Cutaneous leishmaniasis

Richard Reithinger, Jean-Claude Dujardin, Hechmi Louzir, Claude Pirmez, Bruce Alexander, Simon Brooker

Cutaneous leishmaniasis is endemic in the tropics and neotropics. It is often referred to as a group of diseases because of the varied spectrum of clinical manifestations, which range from small cutaneous nodules to gross mucosal tissue destruction. Cutaneous leishmaniasis can be caused by several *Leishmania* spp and is transmitted to human beings and animals by sandflies. Despite its increasing worldwide incidence, but because it is rarely fatal, cutaneous leishmaniasis has become one of the so-called neglected diseases, with little interest by financial donors, public-health authorities, and professionals to implement activities to research, prevent, or control the disease. In endemic countries, diagnosis is often made clinically and, if possible, by microscopic examination of lesion biopsy smears to visually confirm leishmania parasites as the cause. The use of more sophisticated diagnostic techniques that allow for species identification is usually restricted to research or clinical settings in non-endemic countries. The mainstays of cutaneous leishmaniasis treatment are pentavalent antimonials, with new oral and topical treatment alternatives only becoming available within the past few years; a vaccine currently does not exist. Disease prevention and control are difficult because of the complexity of cutaneous leishmaniasis epizology, and the few options available for effective vector control.

*Introduction*

Leishmania parasites are the causal agents of leishmaniasis, a group of protozoan diseases transmitted to mammals, including human beings, by phlebotomine sandflies. Globally, there are an estimated 1·5–2 million new cases and 70 000 deaths each year, and 350 million people are at risk of infection and disease.† Morbidity and mortality because of the leishmaniases cause an estimated 2·4 million disability-adjusted life-years.‡

The leishmaniases are characterised by a spectrum of clinical manifestations: ulcerative skin lesions developing at the site of the sandfly bite (localised cutaneous leishmaniasis [LCL]); multiple non-ulcerative nodules (diffuse cutaneous leishmaniasis [DCL]); destructive mucosal inflammation (mucosal leishmaniasis); and disseminated visceral infection (visceral leishmaniasis). The clinical spectrum observed in patients indicates the complexity of leishmaniasis epizology: several *Leishmania* spp can cause disease (table 1), and many sandfly and mammalian species have been implicated as vectors and reservoir hosts, respectively.

We critically review the most recent data on the burden of the cutaneous leishmaniases, namely LCL, DCL, and mucosal leishmaniasis, their epidemiology, clinical pathology, diagnosis, treatment, prevention, and control. Visceral leishmaniasis has been reviewed elsewhere;§∥ we did not review post kala-azar dermal leishmaniasis, because this is a manifestation seen in patients with visceral leishmaniasis after apparent clinical cure.

*Epidemiology and ecology*

**Disease burden and distribution**

Cutaneous leishmaniasis is endemic in more than 70 countries worldwide, and 90% of cases occur in Afghanistan, Algeria, Brazil, Pakistan, Peru, Saudi Arabia, and Syria (figure 1).¶ Surveillance data indicate that the global number of cases has increased during the past decade, as documented in Afghanistan,¶¶ Bolivia,¶¶ Brazil,¶¶ Colombia,¶¶¶ Peru, and Syria.¶¶¶¶ Such increases can be explained in part by improved diagnosis and case notification,¶¶¶ but are also a result of inadequate vector or reservoir control, increased detection of cutaneous leishmaniasis associated with opportunistic infections (eg, HIV/AIDS),¶¶¶¶ and the emergence of antileishmanial drug resistance.¶¶¶¶¶ However, because

<table>
<thead>
<tr>
<th>New World <em>Leishmania</em> spp</th>
<th>Main clinical pathology</th>
<th>Transmission cycle</th>
<th>Main geographical distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. (Viannia) braziliensis</em></td>
<td>LCL, mucosal</td>
<td>Zoonotic</td>
<td>South America, parts of Central America, Mexico</td>
</tr>
<tr>
<td><em>L. (Viannia) panamensis</em></td>
<td>LCL, mucosal</td>
<td>Zoonotic</td>
<td>Northern South America and southern Central America</td>
</tr>
<tr>
<td><em>L. (Viannia) piroplasm</em></td>
<td>LCL, DCL</td>
<td>Zoonotic</td>
<td>Peru</td>
</tr>
<tr>
<td><em>L. (Viannia) guyanensis</em></td>
<td>LCL</td>
<td>Zoonotic</td>
<td>South America</td>
</tr>
<tr>
<td><em>L. (Viannia) lainsoni</em></td>
<td>LCL</td>
<td>Zoonotic</td>
<td>South America</td>
</tr>
<tr>
<td><em>L. (Viannia) colombiensis</em></td>
<td>LCL</td>
<td>Zoonotic</td>
<td>Northern South America</td>
</tr>
<tr>
<td><em>L. (Leishmania) amazonensis</em></td>
<td>LCL, DCL</td>
<td>Zoonotic</td>
<td>South America</td>
</tr>
<tr>
<td><em>L. (Leishmania) mexicana</em></td>
<td>LCL, DCL</td>
<td>Zoonotic</td>
<td>Central America, Mexico, USA</td>
</tr>
<tr>
<td><em>L. (Leishmania) pifanoi</em></td>
<td>LCL</td>
<td>Zoonotic</td>
<td>South America</td>
</tr>
<tr>
<td><em>L. (Leishmania) venezuelensis</em></td>
<td>LCL</td>
<td>Zoonotic</td>
<td>Northern South America</td>
</tr>
<tr>
<td><em>L. (Leishmania) garnhami</em></td>
<td>LCL</td>
<td>Zoonotic</td>
<td>South America</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Old World <em>Leishmania</em> spp</th>
<th>Main clinical pathology</th>
<th>Transmission cycle</th>
<th>Main geographical distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. (Leishmania) aethiopica</em></td>
<td>LCL, DCL</td>
<td>Zoonotic</td>
<td>Ethiopia, Kenya</td>
</tr>
<tr>
<td><em>L. (Leishmania) killicki</em></td>
<td>LCL</td>
<td>Zoonotic</td>
<td>North Africa</td>
</tr>
<tr>
<td><em>L. (Leishmania) major</em></td>
<td>LCL</td>
<td>Zoonotic</td>
<td>Central Asia, north Africa, middle east, East Africa</td>
</tr>
<tr>
<td><em>L. (Leishmania) tropica</em></td>
<td>LCL</td>
<td>Anthroponotic</td>
<td>Central Asia, middle east, parts of north Africa, southeast Asia</td>
</tr>
<tr>
<td><em>L. (Leishmania) donovani</em></td>
<td>Visceral, LCL</td>
<td>Anthroponotic</td>
<td>Africa, central Asia, southeast Asia</td>
</tr>
</tbody>
</table>

**Old and New World *Leishmania* spp**

| *L. (Leishmania) infantum* | Visceral, LCL | Zoonotic | Europe, north Africa, Central America, South America |

Table 1: *Species* of *Leishmania* that cause human disease.

