

## REVIEW ARTICLE

# Phlebotomine sandflies and the spreading of leishmaniasis and other diseases of public health concern

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**Abstract.** Phlebotomine sandflies transmit pathogens that affect humans and animals worldwide. We review the roles of phlebotomines in the spreading of leishmaniasis, sandfly fever, summer meningitis, vesicular stomatitis, Chandipura virus encephalitis and Carrión's disease. Among over 800 species of sandfly recorded, 98 are proven or suspected vectors of human leishmaniasis; these include 42 *Phlebotomus* species in the Old World and 56 *Lutzomyia* species in the New World (all: Diptera: Psychodidae). Based on incrimination criteria, we provide an updated list of proven or suspected vector species by endemic country where data are available. Increases in sandfly diffusion and density resulting from increases in breeding sites and blood sources, and the interruption of vector control activities contribute to the spreading of leishmaniasis in the settings of human migration, deforestation, urbanization and conflict. In addition, climatic changes can be expected to affect the density and dispersion of sandflies. Phlebovirus infections and diseases are present in large areas of the Old World, especially in the Mediterranean subregion, in which virus diversity has proven to be higher than initially suspected. Vesiculovirus diseases are important to livestock and humans in the southeastern U.S.A. and Latin America, and represent emerging human threats in parts of India. Carrión's disease, formerly restricted to regions of elevated altitude in Peru, Ecuador and Colombia, has shown recent expansion to non-endemic areas of the Amazon basin.

**Key words.** Carrión's disease, Chandipura virus encephalitis, leishmaniasis, phlebotomine sandflies, sandfly fever, summer meningitis, vesicular stomatitis.

## Introduction

Among the most important emerging and resurging vector-borne protozoal diseases, the leishmaniasis are second only to malaria in terms of numbers of people affected [World Health Organization (WHO), 2010]. With few exceptions, phlebotomine sandflies are the unique haematophagous insects proven to transmit leishmaniasis through the bite of infected female that have previously fed on an infected mammal. Exceptions in leishmaniasis infection are rare and include:

(a) venereal transmission (Symmers, 1960); (b) congenital transmission (Eltoum *et al.*, 1992); (c) infection by blood transfusion (Bruce-Chwatt, 1972), and (d) needle transmission among drug users (Alvar *et al.*, 2008). Suggestions that leishmaniasis may be transmitted by the bites of haematophagous arthropods other than phlebotomine sandflies (e.g. fleas, ticks) are not generally supported by convincing experimental evidence (Killick-Kendrick, 1999; Dantas-Torres, 2011), although it should be noted that strong evidence incriminating day-feeding midges (Diptera: Ceratopogonidae)

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as potential vectors of *Leishmania* spp. (Kinetoplastida: Trypanosomatidae) among red kangaroos (*Macropus rufus*) in Australia has been presented (Dougall *et al.*, 2011).

Sandflies are also known to be vectors of other human pathogens, such as *Bartonella* spp. (Carrión's disease), and a number of viral agents causing sandfly fever, summer meningitis, vesicular stomatitis and Chandipura virus encephalitis (Depaquit *et al.*, 2010).

Over the last decades, there has been significant resurgence of several long-known, vector-borne diseases. Incidences of malaria, leishmaniasis, dengue and plague have increased in numerous foci, in some of which they were thought to have been brought under effective control. In most instances, the appearance of new and the resurgence of old diseases and pathogens can be associated with ecological and climatic changes that have favoured an increase in vector densities. Irrigation, dam construction and other development projects, deforestation and urbanization have all resulted in changes in vector population densities. Furthermore, the increase in human travel has enabled the spread of infectious agents of human and animal origin (e.g. pets) by introducing them into areas from which they had been hitherto absent (Colwell *et al.*, 2011).

Although the close relationships among climate conditions, phlebotomine sandfly seasonality and leishmaniasis are well documented, limited investigations have attempted to link inter-annual fluctuations in the incidence of leishmaniasis to climate cycles (WHO, 2010). Apart from predictive studies on the possible expansion of leishmaniasis endemic zones to central Europe (Fischer *et al.*, 2011) and North America (Gonzales *et al.*, 2010), few field studies have been carried out on the effects of longterm climate change on disease dissemination. Epidemiological investigations in Sudan (Thomson *et al.*, 1999) and Tunisia (Ben-Ahmed *et al.*, 2009) have shown that the annual incidence of visceral leishmaniasis (VL) over a certain period was correlated with annual rainfall in previous years. Epidemic waves of VL in northeastern Brazil have been associated with human migration to urban areas after prolonged periods of aridity. In addition, inter-annual fluctuations in incidences of leishmaniasis in Bahia (Brazil), Costa Rica and Colombia may be associated with El Niño southern oscillation indices (Franke *et al.*, 2002; Cardenas *et al.*, 2006; Chaves & Pascual, 2006). Finally, the recent northward spread of leishmaniasis in Italy was correlated with a 30-year expansion of its vectors towards northern latitudes (Maroli *et al.*, 2008).

In this paper, we review the roles of phlebotomine sandflies in the transmission and spreading of leishmaniases, sandfly fever, summer meningitis, vesicular stomatitis, Chandipura virus encephalitis and Carrión's disease.

## Phlebotomine sandfly taxonomy, distribution and biology

### Taxonomy

The name 'sandfly' can be misleading, as it wrongly suggests to laypeople that they may be at risk of vector-borne disease while on holiday on the beach. Actually, the English

denomination refers to the pale (sandy) colour of this insect. There is further confusion because in certain parts of the world, midges of the genus *Culicoides* (Diptera: Ceratopogonidae) and blackflies (Diptera: Simuliidae) are referred to by the same name. A distinction must therefore be made for the vectors of the leishmaniases and other diseases of public health concern, which are correctly termed 'phlebotomine sandflies' (Killick-Kendrick, 1999). To date, over 800 species have been estimated to exist in different regions of the world. They are grouped in the suborder Nematocera of the order Diptera, family Psychodidae, subfamily Phlebotominae. Phlebotomine sandflies share the family Psychodidae with the non-vector, non-biting moth flies (subfamily Psychodinae), often seen around shower drains.

Currently, the classification of phlebotomine sandflies remains controversial, cumbersome and far from being definitive. Based on the pioneering classification of Theodor (1948, 1958), Lewis *et al.* (1977) proposed two genera for Old World species, *Phlebotomus* Rondani and *Sergentomyia* França, and three for New World species, *Lutzomyia* França, *Brumptomyia* França & Parrot, and *Warileya*, Hertig. The genus *Chinius* Leng, 1987 is a distinct taxon used for some Chinese sandfly species with primitive characters (Leng, 1987). These three genera (*Phlebotomus*, *Sergentomyia* and *Chinius*) are widely accepted by modern Old World taxonomists. A few other species have been or are about to be named, but so far these are of unknown medical importance (WHO, 2010). In the genus *Phlebotomus*, 11 subgenera, 96 species and 17 subspecies have been recognized by Lewis (1982).

For the Neotropical sandflies, most entomologists still follow the classification of Lewis *et al.* (1977), later reviewed by Young & Duncan (1994), who recognize the three genera named above, *Lutzomyia*, *Brumptomyia* and *Warileya*, which include 15 subgenera and 11 species groups. More recent revisions have been proposed, but none has been universally accepted. The most recent and comprehensive is that by Galati (2003), who recognized 464 species of Neotropical phlebotomine sandflies grouped into 23 genera, 20 subgenera, three species groups and 28 series (WHO, 2010).

### Distribution

Phlebotomine sandflies are principally present in the warm zones of Asia, Africa, Australia, southern Europe and the Americas (Killick-Kendrick, 1999). Their distribution extends northwards to just above a latitude of 50° N in southwest Canada (Young *et al.*, 1984) and just below this latitude in northern France and Mongolia (Lewis, 1982). Their southernmost distribution ends at a latitude of 40° S, but they are absent from New Zealand and the Pacific islands (Lane, 1993). Their altitudinal distribution extends from below sea level (Dead Sea) (Lane, 1993) to 3300 m a.s.l. in Afghanistan (Artemiev, 1980).

### Biology

Phlebotomine sandflies undergo complete metamorphosis through four developmental stages: egg; larva (four instars);

pupa, and adult. The immature stages, unlike those of mosquitoes, do not require standing water to complete their development, although they need relatively moist and warm habitats. The eggs are laid by adult females in a suitable habitat rich in organic content, such as animal excreta and soil, which provides the newly emerged larvae with shelter, nutrition and moisture. Eggs (0.3–0.5 mm in length) are initially white or light grey in colour but often turn dark brown or black within a few hours of oviposition. Egg hatching is highly temperature-dependent and subsequent larval development is generally slow. Embryonic and larval development periods were recently determined over a 1-year period for nine sandfly species belonging to six genera or subgenera, by the study of 15 laboratory colonies. After the female has taken a blood-meal and completed oviposition, first-instar larvae emerge in 12–19 days, pupae in 25–59 days, and adults in 35–69 days (Volf & Volfova, 2011).

Larvae are caterpillar-shaped with head capsules and small leaf-like antennae. They have long caudal setae that can help in their identification as sandfly larvae, although these are not usually employed in taxonomy because they are rarely collected in nature (Feliciangeli, 2004) (Fig. 1). The larvae move very little distance from the oviposition site. Pupae are similar to small chrysalises in which the fourth-stage larval exuvia are attached at one end to a solid substrate.

Adults are small and seldom exceed 3.5 mm in body length (Molyneux & Ashford, 1983). They are covered with dense hairs and hold their wings in a characteristic 'V' shape over their backs when at rest (Fig. 2). They range in colour from almost white to almost black. The legs are very long and delicate. Both males and females feed on sugary secretions from plants or from honeydew produced by homopterous aphids (Hemiptera: Aphidoidea). Females require at least one bloodmeal in order to complete development of egg batches. Only a few phlebotomine sandfly species are able to produce viable eggs without a bloodmeal. Unlike mosquitoes, their attack on the host is silent. Adults are mainly active in the evening, at night and in the early morning, although they can bite during the day if disturbed.

The flight speed of phlebotomines is considerably slower than that of mosquitoes and is <1 m/s (Killick-Kendrick



**Fig. 2.** Blood-fed female sandfly (*Phlebotomus papatasi*). Note the dense hairs and characteristic V-shape in which the wing is held.

*et al.*, 1986). They are unable to fly at wind speeds higher than this rate, which is the main factor limiting the range of their dispersal. Their flight range is typically very short (about 300 m) and thus adult activities are usually restricted to the vicinity of larval breeding sites. Evidence from mark–release–recapture studies indicates that forest species disperse at shorter distances than peridomestic ones. For example, *Phlebotomus ariasi* may disperse over 2 km (Rioux *et al.*, 1979; Killick-Kendrick *et al.*, 1984). By contrast, Neotropical forest species seldom appear to disperse over distances of >1 km (WHO, 2010). Mating (Fig. 3) occurs at or near the host. The males congregate in leks on or near the host and produce sex pheromones. Vibration of the wings by males can be important in encouraging females to mate (Oliveira *et al.*, 2001). Resting sites are often near to larval breeding sites and consist of cool, humid and dark micro-habitats (Killick-Kendrick, 1999). The seasonal activity of adult sandflies is affected mainly by temperature and rainfall.



