J Vector Borne Dis 45, December 2008, pp. 255–272

Review Articles

Insect vectors of *Leishmania*: distribution, physiology and their control

Umakant Sharma & Sarman Singh

Division of Clinical Microbiology, Department of Laboratory Medicine, All India Institute of Medical Sciences, New Delhi, India

Abstract

Leishmaniasis is a deadly vector-borne disease that causes significant morbidity and mortality in Africa, Asia, Latin America and Mediterranean regions. The causative agent of leishmaniasis is transmitted from man to man by a tiny insect called sandfly. Approximately, 600 species of sandflies are known but only 10% of these act as disease vectors. Further, only 30 species of these are important from public health point. Fauna of Indian sub-zone is represented by 46 species, of these, 11 belong to Phlebotomine species and 35 to Sergentomyia species. *Phlebotomus argentipes* is the proven vector of kala-azar or visceral leishmaniasis in India. This review gives an insight into the insect vectors of human leishmaniasis, their geographical distribution, recent taxonomic classification, habitat, and different control measures including indoor residual spraying (IRS), insecticide-treated bednets (ITNs), environmental management, biological control, and emerging resistance to DDT. Role of satellite remote sensing for early prediction of the disease by identifying the sandflygenic conditions cannot be undermined. The article also underlines the importance of synthetic pheromones which can be used in near future for the control of these vectors.

Key words Geographic distribution – *Leishmania* – *Lutzomia* – *Phlebotomus* – sandfly – taxonomy –vector control

Introduction

Leishmaniasis is one of the most diverse and complex of all vector borne diseases. Because it involves several overlapping species and sandfly vectors, the disease has a complex ecology and epidemiology. It is caused by an obligate intramacrophage protozoan, characterized by diversity and complexity. A total of about 21 *Leishmania* spp. have been identified to be pathogenic to human[1]. *Leishmania* are one of the several genera within the family Trypanosomatidae, and are characterized by the possession of a kinetoplast, a unique form of mitochondrial DNA. In most instances, they cause disease in animals, and humans become infected incidentally when they enter an area of endemicity.

Leishmaniasis presents mainly in three clinical forms, of which visceral leishmaniasis (VL) is the most severe form. Leishmaniasis has been considered tropical afflictions that together constitute one of the six entities on the World Health Organization/Tropical Disease Research (WHO/TDR) list of most important diseases. The disease is endemic in 88 countries on five continents with a total of 350 million people at risk and annually 12 million cases are re-

ported. Of the 88 endemic countries, 22 are in the New World and 66 in the Old World with an estimated incidence of ~1.5 million cases of cutaneous leishmaniasis (CL) and 500,000 cases of VL per year[1]. More than 90% of the CL cases occur in Iran, Afghanistan, Syria, Saudi Arabia, Brazil, and Peru. Of the 500,000 new cases of VL, more than 90% are reported from India, Nepal, Bangladesh, southern Sudan and north-east Brazil. Despite this widespread geographic distribution, human leishmaniasis is often very focal within an endemic area, leading to 'hotspots' of disease transmission. Leishmaniasis is transmitted through the bite of *Phlebotomus* sandflies in the Old World and *Lutzomyia* in the New World[1].

The VL elimination strategy comprises a combination of early case detection and management, and vector control. The strategy of case-finding and treatment is, thus, one of the mainstays of the elimination initiative. It is so far mainly 'passive', as it targets only those patients who consult health services with symptoms. In the absence of any effective vaccine and ideal drug (i.e. oral, least side effects and cost-effective), the best method to interrupt any vector borne disease is to reduce man-vector contact. In this review, we focus on the second pillar of the leishmaniasis elimination strategy, i.e. vector control. Many methods exist at present for leishmaniasis control which can be used individually or in combination. The selection of the method or combination of methods depends on the type of leishmaniasis to be controlled and also the method should be situation-specific. Control of VL mainly depends on its epidemiological features. In the zoonotic foci, where carriers are involved and dogs are the main vertebrate host, the effective methods include destruction of dogs and elimination of sandflies. In India, Bangladesh and Nepal, where VL is anthroponotic, the only choice is chemical and environmental control.

In this review, attempts have been made to discuss the geographical distribution, recent taxonomic classification, habitat of sandfly, conventional and latest technologies of vector control measures being used worldwide.

Leishmania: The causative agent

The *Leishmania* belongs to the kingdom: Protista, phylum: Euglenozoa and family: Trypanosomatidae. The *Leishmania* parasite which has adapted to a varied and heterogeneous environments, e.g.: (i) temperature—from 37°C in mammalian host to ambient temperature in sandfly and *in vitro*; (ii) pH—from neutral to highly acidic in sandfly stomach and the macrophage phagolysosome; (iii) nutrients and oxygen contents; and (iv) to immune attack—complement, antibodies and T-lymphocytes. This rapid adaptation to the environment must have been due to the ability of *Leishmania* to modulate the gene expression, which probably occurs by the specific gene amplification or by having several tandem repeats[2].

Life cycle of *Leishmania*

The life cycle of *Leishmania* is simple and it involves two stages without sexual stage. In insect vector, the parasite takes a promastigote form which is characterized by elongated, motile and an extracellular stage, while in vertebrates the parasite is found in amastigote form. The amastigotes are ovoid, nonmotile and intracellular stage. The insect vector injects promastigotes into the host's skin and soon after the parasite is taken-up by skin macrophages where the promastigotes transform into amastigote form within 12–24 h of inoculation. After transformation, the amastigotes multiply within the macrophage and ultimately the macrophage bursts releasing the amastigotes to infect other macrophages. This stage is chronic in nature and may continue for months to years and even for the life time without noticeable signs and symptoms, depending upon the host susceptibility and its immune status. The infected macrophages may remain localized to the skin, as in case of CL leading to ulcer formation, or may disseminate to other organs, as in VL or to the mucosa

as in mucocutaneous leishmaniasis (MCL). Further, depending on the competence of the host immune system this paradigm may change.

The opportunity to transmit the amastigotes from infected host to uninfected host of the same species or other species is provided by sandfly insect vector. The sandfly of 2–3 mm penetrates the host skin with its sharp cutting mouth parts from where small pools of blood oozes out. The sandfly feeds on this oozed-out blood pool. It is postulated that in cases of CL, the infected macrophages also ooze out with the pool of blood and are taken up by the sandfly. However, it is debatable in cases of VL, where the parasite is concentrated in the spleen, liver and bone marrow, but how it is made available to the sandfly which can penetrate only skin deep. It is believed that some infected macrophages are released in the blood circulation and it is a chance that the same macrophage is taken up by the sandfly. This chance factor holds further strong as only a few sandflies will be found infected even in a kala-azar household. Vice-versa is also true. In spite of, the fact that the sandfly will remain infected for whole life (few weeks), it can successfully transmit the infection only to a few patients.

As the amastigotes are taken up by the sandfly, the transformation of amastigotes to promastigotes starts within hours of ingestion and completely transformed into motile promastigotes within 24–48 h and keep on dividing by binary division.The mature metacyclic promastigotes are accumulated in the midgut and foregut. The sandfly transmit the infection during the another blood meal on the same or another host species.

Beside humans, numerous rodent and canine species have been incriminated as reservoirs. Several animal reservoirs have been identified in different countries for leishmaniasis. There are 500 species of phlebotomine species, of these about 30 species of the female *Phlebotomous* belonging to six genera are suspected or proven vectors transmitting parasites from animal to animal, animal to man, and man-to-man. In India, the species *Phlebotomous argentipes* transmits the disease from man-to-man[1].