---

†Morbidity and mortality because of the leishmaniases cause an estimated 2·4 million disability-adjusted life-years.

‡We critically review the most recent data on the burden of the cutaneous leishmaniases, namely LCL, DCL, and mucosal leishmaniasis, their epidemiology, clinical pathology, diagnosis, treatment, prevention, and control. Visceral leishmaniasis has been reviewed elsewhere;§∥ we did not review post kala-azar dermal leishmaniasis, because this is a manifestation seen in patients with visceral leishmaniasis after apparent clinical cure.

¶Surveillance data indicate that the global number of cases has increased during the past decade, as documented in Afghanistan, Bolivia, Brazil, Colombia, Peru, and Syria (figure 1).¶ Surveillance data indicate that the global number of cases has increased during the past decade, as documented in Afghanistan, Bolivia, Brazil, Colombia, Peru, and Syria (figure 1).

¶¶Global surveillance systems are currently insufficient to monitor and control tropical diseases. Surveillance must be strengthened, and research and development in disease control methods accelerated.

¶¶¶Data on the burden of the cutaneous leishmaniases, namely LCL, DCL, and mucosal leishmaniasis, their epidemiology, clinical pathology, diagnosis, treatment, prevention, and control. Visceral leishmaniasis has been reviewed elsewhere;§∥ we did not review post kala-azar dermal leishmaniasis, because this is a manifestation seen in patients with visceral leishmaniasis after apparent clinical cure.

¶¶¶¶Such increases can be explained in part by improved diagnosis and case notification,¶¶¶ but are also a result of inadequate vector or reservoir control, increased detection of cutaneous leishmaniasis associated with opportunistic infections (eg, HIV/AIDS),¶¶¶¶ and the emergence of antileishmanial drug resistance.¶¶¶¶¶ However, because

---

LCL=localised cutaneous leishmaniasis. DCL=diffuse cutaneous leishmaniasis. *Subgenus is given in parentheses.
many infections are symptomless or misdiagnosed, the global burden of cutaneous leishmaniasis is likely to be underestimated.

Transmission cycles are adapting to peridomestic environments and are spreading to previously non-endemic areas as a result of urbanisation and deforestation, with domestic animals as potential reservoirs. Additionallly, economic hardship, natural disasters, armed conflict, and tourism cause susceptible populations to migrate to areas endemic for cutaneous leishmaniasis, where exposure to infection results in noticeable epidemics. For example, whereas cutaneous leishmaniasis caused by Leishmania tropica was rare in Kabul, Afghanistan, before 1990, more than 25 000 mainly autochthonous cases were treated in 2003 (Reithinger R, unpublished data), with incidence estimated to be up to 67 500 new cases per year. New foci of L. tropica are also reported in Morocco, Israel, Syria, Iran, and Pakistan.

Epidemiology

Several features characterise cutaneous leishmaniasis epidemiology. In established endemic areas, cutaneous leishmaniasis prevalence typically increases with age up to 15 years, after which prevalence levels off, presumably because of the acquisition of immunity. The infection can cluster within households, which is indicative of the short flight range of sandflies, anthropogenic transmission, or genetic susceptibility. Risk factors of disease commonly include sex (eg, sex bias usually points to behavioural patterns that increase vector exposure), age, household design and construction material, and presence of domestic animals.

Recent use of geographical information systems and remote sensing has allowed investigations of large-scale distributions and geographical risk factors of disease, but so far such research on cutaneous leishmaniasis has been scarce, partly because of the complexity of the transmission cycle (figure 2). Better understanding of the relations between environmental factors and distributions of sandflies and infection in a wide range of transmission settings would contribute to currently anecdotal or laboratory-based, information on the importance of the environment to transmission (figure 2).

Sandflies and the epizootology of the cutaneous leishmaniases

Leishmania infections typically originate via the bite of sandflies belonging to either Phlebotomus spp (in Europe, North Africa, the middle east, and Asia; figure 3) or Lutzomyia spp (from southern USA to northern Argentina; figure 3). Non-vector transmission (eg, by accidental laboratory infection) is rare. Cutaneous leishmaniasis transmission is either anthropogenic or zoonotic, depending on whether human beings are the main reservoir host (table 1).

Approximately 30 species or subspecies of sandflies are proven vectors, with more than 40 additional species probably involved in transmission. Perhaps the most striking difference between so-called Old World
(ie, Africa, Europe, and Asia) and New World (ie, the Americas) cutaneous leishmaniasis is the ecological context of their respective transmission cycles: whereas Old World cutaneous leishmaniasis usually occurs in open semi-arid or even desert conditions, New World cutaneous leishmaniasis is still mostly associated with forests (figure 3). Cutaneous leishmaniasis foci have wide ecological variation and sandflies are able to find cool, shaded, humid microhabitats in each of them (eg, rock crevices or animal burrows in dry areas; tree buttress roots or leaf litter in forests). Although transmission of most cutaneous leishmaniasis-causing Leishmania spp is zoonotic (table 1), man-made environmental modifications have led to the disease now being acquired within various ecological settings, including settlements established adjacent to primary forest,31 large-scale cultivation of agricultural crops (eg, coffee),11 and marginal neighbourhoods of cities.13 Sandflies will generally take blood from various hosts, and the loss of mammalian biodiversity as a result of deforestation, agricultural practices, and urbanisation can concentrate leishmania transmission by forcing vectors to feed on human beings and a progressively smaller number of synanthropic reservoirs (eg, domestication of Lutzomyia whitmani in Brazil).12

Because mammals of several orders can be infected by the same Leishmania sp, it seems that more selective pressure is exerted on the parasite by the vector than the host. Natural leishmania infections are found in a range of non-human mammal hosts (mainly marsupials, rodents, edentates, and carnivores). So far, only a handful of reservoir hosts for the main Leishmania spp (ie, L infantum, L peruviana, L amazonensis, L mexicana, L guyanensis, L panamensis, L major, and L aethiopica) have been reported,2,35 the reservoir hosts of L braziliensis remain to be identified conclusively. Reservoir implication is difficult because it is often specific to the local epizoological context and depends on many variables (eg, host abundance and distribution, infectiousness to the sandfly vector), which are rarely investigated.

