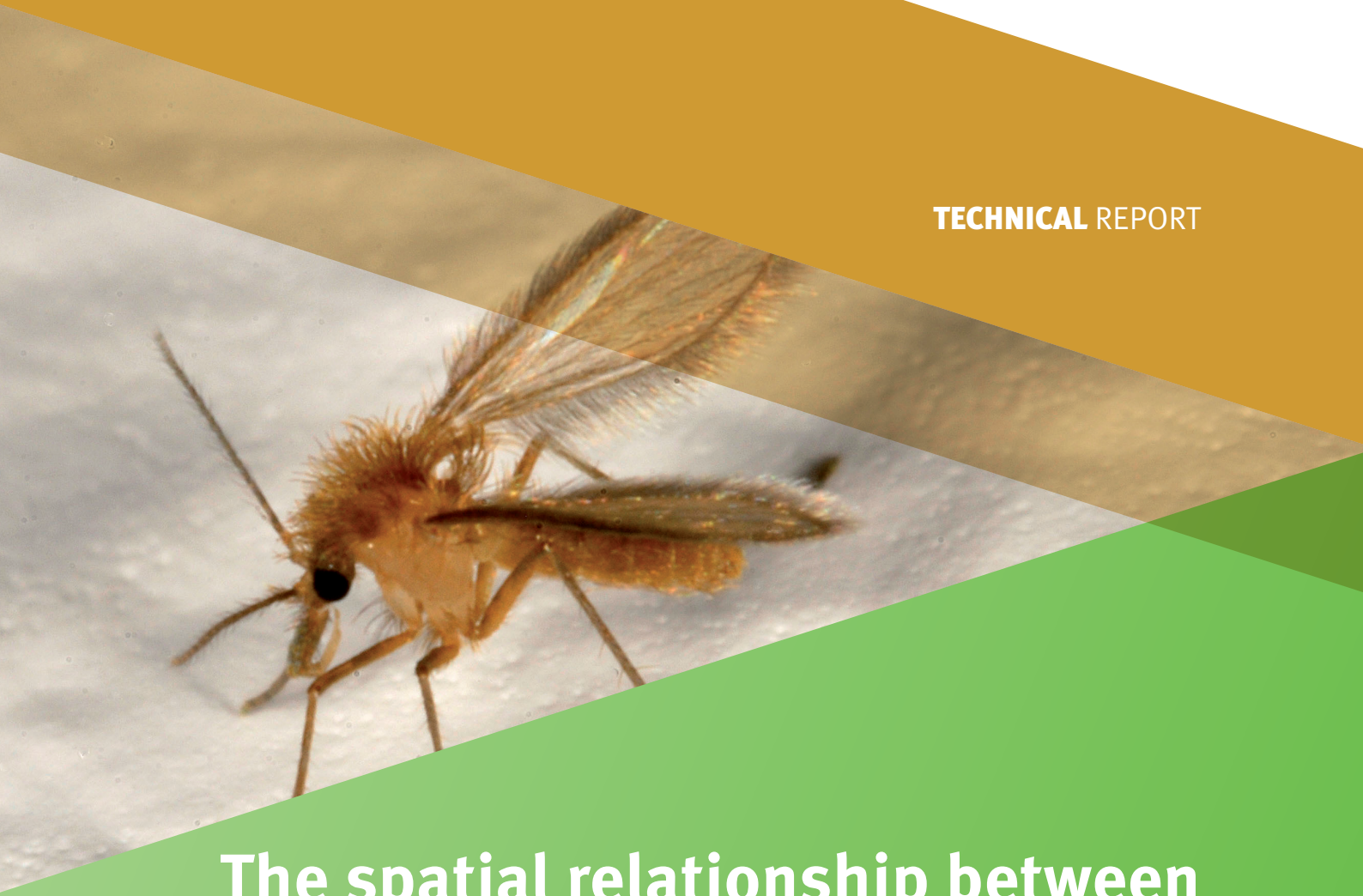
EUROPEAN FOOD SAFETY AUTHORITY

EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

**TECHNICAL** REPORT



# The spatial relationship between the presence and absence of *Leishmania* spp. and leishmaniasis, and phlebotomine sand fly vectors in Europe and neighbouring countries

www.ecdc.europa.eu

**ECDC** TECHNICAL REPORT

# The spatial relationship between the presence and absence of *Leishmania* spp. and leishmaniasis, and phlebotomine sand fly vectors in Europe and neighbouring countries