Causative species, associated vectors and clinical manifestations

The clinical manifestations of leishmaniasis depend on complex interactions between the virulence characteristics of the infecting *Leishmania* species and the immune responses of its human host. The result is a spectrum of disease ranging from localized skin lesions to diffuse involvement of the reticuloendothelial system. Human leishmaniasis presents in four different forms with a broad range of clinical manifestations. Though all forms can have devastating consequences; but VL, also known as kala-azar (KA), is the most severe form of the disease, which if untreated, has a mortality rate of almost 100%. It is caused by the species of *Leishmania donovani* complex that consists mainly of *L. (d) infantum, L. (d) donovani* and *L. (d) chagasi.* The MCL or *espundia*, produces lesions, which can lead to extensive and disfiguring destruction of mucous membranes of the nose, mouth and throat cavities. The causative species of MCL are *L. (viannia) braziliensis* and *L. (viannia) guyanensis*. The CL can produce large number of skin ulcers, as many as 200 in some cases, on the exposed parts of the body. The causative species of CL are, *L. major, L. tropica, L. mexicana* and *L. amazonensis*. The fourth form is diffuse cutaneous leishmaniasis (DCL). It is an anergic variant of localized CL in which lesions are disseminated, resembling lepromatous leprosy. The disease is caused by *L. (mexicana) amazonensis* and *L. aethiopica*. The detailed list of causative species, their associated vectors and geographical distributions are depicted in Tables 1 and 2, respectively.

Taxonomy of sandfly and geographic distribution

The vector of various leishmaniasis world over belongs to Order: Diptera; Class: Insecta; Family: Psy-

Table 1. Human pathogenic species of *Leishmania* and their vectors in New World and clinical manifestations and their geographical distribution

Country	Species of *Leishmania*	Disease caused	Sandfly vector
Argentina	*L. (L.) chagasi*	VL	*Lu. longipalpis*
	L. (V.) braziliensis s.l.	CL	*Lu. intermedia*
Belize	*L. (L.) mexicana*	CL	*Lu. olmeca olmeca*
	L. (V.) braziliensis s.l.	CL	*Lu. ovallesi*
Bolivia	*L. (L.) amazonensis,*	CL, ADCL	*Lu. flaviscutellata,*
	chagasi	VL	*Lu. longipalpis,*
	L. (V.) braziliensis s.l.	CL, MCL	*Lu. carrerai carrerai,*
	yucumensis and *llanosmartini*		
Brazil	*L. (L.) amazonensis,*	CL, ADCL, MCL & VL	*Lu. flaviscutellata*
	chagasi	VL	*Lu. longipalpis*
	L. (V.) braziliensis,	CL, MCL	*Lu. wellcomei*
	guyanensis,	CL, MCL	*Lu. umbralitis*
	lainsoni,	CL	*Lu. ubiquitalis*
	naiffi, and	CL	*Lu. ayrozai*
	shawi	CL	*Lu. whitmani*
Colombia	*L. (L.) amazonensis,*	CL, ADCL	*Lu. flaviscutellata*
	chagasi &	VL	*Lu. evansi*
	mexicana	CL, ADCL	*Lu. columbiana*
	L. (V.) braziliensis s.l.,	CL, MCL	*Lu. spinicrassa*
	colombiensis,	CL	*Lu. hartmanni*
	guyanensis &	CL	*Lu. umbratilis*
	panamensis	CL, MCL	*Lu. trapidoi*
Costa Rica	*L. (L.) mexicana*	CL	*Lu. olmeca olmeca*
	L. (V.) braziliensis s.l. &	CL, MCL	*Lu. trapidoi*
	panamensis	CL	*Lu. trapidoi*
Dominican	*L. (L.) mexicana*-like	ADCL	unknown
Ecuador	*L. (L.) mexicana*	CL	*Lu. ayacuchenisis*
	L. (V.) braziliensis s.l.	CL, MCL	*Lu. gomezi* & *Lu. trapidoi*
El Salvador	*L. (L.) chagasi* & *mexicana*	VL & CL	*Lu. longipalpis*
French Guyana	*L. (L.) amazonensis*	CL, ADCL	*Lu. flaviscutellata,*
	L. (V.) braziliensis s.l.,	CL, MCL	*Lu. whitmani*
	guyanensis &	CL	*Lu. umbretilis*
	naiffi	CL	*Lu. squamiventris*
		Lu. paraensis	
Guadeloupe	*L. (L.) chagasi*	VL	*Lu. longipalpis*
Guatemala	*L. (L.) chagasi*	VL,	*Lu. olmeca olmeca*
	L. (L.) mexicana	CL	*Lu. ylephiletor, Lu. olmeca* & *cruciata*
	L. (V.) braziliensis s.l.	CL	*Lu. ovallesi*
Guyana	*L. (V.) guyanensis* &	CL	*Lu. umbretilis, anduzei*
	Leishmania sp.	MCL	*Lu. whitmani*

contd...

Table 1. *(contd.)*

Honduras	*L. (L.) chagasi* &	VL, CL	*Lu. olmeca olmeca*
	L. (L.) mexicana	CL, ADCL	*Lu. olmeca olmeca*
	L. (V.) braziliensis s.l.	CL, MCL	*Lu. ovallesi*
	L. (V.) panamensis	CL, MCL	*Lu. hartmanni*
Martinique	*L. (L.)* sp.	CL	*Lu. whitmani*
Mexico	*L. (L.) chagasi*, *mexicana* & other sp	VL, CL, ADCL	*Lu. longipalpis, Lu. olmeca olmeca*
Nicaragua	*L. (L.) chagasi*	VL	*Lu. longipalpis*
	L. (V.) braziliensis s.l.	CL, MCL	*Lu. ylephiletor*
	L. (V.) panamensis	CL, MCL	*Lu. panamensis*
Panama	*L. (L.) aristedesi*	CL	*Lu. trapidoi*
	L. (V.) braziliensis s.l.,	CL	*Lu. ovallesi*
	panamensis	CL	*Lu. gomezi*
	other *Leishmania* sp.	MCL	*Lu. panamanensis*
Paraguay	*L. (L.) amazonensis*	CL, ADCL	*Lu. flaviscutellata*
	L. (L.) chagasi	VL	*Lu. longipalpis*
Peru	*L. (V.) braziliensis s.l.*	CL, MCL	*Lu. whitmani*
	L. (V.)peruviana	CL	*Lu. peruensis* & *verrucarum*
Surinam	*Leishmania* sp.	CL	*Lu. flaviscutellata*
USA	*L. (L.) mexicana*	CL, ADCL	*Lu. olmeca olmeca*
Venezuela	*L. (L.) infantum chagasi*	VL	*Lu. evansi*
	L. (L.) garnhami,	CL	*Lu. youngi,*
	L. (L.) pifanoi	CL, ADCL	*Lu. olmeca bicolor*
	L. (L.) venezuelensis	CL	*Lu. spinicrassa*
	L. (V.) braziliensis s.l.	CL, MCL	*Lu. umbralitis*
	L. (V.) colombiensis,	VL	*Lu. hartmanni*
	L. (V.) guyanensis	CL	*Lu. ovallesi*

Table 2. Human pathogenic species of *Leishmania* and their vectors in Old World and clinical manifestations and their geographical distribution

Geographical distribution	Causative species	Disease form	Sandfly vector
North Africa, central and west Asia	*L. major*	Rural, zoonotic, cutaneous leishmaniasis, or oriental sore	*P. papatasi, P. duboscqi, P. salehi*
Central & west Asia and western India	*L. tropica*	Urban, anthroponotic cutaneous oriental sore	*P. sergenti*
Ethiopia and Kenya	*L. aethiopica*	Cutaneous leishmaniasis, diffuse cutaneous leishmaniasis	*P. longipes, P. pedifer*
Indian subcontinent, (India, Nepal, Bangladesh) and east Africa	*L. donovani*	Visceral leishmaniasis, kala-azar, post-kala-azar dermal leishmaniasis (PKDL)	*P. argentipes, P. orientalis, P. martini*
Mediterranean basin, central & west Asia	*L. infantum*	Infantile visceral leishmaniasis	*P. ariasi, P. perniciosus*

Phlebotomus argentipes

Lutzomyia longipalpis

chodidae; and Phylum: Arthopoda[3]. The parasite is transmitted by the bite of infected female sandflies: *Phlebotomus* in the Old World and *Lutzomyia* in the New World (central and south America). Morphologically they resemble very closely with each other. The name 'sandfly' can be confusing as this name is sometimes used for other species as well. Sandflies in the genus *Phlebotomus* are vectors of a bacterium (*Bartonella bacilliformis*) that causes Carrion's disease (oroyo fever) in south America. In parts of Asia and north Africa, they spread a viral agent pappataci virus (an arbovirus) that causes sandfly fever (pappataci fever) as well as protozoan pathogens (*Leishmania* spp.) that causes leishmaniasis. Only some 10% of the approximately 600 known species of sandflies are vectors, and only 30 of these are important. Fauna of Indian sub-zone is represented by 46 species, of these 11, belong to Phlebotomine species and 35 to Sergentomyia species[3]. *Phlebotomus argentipes* is the proven vector of kala-azar in India[4].

