Leishmaniasis emergence in Europe

P D Ready (P.Ready@nhm.ac.uk)[1]
1. Department of Entomology, Natural History Museum, London, United Kingdom

Citation style for this article:
Citation style for this article: Ready PD. Leishmaniasis emergence in Europe. Euro Surveill. 2010;15(10):pii=19505. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19505

This article has been published on 11 March 2010

Leishmaniasis emergence in Europe is reviewed, based on a search of literature up to and including 2009. Topics covered are the disease, its relevance, transmission and epidemiology, diagnostic methods, treatment, prevention, current geographical distribution, potential factors triggering changes in distribution, and risk prediction. Potential factors triggering distribution changes include vectorial competence, importation or dispersal of vectors and reservoir hosts, travel, and climatic/environmental change. The risk of introducing leishmaniasis into the European Union (EU) and its spread among Member States was assessed for the short (2-3 years) and long term (15-20 years). There is only a low risk of introducing exotic *Leishmania* species because of the absence of proven vectors and/or reservoir hosts. The main threat comes from the spread of the two parasites endemic in the EU, namely *Leishmania infantum*, which causes zoonotic visceral and cutaneous leishmaniasis in humans and the domestic dog (the reservoir host), and *L. tropica*, which causes anthroponotic cutaneous leishmaniasis. The natural vector of *L. tropica* occurs in southern Europe, but periodic disease outbreaks in Greece (and potentially elsewhere) should be easily contained by surveillance and prompt treatment, unless dogs or other synanthropic mammals prove to be reservoir hosts. The northward spread of *L. infantum* from the Mediterranean region will depend on whether climate and land cover permit the vectors to establish seasonal biting rates that match those of southern Europe. Increasing dog travel poses a significant risk of introducing *L. infantum* into northern Europe, and the threat posed by non-vectorial dog-to-dog transmission should be investigated.

Leishmaniasis

Leishmaniasis (or 'leishmaniosis') is a complex of mammalian diseases caused by parasitic protozoans classified as *Leishmania* species (Kinetoplastida, Trypanosomatidae) [1,2]. Natural transmission may be zoonotic or anthroponotic, and it is usually by the bite of a phlebotomine sandfly species (order Diptera, family Psychodidae; subfamily Phlebotominae) of the genera *Phlebotomus* (Old World) and *Lutzomyia* (New World) [2,3]. Primary skin infections (cutaneous leishmaniasis) sometimes resolve without treatment, with the host developing acquired immunity through cellular and humoral responses [4], but the infection can spread to produce secondary lesions in the skin (including diffuse cutaneous leishmaniasis), the mucosa (muco-cutaneous leishmaniasis) and the spleen, liver and bone marrow (visceral leishmaniasis, which is usually fatal if untreated) [1]. Worldwide, at least 20 *Leishmania* species cause cutaneous and/or visceral human leishmaniasis (HumL) [1,5]. Most foci occur in the tropics or subtropics, and only zoonotic *L. infantum* is transmitted in both the eastern and western hemispheres [5] (Table 1).

Worldwide and European relevance of leishmaniasis

The World Health Organization (WHO) reports that the public health impact of leishmaniasis worldwide has been grossly underestimated for many years [1]. In 2001 and 2004, Desjeux reported that in the previous decade endemic regions had spread, prevalence had increased and the number of unrecorded cases must have been substantial, because notification was compulsory in only 32 of the 88 countries where 350 million people were at risk [5,6]. About two million new cases of HumL (half a million visceral) are considered to occur every year in the endemic zones of Latin America, Africa, the Indian subcontinent, the Middle East and the Mediterranean region [1].

Risks of emergence or re-emergence of leishmaniasis in Europe are associated with three main scenarios:

1) the introduction of exotic *Leishmania* species or strains into Europe via the increasing worldwide travelling of humans [6] and domestic dogs [7],
2) the natural spread of visceral and cutaneous leishmaniasis caused by *L. infantum* and *L. tropica* from the Mediterranean region of Europe, where these species are endemic [1,8,9], to neighbouring temperate areas where there are vectors without disease [2],
3) the re-emergence of disease in the Mediterranean region of Europe caused by an increase in the number of immunosuppressed people.

The high prevalence of asymptomatic human carriers of *L. infantum* in southern Europe [10-13] suggests that this parasite is a latent public health threat. This was demonstrated by the increase of co-infections with

human immunodeficiency virus (HIV) and *Leishmania* that has been observed since the 1980s [14], with leishmaniasis becoming the third most frequent opportunistic parasitic disease after toxoplasmosis and cryptosporidiosis [15].

Disease transmission and epidemiology

Visceral leishmaniasis (VL) is usually fatal if untreated, and so it is distinguished from cutaneous leishmaniasis (CL) in all sections of the current review. If untreated, uncomplicated CL is often disfiguring, but not fatal. In contrast, muco-cutaneous and diffuse cutaneous disease can lead to fatal secondary infections even if treated. Patient immunodeficiency is one factor for this, but in Latin America these diseases are associated with regional strains of the *L. braziliensis* and *L. mexicana* species complexes [1,5].

A female sandfly ingests *Leishmania* while blood-feeding, and then transmits the infective stages (usually accepted to be the metacyclic promastigotes) during a subsequent blood meal [16]. The infective promastigotes inoculated by the sandfly are phagocytosed in the mammalian host by macrophages and related cells, in which they transform to amastigotes and often provoke a cutaneous ulcer and lesion at the site of the bite.

There are only two transmission cycles with proven long-term endemism in Europe [2,17]: zoonotic visceral and cutaneous HumL caused by *L. infantum* throughout the Mediterranean region; and, anthroponotic cutaneous HumL caused by *L. tropica* now occurring sporadically in Greece (Table 1, Figure 1).

Worldwide, most transmission cycles are zoonoses, involving reservoir hosts such as rodents, marsupials, edentates, monkeys, domestic dogs and wild canids [2,5,6,18] (Table 1). However, leishmaniasis can be anthroponotic, with sandflies transmitting parasites between human hosts without the involvement of a reservoir host. Anthroponotic transmission is characteristic of species of the *L. tropica* complex and, except for *L. infantum*, of the *L. donovani* complex. One species (*L. donovani sensu stricto)* or two species (*L. donovani* and *L. archibaldi*) cause periodic epidemics of anthroponotic visceral leishmaniasis ('Kala-azar') in India and northeast Africa, respectively [19]. Sandfly vectors of both complexes (*L. donovani* and *L. tropica*) are abundant in southern Europe.

The domestic dog is the only reservoir host of major veterinary importance, and in Europe there is a large market for prophylactic drugs and treatment of canine leishmaniasis (CanL) caused by *L. infantum* [2]. Domestic cats might be secondary reservoir hosts of *L. infantum* in southern Europe [13], because they are experimentally infectious to sandflies [20] and natural infections can be associated with feline retroviruses [21].

