristically small, separate foci near reservoir host habitats. Knowledge of the flight range was used in the control of zoonotic CL in the Jordan Valley, where *Ph. psammomys* burrows were destroyed by flooding and ploughing in a radius of 1–1.5 km around two small towns *(47)*.

[1] Although the single genus name *Lutzomyia* is maintained for all species in the Americas, there is consensus among Galati E and other entomologists *(43)* that it comprises different genera.

2.3 Lessons

- Appropriate, effective vector control must take into consideration the biology and behaviour of sand flies as well as their temporal and spatial distributions.
- The primary methods used for leishmaniasis vector control worldwide are IRS, ITNs, insecticide-impregnated collars and targeting reservoir hosts. Selection of a method depends on the relative importance of indoor and outdoor transmission of disease.
- To ensure judicious use of available resources, selected vector control interventions should coincide with peaks in vector abundance.
- The full potential and maximal impact of vector control for leishmaniasis elimination or control has not yet been realized in many regions.
- Vector control programmes should be realigned to optimize delivery of interventions in local contexts and be part of an integrated vector management framework, as discussed below.

3. Vector surveillance

3.1 Vector surveillance in leishmaniasis control and elimination

Purpose of vector surveillance

One of the four pillars of action in the *Global vector control response 2017–2030* is enhancement of vector surveillance and monitoring and evaluation of interventions *(48)*. The aim of vector surveillance with respect to leishmaniasis is to:

- determine the geographical distribution of sand fly species;
- study vector bionomics in different eco-epidemiological settings in relation to the programme, such as seasonal prevalence, resting behaviour (endophily, exophily), feeding behaviour and host preferences (anthropophily, zoophily);
- incriminate vectors (pathogen detection and identification);
- monitor insecticide resistance and detect resistance mechanisms;
- measure temporal changes in the presence and abundance of sand fly vector species, particularly in view of climatic and environmental changes caused by natural events and/or development;
- assess vectorial potential and level of transmission;
- monitor the durability of interventions, e.g., of ITNs and insecticide-treated dog collars, and of the residual action of IRS;
- monitor and evaluate the impact of vector control interventions on vector populations and thus disease transmission and outbreak containment; and
- assess the risk of enhanced transmission of leishmaniasis in areas of low endemicity or of reintroduction of transmission in disease-free areas.

Integration of vector surveillance

The operational feasibility of integrating vector surveillance can be explored in areas where leishmaniasis is co-endemic with other vector-borne diseases such as malaria and dengue. Where possible and required, activities that could be integrated include identification and calculation of the indices of various vectors and sand flies, monitoring insecticide resistance and assessment of the entomological impact of interventions such as IRS and ITNs.

Similarly, the health workforce engaged in sand fly surveillance can be used for entomological surveillance for other diseases, with clear reporting indicators. An integrated national report on vector control interventions and entomological surveillance for several vector-borne diseases can also strengthen and promote the concept of integrated vector surveillance.

A leishmaniasis control programme should be carefully monitored and reviewed and be responsive to relevant findings from basic and applied research on entomology and vector control. The requirement for entomological and cross-sectoral workforces for vector surveillance should be appraised in a needs assessment *(49)*, and a national vector control strategy should be developed *(50)*. When the prevalence of a targeted disease decreases, the cost of sustained surveillance and control to protect each person at risk will increase. It is therefore crucial to maximize the use of resources by using an integrated approach, e.g., by deploying the same personnel and choosing interventions that could be used against more than one vector-borne disease.

3.2 Methods and procedures

Selection of better vector surveillance methods depends on the operational questions asked; the feasibility, accuracy and efficacy of the method used; the frequency of sampling; the availability of sampling tools; the duration of collection required and the number of sentinel or randomly selected sampling locations. Other considerations include the biology and behaviour of vectors, housing conditions and human behaviour, the environmental conditions, absence or position of breeding sites, insecticide spraying and presence of domestic animals *(51)*. The relative cost–effectiveness of continuous surveillance or targeted monitoring is another important consideration. The sand fly sampling methods can be qualitative and/or quantitative. Not all entomological parameters are required to be monitored or can be feasible under programme conditions.

The main surveillance methods are described briefly below; further details are provided elsewhere *(51, 52)*, and additional resources are listed in **Annex 6**. Comprehensive manuals on entomological surveillance and control activities required for both VL and CL developed in consultation with entomologists and the Pan American Health Organization may also be useful and applied in other regions *(53, 54)*.

3.2.1 Field sampling methods for adult sand flies

Aspirator collections

Live female and male adult sand flies can be collected with, preferably, a battery-operated mechanical aspirator and a torch light while they are resting on indoor surfaces in human dwellings, animal shelters and buildings *(51, 52)*. Mouth aspirators can also be used but have the disadvantage of sucking dust and allergens from walls. Battery-operated mechanical aspirators function similarly to mouth aspirators but are safer for the operator. This method is suitable for collecting live specimens of wild sand flies for species identification and testing for susceptibility to insecticides *(51, 52)*. It is, however, relatively labour-intensive and, if aspiration is not done carefully, can damage specimens being sucked into the aspirator tubes. To avoid exposure to allergens, battery-operated aspirators with low suction pressure can be used. Collected sand flies can be released into paper cups lined with fine mesh netting for further study. The peak collection times range from dusk to dawn but can be timed to determine where and when sand flies rest *(51, 52)*.

Light traps

Battery-powered light traps are commonly used to collect host-seeking, moving sand flies or gravid females and males overnight (dusk to dawn) *(51, 52)*. The method can be used to collect sand flies both indoors and outdoors, and sand flies can be separated by hour of collection *(51, 52)*. Sand flies are attracted by a light source in a trap usually placed about 1 m above the ground *(55)*, with the bottom of the collecting device about 15 cm from the ground *(56)*, in the room in which most inhabitants sleep, thus attracting more vertically resting or active sand flies. Light trap collections capture mainly blood-searching females; therefore, population densities may be overestimated, and both live and damaged specimens are collected *(51, 52)*. This method can provide quantitative estimates and can be used for longitudinal assessment of the impact of vector control interventions. Light bulbs can be removed to reduce capture bias *(51, 52)*.

Sticky traps

Sand flies are collected on paper or cardboard soaked in mineral or vegetable oil near their main breeding or resting habitats or on interception traps along flight paths placed vertically off the ground or horizontally at ground level overnight *(51, 52)*. This is an inexpensive, quantitative method. Its disadvantages are that no live specimens can be collected, and high relative humidity can affect longitudinal collection of sand flies; it does, however, avoid trap bias due to the attraction to light in CDC traps. This method has low sensitivity, as many sticky traps are required to collect sand flies *(51, 52)*.

Animal-baited traps

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 animals used as a bait. The method is useful for collecting specimens for entomological studies such as host preference studies (by careful methodology) and insecticide resistance testing *(51, 52)*. Alternative designs are commercially available. A potential disadvantage of this procedure is that an animal is required as a bait *(51, 52)*.

Human landing catches

This method is not recommended under operational programme conditions but can be useful for measuring human biting rates for vectorial capacity assessment or experimental hut trials of insecticidal products such as ITNs and IRS products, provided ethical approval of use of the method is obtained *(51, 52)*. Female sand flies seeking human blood meals are collected with aspirators as they attempt to land on a volunteer's exposed arm or leg. Use of the method is discouraged for ethical reasons, as infective sand flies attempting to feed on a human volunteer could inoculate parasites when blood-feeding *(51, 52)*. A proxy approach is to use a light trap set up near a mosquito net under which a human volunteer sleeps during the collection. The volunteers should preferably be from the same area in which the collections are being made because they are habituated to local sleeping places and are expected to have greater immunity due to past exposure than non-immune people from outside areas who will be at greater risk of infection *(51, 52)*.

3.2.2 Identification and preservation of adult sand flies

Correct identification of phlebotomine sand flies is a critical first step in processing samples collected in the field during vector surveillance. Usually, collected sand flies are preserved for studies in 70% non-denatured alcohol at room temperature *(51)*. Dead sand flies can be preserved in plastic tubes or glass vials with silica gel crystals for further investigation. Detailed information on taxonomic identification and preservation of sand flies is beyond the scope of this manual.

3.2.3 Field sampling methods for immature sand flies

Field sampling of immature sand flies is not suitable or preferred under programme conditions but may be used for research on the biology and ecology of sand flies. It involves placing emergence traps over potential breeding grounds or collecting soil for laboratory emergence of sand fly adults *(51, 52)*.

3.3 Organizing vector surveillance

3.3.1 Selection and identification of surveillance sites

Entomological surveillance should be organized in an integrated fashion with other prevalent vector-borne diseases to allow optimal use of resources, better surveillance and monitoring data and the possibility of combining interventions with those for other vector-borne diseases. The following points should be considered in selecting surveillance sites.

- transmission ecology (detection of primary and secondary vectors in studies of vector competence)
- the endemicity of disease
    - high-transmission or high-incidence areas
    - low- and moderate-transmission areas
    - areas of significant economic importance (e.g., agro-forestry schemes, tourist areas, mining, urban areas)
- areas at risk of disease outbreak; e.g., new human settlements in endemic areas due to migration or disease-free areas near or contiguous with endemic areas
- prevalence of other vector-borne diseases in targeted areas

The following operational factors should also be considered.

- cost and availability of resources
- logistics, including transport
- availability of trained entomological staff
- capacity of laboratories and entomological staff to perform taxonomy and respond rapidly to surveillance by identifying collected vectors

- equipment and supplies for vector collection and conservation, e.g., light traps, tents/nets for baited trap collection, kits for testing insecticide susceptibility, cages
- accessibility of sand fly collection sites.

If vector surveillance sites selected in areas with high transmission or high disease incidence currently have low or moderate transmission or disease incidence, depending on resources, one or more sites in high-transmission areas should be identified to monitor trends and factors responsible for high transmission or in outbreak areas, e.g., occurrence of the first case of VL in a human or dog.

Site selection could be per unit population or administrative unit (e.g., district), or the entire ecological area could be divided into entomological zones (e.g., groups of districts with similar agro-ecological features).

3.3.2 Frequency of surveillance

Data should be collected for comparison of surveillance sites at least annually, although the frequency of surveillance depends on the:

- ecology of the natural cycle of leishmaniasis
- seasonality of the vector population
- environmental factors (e.g., prolonged drought or prolonged, excessive or untimely rain)
- demographic and socioeconomic factors, e.g., population density and large agglomerations due to socio-cultural reasons or new economic development
- expected duration of efficacy of the vector control intervention, e.g., the residual lethal action of IRS on vector populations might have to be monitored for several weeks, or the durability of ITNs might have to be monitored for ≤ 3 years.

3.3.3 Type of surveillance activities

Although many programmes lack entomological expertise and capacity, it is crucial to conduct routine vector surveillance, monitoring and evaluation of interventions at various stages to: obtain baseline data (preliminary surveys), evaluate the programme (trend investigations), identify hotspots of infection, monitor insecticide resistance (spot checks) and check for new or reintroduced vectors *(57)*.

The vector surveillance activities defined for integrated vector management in sub-Saharan Africa (**Table 1**) are generally applicable for leishmaniasis, with measurement of similar parameters (**Table 2**).

Table 1. Vector surveillance activities required for different programme objectives

	Preliminary survey	Trend or regular observations		Focus investigation	Spot check
Definition	Short-term survey with a limited number of techniques in areas for which there is little or no recent information on vector(s)	Long-term observations in fixed locations to follow trends in vector density, species distribution and behaviour over time		Short-term investigation in established foci of transmission	Rapid survey with a single technique to detect vector resurgence or transmission potential
Objectives	Delineate areas with vector-borne diseases Initiate collection of baseline data before an intervention Plan control measures	Areas with no vector control measures: establish baseline information on role of vector in transmission, geographical and seasonal distribution, feeding and resting behaviour and susceptibility to insecticides.	Areas with vector control measures: monitor and evaluate the effect of control measures on entomology)	Reactive measures as part of entomological investigation to determine the reasons for: lack of response to vector control measures; persistence of vector-borne disease transmission or recurrence, and unexpected increase in case incidence (outbreak)	Proactively identify areas with operational shortcomings or detect changes in effectiveness of control measures due, e.g., to insecticide resistance. Check existence and/or density of vectors in receptive and vulnerable areas.
Parameters measured	Vector density Vector geographical distribution	Vector density (seasonal) Vector feeding and resting behaviour Vector habitats Infection of vectors Susceptibility to insecticides	Changes in vector density Changes in vector infection rate Susceptibility to insecticides	Vector density Vector feeding and resting behaviour Vector infection rate Susceptibility to insecticides	Vector presence and density or absence Vector geographical distribution Infection of vectors Susceptibility to insecticides
Where to be implemented	1. Areas designated for vector-borne disease control 2. Areas for which little or no recent information on vectors is available	In fixed sentinel sites (villages) identified in a preliminary survey. Villages should be sited in a larger area in which parasitological observations have been made.	In same sentinel sites (villages) as for baseline	1. In representative localities in areas with no response to vector control measures 2. In all locations with persistent or recurrent transmission	1. Prioritize areas with high transmission potential and weakness in control measures are suspected or where recurrence has been detected 2. In selected locations in areas suspected of high transmission potential

Table 1 (cont'd). Vector surveillance activities required for different programme objectives

	Preliminary survey	Trend or regular observations		Focus investigation	Spot check
When to be implemented	Start in season of expected high vector prevalence	As soon as information from the preliminary survey is available	After application of vector control measures	As soon as the epidemiological investigation indicates the presence of active foci of transmission (for 1) or persistence or recurrence of disease transmission (for 2)	1. During season of high prevalence of vectors, with a lapse after application of control measures 2. During the period of high vector prevalence and during the period of influx of sources of infection into areas

Source: adapted from references *57* and *58*

Short-term: an arbitrary period, which can be as short as one time activity.

Long-term: an arbitrary period in which surveys are done on a regular basis depending upon resources but at-least once on annual basis.

Table 2. Sand fly parameters to be measured in vector surveillance or entomological assessment

Parameter	Questions answered	Measurement method
Vector density	Presence and geographical distribution of vector species	Catches of adult sand flies by aspirator, light trap, sticky trap Longitudinal density surveys Species identification (morphology, molecular and MALDI-TOF MS[a])
	Effects of season on vector species prevalence and relative abundance of gravid females	
	Impact of intervention on vector abundance	
Vector feeding and resting behaviour	Preference for human or animal blood (domestic/other)	Animal- or human-baited traps (ethical considerations and trap placement should be taken into account during analysis) Laboratory test, e.g., ELISA, to determine the origin of blood meals
	Indoor or outdoor feeding	Indoor vs outdoor biting rates in human landing catches or human baited net-traps (ethical considerations required) or double tent traps
	Frequency of feeding. Timing of feeding	Vector density catches over 24 h
	Resting sites of vectors	Adult resting catches, e.g., in traps or mechanical aspirators
Transmission of disease	Sand flies (proven vector) infected with pathogen	Microscopic examination or molecular and other experimental studies
	Sand flies (proven vector) infectious with pathogen	
Insecticide resistance	Phenotypic resistance profile of the vector(s)	Bioassays (adapt from the WHO tube test or WHO bottle bioassay for mosquitoes (*59*, *60*)
	Insecticide resistance of sand flies	Synergist assays Biochemical enzyme assays Molecular diagnostic assays, where available, e.g., with PCR or quantitative PCR
Efficacy of insecticide interventions	Insecticide active ingredient on sprayed walls or in ITNs	High-performance liquid chromatography WHO common analytical method (*61*) WHO cone bioassays (*59*)
	Residual efficacy of sprayed walls; durability of ITNs	

[a]MALDI-TOF MS: matrix-assisted laser desorption ionization–time-of-flight mass spectrometry; ELISA, enzyme-linked immunosorbent assay; PCR: polymerase chain reaction.

3.4 Monitoring insecticide resistance

3.4.1 Insecticide susceptibility test methods

The most common assessment tools are laboratory bioassays for assessing the response of live insects to insecticide. In the absence of sand fly-specific WHO susceptibility test procedures and impregnated papers, phenotypic bioassays with wild sand flies are conducted with WHO susceptibility test kits with procedures developed for mosquitoes *(59, 60)*. The WHO tube test is currently the standard method for monitoring resistance in sand flies in the field. WHO susceptibility test kits are available from a WHO-coordinated facility in Malaysia *(62)*.

Another phenotypic assay is the bottle bioassay developed by the CDC for mosquitoes (63), which involves coating glass bottles with insecticide. It is not widely used operationally and has not been validated with sand flies. The insecticide concentrations are prepared by and easily varied by the user, rather than use of pre-prepared insecticide-impregnated papers in WHO bioassays. The results of the two bioassays may not be directly comparable. As originally proposed, the CDC assays measure knockdown at the end of a predefined time, whereas WHO bioassays measure mortality at 24 h (or 72 h for slow-acting compounds). These two end-points are difficult to compare, but comparability improves if a 24-h mortality end-point is used. Comparability nevertheless remains far from complete *(64)*.

Phenotypic assays may be supplemented by generic tests for the presence of resistance mechanisms in either synergist bioassays, e.g., with piperonyl butoxide, in which pre-exposure to a non-insecticidal compound is used to block the action of enzyme families linked to resistance *(65)*. Although easy to apply, interpretation of synergist bioassays can be difficult because of uncertainty about the enzyme families affected. Biochemical assays detect increases of activity of enzyme families, such as monooxygenases, glutathione-S-transferases and esterase, in wild samples as compared with a laboratory-susceptible strain. This, and the requirement for a cold chain, limit application in the field. Biochemical assays also lack sensitivity, because, although only a few members of an enzyme family might be involved in resistance, the activity of the entire family is assessed.

Molecular diagnostic tests for specific resistance-associated genes or mutations may be the most sensitive for assessing early-stage resistance and resistance dynamics. DNA markers are the most readily applicable in the field but are rarely available for mechanisms other than target site mutations. Considerable work in discovery and calibration work may be required to identify specific resistance-linked variants for assay design *(66)*.

Technical and operational challenges and a way forward for testing and monitoring insecticide resistance in sand flies are summarized in Box 2.

Box 2. Challenges and way forward in monitoring resistance in sand flies

Assessment of the susceptibility and resistance of sand flies to insecticides has been hampered by a lack of validated data on diagnostic doses or concentrations, except for suggested concentrations of DDT and dieldrin to assess resistance in *Phlebotomus* spp. *(67)*. Although it is unclear how broadly applicable these concentrations are to all species, the concentration and exposure time for DDT (4% for 1 h) has become widely used and accepted *(68)*. Denlinger et al. *(69)* used long-established susceptible strains of *Lu. longipalpis* and *Ph. papatasi* from the Walter Reed Institute to establish diagnostic concentrations and exposure times for a range of insecticides in CDC bottle bioassays. For most of the insecticides assessed, the concentration for 100% knockdown was similar to or less than that required to cause 100% mortality at 24 h. For DDT, 100% mortality was two orders of magnitude higher, however, highlighting the problem of using knockdown as the end-point *(69)*. Although the bioassay bottles used were larger than standard bottles of 250 mL and only single strains were tested, the study provides valuable baseline data.

An alternative used in some studies has been to establish a baseline concentration or time with field samples early in monitoring work for comparison of future collections (e.g., *(46)*). This allows identification of changes in phenotypes but may result in an underestimate of resistance if the samples with which the baseline was set were not fully susceptible. If, using this approach, it is better to perform tests prior to (re)introducing widespread use of any class of insecticide. An analogous approach is to compare collections from different areas to find significant differences in resistance ratios (e.g., *(70)*). As with temporal variation, significant spatial variation suggests development of resistance; however, if the least resistant population examined is not truly susceptible, resistance may again be underestimated. Another approach adopted for some studies was to use discriminating concentrations recommended by WHO for mosquitoes; however, its operational applicability for sand flies is unclear, as in this approach it is implicitly assumed that the tested sand fly species is as or more susceptible than *Anopheles*. This may be true for DDT and dieldrin *(67)* and might be the case more generally, because sand flies are likely to fly less than mosquitoes in bioassays and spend more time in contact with the substrate *(71)*.

Lack of understanding of the mechanisms of resistance of sand flies to insecticides is also due to the paucity of diagnostic markers of resistance. Three knockdown resistance (*kdr*) mutations in the para voltage gated sodium channel were identified in *Ph. argentipes* in Bihar, India, which change the wild-type leucine at codon 1014 to either serine or phenylalanine, encoded by either of two nucleotide variants *(72)*. The mutations appear to be largely recessive, and the markers, for which TaqMan qPCR assays are available, are good predictors of resistance to DDT and tolerance of pyrethroids *(72)*. These markers are used in a sensitive, specific diagnostic test for high-throughput screening of samples to monitor changes in resistance. Sequencing of the same area of the gene resulted in identification of the 1014F *kdr* mutation in Turkish, but not Greek, *Ph. papatasi* samples *(73)*; an association of the mutation with resistance phenotypes is probable but remains to be determined.