Habitat and behaviour of sandfly

In general, the Old World sandfly species live in desert or semi-arid ecosystems and the New World species in forest dwelling. Some of the Old World species breed in peridomestic situations and enter human habitations, whereas disease transmission in the New World is associated with humans living or working near the forest. The insect vector of leishmaniasis, the phlebotomine sandfly, is found throughout the world's inter-tropical and temperate regions. The sandflies are small (approximately 2–3 mm in length), hairy and soundlessly flying insects. They are found around human habitations and breed in specific organic wastes such as feces, manure, rodent burrows, leaf litter and in dark corners in the crevices of the walls having high humidity and temperature, although they can be observed in dry regions with a favourable local microclimate (crevices, termite mounds, caves, hollows and holes in tree roots, *etc.*) where 15 to 80 tiny eggs can be laid. So far, knowledge on the breeding sites of *P. argentipes* is poor. The larval stages of sandfly present in alluvial or alkaline soil. The damp and dark corners of cattle-sheds, where humus is present, and the cracks and crevices in the walls are favourable conditions for *P. argentipes* breeding. The larvae cannot survive drying out, they will feed on organic waste and then pupate. The female sandfly lays its eggs in the burrows of certain rodents, in the bark of old trees, in ruined buildings, in cracks of house walls, in animal shelters and in household rubbish, or in such environments where the larvae can find the organic matter, heat and humidity which are necessary for their development. The body and the small wings are very hairy and when at rest the insects hold their wings upright in a V-shape above them. They are poor flyers and have a flight range of a few kilometers, usually fly quite low and remain in the vicinity of their breeding ground. They are unable to fly in the presence of any wind produced by fan or ventilator also. They are usually most active at dawn and dusk.

Physiology of sandfly

The female sandfly needs blood in order to obtain the protein necessary to develop its eggs. In its search for blood they cover a radius of a few to several hundred metres around its habitat. They bite especially at night and dusk, there are exceptions to this such as *Lutzomyia wellcomei*, which bites mainly during daytime. They have short mouthparts and are pool feeders. The bite produces a rose-coloured papule surrounded by erythematous area about 10–20 mm in diameter. They can suck blood both from animals (cats, dogs, various rodents, cattle, birds and lizards,

etc.) and human. Because of their small dimensions, they can get through standard mosquito nets. Mosquito nets with a very fine mesh have the disadvantage that they make ventilation difficult, which is unpleasant in warm conditions.

As vector density is sensitive to climate variability, with vector densities varying seasonally. Parasite developmental time in vectors is also sensitive to environmental conditions, decreasing with high temperatures. We can also expect there to be contextual effects of climate on transmission, such as those mediated by natural disasters, which could increase the risk of acquiring an infectious disease[5].

Identification of sandfly species and *Leishmania* infection

Epidemiological studies on leishmaniasis often begin with vector identification, though taxonomic identification of adult insects is difficult. Because of the wider breeding distribution and large species diversity of sandflies, it is important to combine multiple collection methods in a survey. Commonly used methods include castor oil sticky traps, light-traps, emergence traps, Shannon traps, human bait landing collection, human mouth aspirators on resting sites, household insecticide knockdown collection, and malaise traps. Conventional microscope is commonly used to identify the sandfly species. Closely related species can be morphologically differentiated in one sex only. In laboratory, live sandflies were frozen to death. Later, all sandflies were stained with 20% carbol fuchsin solution and then identified to species by microscope-based on some typical morphologic characteristics: mainly internal structures (such as hair on abdominal tergites, buccal capsule, pigment patch, pharyngeal basket and spermatheca, ciborium, pharynx for females and terminal genitalia for males). This method requires refined storage conditions for samples, a highly skilled technique, and taxonomic expertise[6]. Morphologically identical species can sometimes be differentiated only with sophisticated techniques (e.g. analysis of the cuticle hydrocarbons, polymerase chain reaction (PCR), isoenzymes, *etc.*). Understanding the genetic variability of the vectors is still in its infancy. In recent years, molecular techniques have been used to differentiate the sibling species of sandflies that are similar in morphology[7]. The sandfly species can be identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of the 18S rRNA gene using the individual specimen. The method requires minimum effort and thus may be a powerful tool for research on prevalent sandfly species and the relationships between *Leishmania* species and the vectors.

Similarly, the infection of sandflies with *Leishmania* promastigotes has usually been examined by dissecting individual sandflies under a microscope. The sandflies should be fresh, and considerable skill and expertise are needed for the study of tiny individuals. Although the procedure takes a relatively long time, a large number of specimens have to be examined to obtain informative data for each area, because the rate of infection of sand flies with *Leishmania* is generally very low (0.01–1%)[8], even in endemic areas. In recent years, molecular techniques such as PCR-RFLP of the 18S rRNA gene[6], kDNA-PCR[9], fluorescent quantitative PCR[6] and mini exon PCR assay[10] are used to identify *Leishmania* infections both in experimentally infected and field-captured phlebotomine sandflies, and could be a useful tool in epidemiological studies and strategic planning for the control of human leishmaniasis. In addition, a real time PCR can also be used to detect the sandfly infection of *Leishmania*[11].

Vector control measures

Best method to interrupt any vector-borne disease is to reduce man-vector contact. Vector reduction may also be a viable strategy for the control of leishmaniasis. Although vector-reduction strategies are becoming more prominent, most previous infection-control strategies have focused on the vector. Vector-targeted

strategies are particularly attractive, since the vectorial capacity to transmit infectious diseases to humans is related to vector density and in an exponential way, to vector survival. In this case, a comprehensive plan that included drainage of standing pools of water, cutting of grass and brush, oiling of ponds and swamps to kill larvae, and capture of sandflies will be useful for substantial reduction in the cases of leishmaniasis[12]. The main sandfly vector control methods are: chemical control, environmental management, and biological control.

Chemical control

The main chemical control methods to combat sandflies are indoor residual spraying (IRS) of insecticides, personal protection through application of repellents/insecticides to skin or fabrics and the use of insecticide-impregnated materials such as sheets, veils, curtains and bednets. Another promising chemical control method is the use of synthetic pheromones to attract adult sandflies into traps, but evidence is not yet available[13].