*Leishmania* and vector distribution in Europe and neighbourhood

This report was commissioned by the European Centre for Disease Prevention and Control (ECDC) Specific contract No 3 ECD.11885-ID.12508 implementing inter-agency framework contract for services No ECDC/2019/020, coordinated by Olivier Briët.

*Contributing authors*
Eduardo Berriatua, (University of Murcia, Spain); Pedro Pérez-Cutillas, (University of Murcia, Spain); Aurora González Vidal, (University of Murcia, Spain); Olivier Briët (ECDC, Stockholm).

*Acknowledgments*

We thank William Wint for providing help with organising the databases and we thank Wim Van Bortel, Celine Gossner and Marieta Braks for critically reviewing this report.

Suggested citation: European Centre for Disease Prevention and Control. The spatial relationship between the presence and absence of *Leishmania* spp. and leishmaniasis, and phlebotomine sand fly vectors in Europe and neighbouring countries. Stockholm: ECDC; 2023.

Stockholm, March 2023

PDF
ISBN 978-92-9498-617-7
doi: 10.2900/038177
Catalogue number TQ-03-23-074-EN-N

# Contents

# Abbreviations

| | |
|---|---|
| CL | Cutaneous leishmaniasis |
| LRTs | Likelihood ratio tests |
| VBORNET | European Network for Arthropod Vector Surveillance for Human Public Health |
| VectorNet | European network for medical and veterinary entomology |
| VL | Visceral leishmaniasis |

# Executive summary


## Objectives

To provide an analysis of the spatial relationship between the presence and absence of autochthonous *Leishmania* spp. (*L. infantum*, *L. tropica*, *L. major* and *L. donovani* sensu stricto) and clinical cases in humans and animals, and the presence and absence of their confirmed and suspected respective *Phlebotomus* spp. vectors, in Europe and neighbouring countries.

## Methods

The geographical location of *Leishmania* spp. infections and clinical cases at ECDC territorial polygon resolution (equivalent to NUTS-3 and GAUL-2 in most countries) was obtained from a review of the scientific literature published between January 2009 and July 2020 [1], and vector distribution data came from the February 2021 ECDC-VectorNet database update. The review excluded leishmaniasis cases explicitly described as imported (from another country or region). However, as the place of infection could not be established in most instances, it is possible that some areas without autochthonous transmission may have been classified as having presence of leishmaniasis. The geographical area analysed was defined by the combined area of leishmaniasis and vector distribution areas, which were estimated by joining the centroids of the outermost consecutive ECDC territorial polygons where they were reported as present, using a geographical information system. The frequencies of polygons where leishmaniasis and vectors were present were calculated and the agreement between distributions was assessed using Cohen's kappa coefficient and logistic regression, not accounting for spatial auto-correlations.

## Results

Most of the parasite and vector spatial relationships tested were statistically significant ($p<0.05$), although Cohen's kappa indicated slight agreement for *L. donovani* s.s., *L. tropica* and *L. major* spatial distributions and the distributions of their respective vectors, and fair agreement for *L. infantum* distribution and its 11 confirmed and suspected *Phlebotomus* spp. In regression analysis, McFaddens adjusted pseudo-R-squared statistic indicated that the spatial distributions of *P. perniciosus, P. tobbi, P. alexandri* and *P. kandelakii* significantly explained 9.4% of the observed variation in the combined L. infantum and visceral leishmaniasis distribution, with *P. perniciosus* and *P. tobbi* accounting for 75% and 22% of this, respectively. For *L. donovani* s.s., *P. major* s.l. and *P. halepensis* explained 25% of the observed variation with *P. major* s.l. accounting for 69%. For L. tropica, *P. sergenti* explained 5.5% of the variation, and for *L. major*, *P. papatasi* explained 9.8%.