In the absence of discriminating concentrations of most insecticides for sand fly vectors, WHO is conducting a multi-centre study to determine discriminating concentrations of certain insecticides for monitoring resistance in sand flies.[1]

[1] The report of the study and recommendations on discriminating concentrations for sand fly species are expected to be available in due course from: *https://www.who.int/teams/control-of-neglected-tropical-diseases/interventions/strategies/vector-control/insecticide-resistance*). This will address a major technical gap.

3.4.2 Selection of sites

Routine monitoring of insecticide resistance should be conducted with WHO tube tests or bottle bioassays at carefully chosen sentinel areas or sites in areas of significant disease incidence and, in some cases, variation in habitat. In areas where different interventions are conducted or there are intervention and non-intervention sites, all should be covered in order to compare the effect of the selected insecticide on resistance. Within selected areas, sites for monitoring resistance should be selected according to eco-epidemiology, geographical access and abundance of sand fly populations, especially during the main transmission seasons. Sand flies from each sentinel site should be tested separately. Susceptibility testing should be conducted at least annually.

3.4.3 Insecticides for testing susceptibility

The insecticides to be tested depend on past and current use of different insecticidal products for control of vector-borne diseases and also the insecticides used in agriculture. It is advisable to test the susceptibility of at least one compound in each insecticide class. 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 for insecticide resistance management (IRM).

3.4.4 Collecting and handling wild sand flies for testing

Adult wild sand flies are collected from houses, outbuildings and, if possible, other nearby shelters, such as bamboo stubs and animal burrows, preferably with mechanical aspirators and targeting non-blood-fed females when possible. The flies are transported to a laboratory, where they should be maintained in cages or paper cups with access to 10% sugar solution on soaked cottonwool pads. Owing to the difficulty of rearing sand flies in laboratories to obtain F1 females, testing is usually done with wild-caught females. This has the advantage of being faster and logistically easier as well as more operationally relevant in terms of the age distribution of the collections and for linking results to the response to the insecticides used for vector control. The age, physiological condition and previous exposure of the sand flies is, however, unknown, which is likely to lead to greater variance in the results.

Wild-caught females may also be infected with *Leishmania* parasites and should be handled carefully. Where feasible, as an alternative, F1 progeny from wild-caught females can be used. This has the advantages of a standard age (usually 2–7 days) and ensuring sufficient numbers for testing when the density is low. The major disadvantages are the difficulty of culture and the time required; furthermore, if only a few females are collected in the field, there will be many closely related individuals, thereby reducing the statistical independence of lots of test insects.

When several species of sand fly are collected simultaneously, they should be identified morphologically when possible before testing and should be tested separately, or the species of interest partitioned for testing. If this is not possible, morphological identification should be conducted at the end of the bioassay. Larger numbers of sand flies may be tested to ensure adequate representation of the species of interest.

3.4.5 Susceptibility testing

Tests are run in a laboratory at 27 ± 2 °C and 75 ± 10% relative humidity, which are recorded at the time of testing, as they may affect bioassay results. Wide deviations should be avoided and the climatic conditions in the testing laboratory maintained. For each insecticide, batches of 20–25 female sand flies should be tested, with four or five replicates, for a target of 100 females tested per species and insecticide. In bioassays, batches of sand flies are held for 1 h to acclimatize in holding tubes marked with a green dot. Thereafter, they are exposed to insecticide-impregnated papers in exposure tubes marked with a red dot. Knockdown is recorded at 60 min. All live and dead sand flies are carefully returned to the holding tubes and maintained with sugar solution, and mortality is recorded at 24 h (or 72 h for slow-acting insecticides) after 1 h of exposure. Two control tubes with 25 females per tube are run at the same time.[1]

3.4.6 Recording and reporting mortality

Sand flies are recorded as dead or knocked down if they are lying on their backs or cannot stand or move in a coordinated manner. Sand flies are recorded as live if they are capable of coordinated movement and flight. Percentage mortality is recorded as:

(number dead / number tested) x 100.

Control mortality < 5% can be ignored, but, if it is between 5% and 20%, Abbott's formula *(59)* should be used to correct the mortality results. Corrected percentage mortality is calculated as:

((% test mortality – % control mortality) x 100)/100 – % control mortality)

If control mortality exceeds 20% in any replicates of tests, the results from that replicate should be recorded as invalid and discarded and the tests repeated.

Reported mortality should always include the number of sand flies tested and preferably also the 95% confidence interval.

The results are interpreted according to the WHO guidelines for mosquitoes *(59)*:

- Mortality in the range 98–100% indicates susceptibility.
- Mortality of 90–97% suggests possible resistance, and confirmation is required in additional tests or in molecular assays for known resistance mechanisms. If at least two additional tests consistently show mortality < 98%, resistance is confirmed.
- Mortality of < 90% confirms the presence of resistance.

3.4.7 Reporting data to WHO

There is no global database on the resistance of sand flies to insecticides. This is due primarily to the fact that data are not reported to WHO. High-quality data are necessary on the resistance of CL and VL vectors to insecticides, obtained according to WHO guidance and standard operating procedures. The data should be reported to the respective WHO country office to create regional databases and a global database, so that interactive threats maps can be prepared (see section 6).

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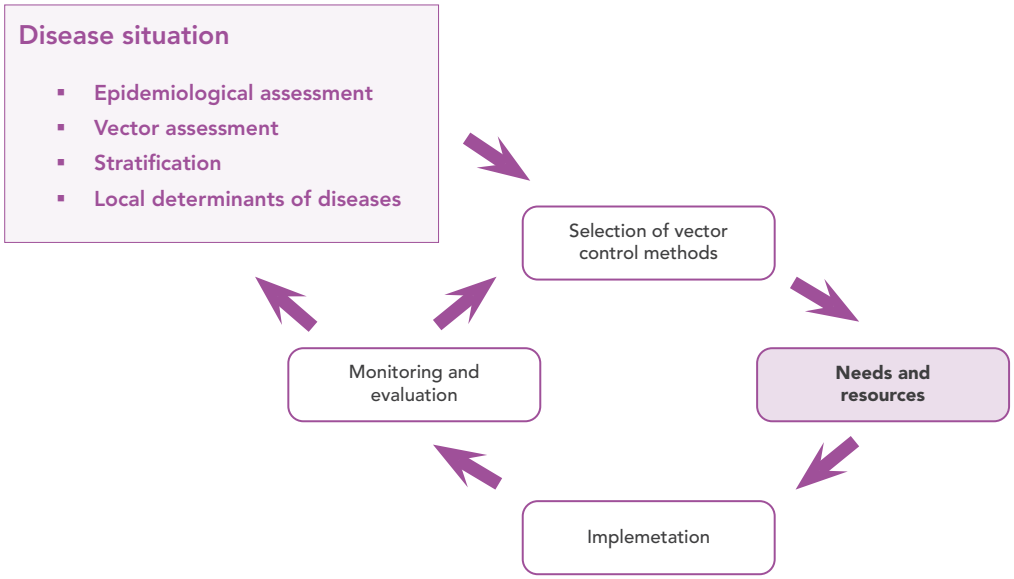
[1] WHO standard operating procedures for testing the susceptibility of sand flies to insecticides in WHO tube test and bottle bioassay will be available in due course from: *https://www.who.int/teams/control-of-neglected-tropical-diseases/interventions/strategies/vector-control/insecticide-resistance*).

3.5 Core capacity required for vector surveillance and control

After selection of locally appropriate vector control interventions, a needs assessment for vector control should be conducted to define the financial resources, human resources and infrastructure (research, training, technical and operational facilities) required for the programme (see **Fig. 1**) *(57)*. Stakeholders should be identified at the beginning and consulted throughout the intervention to avoid any duplication of effort and ensure a greater impact of limited resources. WHO has published a framework for needs assessment in vector control programmes (**Fig. 1**) *(49)*.

Fig. 1. Example of a needs assessment procedure



Source: reference *57*

3.5.1 Financial resources

The adoption and maintenance of any new vector control method will require significant financial resources, at both the outset of the programme and throughout its implementation. All new vector control activities should be conducted within integrated vector management to ensure efficient spending for vector-borne diseases and to prevent duplication of costs.

The exact financial requirements will depend on the vector control method selected; some interventions, such as IRS, require a high annual financial commitment, whereas others, such as LLINs, require a large initial investment but lower maintenance costs until the next distribution campaign. For IRS, the financial resources should cover at least the following:

- salaries, training and supervision of personnel
- procurement of the insecticide and quality control
- procurement and maintenance of hand compression sprayers

- personal protective equipment for spray personnel
- insecticide mixing and storage facilities and equipment
- transport
- procurement of paints and equipment for marking houses
- community information, education and coordination
- computer(s) for data management.

As two or more rounds of IRS are usually required within a single year, spray equipment will have to be replenished and maintained regularly, representing a high continuing financial commitment.

3.5.2 Human resources

Leishmaniasis vector control requires skilled staff centrally and locally and partnerships with other government departments, nongovernmental organizations (NGOs), industry and research institutes. While strong leadership is essential, it is important that the programme not rely on only a few key individuals.

Collaboration among control programmes and research institutes and other partners should be strengthened, with formal agreements where required. When a programme is implemented in an integrated vector management framework, human resources can be shared, increasing the impact and career structures of staff, which will improve retention in the control programme (see **Fig. 2**).

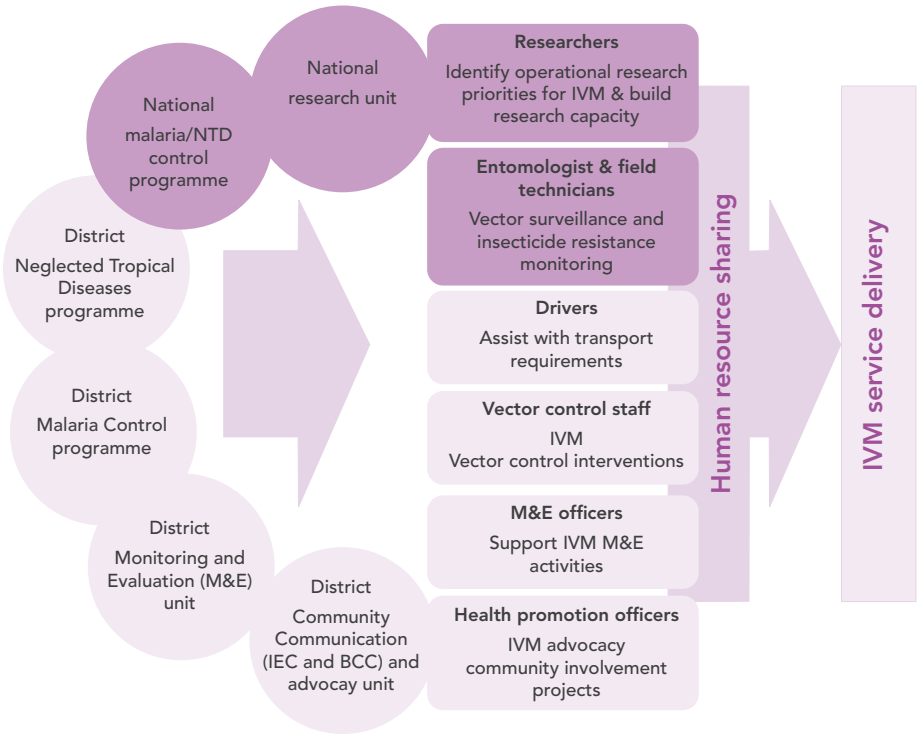
The human resource requirements of the programme depend on the vector control tool(s) selected. For example, programme managers must have the capacity to plan and manage the programme, including procurement and quality control of the insecticide to be used or ITNs to be supplied. IRS requires, at a minimum, trained personnel capable of the following functions:

- selecting target areas
- applying the insecticide in targeted areas (spray teams)
- supervising spray teams and team movement
- maintaining spray equipment
- maintaining records
- monitoring resistance
- assessing impact.

Substantial financial and logistical resources are required to support these roles, including investment in the training and retainment of spray personnel (if IRS method is used). Where appropriate, community members and other stakeholders can be engaged to support these functions.

The WHO manual *Core structure for training curricula on integrated vector management (74)* can be used to guide general training of new staff members. WHO has also published other resources and training manuals for vector control interventions, including IRS *(57, 75, 76)*, that can be adapted to local leishmaniasis control programmes.

Fig. 2. Example of sharing resources for maximum impact in national programmes for control of malaria and/or neglected tropical diseases



Source: reference *57*

BCC: behavioural change communication; IEC: information, education and communication; IVM: integrated vector management: M&E: monitoring and evaluation; NTD: neglected tropical diseases.

3.5.3 Infrastructure and capacity-building

Training should be guided by the needs assessment conducted at the onset of the programme to ensure that it is directly relevant. Gaps in capacity are often not limited to entomology and epidemiology but may include project management, geographical information systems and information technology for effective data collation and response, as seen in malaria control. These skills are essential for better targeting of resources spatially and temporally. Capacity development in entomological surveillance is also essential to ensure that interventions are conducted in accordance with vector distribution, behaviour and ecology and for evaluating the impact of interventions, including insecticide resistance.

Local and international institutions can support capacity-strengthening and any operational research required through a network of mentoring and training opportunities for staff, including entomologists and evaluation staff. Offering these opportunities is essential to retaining staff and encouraging a high quality of work. Staff retention is essential for developing institutional memory; when staff turnover is high, capacity will remain low.

Cross-border collaboration between states (when control activities are coordinated at state, rather than national, level) and/or countries can be useful for sharing experiences and training resources.

Implementation of *Leishmania* vector control within an integrated vector management framework also ensures that infrastructure is shared for different diseases and that duplication and financial waste are minimized. The infrastructure required, in addition to the control method(s) selected, is therefore dictated in part by what is already available for other vector-borne diseases, such as malaria.

3.6 Lessons

- A needs assessment of the financial, human and infrastructure resources required at national and subnational levels, guided by the WHO *Framework for a national vector control needs assessment (47)*, should be conducted at the onset of any new vector control programme.
- Vector control activities should be implemented within an integrated vector management framework to minimize duplication and resource waste.
- Multisectoral partnerships involving health and non-health government departments, NGOs, research institutes and industry should be established to foster sharing of resources and knowledge and to maximize the impact of the programme.
- Staff retention is essential to maintaining the capacity of a vector control programme.
- Any new vector control activities will require significant financial resources. Maintenance costs differ significantly according to the control method selected.
- Given the heavy reliance on insecticidal interventions – primarily ITNs and IRS – the resistance of local vectors to insecticides is a key consideration in planning and implementation.

4. Vector control and management of insecticide resistance

4.1 Policy considerations

For effective sand fly control, an integrated vector management approach should be adopted, which includes the following components:

- a vector assessment for evidence-based planning of control operations, which requires knowledge of local epidemiology *(77)*;
- capacity-building for vector control planning, management, monitoring and evaluation;
- use of safe, cost–effective vector control tools and methods targeting leishmaniasis and other co-endemic vector-borne diseases. (In practice, however, coordination among malaria, leishmaniasis and arbovirus/dengue control programmes in countries may be minimal *(77)* and should be improved to include the biology of each vector group or species.);
- intra- and inter-sectoral collaboration among health and non-health sectors;
- a favourable regulatory environment, e.g., for evaluation, procurement, registration, quality control, storage, distribution and sales, procurement and quality control of spray equipment, use of pesticides in agriculture;
- a national vector control policy, prioritization of vector-borne disease control within the broader context of communicable disease and other health initiatives (e.g., maternal and child health); whether there is guidance on vector control and, if so, the contextual relation between the vector control policy and the larger national vector-borne disease control policy *(49)*;
- national housing development policy, as house improvement may contribute to prevention of leishmaniasis;
- environment policy as it relates to rural and urban sanitation; and
- advocacy to ensure appropriate allocation of resources, legislation to strengthen regulatory control where necessary and community engagement.

The following sections present the available evidence for determining if, when and where to use tools against adult endophilic/endophagic or endophagic/exophilic sand flies (see also **Annex 4**).

4.2 Interventions

As little information is available on the breeding sites of immature stages of all sand fly species, vector control interventions focus on the adult stage. The main interventions used in country programmes for leishmaniasis control and the tools used for personal protection from sand fly bites in specific situations are described below. An analysis of current evidence on the efficacy or effectiveness of tools and those for which more basic and operational studies are required, as well as new tools under development, is presented in **Annex 5**.

4.2.1 Indoor residual spraying

In areas where IRS is known to be effective, the aim is to reduce the abundance, lifespan and human biting of female sand flies by applying an insecticide with residual action to the interior walls of houses and other permanent structures suitable for spraying. It is appropriate where sand flies are endophagic and endophilic, most of the dwellings are suitable for IRS, and people sleep mainly indoors at night. It is not appropriate in settings where the vector is strongly exophagic and exophilic and is unlikely to come frequently into contact with treated surfaces. It is essential to know the biology and behaviour of vector sand flies in advance to decide whether IRS will be effective in a context. Any change in resting or feeding behaviour developed as a consequence of long-term insecticide use should be monitored. The mass killing effect can rapidly reduce disease transmission. Where IRS is applied in the shortest possible time just before the ascending period of vector density, with high coverage, it acts throughout a community and also on individual households. Therefore, sustained, high population coverage is required. General guidance on IRS procedures and programme management is provided in WHO manuals *(75, 76)*. Specific guidance on IRS for any sand fly species has not been formulated.

IRS strategies and targeting areas

Programmes can target areas for IRS on the basis of reports of leishmaniasis cases, vector attributes, logistics and costs. Three primary IRS strategies have been defined to apply broadly to different epidemiological contexts or transmission settings: universal, focal and reactive spraying.

Universal (blanket) spraying

All households or structures in highly endemic areas are sprayed, irrespective of the risk of leishmaniasis transmission in populations in the target area. Therefore, certain villages or communities with low transmission risk or disease incidence will also be sprayed to achieve blanket coverage. This approach is best used at the beginning of an elimination programme in highly endemic areas; as it is a logistically and financially demanding strategy, it is not appropriate for long-term vector control. As programmes move towards leishmaniasis elimination and transmission becomes focal, use of focal IRS is more appropriate when coupled with improved surveillance to identify transmission foci.

Focal or selective spraying

All households or structures in a spatially or temporally defined location in low- to moderate-transmission areas are sprayed to reduce seasonal peaks of vector density and prevent disease outbreaks. Generally, a village in which a new case has been reported is sprayed, while villages or communities in the target unit (e.g., district or a sub-district) that have reported no cases in the previous 3 years are not sprayed. This situation often

occurs during the disease elimination phase, when many villages report no new cases and technically require no IRS, in order to save resources. While this selective approach is more cost–effective than the universal spray approach, it is also both financially and logistically demanding. Indirect evidence obtained by modelling the role of VL case proximity in transmission in Bangladesh suggested that a radius of ≥ 300 m around the household of a new case is required for use of interventions such as focal IRS *(78)*.

Reactive (targeted) spraying

New cases may suddenly appear in areas with low endemicity or after elimination. In response to such a trigger, i.e., a focal outbreak or a hotspot, all households or structures within a defined space (e.g., in a 300-m radius of the household of a new case) are sprayed. Reactive spraying may be included as a component of a rapid response in order to liquidate the focus of new cases to which the health system is alerted.

Before using reactive IRS, thorough consideration should be given to the distance or number of households around each new case that will have to be sprayed, including:

- the dispersal and flying range of the vector species;
- the effectiveness of the insecticide in field conditions;
- the time since the onset of symptoms and commencement of spraying;
- the presence of other symptomatic people in the village and, for VL, unexplained deaths or illness with symptoms consistent with VL;
- the results of a rapid diagnostic survey, if available, to determine the extent of transmission around the new case;
- the proximity of neighbouring households;
- the suitability of surrounding areas as a habitat for the vector; and
- risk analyses conducted in similar transmission settings.

Note: Given the relatively long incubation period, disease outbreaks may occur and be reported during the low- or non-transmission seasons. Therefore, vector control will reduce an epidemic only if active transmission is ongoing at the time of the spraying.

Choice of insecticides

Factors to be considered in selecting insecticides include vector susceptibility, excito-repellency, length of residual efficacy, safety, community acceptability, formulation, local registration and availability of insecticides and the costs of insecticides, equipment and spray operations. **Table 3** lists the insecticide formulations for IRS recommended in the former WHO Pesticide Evaluation Scheme. Most end-use formulations have been prequalified by WHO for malaria vector control after assessment of their efficacy, safety and quality *(79)*. They have also already been used for IRS to control sand flies and can be used when authorized by national regulatory authorities. They include DDT, although WHO recommends use only of insecticides that have been prequalified. Because of widespread resistance, DDT is no longer effective in sand fly control, and DDT for IRS was replaced by pyrethroids on the Indian subcontinent, including in Bihar State, the main epicentre of VL transmission. Currently effective pyrethroids have the same mode of action as DDT, both targeting the voltage-gated sodium channel in insect nerve cells. Knockdown resistance mutations (*kdr*) selected by DDT also confer low-level

resistance to pyrethroids *(72)*, and this is likely to increase under continued selection with pyrethroid use. The efficacy of other IRS insecticides with different modes of action is therefore essential.