Fewer than 50 of the approximately 1,000 species of sandflies are vectors of leishmaniasis worldwide [3]. This is due to the inability of some sandfly species to support the development of infective stages in their gut [16] and/or a lack of ecological contact with reservoir hosts [22]. Our understanding of the fundamentals of leishmaniasis epidemiology has been challenged in the last 20 years. Firstly, HIV/*Leishmania* co-infections were recorded in 35 countries worldwide, and widespread needle transmission of *L. infantum* was inferred in southwest Europe [15], where Cruz *et al.* demonstrated *Leishmania* in discarded syringes [23]. Secondly, leishmaniasis has become more apparent in northern latitudes where sandfly vectors are either absent or present in very low densities, such as in the eastern United States (US) and Canada [24] as well as in Germany [25-27]. Most infections involve CanL, not HumL, and this might be explained by dog importation from, or travel to, endemic regions, followed by vertical transmission from bitch to pup or horizontal transmission by biting hounds [24]. Vertical transmission of HumL from mother to child has rarely been reported [28].

Diagnostic methods

Most diagnoses are only genus-specific, being based on symptoms, the microscopic identification of parasites in Giemsa-stained smears of tissue or fluid, and serology [18,29]. Consequently, the identity of some causative agents has only been known relatively recently, following typing performed during limited eco-epidemiological surveys. For example, it was thought that all cutaneous leishmaniasis cases in Europe were caused by *L. tropica*, until Rioux and Lanotte reported *L. infantum* to be the causative agent in the western Mediterranean region [30].

Rioux and Lanotte used multi-locus enzyme electrophoresis (MLEE) to identify *Leishmania* species and strains [30], which remains the gold standard [1,18]. However, MLEE requires axenic culture [31] in which one strain can overgrow others in mixed infections. It is therefore more practical to identify the isoenzyme strains (or zymodemes) by directly characterising the enzyme genes [32]. Other molecular tests have been used to identify *Leishmania* infections in humans, reservoir hosts and sandfly vectors [33], including in the Mediterranean region [34], but there has been no international standardisation [29]. However, PCR of the internal transcribed spacer of the multi-copy nuclear ribosomal genes is often used [34,35]. A set of carefully chosen criteria must accompany PCR-based diagnosis, especially for immunocompromised patients [14,15]. Monoclonal antibodies have long been available for the identification of neotropical species [36] and the serotyping of Old World species [37] but they are not widely used.

Most sensitive molecular techniques indicate only the presence of a few recently living *Leishmania*, not that the parasites were infectious. Therefore, serology is

often more informative [29]. However, antigens prepared in different laboratories can cause test variation for the frequently used methods [29]: the indirect fluorescent antibody test (IFAT), the enzyme-linked immunosorbent assay (ELISA), the indirect haemagglutination assay (IHA) and the direct agglutination test (DAT). Some antigens are stable and produced commercially, such as the recombinant (r) K39 for a dipstick or strip test. Multi-centre studies of 'Kala-azar' diagnostics [38] showed that both the freeze-dried DAT and the rK39 strip test could exceed the 95% sensitivity and 90% specificity target, but only for the strains found in some regions. Antibody detection tests should complement other diagnostic tests, because they do not usually distinguish between acute disease, asymptomatic infections, relapses and cured cases [38].

Delayed hypersensitivity is an important feature of all forms of human leishmaniasis [4] and is often measured by the leishmanin skin test (or Montenegro reaction) [29]. False-positivity is approximately 1% in otherwise healthy people. Other problems with this test include the absence of commercially available leishmanins, that there is complete cross-reactivity among most species and strains of *Leishmania*, and that for VL its applicability is limited to the detection of past infections, because a complete anergy is found during active disease.

Treatment

Pentavalent antimonials were the first-choice drugs for leishmaniasis worldwide [39,40]. Miltefosine, Paromomycin and liposomal Amphotericin B are gradually replacing antimonials and conventional Amphotericin B in some regions [40,41], especially where there is drug resistance or the need to develop combination therapy to prevent the emergence of resistance to new drugs [41].

Highly active anti-retroviral therapy (HAART) treatment has reduced the incidence of co-infections with *Leishmania* and HIV by preventing an asymptomatic infection with *L. infantum* from becoming symptomatic, but unfortunately it is not good at preventing visceral leishmaniasis relapses. The benefits of treatment are not as clear-cut as they are for other opportunistic diseases [42].

Prevention

Most research on vaccines is strategic, not applied, for example targeting secretory-gel glycans of *Leishmania* [43] and some sandfly salivary peptides [44], both of which are injected into the mammalian host by the female sandfly during blood feeding. Therapeutic vaccine trials continue to use killed cultured parasites (often with BCG as adjuvant) in combination with anti-leishmanial drugs but with only 0-75% efficacy [45]. One second generation recombinant vaccine contains a trifusion recombinant protein (Leish-111f), and some of its epitopes are shared by *L. donovani* and *L. infantum* [46].

Research and development of vaccines against CanL has been stimulated by the economic importance of dogs and their role as reservoirs of HumL caused by *L. infantum* in the Americas and the Mediterranean region. Leishmune is the first licensed vaccine against CanL. It contains the fucose-mannose ligand (FML) antigen of *L. donovani* and has a reported efficacy of 76-80% [47]. The industrialised formulation of FML-saponin underwent safety trials in Brazil [48]. The vaccine LiESAp-MDP (excreted/secreted antigens with adjuvant) was reported to have an efficacy of 92% when tested on naturally exposed dogs in the south of France [49,50]. More recently, a modified vaccinia virus Ankara (MVA) vaccine expressing recombinant *Leishmania* DNA encoding tryparedoxin peroxidase (TRYP) was found to be safe and immunogenic in outbred dogs [51].

One means of controlling transmission is to reduce the biting rate of peri-domestic sandfly vectors of visceral HumL and CanL. This has been effective locally, by using repellents [52], insecticide-impregnated nets and bednets [52], topical applications of insecticides [53] and deltamethrin-impregnated dog collars [54,55]. The latter are favoured by many dog owners in southern Europe.

Current geographical distribution

Outside the European Union

Table 1 (updated from Ready [2]) relates the distributions of each form of HumL to causative species and known reservoir hosts [1,5,6,17,18]. Most VL foci occur in India and neighbouring Bangladesh and Nepal, and in Africa (Sudan and neighbouring Ethiopia and Kenya), where anthroponotic 'Kala-azar' is caused by *L. donovani* and in north-eastern Brazil and parts of Central America, where zoonotic infantile visceral leishmaniasis is caused by *L. infantum*. Most CL foci occur in Latin America, North Africa, and the Middle East, and muco-cutaneous and diffuse cutaneous disease are frequent in South America [56].