## Discussion

The analysis showed that the distributions of a small number of vector species out of all confirmed and suspected vectors could explain some of the variation in the spatial distribution of *Leishmania* species and clinical forms. This result may be considered (weak) epidemiological evidence of differences in vectorial capacity among vector species that should be further investigated.

The overall low degree of agreement between parasite and vector distributions can be attributed to (i) *Leishmania* vectors being reported beyond the geographical limits of autochthonous *Leishmania* spp. infections; (ii) scarce and spatially heterogeneous vector distribution information, (iii) *Leishmania* species and clinical forms being frequently underreported, and (iv) the administrative units used in the study not necessarily reflecting sandfly and *Leishmania* ecology and possibly being unbalanced.

This study highlights a number of areas where *Leishmania* species and/or clinical forms have been diagnosed but no vectors have been reported so far, supporting the need for enhanced vector surveillance. Considering enhanced *Leishmania* spp. surveillance in periendemic areas where vectors are present may facilitate detection of parasite introduction via movement of infected people and animals.

# Background

The leishmaniases are a group of diseases caused by *Leishmania* spp., transmitted by phlebotomine sand flies, that, in Europe and its neighbourhood, are endemic in countries bordering the Mediterranean Sea and the Black Sea. Species of *Leishmania* described in this area are *Leishmania* donovani sensu lato (s.l.) (which is a species complex that includes both *Leishmania infantum* and *L. donovani* sensu stricto (s.s.)), *Leishmania major* and *Leishmania tropica* [2]. The most common clinical presentations of leishmaniasis are visceral leishmaniasis (VL) caused by *L. donovani* s.l., and cutaneous leishmaniasis (CL) caused by all four species. Whilst VL is life-threatening unless adequately treated, CL is characterised by the presence of one or more long-lasting skin nodules and ulcers that mostly respond to treatment, but are an important cause of stigma and working disability [3]. The incidence of CL is considerably higher than that of VL, particularly in Northern Africa, Middle-East and Türkiye, where it is mainly associated with *L. major* and *L. tropica* [4]. *Leishmania major* and *L. tropica* are not endemic in Europe itself. *Leishmania donovani* s.s. is widely distributed in the Indian subcontinent and Eastern Africa and it is rare in the study area, having been described in some areas of Türkiye, Cyprus and the Middle East [5-7]. *L. infantum* causes VL and is endemic in all countries bordering the Mediterranean Sea and the Black Sea with variable prevalence.

*Leishmania* spp. endemicity relies on the presence of specific vectors and reservoir host species. Sand flies breed in terrestrial sites. They are frail insects with a relatively limited flying capacity. For these reasons they are not considered invasive insects, able to colonise new distant areas by means of passive transportation. Proven or suspected vectors of *Leishmania* spp. in mainland Europe and neighbouring countries include *Phlebotomus alexandri, Phlebotomus ariasi, Phlebotomus balcanicus, Phlebotomus halepensis, Phlebotomus kandelakii, Phlebotomus langeroni, Phlebotomus major* s.l., *Phlebotomus mascittii, Phlebotomus papatasi, Phlebotomus perfiliewi, Phlebotomus perniciosus, Phlebotomus similis Phlebotomus sergenti*, and *Phlebotomus tobbi* (Table 1) [8,9]. *Phlebotomus major* s.l. include *P. major* s.s., *Phlebotomus neglectus* and other less-well characterised species [10]. *Leishmania major* and *L. tropica* are transmitted by 'non-permissive' vectors (i.e., these do not support development of multiple *Leishmania* spp.), *P. papatasi* in the first case, and by *P. sergenti* and *P. similis* in the second case. In contrast, proven or suspected vectors of *L. infantum* and presumably *L. donovani* s.s. also, include the remaining 'permissive' 11 vector species [11]. *Leishmania infantum and L. major* have zoonotic transmission cycles, with dogs and wild rodent species as primary reservoirs of infection, respectively. The cycle of *L. donovani* s.s. is anthroponotic with humans as the main reservoir of infection, and L. tropica has both anthroponotic and zoonotic cycles with rodents and hyraxes as animal reservoirs of infection [12].