The organophosphate insecticides malathion and pirimiphos-methyl and the carbamate bendiocarb are alternatives that target the insect acetylcholinesterase and are being used successfully in malaria control programmes in Africa against DDT- and pyrethroid-resistant Anopheles populations *(80, 81)*. Another alternative is clothianidin (a neonicotinoid), alone or mixed with a pyrethroid (deltamethrin), which have been added to the list of WHO prequalified IRS products *(79)*.

Table 3. Insecticides for indoor residual spraying. Most (see footnote [b]) have been prequalified by WHO for malaria vector control *(79)* and can be used for sand fly control if authorized by the national pesticide regulatory agency

Insecticide class group	Insecticide compounds and formulations[a]	Application rate (dosage) of insecticide active ingredients (a.i.)		Mode of action	Duration of effective action (months)
		g a.i./m²	mg a.i./m²		
Pyrethroids	Alpha-cypermethrin WP, SC	0.02–0.03	20–30	Contact	4–6
	Alpha-cypermethrin WG-SB	0.02–0.03	20–30	Contact	up to 4
	Bifenthrin WP	0.025–0.05	25–50	Contact	3–6
	Cyfluthrin WP	0.02–0.05	20–50	Contact	3–6
	Deltamethrin SC-PE	0.02–0.025	20–25	Contact	6
	Deltamethrin WP, WG, WG-SB	0.02–0.025	20–25	Contact	3–6
	Etofenprox WP	0.10–0.30	100–300	Contact	3–6
	Lambda-cyhalothrin WP, CS	0.02–0.03	20–30	Contact	3–6
Neonicotinoids	Clothianidin WG	0.30	300	Contact	3–8
	Clothianidin + deltamethrin WP-SB	0.225	225	Contact	6–8
Carbamates	Bendiocarb WP, WP-SB	0.10–0.40	100–400	Contact & airborne	2–6
	Propoxur WPb	1.00–2.00	1000–2000	Contact & airborne	3–6
Organophosphates	Pirimiphos-methyl WP, EC	1.00–2.00	1000–2000	Contact & airborne	2–3
	Pirimiphos-methyl CS	1.00	1000	Contact & airborne	4–6
	Malathion WP[b]	2.00	2000	Contact	2–3
Organochlorines	DDT WP[b]	1.00–2.00	1000–2000	Contact	> 6

CS: capsule suspension; EC: emulsifiable concentrate; SC: suspension concentrate; SC-PE: polymer enhanced suspension concentrate; WG: water dispersible granules; WG-SB: water dispersible granules in sealed water soluble bags; WP: wettable powder; WP-SB: wettable powder in sealed water-soluble bags.

[a] See updated list (excluding propoxur, malathion and DDT) at https://extranet.who.int/pqweb/vector-control-products.

[b] Not prequalified by WHO but can be used for IRS if authorized by the national registration authority.

The insecticide formulations available for IRS are wettable powders, water-dispersible granules, suspension concentrates, polymer-enhanced suspension concentrates, capsule suspensions and emulsifiable concentrates. As stated earlier, procurement and use of insecticides packaged in sealed, water-soluble bags or sachets should be preferred to other formulations. They reduce human exposure and ensure accurate measurement, as they are pre-weighed (do not have to be weighed in the field) and are relatively easy to handle, ensuring operator safety.

Residuality

Long residual efficacy and cost–effectiveness are key factors in sustaining an IRS programme. Thus, IRS formulations must be effective throughout the sand fly transmission season. Table 3 lists the general duration of effective residual action, which varies from 2 to 8 months for different products. The persistence of IRS depends on the type of surface sprayed, the type of insecticide chosen and the formulation used *(82, 83)*. Several factors, such as poor-quality products, improper preparation of spray liquid, inappropriate discharge rate from spray equipment, partial coverage of targeted households and plastering of sprayed surfaces can shorten the duration of residual action of an insecticide. Therefore, high-quality, preferably WHO-prequalified, products should be registered and used. Mud- and cement-plastered and brick surfaces are generally more absorbent than thatched, painted or wood surfaces. Thus, where the surfaces are uniform, the choice of formulation improves the effectiveness of IRS. Solid formulations (wettable powder and water-dispersible granules) are suitable for application on porous surfaces, while liquid formulations (suspension or emulsifiable concentrates) are advised to be applied on smooth or painted surfaces. As the residual efficacy of insecticides on absorbent surfaces is 10–20% less than on non-absorbent surfaces, it is important to ensure that the right concentration of the recommended dose is sprayed on non-absorbent surfaces *(76)*. To avoid run-off of spray suspension on smooth surfaces, sprayers with control flow valves that regulate the discharge and provide a uniform spray should be used.

The expected duration of residual action of insecticides is stated on the product label. The residual action on sand fly populations of insecticides applied to different surfaces can be monitored by cone bioassays in households in sentinel villages in sprayed areas. The WHO cone is a 12-cm diameter plastic device with a hole to introduce sand flies. To perform the bioassays, five cones are fixed on different surfaces in a selected room, and 20 non-blood-fed female sand flies aged 2–7 days are introduced into each cone and exposed for 30 min. They are then removed and held for 24 h (or longer for slow-acting insecticides) at 27 ± 2 °C and 75 ± 10% relative humidity. Mortality is recorded 24 h after exposure. The first cone bioassay should be conducted 1–2 weeks after the targeted households have been sprayed, the second 30 days after spraying and then every 30 days until the mortality rate of sand flies remains ≥ 80%. When the rate decreases to < 80%, a confirmatory cone bioassay is performed within 2 weeks. This procedure shows the actual residual action of insecticides in the local situation and can be used as a basis for deciding whether another round of spraying is necessary to cover the entire main transmission period.

When to apply IRS

The length of leishmaniasis transmission and the duration of residual action of insecticide formulations are the main factors to be considered in deciding the number of spray rounds per year. Therefore, the insecticide dose that best suits the length of transmission should be chosen. The timing of each IRS round is critical for effective reduction of vector populations. Each spray round should be completed in less than 2 months and just before the ascending phase of the seasonal vector density peaks or at the beginning of the transmission period. In areas with one seasonal transmission and a single incidence peak, one spray round per year should be adequate and should be completed before the start of the transmission. If the transmission has bimodal peaks, two spray rounds are required.

When to stop IRS operations

In endemic areas where significant progress has been made to eliminate VL, programme managers are often confronted with deciding when to cease IRS operations. While factors for initiating IRS in areas of anthroponotic peridomestic transmission of VL in South Asia are well known, no single factor determines when to stop IRS. The following points should be taken into consideration in deciding to stop IRS for anthroponotic transmission of *Le. donovani* in South Asia.

Ongoing transmission

- In endemic areas, most *Leishmania* infections remain asymptomatic, and only a minor proportion eventually develop into clinical VL. In these areas, the disease tends to be chronic, with the highest case incidence in children and young adults. Malnutrition and immune suppression, notably due to HIV infection, predispose infections to manifest as clinical disease. Therefore, VL can present in endemic, sporadic or epidemic forms, with different clinical features in each situation.
- Infections are usually not evenly distributed in a population because of clustering (micro-foci). For example, in a study in Bihar, India, > 80% of VL cases reported in 2018 were in villages from which cases had been reported in the preceding 5 years (2013–2017) *(84)*. Infective sand flies show increased biting persistence and multi-host feeding behaviour when infective stages are present, thus contributing to the clustering of cases *(85)*.
- In a 2021 xenodiagnosis study in Bihar, none of the serologically positive asymptomatic individuals were infectious to sand flies *(86)*. Clinical VL (primary VL, relapse, VL–HIV coinfections) and PKDL have been found to be infectious to sand flies and act as reservoirs of parasites *(87, 88)*.
- Continued occurrence of new cases in an area is one of the most important reasons for continuing IRS. Hence, a decision to stop IRS in an area should be based on consideration of past cases and the current incidence.

Status of surveillance

- The status of surveillance in endemic areas should be determined to rule out the possibility of hidden cases, poor or nil case reporting in areas that have achieved the elimination threshold, treated VL–HIV coinfected cases presenting with multiple episodes of relapses that are missed in follow-up and the absence of diagnostic services in endemic areas, which poorly reflects the actual prevalence of the disease and lack of surveillance in areas of sporadic cases.

Factors that affect the performance and impact of IRS

- The coverage of IRS in spray rounds depends on factors such as:
  - the criteria for selecting villages or sites; e.g., in India, a village that reports new VL and/or PKDL cases in the preceding 3 years is included in the spray plan;
  - houses that were not sprayed because of a poor spray plan; e.g., in a village in Bihar, only half the households were fully sprayed, resulting in an intense focal outbreak of VL *(89)*;
  - inadequate quantity of insecticide for the number of houses to be sprayed;
  - locked houses that could not be sprayed;
  - high rate of refusal by a community to spray their houses.
- Timing of spray operations: Lack of information about the peak vector density in the target area can result in poor timing of the start of IRS operations, missing the peak sand fly abundance.
- Long or extended duration of spray operations: Ideally, 80–85% of targeted houses and villages should be sprayed in the shortest time (about 40–60 days) before the beginning of the ascending phase of vector density to maximize the insecticidal impact. Delaying spray operations to when the vector density has already increased will reduce the impact.
- Application of poor-quality insecticide and operational factors that affect the quality of IRS can reduce the overall impact.
- Poor community awareness about the importance of spraying and residual action can result in low community participation, refusal of house spraying and covering of sprayed surfaces such as with mud plastering and white-washing.

Access to services

- In endemic areas, access to preventive and case management services can be adversely affected by factors such as flooding after heavy rains, mountainous and other hard-to-reach areas and weak public health systems with inadequate health infrastructure, human resources and capacity.
- Poor health-seeking behaviour of a community that affects acceptance of IRS and preference for local (informal) health providers.

Other conditions

- Movement of populations: Inward seasonal migration of nonimmune populations into endemic areas or outward movement of infected individuals to non-endemic areas where the vector is present are risk factors for transmission. People may be infected in one place and clinically manifest the disease in another.
- Risk of outbreaks: The risk of an outbreak should be carefully assessed before stopping IRS. Mathematical modelling can be useful for predicting an outbreak in various scenarios.
- All the conditions favourable for sand fly breeding and abundance are risk factors for transmission *(90)*.
- Improvement of the socioeconomic conditions of communities at risk will reduce their risk.

Experience from large VL elimination programmes such as in India, in which annual IRS plans exclude villages that have reported no new VL or PKDL cases in the preceding 3 years, are an operational example for deciding when to stop IRS through the routine surveillance system. In India, the target units for IRS are villages in VL-endemic blocks, the sub-district administrative units. Each year, villages that report no confirmed VL cases in previous 3 years or more are removed from the annual IRS planning cycle, while villages that have reported cases are added. As a result of a significant decrease in case incidence during 2013–2021 *(91)*, the number of target IRS areas has decreased significantly.

Equipment, maintenance and spare parts

Spray equipment (hand-operated compression sprayers) that complies with WHO specifications *(92)* should be used for IRS application (**Fig. 3**). A hand-compression sprayer consists of a tank for holding a liquid insecticide suspension, which is pressurized by means of an in-built hand-operated pump. The compressed air forces the liquid out of the tank via a hose control flow valve, a lance and a nozzle. WHO specifications require the sprayers to be equipped with 1.5-bar control flow valves to ensure a persistently uniform spray and discharge droplets of > 50 µm (volume median diameter), which reduce the risk of spray operators for inhalation. Control flow valves also result in use of 25% less water to spray the same surface area.

Fig. 3. Indoor residual spraying with a hand-operated compression sprayer fitted with a control flow valve

Source: reference *92*

The assembled sprayer and fittings must have no sharp edges or projections that might injure workers during normal operation. To ensure good ergonomics, sprayers should have about 11.5 L total tank volume to hold 7.5 or 8 L of insecticide suspension in water, leaving enough space for air. The estimated total weight of loaded pumps is about 12 kg (i.e., 7.5–8 L suspension plus about 4 kg of sprayer). Sprayers with > 11.5 L volume can tire operators; e.g., spray pumps with about 15 L capacity can hold 10 L of suspension but are heavier.

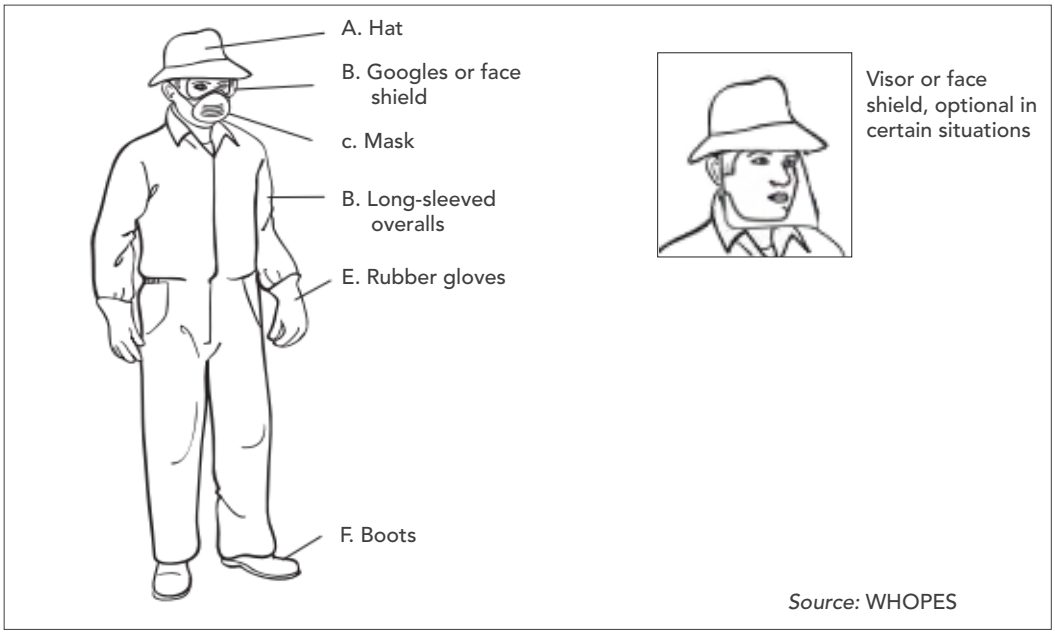
The detailed WHO instructions for IRS procedures and proper use and maintenance of spray equipment *(75, 76)* should be followed. An inventory of equipment should be maintained during spraying, and any repairs, replacements or other requirements should be identified. Routine daily and weekly cleaning and monthly maintenance will maximize sprayer performance and lengthen the lifespan of the equipment. An adequate supply of spare parts, nozzles of WHO specification 8002E and control flow valves (1.5 bar) must be assured. Ceramic and polyacetal nozzles are more durable than nozzles made of stainless steel, brass or plastic.

Personal protective equipment

Insecticide is absorbed mainly through the skin, lungs and mouth. Therefore, IRS operators must wear specific protective clothing in accordance with the safety instructions on the product label *(75, 93)*. Standard personal protective equipment includes (**Fig. 4**):

- a broad-rim, non-absorbent, waterproof hat to protect the head, face, neck and ears from spray droplets;
- goggles or a face shield to protect the face and eyes against spray fall-out;
- a face mask (particulate air filter mask) to protect the nose and mouth from airborne particles of spray fall-out;
- a face shield or a transparent plastic visor that provides comprehensive protection to the face and eyes from splashes, particularly during opening of containers and mixing and filling sprayers. It may also be worn at other times when spray or splashes of pesticide could occur. They are often preferred to goggles and safety glasses as they provide protection for the whole face.
    - long-sleeved overalls, worn outside boots;
    - rubber or nitrile gloves; and
    - boots.

Fig. 4. Personal protective equipment for spray operators



Source: reference *75* and *93*

Training of spray personnel

For an IRS campaign to be successful, an adequate, uniform dose of insecticide must be applied on all possible resting places of adult female sand flies to ensure that they are not exposed to sub-lethal doses of insecticide. Before each round of spraying, spray operators, team leaders and group team leaders or supervisors, as well as subdistrict and district coordinators, should be trained. Training should cover safe handling of insecticides, proper application techniques, maintenance of equipment, disposal of insecticide waste and empty bags and keeping records of spray coverage. Various manuals offer expert guidance for training IRS operatives in the context of malaria vector control, which are generally applicable to VL *(75, 76)*.

In general, training is required on the following topics:

- basic understanding of IRS and why, where and when it is used;
- the role of baseline entomological surveys;
- conducting a census of spray areas and houses, structures and population;
- insecticides used for IRS and the related safety precautions;
- spray application equipment and its maintenance and inventory;
- procurement, distribution and storage of insecticide products;
- developing an IRS plan of action;
- conducting a house spray;
- tracking, supervising and implementing spray rounds;
- reporting on progress and the performance of an IRS campaign; and
- the principles and requirements for safe, appropriate insecticide management.

Handling of insecticides and environmental safety

Insecticides pose risks to the environment and human safety unless they are used correctly according to good labelling practices. Programmes must comply with the procedures and specific standards set by the national pesticide regulatory agency. Only WHO-prequalified insecticides should be used for IRS. The WHO prequalification process involves assessment of risks to humans during and after indoor application of insecticides. Advance information should be given to the community to avoid undue exposure of people in sprayed households, especially to prevent exposure of pregnant women and young children. Spray equipment that complies with WHO specifications should be used. Insecticides should be handled with care and their use managed according to WHO technical guidance for management of public health pesticides *(94)*.

Proper cleaning of sprayers and empty insecticide containers at the end of a day is an important safety step. Sprayers and empty containers should be triple-rinsed and the rinsate (wash liquid) used in preparing spray suspension the next day. Alternatively, the rinsate can be sprayed onto the outer walls or eaves of houses. Rinsate disposal pits can be designed but would add to costs.

Shelf-life and disposal of obsolete stockpiles and empty containers

Generally, insecticide formulations should not be stored for more than 2 years, and the WHO specifications are not intended to apply to longer storage *(95)*. When a formulation has been stored for a long time or under adverse conditions (e.g., prolonged high temperature, humidity, exposure to sunlight) and its shelf-life has expired, samples should be analysed for physical and chemical properties according to the WHO specifications to assess its suitability for use. If the product meets the full specifications, its use could be authorized for a further period of up to 2 years, provided it is stored under appropriate conditions and is used within the extended period.

To prevent expired stocks, programme managers should assess the necessary procurement and procure no excess amount. Available inventory stocks should be used within the shelf-life according to the principle of "first in, first out", or stock that is about to expire should be sent to other districts such that it will be used in time.

If some stocks do expire or are found to be of substandard quality on chemical analysis, they must be managed and disposed of according to the guidance of the Food and Agriculture Organization of the United Nations (FAO) on prevention and disposal of obsolete pesticides *(96)*.

Empty metal or plastic containers can be disposed of as usual metal or plastic scrap after triple rinsing.

Community acceptance

Community acceptance of spraying is important and requires advance notification about the dates of the spray campaign and health education messages.

4.2.2 Insecticide-treated nets

Long-lasting insecticidal nets (LLINs) are factory-treated mosquito nets that are expected to retain their biological activity for at least 20 standard WHO washes under laboratory conditions and 3 years of recommended use under field conditions. Although WHO recommends use of insecticide-treated nets (ITNs) for malaria control on the basis of

high-certainty evidence, systematic evidence for the use of ITNs in leishmaniasis control is inadequate (see details in **Annex 5**).

ITNs protect against nocturnal-feeding, endophagic sand flies at both individual and community level by:

- promoting contact mortality (community protection), as sand flies trying to blood-feed are often attracted to the CO2 emitted by sleepers under ITNs and come into contact with the insecticide on the netting *(71)*;
- creating a physical barrier between the user and female sand flies seeking a blood-meal, thus providing personal protection to net users;[1] and
- causing excito-repellency at close ranges, inhibiting blood-feeding.

Currently, pyrethroids (alone or combined with the synergist piperonyl butoxide or with pyriproxyfen or chlorfenapyr) are the only class of insecticide prequalified by WHO for use in ITNs because of their very low mammalian toxicity and their effectiveness against mosquitoes (**Table 4**). WHO has targeted access to ITNs of ≥ 80% of all at-risk people in areas for malaria control *(97)*. A similar target on access to and use of ITNs could be considered for leishmaniasis vector control when they are the main vector control intervention, such as in following situations:

- for personal protection of patients with VL, VL coinfected with HIV and PKDL;
- for outbreak control, when they can be distributed rapidly to populations at risk without requiring specialized training or equipment and can therefore be used where there are no skills or infrastructure for IRS; and
- for long-term community protection where they are widely considered to be socially and culturally acceptable, as they are minimally invasive and need replacement only every 2–3 years. They are therefore more cost–effective, socially appropriate and less resource-intensive than IRS for long-term leishmaniasis vector control.

For control of VL or anthroponotic CL, they are distributed in campaigns to targeted populations in an endemic area and to newborns and people with HIV-VL or PKDL for personal protection in a continuous system through antenatal clinics or health centres. They are replaced when most are torn, usually every 3 years. Monitoring the durability of nets distributed in campaigns is used to plan replacement of the nets in programmes.