Inside the European Union: main biomes

Only two transmission cycles have been endemic in the European Union (EU) for a long time, and both are widespread in the adjoining Middle East and in North Africa: zoonotic cutaneous and visceral leishmaniasis caused by *L. infantum* throughout the Mediterranean region and anthroponotic cutaneous leishmaniasis caused by *L. tropica*, which occurs sporadically in Greece and probably neighbouring countries and poses a high risk of introduction by migrants and travellers into the rest of the EU [2,6,17] (Table 1, Figure 1). The former is endemic and sandfly-borne only in the Mediterranean region of the EU ('Mediterranean forests' biome), where its epidemiological significance is clear from published serological surveys [7,8]. However, the vectors of *L. infantum* [57] (Figure 2, Table 2, updated from Ready [2]) are also abundant in the adjoining parts of the temperate region (Temperate broadleaf forests' biome), in northern Spain [58] and central France [59],

Table 1

European distribution of parasites causing most human leishmaniasis worldwide up to 2009

Human disease	(Diffuse and muco-) cutaneous leishmaniasis	Cutaneous leishmaniasis	Cutaneous leishmaniasis	Visceral leishmaniasis	Visceral leishmaniasis	Cutaneous leishmaniasis	Muco-cutaneous leishmaniasis	Diffuse cutaneous leishmaniasis
Leishmania species	*L. tropica* species complex	*L. major*	*L. infantum (= L. chagasi* in Neotropics*)*	*L. infantum (= L. chagasi* in Neotropics*)*	Other members of *L. donovani* species complex	*L. braziliensis* species complex.; *L. mexicana* species complex	*L. braziliensis*	*L. mexicana* species complex
Reservoir hosts (zoonosis) or anthroponosis	Often anthroponotic	Rodents	Domestic dogs, wild canids	Domestic dogs, wild canids	Anthroponotic	Edentates, primates, rodents, marsupials	Rodents, marsupials	Rodents, marsupials
World ecozone	Palaearctic, Afrotropical, Indo-Malayan	Palaearctic, Afrotropical, Indo-Malayan	Palaearctic, Afrotropical, Neotropical	Palaearctic, Afrotropical, Neotropical	Afrotropical, Indo-Malayan	Neotropical, Nearctic	Neotropical	Neotropical
EU biome	Mediterranean forests	Absent	Mediterranean forests, temperate broadleaf forest	Mediterranean forests, temperate broadleaf forest	Absent	Absent	Absent	Absent
EU: Cyprus	Absent	Absent	Present	Present	Present	Absent	Absent	Absent
EU: France	Absent	Absent	Present	Present	Absent	Absent	Absent	Absent
EU: Germany	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
EU: Greece	Sporadic	Absent	Present	Present	Absent	Absent	Absent	Absent
EU: Hungary	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
EU: Italy	Absent	Absent	Present	Present	Absent	Absent	Absent	Absent
EU: Malta	Absent	Absent	Present	Present	Absent	Absent	Absent	Absent
EU: Portugal	Absent	Absent	Present	Present	Absent	Absent	Absent	Absent
EU: Romania	Formerly sporadic? (untyped parasites)	Absent	Formerly sporadic? (untyped parasites)	Formerly present	Absent	Absent	Absent	Absent
EU: Spain	Absent	Absent	Present	Present	Absent	Absent	Absent	Absent
EU Overseas Territories: French Guyana	Absent	Absent	Absent	Absent	Absent	Present	Present	Present
EU candidate: Former Yugoslav Republic of Macedonia	Formerly sporadic? (untyped parasites)	Absent	Formerly sporadic? (confirmed in Croatia)	Present	Absent	Absent	Absent	Absent
EU candidate: European Turkey (Asiatic Turkey)	Absent? (present)	Absent (absent)	Absent? (present)	Absent? (present)	Absent	Absent	Absent	Absent
Other Europe: Albania	Absent	Absent	Present	Present	Absent	Absent	Absent	Absent
Other Europe: Switzerland	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Adapted from a contribution [2] published in the OIE (World Organisation for Animal Health) Scientific and Technical Review: In Climate change: the impact on the epidemiology and control of animal diseases (S. de la Roque, ed.). Rev Sci Tech Off Int Epiz. 2008;27(2):399-412.

and small numbers occur as far north as Paris [60] and the upper Rhine valley in Germany [26]. The occurrence of 'vectors without disease' poses a significant risk for the emergence of leishmaniasis in temperate regions of Europe [2].

Potential factors triggering changes in distribution

Climate change

Most transmission of *Leishmania* is by the bite of permissive sandflies, and so climate change might affect leishmaniasis distribution directly, by the effect of temperature on parasite development in female sandflies [16], or indirectly by the effect of environmental variation on the range and seasonal abundances of the vector species. Female sandflies seek sheltered resting sites for blood meal digestion, and in southern Europe the temperatures of these micro-habitats are buffered but vary significantly with the external air temperature [2].

Based on molecular markers, European vectors of leishmaniasis have extended their ranges northward since the last ice age (approximately 12,000 years ago) [61,62], and the mapping of statistical measures of climate has permitted transmission cycles to be loosely associated with some Mediterranean bioclimates [63]. However, bioclimate zones and their vegetation indicators vary regionally, and ongoing climate change may alter the patterns of land cover and land use. The geographic information system (GIS)-based spatial modelling of the Emerging Diseases in a changing European Environment (EDEN) project is permitting an analysis of changes in climate and land cover [64] and their effects on sandflies.

The project 'climate Change and Adaptation Strategies for Human health in Europe' (cCASHh) concluded: "There is no compelling evidence, due to lack of historical data, that sand fly and VL distributions in Europe have altered in response to recent climate change" [9]. There is now a published analysis of the northward spread of CanL and its vectors in Italy [65], but an association with climate change was only surmised.

Capacity and competence of vectors in Europe

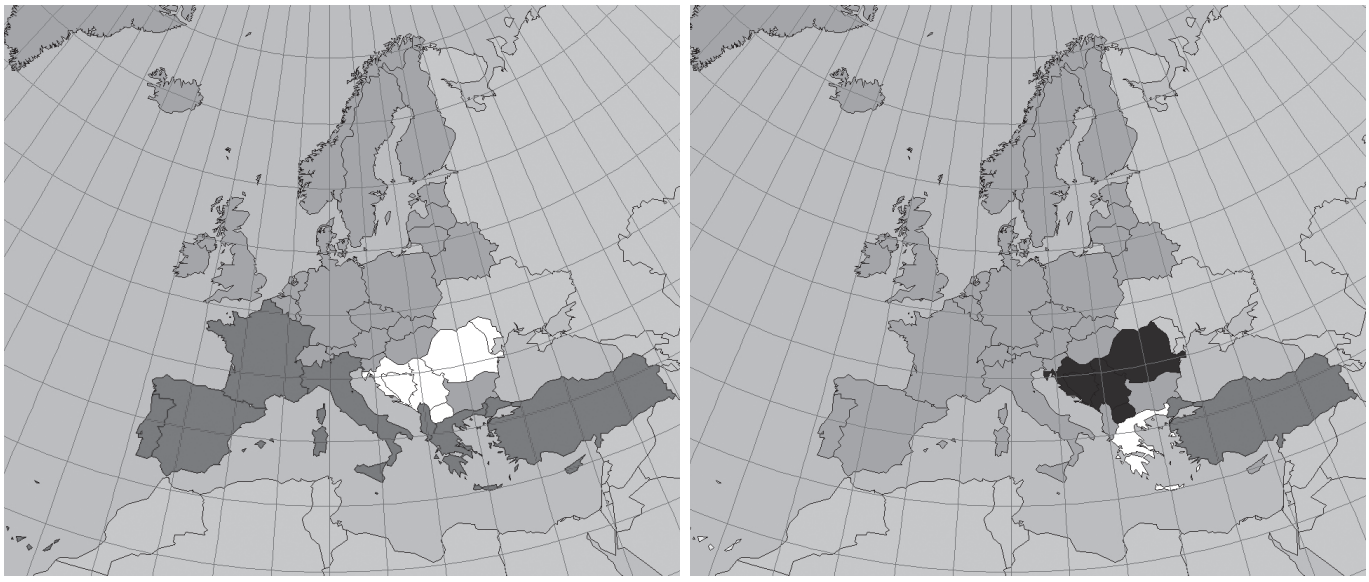
Vectorial capacity has only been calculated indirectly. The average number of gonotrophic cycles (i.e. egg development following a bloodmeal) completed by *P. ariasi* in the south of France was only a little greater than one [66]. Therefore, relatively small changes in temperature could have a large effect on vectorial capacity, because transmission occurs only during the second or subsequent bloodmeals and temperature affects the level of activity of the sandfly and therefore the frequency of the bloodmeals.