Knowledge of the spatial distribution of the parasites and vectors is key to understand the epidemiology of leishmaniasis, assess the risk of infection, and develop evidence-based control programs. Since 2008, ECDC, has funded projects such as VBORNET (2010-13) and VectorNet (2014-present), that have collected and mapped the presence and absence of *Leishmania* spp. vectors in Europe and its neighbouring countries based on a comprehensive review of the scientific literature, and in some cases from unpublished surveillance information. In 2020, ECDC commissioned a review of the epidemiology of leishmaniases in this region, which included the mapping of reported presence of *Leishmania* spp. infections and clinical forms; this study was based on a review of peer-reviewed and grey literature published from 2009 through 2020. The records of *Leishmania* spp. infections and clinical forms were used in the study presented in this technical report to analyse the relationship between the spatial distribution of autochthonous *Leishmania* spp. infections and its clinical forms and sand fly vector species presence. This analysis should highlight areas where vector and *Leishmania* spp. surveillance is missing, and may provide some epidemiological evidence of differences in vector competence. Information deriving from targeted parasite and vector investigations can be used to improve and develop new existing statistical and mathematical environmental niche models for predicting vector and *Leishmania* spp. occurrence and density in Europe and neighbouring countries [13-19]. Environmental modelling information may then be taken one step further towards the development of an early warning system for sand fly-borne diseases in Europe and neighbouring countries. Such an endeavour is currently being developed for mosquito-borne infections in the European Union (http://beyond-eocenter.eu/index.php/web-services/eywa).

The analysis in the present study was performed for all possible combinations of suspected and confirmed competent vectors. The specific null hypotheses tested were:

- The presence/absence of *Leishmania* spp. and/or their clinical forms, and the presence/absence of vector species are not statistically associated with each other.
- There is no degree of agreement (concordance) between the presence/absence of *Leishmania* spp. and/or their clinical forms, and the presence/absence of vector species.

# Methods

## Sand fly vector and *Leishmania* spp. data

Vector distribution data were extracted from the VectorNet database and included the distribution status of the 14 confirmed and suspected *Leishmania* spp. vector species in the 1 506 territorial units or 'mapping polygons' (polygons)(https://www.ecdc.europa.eu/en/disease-vectors/surveillance-and-disease-data/phlebotomine-maps) based on NUTS3 or GAUL2 units. Sand fly vector species considered were *P. alexandri, P. ariasi, P. balcanicus, P. kandelakii, P. halepensis, P. langeroni, P. major* s.l.*, P. mascittii, P. papatasi, P. perfiliewi, P. perniciosus, P. sergenti, P. similis* and *P. tobbi*. The categories in the vector distribution status variable were: 'observed presence', 'observed absence', 'presumed absence', 'unknown presence' and 'no data'. For the purpose of this study, 'observed absence' and 'presumed absence' were combined into one 'absent' class and 'unknown presence' was lumped with 'no data'. Thus, vector distribution categories used were 'present', 'absent' and 'no data' only. Furthermore, we assumed species to be absent from any polygon where at least one sand fly trapping study had been carried out and the species not found, regardless of the sampling effort.

Mapping polygon-based presence of autochthonous *Leishmania* species and clinical forms (CL and VL) data were obtained from the above-mentioned ECDC leishmaniasis review, which involved 1 026 scientific articles and 120 additional documents, including 46 PhD and MSc theses reporting *Leishmania* species and clinical cases in humans, animals and vectors, and the databases from the National Epidemiological Surveillance networks of Bulgaria, France and Greece and the Centralized Hospital Discharge records of Italy, Malta, Portugal and Spain. Mapping polygons where no *Leishmania* spp. or leishmaniasis cases had been reported were considered as 'absence'. In some instances, only the clinical form of leishmaniasis was reported and not the species causing it, and vice versa. Here we describe and analyse species and clinical forms separately and when possible, also in combination; for example, *Leishmania* spp. and CL because all species can cause this clinical form, and similarly, L. infantum and VL together since this is the species causing this clinical form in the study area, except in some areas in Türkiye which were excluded from this analysis.