4.2.3 Personal protective measures

Although no epidemiological or public health impact of personal protective measures has been demonstrated, certain topically applied repellents are efficient and could be used in temporary settlements such as camps for seasonal migrant labourers and refugees. The repellents include topically applied DEET (*N,N*-diethyl-*m*-toluamide) and permethrin, which provide good protection against various species of sand fly for 4–8 h, depending on the type of formulation and when used according to the instructions on the label *(99)*; and insecticide-treated curtains reduced sand fly biting density and

[1] Despite the smaller size of sand flies than mosquitoes, the standard mesh size of ITNs (e.g., 24 holes/cm^2) do not allow sand flies to easily pass through the holes without coming into contact with insecticide on the netting. Although the physical barrier effect may be increased with finer-mesh nets, they may be less acceptable to users and should be evaluated further *(98)*.

Table 4. Insecticide-treated nets prequalified by WHO

Brand name[a]	Active ingredient	Netting polymer and treatment technology
Interceptor	Alpha-cypermethrin	Coated on polyester
Interceptor G2	Alpha-cypermethrin + chlorfenapyr	Coated on polyester
SafeNet	Alpha-cypermethrin	Coated on polyester
Veeralin	Alpha-cypermethrin + piperonyl butoxide	Incorporated into polyethylene
Duranet Plus	Alpha-cypermethrin + piperonyl butoxide	Incorporated into polyethylene
Duranet	Alpha-cypermethrin	Incorporated into polyethylene
MAGNet	Alpha-cypermethrin	Incorporated into polyethylene
MiraNet	Alpha-cypermethrin	Incorporated into polyethylene
Royal Sentry	Alpha-cypermethrin	Incorporated into polyethylene
Royal Sentry 2.0	Alpha-cypermethrin	Incorporated into polyethylene
Royal Guard	Alpha-cypermethrin + pyriproxyfen	Incorporated into polyethylene
Tsara	Alpha-cypermethrin	Incorporated into polyethylene
PermaNet 2.0	Deltamethrin	Coated on polyester
Yahe	Deltamethrin	Coated on polyester
Yorkool	Deltamethrin	Coated on polyester
Tsara Soft	Deltamethrin	Coated on polyesterr
Tsara Plus	Deltamethrin + piperonyl butoxide (roof panel); Deltamethrin (side panels)	Incorporated into polyethylene (roof); coated on polyester (sides)
Tsara Boost	Deltamethrin + piperonyl butoxide	Incorporated into polyethylene
PermaNet 3.0	Deltamethrin + piperonyl butoxide (roof panel); Deltamethrin (side panels)	Incorporated into polyethylene (roof); coated on polyester (side panels)
Panda Net 2.0	Deltamethrin	Incorporated into polyethylene
Olyset Net	Permethrin	Incorporated into polyethylene
Olyset Plus	Permethrin + piperonyl butoxide	Incorporated into polyethylene

[a] Latest updates are available from: *https://extranet.who.int/pqweb/vector-control-products*

protected people from CL in the Bolivarian Republic of Venezuela, Burkina Faso and Colombia, although concern was raised about the quality of the data because of the study design used *(100)*. An area repellent system with a spatial repellent, cis–trans-allethrin, reduced the number of bites by *Ph. papatasi* by > 11 times in a field study in Türkiye *(101)*. A clip-on fan vaporizer device for releasing metofluthrin to protect against predominantly *Ph. sergenti* sand flies in the Judean Desert, Israel, however, had no spatial repellency effect but did have insecticidal activity *(102)*.

4.2.4 Targeting domestic reservoir hosts

In large-scale intervention trials, insecticide-treated dog collars to target sand flies feeding on reservoir hosts reduced the incidence of zoonotic VL canine infection, human infection and clinical VL. As discussed in section 2.2, the Brazilian Ministry of Health and municipalities are using 4% deltamethrin-impregnated dog collars in zoonotic VL control in areas with intense, very intense or high VL transmission. The dog collars prevented sand fly bites and are considered a more ethical, effective means for reducing the risk of *Le. infantum* infection in uninfected dogs and its spread from already infected dogs than dog culling *(103)*. Furthermore, 4% deltamethrin-impregnated dog collars were more cost–effective than culling infected dogs or IRS, thereby offering a viable alternative public health measure, depending on the VL transmission intensity *(104)*. Widescale use of 4% deltamethrin-treated dog collars prevented canine infection *(24, 105)*, and the reduction in VL incidence was sustained for 1 year after use of the collar was discontinued *(24)*.

The effectiveness of impregnated dog collars has also been evaluated in other countries. A meta-analysis of 14 studies covering 3786 collared dogs and 3428 uncollared dogs showed that deltamethrin-impregnated collars were 54% (95% confidence interval [CI], 35–65%) effective, while collars impregnated with 10% imidacloprid and 4.5% flumethrin were 90% (95% CI, 80–96%) effective against canine infection *(106)*. In order to protect public health, dog collars should be used in entire communities rather than for individual dogs or households. Two cluster randomized trials of collars in communities have been conducted, one against infection *(17)* and the other against clinical VL incidence in children (the high-risk group) *(107)*. In the trial of effectiveness, conducted by the Leishmaniasis Control Programme in north-west Islamic Republic of Iran in 2002–2006, deltamethrin-impregnated dog collars provided 50% (95% CI, 17.8–70.0%) protection against infantile VL *(108)*. The minimum coverage threshold (proportion of dogs collared per unit area) required to provide herd immunity to unprotected dogs and humans has yet to be established.

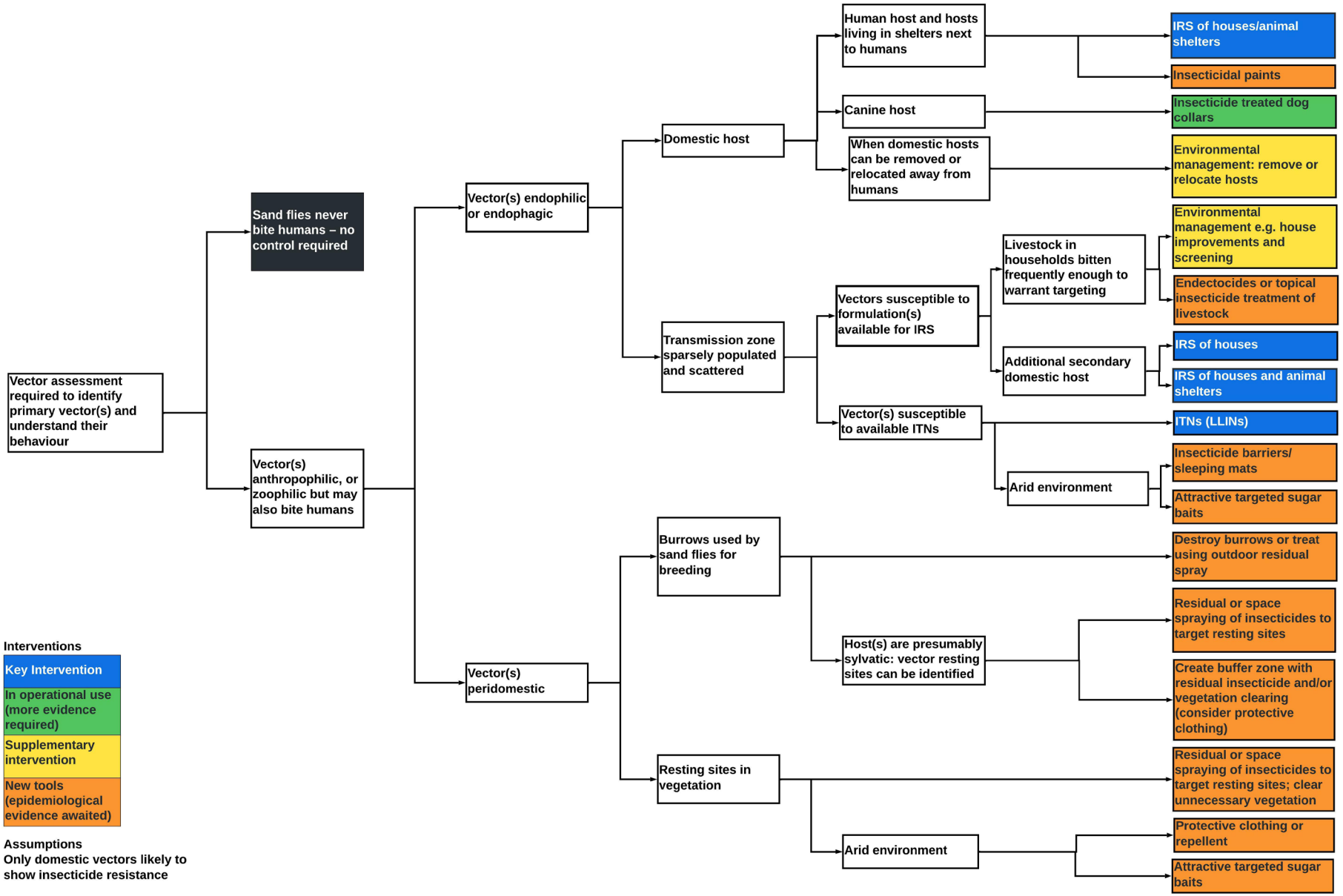
4.2.5 Effectiveness of interventions and new tools

Annex 5 summarizes current evidence on the efficacy and effectiveness of vector control tools and descriptions of new or novel tools for controlling leishmaniasis. The tools may be effective against sand flies or for use in disease control in certain situations if a review of the evidence base supports their effectiveness.

4.2.6 Criteria for selecting interventions

A needs assessment for vector control should be conducted before selecting an intervention, to ensure that it is appropriate to where vectors rest and/or bite and the measures are suitable for the transmission setting. **Fig. 5** illustrates a decision tree for identifying appropriate tools for specific settings.

Fig. 5. **A decision tree for identifying appropriate control methods on the basis of an assessment in the same setting to determine where sand flies rest and/or bite**



IRS: indoor residual spraying; ITN: insecticide-treated net; LLIN: long-lasting insecticidal net

4.3 Management of insecticide resistance

Insecticide resistance is a growing problem, which challenges the control of insect vectors of disease *(108)*. The aim of IRM is to prevent insect populations from reaching the level of operationally significant insecticide resistance that results in control failure. Ideally, insecticide resistance should be detected early by monitoring, well before establishment of resistance in populations, so that pre-emptive mitigation strategies can be used. Otherwise, convincing identification of control failure, measured with epidemiological data, is extremely difficult *(109)*.

The first step in an IRM programme is to identify the susceptibility of the populations to be controlled to the insecticides that could be used. Methods for monitoring resistance are described in section 3.4. A brief overview of insecticide resistance in sand flies is presented in **Box 3**.

Box 3. Status of insecticide resistance in sand flies

There is no global database on insecticide resistance in sand fly vectors, which may be due to inadequate programme capacity and resources. A system for reporting and maintaining a database should be developed as part of insecticide resistance management plans, with threat maps for selecting appropriate, effective interventions.

Insecticide resistance in sand flies was first reported in Bihar, India, in the late 1970s, when *Ph. papatasi* showed a high survival rate after exposure to 4% DDT for 1 h *(110)*. Resistance to DDT of *Ph. papatasi*, *Ph. argentipes* and *Sergentomyia* spp. has since been reported repeatedly in India *(71, 111)*, and has been linked to past use of DDT for malaria vector control and subsequently for control of *Ph. argentipes (112, 113)*. The frequency of the *kdr* marker was significantly higher in frequently sprayed areas endemic for VL than in non-endemic areas *(72)*. Resistance to non-organochlorine insecticides is far less common in India, although confirmed resistance to pyrethroids was reported in strains of both *Ph. papatasi* and *Ph. argentipes* in Pondicherry *(114)*. Ph. argentipes remained susceptible to pyrethroids in spite of > 5 years of indoor applications for VL elimination *(113)*.

Elsewhere than South-East Asia, resistance appears to be quite rare and, when detected, is generally of low frequency. A low prevalence of resistance to DDT has been reported for many years in *Ph. papatasi* in the Islamic Republic of Iran *(46)* but has apparently not escalated *(115, 116)*. In Türkiye, a mixed population of *Ph. tobbi* and *Ph. papatasi* showed slight resistance to pyrethroids *(117)*. Resistance of *Ph. papatasi* to malathion and propoxur was found in Khartoum State, Sudan, but was apparently highly focal *(118)*. Fewer tests have been conducted of resistance in *Lutzomyia* spp., but significant differences in pyrethroid resistance between two Brazilian *Lu. longipalpis* populations suggested at least less susceptibility *(70)*, while Venezuelan *Lu. youngi* showed resistance to deltamethrin and propoxur *(71)*.

As is generally the case for disease vectors, there has been no epidemiological demonstration of operationally significant resistance, and this will be difficult in VL pre-elimination settings where there are few cases. Suggestive evidence is provided by reports of the return of DDT-resistant *Ph. papatasi* to houses in West Bengal within 1 month of IRS, whereas susceptible *Ph. argentipes* were not recorded for 6–9 months *(119, 120)*. Moreover, DDT resistance in *Ph. argentipes* appears to be at least potentially operationally significant, as the level of resistance (seen from prevalence) in Bihar is sometimes extremely high *(121, 122)*. Moreover, the residual efficacy of DDT after spraying was shorter than expected, although poor spraying may have been a problem *(113)*.

In view of the difficulty of ensuring effective larval control and the limited number of insecticides available for adult control (which may be restricted further by local legislation), effective IRM is crucial to withdraw selection pressure from targeted populations, so that any resistance that has developed in the vector population will die out. The rates at which resistance develops initially and then recedes depend on a number of biological, genetic and environmental factors. Resistance is expected to emerge faster in vectors with a rapid life-cycle and high fecundity. Sand flies develop relatively slowly, with generation times of 6–12 weeks, depending on the temperature, and their fecundity is more moderate than that of mosquitoes *(123)*. Given the rarity of mutation, resistant alleles are expected to emerge more frequently from variation or immigration. The origins of the few known resistance mutations in sand flies are unclear, and, while dispersal is usually limited, host-seeking females can disperse over much longer distances *(124)*, suggesting that greater spread of resistance may be possible. Nevertheless, DDT resistance in a Nepalese (non-DDT-sprayed) area bordering Bihar suggests that local spread of resistance must be considered. More studies are required of the spread of specific mechanisms for which the source and recipient populations can be identified. The frequency of resistance alleles also increases much more rapidly if they are dominant or semi-dominant. This does not appear to be the case at least for kdr mutations, and their expression appears to be largely recessive, making progression slower and tracking easier *(72)*.

Another crucial factor in resistance dynamics and a key assumption for IRM is that fitness carries a cost in the absence of exposure to insecticide. Studies of the costs of resistance in the field are difficult because of confounding factors, and laboratory studies may not be representative. The lack of field studies in sand flies results in an important knowledge gap, although it is possibly due to lack of resistance in most species. Reversion of resistance also depends on the capacity of a control programme to withdraw insecticidal selection pressure. Inadvertent control of sand flies and leishmaniasis in the past by DDT spraying for malaria control in India and elsewhere *(71)* clearly illustrates that withdrawal of selection pressure may not always be possible. The more prescient threat to IRM in sand flies is likely to be agricultural use of pesticides, and inter-sectoral partnerships should be formed to avoid use of the same insecticides when possible.

Three general strategies have been used to manage insecticide resistance in insect disease vectors: insecticide rotation, use of combinations or mixtures and mosaics. The concept behind each is use of insecticides with alternative modes of action, with different targets in the insect *(125)*. Thus, both DDT and pyrethroids modulate the para voltage gated sodium channel, while organophosphates and carbamates both inhibit the neurotransmitter acetylcholinesterase *(125)*.

Rotation involves periodic substitution of two or more insecticides with different modes of action. The inherent assumption is that, if resistance is rare, it will have insufficient time to develop before application of the next insecticide, and any resistance that has developed will no longer be favoured and may even be selected against by the alternative mode of action (negative cross-resistance). The frequency of rotation should be short enough to prevent the build-up of resistance in the population. For disease vectors – primarily *Anopheles* – annual rotation is currently considered best practice in resistance management *(126)*. Although rotation might be applied in several different types of insecticidal intervention, at present it is used only on a large scale for IRS.

Use of mixtures of insecticide is based on the assumption that development of resistance to two insecticides with contrasting modes of action is unlikely. The mixing of

two formulations in a single spray tank raises issues of both safety and efficacy and has been used in vector control only rarely. Mixtures of insecticides with unrelated modes of action in a single chemical formulation for ITNs and IRS have become available for vector control only recently *(127–129)*. Combinations of unrelated insecticides for example in pyrethroid ITNs and IRS might be effective for managing resistance, although the cost of implementing two interventions simultaneously might be a deciding factor.

Use of mosaics involves spatial alternation of insecticides with different modes of action or, in some cases, leaving areas untreated to allow susceptibility to persist. Although a trial of mosaics (and rotation) against An. albimanus in Mexico was successful *(130)*, the operational difficulties of procuring and accurately covering different areas on a fine scale with different interventions has precluded adoption.

To date, there is limited evidence on IRM in sand fly control. The recent switch from DDT to pyrethroid in Bihar was driven, at least in part by growing resistance to DDT *(113)*, but this was a reactive change from use of a compound likely to be losing efficacy, rather than a planned change or rotation. In Nepal, use of different insecticides for IRS *(131)* also lacked a key element of resistance management, because the active ingredient of all the formulations used was type-2 pyrethroids. The absence of proactive IRM is not unusual in disease vector control programmes *(132),* but control and elimination targets could be improved by considering more sustainable insecticide use. An important step is to devise a proactive plan for resistance management, which will ensure that the necessary capacity is in place for robust, regular monitoring and use of formulations with new active ingredients as they become available.

IRS is the main intervention for control of endophilic, endophagic sand flies. Because of the widespread resistance of sand flies, DDT is no longer effective; however, their widespread susceptibility to other classes of insecticides prequalified by WHO for malaria vector control can be used for sand fly control (80). Current evidence indicates the following resistance management options:

- One or more annual rounds of IRS, depending on the length of leishmaniasis transmission, with a pyrethroid insecticide used in the sand fly control programme and at least annual monitoring of resistance of sand fly populations in the sprayed areas. If resistance appears, the insecticide is changed in a planned rotation scheme or a mixture or combination of products is used with an insecticide from alternative classes, e.g., an organophosphate (pirimiphos-methyl), a carbamate (bendiocarb) or even a neonicotinoid (clothianidin alone or in combination with deltamethrin). A pyrethroid formulation should not be replaced by another type of pyrethroid.
- Use of pyrethroid-impregnated LLINs alone in the targeted areas with no IRS;
- Combined interventions:
    - In areas in which pyrethroid- impregnated LLINs are used for malaria or leishmaniasis control, IRS with a non-pyrethroid formulation could be considered as an alternative or complementary strategy if resistance to pyrethroids appears.
    - In areas in which IRS with a pyrethroid is used for disease control, use of pyrethroid-impregnated LLINs should be avoided to avert rapid selection of pyrethroid resistance.

There are several strategies for controlling sand flies; however, the three main vector control methods, IRS, ITNs and insecticide-impregnated dog collars for zoonotic transmission, are all based on insecticides. When possible in resistance management, insecticide and non-insecticidal approaches should be used in an integrated vector management strategy.

4.4 Lessons

- Vectors should be assessed to provide evidence for selecting control methods.
- IRS has been a popular intervention for containment of epidemics of CL and VL and other vector-borne diseases because it can be implemented rapidly and extended to a broad range of epidemiological and ecological settings. The optimal timing and number of IRS rounds conducted annually depend on the seasonality of the sand fly species, the residual action of the selected insecticide and the length of transmission; at least two spray rounds per year are often necessary. IRS is both financially and logistically demanding, as it is a long-term vector control method. There is limited epidemiological evidence of the effectiveness of IRS in controlling leishmaniasis.
- ITNs or LLINs are most suitable for protection against endophilic sand flies with a nocturnal peak in biting activity. ITNs are less effective when they are used infrequently by individuals at risk, and any social factors that limit their uptake should be carefully monitored and addressed throughout the programme. ITNs are currently not widely used specifically for CL or VL control in endemic countries, and further, high-quality studies of their epidemiological impact should be conducted in various eco-epidemiological settings. Distribution of ITNs is usually more cost–effective and acceptable for long-term vector control than IRS.
- Only weak evidence is available for the efficacy of supplementary tools, such as environmental management, space spraying with ultra-low-volume sprays, outdoor residual spraying, insecticidal barriers created by painting or spraying vegetation or fencing and attractive targeted sugar baits (**Annex 5**), although they could be considered as part of an integrated vector management programme for certain vector species and transmission situations.
- Ideally, IRM should be part of routine operations, rather than implemented once resistance has spread or increased and control failure is suspected or confirmed.
- Collaboration between programmes and research institutions can be useful for evaluating new tools and alternative classes of insecticides, and coordination with national pesticide regulatory agencies is essential for registration of new insecticides.

5. Monitoring and evaluation of vector control interventions

5.1 Quality assurance

5.1.1 Quality assurance in procurement

Quality assurance was developed for IRS in the context of malaria control. WHO has provided best management practices that are generally applicable to the design and implementation of high-quality IRS *(75, 76)*.

The insecticides procured should conform to WHO specifications and be registered in the country of use to ensure high-quality insecticides that are safe for human and to exclude suppliers who cannot guarantee the quality and performance of their products. Compliance with the quality of the solid insecticide formulations (wettable powders and granules) is particularly important to ensure that they do not sediment rapidly in suspension in water and do not block sprayer nozzles during application.