Disease presentation and pathogenesis

Clinical symptoms

Several Leishmania spp can cause cutaneous leishmaniasis in human beings, although most infections probably remain symptomless.1 The first sign of an infection is typically a small erythema that develops after a variable prepatent period at the site where an infected sandfly has bitten the host. The erythema develops into a papule, then a nodule that progressively ulcerates over a period of 2 weeks to 6 months to become the lesion that is characteristic of LCL.25 LCL lesions vary in severity (eg, lesion size), clinical appearance (eg, classic LCL vs disseminated leishmaniasis16 vs leishmaniasis recidivans17), and time to (spontaneous) cure (figure 4). Lymphatic spread and lymph-gland involvement, which may precede lesion development,18 are common and there is a variable tendency for lesions to self-cure within approximately 2–6 months (eg, L major),18–20 3–9 months (eg, L mexicana),22–24 or 6–15 months (eg, L tropica,25 L braziliensis,26–28 L panamensis29–31) of disease onset.

Figure 3: Diversity of habitats endemic for the cutaneous leishmaniases

The distribution of the disease is dependent on the distribution and abundance of the sandfly vector, which in turn is strongly dependent on environmental factors. Sandflies (A: Lutzomyia longipalpis, vector of Leishmania infantum) and, hence, leishmaniasis, can be found in urban (B: Kabul, Afghanistan) or rural areas, in deserts, agricultural areas (C: coffee plantations in Colombia), or tropical rainforests, or below sea level or at high altitudes (D: Andean cordillera, Peru).

Figure 4: Clinical spectrum of the cutaneous leishmaniases

The disease encompasses a range of clinical symptoms, including large ulcers (A: lesion caused by L tropica in Kabul, Afghanistan), destruction of the mucosae (B: mucosal leishmaniasis caused by L braziliensis in Cochabamba, Bolivia), and various secondary diseases (C: leishmaniasis recidivans caused by L tropica in Kabul, Afghanistan). Most lesions are on areas exposed to the sandfly vectors (ie, face, hands, and feet). Clinical diagnosis can be difficult because of other causes leading to similar pathologies or home-based remedies that change the clinical picture typical of leishmaniasis (D: lesion treated with battery acid, Peru).
Spontaneous healing usually results in lifelong protection from disease, which may or may not be restricted to the same Leishmania spp. Resolution of disease results in a lifelong cutaneous scar, which, depending on its size and location, may cause substantial trauma in affected individuals.\textsuperscript{54}

In DCL, which is seen rarely in parts of South and Central America, Ethiopia, and Kenya, parasite-laden, non-ulcerative nodules disseminate from the initial site of infection and may cover a patient’s entire body.\textsuperscript{35} Compared with LCL, DCL is difficult to treat and patients do not self-cure.

Although mucosal leishmaniasis can be caused by L panamensis,\textsuperscript{55} L guyanensis,\textsuperscript{56} L amazonensis,\textsuperscript{57} L major,\textsuperscript{58} L tropica,\textsuperscript{59} and L infantum,\textsuperscript{60} it is most commonly associated with L braziliensis;\textsuperscript{61} thus, barring exceptions,\textsuperscript{58–60} it is usually limited to South America. Mucosal involvement is the most serious complication in L braziliensis infections and can lead to disfiguring and life-threatening mucosal leishmaniasis (also known as espundia) in a varying proportion of patients. In most endemic areas, 1–10% of LCL infections result in mucosal leishmaniasis 1–5 years after LCL has healed,\textsuperscript{7,61} but reports do exist for which mucosal leishmaniasis presented at the same time as LCL,\textsuperscript{62} or for which up to 25% of LCL infections resulted in mucosal leishmaniasis.\textsuperscript{63} Mucosal leishmaniasis is characterised by the ability of the parasite to metastasise to mucous tissues by lymphatic or haematogenous dissemination. It typically begins with nasal inflammation and stuffiness (ie, mild mucosal leishmaniasis), followed by ulceration of the nasal mucosa and perforation of the septum. In some cases, the lips, cheeks, soft palate, pharynx, or larynx are also involved (ie, severe mucosal leishmaniasis; figure 4). Mucosal leishmaniasis never heals spontaneously, is very difficult to treat, with secondary bacterial infections common, and is potentially fatal.\textsuperscript{61}

Disease pathogenesis and immunology

The life cycle of leishmania parasites, whether in the sandfly vector or the human host is shown in figure 5. The establishment of the primary leishmania infection and
Acquired immune responses (eg, macrophages, neutrophils, natural killer cells, dendritic cells). These inflammatory responses mediate disease expression and may result in either symptomless or subclinical infection, self-healing LCL, or chronic leishmaniasis (eg, DCL, mucosal leishmaniasis, leishmaniasis recidivans). Clinical cure ensues when macrophages become activated to a leishmanicidal state. This is mainly mediated by the T-helper cell type 1 (Th1) response, which also prevents recrudescence of latent chronic infection. The Th1 response is characterised by antigen-presenting dendritic cells, responding CD4 and CD8 T cells, and secretion of proinflammatory cytokines (eg, interferon γ, tumour necrosis factor α [TNFα]).

The Th2-type response is inactivated by antigen-presenting dendritic cells, responding CD4 and CD8 T cells, and secretion of T-helper cell type 2 (Th2) cytokines. characterise the Th2 response, which deactivates macrophages and prevents excessive production of protective cytokines. Although the Th2 response probably prevents extensive tissue destruction, it promotes intracellular infection.