Alone, PCR detection of a natural infection of *Leishmania* in a sandfly does not identify a vector. It only indicates that the sandfly has fed on an infected mammalian host [35] because many parasites do not survive in a non-permissive sandfly after bloodmeal defecation [16].

Vectorial competence has been tested [67] or inferred based on finding naturally infected females of the more abundant human-biting species [3,57,68], from which it

Figure 1

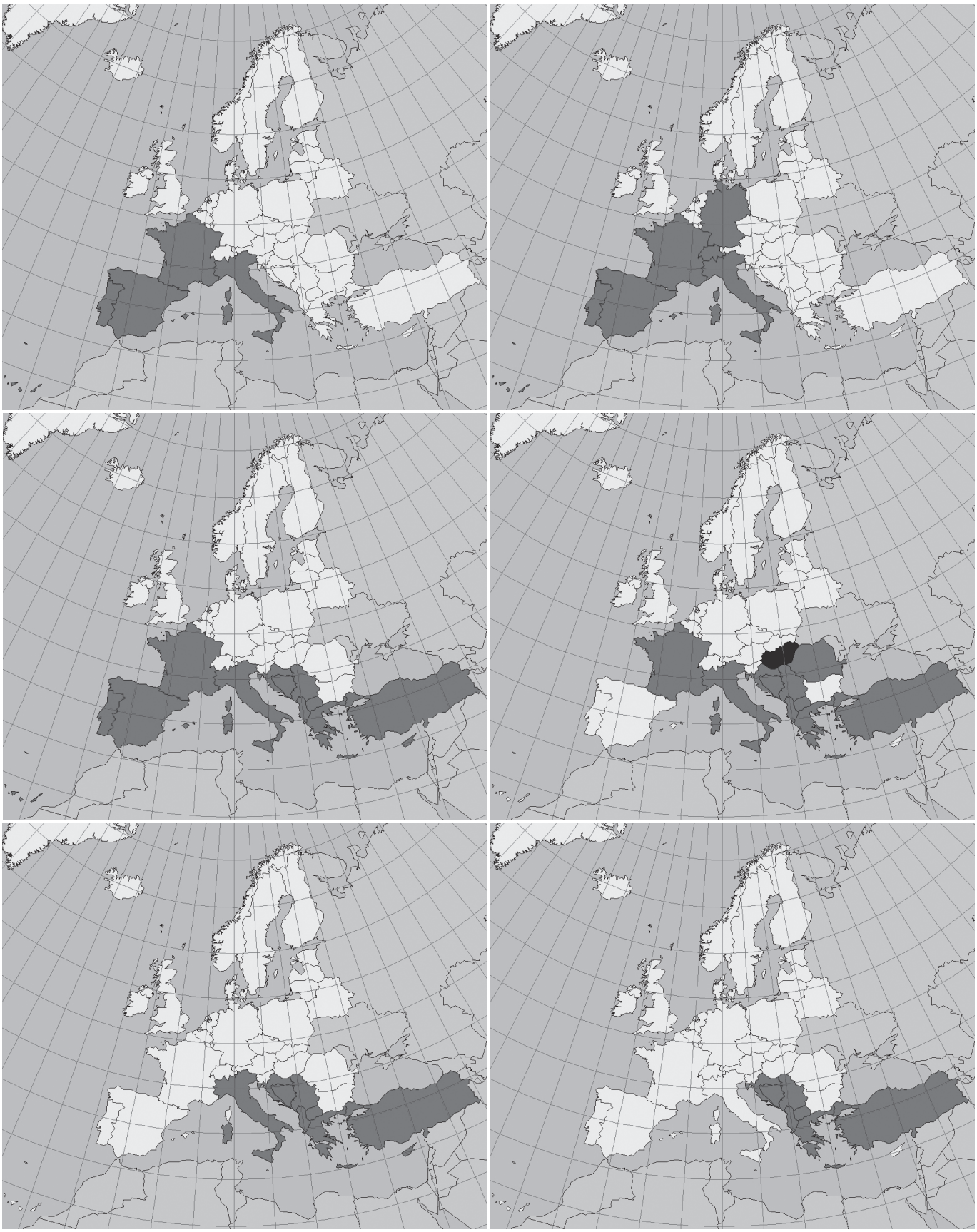
Distribution by country of *Leishmania* species transmitted by phlebotomine sandflies in Europe up to 2009



Left panel: *L. infantum*; right panel: *L. tropica*.
Grey: absent; dark grey: present; white: sporadic or untyped infections; black untyped infections.
Presence in North Africa and Middle East not depicted.
Source: V-borne project; reproduced with permission from the European Centre of Disease Prevention and Control.

Figure 2

Distribution of vectors of leishmaniasis in European countries up to 2009



From left to right and top to bottom: (a) *Phlebotomus ariasi*, (b) *P. perniciosus*, (c) *P. sergenti*, (d) *P. perfiliewi*, (e) *P. neglectus*, (f) *P. tobbi*.
Dark grey: present; light grey: absent; black: old record.
Presence in North Africa and Middle East is not depicted.
Source: V-borne project; reproduced with permission from the European Centre of Disease Prevention and Control.

is concluded that the principal vectors of *L. infantum* in the Mediterranean region are members of the subgenus *Larroussius* (Table 2, Figure 2). The vectorial competence of *Phlebotomus (Transphlebotomus) mascittii* should be tested because this species is now known to be widespread in northern France, Belgium and Germany [69]. However, low rates of biting humans and autogeny (the ability to produce eggs without a blood-meal) cast doubt on its epidemiological importance [2]. Based on distribution and vectorial competence elsewhere, *P. sergenti sensu lato* is likely to be the main vector of *L. tropica* in southern Europe [3].

Importation or dispersal of vectors and reservoir hosts

The importation or inter-continental dispersal of vectors is unlikely because sandflies are not as robust as some mosquitoes and are not known to be wind-dispersed [3]. Any importations are unlikely to be significant for several reasons: The natural vectors of Old World leishmaniasis are already abundant in Mediterranean Europe (Table 2, Figure 2); most American sandflies are believed to be poor vectors of Old World *Leishmania* species [3]; and *Leishmania* species native to the Americas have hosts that do not occur in Europe [56].

The vector of *L. major* in North Africa and the Middle East is *P. papatasi*, which is locally abundant in southern Europe. However, the natural reservoir hosts of this parasite are usually gerbil species not present in EU countries [18] and the risks of them dispersing into southern Europe or surviving accidental/deliberate release by humans have not been assessed.

Importance of travel within Europe (mainland and overseas territories) and internationally

Travel has led to increasing numbers of HumL cases that need to be treated, e.g. in France [70], Germany [25], Italy [71] and the United Kingdom [72]. Leishmaniasis in Guyana (overseas region of France) is a major source of exotic cases imported to mainland France, and *L. infantum* has travelled in the reverse direction in a dog [73]. Isoenzyme [12] and molecular markers [32,34] can sometimes identify the origins of *Leishmania* strains.