Testing of the quality of procured insecticides and ITNs before and after shipment should be a requirement of tenders. The testing should include determination of the content of active ingredients and of physical and chemical parameters of products, including relevant impurities and stability during storage for the shelf life, as detailed in product specifications *(95)*. For such testing, random samples are taken from different batches of a consignment and analysed for all specifications in laboratories certified by the International Organization for Standardization or for good laboratory practice. WHO guidance on quality control of public health pesticides should be consulted *(133)*. The WHO analytical method developed in 2021 should be used for determining the active ingredient content of insecticides on filter papers collected from experimental huts or operational research trials *(61)*.

5.1.2 Quality assurance in IRS

Poorly implemented vector control reduces its effectiveness. The efficacy of IRS is maximized when coverage is sufficiently extensive and the correct dose of insecticide active ingredient is applied to kill the targeted insect population. Spraying with sub-lethal doses of insecticide reduces the effect on the disease vector and facilitates evolution of resistance. Recommended approaches for quality assurance of IRS in vector control programmes are pre-spray, during spray and post-spray. Pre-spray checks involve stock auditing, physical checking of the state of spray equipment, servicing equipment at least once a year and rigorous training of IRS spray operators before IRS is started *(76)*. Team leaders should closely supervise spray operators during IRS operations to ensure an optimal spray application technique and to correct any malfunctioning of equipment.

After IRS, WHO recommends cone bioassays to determine the quality of spraying; however, this is not generally feasible for sand flies, which are difficult to rear, and is

impractical on a large scale. For experimental product trials, WHO recommends that post-spray sampling be conducted by placing several filter papers (Whatman 5 x 5 cm) at different heights on walls before spraying and analysing them after spraying by high-performance liquid chromatography *(61, 134)*. At present, there is no method for quantifying insecticide that is applicable in the field. Alternative options include extracting surface residues by scraping *(135)* or by removal on sticky tape, but these methods are less efficient for extraction from various surfaces than more absorbent microencapsulated products. An operationally more convenient and practical method is proxy measurement of the quantity applied and of the number of targeted households or populations, which can be used to check whether spray teams have used the right amount of insecticide.

5.1.3 Monitoring IRS coverage

As in malaria control programmes, it is estimated that > 85% of households in each spray round must be sprayed within acceptable target concentration limits for IRS to be effective by mass killing of vector species *(76)*. Where vectors are attracted to domestic animals, such as cattle in the case of *Ph. Argentipes* in India, a household unit includes adjoining or close animal shelters. The population or the number of households to be protected by IRS should be estimated during planning. The numbers of houses or structures that have been sprayed and those that were not sprayed for logistical reasons are reported daily and weekly during spray operations. IRS coverage is calculated as the percentage of the total number of houses or structures that were targeted or found suitable for spraying that were actually sprayed *(76)*. The amount of insecticide used is also a useful indirect indicator of the estimated number of structures sprayed.

5.1.4 Monitoring durability, coverage and use of ITNs

LLINs are ITNs used mainly for malaria control, and the same principles and procedures apply in their distribution and use for control of VL, CL and other vector-borne diseases. Universal coverage and high rates of use of ITNs (≥ 80%) are the aim *(97)*, although the value of universal coverage as an indicator has been questioned *(136)*.

It is important to monitor the durability of the nets distributed in a campaign in order to plan replacement of torn nets in the programme. According to the WHO guidelines for monitoring the durability of LLINs under operational conditions, the three elements to be considered in assessing net durability are net survivorship, fabric integrity and insecticidal activity or bioefficacy *(137)*. These are determined partly by factors intrinsic to the manufacture of the net (e.g., material composition, knitting or weaving pattern, quality of finishing, insecticide type and content, binders or additives and treatment technology) and partly by extrinsic factors that cause wear and tear.

The elements of durability defined below should be monitored during use of nets in households:

- Survivorship: the proportion of distributed nets still usable as intended in the households to which they were given after a defined period of use, e.g., 1, 2, 3 or more years;
- Attrition rate: the proportion of nets lost and or no longer used as intended after a defined period after their distribution to households;

- Physical or fabric integrity: the number, location and size of holes in each net; and
- Insecticidal activity (bioefficacy): the degree of knock-down, mortality or inhibition of blood-feeding susceptible insect vectors, as determined by standard WHO test procedures and criteria (i.e., cone bioassay, tunnel test). Insecticidal activity is associated with the type and content or availability of insecticide. The insecticide content is expressed as g a.i./kg or mg a.i./m^2 of the netting fabric and is determined by the method outlined in WHO specifications guidelines *(95)*. This information is valuable for interpreting data on bioefficacy. Insecticidal activity can be assessed as a function of length of use.

A questionnaire for monitoring the durability of nets is available in the WHO guidelines *(137)*.

5.2 Core entomological indicators

A complete set of entomological indicators should include at least one indicator per programme or project. Programme indicators should be:

- reliable
- precise
- measurable
- timely
- valid
- programmatically important.

Proposed indicators of the entomological efficacy of IRS and ITNs are listed in **Tables 5** and **6**, respectively.

Table 5. Core entomological indicators of the efficacy of IRS

Indicator[a]	Frequency	Level	Outcome	Explanation
Adult sand fly occurrence and density				
Occurrence	Once a year		Adult female sand fly species present or absent	Presence of sand fly species known to support development of the parasite; important in areas that report VL cases for the first time
Vector density	Pre- and post-IRS; monthly	Sentinel sites	Number of adult female sand flies collected (per sampling method and unit time)	Seasonal prevalence and differs from year to year
Adult sand fly vector behaviour				
Abdominal, physiological conditions (blood digestion stage)	Pre- and post-IRS	Sentinel sites	Proportion of female sand flies unfed, freshly blood-fed, half-gravid and gravid per sample collection	Abdominal stages of unfed, freshly blood-fed, half-gravid and gravid. Reflects ovarian maturation. Human and animal blood meal identification may be considered.
Larval source management				
Insufficient data for evidence-based recommendations	N/A	N/A	N/A	N/A
Insecticide resistance in adults				
Occurrence	Once a year		Adult female sand fly species present or absent	Presence of sand fly species known to support development of the parasite; important in areas that report VL cases for the first time
Status	Annually	Sentinel sites	Proportion of adult female sand flies alive at the end of the standard holding period after a standard length of exposure (1 h) to insecticide in bioassays	Classification based on proportion of female sand flies dead or incapacitated after exposure to a discriminating concentration of insecticide in a standard bioassay, whereby: ▪ < 90% mortality = confirmed resistance; ▪ 90–97% mortality = possible resistance; and ▪ ≥ 98% mortality = susceptibility.
Intensity	Annually	Sentinel sites	Classification of adult female sand fly vector population as having high, moderate or low intensity resistance	Classification based on proportion of sand flies dead or incapacitated after exposure to 5 and 10 concentrations of an insecticide in a standard bioassay, whereby: ▪ < 98% mortality after 10 exposure = high resistance; ▪ ≥ 98% after 10x exposure but < 98% after 5 exposure = moderate intensity resistance; and ▪ ≥ 98% after 10 and 5 exposure but < 98% after 1 exposure = low intensity resistance.

IRS: indoor residual spraying; N/A: not applicable

Table 5 (cont'd). Core entomological indicators of the efficacy of IRS

Indicator[a]	Frequency	Level	Outcome	Explanation
Coverage of vector control intervention				
IRS programme planning				
Total no. of implementation units requiring IRS as per the national vector control policy	Per round of IRS	At national, sub-national level. Only national level data should be shared with WHO	No. of implementation units included in the IRS rounds	To reach the recommended ≥ 85% coverage (see section 5.1)
Total no. of villages in implementation units requiring IRS as per the technical criteria of the programme	Per round of IRS	At national and sub-national levels Only national data should be shared with WHO	No. of villages sprayed per round	To reach the recommended ≥ 85% coverage (see section 5.1)
Total no. of households requiring IRS intervention (i.e., targeted population in the implementation units requiring IRS)	Per round of IRS	At national and sub-national levels Only national data should be shared with WHO	Total no. of targeted population covered	To reach the recommended ≥ 85% coverage (see section 5.1)
IRS coverage	Per round of IRS	Per implementation unit (at national and first sub-national level). Only national data should be shared with WHO.	Proportion of coverage of targeted number of houses and or population	Determines the coverage of the intervention
			Proportion of coverage of targeted number of structures Proportion of coverage of targeted no. of households at risk (village level)	
	Per round of IRS	Per implementation unit (at national and first sub-national level). Only national data should be shared with WHO.	No. of households covered by IRS according to household survey (reported vs surveyed coverage)	Based on independent surveys

IRS: indoor residual spraying

Table 5 (cont'd). Core entomological indicators of the efficacy of IRS

Indicator[a]	Frequency	Level	Outcome	Explanation
Impact of IRS intervention				
Cone bioassay tests on sprayed surfaces[b]	Monthly after each IRS round (for the duration of expected residual action)	Sentinel sites	Proportional mortality of the target vector exposed to the sprayed surface at intervals of weeks or months after spraying	Determines residual efficacy of an insecticide on common indoor sprayed surfaces
Other process indicators				
Requirement for insecticide vs targeted population per endemic district	Annually (total of all spray rounds)	Per district		To estimate the quantity of insecticide necessary to cover the targeted population
Use of insecticide vs quantity supplied or procured per endemic district	Per spray round	Per district	Proportion of insecticide used against supplied	To estimate consumption rate per round
No. of functional hand compression sprayers available (with operational control flow valve)	Annually	Per district per round		To estimate no. of pumps required to complete planned IRS rounds

IRS: indoor residual spraying.

[a] The process and the impact indicators appropriate for monitoring VL and CL vector control programmes depend on the region, the intervention and the unit of implementation (e.g., sub-district, district, state or national).

[b] Bioassay and resistance test kits are available from Universiti Sains Malaysia (*https://www.who.int/teams/control-of-neglected-tropical-diseases/interventions/strategies/vector-control/insecticide-resistance*).

Table 6. Specific core indicators for ITNs and LLINs

Indicator[a]	Frequency	Level	Outcome	Explanation
Coverage of vector control interventiony				
No. of LLINs[b] distributed to populations at risk in the past 2 years (in areas co-endemic for malaria, as a supplementary intervention or for personal protection of people with HIV–VL coinfection and PKDL)	In a campaign, continuous	Records	Proportion of population at risk to whom LLINs were distributed	Determines the coverage of the intervention
Percentage of population at risk who slept under an LLIN the previous night	Annually	Household survey	LLIN use rate	Determines use

CL: cutaneous leishmaniasis; ITN: insecticide-treated net; LLIN: long-lasting insecticidal net; PKDL: post-kala-azar dermal leishmaniasis; VL: visceral leishmaniasis.

[a] The process and impact indicators appropriate for monitoring VL and CL vector control programmes depend on the region, the intervention and the unit of implementation (e.g., sub-district, district, state or national).

[b] In many countries, LLINs are expected to be distributed as a core intervention against malaria and may have a collateral impact on VL and or CL in areas where these diseases are both prevalent.

6. Data management, repository and reporting

Consistent record-keeping, reporting, surveillance and periodic surveys of epidemiological and entomological indicators of leishmaniasis are essential to monitor the performance of a control programme. Data can be used to verify whether activities have been implemented as planned and indicate any problems so that corrective measures can be implemented.

Evaluation is closely linked to monitoring but consists of periodic investigation of the impact of a control programme. The objectives may be to determine the effectiveness of a programme, measure the value of a particular component or link changes in an epidemiological or entomological outcome to an intervention.

Users of data and information from monitoring and evaluation of leishmaniasis control range from operational staff and programme managers who are directly involved in implementation of vector control to national policy-makers, donors and NGOs. Data and reporting must therefore be carefully managed and accessible to all parties.

6.1 Data generation and flow

Data are generated in various ways (Table 7). The frequency of data collection is shown in Table 5 and data sources in section 6.2.

Table 7. Where and how entomological data are generated

Place of collection	Type of data
In the field	Sand fly collection
	IRS coverage
	WHO cone bioassays on IRS-sprayed walls
	IRS quality monitoring
	ITN durability assessment (survivorship, physical integrity)
	Changes in vector behaviour and host preference
In laboratories	Morphological identification of species
	▪ Insecticide resistance monitoring (WHO tube test and bottle bioassays) ▪ Standard laboratory molecular tests (e.g., PCR)
	▪ WHO cone bioassays on ITNs (for net efficacy and durability)

IRS: indoor residual spraying; ITN: insecticide-treated net; PCR: polymerase chain reaction.

Data should be collated at the lowest administrative level possible, ideally in districts or lower levels, and sent to regional and national offices. A feedback system should ensure that all information collated by national offices is communicated to regional and district programmes to inform vector control activities. External agencies, academic institutions and NGOs involved in vector control and leishmaniasis surveillance and independent researchers should be encouraged to send their data regularly to the national programme for inclusion in the centralized system to ensure that monitoring results are not biased and that all relevant information are used to inform vector control.

6.2 Data types

The types of data (Table 8) required in a leishmaniasis control programme vary and are often located in different databases and systems. This fragmentation of data sources may make it difficult for control programmes to bring all the necessary data together to make informed decisions.

Table 8. Common data types and sources

Data	Possible sources
Disease morbidity and mortality	Data on cases are usually obtained from a health management information system, such as DHIS2. They are often collected passively, e.g., when patients present to local health centres, and are compiled weekly or monthly. Data on diagnoses must be confirmed and reported rapidly, especially for a reactive or outbreak response. When an outbreak is considered to be occurring, active case detection may also be implemented, and such data must be recorded in the health management system, such as DHIS2.
Intervention coverage	Data on coverage comprise case management, i.e., diagnosis, treatment and treatment outcomes, and coverage of vector control interventions such as IRS and ITNs to monitor impact. The source of these data will depend on the intervention method used. For interventions such as IRS and outdoor spraying, daily or weekly reports can be used to calculate coverage. For ITNs and, e.g., impregnated dog collars, the number of units distributed and the method should be used.
Quality assurance	Quality assurance prevents sub-standard delivery of services or products. It is essential to the potential success of a control programmes. According to the intervention method, data for quality assurance data are derived from the results of WHO cone bioassays, physical–chemical properties, including the content of active ingredient (for IRS and ITNs) and assessment of fabric integrity (ITNs).
Cost–effectiveness	Such an analysis determines whether mortality and morbidity rates could be reduced at a lower cost. For accurate calculation of cost–effectiveness, all costs associated with the intervention, including physical materials, community engagement campaigns, personnel and training, should be recorded. As these costs may differ by region, the data should be collected at the most disaggregated level possible and reported to regional and national levels. This is generally done within operational research.

DHIS: district health information system (version 2); IRS: indoor residual spraying; ITN: insecticide-treated net.

6.3 Data management

A centralized data reporting and management system is necessary to collect and collate data from all relevant sources (e.g., data on vector control from the programme, sentinel sites and research sites). It should be integrated with epidemiological and entomological indicators and maintained to generate periodic reports according to the requirements of the programme. The data management system must allow importation and exportation of data from and to other systems and respond to questions about the programme (e.g., the coverage of IRS in a district in the most recent IRS round). The responses should be in the form of reports or maps that can be easily read and interpreted by both operational staff and policy-makers. They can be used to identify deficiencies in the programme, inform decisions and included in reports to funders for advocacy purposes.

Data collected and reported in the management system must be linked to the indicators used in the leishmaniasis control programme for monitoring and evaluation and should include the source of the numerator and of the denominator and the frequency of collection. For example, for IRS coverage, the number of structures targeted would be the denominator and the number of structured sprayed the numerator. To measure leishmaniasis incidence in an administrative unit (e.g., an implementation unit in an elimination context or its equivalent administrative unit, such as a district), the district population would be the denominator and the number of cases presenting at district health centres the numerator.

6.4 Data collection

At the lowest administrative levels, data are often still collected on paper, although some programmes are moving towards digital platforms to increase efficiency and speed of access and to reduce the chance of errors. The tool used should be simple and efficient for the relevant data.

Training in data collection, filling in forms, analysis and interpretation should be provided at all levels of a control programme. If a digital data collection system is to be used, training in entering data into a computer and/or a digital platform should also be provided. The level at which data are collated into computerized software will depend on the budget and infrastructure of the control programme, the ability of staff to enter data, the technical assistance necessary to maintain the system and compatibility with other software. Human resources to support the system must be available.

Forms for collecting data on spray coverage in villages, primary health care centres and districts in anthroponotic VL-endemic areas in South Asia, where IRS is the main intervention for vector control, are presented in **Tables 9–11** as examples to be adapted to the local context. A questionnaire for monitoring the durability of LLINs in field is also available *(137)*. Suitable data recording forms should be developed for areas with extra-domiciliary transmission, such as in South America.

Table 9. Indoor residual spraying: form for collecting data in villages or urban wards

Reporting format for indoor residual spraying (village or ward; format 1)							**Date of data collection:**		
Name of the village or urban ward:			Name of the administrative unit (country, ward, primary health centre):				Insecticide and percentage formulation used:		
Spray round (1st or 2nd):			Population at risk:			Population targeted for IRS:			
No. of targeted structures (houses or other dwellings):			No. of targeted animal shelters:	No. of spray squads:	No. of people per squad:		No. of spray operators: No. of helpers: No. of field supervisors:		
Date of start of spray operation (dd/mm/yyyy):			Date of completion of spray operation (dd/mm/yyyy):		Quantity of insecticide required (kg):		Name of team supervisor:		
Details of sprayed structures									
	Name of head of household	Total no. of people in the household	No. of targeted structures (houses and animal shelters) sprayed	No. of houses fully sprayed	No. of houses partially sprayed	No. of rooms fully sprayed	No. of rooms partially sprayed	No. of rooms for which spraying was refused	No. of closed rooms	Remarks
	1	2	3	4	5	6	7	8	9	10
1										
2										
3										
4										
5										
6										
Daily summary:										
No. of structures sprayed:			No. of houses closed:							
Total no. of houses:			Quantity of insecticide received (kg):					Signature of supervisor:		
No. of animal shelters:			Quantity of insecticide used (kg):							
No. of persons covered:			Balance available (kg):							

Table 10. Indoor residual spraying: form for collecting data in primary health care centres or equivalent

Reporting format for indoor residual spraying (PHC or equivalent). Name of district:																				
Round (1st or 2nd):						Insecticide used (%):						Spray period: from (dd/mm/yyyy): to:								
						Coverage												Insecticide stock		
	Name of PHC or equivalent unit	Total population of the PHC or equivalent	Population targeted	No. of villages	**No. of villages targeted for IRS**	No. of villages	No. of targeted structures (houses and animal shelters)	No. of targeted structures sprayed	No. of houses sprayed	% of houses sprayed	No. of rooms	No. of rooms fully sprayed	No. of houses partially sprayed	Percentage of rooms completely sprayed	Population in sprayed houses	Percentage of population protected	Estimated quantity required (kg)	Quantity used (kg)	Unused quantity (kg)	Remarks
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1																				
2																				
3																				
4																				
5																				
Total																				
Daily summary:																				
No. of all structures covered:					No. of houses closed:															
No. of houses sprayed:					Quantity of insecticide received (kg):															
Percentage of houses sprayed:					Quantity of insecticide used (kg):															
No. of people covered:					Balance available (kg):										Signature of supervisor:					

PHC: primary health centre.

Table 11. Indoor residual spraying: form for collecting data in districts

Reporting format for indoor residual spraying (district). Name of district:																				
Round (1st or 2nd):						Insecticide used (%):							Spray period: From (dd/mm/yyyy): To:							
					Targeted		Coverage												Insecticide stock position		
	Name of district	Population of district	Targeted population	No. of PHCs or equivalent in the district	No. of PHCs	No. of villages	No. of PHCs or equivalent	No. of villages	No. of targeted structures (houses and animal shelters)	No. of targeted structures (houses and animal shelters) sprayed	No. of houses sprayed	% of houses sprayed	No. of rooms	Rooms fully sprayed	No. of houses partially sprayed	% of rooms fully sprayed	Population in sprayed houses	% of population protected	Estimated quantity required (kg)	Quantity used (kg)	Unused quantity (kg)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1																					
2																					
3																					
4																					
5																					
6																					
Total																					
Summary:																					

PHCs: primary health centres.

6.5 Data dissemination

In disseminating data, consideration should be given to the users, the information to be disseminated, how often and in what format (e.g., a written report or a formal meeting). At each level, district, regional and national, data should be interpreted for operational decision-making

6.6 Lessons

- A leishmaniasis control programme must track progress in achieving its objectives and targets.
- A set of indicators is required to monitor and evaluate the objectives and targets.
- Data for the indicators are derived from combined sources to form a large data set, which must be managed correctly.
- Data management is essential for monitoring and evaluation; data on disease surveillance, entomological surveillance, intervention coverage, quality assurance and meteorological information should be integrated.

7. Operational research required

The operational research required should be assessed regularly in national programmes in leishmaniasis-endemic countries. The research priorities identified by the peer reviewers of this document are presented below by WHO region. The list is not exhaustive.