**Fig. 1.** Fourth-larval stage of the sandfly *Phlebotomus perniciosus*.



**Fig. 3.** *Phlebotomus perniciosus* mating before a blood meal taken by the female in laboratory.



## Leishmaniases

### *Phlebotomine sandfly species involved in Leishmania spp. transmission*

Among the over 800 phlebotomine sandfly species estimated to exist, only 98 species of *Phlebotomus* and *Lutzomyia* genera are currently proven or suspected vectors of human leishmaniases (Tables 1 and 2). The role of species belonging to the genus *Sergentomyia* in *Leishmania* spp. transmission among mammal hosts needs to be elucidated (Mukherjee *et al.*, 1997; Senghor *et al.*, 2011). Tables 1 and 2 are based on data collated from Killick-Kendrick (1999) and the WHO (2010), integrated with information in the most recent literature and personal evaluations in cases of doubtful reports. Only countries that have reported indisputable endemic human leishmaniases are listed. In the Old World, proven or probable vectors account for a total of 42 species, of which 20 are implicated in the transmission of *Leishmania infantum*, six in the transmission of *Leishmania donovani*, seven in the transmission of *Leishmania major*, seven in the transmission of *Leishmania tropica* and three in the transmission of *Leishmania aethiopica*. Each species appears to be involved in the transmission of one *Leishmania* agent only, except *Phlebotomus sergenti*, which has been incriminated in the transmission of both *L. tropica* and *L. aethiopica* in parts of Ethiopia (Gebre-Michael *et al.*, 2004), and *Phlebotomus alexandri*, the role of which in the transmission of both recognized species of the *L. donovani* complex (*L. donovani s.s.* and *L. infantum*) in parts of China, and probably in other countries, is still to be ascertained. Among the phlebotomine sandflies recorded in the New World, 56 species, all of which belong to the genus *Lutzomyia*, are involved in the transmission of 15 *Leishmania* species, namely, *L. infantum* (= *Leishmania chagasi*), *Leishmania braziliensis*, *Leishmania guyanensis*, *Leishmania mexicana*, *Leishmania amazonensis*, *Leishmania panamensis*, *Leishmania peruviana*, *Leishmania lainsoni*, *Leishmania shawi*, *Leishmania naiffi*, *Leishmania garnhami*, *Leishmania pifanoi*, *Leishmania lindenbergi*, *Leishmania venezuelensis* and *Leishmania colombiensis*. By contrast with *Phlebotomus* spp., some *Lutzomyia* species are probably able to transmit more than one *Leishmania* species; for example, *Lu. migonei* has been found to be infected with four different parasite species (see below).

The incrimination of a species as a vector is based on a series of widely accepted criteria (Killick-Kendrick, 1990; WHO, 2010): (a) the vector must feed on humans; (b) in zoonotic entities of leishmaniasis, the vector must also bite the reservoir host(s); (c) the vector must be infected in nature with the same *Leishmania* species as occurs in humans, and this must be ascertained by comparison of isolates using isoenzymes or DNA; (d) the vector must support the complete development of the parasite after the infecting bloodmeal has been digested, and (e) the vector must be able to transmit the parasite by bite to a susceptible host while taking a bloodmeal.

With regard to the 'degree' of incrimination (species can be 'proven', 'strongly suspected' or 'suspected' vectors), it must be admitted that even these reference standard criteria may be subject to interpretation or may be extremely difficult to meet. An example refers to the fourth criterion concerning

the ability of a species to support parasite development: the description of permissive vectors by Myskova *et al.* (2007) indicates that no rule should be taken into account separately and isolated from an epidemiological context; these authors detected massive promastigote infections after blood digestion in 80% of Neotropical *Lutzomyia longipalpis* artificially fed on blood containing Old World wild-type *L. major*. Furthermore, meeting the fifth criterion by (laboratory) demonstration of *Leishmania* spp. transmissibility to susceptible hosts is notoriously difficult because sandflies must first be infected 'naturally' through the ingestion of the appropriate stage and number of parasites (the best option being by biting on infected reservoir hosts), and those that survive in laboratory conditions after blood digestion must be induced to feed again on a naïve susceptible host, a procedure that is quite difficult to accomplish (Pozio *et al.*, 1985).

Given these limitations, the present analysis of the literature takes into account the following minimal requirements for robust vectorial incrimination: (a) epidemiological evidence indicated by the overlapping of the geographical distributions of the vector and the human disease; (b) evidence that the vector feeds on humans, and (c) evidence that the vector supports natural gut infections with promastigotes of the same *Leishmania* species as occurs in humans. Evidence for species incrimination is further reinforced in endemic settings from which the usual proven vectors are apparently absent, or in which species meeting the criteria listed here are the only human-biting phlebotomine sandflies. Accordingly, we have produced an updated list of proven vector species by endemic country where data are available, which are shown marked with asterisks in Tables 1 and 2.

In the Old World, updated evidence suggests *Phlebotomus argentipes* as a possible vector of *L. donovani* cutaneous leishmaniasis (CL) in Sri Lanka (Senanayake *et al.*, 2011) and confirms the vectorial role of *Phlebotomus orientalis* for *L. donovani* in Kenya (Ngumbi *et al.*, 2010), *Phlebotomus salehi* for *L. major* (Davami *et al.*, 2011) and members of the *Phlebotomus major* complex for *L. infantum* in Iran (Azizi *et al.*, 2008; Emami & Yazdi, 2008). Furthermore, *P. sergenti* has been confirmed as the vector of CL caused by *L. tropica s.l.* in Algeria (Boubidi *et al.*, 2011) and Tunisia (Tabbabi *et al.*, 2011). With regard to the identity of the leishmanial agent *Leishmania killicki*, to which the latter two records actually refer, we find that Pratlong *et al.* (2009) provided clear evidence that this species belongs to the largely polymorphic *L. tropica* taxon. Finally, we were convinced by the evidence provided by Leng & Zhang (2001) and Zhang & Leng (2002) that *Phlebotomus chinensis* and *Phlebotomus sichuanensis* are indeed two separate species, both of which are involved in *L. infantum* transmission, although in different VL endemic areas of China.

In the New World, recent evidence incriminates *Lutzomyia forattinii* and *Lu. migonei* as new potential vectors of VL. In the state of Mato Grosso, Brazil, Pita-Pereira *et al.* (2008) found *Lu. forattinii* together with the isomorphic *Lutzomyia cruzi*, which was macroscopically distinguishable by its external coloration; both species were naturally infected by parasites identified as *L. infantum* by molecular methods. *Lutzomyia migonei*, recently suspected to be the VL vector

**Table 1.** Phlebotomine species of the genus *Phlebotomus* that act as vectors of Old World leishmaniases.

Proven or suspected vector species	Country*	<i>Leishmania</i> species	Clinical form in humans	
			Most common	Rare
<i>P. argentipes</i>	Bangladesh, India*, Nepal*, Sri Lanka	<i>L. donovani</i>	VL, PKDL	LCL
<i>P. celiae</i>	Ethiopia*, Kenya			
<i>P. longiductus</i>	China			
<i>P. martini</i>	Ethiopia*, Kenya*, Somalia, Uganda			
<i>P. orientalis</i>	Chad, Ethiopia, Kenya, Saudi Arabia, Yemen, Sudan*			
<i>P. vansomeranae</i>	Kenya			
<i>P. alexandri</i>	China*, Iran, Iraq, Oman	<i>L. donovani s.l./L. infantum</i>	VL	LCL, DCL†
<i>P. ariasi</i>	Algeria, France*, Italy, Portugal*, Spain*, Morocco	<i>L. infantum</i>	VL	LCL, DCL†
<i>P. balcanicus</i>	Armenia, Georgia*			
<i>P. chinensis</i>	China*			
<i>P. galileus</i>	Syria			
<i>P. halepensis</i>	Azerbaijan, Georgia, Syria			
<i>P. kandelakii</i>	Armenia, Azerbaijan, Georgia*, Iran			
<i>P. langeroni</i>	Egypt*, Spain, Tunisia*			
<i>P. longicuspis</i>	Algeria, Morocco, Tunisia			
<i>P. longiductus</i>	Kazakhstan*, Kyrgystan, Ukraine, Uzbekistan			
<i>P. major s.l.</i>	Iran*			
<i>P. neglectus</i>	Albania*, Cyprus, Croatia, Greece*, Kosovo, Italy, Republic of Macedonia, Montenegro, Romania, Slovenia, Turkey, Ukraine			
<i>P. perfiliewi</i>	Albania, Algeria*, Croatia, Greece, Israel, Italy*, Malta, Morocco, Palestine, Republic of Macedonia, Romania, Tunisia, Turkey			
<i>P. perniciosus</i>	Algeria*, France*, Italy*, Malta*, Monaco, Morocco, Portugal*, Spain*, Tunisia			
<i>P. sichuanensis</i>	China*			
<i>P. smirnovi</i>	China*, Kazakhstan			
<i>P. syriacus</i>	Greece, Israel, Lebanon, Palestine, Syria, Turkey			
<i>P. tobbi</i>	Albania*, Croatia, Cyprus*, Greece, Israel, Syria, Turkey*			
<i>P. transcaasicus</i>	Azerbaijan, Iran*, Turkey			
<i>P. turanicus</i>	Turkmenistan*			
<i>P. wui</i>	China*			
<i>P. aculeatus</i>	Kenya	<i>L. tropica</i>	LCL, LR	VL†
<i>P. arabicus</i>	Israel*, Ethiopia			
<i>P. chabaudi</i>	Morocco, Tunisia			
<i>P. guggisbergi</i>	Kenya*			
<i>P. rossi</i>	Namibia*			
<i>P. saevus</i>	Ethiopia*			
<i>P. sergenti</i>	Afghanistan*, Algeria*, Azerbaijan, Ethiopia*, Greece, Iran*, Iraq, Israel*, Jordan, Libya, Morocco*, Pakistan, Palestine, Saudi Arabia*, Syria, Tunisia*, Turkey, Turkmenistan, Uzbekistan, Yemen			
<i>P. ansarii</i>	Iran	<i>L. major</i>	LCL	DCL†
<i>P. caucasicus</i>	Afghanistan, Iran*			
<i>P. duboscqi</i>	Burkina Faso, Cameroon, Chad, Ethiopia*, Gambia, Ghana, Guinea, Guinea-Bissau, Kenya*, Mali*, Mauritania, Niger, Nigeria, Senegal*, Sudan, Yemen			
<i>P. mongolensis</i>	Kazakhstan			
<i>P. papatasi</i>	Afghanistan*, Algeria*, Azerbaijan, Egypt*, Iran*, Iraq, Israel*, Jordan*, Kazakhstan, Libya, Morocco*, Oman, Pakistan, Palestine, Saudi Arabia*, Sudan, Syria, Tunisia*, Turkmenistan*, Uzbekistan*, Yemen			

**Table 1.** Continued

Proven or suspected vector species	Country*	<i>Leishmania</i> species	Clinical form in humans	
			Most common	Rare
<i>P. salehi</i>	India*, Iran*, Pakistan			
<i>P. bergeroti</i>	Burkina Faso, Chad, Egypt, Iran, Mauritania, Oman, Yemen			
<i>P. longipes</i>	Ethiopia*	<i>L. aethiopic</i>	LCL	DCL
<i>P. pedifer</i>	Kenya*, Ethiopia*			
<i>P. sergenti</i>	Ethiopia*			

\*Countries in which the sandfly species is a proven vector (for criteria, see text). Elsewhere, a sandfly species is suspected to be a vector on the basis of epidemiological evidence or because it is a proven vector elsewhere.