Travel poses the risk of the emergence in southern Europe of anthroponotic *L. donovani* [74] and *L. tropica* (see above), and the introduction to northern Europe of *L. infantum* in dogs taken to the Mediterranean region on holiday or rescued from there as strays [7].

Table 2

European distributions of sandfly vectors of human leishmaniasis up to 2009 (unproven role throughout range)

***Leishmania* species**	***L. tropica* species complex - Greece only**	***L. major***	***L. infantum* (= *L. chagasi* in Neotropics) - Mediterranean region only**	***L. infantum* (= *L. chagasi* in Neotropics) - Mediterranean region only**
Human disease	**(Diffuse and muco-) cutaneous leishmaniasis**	**Cutaneous leishmaniasis**	**Cutaneous leishmaniasis**	**Visceral leishmaniasis**
EU biome	Mediterranean forests	Absent	Mediterranean forests, Temperate broadleaf forest	Mediterranean forests, Temperate broadleaf forest
EU: Cyprus	*P. sergenti s.l.*	*P. papatasi*	*P. perfiliewi s.l., P. tobbi*	*P. perfiliewi s.l., P. tobbi*
EU: France	*P. sergenti s.l.*	*P. papatasi*	*P. ariasi, P.perniciosus, P. perfiliewi ?*	*P. ariasi, P. perniciosus*
EU: Germany	No vectors	No vectors	*P. perniciosus*	*P. perniciosus*
EU: Greece	*P. sergenti s.l.*	*P. papatasi*	*P. perfiliewi s.l., P. tobbi*	*P. perfiliewi, P. tobbi, P. neglectus*
EU: Hungary	No vectors?	No vectors?	*P. neglectus, P. perfiliewi ?*	*P. neglectus, P. perfiliewi ?*
EU: Italy	*P. sergenti s.l.*	*P. papatasi*	*P. ariasi P. perfiliewi, P. perniciosus, P. neglectus*	*P. ariasi, P. perfiliewi, P. perniciosus,P. neglectus*
EU: Malta	*P. sergenti s.l.*	*P. papatasi*	*P. perfiliewi, P. perniciosus, P. neglectus*	*P. perfiliewi, P. perniciosus, P. neglectus*
EU: Portugal	*P. sergenti s.l.*	*P. papatasi*	*P. ariasi, P. perniciosus*	*P. ariasi, P. perniciosus*
EU: Romania	No vectors?	*P. papatasi*	*P. perfiliewi, P. neglectus*	*P. perfiliewi*
EU: Spain	*P. sergenti s.l.*	*P. papatasi*	*P. ariasi, P. perniciosus*	*P. ariasi, P. perniciosus*
EU candidate: Former Yugoslav Republic of Macedonia	*P. sergenti s.l.*	*P. papatasi*	*P. perfiliewi, P. tobbi, P. neglectus*	*P. perfiliewi, P. tobbi, P. neglectus*
EU candidate: European Turkey (Asiatic Turkey)	*(P. sergenti s.l.)*	*(P. papatasi)*	*(P. perfiliewi s.l., P. tobbi, P. neglectus)*	*(P. perfiliewi s.l., P. tobbi, P. neglectus)*
Other Europe: Albania	*P. sergenti s.l.*	*P. papatasi*	*P. perfiliewi, P. tobbi, P. neglectus*	*P. perfiliewi, P. tobbi, P. neglectus*
Other Europe: Switzerland	No vectors	No vectors	*P. perniciosus*	*P. perniciosus*

Adapted from a contribution [2] published in the OIE (World Organisation for Animal Health) Scientific and Technical Review: In Climate change: the impact on the epidemiology and control of animal diseases (S. de la Roque, ed.). Rev Sci Tech Off Int Epiz. 2008;27(2):399-412.

Changes in environments (e.g. urbanisation, deforestation) and socio-economic patterns

Deforestation and urbanisation are known to affect leishmaniasis worldwide [6] because of the associations of many vectors and reservoirs with natural or rural areas. Based on the EDEN partners' findings, most Mediterranean regions have at least one vector associated more closely with rural or peri-urban zones [64]. From 1945, most of the socio-economic changes favoured a reduction in 'infantile visceral leishmaniasis' (caused by *L. infantum*) in southern Europe, including better nutrition, widespread insecticide spraying (against malaria-transmitting mosquitoes), better housing and a reduction in the rural population. The last 20 years have seen changes that have increased contact with the Mediterranean vectors, including more holidays and second homes for northern Europeans, unforeseen modes of transmission (among intravenous drug users), and immunosuppression (HIV/*Leishmania* co-infections). The latter is highest in south-western Europe [15].

Risk prediction models

The logic of visceral leishmaniasis control

Based on compartmental mathematical (R_0) models, Dye [75] concluded that insecticides can be expected to reduce the incidence of HumL caused by *L. infantum* even more effectively than they reduce the incidence of CanL, but only where transmission occurs peridomestically and the sandfly vectors are accessible to treatment, as in parts of Latin America. For control of HumL and CanL in Europe, Dye [75] concluded that a dog vaccine is highly desirable, because sandfly vectors here are less accessible to insecticide treatment. In Europe, CanL is a veterinary problem with socio-economic importance and a vaccine is more likely to be afforded than elsewhere.

Risk assessment of introduction, establishment and spread in the European Union (EU) for the short term (2-3 years)

'Oriental sore' caused by *L. tropica* is usually anthroponotic, and it is sporadically endemic in Greece and endemic in neighbouring countries to the EU. The principal vector (*P. sergenti s.l.*) is locally abundant in southern Europe, where new foci could be initiated by people infected in North Africa and the Middle East, including members of the European armed forces based in Iraq and Afghanistan [76,77]. Recently, *L. donovani* has been introduced to Cyprus [74]. Good surveillance, followed by prompt diagnosis and treatment should be extended to all areas of high risk, in order to help prevent the emergence of anthroponotic leishmaniasis.

Cutaneous leishmaniasis caused by the Old World parasite *L. major* has a low risk of emergence as a sandfly-borne disease in southern Europe in the short and long term, even though its principal vector (*P. papatasi*) is locally abundant, because its main gerbil reservoir hosts are absent.

Cutaneous leishmaniases caused by the American parasites of the *L. braziliensis* and *L. mexicana* complexes have low risks of emergence as sandfly-borne diseases in southern Europe in the short and long term because of the absence of their exotic vectors and mammalian reservoir hosts.

However, all these parasites pose a significant risk of introduction to Europe by intravenous drug users (IVDUs) and the establishment of local transmission by syringe needles, especially if these patients have HIV co-infections. This is based on the experience with endemic visceral leishmaniasis caused by *L. infantum* [15,42].

Risk assessment of introduction, establishment and spread in the European Union for the long term (15-20 years)

Leishmania infantum is currently the only significant causative agent of visceral and cutaneous HumL endemic in Europe. Its high prevalence in asymptomatic humans and in the widespread reservoir host (the domestic dog) means there is a high risk of emergence in parts of Europe further north, as demonstrated in northern Italy [65]. In addition to risk factors [78] and statistical models [64] with associated risk maps, EDEN is producing Ro mathematical models as part of research to explain why large regions of temperate Europe have sandflies without HumL in the presence of imported CanL. Some of the key data come from questionnaires to veterinary clinics, validated by prospective serological surveys of CanL, from northern and southern areas with a wide range of disease prevalence.