To maintain a memory cell response dependent on continual antigen presentation, lifelong protection against reinfection may involve live parasite persistence (eg, Leishmania subgenus Viannia DNA is detected in scars and blood of clinically cured patients) and repeated challenges by parasites via new bites of infected sandfly vectors. Recurrence caused by reactivation of persistent infections or trauma may occur, and some patients develop a second cutaneous lesion at a different site after their primary lesion has healed.

Our knowledge of the immune response to leishmania infection mainly stems from studying leishmania infection in various experimental models, of which the L. major murine model has been the most dominant, and has been extensively reviewed. This response can be summarised as follows: (1) disease resolution is mediated by the cell-mediated response rather than the humoral immune response; (2) the primary activation of T-cell subsets is important for the development of Th1 and Th2 responses and the subsequent course of infection; and (3) there is strong correlation between activation of different T-cell subsets and outcome of disease.

Studies of the cellular immune responses in human beings have mostly been descriptive, because of the difficulties in defining the immunopathological and protective mechanisms in leishmania infections, the necessity to do longitudinal studies, and by the genetic heterogeneity of human and parasite populations. Although epidemiological data from surveys of patients seem to confirm the Th1/Th2 dichotomy shown in experimental animal models, other studies show that the human immunological response is not exclusively explained in terms of Th1/Th2 subsets.

LCL patients, who present limited and ulcerated skin lesions, represent the so-called healing form of the disease. Their peripheral blood mononuclear cells (PBMCs) proliferate and produce Th1-type cytokines, including interferon γ, when stimulated with leishmanial antigens in vitro. Their delayed-type hypersensitivity (DTH) response is positive, with Montenegro skin test induration size correlated to lesion size and occasionally to lesion number. By contrast, patients with recurrent infections have a weaker DTH response than patients with subclinical infections, as do patients with relapses when compared with those with reinfections; their PBMCs produce low concentrations of interferon γ and high concentrations of interleukin 4 when stimulated with leishmanial antigens, emphasising the role of a Th2-type response in chronic infections. Cytokine profiles may vary with time during the course of infection: although large concentrations of interferon γ are produced on leishmanial antigen presentation in situ (eg, as detected in lesion biopsies), during the early phase of infection (<60 days) interferon-γ production may be downregulated by high concentrations of interleukin 10, which may account for a transient period of high local parasite multiplication. There is no evidence, however, that patients with low initial interferon-γ production are at risk of developing larger lesions or parasite dissemination; in fact, they may have a better response to therapy with pentavalent antimonials.

DCL patients display a predominantly Th2-type cytokine response, in that DCL patients have a complete anergy to leishmanial antigen, with a negative DTH response and lymphocytes non-responsive to leishmanial antigen. DCL patients have low concentrations of interferon γ and interleukin 12, but substantial serum concentrations of interleukin 4, interleukin 5, and TNFα.

Patients with mucosal leishmaniasis show a mixture between Th1 and Th2 cytokine responses (with high levels of interleukin 2, interleukin 4, interleukin 5, and TNFα), which could explain non-resolution of disease, because the Th2-type response tends to dominate when both types of responses are activated. Mucosal leishmaniasis patients tend to have a larger DTH response than LCL patients, with comparatively high serum concentrations of interferon γ and interleukin 2, as well as interleukin 5 and TNFα. So far, there are no known immunological markers that may help to identify those LCL patients who are at risk of developing mucosal leishmaniasis. Indeed, studies showing differences in the immune response to different parasite strains or species are scarce. For example, the DTH response to leishmanial antigen is greater in L. braziliensis-infected patients than in L. panamensis-infected patients.
even after adjusting for time of evolution and lesion type (ie, LCL or mucosal leishmaniasis).17

Only a few studies have reported on parasite-specific cytotoxic T-cell responses.86,87 Several reports implicate natural killer cells and CD8 T cells in interferon-γ production and immunity in human cutaneous leishmaniasis.88,89 By using granzyme B as a surrogate marker of leishmaniasis-specific cell-mediated cytotoxicity, Bousoff ara and colleagues90 recently showed that parasite-specific cytotoxic immune responses are developed by individuals living in areas of L major transmission, and play a crucial part in resistance to re-infection (Louzir H, unpublished data). Finally, the role of regulatory T cells in cutaneous leishmaniasis has only recently been investigated, with distinct subpopulations of CD4 CD25 regulatory T cells shown to stimulate TGFβ1 production by PBMCs from healthy individuals when incubated with L guyanensis parasites.91 Regulatory T cells can also be found in skin lesions of patients with cutaneous leishmaniasis caused by L braziliensis, which produce large amounts of interleukin 10 and TGFβ1.92 These findings suggest that, like in the mouse model,71,72 functional regulatory T cells accumulate at sites of leishmania infection in human beings and possibly contribute to the local control of effector T-cell functions and thereby affect parasite persistence.

Parasite effects and factors

The contribution of the parasite to the clinical cutaneous leishmaniasis pleomorphism has been supported for years at species and intraspecies levels by several studies showing a correlation between specific genotypes and clinical forms (eg, L infantum zymodemes causing visceral or cutaneous disease).93 Other studies have, however, failed to identify such correlation,94 and underscore the complementary role of host and other factors in clinical sequelae.

Several determinants of parasite virulence have been identified experimentally, all of which may help the parasite to evade the host’s immune system. These can be classified into three main categories: (1) invasive or evasive determinants (eg, lipophosphoglycans, leishmanolysin, or cysteine proteases), which are crucial for infection, but unable to produce pathology in the host; (2) pathoantigenic determinants (eg, histones, chaperones, or proteasomes), which lead to host immunopathology as the principal cause of clinical symptoms; and (3) protective determinants (to be identified), which seem to lead to clinical cure.95 Of note is that most virulence studies are based on a single Leishmania sp strain in well-controlled models in vitro or in vivo, which may not be applicable to human pathology.96 Indeed, genetic diversity is a major advantage to the parasite, and it seems that Leishmania spp differ in their approach to tackle the host immune system.97 For example, lipophosphoglycans are a clear virulence factor in L major, but not in L mexicana,98 and distinct L braziliensis isolates induce different paces of chemokine expression patterns.99 In this context, molecular epidemiologists should consider alternative markers to the established neutral markers (eg, isoenzymes or ribosomal DNA internally transcribed spacers) for genotyping natural populations, and focus on the polymorphism of virulence determinants.100 The informative power of such an approach is shown by the finding that L peruviana (reportedly of high pathogenicity but low virulence) differs from L braziliensis (reportedly of low pathogenicity and high virulence) by the deletion of half the leishmanolysin genes,101 and that leishmanolysin genes are highly polymorphic in leishmania populations, particularly in immunodominant B-cell and T-cell epitopes.102 PCR-based assays targeting leishmanolysin and other virulence genes are becoming available for such a molecular epidemiology approach,103,104 but should be complemented by studies at transcriptomic and proteomic levels.