## Delimiting areas for vector and leishmaniasis presence

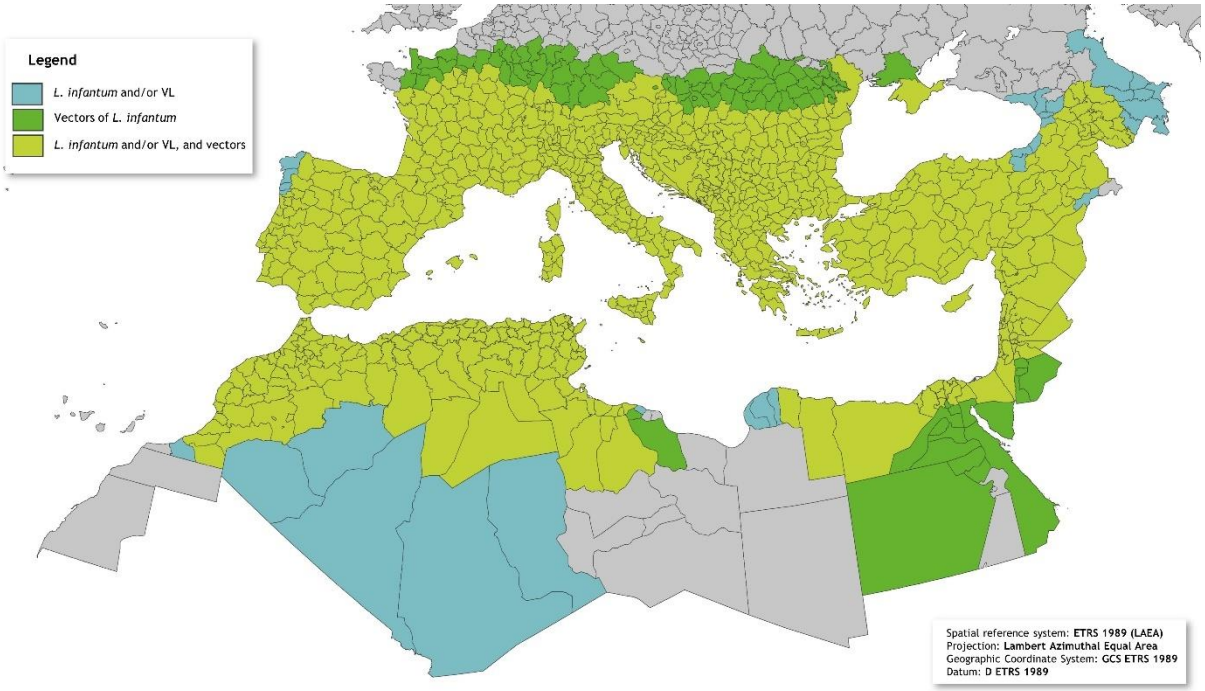
Areas representing the combination of the presumed distribution of *Leishmania* species and/or clinical forms and their vectors were defined to analyse their spatial relationship and agreement.

Eleven presumed distribution areas for *Leishmania* species and/or clinical forms were delimited: one for each of the four *Leishmania* spp., one for all *Leishmania* spp. together, one for VL cases, one for CL cases, one for both VL and CL cases, one for CL cases in North Africa, Middle East and Türkiye since this area has an outstandingly high prevalence of CL, one for L. infantum and VL cases, and one for all *Leishmania* species and clinical forms together.

Similar delimited vector areas included one for all fourteen *Leishmania* spp. vectors, one for the eleven *L. donovani* s.l. vector species, one for the two *L. tropica* vectors, one for the *L. major* vector (Table 1).

Delimited areas for *Leishmania* species, clinical forms and vectors were created by joining the centroids of the outermost consecutive polygons where they were reported (as present), using the geoprocessing 'Aggregate Points' in the generalisation toolset in ArcGIS v 10.5. This tool delimits areas around clusters of proximate point features (three or more points) within an aggregation distance. In our case, the aggregation distance was set at 10° of geographical coordinate units (equivalent to approximately 1 110 km). This length, which represents a third of the total longitudinal distance in the study area, allowed the generation of sufficiently compact groupings of points. Figure 1 shows an example of the delimited areas for *L. infantum* and VL and their vectors, and the study area resulting from combining both (the sum of the presence areas of both).