†In cases of severe immunosuppression (e.g. HIV co-infection).

DCL, disseminated or diffuse cutaneous leishmaniasis (the two forms are treated together in this review); LCL, localized cutaneous leishmaniasis; LR, leishmaniasis recidivans; PKDL, post-kala-azar dermal leishmaniasis; VL, visceral leishmaniasis.

in La Banda, Argentina (Salomón *et al.*, 2010), has also been indicated as a possible vector in Brazil (Pernabuco state) because *L. infantum* DNA has been detected in wild-caught specimens. This finding suggests that this species may be responsible for the transmission of the disease in areas from which the usual VL vector, *Lu. longipalpis*, is absent (de Carvalho *et al.*, 2010). As far as the vectors of CL, we add to previous lists the following information: (a) *Lutzomyia nuneztovari anglesi* is a vector of *L. amazonensis* in Bolivia, as confirmed by anthropophily, biochemical identification of wild isolates and successful experimental infection (Martinez *et al.*, 1999); (b) *Lutzomyia ayacuchensis* was recently found in Peru naturally infected by promastigotes typed as *L. guyanensis* (Córdova *et al.*, 2011); (c) *Lutzomyia fischeri* is included as a proven vector because of repeated observations in Brazil of natural promastigote infections identified as *L. braziliensis*, associated with anthropophily and a spatial distribution related to human CL (Margonari *et al.*, 2010; Rocha *et al.*, 2010; Pita-Pereira *et al.*, 2011); (d) in Venezuela, *Lu. migonei* has recently been reported as a putative vector of *L. guyanensis* and *L. mexicana* (Felicangeli *et al.*, 2011), and past reports have incriminated *Lutzomyia gomezi* as a proven vector of *L. braziliensis* and *Lutzomyia ovallesi* as responsible for the transmission of not only *L. braziliensis*, but also *L. mexicana* (Felicangeli *et al.*, 1994; Jorquera *et al.*, 2005); (e) in the Yucatan peninsula of Mexico, *Lutzomyia cruciata*, *Lutzomyia panamensis*, *Lutzomyia shannoni* and *Lutzomyia ylephiletor* are considered to represent possible vectors of *L. mexicana* because recent investigations using molecular techniques have detected natural infections (Pech-May *et al.*, 2010).

#### Role of phlebotomine sandflies in the pathogenesis of leishmaniasis

As well as acting as *Leishmania* spp. vectors in the parasite lifecycle, phlebotomine sandflies may also be directly involved in the pathogenesis of leishmaniasis. During the feeding process on the vertebrate host's skin, the salivary gland content is injected into the haemorrhagic pool upon which a female sandfly feeds. Sandfly saliva contains a variety of pharmacologic agents, such as anticoagulants,

vasodilators, anti-platelet agents and immunomodulatory and anti-inflammatory molecules (Andrade *et al.*, 2007). Probably as a result of co-evolutionary mechanisms, the haemostatic and immune modifications of the feeding site play some part in *Leishmania* spp. transmission as they affect parasite establishment. Since the early description of *L. major* infection enhancement by the vasodilator and immunomodulator maxidilan, a 6.5-kDa peptide from *Lu. longipalpis* saliva (an unnatural parasite–vector combination) (Titus & Ribeiro, 1988), several studies have been performed mainly in *Lu. longipalpis* and *Phlebotomus papatasi* and, more recently, in *Lutzomyia intermedia*. The immunomodulatory effects, including the enhancement of parasite burden or protection from exacerbated disease, have been reviewed by Rohousova & Volf (2006). In naïve hosts (human *ex vivo* cells and *in vivo* murine models), *Lu. longipalpis* saliva or maxidilan alone promotes downregulation of tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) secretion, thereby affecting blood coagulation, major histocompatibility complex molecule expression, cytotoxicity against infected cells, and neutrophil migration, while upregulating secretion of interleukin 6 (IL-6), a type 2 T helper cell (Th2) cytokine promoting humoral responses. It also suppresses T cell proliferation *in vitro* and delayed-type hypersensitivity (DTH) *in vivo*, and exerts anti-complement activity. *Phlebotomus papatasi* saliva has effects similar to those of *Lu. longipalpis* saliva in terms of decreasing TNF- $\alpha$  synthesis, increasing IL-6 production and inhibiting lymphoproliferation, but specifically downregulates nitric oxide synthesis by macrophages and upregulates the Th2 cytokine IL-4, a strong suppressor of protective Th1 responses. Such sandfly species-specific responses have been recently highlighted by the demonstration that *Lu. intermedia* enhances rather than decreases TNF- $\alpha$  production (Menezes *et al.*, 2008).

When human and canine hosts are repeatedly exposed to bites of uninfected phlebotomine sandflies, they develop specific immunoglobulin G (IgG) antibodies against salivary proteins: this occurs less frequently and mostly within a range of 43–45 kDa proteins for *Lu. longipalpis* (Gomes *et al.*, 2002; Hostomska *et al.*, 2008) and more consistently to a 35-kDa protein for *P. papatasi* saliva (Rohousova *et al.*, 2005). Canine hosts exposed to *Phlebotomus perniciosus* bites develop IgG1

**Table 2.** Phlebotomine species of the genus *Lutzomyia* that act as vectors of New World leishmaniasis.

Proven or suspected vector species	Country*	<i>Leishmania</i> species	Disease in humans	
			Most common	Rare
<i>Lu. almerioi</i>	Brazil*	<i>L. infantum</i>	VL	LCL, DCL†
<i>Lu. cruzi</i>	Brazil*			
<i>Lu. evansi</i>	Colombia*, Costa Rica, Mexico, Nicaragua, Venezuela*			
<i>Lu. forattinii</i>	Brazil			
<i>Lu. longipalpis</i>	Argentina*, Bolivia*, Brazil*, Colombia*, Costa Rica, El Salvador, Guatemala, Honduras*, Mexico, Nicaragua, Paraguay, Venezuela*			
<i>Lu. migonei</i>	Argentina, Brazil			
<i>Lu. pseudolongipalpis</i>	Venezuela			
<i>Lu. sallesi</i>	Brazil			
<i>Lu. ayrozai</i>	Bolivia	<i>L. braziliensis</i>	LCL	MCL, DCL†
<i>Lu. c. carrerai</i>	Bolivia*			
<i>Lu. colombiana</i>	Colombia			
<i>Lu. complexa</i>	Brazil*			
<i>Lu. cruciata</i>	Mexico			
<i>Lu. edwardsi</i>	Brazil			
<i>Lu. fischeri</i>	Brazil*			
<i>Lu. gomezi</i>	Venezuela*			
<i>Lu. intermedia</i>	Brazil, French Guyana, Paraguay			
<i>Lu. llanosmartinsi</i>	Bolivia*			
<i>Lu. migonei</i>	Argentina, Brazil*, Paraguay			
<i>Lu. neivai</i>	Argentina, Brazil*			
<i>Lu. nuneztovari anglesi</i>	Bolivia*			
<i>Lu. ovallesi</i>	Belize, Colombia, Guatemala*, Honduras, Mexico, Nicaragua, Panama, Venezuela*			
<i>Lu. panamensis</i>	Guatemala*, Honduras, Nicaragua, Panama, Venezuela*			
<i>Lu. paraensis</i>	Brazil			
<i>Lu. pescei</i>	Peru			
<i>Lu. pessoai</i>	Brazil			
<i>Lu. pia</i>	Colombia			
<i>Lu. shawi</i>	Bolivia*			
<i>Lu. spinicrassa</i>	Colombia*, Venezuela*			
<i>Lu. tejadai</i>	Peru			
<i>Lu. townsendi</i>	Colombia			
<i>Lu. trinidadensis</i>	Venezuela			
<i>Lu. ylephiletor</i>	Guatemala*, Honduras			
<i>Lu. youngi</i>	Costa Rica, Venezuela, Colombia			
<i>Lu. yucumensis</i>	Bolivia*			
<i>Lu. wellcomei</i>	Brazil*, French Guyana			
<i>Lu. whitmani</i>	Argentina, Brazil*, Paraguay			
<i>Lu. anduzei</i>	Brazil*, French Guyana, Guyana, Suriname, Venezuela	<i>L. guyanensis</i>	LCL	MCL
<i>Lu. ayacuchensis</i>	Peru*			
<i>Lu. longiflocosa</i>	Colombia*			
<i>Lu. migonei</i>	Venezuela			
<i>Lu. shawi</i>	Bolivia*			
<i>Lu. umbratilis</i>	Brazil*, Colombia*, French Guyana, Guyana, Suriname, Venezuela			
<i>Lu. whitmani</i>	Brazil*			
<i>Lu. anthophora</i>	U.S.A.	<i>L. mexicana</i>	LCL	MCL, DCL†
<i>Lu. ayacuchensis</i>	Ecuador*			
<i>Lu. colombiana</i>	Colombia			
<i>Lu. cruciata</i>	Mexico, Nicaragua			
<i>Lu. diabolica</i>	U.S.A.			
<i>Lu. migonei</i>	Venezuela			