Host effects and factors

Susceptibility to cutaneous leishmaniasis can be greatly influenced by malnutrition,105 immunosuppression (eg, HIV),106 and host genetic background.107 Comparative studies focusing on different ethnic groups (eg, mucosal leishmaniasis caused by L braziliensis in Bolivia),108 natives and migrants (eg, LCL caused by L major in Saudi Arabia),109 or by familial clustering studies (eg, mucosal leishmaniasis caused by L braziliensis in Brazil),110 have shown that human genetic components control cutaneous leishmania susceptibility and resistance. Identification of candidate genes or regions involved in the genetic control of leishmaniasis has been made possible by a mouse-to-human approach, whereby susceptibility or resistance genes have been identified in murine models,111 and refined by the knowledge of the human immune response to leishmaniasis and genetic studies of other intramacrophage pathogens. Thus, studies in human beings indicate a role of HLA molecules in LCL and mucosal leishmaniasis,112 and the role of TNFα in developing mucosal leishmaniasis.113 However, a strong imbalance exists between the number of experimental analyses in mice and studies in a natural human context. In consideration of the diversity in the above-mentioned parasite approach of the host, whether host genetic determinants of leishmaniasis will be the same for different Leishmania spp remains to be established.

Sandfly vector effects and factors

In the past decade, it has become clear that sandfly saliva is crucial in the establishment of infection and disease pathogenesis.114 Sandfly saliva is vasodilatory and enhances erythema (caused by the maxadilan peptide in Lutzomyia longipalpis); increases parasite burden, lesion size, and persistence after co-inoculation with L major, L amazonensis, and L braziliensis; and intraspecific variation in saliva
components determines clinical outcome after *L. infantum* infection. The immunological basis for these findings is not fully understood, but it seems that saliva proteins can shift the adaptive immune response from a Th1 to a Th2 cell-mediated immune response (eg, by increasing the production of interleukin 4 and interleukin 6, or by inhibiting TNFα, interferon γ, interleukin 12, and nitric oxide production). Furthermore, experimental or natural pre-exposure to sandfly saliva cancels any enhancing effect from subsequent co-inoculation of saliva with *L. major*, reducing parasite load and lesion size, as well as increasing the DTH response and reducing interleukin-4 production. This protective effect seems to be mediated by anti-saliva antibodies produced after saliva exposure. If this phenomenon exists under natural conditions, it may explain why cutaneous and mucosal leishmaniasis host susceptibility declines with age, as observed in people in cutaneous leishmaniasis-endemic areas.

** Diagnosis and treatment**

**Diagnosis**

The broad clinical spectrum of cutaneous leishmaniasis makes diagnosis of present and past cases difficult. Differential diagnosis is important because diseases of other causes but with a similar clinical spectrum to leishmaniasis (eg, leprosy, skin cancers, tuberculosis, cutaneous mycoses) are common in leishmaniasis-endemic areas.

Parasitological diagnosis remains the gold standard in cutaneous leishmaniasis diagnosis, because of its high specificity. It includes microscopic examination of Giemsa-stained biopsy smears or aspirates, histopathological examination of fixed lesion biopsies, or culture of biopsy triturates or aspirates. Microscopic examination is probably the most common diagnostic approach used, because more sophisticated techniques are expensive and rarely available at primary, secondary, and tertiary health-care levels in endemic areas. Culture methods are probably the most informative, allowing species identification and characterisation, but require a wealth of technical expertise, and are time-consuming and expensive. The sensitivity of these techniques, however, tends to be low and can be highly variable, depending on parasite number and dispersion in biopsy samples, technical expertise, and culture media. Molecular parasitological diagnosis for cutaneous leishmaniasis was developed extensively during the past decade, and has been recently reviewed. It is essentially done by PCR-based methods and is particularly useful in cases with low parasite load (eg, mucosal leishmaniasis); potentially, therapy of cutaneous leishmaniasis patients could also be monitored. Whereas reported specificity is 100%, sensitivity is improved by 20–30% in LCL and 55–70% in mucosal leishmaniasis when compared with conventional parasitological diagnosis. Although there has been substantial effort in applying molecular diagnostics in the field (eg, successful detection of parasite DNA in blood or tissue smears; development of rapid PCR oligochromatography), its widespread use is still hampered by the requirement of substantial laboratory infrastructure, technical expertise, and cost. Until these hurdles can be overcome, molecular diagnosis will be limited to well-established reference laboratories, or travel medicine clinics.

Serological diagnosis is rarely used in cutaneous leishmaniasis diagnosis because of variable sensitivity and specificity. The Montenegro skin test is occasionally used in diagnosis of cutaneous disease (eg, in epidemiological surveys), because of its simple use and high sensitivity and specificity; however, it fails to distinguish between past and present infections.

**Treatment**

Although non-fatal, cutaneous leishmaniasis is treated to accelerate cure to reduce scarring, especially in cosmetic sites, and to prevent parasite dissemination (ie, mucosal leishmaniasis) or relapse. Treatment is commonly given for persistent (>6 months duration), multiple, or large lesions, and for lesions located on joints or on the face. In most leishmaniasis-endemic countries, official Ministry of Health policy is to provide free treatment to all patients. This is often not feasible in practice, because drugs may be in limited supply, particularly in the mostly rural areas where the disease occurs. Thus, self-help patient associations or non-governmental organisations may facilitate diagnosis and treatment of patients (eg, Bolivia, Peru, and Afghanistan).