**Figure 1. *Leishmania infantum* and visceral leishmaniasis and *Leishmania infantum* vector-delimited areas and the resulting study area**




# Statistical analysis and mapping

The frequency of polygons where *Leishmania* species and/or clinical forms and vectors were present were calculated. Bivariate logistic regression (without correction for spatial autocorrelation) and Cohen's kappa coefficient (k) were used to analyse the statistical relationship and the degree of agreement, respectively, between *Leishmania* species and/or clinical forms and vector distribution variables in study areas [20,21]. Tests were done on a total of 6 148 bivariable comparisons of *Leishmania* species (*L. infantum, L. donovani, L. tropica* and *L. major*), clinical forms (VL and CL) and vector variables, where a vector variable was a single species or a composite of two or several species, marking presence if one or more of the species in the composite was present, and absent otherwise. Comparisons included: (i) one between all *Leishmania* spp. and all 14 Phlebotomus spp., (ii) one between all *Leishmania* spp. and clinical forms (VL and CL), and all 14 *Phlebotomus* spp., (iii) one between CL in North Africa, Middle East and Türkiye, and all 14 Phlebotomus spp., (iv) one between L. major and its vector *P. papatasi*, (v) three between *L. tropica* and *P. sergenti* and *P. similis*, (vi) 2 047 between *L. donovani* s.s. and all combinations of its 11 *Phlebotomus* spp. vectors, (vii) 20 47 between *L. infantum* and all combinations of its 11 *Phlebotomus* spp. vectors, and (viii) 2 047 between *L. infantum* and VL, and all possible combinations of their 11 *Phlebotomus* spp. vectors.

Kappa coefficient value ranges and interpretations were as follows: kappa<0: less than random chance agreement, kappa=0.01-0.20: slight agreement, kappa=0.21-0.40: fair agreement, kappa=0.41-0.60: moderate agreement, kappa=0.61-0.80: substantial agreement and kappa=0.81-0.99: almost perfect agreement. Bivariate logistic regression between *leishmania* outcome variables and all sand fly vector combinations was carried to assess significance and obtain McFadden pesudo-R2 values. Subsequently, multivariate logistic modelling was performed to explain the spatial distributions of *L. infantum* plus VL (with up to 11 vectors), *L. infantum* alone (with up to 11 vectors), *L. donovani* s.s. (with up to 11 vectors), and *L. tropica* (with up to two vectors), where, instead of a composite, vector distributions were assessed as independent explanatory variables. Models were selected with the highest McFadden pseudo R2 values for, one, two, three or more vector species, and increasingly large, nested models were compared using likelihood ratio tests (LRTs). Models where the coefficients for at least one of the vectors was negative were excluded from selection.

All calculations and analysis were performed in the R statistical computer package. Significance was considered for $p<0.05$ for a two-sided test. Maps to represent the distribution of vector and *Leishmania* species and clinical forms were generated using ArcGIS v 10.5.

# Results

## Frequency distributions and maps of *Leishmania* species, clinical forms and vectors

Maps of the presence and absence of vector and *Leishmania* species and clinical forms are provided as supplementary material (Figs. S1-S25[i]). Table 1 presents the frequencies of polygons where *Leishmania* species, clinical forms and vectors were reported from as absolute numbers and relative to the number of polygons in delimited (Section 3.2) and VectorNet areas. Overall, *Leishmania* species and clinical forms were reported from 570 polygons, representing 69% and 38% of those in the delimited and VectorNet areas, respectively. Similarly, vectors of *Leishmania* spp. were reported from 522 polygon corresponding to 58% and 35% polygons the delimited and VectorNet area, respectively (Table 1).