Indoor residual spraying (IRS)

Indoor residual spraying is a simple and cost-effective method of controlling vector. It involves coating the walls and other surfaces of a house, human as well as animal dwellings and space-spraying with a residual insecticide. Insecticides include products such as organochlorines (DDT and dieldrin), organophosphates (malathion), carbamates (propoxur) and synythetic pyrethroids (permethrin and deltamethrin). For several months, the insecticide will kill all susceptible insects that come in contact with these surfaces. IRS prevents VL transmission by decreasing the sandfly survival, but it has no barrier effect. Most intense transmission of *L. donovani* in the Indian subcontinent occurs during two periods: a pre-winter peak in September–November and a post-winter peak in March–May. During the rainy season (monsoon) from June–September, the numbers of sandflies are low, in contrast to other insects. Timing of spraying is important. The residual activity of the insecticide must last through the periods of intense VL transmission or the spraying has to be repeated. To obtain a mass effect, i.e. protecting also persons in houses which were not sprayed, IRS must be applied to >70% of households in that area. The Indian Kala-azar Control Programme has applied the strategy of two rounds of DDT spraying per year since 1991 keeping in mind the long incubation period of kala-azar. Because the breeding sites of sandflies are generally unknown, control measures that act specifically against sandfly larvae are not feasible. Reports of insecticide-resistance refer to only three sandfly species (*P. papatasi*, *P. argentipes* and *S. shorttii*) against DDT in India, although there are reports of DDT-tolerance by most of the sandfly species from other countries[14].

Insecticide of choice: DDT still remains the insecticide of choice because of its low cost, high efficacy, long residual action and relative safety when used for IRS. Dosage schedule of 1 or 2 g/m^2 or 100–200 mg/ft^2 has been found to be quite effective; 5% emulsified suspension of DDT is the choice. DDT still being the choice insecticide is going to be used extensively in the kala-azar control programme in India. However, some workers have reported development of tolerance in *P. argentipes* against DDT, therefore, its sensitivity level needs to be taken into consideration while formulating any control strategies. Development of tolerance against DDT in *P. argentipes* has been reported from Samstipur[15], Bakhtiyarpur[16], Darbhanga (Assam, India), Vaishali and Muzaffarpur districts (Bihar, India)[17,18]. Thus, the magnitude of the problem is well-recognized, warranting new approaches by developing more effective and feasible control measures. Use of slow release formulation of polyvinyl acetate-based malathion SRES may be feasible proposition since this formulation is expected to give desired effect for a longer time. Malathion SRES, paint formulation has been tried against triatomid bugs which proved to be very effective in

control of Chagas disease in Brazil[19]. On the basis of house index and man hour density (MHD) of the vector species, the study revealed the effectiveness of malathion SRES, for 25–26 months. Oleveira-Filho *et al.* [20,21] reported the polyvinyl acetate-based suspension of malathion quite effective against *Lutzomyia longipalpis* and gave estimated cost ratio of 0.9 becoming cheaper than DDT due to its long residual effect. The effectiveness of spraying is not the only issue of concern, other problems are the side effects on human health and environment and their sustainability. Several factors such as cost of the insecticides, the logistic constraints, low acceptance by the community, low community participation and the emergence of resistance affect the long-term effectiveness and sustainability of these interventions.

Spraying procedure: Spraying of DDT in the indoor human dwellings including the roof structure should be done. It should also cover animal shelters (especially cow-sheds) and other structures in peridomestic situations as sandflies have been recovered both from human as well as animal dwellings. People living in mixed dwellings are at the greatest risk of contracting the disease. Special care should be taken to spray into cracks/crevices in which sandflies seek shelter. Before spraying, evaluation of technical know-how among spraying personnel, spraying technique, stroke of spraying machines, handling of pumps, total area of coverage by a given amount of insecticide, *etc.* should be undertaken. DDT should be mixed in the proportion of 3.3 pounds/3 gallons (1.1 kg/15 L) resulting in a 5% suspension at the flow rate of 750 ml/min and 45 mm distance from the target, using single nozzle. According to WHO recommendation, 2 L of 5% water dispersible powder (3.3 pounds) DDT suspension sprayed at the rate of 100 mg/ft^2 should cover 1000 square feet[22].

Entomological evidence: House spraying is reasonably effective against endophilic sandfly species such as *Lutzomyia verrucarum* and *Lu. peruensis*, *Lu. longipalpis*, *Lu. ovallesi* and *Lu. intermedia* in the New World[23], and *P. papatasi* and *P. sergenti* in the Old World[24]. In contrast, blanket house spraying failed to reduce the abundance of exophilic sandflies such as *Lu. nuneztovari* in Bolivia[25], which have a relatively low probability of contact with the treated surfaces (walls and ceilings). *P. argentipes*, in the Indian subcontinent, is endophilic. When in the early 1990s, in the VL-endemic states like Bihar and West Bengal in India, a new vector control strategy was introduced, based on two rounds of indoor residual DDT spraying, entomological studies from that time confirmed the reduction in vector abundance after spraying. It is believed that, despite reports of resistance, *P. argentipes* is still highly susceptible to DDT in most of the endemic areas. In future, the choice of insecticide will thus be important as susceptibility of sandflies is likely to vary from one village to another depending on its history of spraying and frequency of sprays.

Epidemiological data: In the Peruvian Andes, the incidence of susceptible householders acquiring zoonotic CL was significantly reduced by 54% as a result of spraying interior walls and ceilings with lambda-cyhalothrin (25 mg/m^2)[23]. In a trial with residual pyrethroid spraying with lambda-cyhalothrin (30 mg/m^2) in Afghanistan, the incidence of anthroponotic CL was reduced by 59%[24]. In Nepal, malaria and VL used to be a serious public health problem before the 1950s. Intensive DDT spraying undertaken in the 1960s and 1970s to eradicate malaria apparently was effective on VL. During the mid 1970s the insecticide spraying programme was stopped. As of 1980 cases of VL reappeared, probably facilitated by migration of people between Nepal and the neighbouring Indian state of Bihar. In 1992 and the following years, IRS programme policy consisted of spraying all endemic districts with DDT, malathion and lambda-cyhalothrin[26]. Nevertheless, Siraha district in the south-east Nepal, which had received annual residual insecticide spraying for over 10 years (1991–2001) has been continuously and severely affected by VL. The trends of disease incidence even

show an increase in cases and in geographical spread[26].

In India, there is no definite control programme for kala-azar but control was only as a by-product of antimalaria activities. Under the National Malaria Control Programme (NMCP) in 1953 and later National Malaria Eradication Programme (NMEP) now National Vector Borne Disease Control Programme (NVBDCP), DDT was extensively used. The DDT spray operation reduced the sandfly population to the very low levels resulting in interruption of kala-azar transmission and virtual elimination of the disease. Spraying of residual insecticide was withdrawn under NMEP in phased manner from different areas from 1962. In 1970s, kala-azar cases started being reported from Bihar signaling a simmering outbreak[13,26]. Kala-azar incidence recorded an increasing trend after resurgence till 1992. However, during 1990–91 planning commission approved a centrally sponsored Kala-azar Control Scheme which was implemented in both the endemic states, namely Bihar and West Bengal. After the implementation of this scheme, kala-azar cases showed a sharp decline from 1993 and continued till 1999. However, since 2000 the number of cases significantly increased again, which raises doubts on the continued effectiveness of the vector control strategy. Other explanations may be increased incidence of treatment failure or resistance to antimonials and the emergence of HIV-co-infections.

Feasibility, cost and acceptability

In the last decade, VL epidemiological data indicate that the impact of IRS in the Indian-Nepalese VL endemic region decreased. Several factors might contribute to this: (i) the timing and the number of rounds of insecticide spraying may not have been optimal to control *P. argentipes*; (ii) long gaps between two rounds and the short residual effect of the insecticides may have allowed the vector to increase its numbers and in this way resistance may have developed; (iii) 'Patchy' geographical coverage of spraying; (iv) community-related factors, such as poor user acceptance and low community participation during the spraying campaigns; (v) programme-related issues, such as cuts in public spending, lack of trained manpower, managerial problems, corruption and mismanagement of the stocks of insecticide product including its diversion to the black market for agricultural purposes[26]. The effectiveness of these spraying programmes is not the only issue for concern but their side effects are also important on health and environment, and their potential for sustainability, which depends on the cost of the insecticides and their application, in addition to the above mentioned factors.