Except for the immunotherapy policy in Venezuela, and the pentamidine treatment policies in French Guyana and Suriname, WHO recommends treating cutaneous leishmaniasis with pentavalent antimonial drugs (ie, sodium stibogluconate or meglumine antimonate) at 20 mg/kg per day for 20–28 consecutive days. Barring one exception, this regimen has been shown to be more efficient than a daily dose of 10 mg/kg, 13 mg/kg, or 15 mg/kg in treating LCL.

The main problems in treating cutaneous leishmaniasis are that clinical diagnosis is difficult in the absence of microscopy at the basic health-care level, and pentavalent antimonial drugs can have serious, although usually reversible, side-effects (eg, musculoskeletal pains, renal failure, hepatotoxicity, and cardiotoxicity) and are of variable efficacy against mucosal leishmaniasis. Drugs and medical attention because of the side-effects make treatment expensive, and reports on patients non-responsive to the drugs either because of drug-resistant parasite strains (although a recent study has questioned the definition of true parasite resistance) or to immunosuppression (eg, caused by HIV) are increasing. Moreover, the invasiveness of the standard treatment protocol (ie, a lengthy course of intramuscular or intravenous injections) means that many patients fail to complete their full course of treatment. Hence, to reduce systemic toxic effects, economic cost, and poor
treatment compliance, most research in the past decade has focused on the development of alternative dosage schedules, modes of delivery (ie, parenteral vs local, or topical vs oral), or treatments.125

Recommended and alternative treatment regimens are shown in table 2, categorised by treatment modality and Leishmania cause. We reviewed currently available data and conclude that for cutaneous leishmaniasis, pentavalent antimony, given parenterally or intralesionally, remains the first-line treatment approach. Alternative treatment regimens include amphotericin B, especially for mucosal leishmaniasis, and pentamidine. Several studies have shown the efficacy of miltefosine and thermotherapy, which should also be considered as alternative treatments, depending on leishmania cause and clinical manifestation. For other treatment regimens, not enough consistent data exist in our view to show their efficacy against cutaneous leishmaniasis, and these are not recommended for use in routine clinical practice.

A few groups of studies are worth mentioning because of their potential relevance for antileishmanial treatment policy. First, several studies on patients infected with L panamensis in Colombia,126 L braziliensis in Guatemala,127 and L tropica in the USA,128 have shown no substantial difference when reducing the treatment duration with 20 mg/kg per day pentavalent antimony from 20 days to 10 days. Extending the treatment from 28 days to 40 days does not lead to an increase in clinical cure in patients with mucosal leishmaniasis, with proportions being the same for both patient groups.129

Thus, there seems to be room to reduce treatment time when using antimony, particularly if there is no increased risk of secondary leishmanial diseases (eg, mucosal leishmaniasis or leishmaniasis recidivans), the advantage being a reduction in systemic toxicity and treatment cost.

Second, numerous studies have shown that intraleseional pentavalent antimony administration (figure 6) can be very effective in treating patients with LCL caused by L major,130 L tropica,131 L braziliensis,132 or L panamensis.133 The advantages of this approach are that a higher drug concentration targets the site of infection, reducing

<table>
<thead>
<tr>
<th>Application</th>
<th>Pathology</th>
<th>Clinical efficacy against Leishmania spp</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>First-line treatment</td>
<td>Parenteral</td>
<td>All species: 36–96%</td>
<td>Toxic side-effects; not recommended for pregnant women; shorter course can be effective</td>
<td>53, 124, 129-132</td>
</tr>
<tr>
<td>Pentavalent antimony</td>
<td>Parenteral, intramuscularly or intravenously</td>
<td>LCL</td>
<td>Toxic side-effects; not recommended for pregnant women</td>
<td>124</td>
</tr>
<tr>
<td>20 mg/kg daily for 20 days</td>
<td>Parenteral, intramuscularly</td>
<td>LCL</td>
<td>Several species: &gt;75%</td>
<td>Toxic side-effects; not recommended for pregnant women</td>
</tr>
<tr>
<td>10–15 mg/kg daily for 20–30 days</td>
<td>Parenteral, intramuscularly</td>
<td>LCL</td>
<td>L mexicana: 73%; L panamensis: 91%; L braziliensis: 53%</td>
<td>Toxic side-effects, especially at higher doses; first-line therapy against L guyanensis in French Guiana and Suriname</td>
</tr>
<tr>
<td>20 mg/kg daily for 28 days</td>
<td>Parenteral, intramuscularly or intravenously</td>
<td>LCL</td>
<td>L braziliensis: 91%; L panamensis: 95%; L mexicana: 35%; L guyanensis: 20–75%</td>
<td>Toxic side-effects, especially at higher doses</td>
</tr>
<tr>
<td>Protocol variable*</td>
<td>Local, intralesionally</td>
<td>LCL</td>
<td>L major: 73%; L tropica: 75%</td>
<td>No systemic toxic side-effects; substantially increases patient compliance; substantial reduction in pentavalent antimony used and cost of therapy</td>
</tr>
</tbody>
</table>

Alternative treatments

Amphotericin B

| Application | Pathology | LCL, ML | L braziliensis: unknown | Toxic side-effects, given for antimony-unresponsive patients; case studies only | 124 |

Pentamidine isetionate

| Application | Pathology | LCL | L panamensis: 95%; L braziliensis: 35% | Toxic side-effects, especially at higher doses; first-line therapy against L guyanensis in French Guiana and Suriname | 52, 121, 122, 135 |

Miltefosine

| Application | Pathology | LCL | L panamensis: 91%; L braziliensis or L mexicana: 53% | Limited toxic side-effects; teratogenicity in animals, contraindicated for pregnant women or women of childbearing age; registered for cutaneous leishmaniasis treatment in Colombia in 2005; also seems effective for treatment of ML | 45, 137 |

Thermotherapy†

| Application | Pathology | LCL | L tropica: 69% | No systemic toxic side-effects; increases patient compliance; use depends on lesion size and number; Ministry of Public Health approval as alternative cutaneous leishmaniasis treatment in Afghanistan | 128 |

(Continues on next page)
systemic toxic effects, improving healing time, and reducing cost (eg, in Afghanistan, the cost of successfully treating cutaneous leishmaniasis can vary substantially, depending on whether a patient is treated intramuscularly or intramuscularly).