**Table 2.** Continued

Proven or suspected vector species	Country*	<i>Leishmania</i> species	Disease in humans	
			Most common	Rare
<i>Lu. olmeca olmeca</i>	Belize, Guatemala, Mexico*			
<i>Lu. ovallesi</i>	Venezuela*			
<i>Lu. panamensis</i>	Mexico			
<i>Lu. shannoni</i>	Mexico			
<i>Lu. ylephiletor</i>	Guatemala, Mexico			
<i>Lu. flaviscutellata</i>	Brazil*, Bolivia, Colombia, Ecuador, French Guyana, Suriname, Venezuela	<i>L. amazonensis</i>	LCL	DCL
<i>Lu. longipalpis</i>	Brazil*			
<i>Lu. nuneztovari anglesi</i>	Bolivia*			
<i>Lu. Olmeca reducta</i>	Brazil*, Venezuela			
<i>Lu. Olmeca nociva</i>	Brazil*			
<i>Lu. cruciata</i>	Nicaragua, Panama	<i>L. panamensis</i>	LCL	MCL
<i>Lu. gomezi</i>	Colombia*, Ecuador, Panama			
<i>Lu. hartmanni</i>	Ecuador*			
<i>Lu. panamensis</i>	Colombia*, Costa Rica, Honduras, Nicaragua, Panama*			
<i>Lu. sanguinaria</i>	Panama			
<i>Lu. trapidoi</i>	Colombia*, Costa Rica, Ecuador, Honduras, Nicaragua, Panama*			
<i>Lu. ylephiletor</i>	Costa Rica, Guatemala, Honduras, Nicaragua, Panama			
<i>Lu. yuilli</i>	Colombia*			
<i>Lu. ayacuchensis</i>	Peru*	<i>L. peruviana</i>	LCL	MCL
<i>Lu. peruensis</i>	Peru*			
<i>Lu. verrucarum</i>	Peru*			
<i>Lu. nuneztovari anglesi</i>	Bolivia*	<i>L. lainsoni</i>	LCL	
<i>Lu. ubiquitalis</i>	Brazil,* Peru			
<i>Lu. whitmani</i>	Brazil*	<i>L. shawi</i>	LCL	
<i>Lu. amazonensis</i>	Brazil	<i>L. naiffi</i>	LCL	
<i>Lu. ayrozai</i>	Brazil*			
<i>Lu. paraensis</i>	Brazil			
<i>Lu. squamiventris</i>	Brazil			
<i>Lu. antunesi</i>	Brazil	<i>L. lindenbergi</i>	LCL	
<i>Lu. youngi</i>	Venezuela	<i>L. garnhami</i>	LCL	MCL
<i>Lu. flaviscutellata</i>	Venezuela	<i>L. pifanoi</i>	DCL	
<i>Lu. Olmeca bicolor</i>	Venezuela	<i>L. venezuelensis</i>	LCL	DCL
<i>Lu. hartmanni</i>	Colombia*	<i>L. colombienseis</i>	LCL	
<i>Lu. gomezi</i>	Panama			
<i>Lu. panamensis</i>	Panama			

\*Countries in which the sandfly species is a proven vector (for criteria, see text). Elsewhere, the sandfly species is suspected to be a vector on the basis of epidemiological evidence or because it is a proven vector elsewhere.

†In cases of severe immunosuppression (e.g. HIV).

DCL, disseminated or diffuse cutaneous leishmaniasis (the two forms are treated together in this review); LCL, localized cutaneous leishmaniasis; MCL, mucocutaneous leishmaniasis; VL, visceral leishmaniasis.

and IgG2 responses to a broad range of nine to 11 saliva proteins in the range of 75–14 kDa (Vlkova *et al.*, 2011). Bitten hosts also develop strong cellular responses against saliva components. Natural DTH responses in humans are well known after repeated *P. papatasi* bites; in sensitized mouse models, very few molecules from the saliva of this species were found to elicit DTH reactions, but this was also dependent on the genetic background of the mouse strain.

It is well known that injection into the skin of susceptible hosts of a relatively small number of cultured promastigotes does not usually result in disease; however, the same

number of parasites readily provokes an established infection when they are transmitted by phlebotomine sandflies. The components promoting infection have been identified in the promastigote secretory gel, a matrix formed in the insect foregut containing metacyclics (Rogers *et al.*, 2004), and in saliva components. In various murine and canine experimental models, the co-injection of *Leishmania* spp. with natural or unnatural sandfly vector saliva increased the chance of successful infection and resulted in greater lesion size in CL models and a higher parasite burden in relevant tissues for all disease models (Rohousova & Volf, 2006). Different



combinations of saliva origin and parasite species have been tested, such as *Lu. longipalpis/L. major* (unnatural), and *Lu. longipalpis/L. infantum*, *P. papatasi/L. major* and *P. perniciosus/L. infantum* (natural). Among the mechanisms proposed to explain these effects, the functional alteration of antigen-presenting cells and the IL-4-driven development of Th2 immune responses are the most plausible.

Apparently a paradox, the immunity elicited by sandfly saliva in some experimental models appears to be protective against *Leishmania* spp. infection (Kamhawi, 2000). Salivary gland lysates/sonicates, purified salivary proteins or bites by uninfected *P. papatasi* or *Lu. longipalpis* reduced the severity of CL in treated mice challenged with *L. major* or *L. amazonensis*. Analogously, treatment of hamsters with DNA plasmids coding for *Lu. longipalpis* salivary proteins protected the animals against fatal VL following *L. infantum* challenge (Gomes *et al.*, 2008). In combination, these findings suggest that vaccination against vector antigens may represent a novel method for controlling leishmaniasis. This protective effect, however, was not shown in a *Lu. intermedia/L. braziliensis* challenge in a murine model reported by Andrade *et al.* (2007). Given both the large diversity of vector–parasite natural associations and the fact that phlebotomine sandfly species differ in salivary antigens [and that differences may be present within a species, as recently shown by Rohousova *et al.* (2012) in three *Phlebotomus* species], any protective effects may also be extremely specific, which lowers the likely feasibility of worldwide vector-based vaccines. Furthermore, recently Rohousova *et al.* (2011) used an experimental murine model involving *Phlebotomus duboscqi* exposure with an *L. major* challenge to show that short-term exposure to bites shortly before challenge is indeed protective, whereas both long-term exposures or a long delay prior to challenge after short-term exposure are not. This explains the persistence of severe *Leishmania* spp. infections in endemic areas in which individuals are repeatedly exposed to bites and/or sandfly activity is seasonal. Nevertheless, the protective effects of saliva may play an important role in the dynamics of clinico-epidemiological patterns (e.g. in the patterns of asymptomatic vs. symptomatic infections) found in endemic settings.

## Current spreading of leishmaniases

### Overview

About 20 named *Leishmania* species and subspecies are pathogenic for humans, in whom leishmaniases have diverse clinical manifestations. Visceral leishmaniasis, caused by *L. donovani* in the Old World and *L. infantum* in both the Old and New Worlds, is the most severe form, with an estimated yearly incidence of 500 000 cases (Desjeux, 1996). Several species of *Leishmania* cause tegumentary diseases in which the clinical spectrum varies from localized, disseminated or diffuse CL (treated together in this review), to mucocutaneous leishmaniasis and pathological sequelae following *L. donovani* VL (post-Kala-azar dermal leishmaniasis) or *L. tropica* CL (leishmaniasis recidivans). Disfigurement, social and psychological stigma are severe consequences of

the diseases, for which the estimated yearly incidence is 1–1.5 million cases (Desjeux, 1996).

Each parasite species circulates in natural foci of infection where susceptible phlebotomine species and mammals coexist. Nosogeographical entities of leishmaniasis can be categorized in two main epidemiological groups of, respectively, zoonotic (representing the large majority of such entities) and anthroponotic leishmaniases. Traditionally, Old and New World nosogeographical entities have been considered separately because they involve different parasites (with the exception of *L. infantum*), vectors and ecosystems. However, common risk factors for human leishmaniases are found worldwide and include poverty and poor housing, conflict, human migration, deforestation and urbanization.

### Old World leishmaniases

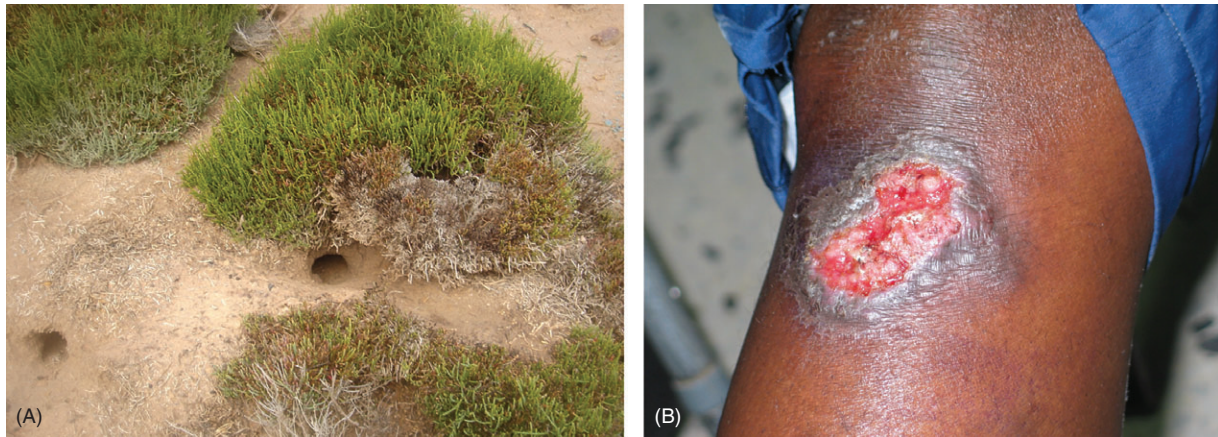
Autochthonous cases of human leishmaniases are currently reported from 80 countries, by contrast with the 66 countries recorded by Desjeux (2001). However, despite the endemic nature of disease in these countries, the *Leishmania* species involved were not determined in nine countries and the phlebotomine vector species, either proven or suspected, were not identified in 16.

*Zoonotic leishmaniases.* Three well-recognized Old World *Leishmania* species have a zoonotic nature. Zoonotic VL caused by *L. infantum* is widespread in countries of the Mediterranean basin and central Asia. Cutaneous leishmaniasis infections by this parasite are also found within the same endemic range, where they are usually sporadic, although in some foci they may show hyperendemic patterns (Corradetti, 1952; Svobodova *et al.*, 2009). Several vector species are involved, most of which belong to the subgenus *Phlebotomus* (*Larroussius*). Dogs are the main domestic reservoirs, and foxes, jackals and wolves represent sylvatic reservoirs (Fig. 4). The classical zoonotic CL is caused by *L. major*, a parasite widely distributed in arid and savannah areas in which several rodent species act as reservoir hosts. Proven vectors belong to the subgenus *Phlebotomus* (*Phlebotomus*) and *P. papatasi* is the principal vector over a wide geographical range that extends from northern Africa to India (Fig. 5). A third zoonotic agent of CL, *L. aethiopica*, is limited to the highlands of Ethiopia and Kenya. It is a classical parasite of the Hyracoidea (e.g. *Procapra capensis*) transmitted by the *Larroussius* species *Phlebotomus longipes* and *Phlebotomus pedifer*.