7.1 African and Eastern Mediterranean regions

- Region-wide surveys to map the distribution of leishmaniasis vectors and the phylogenetic and molecular characterization of the vector population
- Large-scale evaluation of the epidemiological impact of outdoor residual spraying of house fences on populations of *Ph. orientalis* in countries in the Horn of Africa
- Assessment of the impact of environmental management on sand fly abundance
- Assessment of the impact of area-wide habitat modification
- Evaluation of the effects of new tools, such as attractive targeted sugar baits, ivermectin and treatment of animals with other insecticides, rodent feed-through insecticides and pyrethroid-impregnated dog collars
- Status of insecticide resistance in sand fly vectors of leishmaniasis in foci that have been subjected to intensive IRS, space spray and LLIN use.

7.2 Region of the Americas

- Evaluation of the efficacy of new insecticides (active ingredients and formulations) for control of vector sand fly species in South America, especially *Lu. longipalpis*
- Evaluation of the effectiveness of attractants for entomological monitoring in areas with unknown vectors
- Assessment of environmental management for indirect control of larval stages
- Monitoring of the feeding behaviour of sand flies in areas where dog collars impregnated with deltamethrin are used to determine any change in the behaviour of Lu. longipalpis and whether other domestic animals will become sources of infection
- Evaluation of critical coverage thresholds for use of insecticide-treated dog collars to achieve community protection (herd immunity) in uninfected canine reservoirs and human populations at risk of zoonotic VL
- Evaluation of vectorial capacity and competence of species in endemic areas or areas with no primary vectors in South America
- Assessment of the effectiveness of ITNs for control of VL and CL in South America

- Assessment of the susceptibility and resistance of *Lu. longipalpis* to insecticides in areas in which dog collars impregnated with insecticide are used
- Assessment of the susceptibility of vectors to insecticides in areas of continuous IRS
- Evaluation of use of repellents for individual protection against sand flies for VL and CL control
- Evaluation of traps with kairomones and/or pheromones for surveillance and control of VL
- Evaluation of zooprophylaxis for control of VL and CL
- Development of an information system on vectors to strengthen surveillance and control of leishmaniasis
- Development of strategies for socio-environmental and entomological monitoring of the risk for anthroponotic CL outbreaks and integrated control in areas of land-use changes ("edge effect") or exposure
- Development of cost–effective tools and strategies for integrated anthroponotic VL control in areas of recent vector colonization or low VL transmission
- Environmental management strategies for VL and CL in areas that include several household units.

7.3 South-East Asian Region

- Contribution of exophagic, exophilic vectors in transmission of VL in South-East Asia and its relevance to VL elimination programmes
- Potential contribution (and acceptability) of LLINs and ITNs to reduce transmission in persistent hotspots of VL transmission
- Evaluation of use of integrated vector control and surveillance
- Assessment of the effectiveness of alternative or supportive vector control interventions for elimination of VL in South-East Asia
- Assessment of the possible role of new vector species in newly or suspected newly endemic areas in South-East Asia for VL
- Role of reservoir hosts in VL transmission in Sri Lanka
- Role of non-vector species (or yet-to-be-proven vectors) of sand flies on the transmission cycle in Sri Lanka
- Effect of insecticide application for dengue control on the distribution of sand fly vector species and VL in Sri Lanka.

7.4 European Region

- Evaluation of the cost–effectiveness of interventions in cheap (< US$ 25 000) randomized controlled trials with only entomological outcomes

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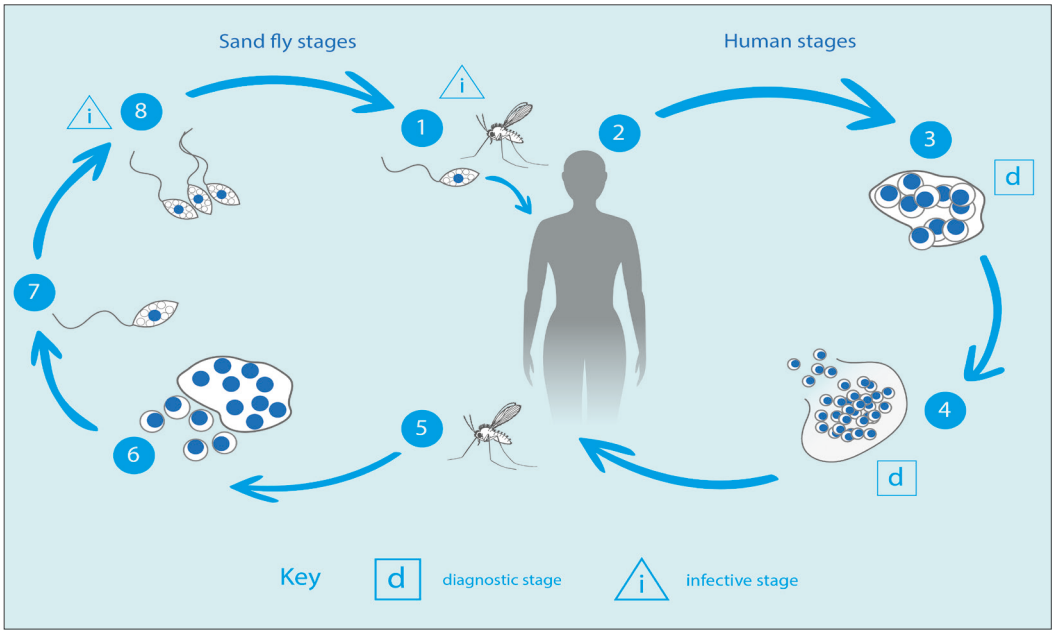
Annexes

Annex 1. Life-cycles of *Leishmania* and sand flies

A1. *Leishmania* life-cycle and transmission

The life-cycle of anthroponotic leishmaniasis is shown in **Fig. A1.1**.

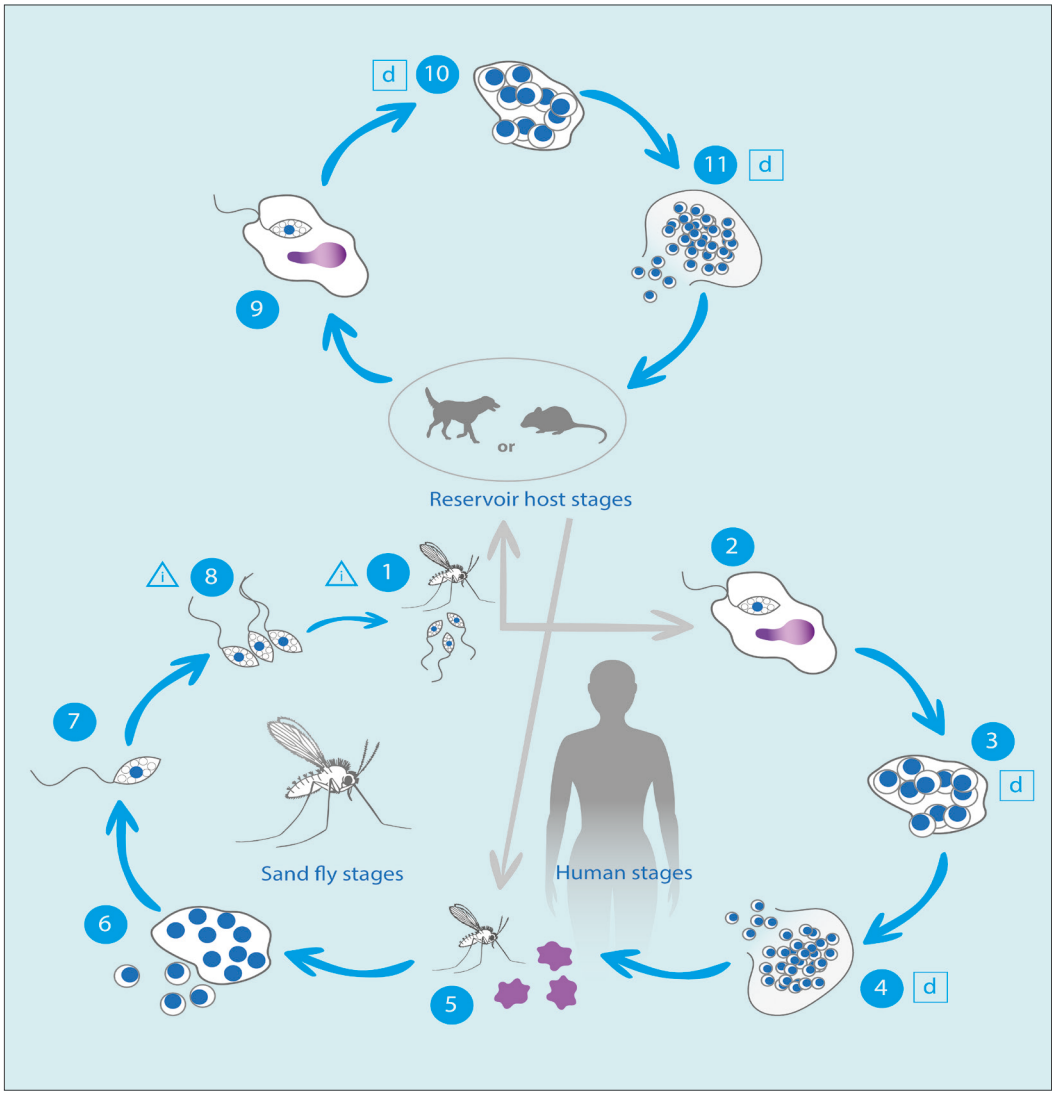
Fig. A1.1. Life-cycle of anthroponotic leishmaniasis



1. An infected sand fly takes a blood meal from a human or reservoir host (injects metacyclic stages of promastigotes into the skin). 2. Inside the body, promastigotes are phagocytosed by macrophages (or other types of mononuclear phagocytic cells). 3. Promastigotes transform into amastigotes. 4. Amastigotes multiply in cells of various tissues and infect other cells. 5. A sand fly takes a blood meal containing amastigotes. 6. The amastigotes survive within the peritrophic membrane that forms around the blood meal. 7. The amastigotes transform into different promastigote stages in the gut of the sand fly after the peritrophic membrane breaks down. 8. The promastigotes go through a series of transformations and multiply in the sand fly gut, then the metacyclic promastigotes (infective stages to humans) move towards the anterior of the gut and proboscis ready to be regurgitated by the sand fly during her next blood meal. This process takes 7–14 days to complete, depending on the *Leishmania* and sand fly species.

The life-cycle of zoonotic leishmaniasis is similar but involves an animal host as well as a human host. Steps 1–8 are the same, but additional multiplication of amastigotes occurs in steps 9–11: the promastigotes regurgitated by an infected sand fly when she takes a blood meal are taken up by macrophage cells, develop into amastigotes, multiply and invade new cells (**Fig. A1.2**).

Fig. A1.2. Life-cycle of zoonotic leishmaniasis

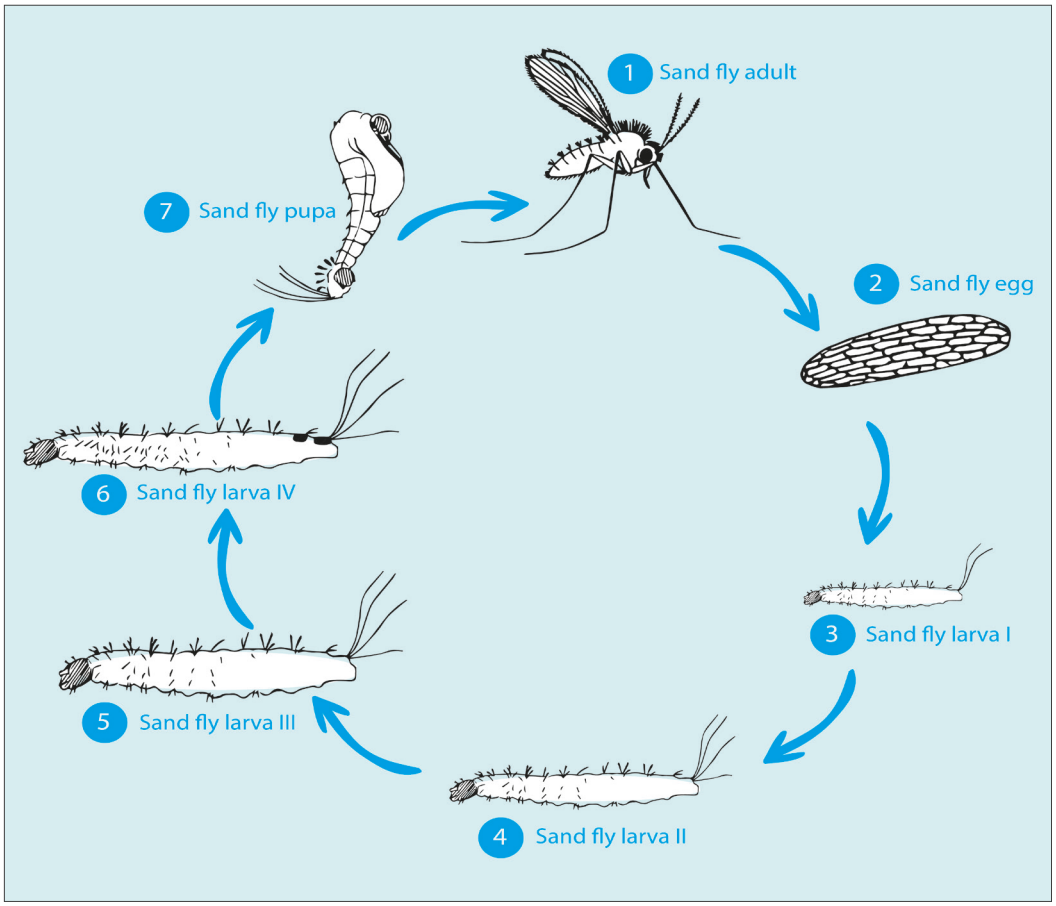


1. An infected sand fly takes a blood meal from a human or reservoir host (injects metacyclic stages of promastigotes into the skin). 2. Inside the human body, promastigotes are phagocytosed by macrophages (or other types of mononuclear phagocytic cells). 3. Promastigotes transform into amastigotes. 4. Amastigotes multiply in cells of various tissues and infect other cells. 5. A sand fly takes a blood meal containing amastigotes. 6. The amastigotes survive within the peritrophic membrane that forms around the blood meal. 7. The amastigotes transform into different promastigote stages in the gut of the sand fly after the peritrophic membrane breaks down. 8. The promastigotes go through a series of transformations and multiply in the sand fly gut, then the metacyclic promastigotes (infective stages to humans) move towards the anterior of the gut and proboscis ready to be regurgitated by the sand fly during her next blood meal. 9. Inside the body of the reservoir host, promastigotes are phagocytosed by macrophages (or other types of mononuclear phagocytic cells). 10. Promastigotes transform into amastigotes. 11. Amastigotes multiply in cells of various tissues and infect other cells. This process takes 7–14 days to complete, depending on the Leishmania and sand fly species.

A2. Life-cycle of sand flies

All species of sand fly have the same life-cycle components (**Fig. A1.3**).

Fig. A1.3. Life-cycle of sand flies



Adult females (1) lay their eggs (2) in humid, nutrient-rich terrestrial environments, such as holes in the ground and among tree roots. Depending on species and climatic conditions, the eggs usually hatch into first-instar larvae (3) after 6–10 days; this may take longer, particularly at low temperatures. Over 15–30 days (depending on the species, climatic conditions and availability of nutrients), the larvae feed on organic matter and develop into second- (4), third- (5) and fourth-instar larvae (6). They then enter an inactive pupal stage (7) for approximately 7–10 days, before emerging as adults. After emergence, males and females mate once, and the female seeks to take a blood meal from either an animal or a human host for development of her eggs. Both males and females feed on sugar sources throughout their life; males do not take blood meals.

Annex 2. The main vectors responsible for transmitting human leishmaniasis in WHO regions

The main vectors responsible for transmitting human leishmaniasis in WHO regions *(1–3)* are shown in **Table A2.1**. This is not an exhaustive list, and local resources should be consulted.

Table A2.1. Main vectors of human leishmaniasis in the WHO African Region and the Region of the Americas

Region	Endemic country or area	Main vectors	Habitat; reservoir(s)	Form of disease; species of *Leishmania* transmitted
African	Ethiopia, Kenya, Uganda	*Phlebotomus (Synphlebotomus) martini*	Savannah with termite mounds; humans?	AVL; *Le. (Le.) donovani*
	Ethiopia, Kenya	*Ph. (Larroussius) longipes Ph. (La.) pidifer*	Rocky highlands; hydraxes	ZCL, MCL; *Le. (Le.) aethiopica*
	Sub-Saharan Africa	*Ph. (Ph.) duboscqi*	Dry savannah; rodents	ZCL; *Le. (Le.) major*
	Algeria	*Ph. (Ph.) papatasi*	*Ph. (Ph.) papatasi*	ZCL; *Le. (Le.) major*
Americas[a]	Central and South America	*Lutzomyia (Lu.) longipalpis* s.l.	Peridomestic; dogs	ZVL; *Le. (Le.) infantum*
		Lu. (Nyssomyia) spp. *(syn Nyssomyia intermedia; Ny whitmani; Ny neivai); Lu. migonei (syn = Migonomyia migonei); Lu. fischeri (syn = Pintomyia fischeri); Lu. pessoai (syn = Pi. pessoai) Lu. (Pifanomyia)* spp. *(= Pi. (Pifanomyia) verrucarum))* and *Lu. (Psychodopygus)* spp. *(Psychodopygus wellcomei)*	Peridomestic and sylvatic; rodents, marsupials & dogs, edentulous (sloth, armadillo and anteater)	ZCL and MCL; *Le. (Vi.) braziliensis*
		Lu. (Nyssomyia) spp.	Sylvatic; tree edentates and others	ZCL; *Le. (Vi.) panamensis Le. (Vi.) guyanensis*
	South America	*Lu. (Pifanomyia) evansi (= Pi (Pifanomyia) evansi))*	Peridomestic; dogs	ZVL; *Le. (Le.) infantum*
		Lu. (Nyssomyia) flaviscutellata (= Bichromomyia flaviscutellata) and *Lu. (Pifanomyia) longiflocosa*	Sylvatic, rodents and marsupials	ZCL; *Le. (Le.) amazonensis CL Le. (Vi.) guyanensis*
	Peru	*Lu. (Helcocyrtomyia)* spp. *(= Lutzomyia (Helcocyrtomyia) peruensis)*	Sylvatic, rodents and marsupials	ZCL; *Le. (Le.) mexicana* s.l.
	Many countries	Suspected vectors in one or more of the 26 subgeneric groups of *Lutzomyia* (Young and Duncan classification *(5))*	Multiple and unknown reservoirs	American parasites (above); plus *Le. (Le.) infantum* in North American foxhounds

ACL: anthroponotic cutaneous leishmaniasis; AVL: anthroponotic visceral leishmaniasis; MCL: mucocutaneous leishmaniasis; ZCL: zoonotic cutaneous leishmaniasis; ZVL; zoonotic visceral leishmaniasis.

[a] The table does not list all species in South America; a detailed list is available in Table 23 of reference *4*.

Table A2.2. Main vectors in the WHO Eastern Mediterranean, European, South-East Asian and Western Pacific regions

Region	Endemic country or area	Main vectors	Habitat; reservoir(s)	Form of disease; species of *Leishmania* transmitted
Eastern Mediterranean	Djibouti, Egypt, Saudi Arabia, Somalia, Sudan, Yemen	*Ph. (La.) orientalis*	Sylvatic (Acacia-Blamites forest); humans and mongooses?	AVL, MCL or ZVL; *Le. (Le.) donovani*
	Somalia, Sudan	*Ph. (Synphlebotomus) martini*	Savannah with termite mounds; humans?	AVL; *Le. (Le.) donovani*
	Morocco, Tunisia	*Ph. (La.) ariasi*	Peridomestic rural, dogs	ZVL; *Le. (Le.) infantum infantum*
	Lebanon, Libya, Morocco, Syrian Arab Republic, Tunisia	*Ph. (La.) perniciosus*	Peridomestic rural, dogs	ZVL; *Le. (Le.) infantum infantum*
	Afghanistan, Islamic Republic of Iran, Middle East, North Africa	*Ph. (Paraphlebotomus) sergenti*	Peridomestic urban; humans	ACL; *Le. (Le.) tropica*
	North Africa	*Ph. (Adlerius) arabicus* *Ph. (La.) guggisbergi*	Rocky arid; hydraxes and rodents?	ZCL; *Le. (Le.) tropica*
	North Africa	*Ph. (Ph.) papatasi*	Arid; Gerbils and rodents	ZCL; *Le. (Le.) major*
	Sub-Saharan Africa, Yemen	*Ph. (Ph.) duboscqi*	Dry savannah; rodents	ZCL; *Le. (Le.) major*
European	Mediterranean Europe	*Ph. (La.) ariasi; Ph. (La.) perniciosus; Ph. (La.) tobbi*	Peridomestic; dogs	ZVL; *Le. (Le.) infantum*
	Middle East and Central Asia	*Ph. (Ph.) papatasi*	Arid; Gerbils and rodents	*ZCL; Le. (Le.) major*
South-East Asian	Bangladesh, India, Nepal,Sri Lanka	*Ph. (Euphlebotomus) argentipes*	Peridomestic; humans	AVL; *Le. (Le.) donovani*
	Sri Lanka	*Ph. (Eu.) argentipes*	Peridomestic; humans?	ACL; *Le. (Le.) donovani*
	Northwest India	*Ph. (Ph.) papatasi*	Unknown	ZCL; *Le. (Le.) major*
Western Pacific	China	*Ph. (Larroussius) spp.*	Unknown	*ZVL; Le. (Le.) i. infantum*

ACL: anthroponotic cutaneous leishmaniasis; AVL: anthroponotic visceral leishmaniasis; MCL: mucocutaneous leishmaniasis; ZCL: zoonotic cutaneous leishmaniasis; ZVL: zoonotic visceral leishmaniasis.