The drawbacks are that there is no set protocol (ie, the drug amount used is dependent on lesion number, size, and location), and treatment administration requires substantial technical expertise.

Third, several less toxic formulations of amphotericin B have been developed (eg, AmBisome, Amphocil, and Abelcet). These have been tested in vitro and in vivo, but unlike for visceral leishmaniasis, their cost has restricted their use in cutaneous leishmaniasis treatment to a handful of (successful) case studies.

Fourth, several studies have shown the efficacy of oral (eg, ketoconazole, fluconazole, miltefosine) or topical (eg, paromomycin cream, thermotherapy) treatment regimens (table 2). Whereas certain regimens have been pursued more vigorously and consistently (eg, miltefosine), others have been less so, probably as a result of the high costs of the branded drug (eg, ketoconazole), cream (eg, paromomycin), or hardware (eg, radiofrequency generator used in thermotherapy). However, some of these treatment alternatives may substantially reduce treatment duration and non-compliance of patients, and, hence, ultimately prove cost-effective.

Finally, there is increasing evidence that the treatment response of patients with cutaneous leishmaniasis depends on the infecting *Leishmania* spp. For example, miltefosine was shown to have high efficacy in treating *L panamensis* patients in Colombia, but had a reduced...

...
efficacy for patients infected with \textit{L. braziliensis} in Guatemala,\textsuperscript{6} with the possibility for a species-specific tolerance to miltefosine supported by susceptibility data in vitro.\textsuperscript{25}

Despite the numerous clinical trials that have tested different treatment approaches for cutaneous leishmaniasis, comparisons between studies are problematic. First, cutaneous leishmaniasis lesions can self-cure. Failure to include either negative (placebo) or positive (recommended standard treatment, such as pentavalent antimony) controls in the studies makes the interpretation of an effect of different drugs, doses, or schedules impossible, especially if small numbers of patients are used to assess treatment response. Second, infecting parasite species and strains clearly vary in their sensitivities to drugs, and cure rates of cutaneous leishmaniasis patients with moderate or severe disease (LCL \textit{vs} mucosal leishmaniasis) are very different; infecting parasites should be characterised if financially and logistically feasible. Healing rates also depend on host factors, such as localisation and chronicity of lesions, underlying illness or concomitant infection, and acquired resistance to leishmanial infection; thus, such data should be collected. Third, studies vary in experimental protocol (eg, study design, duration of follow-up) and in particular in their definition of clinical cure. For example, clinical cure may be defined as, “when lesions have more than 80% re-epithelialised by the first follow-up at 1·5 months”,\textsuperscript{13} or as, “complete re-epithelialisation of all lesions at the end of treatment and no reactivation or mucosal involvement during follow-up”.\textsuperscript{13} Standardised endpoints should be established.

**Disease control**

**Vector and reservoir control**

Because the strategies available are expensive and labour intensive, and because cutaneous leishmaniasis is a non-fatal disease, prevention and control strategies have mainly focused on treatment of the human disease, rather than on the elimination of reservoirs or reduction of human–vector contact.\textsuperscript{162} Hence, most approaches have been limited to pilot research studies and only a few have been brought up to operational scale.\textsuperscript{53}

Sandflies are highly susceptible to insecticides. Although they possess the necessary biochemical mechanisms,\textsuperscript{34} reports of resistance are few.\textsuperscript{82} Anecdotal evidence from Peruvian and Iranian malaria eradication campaigns in the 1950s suggested that residual spraying of houses is effective against endophilic and endophagic sandfly vectors, which was subsequently shown in controlled studies.\textsuperscript{82,135} Measures involving the participation of the at-risk human population focus on personal protection from cutaneous leishmaniasis (figure 6), including insecticide-impregnated materials (eg, bednets, curtains, clothes, or bed-sheets)\textsuperscript{165} and repellents,\textsuperscript{162,170} which may offer an alternative in places with poor health-service infrastructure and peri-domestic leishmania transmission. Several studies have shown that pyrethroid-treated bednets provide 50–65% protection against infection or disease.\textsuperscript{162,163,165–168}

However, similar to house spraying, the long-term feasibility of insecticide-treated materials is debatable, because of logistical constraints (eg, re-impregnation of materials) and the intervention’s economic cost. Opportunities for cost-effective scale-up of cutaneous leishmaniasis prevention and control through insecticide-treated materials are the recent development of long-lasting insecticide-treated nets (ie, WHO-approved Olyset [Sumitomo Chemical, Tokyo, Japan] and PermaNet [Vestergaard Frandsen, Lausanne, Switzerland] nets, which are already extensively used in malaria control) or long-lasting insecticide formulations (ie, K-O TAB, Bayer Environmental Science, Monheim am Rhein, Germany). In forested environments (ie, in most endemic areas of South and Central America) health authorities are usually limited to treating human cutaneous leishmaniasis cases. Although prevention and control strategies (eg, environmental management, spraying of sandfly resting sites) have been explored,\textsuperscript{82} targeting the sandfly vector effectively in these habitats is difficult.
We know of only one reported example of reservoir control as a cutaneous leishmaniasis prevention and control strategy, in which zoonotic LCL caused by *L major* was controlled by destroying burrows of the rodent LCL reservoir.11 In endemic areas where dogs are domestic reservoirs of cutaneous leishmaniasis,16 deltamethrin-impregnated dog collars could be an effective and feasible strategy, especially if these areas are sympatric for visceral leishmaniasis or Chagas disease.172 To be sustainable in the long-term, cutaneous leishmaniasis control strategies will have to be integrated into a strategy addressing other vector-borne diseases (eg, malaria or Chagas disease).