Biochemical analyses of insecticide resistance

Sandflies have also developed resistance to the chemicals, mainly to DDT and in some cases to malathion and pyrethroids. Any proposed intervention aimed specifically at sandfly populations must then assess extent resistance, in order to design an effective control programme. Moreover, it is important to determine the biochemical and molecular basis of any such resistance, since alterations in one group of resistance-associated enzymes may confer cross-resistance to other classes of insecticide. By examining the involvement of various resistance mechanisms using standard biochemical assays for monitoring insecticide resistance, the potential effectiveness of alternative insecticides can be predicted. Some of the resistance-associated enzymes are cytochrome p450 mono-oxygenases and glutathione-s-transferases (GST), acetylcholinesterase (AChE), non-specific carboxylesterases, sodium channels and the γ-aminobutyric acid (GABA) type A receptor. The majorities of these resistance-associated esterases are not membrane-bound and can be readily measured without the need to solubilize them with a detergent. Insensitive AChE confers resistance to both organophosphorus (OP) and carbamate insecticides which is caused by one or more point mutations within the

ace gene(s). Malathion resistance can also be conferred by malathion-specific carboxylesterases alone or in combination with elevated esterases. Mutations in the voltage-gated sodium channel confer resistance to DDT and to pyrethroids, whilst an alteration in the GABA receptor confers resistance to cyclodienes (e.g. dieldrin) and to phenyl-pyrazoles such as fipronil[27].

Insecticide impregnated bednets

Insecticide-treated bednets (ITNs) are one of the most effective methods of reducing man-vector contact in intra and peridomiciliary transmission of vector-borne diseases. The principle of ITNs is to act as 'baited traps' with the odour of the sleeper as bait, alongside a deterrent and repellent effect. The effectiveness of untreated bednets as a tool for prevention of parasite transmission depends on mesh size, behaviour of the vector in terms of biting habits, and on sleeping habits. *Phlebotomus argentipes* sandflies live in and around houses and biting occurs at night, mainly during 2100–0100 hrs peaking at 2300–2400 hrs. Bednets could thus be a useful tool in VL control, however, in order to be physically sandfly-proof bednets need to have a finer mesh (>200 holes/inch^2) than those used against malaria mosquitoes. In eastern Sudan, where another sandfly *P. orientalis*, transmits VL, the mean total number of bites/man/night was investigated with human volunteers, staying either under an impregnated bednet (156 mesh, lambda-cyhalothrin 10 mg/m^2), an untreated bednet or without a bednet. Sandfly biting was zero for persons using impregnated bednets, but was also significantly reduced for persons staying under untreated bednets (6.92 ± 2.71 bites/man/night) while persons without bednets was bitten many times (32 ± 8.3 bites/man/night)[28]. In most studies, the insecticides used were synthetic pyrethroids (permethrin, deltamethrin, lambda-cyhalothrin), which combine the properties of low to moderate mammalian toxicity, low volatility and high insecticidal activity[29]. Insecticide-treated nets combine the individual protection of a bednet with the effect of insecticide. Due to the deterrent and repellent effect of the insecticide, mesh size does not matter as long as the insecticide remains active. As with residual spraying, vector abundance inside houses is expected to be reduced, giving relative protection to people inside the room but outside the net. In terms of acceptability, ITNs theoretically have the advantage that less insecticide is used and that the household exerts control over its application, thus depending less on the performance of a top-down planned disease control programme.

Entomological evidence: Studies carried out in Italy[30,31], Burkina Faso[32], Sudan[33], Kenya[34,35], Afghanistan[28], Iran[36], Syria[37,38], Turkey[39], Bolivia[40], Colombia[41] and Venezuela[42] showed that insecticide-treated materials have high degrees of toxicity on contact with sandflies. Insecticide resistance of *P. argentipes* against pyrethroids has been reported in Pondicherry, India[43]. In contrast, the repellent effect of insecticide-treated materials does not seem to be systematic. While curtains treated with permethrin reduced indoor density of *P. duboscqi* and *Sergentomyia* spp in Burkina Faso and of *P. perfiliewi* in Italy. *P. papatasi* indoor density in Khartoum did not differ between rooms with and without permethrin curtains. In field studies in Iran[36], Syria[38] and Turkey[39], the presence of deltamethrin (25 mg/m^2) in bednets and curtains seemed to have no effect indoors and outdoors, on the density of *P. papatasi* or *P. sergenti.* However, caution needs to be taken in the interpretation of the results, sticky traps may not be the best technique to evaluate repellence and results may be affected by the design of houses, e.g. in Syria, bedrooms are large with high ceilings, meaning that sandflies have plenty of space in which to escape when repelled by the insecticide. As with IRS, epidemiological rather than entomological data should provide the strongest indication as to the efficacy of bednets on transmission.

Epidemiological evidence: There are some studies (trials and retrospective analyses)[24] that looked spe-

cifically at the impact of ITNs on the incidence of CL, and two on VL. In Afghanistan[24] (ACL transmitted by *P. sergenti*), a household study in Kabul compared permethrin-treated bednets with two other treatments (house spraying and impregnated bedsheets). There was a marked reduction on CL incidence, from 7.2 to 2.4% showing 65% protective efficacy, while in the arm of sprayed houses, the incidence was 4.4%. In Iran, bednets impregnated with deltamethrin reduced incidence of ACL with 60% in Bam, while 97% reduction was reported in Shiraz and Sedeh with permethrin-treated long-lasting bednets (Olyset Net)[36]. In Isfahan, the incidence of zoonotic CL (*P. papatasi*) dropped to zero with deltamethrin-treated bednets plus curtains and in Mashad, the incidence of ACL (*P. sergenti*) dropped from 3.3 to 0.69%. In Turkey, deltamethrin-treated bednets reduced ACL incidence from 1.87 to 0.035% in Yenice and from 2.3 to 1.32% in Seruc, while incidence increased in control areas and areas provided with non-impregnated bednets[39]. Finally, in Syria the CL incidence dropped from 5.1% (103/2035) to 3.1% (59/1910) ($p < 0.05$), compared to control villages which showed an increase instead[38]. This was confirmed a few years later by a matched cluster randomized trial in 10 other villages, showing a protective efficacy of about 85%[37].

In 1995, inhabitants of Galabat province (Gaderef state) using bednets impregnated with lambda-cyhalothrin insecticide had a significantly lower incidence of VL, caused by *P. orientalis*, from 12.4 to 1.6%[44]. The ratio of clinical to sub-clinical infections of *L. donovani* changed from 7:1 in the non-intervention village to 1:3 in the intervention villages. A second evaluation report from Sudan demonstrated a significant reduction of VL by 59% using ITNs[45] (small mesh deltamethrin 25 mg/m^2 bednets) during an epidemic of VL in 1999–2001. There is no evidence on the impact of ITNs on VL incidence in south-east Asia, while ITNs are increasingly being promoted and supplied in the fight against malaria worldwide. In India, Bangladesh and Nepal this has been largely limited to those areas that are high-priority in terms of malaria transmission. These areas do not overlap with those with high kala-azar attack rates. Therefore, so far little evidence is available about potential implications of ITNs in regions where both diseases are co-endemic[28].

.

Feasibility, cost and acceptability: Till now, no studies measured the cost and cost-effectiveness of impregnated bednets in the prevention of leishmaniasis. The cost of intervention of long-lasting insecticidal nets (LLIN) has been calculated for malaria programmes. Assuming three years duration and a standard cost of 5 US$ per LLIN, the average annual economic cost per ITN distributed in five different African countries varied from 3.47 to 7.64 US$[46]. Acceptability studies, conducted in Africa and Latin-America have shown that people generally accept nets on the basis of their effectiveness in reduction of the nuisance of the mosquitoes rather than as a device to prevent disease and preventive behaviour. In hot weather, bednets with fine mesh have been described as unpleasant to use as they are poorly ventilated. By the repellent effect of insecticide coated on or in the fabric, a wider mesh can be used overcoming this problem.