Increasing dog travel poses a significant risk of introduction of *L. infantum* into northern Europe from the Mediterranean region. There is also a risk of establishment of non-vector transmission and spread as has been observed in North America [24]. Non-vector transmission might explain the autochthonous cases of CanL in Germany [25, 26].

L. tropica has been isolated from both the domestic dog and the black rat [5,8], and so the risk of introduction and spread of CL caused by this parasite in the EU should be re-assessed if either these mammals or related synanthropic species were found to be reservoir hosts (rather than dead-end hosts) in the disease foci in North Africa and southwest Asia [35].

Assessment of whether the existing data sources are adequate and, if not, identification of missing key data needed for conducting risk assessment studies

Research data about leishmaniasis and its spatial distribution in Europe and the Mediterranean region are being enhanced [79] and made accessible online by EDEN and another EU-funded project, LeishRisk, which has collaborated with the WHO to produce an E-compendium, a compilation of peer-reviewed literature on leishmaniasis epidemiology [1,80].

However, public health and veterinary surveillance data are more fragmentary, which undoubtedly caused the public health impact of leishmaniasis to be underestimated for many years in Europe as well as worldwide [1]. The WHO has concluded that more surveillance is necessary in Europe to assess an emergence of leishmaniasis [9], but the partners of the EDEN leishmaniasis sub-project have stressed the need for better coordination of existing surveillance, including linking human health and veterinary data for the zoonotic disease. Currently (EDEN partners, personal communications), HumL is notifiable in Greece, Italy, Portugal and Turkey, and in endemic autonomous regions in Spain. CanL is notifiable in Greece and at municipality level in the endemic regions of the four other countries mentioned above. Neither disease is notifiable in France. At international level, WHO organised a meeting of Eurasian countries in 2009 (J. Alvar, personal communication), aimed at standardising surveillance and reporting, and CanL is reported as a listed disease ('other diseases') of the World Organisation for Animal Health (OIE) [81]. The monitoring of dog travel [7] should continue to be improved and standardised.

Acknowledgements

This review is based on an extensive literature study conducted as part of the European Centre for Disease Prevention and Control funded V-borne project: "Assessment of the magnitude and impact of vector-borne diseases in Europe", tender n°o. OJ/2007/04/13-PROC/2007/003. It was compiled using EDEN funding, EU grant GOCE-2003-010284 EDEN. The paper is catalogued by the EDEN Steering Committee as EDEN0199 (http://www.eden-fp6project.net/). The contents of this publication are the responsibility of the author and do not necessarily reflect the views either of the ECDC or of the European Commission. I thank all partners of the EDEN leishmaniasis sub-project for information and support.

References

1. World Health Organization (WHO). Leishmaniasis: background information. A brief history of the disease. WHO. 2009. Available from: www.who.int/leishmaniasis/en/
2. Ready P.D. Leishmaniasis emergence and climate change. In: S de la Roque, editor. Climate change: the impact on the epidemiology and control of animal diseases. Rev Sci Tech Off Int Epiz. 2008;27(2):399-412.
3. Killick-Kendrick R. Phlebotomine vectors of the leishmaniases: a review. Med Vet Entomol. 1990;4(1):1-24.
4. Peters N, Sacks D. Immune privilege in sites of chronic infection: Leishmania and regulatory T cells. Immunol Rev. 2006;213:159-79.
5. Desjeux P. Leishmaniasis: current situation and new perspectives. Comp Immunol Microbiol Infect Dis. 2004;27(5):305-18.
6. Desjeux P. The increase in risk factors for leishmaniasis worldwide. Trans R Soc Trop Med Hyg. 2001;95(3):239-43.
7. Trotz-Williams LA, Trees AJ. Systematic review of the distribution of the major vector-borne parasitic infections in dogs and cats in Europe. Vet Rec. 2003;152(4):97-105.
8. Gramiccia M, Gradoni L. The leishmaniases in Southern Europe. In: W Takken, BGJ Knols, editors. Emerging pests and Vector-Borne Diseases, Ecology and control of vector-borne diseases, Vol. 1. Wageningen Academic Publishers. 2007:75-95.
9. World Health Organization (WHO). Regional Office for Europe. Vectorborne and rodentborne diseases. WHO. 29 September 2009. Available from: http://www.euro.who.int/globalchange/Assessment/20070216_10
10. Moral L, Rubio EM, Moya M. A leishmanin skin test survey in the human population of l'Alacantí region (Spain): implications for the epidemiology of Leishmania infantum infection in southern Europe. Trans R Soc Trop Med Hyg. 2002;96(2):129-32.
11. Martín-Sánchez J, Pineda JA, Morillas-Márquez F, García-García JA, Acedo C, Macías J. Detection of Leishmania infantum kinetoplast DNA in peripheral blood from asymptomatic individuals at risk for parenterally transmitted infections: relationship between polymerase chain reaction results and other Leishmania infection markers. Am J Trop Med Hyg. 2004;70(5):545-8.
12. Pratlong F, Rioux JA, Marty P, Faraut-Gambarelli F, Dereure J, Lanotte G, et al. Isoenzymatic analysis of 712 strains of Leishmania infantum in the south of France and relationship of enzymatic polymorphism to clinical and epidemiological features. J Clin Microbiol. 2004;42(9):4077-82.
13. Marty P, Izri A, Ozon C, Haas P, Rosenthal E, Del Giudice P, et al. A century of leishmaniasis in Alpes-Maritimes, France. Ann Trop Med Parasitol. 2007;101(7):563-74.
14. Alvar J, Cañavate C, Gutiérrez-Solar B, Jiménez M, Laguna F, López Vélez R, et al. Leishmania and human immunodeficiency virus co-infection: the first 10 years. Clin Microbiol Rev. 1997;10(2):298-319.
15. Desjeux P, Alvar J. Leishmania/HIV co-infections: epidemiology in Europe. Ann Trop Med Parasitol. 2003;97 Suppl 1:3-15.
16. Bates PA. Transmission of Leishmania metacyclic promastigotes by phlebotomine sand flies. Int J Parasitol. 2007;37(10):1097-106.
17. Desjeux P. Information on the epidemiology and control of the leishmaniases by country or territory. WHO/LEISH/91.30. Geneva: World Health Organization. 1991.
18. World Health Organization. Control of the Leishmaniases. Report of a WHO Expert Committee. World Health Organ Tech Rep Ser. 1990;793:1-158.
19. Mauricio IL, Gaunt MW, Stothard JR, Miles MA. Glycoprotein 63 (gp63) genes show gene conversion and reveal the evolution of Old World Leishmania. Int J Parasitol. 2007;37(5):565-76.
20. Maroli M, Pennisi MG, Di Muccio T, Khoury C, Gradoni L, Gramiccia M. Infection of sandflies by a cat naturally infected with Leishmania infantum. Vet Parasitol. 2007;145(3-4):357-60.
21. Solano-Gallego L, Rodríguez-Cortés A, Iniesta L, Quintana J, Pastor J, Espada Y. Cross-sectional serosurvey of feline leishmaniasis in ecoregions around the Northwestern Mediterranean. Am J Trop Med Hyg. 2007;76(4):676-80.
22. Ready P. Sand fly evolution and its relationship to Leishmania transmission. Mem Inst Oswaldo Cruz 2000;95(4):589-90.
23. Cruz I, Morales MA, Noguer I, Rodríquez A, Alvar J. Leishmania in discarded syringes from intravenous drug users. Lancet. 2002;359(9312):1124-5.
24. Duprey ZH, Steurer FJ, Rooney JA, Kirchhoff LV, Jackson JE, Rowton ED, et al. Canine visceral leishmaniasis, United States and Canada, 2000-2003. Emerg Infect Dis. 2006;12(3):440-6.
25. Harms G, Schönian G, Feldmeier H. Leishmaniasis in Germany. Emerg Infect Dis. 2003;9(7):872-5.
26. Naucke TJ, Schmitt C. Is leishmaniasis becoming endemic in Germany? Int J Med Microbiol. 2004;293 Suppl 37:179-81.
27. Mettler M, Grimm F, Naucke TJ, Maasjost C, Deplazes P. [Canine leishmaniosis in Central Europe: retrospective survey and serological study of imported and travelling dogs]. Berl Munch Tierarztl Wochenschr. 2005;118(1-2):37-44. German.
28. Meinecke CK, Schottelius J, Oskam L, Fleischer B. Congenital transmission of visceral leishmaniasis (Kala Azar) from an asymptomatic mother to her child. Pediatrics. 1999;104(5):e65.
29. World Organisation for Animal Health (OIE). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. OIE. 2004. Available from: http://www.oie.int/fr/normes/mmanual/a_00050.htm
30. Rioux JA, Lanotte G. Leishmania infantum as a cause of cutaneous leishmaniasis. Trans R Soc Trop Med Hyg. 1990;84(6):898.
31. Evans D, editor. Handbook on Isolation, Characterization and Cryopreservation of Leishmania. Geneva: World Health Organization; 1989.
32. Lukes J, Mauricio IL, Schönian G, Dujardin JC, Soteriadou K, Dedet JP, et al. Evolutionary and geographical history of the Leishmania donovani complex with a revision of current taxonomy. Proc Natl Acad Sci USA. 2007;104(22):9375-80.
33. Alvar J, Barker JR. Molecular tools for epidemiological studies and diagnosis of leishmaniasis and selected other parasitic diseases. Trans R Soc Trop Med Hyg. 2002;96:1-250.