**Vaccines**

The rationale for vaccine development is provided by the evidence that most individuals that had leishmaniasis or symptomless infection are resistant to subsequent clinical infections. As outlined in recent reviews,173–75 substantial effort has been spent in developing a leishmania vaccine, an effort that has so far remained fruitless. The only proven cutaneous leishmaniasis vaccine (practised for centuries) is the deliberate inoculation of virulent leishmania parasites, so-called leishmanisation.16 However, for several basic and logistic problems (eg, difficulties in maintaining parasite virulence, risk of unacceptable lesions in some recipients), leishmanisation is not currently recommended by WHO. Its use is restricted to a few countries (eg, Uzbekistan), notably as an evaluation method of new leishmania vaccines. With the support of WHO’s Research and Training in Tropical Diseases programme, several vaccines, based on killed parasites, have been developed and assessed for their immunogenicity and efficacy in South and Central America, Sudan, and Iran.173–175 In all these studies, the Montenegro skin test, PBMC proliferation, or interferon-γ production were used as indicators of Th1 response for the selection of naive individuals and as a correlate for protection. Although tested vaccines were safe and immunogenic (ie, in terms of leishmanin skin test conversion or increase of specific interferon-γ production by PBMC), significant, long-lasting protection could not be shown. It seems that in leishmania vaccine studies, the specific DTH reaction induced by vaccination is not predictive of protection. These observations contradict the protective effect of leishmanin skin test reactivity in naturally infected individuals,72,177 and emphasise the complexity of *Leishmania* spp susceptibility and resistance mechanisms.

New approaches are now being investigated in the experimental leishmaniasis mouse models, with several *Leishmania* spp and sandfly saliva proteins having been identified as candidate vaccines.178–80 It is hoped that the recent completion of the genome sequence for *L major*,80 and soon *Lu longipalpis*,80 will yield novel strategies for vaccine development that take advantage of recent progress in molecular biology, immunology, and post-genomics.

**Panel: Priorities for research and public-health policy**

**Research**

**General approaches**

- Standardise protocols of experimental, clinical, and epidemiological studies, so that comparisons between studies can be made (eg, clinical trials must include placebo or antimony controls and should have the same clinical endpoints)

**Diagnosis, pathology, and immunology**

- Investigate relevance and cost-effectiveness of including species typing in the routine diagnosis in endemic countries with sympatric species
- Understand how innate immune system shapes adaptive antileishmanial immunity

**Treatment and vaccines**

- Assess the clinical efficacy of antileishmanial drugs that have recently become available in their generic forms (eg, fluconazole)
- Assess the effect of true parasite drug resistance on treatment efficacy

**Epidemiology, prevention, and control**

- Reassess the global burden of cutaneous leishmaniasis because current figures are based on poor notification data and do not include social impact (disability) caused by scarring of lesions
- Investigate associations between disease distribution, sociodemographic, and environmental risk factors at both small and larger levels so that rational prevention and control strategies can be developed
- Support field studies to investigate sandfly ecology, because they are fundamental in developing putative prevention and control strategies

**Public-health policy**

- Harmonise leishmaniasis notification, prevention, and control guidelines in endemic countries to allow better estimates of burden of disease
- Expand use of generic sodium stibogluconate, because it has shown to be as effective as branded antimony for treating cutaneous leishmaniasis and at a fraction of the economic cost
- Expand use of local, oral, and topical antileishmanial therapies, especially in areas where patient compliance and drug supply are problematic
- Develop cost-effective prevention and control strategies, especially if these can be integrated into programmes to control other diseases (eg, malaria or Chagas disease)

**Conclusions**

The leishmaniases are a complex group of diseases and although we know much more than we did a decade ago, we are no nearer to the prevention or control of this neglected disease, which mainly affects the world’s poorest populations.16 To do so requires professional and financial commitment, focusing on key research and policy areas (panel). Over the past decade, several reviews and reports have identified priorities in research and public-health policy with regard to cutaneous leishmaniasis. These have ranged from increasing efforts in vaccine research and development of antileishmanial combination therapies to the encouragement of multidisciplinary studies to consider the tremendous diversity of natural leishmania populations in protocol design and to maximise project output. Although some of these priorities are still relevant today, we believe that others should be included to bring the field significantly forward.

Management of patients can be substantially improved, by developing better approaches to case detection and treatment. Better case detection and epidemiological
surveillance are also required to better quantify the disease burden of cutaneous leishmaniasis. Several epidemiological aspects deserve further study. The relative association and contribution of environmental factors, parasite and vector species, exposure and susceptibility factors generating predisposition to disease, and distributions of infection and disease are poorly understood, and need to be elucidated for the design of any control strategy. If, for example, susceptibility factors are dominant, then comprehensive research on chemotherapy and vaccines should focus on how best to protect susceptible individuals; if exposure factors are more important, then the identification of risk factors would help to guide the design of prevention strategies.

Conflicts of interest
RR has been a consultant to Thermosurgery Technologies Inc, received conference travel funds from Zentaris and Intervet, was provided free dog collars from Intervet to carry out field trials in Brazil, and was provided free miltefosine by Zentaris to carry out a clinical trial on its use.

Acknowledgments
We are grateful to Rupert Quinnell for valuable comments on the manuscript and various colleagues who contributed to helpful discussions and provided photographs for figure 3A (Ray Wilson) and figure 6C (Mark Rowland); we also thank three anonymous reviewers for constructive comments on the submitted manuscript. Our work is and has been supported by grants from the Albert Berman Foundation, Afghan Research Evaluation Unit, Belgian Agency for Cooperation and Development, Conselho Nacional de Pesquisa, European Union (European Commission [EC], EC INCO Programme, EC Humanitarian Office), Gesellschaft für Technische Zusammenarbeit, Hilda Leeven Family Fund, Office of the United Nations High Commissioner for Refugees, Sir Halley Stewart Trust, United Nations Aid Mission to Afghanistan, Wellcome Trust, and WHO. SB is supported by a Wellcome Trust Advanced Training Fellowship (071656). The opinions expressed in this Review are those of the authors and may not reflect the position of their employing organisations nor of their work’s sources of funding.

References
Review


123 Oliveira-Neto MP, Schubach A, Mattos M, Goncalves-Costa SC, Pirmez C. Treatment of American cutaneous leishmaniasis: a comparison between low dosage (5 mg/kg/day) and high dosage (20 mg/kg/day) antiymin regimens. *Pathol Biol (Paris)* 1997; 45: 86–89.


130 Palacios R, Osorio LE, Grajalew LF, Ochoa MT. Fidelity of a short course (10 days) of high-dose meglumine antimonate with or without interferon-gamma in treating cutaneous leishmaniasis in Guatemala. *Clin Infect Dis* 1994; 18: 381–84.