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Annex 3. Case studies: factors related to etiology and vector bionomics

Tables A3.1–4 list factors to be considered before control according to the type of transmission.

Table A3.1. Etiology of VL in the South-East Asian, Americas and Eastern Mediterranean regions

Factor	Case 1. Peridomestic, anthroponotic VL in the South-East Asian Region	Case 2. Domestic, zoonotic in the Americas Region	Case 3. Sylvatic, zoonotic VL in the Eastern Mediterranean
Parasite	*Le. donovani*	*Le. infantum* (syn. *Le. chagasi*)	*Le. donovani*
Vector(s)	Only one sand fly vector, *Ph. argentipes*. Found in Bangladesh, India, Nepal and Sri Lanka	Main vector throughout the Americas is *Lu. longipalpis (1)*. Other vectors are *Lu. evansi (= Pintomyia (Pifanomyia) evansi) and Lu. cruzi (2)*.	*Ph. orientalis* in Ethiopia, Kenya and Sudan *(3, 4)*, Ph. martini in southern Ethiopia. Kenya, Somalia and Uganda and also *Ph. celiae* in southern Ethiopia and Kenya *(3, 5, 6)*
Reservoir	Cases of VL and PKDL are sources of infection. Non-human reservoir hosts are not known to contribute to disease transmission.	Domestic dog, *Canis familiaris*, is the key urban reservoir.	The contribution of PKDL and HIV-VL coinfections to transmission is unknown. There may also be zoonotic transmission from domestic dogs and/or rodents with recorded seroprevalences of 6–28% and blood-meals of canine origin, although the epidemiological significance of non-human hosts is unknown *(7–11)*.
Local context	Mainly transmitted in or around houses in rural areas. In Bangladesh, VL is most common in Mymensingh district. Since 2017, all sub-districts in Bangladesh have reported incidence rates below the elimination threshold. In India, transmission is restricted to the states of Bihar, Jharkhand, Uttar Pradesh and West Bengal. In Nepal, cases are most frequently reported in the southern Terai region bordering Bihar (India), but outbreaks were reported in the country's eastern hilly regions *(12)*. All sub-districts currently report an incidence rate below the elimination threshold.	Traditionally a disease of rural environments, VL has extended into more urban settings in Argentina, Brazil, Paraguay and Uruguay *(13–16)*.	VL associated with *Ph. orientalis* is predominant in low-altitude *Acacia seyal*, *Balanites aegyptiaca* and *Combretum* savannah and woodland habitats, typically on black cotton-clay soils that crack in the dry season, providing daytime resting sites for sand flies. VL associated with *Ph. martini* and *Ph. celiae* occurs in areas of red soil, where they breed in humid habitats such as *Macrotermes* termite mounds *(17)*. Living near infested termite mounds is a recognized risk factor for infection *(4)*.

Table A3.2. Vector bionomics relevant to control and surveillance of VL in the South-East Asian, Americas, Eastern Mediterranean and African regions

Factor	Case 1. Peridomestic, anthroponotic VL in the South-East Asian Region	Case 2. Domestic, zoonotic in Americas Region	Case 3. Sylvatic, zoonotic VL in Eastern Mediterranean and African regions
Seasonality	Two peaks in *Ph. argentipes* abundance vary both spatially and temporally *(18–20)*. Density falls dramatically in winter due to cool temperatures *(20)*.	Varies geographically: all year round in tropical areas with peaks at the end of the dry season in Amazonian Brazil *(21, 22)* but peaks at the end of the rainy season in seasonal regions of Brazil and Colombia *(23)*.	Intense biting activity from March/ April to June in eastern Sudan and northern Ethiopia but starts 1 month earlier in South Sudan (February–June). In southern Ethiopia, *Ph. martini* and *Ph. celiae* densities rise during the rainy season, which is considered to be the time of highest risk of infection with *Le. donovani*;. Only *Ph. celiae*, but not Ph. martini, exhibits seasonality, showing greatest abundance in the rainy season.
Resting behaviour	Considered endophilic, but the relative importance of exophily vs endophily has received increased attention in operational research, as there is evidence of resting in vegetation as well as in houses and cattle sheds *(24–26)*.	*Lu. longipalpis* is considered more exophilic than endophilic, although its behaviour may vary across its geographical range.	*Ph. orientalis* generally occurs outside houses, in household compounds and in sylvatic locations *(27)*.
Blood-feeding behaviour	The extent to which *Ph. argentipes* feed on humans and diurnal biting times, varies across the region and with the design of the study. For example, the proportion that feeds on humans and on cattle depends on whether the sand flies are captured inside houses or in cattle sheds.	Blood-feeding preferences of *Lu. longipalpis* are largely based on host biomass and host accessibility: more blood-feeding occurs outside houses, particularly in animal shelters, near where dogs sleep and poultry are found. Humans probably receive most infectious bites in the early evening outside houses, although the extent of anthropophagy varies across the vector's geographical range.	*Ph. orientalis* are opportunistic blood-feeders, predominantly zoophagic. Blood-feeding on humans occurs close to dwellings where inhabitants habitually sleep outside and/or under an acacia tree at night. Blood-fed flies remain in the peridomestic vicinity after feeding.
Dispersal	Most *Ph. argentipes* do not disperse further than 100 m from marked sites, although the proportion that fly >100 m is higher for females than males, 15.7% versus 3.1%, respectively *(28)*.	Mark–recapture studies in Brazil and Colombia show that the *Lu. longipalpis* dispersal range is limited, the vast majority being recaptured < 300 m from the site of release *(29)*, including after insecticide treatment of chicken sheds *(30)*.	A single mark–recapture study indicated that *Ph. orientalis* do not disperse widely, most being recaptured within 300 m of the release site *(31)*.
Insecticide resistance	Reports of *kdr* mutation L1014F/S detected in bioassays *(32)*	Resistance detected in a bioassay *(33)*.	No reports

Table A3.3. Etiology of CL in the Eastern Mediterranean, European and Americas regions

Factor	Case 4. Anthroponotic CL in the Eastern Mediterranean and European regions	Case 5. Peridomestic or sylvatic zoonotic CL in the Americas region	Case 6. Sylvatic, zoonotic CL in the Eastern Mediterranean and European regions
Parasite	*Le. tropica*	Peruvian Andes: *Le. (Vianna) peruviana*; east of the Andes and Central America: *Le. (Vi.) guyanensis, , Le. (Vi.) braziliensis* and *Le. (Le.) amazonensis*; west of the Andes: *Le. (Vi.) panamensis and Le. (Le.) mexicana*	*Le. major*
Vector(s)	Main vector is *Ph. sergenti (4)*	Several[a], e.g., *Lu. intermedia (= Nyssomyia intermedia)* (northeast and southeast Brazil); *Lu. neivai (= Ny. neivai)* (central-west, south and southeast Argentina, Brazil and Paraguay); *Lu. whitmani (= Ny. whitmani)* (northeast, central-west and southeast Brazil, adjoining trifinium with Argentina and Paraguay) and *Lu. migonei (= Mignomyia migonei)* (all regions); Psychodopygus wellcomei (Amazon region) *(35)*. In the Peruvian Andes, *Lu. ayacuchensis, Lu. peruensis (= Lutzomyia (Helcocyrtomyia) peruensis), Lu. tejadai* and *Lu. verrucarum sensu lato (= Pi. (Pifanomyia) verrucarum) (36)*.	Main vector is *Ph. papatasi*. Widely distributed, from the Atlantic Ocean in the west to eastern India; North to Aral Sea (in central Asia) and south to South Sudan and southern India
Reservoir	Humans considered the principal reservoir hosts in most foci, but dogs and the rock hyrax *(Procavia capenensis)* may be secondary reservoirs, especially at the beginning of an epidemic or during a resurgence of cases *(37)*.	Wild rodents, marsupials and domestic dogs for *Le. periviana (38, 39)*.	*Rhombomys opimus, Psammomys obesus, Tatera indica* and some species of *Meriones*. In the Islamic Republic of Iran, dogs are considered secondary reservoirs. *Mastomys* spp. and the rock hyrax are the suspected reservoir hosts of CL in Senegal and Sudan, and in Yemen, respectively.
Local context	Found in Afghanistan, the Islamic Republic of Iran, Iraq, Jordan, Morocco, Pakistan, Saudi Arabia, Syrian Arab Republic and Yemen	Argentina: Transmission of forest vectors associated with the edge effect in deforestation fronts and other environmental changes *(40)*. Peru: suspected sand fly species are ecologically associated with human communities or regions 800–3200 m above sea level in which CL is endemic. Venezuela (Bolivarian Republic of): intradomiciliary transmission of *Le. braziliensis* by *Lu. ovallesi (41)*.	The proximity of houses to sources of infected sand flies greatly increases the risk of infection. In new urban developments, houses on the periphery, close to "undeveloped" or undisturbed desert, have the greatest number of ZCL cases. As conurbations extend, the risk moves outwards with development. Extensive agricultural projects, either to improve irrigation of established settlements or new "farming-the-desert" schemes, from Morocco to Uzbekistan, have also had highly significant increases in the numbers of ZCL cases. The historical risk of CL is positively associated with the Amazonian, Andean and Savannah clusters in a decreasingly manner but negatively associated with the forest evergreen, forest-crop and forest-populated clusters; the agricultural clusters were not associated with the CL cases *(42)*.

[a] More details of the sand fly vectors of American CL in Brazil, especially of the genus Nyssomyia, are available in the work of Rangel et al. *(35)*.

Table A3.4. Vector bionomics relevant to control and surveillance of CL in the Eastern Mediterranean, European and the Americas regions

Factor	Case 4. Anthroponotic CL in the Eastern Mediterranean and European regions	Case 5. Peridomestic or sylvatic zoonotic CL in the Americas region	Case 6. Sylvatic, zoonotic CL in the Eastern Mediterranean and European regions
Seasonality	Generally, two peaks occur, during June–July and August–September; however, the distribution of sand flies within a focus of disease (i.e., over a few to tens of km) is not uniform *(43)*.	Depends on species and distribution. Peru: Abundance decreases through January–March. Proportionally more *Lu. peruensis (= Lutzomyis (Helcocyrtomyia peruensis)* are found in houses in the wet season but less in outdoor collections. In contrast, more *Lu. verrucarum (= Pi. (Pifanomyia) verrucarum)* are found in houses during the dry season. There is also seasonal variation in diurnal activity patterns (arrive later in April and November but earlier in June–August) *(44)*. Venezuela (Bolivarian Republic of): *Lu. ovallesi (= syn. Pintomyia (Pifanomyia) ovallesi)* is a dry-season species with highest abundance between November and February *(45)*. In the region of the "triple border" countries: Argentina, Brazil and Paraguay: *Lu intermedia (= Nyssomyia intermedia), Lu. whitmani (= Ny. whitmani); Lu. fischeri (= Pintomyia fischeri); Lu. migonei (= Migonomyia migonei)* occur almost all year round, especially in the hottest months and a relative humidity of ~ 70%.	*Ph. papatasi* usually has a bimodal abundance: in the Jordan Valley and Tunisia, it peaks in July and October, with very little activity in August. In Shiraz, Islamic Republic of Iran, the sand fly season is May to mid-November with a peak in June or July and a second in August and September, where rainfall is significantly correlated with the relative density of sand flies. In the Negev desert, (southeast Israel), a single, unimodal peak of activity culminated in August *(46)*. Infection rates of *Ph. papatasi* with *Le. major* vary greatly among foci and time of year *(47)*.
Resting behaviour	In most foci, *Ph. sergenti* is strongly endophilic *(48)*; in the Syrian Arab Republic, however, *Ph. sergenti* was found to be more exophilic than previously reported *(49)*, and in Morocco it was the most abundant *Phlebotomus* species found both inside (49.3%) and outside houses (52.1%) *(50)*.	Varies according to species and distribution. Peru: *Lu. peruensis (= Lutzomyia (Helcocyrtomyia)* peruensis) is markedly endophilic in the wet season *(44)*. Venezuela (Bolivarian Republic of): *Lu. ovallesi (= syn. Pintomyia (Pifanomyia) ovallesi)* has been observed resting on walls.	Most commonly rest in rodent burrows
Blood-feeding behaviour	Peak of biting activity depends on the ecological zone and time of the year. It ranges from a few hours after sunset, e.g., *Ph. sergenti* in Syrian Arab Republic from 20.00–22.00 but 24.00–01.00 in the Sinai peninsula and occasionally just before dawn *(46)*.	Peru: Host-seeking activity varies according to site: in Huanchoc, there is a peak for both *Lu. peruensis (=Lutzomyia (Helcocyrtomyia) peruensis) and Lu. verrucarum (= Pi. (Pifanomyia) verrucarum)* from 18:00–19:00, but is ~20:30 for *Lu. peruensis* in Iscas *(44)*. Venezuela (Bolivarian Republic of): *Lu. ovallesi (= syn. Pintomyia (Pifanomyia) ovallesi)* activity increases from 18.00–20.00, plateaus until 24.00 and then starts to decrease.	*Ph. papatasi* bites both indoors and outdoors. They feed on a wide range of hosts and on animals, which play no part in maintenance of the parasite but help maintain high densities of sand flies. Biting rates can be sufficiently high for sand flies to be a pest (e.g., for *Ph. papatasi* in Khuzestan, Islamic Republic of Iran, biting rates can be as high as 120 bites/h near rodent burrows) *(51)*.
Dispersal	Not investigated	One study suggested that a single human host in a crop does not attract *Lu. peruensis (= Lu. (Helcocyrtomyia) peruensis*) over distances > 5 m *(52)*.	Can fly several kilometres *(53)*. The distance likely to be covered by sufficient infected flies to be of epidemiological importance is probably ≤ 1.5 km, although it is generally accepted that most flies travel < 1 km. Prevailing wind direction influences dispersal *(46)*.
Insecticide resistance	No reports	No reports	Report of *kdr* mutation L1014F/S *(54)*.

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Annex 4. Considerations before deploying interventions

Table A4.1. Indoor residual spraying and insecticide-treated nets

Target	Insecticide	Where and when appropriate	Programme requirements	Other considerations
Indoor residual spraying				
Endophilic species	Several classes available (select an insecticide with no known resistance)	Sand fly vector is endophilic and endophagic. In areas with permanent structures that have both a ceiling and walls and are not frequently re-plastered or otherwise modified and where the majority of the local population sleeps indoors, particularly during peak leishmaniasis transmission seasons, seasonal transmission trends, including the timing and length of transmission peaks, should be investigated to ensure that spraying is done at the optimal time and frequency for the setting.	Sustained political and financial support from government and/or funding bodies. A health system with adequate capacity to deliver the programme Supply of WHO-prequalified insecticides Equipment: hand compression pumps and personal protective clothing Mixing and storage facilities and equipment for the selected insecticide Staff, including highly trained spray squads, supervisory personnel for each spray squad and operations managers Transport Centralized reporting, monitoring and evaluation systems	IRS should be conducted more than once a year; a high level of community engagement and acceptance must be sustained for adequate coverage.
Insecticide treated nets (ITNs)				
ITNs primarily target nocturnal endophagic/endophilic sand flies. When nets can be hung outdoors, they may also be effective against exophagic species.	Synthetic pyrethroids, alone or combined with the synergist (piperonyl butoxide), pyriproxyfen or chlorfenapyr	ITNs are cost–effective and sustainable for leishmaniasis control in regions where the entomological, social and demographic factors are suitable. They are better than IRS when used as the primary intervention for leishmaniasis control, i.e., no specialized equipment or training required for distribution; no treatment of LLINs necessary during their useful life (≤ 3 years). ITNs can be given for personal protection of people at risk (HIV-VL and PKDL cases).	Stockpile of ITNs adequate to cover the target population Storage facilities for surplus nets Transport Distribution centres or channels (e.g., public health centres, antenatal clinics) Staff to carry out initial population surveys and distribute nets Programme management staff Centralized reporting, monitoring and evaluation systems	The effectiveness of ITNs against vector-borne disease transmission requires continuous compliance in terms of both consistency and accuracy by users. No guidelines available on population coverage for either CL or VL, and research should be conducted on the minimum coverage required to reduce leishmaniasis transmission in various eco-epidemiological settings.

Table A4.2. Supplementary interventions

Target	Insecticide	Where/when appropriate	Programme requirements	Other considerations
Insecticide-impregnated dog collars				
Sand flies that bite canine reservoirs of VL	Synthetic pyrethroids	When domestic dogs are the main reservoirs of VL	Dog collars and storage facilities Transport Distribution centres or channels (e.g., public health centres) Staff to conduct initial population surveys, distribute collars and replace lost collars Programme management staff Centralized reporting, monitoring and evaluation systems	Community awareness and engagement are important for sustained success. No guidelines have been issued on population coverage requirements for VL.
Environmental management				
Various species	None	When housing and conditions in surrounding environments provide potential resting or breeding sites Where housing schemes provide improved housing to underprivileged people (e.g., in Bihar, India)	Materials for filling cracks, equipment for removing organic debris and burrows Transport Staff to conduct initial population surveys and engage with the community Programme management staff Centralized reporting, monitoring and evaluation systems Government housing scheme	Community awareness and engagement are important for sustained success. No guidelines on population coverage have been issued for either CL or VL.

Annex 5. Effectiveness of measures to control sand flies

The effectiveness of various control tools and methods have been investigated, mainly by recording entomological parameters; only a few measured the epidemiological impact (effectiveness) on the disease *(1)*. A study design that includes calculation of sample size, an adequate follow-up period and a low risk of bias is considered to provide higher-quality evidence *(2)*. WHO recommends the "grading of recommendations assessment, development, and evaluation" (GRADE) approach for synthesizing and grading evidence on the health effects of interventions against diseases; however, no WHO guidelines are available for interventions against sand flies. A Cochrane review of leishmaniasis vector control methods was published in 2015 *(1)*, and a meta-analysis of systematic reviews was performed of trials in which the following tools or methods were considered: IRS, ITNs, insecticide-treated curtains (and other materials), environmental management, control targeting reservoir hosts and strengthening vector control through education *(3)*. These reviews address the quality of evidence in some of the studies described below.

A5.1 Tools used for community protection

IRS

On the basis of experience with use of IRS for control of malaria and co-endemic VL, IRS has continued to be a key intervention; however, the evidence for its effectiveness if of low certainty. Similarly, although several studies have been performed in various eco-epidemiological settings to determine the effectiveness of IRS against leishmaniasis, few were randomized controlled trials or evaluated the epidemiological impact of IRS. For the 2015 Cochrane review *(1)*, only two randomized controlled trials were found, which addressed the impact of IRS on CL incidence in Afghanistan *(4)* and IRS and VL seroconversion in Brazil *(5)*). In Afghanistan, the incidence of clinical CL was significantly reduced in the intervention arm; in Brazil, however, no difference in the risk for VL seroconversion was found between the two arms. Both studies were at risk of one or more forms of bias *(1)*. A subsequent randomized controlled trial in Morocco in 2016 showed that IRS was more efficient and cost–effective against CL than other interventions, including LLINs *(6)*.

Intervention and observational studies of the entomological impact of IRS have had mixed results within the same sites, indicated only short-term effectiveness and did not show clinical impact. High-quality evidence is required on the effectiveness of IRS in reducing the incidences of VL and CL in a broad spectrum of eco-epidemiological settings.

ITNs (LLINs)

Although WHO strongly recommends use of LLINs for malaria control, on the basis of high-certainty evidence *(7)*, there is limited evidence for the use of ITNs in leishmaniasis control. For the 2015 Cochrane review, two cluster-randomized controlled trials of the impact of ITNs on CL incidence and one on VL incidence were identified *(1)*. Reyburn et al. *(4)* reported a significant reduction in the incidence of CL in Afghanistan after distribution of permethrin-impregnated ITNs; however, Emami et al. *(8)* did not find a statistically significant difference in CL incidence between the intervention and control arms after adjustment for clustering *(1)*, and Picado et al. *(9)* found no statistically significant reduction in VL incidence or seroconversion in India or Nepal. Three cluster-randomized controlled trials of the effect of ITNs on sand fly density were identified by González et al. *(1)*, of which two *(8, 10)* found significant reductions after the intervention, and one *(11)* found no overall difference in Nepal but significant reductions in Bangladesh and India. Mondal et al. *(12)* reported a significant reduction in the number of VL cases identified through active detection in intervention villages and households that received deltamethrin-impregnated bed nets as compared with controls, whereas Picado et al. *(9)* observed no significant reduction in either the risk of infection or sero-conversion in clusters receiving LLINs as compared with controls.