34. Schönian G, Mauricio I, Gramiccia M, Cañavate C, Boelaert M, Dujardin JC. Leishmaniases in the Mediterranean in the era of molecular epidemiology. Trends Parasitol. 2008;24(3):135-42.
35. Parvizi P, Mazloumi-Gavgani AS, Davies CR, Courtenay O, Ready PD. Two Leishmania species circulating in the Kaleybar focus of 'infantile visceral leishmaniasis', northwest Iran: implications for deltamethrin dog collar intervention. Trans R Soc Trop Med Hyg. 2008;102(9):891-97.
36. Shaw JJ, De Faria DL, Basano SA, Corbett CE, Rodrigues CJ, Ishikawa EA, et al. The aetiological agents of American cutaneous leishmaniasis in the municipality of Monte Negro, Rondônia state, western Amazonia, Brazil. Ann Trop Med Parasitol. 2007;101(8):681-8.
37. Ardehali S, Moattari A, Hatam GR, Hosseini SM, Sharifi I. Characterization of Leishmania isolated in Iran: 1. Serotyping with species specific monoclonal antibodies. Acta Trop. 2000;75(3):301-7.
38. Boelaert M, El-Safi S, Hailu A, Mukhtar M, Rijal S, Sundar S, et al. Diagnostic tests for kala-azar: a multi-centre study of the freeze-dried DAT, rK39 strip test and KAtex in East Africa and the Indian subcontinent. Trans R Soc Trop Med Hyg. 2008;102(1):32-40.
39. Ameen M. Cutaneous leishmaniasis: therapeutic strategies and future directions. Expert Opin Pharmacother. 2007;8(16):2689-99.
40. Gradoni L, Soteriadou K, Louzir H, Dakkak A, Toz SO, Jaffe C, et al. Drug regimens for visceral leishmaniasis in Mediterranean countries. Trop Med Int Health. 2008;13(10):1272-6.
41. Croft SL, Sundar S, Fairlamb AH. Drug resistance in leishmaniasis. Clin Microbiol Rev. 2006;19(1):111-26.
42. López-Vélez R. The impact of highly active antiretroviral therapy (HAART) on visceral leishmaniasis in Spanish patients who are co-infected with HIV. Ann Trop Med Parasitol. 2003;97 Suppl 1:143-7.
43. Rogers ME, Sizova OV, Ferguson MA, Nikolaev AV, Bates PA. Synthetic glycovaccine protects against the bite of Leishmania-infected sand flies. J Infect Dis. 2006;194(4):512-8.
44. Valenzuela JG, Belkaid Y, Garfield MK, Mendez S, Kamhawi S, Rowton ED, et al. Toward a defined anti-Leishmania vaccine targeting vector antigens: characterization of a protective salivary protein. J Exp Med. 2001;194(3):331-42.
45. World Health Organization on behalf of the Special Programme for Research and Training in Tropical Diseases (WHO). Eighteenth Programme Report. Progress 2005-2006. Geneva: WHO. 2007.
46. Reed SG, Campos-Neto A. Vaccines for parasitic and bacterial diseases. Curr Opin Immunol. 2003;15(4):456-60.
47. Nogueira FS, Moreira MA, Borja-Cabrera GP, Santos FN, Menz I, Parra LE, et al. Leishmune vaccine blocks the transmission of canine visceral leishmaniasis: absence of Leishmania parasites in blood, skin and lymph nodes of vaccinated exposed dogs. Vaccine. 2005;23(40):4805-10.
48. Parra LE, Borja-Cabrera GP, Santos FN, Souza LO, Palatnik-de-Sousa CB, Menz I. Safety trial using the Leishmune vaccine against canine visceral leishmaniasis in Brazil. Vaccine. 2007;25(12):2180-6.
49. Lemesre JL, Holzmuller P, Cavaleyra M, Gonçalves RB, Hottin G, Papierok G. Protection against experimental visceral leishmanisis infection in dogs immunized with purified excreted secreted antigens of Leishmania infantum promastigotes. Vaccine. 2005;23(22):2825-40.
50. Lemesre JL, Holzmuller P, Gonçalves RB, Bourdoiseau G, Hugnet C, Cavaleyra M, et al. Long-lasting protection against canine leishmanisis using LiESAp-MDP vaccine in endemic areas of France: double-blind randomised efficacy trial. Vaccine. 2007;25(21):4223-34.
51. Carson C, Antoniou M, Ruiz-Argüello MB, Alcami A, Christodoulou V, Messaritakis I, et al. A prime/boost DNA/Modified vaccinia virus Ankara vaccine expressing recombinant Leishmania DNA encoding TRYP is safe and immunogenic in outbred dogs, the reservoir of zoonotic visceral leishmaniasis. Vaccine. 2009;27(7):1080-6.
52. Centers for Disease Control and Prevention (CDC). Parasitic Disease Information. Leishmania Infection. 2008. Atlanta: CDC. Available from: http://www.cdc.gov/ncidod/dpd/parasites/leishmania/factsht_leishmania.htm#prevent
53. Reithinger R, Teodoro U, Davies CR. Topical insecticide treatments to protect dogs from sand fly vectors of leishmaniasis. Emerg Infect Dis. 2001;7(5):872-6.
54. Killick-Kendrick R, Killick-Kendrick M, Focheux C, Dereure J, Puech MP, Cadiergues MC. Protection of dogs from the bites of phlebotomine sandflies by deltamethrin collars for the control of canine leishmaniasis. Med Vet Entomol. 1997;11(2):105-11.
55. Maroli M, Mizzon V, Siragusa C, D'Oorazi A, Gradoni L. Evidence for an impact on the incidence of canine leishmaniasis by the use of deltamethrin-impregnated dog collars in southern Italy. Med Vet Entomol. 2001;15(4):358-63.
56. Lainson R, Shaw JJ, Silveira FT, de Souza AA, Braga RR, Ishikawa EA. The dermal leishmaniases of Brazil, with special reference to the eco-epidemiology of the disease in Amazonia. Mem Inst Oswaldo Cruz. 1994;89(3):435-43.