Data on ITN use in Bihar, India are not available, but data from Nepal and Bangladesh showed that bednet acceptability is unlikely to be a major concern. Notably, in three VL endemic districts of Nepal, an age and gender matched VL case-control study found that more than 70% of the 105 controls reported the regular use of (untreated) bednets[47], and a random survey of 1800 households in six endemic districts found that 76% of households owned at least one (untreated) bednet, while 47% of households reported that all householders used a bednet. In a community study in a highly affected district in Bangladesh, 86% of the population reported sleeping under a bednet and 91% lived in a house that owned at least one net[48]. Nevertheless, the main weakness of an ITN strategy is that, contrary to IRS, its use depends on the individual

decision beyond the control of the programme delivering the tool. There is need to propose culturally sensitive and appropriate recommendations for VL prevention. Any ITN strategy should take into account the factors that motivate a family to acquire and appropriately use bednets. Trials in malaria control have shown that, in order to achieve a mass or community effect in addition to the personal protection, a high percent of coverage of the community is needed. This suggests that free or heavily subsidized provision of treated nets, comparable to a house spraying campaign, is likely to be more cost-effective than trying to market nets to poor, rural populations.

As is the case for all vector control methods, the challenge is to maintain the effort after its initial success. In Syria, after showing the high efficacy of ITNs in preventing ACL, a second study evaluating the impact of interruption on ITN intervention showed a return to pre-intervention prevalence within 1–2 years. Long-lasting insecticidal nets (LLINs) are a major step forwards, as yearly re-impregnation is not necessary, but they also have a limited life span and will eventually need to be replaced.

Environmental control

The principle behind the environmental control is to manage the environment to make it unsuitable for breeding of sandfly. In 1980, Vyokov in USSR successfully controlled leishmaniasis by destroying rodent burrows[49]. In technological control of sandflies, the walls of the resting sites can be plastered filling all the cracks and crevices by mud and lime, and the breeding of sandflies can be stopped. Lime has a powerful water absorbing capacity which makes it unsuitable for the sandfly breeding. In an experiment, Dhiman successfully controlled 70% population of *P. papatasi* by constructing cement skirting of 9" vertically on the wall and 9" horizontally on the floor[50]. A study in Bihar[51] found immature stages of *P. argentipes* mainly in human dwellings, while another study[52] found most positive samples inside cattlesheds, some in mixed dwellings and none in houses without cattle. An environmental management strategy, making in-house breeding of the sandflies impossible by filling cracks and crevices in walls by mud and lime, was implemented in Bihar. This reduced sandfly density, but cracks and crevices reappeared within seven months.

In epidemiological studies in Nepal[47] and in Bangladesh[53], the proximity of cattle has been identified as a protective factor (cattle as preferred blood source). No evidence was found on environmental interventions tackling the proximity of cattle.

Biological control

Very scanty information is available on the biological control of sandfly. In laboratory studies, infecting sandflies with different organisms such as nematodes, bacilli viruses and fungi characteristically kill pre-adult and adult sandflies. De Barjac *et al*[54] first time demonstrated the role of *Bacillus thuringiensis* var. *israelensis* in the control of larvae of *P. papatasi* and *Lu. longipalpis*. Robert *et al*[55] successfully used *Bacillus sphaericus* in the control of *P. martini* in Kenya. They also observed inhibitory effect of *B. sphaericus* on hatching of eggs of *P. duboscqi.* As the application of biolarvicides in the field condition is difficult due to diverse breeding habitat of sandfly, their practical application appears to be of limited use in the control of VL.

Some plants, such as *Solanum jasminoides, Ricinus communis*, or *Bougainvillea glabra*, are toxic for adult sandflies. Certain plants (*Capparis spinosa*, *Ricinus communis*, *Solanum luteum*) used as sources of sugar by sandflies are toxic to *L. major*, and these are able to kill sandflies. Planting these (*Bougainvillea glabra*, *Ricinus communis*, *Solanum jasminoides*) in barrier zones might, therefore, provide a low-cost, sustainable alternative to insecticide use in the control of sandflies and leishmaniasis[56]. Certain plant extracts used by Amazonian Indians to kill fish

are highly toxic to *Lu. longipalpis*, such as dried leaf extracts of *Antonia ovata* (Loganiaceae) and *Derris amazonica* (Papillionaceae) killing 80 and 100% of female sandflies, respectively[56]. These plants could, therefore, represent a readily available alternative to commercial insecticides for sandfly control but this approach requires further evaluation.

Prophylactic methods: These include self protection by use of mosquito nets and of repellents. Use of nets for sandflies of approximately 36–42 mesh would definitely prevent from its biting. N,N-diethyl-meta-toluamide (DEET) is quite effective against *Lu. longipalpis*. Essential lemon oil also gave good results with 70% protection. Soap solution containing 20% DEET and 0.5% permethrin is effective against *Lu. longipalpis*, its effect lasted for 4 h. Sharma and Dhiman found that concentration of 2% neem oil mixed in coconut or mustard oil provided 100% protection against *P. argentipes* throughout the night in the field conditions[57]. Application of mustard oil alone exposed to the uncovered areas acts as repellent against *P. argentipes*[57]. Recently, it was found that 0.1% allethrin (in coil) and 1.6% prallethrin in liquid form can give maximum protection against the bite of *P. argentipes*[13].

Role of community participation in sandfly control

Health education for control of leishmaniasis is of great importance. Simple eradication of all possible resting sites of vector within a locality by the population itself can result in a significant reduction of disease incidence. Like other communicable diseases of public health importance, general and broad basic knowledge about leishmaniasis should be widely disseminated[13].

Remote sensing

In the absence of any suitable 'epidemic prediction tool', it is very difficult to forewarn or predict epidemic outbreak of the disease. With increasing accessibility to new technologies, viz. remote sensing and geographical information system (GIS), it has become possible to monitor land-use features on earth's surface over various time intervals to develop methods for rapid stratification of high susceptive areas and for the design of remedial measures. Satellite remote sensing has been successfully used in the identification of high risk areas for malaria. It is used for identifying and mapping of *P. argentipes* distribution for early prediction of disease with the help of satellite remote sensing in integration with GIS[58,59]. It shows a significant correlation of vector density with variables like temperature, humidity, settlement, crop areas, moist fallow, dry fallow, minimum normalized digitized vegetation index (NDVI) and standard deviation of NDVI. It gave detailed mapping of sandfly density in both endemic and non-endemic sites. It can be used for the distribution and prediction of vector density in other endemic areas of Bihar. Cross *et al*[58] on the basis of NDVI values have advocated the low and high probability of *P. papatasi* zone. In another study, Miranda *et al*[59] in Brazil demonstrated strong co-relation between CL infection and creek and relevant vegetation.

Role of pheromones

Pheromones are chemical substances which help in attracting the insects at a particular site for mating. This property of pheromones is yet to be exploited in the control of vector of leishmaniasis. Some workers have started efforts to explore the role of synthetic pheromones as a potential sandfly control strategy[13].

Conclusion

Kala-azar or VL has been continuing unabated in India for over a century and now considered one of the major health problems in the eastern states mainly Bihar, West Bengal and Uttar Pradesh. Our review shows that IRS with insecticides has been virtually the only strategy for leishmaniasis vector control used

in the Indian subcontinent so far. While there is clear evidence on the effect of IRS on vector abundance, past experience in the region and elsewhere has demonstrated the difficulties in its implementation, leading to poor results and emerging resistance to DDT. Nevertheless, DDT remains the insecticide of choice, but in order to achieve optimal outcomes, meticulous planning, good training, supervision, co-ordination and management will be needed to avoid the pitfalls of the past, and this means heavy logistics and high costs. In some places, alternatives of DDT will be required. When implemented correctly, however, IRS has the potential to effectively protect the whole community.