*Anthroponotic leishmaniases.* Two species, *L. donovani* and *L. tropica*, have exclusively or predominantly anthroponotic transmission patterns that result in several thousands of human cases. However, the presence of mammal reservoirs has been indicated in several endemic settings, suggesting an ancient type of parasite transmission, such as in eastern Sudan for *L. donovani* (Dereure *et al.*, 2003) and in northwest Africa (Maghreb) (Dereure *et al.*, 1991; Boubidi *et al.*, 2011;



**Fig. 4.** Zoonotic visceral leishmaniasis, *Leishmania infantum*. (A) Periurban biotope where the Old World sandfly *Phlebotomus* (*Larrousius*) *neglectus* acts as the main vector (Albania). (B) An infected dog with severe clinical signs of disease.



**Fig. 5.** Old World zoonotic cutaneous leishmaniasis, *Leishmania major* (Libya). (A) Rodent reservoir burrows (*Psammomys obesus*), breeding site for the vector *Phlebotomus* (*Phlebotomus*) *papatasi*. (B) Human lesion.

Jaouadi *et al.*, 2011), northern Israel (Jacobson *et al.*, 2003; Svobodova *et al.*, 2006) and Iran (Mohebbi *et al.*, 2005) for *L. tropica* *s.l.* In the Asian continent, anthroponotic VL caused by *L. donovani* is restricted to northeast India, Bangladesh and Nepal, where *P. argentipes* is the sole vector. In East Africa (Kenya, Ethiopia, Somalia, Sudan and Uganda) and the Arabian peninsula, the distribution of *L. donovani* is associated with those of *P. orientalis* and/or *Phlebotomus martini*. Anthroponotic CL caused by *L. tropica* is highly prevalent in semi-arid subtropical regions extending from the southeast of Turkey to the northwest of India. In well-established foci, CL is transmitted person-to-person through *P. (Paraphlebotomus) sergenti* in urban settings. Large-scale urban migrations influence *L. tropica* transmission patterns and infections may occur in outbreaks that last for some years (Fig. 6). In addition, small and discontinuous foci are also found in northern Africa, Israel, Greece and Saudi Arabia, as well as in sub-Saharan Africa in Kenya and Ethiopia (Hailu

*et al.*, 2006), where *L. tropica* can be transmitted by other phlebotomine sandfly species (Table 1).

Causes of the spreading of Old World leishmaniasis are largely diverse, although they are mostly associated with human social, behavioural and individual factors, such as massive migrations, conflict, man-made environmental changes and immunosuppressive conditions. As such, the contribution of phlebotomine sandfly dynamics to the spread of disease may vary greatly according to the various epidemiological settings in which it occurs. For instance, epidemics of human immunodeficiency virus (HIV) and the increasing use of immunosuppressive therapies have substantially contributed to an increase in numbers of VL cases from southern Europe to India (Alvar *et al.*, 2008), whereas changes in sandfly populations can be assumed to have no specific role in these events. Civilians and soldiers have been substantially affected by leishmaniasis in recent conflicts in Sudan, Iraq, Afghanistan and, recently, Libya (Amro *et al.*, 2012). Furthermore, urbanization and the domestication of zoonotic





**Fig. 6.** Old World anthroponotic cutaneous leishmaniasis, *Leishmania tropica*. (A) Urban biotope in which *Phlebotomus* (*Paraphlebotomus*) *sergenti* is the proven vector (Morocco). (B) Scarring caused by a severe facial lesion in a patient in Syria.

transmission cycles are causing an increase in numbers of cases in parts of northern and sub-Saharan Africa and the Middle East (Desjeux, 2004). In such contexts, it can be assumed that an increase in the density of vector populations resulting from both the interruption of vector control activities and an increase in breeding sites and blood sources may have largely contributed to the increase and spread of the disease. Finally, climatic changes can be expected to directly affect the density and dispersion of sandfly species in the Old World (Fischer *et al.*, 2011) and will thus represent a major cause of the spread of leishmaniasis in some settings.

Recent studies performed in Europe, in regions at the northernmost limit of leishmaniasis endemicity, demonstrate how human behaviour and climatic factors may interact.

This phenomenon was particularly investigated in northern continental Italy, traditionally a *Leishmania*-free territory which shares borders with France, Switzerland, Austria and Slovenia. Since the early 1990s, several VL cases have occurred in patients with no travel history; investigations in dogs have disclosed a number of *Leishmania*-infected pets imported from areas of traditional endemicity, and hundreds of autochthonous cases have been identified among thousands of animals serosurveyed in six regions. Comparisons with previous entomological data for the 1960s and 1970s revealed that two proven vectors of *L. infantum*, *P. perniciosus* and *Phlebotomus neglectus*, have expanded their geographic range northward and were detected for the first time in large parts of the territory during the 1990s and 2000s (Maroli *et al.*, 2008; Morosetti *et al.*, 2009). There are some other examples in Europe which may indicate an expansion in patterns of *L. infantum* transmission towards northern latitudes. In Germany, over the last two decades, cases of human, canine, feline and equine leishmaniasis have been detected, the autochthonous origins of which have been confirmed or are strongly suspected; *Phlebotomus mascittii* is indicated as a probable vector in the affected territories (Naucke *et al.*, 2008). In the French Pyrenees, increases in the incidence and distribution of canine leishmaniasis have been reported in a VL focus in which a recorded increase of 1 °C in mean annual

temperatures over the past 20 years has probably affected the density and diffusion of the two local vectors, *P. ariasi* and *P. perniciosus* (Dereure *et al.*, 2009).

#### New World leishmaniases

Autochthonous cases of human leishmaniases are currently reported from 21 countries in the Americas. *Leishmania* and phlebotomine vector(s) species were not conclusively identified in one country, the Dominican Republic, in which diffuse CL is endemic (WHO, 2010). All clinical forms result from zoonotic transmission from either domestic or sylvatic reservoir hosts. The geographical distribution of the disease spans an area extending from Texas to Argentina.

**Visceral leishmaniasis.** Visceral leishmaniasis is recorded as endemic in 12 countries in the New World. In the U.S.A., *L. infantum* has been isolated from dogs, but no confirmed autochthonous cases have been reported in humans (WHO, 2010). In the past, VL has been considered a rural disease caused by *L. chagasi* in semi-arid tropical areas in which *Lu. longipalpis* was recognized as the sole vector. A recent continent-wide DNA microsatellite analysis of parasite populations has confirmed an early hypothesis that the agent of VL in the New World is indeed *L. infantum* (Kuhls *et al.*, 2011), supporting the theory that the parasite was imported to the Americas by infected dogs brought by Iberian colonizers. It is now accepted that *Lu. longipalpis* is not a single species but a sibling complex, as evidenced by the findings of studies using various approaches, such as breeding experiments (Lanzaro *et al.*, 1993), allozyme analysis (Lampo *et al.*, 1999), male sex pheromone chemotypes (Hamilton *et al.*, 2005) and mitochondrial DNA (Arrivillaga *et al.*, 2002). So far, differences in genetic and morphological characteristics have resulted in the first formal description of a new species from the *Lu. longipalpis* species complex, *Lutzomyia pseudolongipalpis*, from Venezuela (Arrivillaga & Feliciangeli,

2001). A sibling clade of *Lu. longipalpis* that is genetically well separated has been found to predominate in areas that are hyperendemic for VL in the north of northeastern Brazil (Watts *et al.*, 2005).

Most of the VL cases in the New World occur in Brazil. During the 1990s and 2000s, an epidemic in which 3000–4000 cases were reported annually was attributed to urbanization processes in the states of Piauí, Maranhão, Bahia, Ceará, Pará, Rio Grande do Norte and Roraima. The urbanization resulted in the disorderly proliferation of crowded slums with poor sanitary conditions; the presence in such areas of *Lu. longipalpis* led to the onset and establishment of domestic and peridomestic cycles of the disease (Costa, 2008; Maia-Elkhoury *et al.*, 2008; Rangel & Vilela, 2008). Although less massively, urban VL has also occurred in other countries, such as Venezuela (Aguilar *et al.*, 1998; Zerpa *et al.*, 2002) and Paraguay (Canese, 2000).

The recent geographical expansion of VL in South America is indicated by the first appearance of a human VL focus in Argentina in 2006, at the northeastern border with Paraguay and Brazil; the disease was associated to *L. infantum*-infected dogs and *Lu. longipalpis* sandflies (Acardi *et al.*, 2010). The spatial distribution of *Lu. longipalpis* appears to be heterogeneous in Argentina, in which vectors are concentrated in limited patches of high abundance characterized by higher tree coverage and poor urban services (Fernández *et al.*, 2010). In a focus of low VL endemicity from which *Lu. longipalpis* was apparently absent, *Lu. migonei* has been suspected as the vector (Salomón *et al.*, 2010). This finding, along with several reports from other countries that incriminated VL vectors other than *Lu. longipalpis* (Table 2), call attention to a possible widespread adaptation of *L. infantum* to other epidemiologically relevant *Lutzomyia* species.

**Cutaneous leishmaniasis.** Prior to the 1960s, CL was primarily confined to forested areas. That the condition is widely known as ‘ulcera de los chicleiros’ (ulcer developed in gatherers of ‘chicle’, a gummy latex from the forest tree *Manilkara zapota*) in Mexico and is designated ‘guerrilla’s sore’ in Venezuela and Colombia reflects the historically close contact of humans with a sylvatic environment that maintains several species of phlebotomine vectors among wild species of mammalian reservoirs. Hence, hunting, lumbering and mining activities have been associated with the disease. Since the 1960s, transmission has increasingly spread to peridomestic areas. Massive migration from the high plateau to low tropical areas in the Andean region, intensive deforestation and the establishment of new settlements have greatly contributed to the increase in numbers of cases (Desjeux, 2001; Aagaard-Hansen *et al.*, 2010). The impact of environmental changes on the behaviour of vectors has been exhaustively reviewed by Rangel & Lainson (2009) in Brazil, and by Gonzalez *et al.* (2011) in Mexico. Deforestation and the replacement of primary forest by monocultures (e.g. coffee plantations) has been found to be crucial to the establishment of some *Lutzomyia* vectors in Venezuela and Colombia (Scorza, 1985; Alexander *et al.*, 1995). Although deforestation was predicted to reduce human contact with wild reservoirs and sandflies,

and was hoped to lead to the local eradication of important *Leishmania* species (e.g. *L. braziliensis* in parts of Brazil and *L. panamensis* in Panama), human encroachment of wild habitats has resulted in the intrusion of *Lutzomyia* species into the domestic environment, thus radically changing the epidemiological profile. Examples of *Lutzomyia* species that have participated in this invasion include *Lu. nuneztovari anglesi*, in Bolivia, *Lutzomyia verrucarum* and *Lutzomyia peruensis* in Peru, and *Lu. ovallesi* and *Lu. gomezi* in Venezuela (Gómez *et al.*, 1998; Campbell-Lendrum *et al.*, 2001). In Brazil, it has long been observed that environmental modifications in São Paulo state occurred early in the 20th century allowed *Lu. intermedia* to play a predominant role in the transmission of *L. braziliensis* (Tolezano, 1994). Similarly, *Lutzomyia whitmani* spread in most of the municipalities of Tocantins state in which leishmaniasis was endemic, in areas highly degraded by deforestation followed by migration and unplanned settlements (Vilela *et al.*, 2011). In Argentina, the abundance of *Lu. whitmani* in the Iguazú Falls area was found to be associated with economic and leisure activities in primary and secondary forest that led to marked environmental changes (Salomón *et al.*, 2009), and even moderate environmental modifications were reported to have an impact on *Lutzomyia neivai* distribution in Salta, in which CL is hyperendemic (Quintana *et al.*, 2010).