A large multi-year cluster randomized control trial in Morocco found that LLINs had no significant impact on the incidence of anthroponotic CL caused by *Le. tropica* or *Ph. sergenti* sand fly abundance as compared with the control arms *(6)*. In the Syrian Arab Republic, however, where Le. tropica is also transmitted by *Ph. sergenti*, distribution of ITNs resulted in a significant reduction in CL incidence as compared with control villages that received untreated nets in two trials) *(13)*. Gunay et al. *(14)* also observed a significant decrease in the incidence of CL in rural Türkiye after distribution of LLINs, but they found no reduction in the density of *Ph. tobbi* in houses.

Dog collars

Studies of the effectiveness of impregnated dog collars have been performed in several countries, in addition to the evidence obtained in Brazil (section 2.2). Two community-wide cluster randomized trials of collars have been performed, one against the incidence of childhood seroinfection *(15)* and the other against the incidence of childhood clinical VL *(16)*. The trial of effectiveness, conducted by the Leishmaniasis Control Programme in northwest Islamic Republic of Iran between 2002 and 2006, found that dog collars impregnated with 4% deltamethrin provided 50% (95% CI: 17.8–70.0%) protection against infantile VL. The minimum coverage (proportion of dogs collared) required to provide herd immunity has yet to be established.

In a study in Colombia in dispersed rural settlements in the sub-Andean region, use of deltamethrin- and lambda-cyhalothrin-impregnated LLINs led to a significant (74–76%) reduction in the indoor density of *Lu. longiflocosa (17)*.

Environmental management

A high subsoil water level and damp, dark houses and mud walls with cracks and holes are typically found where leishmaniasis is endemic and are risk factors for transmission *(18)*. Mixed results have been obtained in trials, however, and there is concern about whether environmental control can be included in large-scale vector control

programmes or adds any benefit where IRS is used *(19)*. Plastering of house walls with locally available materials (lime, mud, cement, fly ash) reduced the indoor densities of *Ph. argentipes* population in Bangladesh, India and Nepal *(11, 20)*. Deep ploughing of burrows of reservoir hosts associated with zoonotic VL transmission, which removes their favoured food source, and planting of trees in a 2–3-km zone around human settlements reduced the incidence of zoonotic VL in the human population, but concern has been raised about the sustainability of the approach; furthermore, it is less effective against some reservoirs *(21)*.

Interventions to improve housing to mitigate risk factors associated with leishmaniasis, such as damp, dark houses with mud walls that have cracks and crevices, have had mixed results *(18)*. In rural VL-endemic areas on the Indian subcontinent, houses and particularly thatched dwellings have many cracks and crevices in which *Ph. argentipes* can hide *(22)*. Environmental management usually involves filling in cracks and crevices in walls and floors with mud or lime. A small field trial showed a 42% reduction in sand fly density 5 months after treatment of the walls of houses and cattle sheds *(23)*. Another study in India, however, found that plastering of walls was a risk factor *(24)*, and two studies conducted in Bangladesh and India, with monitoring for 12 months, found that mud or lime plastering did not significantly reduce the densities of *Ph. argentipes* as compared with no intervention *(10, 11)*. Furthermore, concern has been raised about the cost–effectiveness of environmental control.

In the state of São Paulo, Brazil, properties that pose a risk for the presence of sand flies include domestic animals, abundant vegetation and accumulation of organic matter in the soil. Environmental management consisting of removal of outside organic matter and trimming trees resulted in small reductions in the numbers of *Lu. longipalpis* in CDC traps located inside and outside structures *(25)*. A study in Rio de Janeiro indicated that environmental management could usefully supplement interventions against anthroponotic CL *(26)*.

Space spraying

Indoor space spraying is done with hand-held foggers, while outdoor space spraying is a method for wide coverage by spraying tiny droplets of insecticide, usually from vehicle-mounted sprayers. Outdoor space spaying for sand fly control is relatively recent. Cold fogging involves use of a water-based formulation, while thermal fogging involves use of heat to vaporize insecticide solutions in kerosene oil or diesel. An advantage of space spraying is that very wide coverage can be achieved, so that the method is potentially suitable for sparsely populated rural areas. Weak or very low-certainty evidence is available, however, for the effectiveness of space spraying against both malaria and leishmaniasis.

Fogging from vehicle-mounted vaporizers was used against *Ph. orientalis* in a forest area in the Upper Nile region of Sudan with either lindane or DDT, DDT resulting in a short-term reduction in density of about 80% *(27)*. During the 1970s, widespread spraying of lindane from aircraft dramatically reduced the numbers and biting rates of *P. major* in a desert area of Inner Mongolia. Although VL rates were reported to have been reduced, however, significant transmission persisted *(28)*. A small study in a Panamian forest showed only a 30% reduction in anthropophilic sand flies as a result of bimonthly fogging with malathion over 9 months *(29)*. In a more recent pilot study in Panama, when thermal fogging with deltamethrin was used both indoors and outdoors (within a 15-m range), the number of sand flies in houses was reduced by up to 50% for

up to 4 months; however, the efficacy differed markedly among vector species, and the abundance of the major CL vector *Lu. trapidoi* showed a slight increase *(30)*. Evidence for the effectiveness of fogging is generally limited. The only situation in which fogging is currently recommended by WHO is for the control of *Le. aethiopica* in East African highlands by focal spraying of areas occupied by the rock hyrax reservoir hosts to target the co-occurring CL vector species *Ph. pedifer* and *Ph. aculeatus (31)*.

On a Kenyan military base, ultra-low volume spraying with malathion was used over distances of ≤ 70 m from the vehicle, which resulted in immediate mortality of almost 100% of caged *Ph. Dubosqui* and 24-h mortality of 60%, with similarly large reductions in the numbers of wild-caught sand flies in CDC traps *(32)*. Control operations on US military bases in Iraq, which included ultra-low-volume spraying, did not reduce the numbers of sand flies *(33, 34)*, and tests in the Kuwait desert suggesting that ultra-low-volume spraying may be too high for optimal impact *(35)*. Before-and-after tests were performed in three rounds over 5 months in a Libyan CL focus, with a comparison of ultra-low-volume-sprayed and an unsprayed village, were more successful, with an almost 50% reduction in abundance of local *Phlebotomus* and *Sergentomyia* species in the sprayed village *(35)*. Truck-mounted ultra-low-volume spraying of dog kennel compounds with deltamethrin in Thessalonki, Greece, resulted in significant reductions in trap catches of *Ph. perfiliewi* but a substantial quantitative impact of application rate (18% vs 66% reductions with 1 or 2 g/ha, respectively), indicating that both height (and seasonal timing) of spraying and dose rates must be carefully optimized *(36)*.

Outdoor residual spraying

Spraying of outdoor walls, caves and vegetation with residual insecticides has been used in integrated programmes to include more exophilic sand fly species, sometimes with IRS and clearing of surrounding vegetation. It is not clear whether outdoor spraying adds value, and there is no evidence of its effectiveness. Spraying of both internal and external walls of houses (and of neighbouring properties) in which cases have been documented may reduce anthroponotic CL caused by *Le. tropica (31)*. Clearing forest for 300 m and spraying insecticide onto tree trunks has been suggested as a short-term measure to protect forest workers from *Le. guyanensis* and *Le. panamensis*, although the economic and environmental sustainability of the intervention should be evaluated *(31)*.

Outdoor residual spraying alone is commonly used in situations in which domestic or peridomestic transmission is due to sylvatic sand fly species. This usually involves creating a barrier between sand fly resting sites and villages by using residual insecticide spraying or painting and sometimes also clearing vegetation *(36–39*). The long-term logistics and environmental impact are, however, significant concerns *(40)*.

A randomized controlled trial in Bangladesh of both internal interventions (ITNs and IRS) and external spraying of potential breeding areas with residual organophosphate suggested that combining ITNs or IRS with spraying of breeding sites provided the best control; however, the added benefit of outdoor spraying was not clearly demonstrated *(41)*. Similarly, a small, short study in Argentina in which a mixture of permethrin and the insect growth regulator pyriproxyfen was sprayed inside henhouses and on the surrounding ground, plants and tree trunks resulted in a huge decrease in the number of *Lu. longipalpis* collected inside the henhouses; the impact of external spraying was not, however, evaluated *(42)*.

In a pilot study conducted near Manaus, Brazil, DDT was applied to the lower trunks of trees in which *Lu. umbratilis*, the local vector of *Le. braziliensis guyanensis* rested. The treatment reduced the abundance and number of gravid females for at least 21 days and potentially up to 11 months *(43)*. Perich et al. *(36)* sprayed a 100-m strip of heavily wooded undergrowth with cyfluthrin in a trial in Guatemala and found a greater than four times reduction in sand fly abundance for over 80 days as compared with a control area. Use of insecticide-treated cloth strips to form a barrier around a village in Israel was less successful, with no significant reduction in sand fly numbers *(37)*. A more labour-intensive control scheme in French Guiana involved mechanical felling of trees to 400 m and daily insecticide spraying of vegetation with an organophosphate to create a barrier *(38)*. The programme was very successful for > 1 year after the intervention in terms of both near-elimination of *Lu. umbratlilis* and leishmaniasis cases. The long-term logistics and environmental impact are, however, significant concerns *(44)*.

Spraying with cyfluthrin of termite mounds and animal burrows, the main larval breeding site and adult resting site for *Ph. martini* in Kenya, reduced the number of adults collected by more than 90%, and some residual effect in the control area persisted for 3 months *(45)*. Targeted outdoor residual spraying of the outside walls of buildings and household boundary reed fencing in Sudan against *Ph. orientalis* may be a promising approach *(46)*.

A5.2 Personal protection methods

Insecticide-treated materials

The evidence for using insecticide-treated materials for leishmaniasis control is weak. For a systematic review and meta-analysis *(39)*, no studies of the efficacy of insecticide-treated curtains against VL were found; however, three studies were found of the efficacy of treated curtains against CL in the Bolivarian Republic of Venezuela, Burkina Faso and Colombia *(47–49)*, which showed percentage reductions in biting density of 98%, 54% and 87%, respectively. Concern has been raised, however, about the quality of the data because of the study design used *(39)*.

Permethrin-impregnated uniforms have been issued to protect soldiers against leishmaniasis in areas of conflict. In one study in Colombia, impregnated informs significantly reduced the risk of CL by 75% *(50)*. No significant effect was observed, however, for soldiers in the Islamic Republic of Iran *(51)*. A study with volunteers indicated that a reduction in sand fly-biting may not be sufficient to protect against CL *(52)*. In Panama, three methods were tested for personal protection against Lu. panamensis, *Lu. gomezi* and *Lu. sanguinaria*. Topical DEET provided protection against biting for a few hours, DEET-treated net jackets provided protection for up to 2 weeks, and permethrin-treated clothing did not offer protection *(53)*.

Topical repellents

Topical repellents are recommended by WHO for personal protection against mosquitoes. The evidence base for using topical repellents for leishmaniasis control is weak. Neem (*Azadirachta indica* L.) and chinaberry (*Melia azedarach* L.) seed oils applied at 5% concentrations provided 98% protection for 9 h and 96% protection for 8 h, respectively, against the bites of *Ph. orientalis* in the laboratory; similar protection was obtained in the field *(54)*. In addition, a soap containing 20% DEET and 0.5% permethrin

gave 100% protection against *Lu. longipalpis* in the laboratory for up to 5 h but only 67% protection after 8 h *(55)*. When used in the field, the soap gave 100% protection against *Lu. youngi* bites immediately after application but only 44% within 4 h.

Spatial repellents and delivery systems

Evidence is lacking to recommend use of spatial repellents for the prevention and control of malaria or leishmaniasis. An area-repellent system containing cis–trans-allethrin was shown to reduce the number of bites by *Ph. papatasi* by more than 11 times in a field study in Türkiye *(56)*. A clip-on fan vaporizer device used to release metofluthrin against predominantly Ph. sergenti sand flies in the Judean desert, Israel, had no spatial repellency effect but showed insecticidal activity *(57)*.

A5.3 New tools under evaluation

Insecticidal paints and durable wall linings

Durable wall linings and insecticidal paints have been tested in Bangladesh, India and Nepal with some success *(58, 59)*. In a randomized controlled trial, the relative efficacy of an insecticidal paint containing 0.7% λ-cypermethrin, 1.0% d-allethrin and 0.063% pyriproxyfen applied to walls with a coverage of 2.43 m^2 was measured as percentage reduction in sand fly density as compared with durable wall linings, ITN and IRS. The insecticidal paint resulted in a remarkable reduction in sand fly density at various times during the 12-month follow-up *(58)*. The authors suggested that national kala-azar elimination programmes could consider use of insecticidal wall paints for sand fly control in subsequent phases of the programme, when longer efficacy is required, but noted that the cost of the paint (US$ 30 per household) as compared with durable wall linings (US$ 50 per household) was high for a national programme.

Attractive toxic sugar baits

Attractive toxic sugar baits may be used by soaking barrier fences in front of rodent colonies, spraying selected patches of vegetation and baiting stations to attract and kill *(60)*. In central Islamic Republic of Iran, barrier fences treated with sugar and boric acid located between zoonotic VL reservoir rodent colonies and village housing reduced *Ph. papatasi* by three times, and spraying of attractive toxic sugar baits onto vegetation resulted in a reduction of more than five times *(61)*. The studies were conducted in arid environments with few sources of sugar, where the toxic baits can cover a significant proportion of feeding sites. An important consideration is therefore whether an effect would be observed when many sources of sugar are available. Qualls et al. *(62)* sprayed attractive toxic sugar baits containing the neonicotinoid insecticide dinotefuran onto vegetation and distributed bait stations in a Morocco river flood plain with sugar-rich and -poor plots. During the 5-week trial, the populations of *Ph. papatasi* and *Ph. sergenti* were reduced by about 80% at all sites. These baits appear to be an extremely promising method of control within integrated vector management, although more tests in less arid environments and studies of environmental and epidemiological impact will be required to confirm their general efficacy.

Sand fly pheromones

Studies of *Lu. longipalpis* lekking behaviour suggest that insecticide sprayed in chicken sheds that kills males, and thus their sex-aggregation pheromone release, may make sprayed sites less attractive to blood-seeking females than unsprayed locations *(63)*, which could increase transmission to unprotected humans and dogs. Field trials of a synthetic copy of *Lu. longipalpis* sex-aggregation pheromone 9-methylgermacrene-B (CAS 183158-38-5) placed in a controlled-release dispenser lure indicated that, when placed with insecticide in experimental chicken sheds, it killed 13–20 times more *Lu. longipalpis* than in sprayed sheds with no synthetic pheromone *(64, 65)* and could "restore" female and male attraction to insecticide-sprayed chicken sheds *(64, 66, 67)*. Furthermore, the synthetic pheromone sprayed with lambda-cyhalothrin insecticide at chicken roosting sites reduced the incidence of confirmed *Leishmania* infection in dogs and their blood parasite loads by 53–56% and reduced the number of blood-seeking female *Lu. longipalpis* in households by 49%, comparable to the results obtained with 4% deltamethrin-impregnated dog collars *(68)*.

A reduction of 54% in vector density was also observed in untreated neighbouring houses as compared with controls when the insecticide was sprayed onto a small patch of household boundary wall *(69)*. Mathematical modelling of these data predicted that the synthetic pheromone could lure 40% of vector bites from humans, dogs and chickens *(70)*.

Systemic insecticides (endectocides)

Endectocides could directly kill sand flies while they feed on various hosts, including reservoirs such as rodents and dogs, and, as the insecticide is excreted in animal faeces, it might also kill larvae feeding on faeces. For example, the endectocide fipronil was effective against *Ph. argentipes* sand fly adults and larvae when administered to lesser bandicoot rats, roof rats and cattle *(71, 72)* and against *Ph. papatasi* feeding on desert jirds under laboratory and field conditions in Tunisia *(73)*. The effects of four endectocides against *Ph. papatasi* feeding on dogs were also evaluated. Only one, fluralaner, significantly increased sand fly mortality when tested 14 and 32 days after treatment *(74)*.

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Annex 6. Useful sources of information

Sand fly sampling methods (includes methods for mosquitoes, biting midges and ticks)

- https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/sp.efsa.2018.EN-1435 (pp. 14–22, with preservation and identification techniques).
- https://www.cambridge.org/core/journals/bulletin-of-entomological-research/article/sampling-strategies-for-phlebotomine-sand-flies-diptera-psychodidae-in-europe/28EABEB4D5F15FFDB7A0EC2DF4F902BC

Sand fly identification resources (also check online resources and the reference lists of published studies)

- https://phlebotominaenhmtypes.myspecies.info/biblio (A catalogue of the American Phlebotominae (Diptera; Psychodidae) types in the collections of the Natural History Museum, London).
- http://www.wrbu.org/VecID_SF.html
- https://www.medilabsecure.com/phlebkeytool.html (Erisoz Kasap O, Depaquit J, Rahola N, Haddad N, Gunay F, Akhoundi M et al. (2021) PhlebKeyTool. Identification of phlebotomine sandfly species in Europe, Mediterranean area and Middle-East, version 1
- https://www.ecdc.europa.eu/en/disease-vectors/facts/phlebotomine-sand-flies
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Methods for rearing sandflies and a list of different species held in colonies at various institutes (with contact details) are available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5687099/.

Networks

- The Global Vector Hub is an international network for operational and basic researchers working on various vector-borne diseases, with a focus on mosquito-borne diseases. Members have access to more resources: https://globalvectorhub.tghn.org/.
- The Gnatwork is an international network for operational and basic researchers working on sand flies, biting midges and blackflies. Their website is active, with profiles of members, and has many useful resources, including standard operating procedures for sticky trap collection of sand flies, basic identification features and identifying blood-meal sources: https://www.gnatwork.ac.uk/resources.
- VectorNet is a European network for medical and veterinary entomology: https://ec.europa.eu/eusurvey/runner/VectorNet_Community_2020. They have published useful review articles, including one on entomological endpoints: https://www.efsa.europa.eu/sites/default/files/2021-07/en-9984.pdf.
- The Anti-Vect (Application of Novel Transgenic Technology & Inherited Symbionts to Vector Control) is a network of molecular biologists, with a useful list of members: https://www.gla.ac.uk/research/az/antivec/membershiplist/

WHO resources

- Manual of procedures for leishmaniasis surveillance and control in the Americas. Washington DC: Pan American Health Organization; 2019 (https://iris.paho.org/handle/10665.2/51838).
- Métodos de vigilancia entomológica y control de los principales vectores en las Américas [Methods for entomological vigilance and control of the main vectors in the Americas]. Washington DC: Pan American Health Organization; 2021 (https://iris.paho.org/handle/10665.2/55241).
- WHO Guidelines for malaria control can be obtained by downloading a user-friendly online platform (MAGICapp) via the following link: https://www.who.int/teams/global-malaria-programme/guidelines-for-malaria.
- Bi-regional consultation on the status of leishmaniasis control and surveillance in East Africa. Geneva: World Health Organization; 2018 (https://apps.who.int/iris/handle/10665/273003).
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- Global leishmaniasis surveillance: 2019–2020, a baseline for the 2030 road map. Geneva: World Health Organization; 2020 (https://apps.who.int/iris/handle/10665/344795.
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- Global vector control response: progress in planning and implementation. Geneva: World Health Organization; 2020 (https://apps.who.int/iris/handle/10665/336658).
- Indoor residual spraying: an operational manual for indoor residual spraying (IRS) for malaria transmission control and elimination, 2nd edition. Geneva: World Health Organization; 2015 (https://apps.who.int/iris/handle/10665/177242).

WHO regional resources

Region of the Americas

- Plan of action to strengthen the surveillance and control of leishmaniasis in the Americas, 2017–2022. Washington DC: Pan American Health Organization; 2017 (https://iris.paho.org/bitstream/handle/10665.2/34147/PlanactionLeish20172022-eng.pdf?sequence=5).

South-East Asia Region (Bangladesh, India and Nepal)

- Indicators for monitoring and evaluation of the kala-azar elimination programme: Bangladesh, India and Nepal. Geneva: World Health Organization; 2010 (https://apps.who.int/iris/handle/10665/331956).

Eastern Mediterranean Region

- Framework for action on cutaneous leishmaniasis in the Eastern Mediterranean Region 2014–2018. Cairo: World Health Organization Regional Office for the Eastern Mediterranean; 2014 (https://apps.who.int/iris/handle/10665/120003).
- Regional plan of action 2019–2023 for implementation of the global vector control response 2017–2030. Cairo: World Health Organization Regional Office for the Eastern Mediterranean; 2019 (https://apps.who.int/iris/handle/10665/325805).
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European Region:

- Gradoni L, López-Vélez R, Mokni M. Manual on case management and surveillance of the leishmaniases in the WHO European Region. Copenhagen: World Health Organization Regional Office for Europe; 2017. (https://apps.who.int/iris/handle/10665/344118)

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