The principle of an ITN combines the effect of individual protection and insect-killing activity while a strong repellent effect could possibly enlarge its efficacy by reducing indoor and peri-domestic vector density. ITNs, therefore, have the potential to achieve individual protection for VL and users are not dependent on a top-down, government-led intervention. The new LLINs make yearly re-impregnation no longer necessary. The use of other insecticide-treated materials such as curtains or wall cloths is highly dependent on the repellent effect on the vector involved. However, there is scarcity of data on the effect of ITNs and other insecticide-treated materials on *P. argentipes* in the Indian subcontinent, and their impact on disease incidence, is not guaranteed. Given the existing difficulties in diagnosis and treatment (human reservoir including asymptomatic infections) and the absence of any vaccine, vector control of *P. argentipes* is one of the key strategies in the fight to eliminate VL from the Indian subcontinent. LLINs may be a valuable alternative to the IRS strategy currently in use, in order to maximize the benefits that can be obtained by vector control. One community trial to test the effectiveness of LLIN on leishmaniasis infection is currently underway in this region and its results are eagerly awaited.

Application of satellite remote sensing, insecticide-impregnated bednets and synthetic pheromones traps are other exciting areas which require attention. Health education about the habitat and breeding of vector species may go a long way in the vector control. As we enter the post-genomic era for many of the pathogens, vectors, and reservoirs of human vector-borne diseases, we are gaining a new understanding of genome-genome intersections that are critical to the maintenance of infectious cycles. The availability of new molecular tools such as small interfering RNA (siRNA) and micro-arrays is allowing scientists to rapidly identify and test promising new candidates for disease-interruption strategies. These strategies offer great hope that targeting specific interactions between a pathogen and either its vector or its reservoir host may lead to new approaches that can reduce human disease with minimal disturbance of the delicate ecosystems in which these pathogens persist. Vigorous research efforts need to be done to develop the larvicide for successful elimination of the vector. More research on alternative vector control methods are needed to achieve long-lasting results in the fight against kala-azar.

Acknowledgement

The author wish to acknowledge the financial assistance in the form of research fellowship to US by Indian Council of Medical Research (ICMR), New Delhi.

References

1. Singh S. New developments in diagnosis of leishmaniasis. *Indian J Med Res* 2006; *123:* 311–30.
2. Singh S, Dey A, Sivakumar R. Applications of molecular methods for *Leishmania* control. *Expert Rev Mol Diagn* 2005; *5:* 251–65.
3. Kalra NL, Bang YH. Manual on entomology in visceral leishmaniasis. Document SEA/VBC/35. New Delhi: World Health Organization 1988.
4. Swaminath CS, Short HE, Anderson LAP. Transmission of Indian kala-azar to man by the bite of *P. argentipes*. *Indian J Med Res* 1942; *30:* 473–7.

5. Collier M, Webb RH. *Floods, droughts and climate change*. Tucson: University of Arizona Press 2002; p.153.
6. Kato H, Uezato H, Gomez EA. Establishment of a mass screening method of sandfly vectors for *Leishmania* infection by molecular biological methods. *Am J Trop Med Hyg* 2007; *77:* 324–9.
7. Mukhopadhyay J, Ghosh K, Braig HR. Identification of cutaneous leishmaniasis vectors, *Phlebotomus papatasi* and *P. duboscqi* using random amplified polymorphic DNA. *Acta Tropica* 2000; *76:* 277–83.
8. Hashiguchi Y, Gomez EAL. A review of leishmaniasis in Ecuador. *Bull Pan Am Health Org* 1991; *25:* 64–76.
9. Guerbouj S, Chemkhi J, Kaabi B. Natural infection of *Phlebotomus* (Larroussius) *langeroni* (Diptera: Psychodidae) with *Leishmania infantum* in Tunisia. *Trans R Soc Trop Med Hyg* 2007; *101:* 372–7.
10. Paiva BR, Secundino NFC, Nascimento JC, Pimenta PFP, Galati EAB, Andrade Junior HF, *et al*. Detection and identification of *Leishmania* species in field-captured phlebotomine sandflies based on mini-exon gene PCR. *Acta Tropica* 2006; *99:* 252–9.
11. Gomez SE, Doud CW, Maroli M. Short report: surveillance of *Leishmania* sp. among sand flies in Sicily (Italy) using a fluorogenic real-time polymerase chain reaction. *Am J Trop Med Hyg* 2005; *72:* 138–41.
12. Klemper Mark S, Unnasch Thomas R, Linden TH. Taking a bite out of vector-transmitted infectious diseases. *N Engl J Med* 2007; *356*(25): 2567–9.
13. Kishore K, Kumar V, Kesari S. Vector control in leishmaniasis. *Indian J Med Res* 2006; *123:* 467–72.
14. Alexander B, Maroli M. Control of phlebotomine sandflies. *Med Vet Entomol* 2003; *17:* 1–18.
15. Sanyal RK. Some observations on epidemiology of current outbreak of kala-azar in Bihar. *J Commun Dis* 1979; *11:* 170–82.
16. Dinesh DS, Ranjan A, Palit A, Kishore K, Kar SK. Seasonal and nocturnal landing/biting behaviour of *Phlebotomus argentipes* (Diptera: Psychodidae). *Ann Trop Med Parasitol* 2001; *95:* 197–202.
17. Singh R, Das RK, Sharma SK. Resistance of sandflies to DDT in Kala-azar endemic districts of Bihar in India. *Bull WHO* 2001; *79:* 793.
18. Dhiman RC, Raghavendra K, Kumar V, Kesari S, Kishore K. Susceptibility status of *Phlebotomus argentipes* to insecticide in Districts Vaishali and Patna. *J Commun Dis* 2003; *35:* 49–51.
19. Oliveira Filho AH. Cost effectiveness analysis in Chagas' diseases vector control inteventions. *Mem Inst Oswaldo Cruz* 1989; *85:* 409–17.
20. Oliveira-Filho AM, Melo MT. Vector control importance in leishmaniasis transmission. *Mem Inst Oswaldo Cruz* 1994; *89:* 451–6.
21. Oliveira-Filho AM, Melo MT. The chemical control of vector of leishmaniasis. *Mem Inst Oswaldo Cruz* 1994; *89:* 461–2.
22. Insecticide resistance and vector control. *WHO Tech Rep Ser* 1970; *443:* p.271.
23. Davies CR, Llanos-Cuentas EA, Campos P. Spraying houses in the Peruvian Andes with lambda-cyhalothrin protects residents against cutaneous leishmaniasis. *Trans R Soc Trop Med Hyg* 2000; *94:* 631–6.
24. Reyburn H, Ashford R, Mohsen M, Hewitt S, Rowland M. A randomized controlled trial of insecticide-treated bednets and chaddars or top sheets, and residual spraying of interior rooms for the prevention of cutaneous leishmaniasis in Kabul, Afghanistan. *Trans R Soc Trop Med Hyg* 2000; *94:* 361–6.
25. Le Pont F, Padilla JM, Desjeux P, Richard A, Mouchet J. Impact of the spraying of deltamethrin in a focus of leishmaniasis in Bolivia. (Impact de pulverisations de deltamethrine dans un foyer de leishmaniose de Bolivie). *Annales de la Socie´te´ Belge de Me´decine Tropicale* 1989; *69:* 223–32.
26. Joshi AB, Bhatt LR, Regmi S, Ashford RW. An assessment of the effectiveness of insecticide spray in the control of visceral leishmaniasis in Nepal. *J Nepal Health Res Council* 2003; *1:* 1–6.
27. Surendran SN, Karunaratne SHPP, Adams Z, Hemingway J, Hawkes NJ. Molecular and biochemical characterization of a sandfly population from Sri Lanka: evidence for insecticide resistance due to altered esterases and insensitive acetylcholinesterase. *Bull Entomol Res* 2005; *95:* 371–80.
28. Elnaiem DA, Elnahas AM, Aboud MA. Protective efficacy of lambda-cyhalothrin-impregnated bednets against *Phlebotomus orientalis*, the vector of visceral leishmaniasis in Sudan. *Med Vet Entomol* 1999; *13:* 310–4.
29. Curtis CF. *Control of disease vectors in the community*. London: Wolfe 1991.
30. Maroli M, Lane RP. The effect of permethrin impregnated nets on *Phlebotomus* (Diptera: Psychodidae) in central Italy. In: Leishmaniasis: the current status and new strategies for control. Hart DT ed. *Proceedings of NATO Advanced Study Institute on Leishmaniasis, Zakinthos, Greece*; New York: Plenum 1989: p. 217–23.