Urbanization has greatly contributed to the emergence and increase of CL in the New World. The disease has been spreading in cities in Brazil, such as Belo Horizonte (Carvalho *et al.*, 2010), Mexico (Sánchez-García *et al.*, 2010), Venezuela (Bonfante-Garrido *et al.*, 1984; Scorza & Rojas, 1990), Colombia (Sandoval *et al.*, 1998; Bejarano *et al.*, 2002; Cortés & Fernández, 2008), Bolivia (García *et al.*, 2009) and Argentina (Salomón *et al.*, 2008) (Fig. 7).

## Viral diseases

### Overview

Phlebotomine sandflies are involved in the transmission of several viral agents, among which the most important are grouped into the *Phlebovirus* genus (family *Bunyaviridae*), which includes the sandfly fever Sicilian and Toscana viruses, and the *Vesiculovirus* genus (family *Rhabdoviridae*), which includes vesicular stomatitis, and the Chandipura and Isfahan viruses.

The risk for infection with sandfly-transmitted phleboviruses has been shown to pertain to very large areas of the Old World (southern Europe, Africa, the Middle East, central and western Asia) in association with the presence of sandfly vectors (Tesh *et al.*, 1976). Recent investigations have indicated that virus diversity in the Mediterranean basin is higher than initially suspected, and that populations living south and east of the Mediterranean Sea have a high risk for infection during their lifetime (Sanbonmatsu-Gómez *et al.*, 2005; Papa *et al.*, 2006; Konstantinou *et al.*, 2007; Carhan *et al.*, 2010; Bahri *et al.*, 2011; Ergunay *et al.*, 2011, 2012; Kocak Tufan *et al.*, 2011).

The International Committee for Taxonomy of Viruses currently recognizes several phleboviruses associated with





**Fig. 7.** New World cutaneous leishmaniasis, *Leishmania braziliensis*. (A) A typical new urban settlement in Latin America (Venezuela). (B, C) Cutaneous leishmaniasis in human patients.

sandflies in the Old World (Plyusnin *et al.*, 2011). These include two virus species: (a) sandfly fever Naples virus, which includes the Naples virus, Tehran virus, Karimabad virus and Toscana virus, (b) and Salehabad virus, which includes the Salehabad and Arbia viruses. A further two virus isolates (sandfly fever Sicilian virus and Corfou virus) are listed, but not included among the nine recognized species of the *Phlebovirus* genus. In addition, recent field and clinical studies have provided increasing evidence that the number of known viruses in the genus *Phlebovirus* may be substantially underestimated (Charrel *et al.*, 2009; Collao *et al.*, 2010; Moureau *et al.*, 2010; Zhioua *et al.*, 2010; Anagnostou *et al.*, 2011).

Among the viral agents belonging to the genus *Vesiculovirus*, at least 28 infect invertebrates and vertebrates (Wunner *et al.*, 1995). Those infecting humans and domestic animals, for which phlebotomine sandflies can be regarded as biological vectors, include the New Jersey, Indiana, Alagoas, Chalchaqui, Chandipura, Cocal, Isfahan and Piry viruses (Table 3). Other vesiculoviruses have not been adequately tested for infectivity or pathogenicity in domestic animals and humans (Letchworth *et al.*, 1999). Vesicular stomatitis viruses causing stomatitis in humans and domestic livestock are largely endemic in the New World, whereas Chandipura encephalitis virus and Isfahan virus are endemic in the Old World in some parts of India (Basak *et al.*, 2007), Iran (Tesh *et al.*, 1977b), and Turkmenistan and other central Asian republics (Gaidamovich *et al.*, 1978). Although serological evidence for human infections has been reported for Isfahan virus (associated with *P. papatasi* transmission), it has not been definitively linked to human illness and has been found to be non-pathogenic in horses, cattle and other ruminants (Marriott, 2005).

#### Sandfly fever

Sandfly fever, also known as *Phlebotomus* fever, pappataci fever or three-day fever, has been an important cause of febrile disease during military operations since at least the Napoleonic Wars (Oldfield *et al.*, 1991). It has also historically

caused significant morbidity among non-native populations in Mediterranean regions (Pick, 1886). An Austrian commission in 1909 reported that the illness was caused by a filterable agent found in the blood of infected soldiers and that the vector was the sandfly *P. papatasi* (Doerr *et al.*, 1909). During World War II (WWII), German troops based in the Mediterranean area suffered from sandfly fever (Hallmann, 1943). Allied forces stationed in the Mediterranean and Middle East reported tens of thousands of cases and attack rates of 3–10% (locally up to 80%) (Sabin, 1951; Hertig & Sabin, 1964). First reports in Tunisian and Algerian regions, where the presence of *P. papatasi* was entomologically established, date from April 1943 (Sabin *et al.*, 1944). In August 1943, after the Allied landing in southern Italy, sandfly fever accounted for at least 25% of cases of fever of unknown origin. Outbreaks of sandfly fever occurred repeatedly in the former U.S.S.R. in the period from 1945 to 1950, predominantly in Crimea, Romania, Moldavia and the central Asian republics. Epidemics related to the activity of *P. papatasi* were reported in northern Africa, southern Europe, the Middle East and central Asia (Sabin, 1951; Hertig & Sabin, 1964).

The observation of two or more attacks in the same individual resulted in the early suggestion that sandfly fever might be caused by distinct viruses (Livschitz, 1937). However, it was almost impossible to distinguish these clinically. Sabin (1951) confirmed the existence of more than one strain of sandfly fever virus. Serum samples collected from soldiers after the landing of the Allied troops in southern Italy during WWII allowed the isolation of two different viruses, respectively named the Naples and Sicilian viruses. Volunteers inoculated with Naples virus developed the typical symptoms, but were not subsequently protected against infection with the Sicilian strain (Sabin, 1955).

*Naples virus.* The Naples virus was first isolated from a febrile patient in Italy in 1944 (Sabin, 1955). Additional recoveries have been made in Egypt, India, Iran, Pakistan, Serbia and the former Soviet Union (Gaidamovich *et al.*, 1974; Goverdhan *et al.*, 1976). In 1976, a founding study extended

**Table 3.** Most common vesiculoviruses infecting domestic animals and humans (from Letchworth *et al.*, 1999).

Virus name	First isolation		References
	Place and year	Host	
Indiana	Indiana, U.S.A., 1925	Bovine	Cotton (1926)
New Jersey	New Jersey, U.S.A., 1926	Equine	Cotton (1927)
Cocal	Trinidad, Brazil, 1964	Insect, rodents	Jonkers <i>et al.</i> (1964)
Alagoas	Brazil, 1964	Equine, bovine, human	Federer <i>et al.</i> (1967)
Chandipura	India, 1965	Human	Bhatt & Rodrigues (1967)
Piry	Brazil, 1973	Opossum	Theiler & Downs (1973)
Isfahan	Iran, 1975	Human	Tesh <i>et al.</i> (1977b)
Chalcaqui	Argentina, 1982	Insects	Calisher <i>et al.</i> (1987)

the known distribution of Naples virus to include Bangladesh, Ethiopia, Greece, Iraq, Morocco, Saudi Arabia, Sudan, the Territory of the Afars and Issas (now Djibouti), Turkey and former Yugoslavia (Tesh *et al.*, 1976). Antibodies to the Naples virus have been found in residents of Turkmenia, Tajikistan, Uzbekistan, Azerbaijan and Moldavia (Gaidamovich *et al.*, 1978). Seroepidemiological studies conducted in areas around the Mediterranean indicate that Naples virus infections have decreased during the last 30 years (Tesh & Papaevangelou, 1977). Although the renewed interest in phlebotomine sandfly-transmitted phleboviruses has produced numerous studies during the last decade, Naples virus has not been isolated or detected by polymerase chain reaction (PCR) since 1987 (Feinsod *et al.*, 1987). This may suggest that the Naples virus became progressively extinct.

*Sicilian virus.* The prototype strain of Sicilian virus was isolated from humans in 1943 (Sabin, 1955). Other isolates were subsequently obtained in Egypt, India, Iran, Pakistan and Afghanistan (R. Taylor & J. Casals, personal communication, 2005; Goverdhan *et al.*, 1976; Tesh *et al.*, 1977a). Sicilian virus is also present in Bangladesh, Greece, Cyprus, Iraq, Morocco, Saudi Arabia, Somalia, Sudan, Tunisia, Turkey, the southern European and central Asian republics of the former U.S.S.R. (Turkmenia, Tajikistan, Uzbekistan, Azerbaijan and Moldavia), former Yugoslavia, France and Portugal (C. Hannoun, personal communication, 2005; Filipe, 1974; Tesh *et al.*, 1976; Gaidamovich *et al.*, 1978; Eitrem *et al.*, 1991).

Epidemiological data are of specific interest in the diagnosis because of the seasonality of vector activity and its intrinsic epidemic nature. Cases of sandfly fever begin to appear in April and gradually build to a peak in September. Thus, the epidemiological pattern of the disease mirrors the lifecycle of *P. papatasi*. The viruses that cause sandfly fever (Naples and Sicilian viruses) have a wide geographical distribution, which parallels that of *P. papatasi*, which was dominant during the first half of the 20th century. DDT-based spraying campaigns (1940–1960) to control the insect vectors of typhus and to eradicate malaria and dengue fever (Dunlap, 1981), as well as the easy access of farmers to agricultural insecticide (WHO, 1979), reduced numbers of *P. papatasi* in regions in which insecticides were sprayed. Later reports of virus isolation and serologic studies indicate that phleboviruses are still present in the Mediterranean coastal regions of Europe and North Africa,

the Nile valley, most of southwest Asia, areas adjacent to the Black and Caspian Seas, and in central Asia including Bangladesh (Tesh *et al.*, 1976).