57. Gállego M, Pratlong F, Fisa R, Riera C, Rioux JA, Dedet JP, et al. The life-cycle of Leishmania infantum MON-77 in the Priorat (Catalonia, Spain) involves humans, dogs and sandflies; also literature review of distribution and hosts of L. infantum zymodemes in the Old World. Trans R Soc Trop Med Hyg. 2001;95(3);269-71.
58. Aransay AM, Testa JM, Morillas-Marquez F, Lucientes J, Ready PD. Distribution of sandfly species in relation to canine leishmaniasis from the Ebro Valley to Valencia, northeastern Spain. Parasitol Res. 2004;94(6):416-20.
59. Houin R, Deniau M, Puel F, Reynouard F, Barbier D, Bonnet M. [Phlebotomine sandflies of Touraine]. Ann Parasitol Hum Comp. 1975;50(2):233-43. French.
60. Rioux JA, Golvan YJ. [Epidemiology of the leishmaniases in the south of France]. National Institute of Health and Medical Research (INSERM) Monography. Paris. 1969;37:1-223. French.
61. Aransay AM, Ready PD, Morillas-Marquez F. Population differentiation of Phlebotomus perniciosus in Spain following postglacial dispersal. Heredity. 2003;90(4):316-25.
62. Perrotey S, Mahamdallie S, Pesson B, Richardson KJ, Gállego M, Ready PD. Postglacial dispersal of Phlebotomus perniciosus into France. Parasite 2005;12(4):283-91.
63. Rioux JA, de la Roque S. [Climates, leishmaniasis and trypanosomiasis.] In: Rodhain F, editor. [Climate change, infectious and allergic diseases]. Éditions scientifiques et médicales, Elsevier. 2003. 41-62.
64. Martinez S, Vanwambeke SO, Ready P. Linking changes in landscape composition and configuration with sandfly occurrence in southwest France. Fourth International Workshop on the Analysis of Multi-temporal Remote Sensing Images, 2007. MultiTemp 2007.
65. Maroli M, Rossi L, Baldelli R, Capelli G, Ferroglio E, Genchi C, et al. The northward spread of leishmaniasis in Italy: evidence from retrospective and ongoing studies on the canine reservoir and phlebotomine vectors. Trop Med Int Health. 2008;13(2):256-64.
66. Dye C, Guy MW, Elkins DB, Wilkes TJ, Killick-Kendrick R. The life expectancy of phlebotomine sandflies: first field estimates from southern France. Med Vet Entomol. 1987;1(4):417-25.
67. Volf P, Hostomska J, Rohousova I. Molecular crosstalks in Leishmania-sandfly-host relationships. Parasite. 2008;15(3):237-43.
68. Rossi E, Bongiorno G, Ciolli E, Di Muccio T, Scalone A, Gramiccia M, et al. Seasonal phenology, host-blood feeding preferences and natural Leishmania infection of Phlebotomus perniciosus (Diptera, Psychodidae) in a high-endemic focus of canine leishmaniasis in Rome province, Italy. Acta Trop. 2008;105(2):158-65.
69. Depaquit J, Naucke TJ, Schmitt C, Ferte H, Leger N. A molecular analysis of the subgenus Transphlebotomus Artemiev, 1984 (Phlebotomus, Diptera, Psychodidae) inferred from ND4 mtDNA with new northern records of Phlebotomus mascittii Grassi, 1908. Parasitol Res. 2005;95(2):113-6.
70. El Hajj L, Thellier M, Carrière J, Bricaire F, Danis M, Caumes E. Localized cutaneous leishmaniasis imported into Paris: a review of 39 cases. Int J Dermatol. 2004;43(2):120-5.
71. Antinori S, Gianelli E, Calattini S, Longhi E, Gramiccia M, Corbellino M. Cutaneous leishmaniasis: an increasing threat for travellers. Clin Microbiol Infect. 2005;11(5):343-6.
72. Lawn SD, Whetham J, Chiodini PL, Kanagalingam J, Watson J, Behrens RH et al. New world mucosal and cutaneous leishmaniasis: an emerging health problem among British travellers. QJM. 2004;97(12):781-8.
73. Rotureau B, Ravel C, Aznar C, Carme B, Dedet JP. First report of Leishmania infantum in French Guiana: canine visceral leishmaniasis imported from the Old World. J Clin Microbiol. 2006;44(3):1120-2.
74. Antoniou M, Haralambous C, Mazeris A, Pratlong F, Dedet JP, Soteriadou K. Leishmania donovani leishmaniasis in Cyprus. Lancet Infect Dis. 2009;9(2), 76-7.
75. Dye C. The logic of visceral leishmaniasis control. Am J Trop Med Hyg. 1996;55(2):125-30.
76. Magill AJ, Grögl M, Gasser RA Jr, Sun W, Oster CN. Visceral infection caused by Leishmania tropica in veterans of Operation Desert Storm. N Engl J Med. 1993;328(19):1383-7.

77. Crum NF, Aronson NE, Lederman ER, Rusnak JM, Cross JH. History of U.S. military contributions to the study of parasitic diseases. Mil Med. 2005;170(4 Suppl):17-29.

78. Martín-Sánchez J, Morales-Yuste M, Acedo-Sanchez C, Baron S, Diaz V, Morillas-Marquez F. Canine Leishmaniasis in southeastern Spain. Emerg Infect Dis. 2009; 15:795-8.

79. Dujardin JC, Campino L, Cañavate C, Dedet JP, Gradoni L, Soteriadou K, et al. Spread of vector-borne diseases and neglect of Leishmaniasis, Europe. Emerg Infect Dis. 2008;14(7):1013-8.

80. LeishRisk [Internet]. Belgium: European Commission. [cited 2009 Mar 16]. Available from: http://www.leishrisk.net/leishrisk/

81. World Organisation for Animal Health (OIE) [Internet]. OIE Listed diseases. France: OIE. [updated 2007 Mar 14; cited 2009 Mar 16]. Available from: www.oie.int/eng/maladies/en_classification2007.htm?e1d7