31. Bongiorno G, Panchetti F, Zaim M, Maroli M. Laboratory study to investigate the efficacy of cyfluthrin EW treated nets against phlebotomine sandflies. *Annali dell'Istituto superiore di sanita*‘ 2005; *41:* 247–52.

32. Majori G, Maroli M, Sabatinelli G, Fausto AM. Efficacy of permethrin-impregnated curtains against endophilic phlebotomine sandflies in Burkina Faso. *Med Vet Entomol* 1989; *3:* 441–4.

33. Elnaiem DA, Aboud MA, El Mubarek SG, Hassan HK, Ward RD. Impact of pyrethroid-impregnated curtains on *Phlebotomus papatasi* sandflies indoors at Khartoum, Sudan. *Med Vet Entomol* 1999; *13:* 191–7.

34. Mutinga MJ, Renapurkar DM, Wachira DW, Basimike M, Mutero CM. A bioassay to evaluate the efficacy of permethrin-impregnated screens used against phlebotomine sandflies (Diptera: Psychodidae) in Baringo District of Kenya. *East Afr Med J* 1993; *70:* 168–70.

35. Basimike M, Mutinga MJ. Effects of permethrin-treated screens on phlebotomine sand flies, with reference to *Phlebotomus martini* (Diptera: Psychodidae). *J Med Entomol* 1995; *32:* 428–32.

36. Impact of Olyset long-lasting nets to control transmission of anthroponotic cutaneous leishmaniasis in central Iran: Final Technical Report. WHO, Eastern Mediterranean Region. *WHO* 2002. Project No. SGS04–76.

37. Jalouk L, Al Ahmed M, Gradoni L, Maroli M. Insecticide treated bednets to prevent anthroponotic cutaneous leishmaniasis in Aleppo Governorate, Syria: results from two trials. *Trans R Soc Trop Med Hyg* 2007; *101:* 360–7.

38. Tayeh A, Jalouk L, Al-Kahaimi A. A cutaneous leishmaniasis control trial using pyrethroid- impregnated bednets in villages near Aleppo, Syria. WHO/LEISH/ 97.41. 1997. WHO, Geneva.

39. Alten B, Caglar SS, Kaynas S, Simsek FM. Evaluation of protective efficacy of K-O TAB impregnated bednets for cutaneous leishmaniasis control in Southeast Anatolia-Turkey. *J Vector Ecol* 2003; *28:* 53–64.

40. Campbell-Lendrum D, Dujardin JP, Martinez E. Domestic and peridomestic transmission of American cutaneous leishmaniasis: changing epidemiological patterns present new control opportunities. *Memo´ rias do Instituto Oswaldo Cruz* 2001; *96:* 159–62.

41. Alexander B, Usma MC, Cadena H. Evaluation of deltamethrin-impregnated bednets and curtains against phlebotomine sandflies in Valle del Cauca, Colombia. *Med Vet Entomol* 1995; *9:* 279–83.

42. Kroeger A, Avila EV, Morison L. Insecticide impregnated curtains to control domestic transmission of cutaneous leishmaniasis in Venezuela: cluster randomised trial. *BMJ* 2002; *325:* 810–3.

43. Amalraj DD, Sivagnaname N, Srinivasan R. Susceptibility of *Phlebotomus argentipes* and *P. papatasi* (Diptera: Psychodidae) to insecticides. *J Commun Dis* 1999; *31:* 177–80.

44. Elnaiem DA. Use of pyrethroid impregenated bednets for the control of visceral leishmaniasis in eastern Sudan. Final Report (WHO ID L3 D 1881 D 47). Geneva: WHO 1996.

45. Ritmeijer K, Davies C, van Zorge R. Evaluation of a mass distribution programme for fine-mesh impregnated bednets against visceral leishmaniasis in eastern Sudan. *Trop Med Int Health* 2007; *12:* 404–14.

46. Yukich J, Tediosi F, Lengeler C. Operations, costs and cost-effectiveness of five insecticide-treated net programs (Eritrea, Malawi, Tanzania, Togo, Senegal) and two indoor residual spraying programs (Kwa-Zulu-Natal, Mozambique). Basel, Switzerland: Swiss Tropical Institute 2007.

47. Bern C, Joshi AB, Jha SN. Factors associated with visceral leishmaniasis in Nepal: bed-net use is strongly protective. *Am J Trop Med Hyg* 2000; *63:* 184–8.

48. Bern C, Hightower AW, Chowdhury R. Risk factors for kala-azar in Bangladesh. *Emerg Infect Dis* 2005; *11:* 655–62.

49. Vyokov VN. Control of sandflies. Gamaleya Institute of Epidemiology and Microbiology. In: WHO Travelling Seminar on Leishmaniasis Control. Moscow 1980: p. 6–16.

50. Dhiman, RC. Effect of minor engineering intervention in the control of breeding of *Phlebotomus papatasi* (Scopoli) sandflies. *Southeast Asian J Trop Med Public Health* 1995; *26:* 368–70.

51. Dhanda V, Shetty PS, Dhiman RC. Studies on phlebotomine sandflies as vectors of kala-azar in Bihar. In: Proceedings of the Indo-UK Workshop on Leishmaniasis. New Delhi : Indian Council of Medical Research, 1983; p. 128–37.

52. Kesari S, Kishore K, Palit A. An entomological field evaluation of larval biology of sandfly in Kala-azar endemic focus of Bihar—exploration of larval control tool. *J Commun Dis* 2000; *32:* 284–88.

53. Bern C, Chowdhury R. The epidemiology of visceral leishmaniasis in Bangladesh: prospects for improved control. *Indian J Med Res* 2006; *123:* 275–88.

54. De Barjac H, Larget I, Killick-Kendrick R. Toxicity of *Bacillus thuringiensis* var. *israelensis* serotype HI4, for

larvae of sandflies, the leishmaniasis vectors. *Bulletin de la Socie´te´ de pathologie exotique et de ses filiales* 1981; *74:* 485–9.

55. Robert LL, Perich MJ, Schlein Y. Phlebotomine sand fly control using bait-fed adults to carry the larvicide *Bacillus sphaericus* to the larval habitat. *J Am Mosq Control Assoc* 1997; *13:* 140–4.

56. Schlein Y, Jacobson RL, Muller GC. Sandfly feeding on noxious plants: a potential method for the control of leishmaniasis. *Am J Trop Med Hyg* 2001; *65:* 300–3.

57. Sharma VP, Dhiman RC. Neem oil as a sandfly (Diptera: Psychodidae) repellent. *J Am Mosq Control Assoc* 1993; *9:* 364–6.

58. Cross ER, Newcomb WW, Tucker CJ. Use of weather data and remote sensing to predict the geographic and seasonal distribution of *Phlebotomus papatasi* in southwest Asia. *Am J Trop Med Hyg* 1996; *54:* 451–6.

59. Miranda C, Marques CC, Massa JL. Satellite remote sensing as a tool for the analysis of the occurrence of American cutaneous leishmaniasis in an urban area of Southeastern Brazil. *Rev Saude Publica* 1998; *32:* 455–63.

Corresponding author: Prof. Sarman Singh, Head, Division of Clinical Microbiology, All India Institute of Medical Sciences, New Delhi–110 029, India.
E-mail: sarman_singh@yahoo.com

Received: 22 October 2008 *Accepted in revised form:* 21 November 2008