The clinical pictures corresponding to infections with the Naples and Sicilian viruses are virtually identical. After an incubation period of 3–6 days, sandfly fever is characterized by the sudden onset of fever, headache, retro-orbital pain, photophobia, generalized aching, malaise and chills. The face can be suffused, with injection of the conjunctivas and scleras, and photophobia is accompanied by intense ocular pain on movement of the eyes. At times, a faint pink erythema is present over the shoulders and thorax, and the spleen is palpable in a small percentage of patients. The duration of fever is 2–4 days in 85% of cases, but may extend to 11 days in extreme cases. Leucopenia is present in most cases at admission to hospital and the lowest counts are recorded in the immediate post-febrile period. The virus is present in the blood of patients 24 h before the onset of fever and during the first 24 h thereafter. The Sicilian and Naples viruses are not recovered from the cerebrospinal fluid and, by contrast with Toscana virus, have not been associated with neurological manifestations. No mortality has been recorded in thousands of clinically observed cases. Complications have not been noted but convalescence is occasionally prolonged for weeks (Sabin, 1955; Bartelloni *et al.*, 1976).

#### *Summer meningitis caused by Toscana virus*

Toscana virus was first isolated from *P. perniciosus* and *Phlebotomus perfiliewi* collected in Italy in 1971 (Verani *et al.*, 1982). The first evidence for the human pathogenicity and neurotropism of Toscana virus was reported more than 10 years after the discovery of the virus (Charrel *et al.*, 2005). Because of the transient viraemic condition in human patients, it was suggested that this phlebovirus might cast the vectors themselves in the role of reservoirs because male sandflies were found to be infected in nature and transovarial transmission was demonstrated in the laboratory (Ciufolini *et al.*, 1985, 1989; Tesh & Modi, 1987). In addition, venereal transmission from infected *P. perniciosus* males to uninfected females was demonstrated (Maroli *et al.*, 1993). However, the progressive decrease in viral infection rates observed from generation to generation in sandfly colonies suggested that this virus could not be maintained indefinitely by vertical or venereal transmission. Consequently, the existence of a reservoir was

considered. Serological studies showed no evidence of viral circulation among domestic or wild animals, although a Toscana virus strain was isolated from the brain of a bat, *Pipistrellus kuhli* (Verani *et al.*, 1988).

The first large Italian study showed that Toscana virus was a prominent cause of summer meningitis in central Italy (Nicolletti *et al.*, 1991). Until recently, its known distribution was limited to Italy and Portugal (Charrel *et al.*, 2005). More recently, as indicated by virus isolation or serological surveys, the geographical distribution of the virus has been extended to include France, Spain, Slovenia, Greece, Cyprus, Elba and Turkey (Charrel *et al.*, 2005; Santos *et al.*, 2007; Sonderegger *et al.*, 2009; Gabriel *et al.*, 2010; Kay *et al.*, 2010; Ergunay *et al.*, 2011, 2012). Some studies have reported the presence of Toscana virus based on serological evidence using immunofluorescence assays (IFAs) or enzyme-linked immunosorbent assays (ELISAs) conducted in Tunisia, Kosovo and Greece (Papa *et al.*, 2010; Bahri *et al.*, 2011; Venturi *et al.*, 2011). Although they may indicate the actual presence of Toscana virus in these regions, antigenic cross-reactivity between some phleboviruses can be misleading, and possibly reflects the presence of phleboviruses other than Toscana virus but more or less closely related to it. Therefore, these studies should be taken as providing preliminary data that merit further confirmation through virus isolation or PCR detection and sequencing confirmation. This is particularly relevant because of the recent report of novel viruses that are closely related to but distinct from Toscana virus in Tunisia (Punique virus), France (Massilia virus) and Spain (Granada virus), the antibodies for which are seen to react against Toscana virus antigens by IFA and ELISA.

#### New phleboviruses

Recently, virological and molecular evidence for the presence of a phlebovirus closely related to but distinct from Sicilian virus was reported in Algeria (Moureau *et al.*, 2010), Tunisia (Zhioua *et al.*, 2010) and Turkey (Carhan *et al.*, 2010; Kocak Tufan *et al.*, 2011). Adria virus (a relative of Arbia virus) was detected, but not isolated, in phlebotomine sandflies collected in Albania and subsequently in a human case (Papa *et al.*, 2010; Anagnostou *et al.*, 2011). Massilia virus was isolated from *P. perniciosus* in southeast France (Charrel *et al.*, 2009). Granada virus was isolated from sandflies (unidentified) in Spain (Collao *et al.*, 2010). Punique virus was isolated in northern Tunisia from *P. perniciosus* and *Phlebotomus longicuspis* in 2008 (Zhioua *et al.*, 2010). The latter three viruses are closely related to but distinct from other members of the sandfly fever Naples virus species. To date, there are no data to support the suggestion that they cause disease in humans.

It is, therefore, a matter of priority to address the public health impacts of these newly described phleboviruses via seroprevalence studies and virological investigations of clinical cases of fever of unknown origin and infections of the central nervous system. Interestingly, both CL and VL are also endemic in most of the regions in which sandfly-associated phleboviruses occur (Tesh *et al.*, 1976). Very recently, the epidemiological link between human

leishmaniasis and phleboviral infections, which has been assumed for a long time, was statistically established in south-east France between *L. infantum* and Toscana virus (Bichaud *et al.*, 2011).

Moreover, recent studies indicate that in relation to previously accepted parameters: (a) the geographic distribution of sandfly-associated phleboviruses is much larger; (b) the number of phleboviruses infecting sandflies is higher; (c) the number of sandfly species involved in transmission may be more important, and (d) the relationship between sandfly-borne phleboviruses and *Leishmania* parasites is tighter. In light of these revisions, it is pivotal to reinforce research programmes that aim to achieve a better understanding of interactions among sandfly-borne phleboviruses, *Leishmania* parasites and sandflies.

#### Vesicular stomatitis disease

Three vesiculoviruses, vesicular stomatitis virus (VSV)-Alagoas, VSV-Indiana and VSV-New Jersey, cause vesicular stomatitis disease in humans and domestic livestock. Features common to these three VSVs include the inability of naturally infected vertebrates to produce a sustained, high-titre viraemia, and the capacity for transovarial transmission in arthropod hosts.

*Infections in humans.* Human VSV is endemic in Mexico, Central America, northern South America and eastern Brazil, as well as in limited areas of the southeastern U.S.A. (Letchworth *et al.*, 1999). Most human infections appear to be subclinical. When symptomatic, the disease in humans is a severe, but uniformly non-fatal, influenza-like illness. In patients with clinical manifestations, the initial symptom is high fever that is often biphasic. Subsequent symptoms are flu-like and include severe malaise, headaches, myalgia, arthralgia, retrosternal pain, eye aches and nausea. Vesicle formation on the oral mucosa, lips and nose is possible, but rare (Patterson *et al.*, 1958). In most rural areas in which VSV is active, residents do not have easy access to medical care and are unlikely to seek attention for such relatively minor complaints, and thus their aetiology is never determined. Therefore, the true incidence of clinical illness caused by infection with these viruses is unknown, and they may be much more widespread than is indicated by the relatively few viral isolations obtained from sick persons and from the limited serosurveys that have been undertaken (Comer & Tesh, 1991). Accidental infections with some VSVs in laboratory personnel have usually produced a mild, self-limiting, flu-like illness characterized by fever, myalgia, headache and malaise of 3–5 days in duration (Comer & Tesh, 1991; Acha & Szyfres, 2003). Interestingly, VSV has been engineered to target cancer cells or to stimulate immunity against diseases such as acquired immune deficiency syndrome (AIDS) or influenza (Lichty *et al.*, 2004).

*Infection in domestic animals.* Vesicular stomatitis virus disease is an important infection of cattle, horses and pigs. Clinical disease presents severe vesiculation and/or ulceration of



the dorsal surface of the tongue, oral tissues, feet and teats, and results in substantial loss of productivity. This is of great practical importance because attack rates in dairies can be as high as 96% (Ellis & Kendall, 1964) and the economic consequences are in the range of US\$100–250 per cow (Letchworth *et al.*, 1999). Except for its appearance in horses, the disease is clinically indistinguishable from foot-and-mouth disease. It occurs seasonally every year in the southeastern U.S.A., southern Mexico, throughout Central America, in northern South America, and in eastern Brazil. In the U.S.A. the disease has two different patterns of occurrence: in the southeastern states (Georgia, Alabama, and North and South Carolina) yearly occurrences of clinical cases in livestock were reported from the early 1900s to the mid-1970s, since when viral activity in the region has been focal and limited to isolated wildlife populations. By contrast, in the southwestern states (New Mexico, Arizona, Utah and Colorado), VSV outbreaks have occurred sporadically at approximately 10-year intervals, with the last cycle of activity occurring during 1995–1998 (Rodríguez, 2002).

**Biological vectors.** Strong evidence supports the role of biting arthropods as vectors of vesiculoviruses and indeed the latter may actually be well-adapted insect viruses that incidentally infect mammals. Among arthropods, midges [*Culicoides* spp. (Ceratopogonidae)] (Perez de Leon *et al.*, 2006), blackflies (Simuliidae) (Mead *et al.*, 2004), mosquitoes [*Aedes* spp. (Diptera: Culicidae)] and other dipteran insects have been implicated in VSV transmission (Stallknecht *et al.*, 1999; de Mattos *et al.*, 2001; Rodríguez *et al.*, 2002; Krauss *et al.*, 2003; Lichty *et al.*, 2004). However, phlebotomine sandflies seem to be the only vectors to have been confirmed biologically. The strongest evidence of their involvement in VSV transmission includes: (a) the isolation of viruses from wild-caught males and females; (b) the demonstration of infection by an oral route, replication, and transmission by bite; (c) temporal and/or spatial associations between infected phlebotomine sandflies and infected vertebrates, and (d) the demonstration of transovarial virus transmission (Comer & Tesh, 1991). It is highly probable that phlebotomine sandflies are the only biological vectors because they have been found infected in the absence of clinical cases in humans or domestic animals. For example, the VSV-Indiana serotype has been isolated repeatedly from pools of *Lutzomyia* spp. and from three pools of unfed female *Lutzomyia trapidoi* in Panama, in an area with no disease cases (Comer & Tesh, 1991). By contrast, blackflies, midges, mosquitoes and other non-haematophagous insects have only been found to be infected during epidemics and probably serve as mechanical vectors (Letchworth *et al.*, 1999). Three species of *Lutzomyia* sandflies have been associated with VSV transmission, namely *Lu. trapidoi*, *Lu. ylephiletor* and *Lu. shannoni* (Comer & Tesh, 1991).

#### *Chandipura virus encephalitis*

Another vesiculovirus, Chandipura virus, has recently emerged as a human pathogen associated with several outbreaks of severe encephalitis in different parts of India.

Although the virus closely resembles the prototype vesiculovirus, VSV, it can be readily distinguished by its ability to infect humans (Basak *et al.*, 2007). Chandipura virus was first isolated in 1965 from two patients suffering from febrile illness in the village of Chandipura in India (Bhatt & Rodrigues, 1967). Although Chandipura virus was later identified as the cause of mild dengue-like symptoms in human patients, and was also isolated from an encephalopathic child in 1980 (Rodrigues *et al.*, 1983), the first evidence for its association with severe human epidemics was obtained only in 2003, when the virus was identified as the cause of an outbreak of acute encephalitis in children, in which the fatality rate was high (183 deaths in 329 cases, 55.6%), in Andhra Pradesh, India (Rao *et al.*, 2004). In 2004, a second outbreak with a fatality rate of >75% was reported in the eastern state of Gujarat (Chadha *et al.*, 2005). More recently, another outbreak of Chandipura virus-associated encephalitis in children aged < 15 years (78 cases, 43.6% fatality rate) was reported in the district of Nagpur in Maharashtra (Gurav *et al.*, 2010).

Chandipura virus was reported to have been isolated from pools of wild-caught *Phlebotomus* spp. sandflies (Dhanda *et al.*, 1972). Recently, Chandipura virus was detected in sandfly specimens belonging to the genus *Sergentomyia* (Geevarghese *et al.*, 2005). Nevertheless, *P. papatasi* is the most suspected vector because it is a dominant anthropophilic and domiciliary species prevalent in several parts of India. Vertical transmission of Chandipura virus in *P. papatasi* has been established, and vertically infected males can transfer the virus to females by venereal (horizontal) transmission (Tesh & Modi, 1983). The virus has also been isolated from phlebotomine sandflies in Senegal (Fontenille *et al.*, 1994) and from a hedgehog (*Atelerix spiculus*) in Nigeria, suggesting a broader geographic distribution.

#### **Bartonellosis**

*Bartonella bacilliformis*, a motile, aerobic and Gram-negative bacterium, lives within cells of the human reticuloendothelial system and attaches to erythrocytes. In humans, it causes a disease known as Carrión's disease, which has two clinically distinct phases: an acute or haematic phase, known as 'Oroya fever', and an eruptive or tissue phase, known as 'Peruvian wart' or 'verruca peruana'. Any infected person can experience either one or both phases, which can occur once or more than once during a lifetime.

That the two phases of this condition represented different manifestations of the same disease was unknown until evidence provided in the late 1800s by Daniel Alcides Carrión, a Peruvian medical student who inoculated himself with material taken from a 'verruca' lesion of a chronic patient while attempting to describe the evolution of the disease (Schultz, 1968). After 3 weeks, Carrión developed classic symptoms of the acute disease phase, thus establishing a common aetiology for these two syndromes. He died from bartonellosis on 5 October 1885 and was recognized as a martyr of Peruvian medicine.

The most common clinical features of the acute phase of bartonellosis are muscle and joint pain, fever, headache,



delirium and, ultimately, coma. It results in death in up to 40% of untreated patients, but mortality can reach around 90% when opportunistic infection with *Salmonella* spp. occurs. The chronic phase is characterized by an eruptive phase, in which patients develop a cutaneous rash produced by a proliferation of endothelial cells. Humans are the only known reservoir for *B. bacilliformis* and asymptomatic bacteraemia occurs in 0.5% of persons in endemic regions.

Carrión's disease is restricted to central Peru, Ecuador and southwestern Colombia. In the past, most reported cases occurred in regions of altitude ranging from 500 m to 3200 m a.s.l. However, recent epidemics have been reported in previously non-endemic heights of the Amazon basin, which suggests that the endemic range of the disease is expanding. Moreover, the El Niño-related phenomenon of 1997–1998 resulted in an up to four-fold increase in *B. bacilliformis* infections in some geographic regions (Chinga-Alayo *et al.*, 2004).

The only known vectors of *B. bacilliformis* are phlebotomine sandflies of the genus *Lutzomyia* (Alexander, 1995). Townsend (1913, 1914) was the first to suggest that sandflies might act as vectors of *B. bacilliformis*, based on evidence derived from the presence of sandflies in the areas in which the disease occurred, their biting habits and knowledge of the infectious nature of the disease. Transmission of *B. bacilliformis* was first documented by the intradermal injection of infected, homogenized *Lu. verrucarum* into monkeys (Noguchi *et al.*, 1929) and later by successful transmission by wild *Lu. verrucarum* induced to feed on macaques (Hertig, 1942). Other studies (Shannon, 1929) provided further evidence that *Lu. verrucarum* is the most probable vector of *B. bacilliformis* in the Rimac Valley of Peru, although other human-biting phlebotomine species in the area, such as *Lutzomyia noguchii* and *Lu. peruensis*, may also be involved. *Lutzomyia verrucarum* appears to be absent from Ecuador and a competent vector is still to be identified (Alexander *et al.*, 1992; Young & Duncan, 1994). The most likely vector in Colombia is *Lutzomyia columbiana*, which is closely related to *Lu. verrucarum*. It is highly anthropophilic and is found in all areas of Colombia in which bartonellosis outbreaks have occurred (Gamarra, 1964).

Based on the data reported on *Bartonella* foci in Ecuador and Colombia, it appears that the epidemiology of bartonellosis is far from being elucidated. Indeed, the disease is not restricted to elevations greater than 800 m, and also occurs in areas from which *Lu. verrucarum* is absent. Outbreaks continue to be recorded in areas in which *B. bacilliformis*, but not *Lu. verrucarum*, is endemic, which implies that other *Lutzomyia* sandflies or even other arthropods may serve as vectors. Ellis *et al.* (1999) demonstrated by PCR analysis that 1% of 104 wild-caught *Lu. peruensis* harboured *B. bacilliformis*. Furthermore, DNA from a potentially novel *Bartonella* sp. resembling *Bartonella grahamii* (96% similarity) was identified in another *Lu. peruensis* sample in that study (Ellis *et al.*, 1999). In Ecuador, an increasing number of atypical cases with mono-phase verrucous cutaneous disease have been recorded in recent times. Studies have confirmed the presence of sporadic, atypical bartonellosis in residents of the lowlands and observations suggest that bartonellosis is significantly under-reported as a result of the existence of mild clinical disease, possibly

associated with less virulent bacterial strains, which are now disseminating or re-emerging in previously disease-free areas (Amano *et al.*, 1997).

## Conclusions

### *The burden of leishmaniasis*

Among the phlebotomine sandfly-borne diseases, leishmaniasis are the most widespread. Leishmaniasis and other tropical infectious diseases, such as Chagas' disease and sleeping sickness, are generally regarded as neglected diseases because of the lack of effective, affordable and easy-to-use drug treatments. As most affected patients live in developing countries, the pharmaceutical industry has traditionally ignored these diseases. Particularly, VL affects the poorest segments of populations in the poorest countries, which find it difficult to access appropriate diagnosis and treatment. There is limited investment in the development of new drugs for VL and the most effective treatments are often unavailable or unaffordable for patients in endemic areas. In most VL-affected areas, the main drugs for therapy remain pentavalent antimony salts, which are drugs developed in the 1940s that require 3–6 weeks of daily intramuscular or intravenous administration, and which carry a risk for potentially serious side-effects. In some areas VL diagnosis is often established on clinical assessment or non-specific laboratory assays. Because most affected populations are poor and rural, the VL burden may not be perceived as a national issue by health care policymakers, and the treatment and control of the disease are not usually accorded high priority. Public investment in treatment and control would decrease the disease burden of VL and help to alleviate poverty. The burden of tegumentary forms of leishmaniasis is even higher in terms of incidence. Despite their relatively benign nature, these diseases are epidemiologically unstable and result in unpredictable fluctuations in numbers of cases; hence, major epidemics are frequent. Drug treatment is difficult and, although these diseases have severe social and psychological consequences, they are even more neglected than VL by national health authorities.

### *Highlights and challenges in phlebotomine research*

In April 2011 Professor Robert Killick-Kendrick gave a speech at the 7th International Symposium on Phlebotomine Sandflies (Killick-Kendrick, 2011). Several issues concerning newly identified facets of *Leishmania* spp. transmission and sandfly biology were mentioned, including: the influence of saliva on the course of *Leishmania* spp. infection; pheromones and mating of sibling species of *Lu. longipalpis*; evidence for cryptic species that do not have the same vector competence; new hypotheses on the mechanism of *Leishmania* spp. transmission by bite; observations on dispersal and flight speed, and the sources of sugars taken in nature and their possible influence on *Leishmania* spp. development in the vector. With regard to other phlebotomine-associated pathogens, important open issues refer to the putative role of vertebrates as reservoirs of phleboviruses and/or other modes of virus that overwinter

in temperate zones, and the existence of other potential vectors of *B. bacilliformis*.

The spread of laboratory sandfly colonies over the past decades has shed light on several aspects of the biology of these insects. However, laboratory conditions are so artificial that laboratory-based observations may bear little relevance to what happens in nature. Nevertheless, it should be noted that recent laboratory studies on the interaction between *Leishmania* species and sandflies have permitted the classification of sandfly species into 'specific' or 'permissive' vectors (Myskova *et al.*, 2007) and, although this concept might need to be refined further, at present it provides a useful classification system for future studies on *Leishmania* species–sandfly associations and the molecules involved in parasite attachment to the sandfly midgut. The broad vectorial competence of permissive sandflies also has important epidemiological consequences because it may permit the successful adaptation of *Leishmania* species to new vector species (Volf & Myskova, 2007).

Regrettably, field biology research worldwide is limited to the work of relatively few groups of entomologists experienced in phlebotomine research. If this trend continues, aspects of sandfly behaviour that might be relevant to target control may remain unknown or neglected. In addition, studies on geospatial and analytical models applied to social and environmental variables relevant to *Leishmania* spp. transmission ecology are still quite limited.

Specific control measures against sandflies have been shown to be promising in pilot control programmes (Alexander & Maroli, 2003), but these are difficult to sustain. Whatever the measure, its application should be based on good knowledge of the disease. As Killick-Kendrick (2010), pointed out, if a mother doesn't think the disease is carried by a biting fly, she will see no reason to ask her children to sleep under a bednet. Educational health programmes seem to have been neglected and, when they have been implemented, they have been poorly evaluated.

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This article is dedicated to the memory of Dr Robert Killick-Kendrick (b. 20 June 1929, Hampton, U.K.; d. 22 October 2011, Sumène, France). He was a protagonist in the establishment of laboratory colonies of phlebotomine sandflies, which led to fundamental studies on *Leishmania* species–vector interactions. His contributions to this area of research are particularly remarkable for studies performed with Professor. J.-A. Rioux in the Cévennes, France, which included the first experimental infection of dogs with the *Leishmania infantum* vector *Phlebotomus ariasi*, the elucidation of sandfly flight ranges by means of capture–tagging–recapture operations, and the description of cyclical promastigote transformation during *L. infantum* intestinal migration in the competent vector. Also remarkable were studies on pyrethroid-impregnated collars, performed with his wife Mireille, which have been highly successful in pilot trials in the prevention of both human and canine leishmaniasis.

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