The frequency of polygon occupation varied significantly between *Leishmania* species, clinical forms and vectors. Among *Leishmania* spp., *L. infantum* and *L. donovani* s.s. were the most and least widely distributed species, reported from 456 polygon and 10 polygons, respectively, and both species coincided in nine out of the 10 polygons with *L. donovani* s.s. present. *Leishmania major* was reported from 77 polygons and *L. tropica* form 92 polygons (Table 1). Visceral and cutaneous clinical forms were reported from 351 polygon and 308 polygons, respectively. Among vectors, *P. papatasi* was reported from the greatest number (307) of polygons, followed by *P. sergenti* in 201 polygons. *Phlebotomus perniciosus* and *P. langeroni* were the *L. infantum* vectors reported from the greatest and lowest number of polygons, 198 polygons and 2 polygons, respectively, and all together *L. infantum* vectors were reported from 457 polygons (Table 1).

**Table 1. Presence of *Leishmania* species and clinical forms, and phlebotomine sand fly vector species in delimited and VectorNet areas in Europe and neighbouring countries**

| Variable | Number of polygons in delimited area | Number of polygons present | % present in its delimited area | % present in VectorNet area (1506 polygons) |
|---|---|---|---|---|
| ***Leishmania* species** | | | | |
| Any species | 830 | 506 | 61 | 34 |
| *L. infantum* | 789 | 456 | 58 | 30 |
| *L. donovani* s.s. | 30 | 10 | 33 | 1 |
| *L. major* | 211 | 77 | 36 | 5 |
| *L. tropica* | 243 | 92 | 38 | 6 |
| **Leishmaniasis clinical forms** | | | | |
| Any form (cutaneous or visceral) | 737 | 436 | 59 | 29 |
| Visceral | 641 | 351 | 55 | 23 |
| Cutaneous (all areas) | 688 | 308 | 45 | 20 |
| Cutaneous in the south (Türkiye, M. East and N. Africa) | 250 | 162 | 65 | 11 |
| ***Leishmania* spp. and clinical forms** | | | | |
| Any species and clinical form anywhere | 830 | 570 | 69 | 38 |
| *L. infantum* and visceral leishmaniasis | 810 | 504 | 62 | 33 |
| **Vector species** | | | | |
| Any vector species | 897 | 522 | 58 | 35 |
| *L. donovani* s.l. vectors: any of 11 species | 873 | 457 | 52 | 30 |
| *P. alexandri* | 873 | 83 | 10 | 6 |
| *P. ariasi* | 873 | 80 | 9 | 5 |
| *P. balkanicus* | 873 | 39 | 4 | 3 |
| *P. halepensis* | 873 | 27 | 3 | 2 |
| *P. kandelakii* | 873 | 13 | 1 | 1 |
| *P. langeroni* | 873 | 2 | 0 | 0 |
| *P. major* s.l. | 873 | 163 | 19 | 11 |
| *P. major* s.s. (*part of P. major* s.l.) | 873 | 27 | 3 | 2 |
| *P. mascittii* | 873 | 106 | 12 | 7 |

[i] Supplementary tables and figures can be found here: https://www.ecdc.europa.eu/en/publications-data/spatial-relationship-between-presence-and-absence-leishmania-spp

| Variable | Number of polygons in delimited area | Number of polygons present | % present in its delimited area | % present in VectorNet area (1506 polygons) |
|---|---|---|---|---|
| *P. neglectus* (part of *P. major* s.l.) | 873 | 148 | 17 | 10 |
| *P. perfiliewi* | 873 | 188 | 22 | 12 |
| *P. perniciosus* | 873 | 198 | 23 | 13 |
| *P. tobbi* | 873 | 118 | 14 | 8 |
| *L. major* vector: 1 species: *P. papatasi* | 723 | 307 | 42 | 20 |
| *L. tropica* vectors: any of 2 species | 566 | 221 | 39 | 15 |
| *P. sergenti* | 566 | 201 | 36 | 13 |
| *P. similis* | 566 | 57 | 10 | 4 |

# Relationship between the presence of *Leishmania* species and clinical forms and vectors

Figures S26 to S37 show the spatial distributions and Table 2 the degree of agreement using Cohen's kappa statistic, of *Leishmania* species and/or clinical forms and their vectors. The presence of *Leishmania* spp. and/or clinical forms was significantly associated to their respective (composite) vector distributions ($p<0.05$), except for *L. donovani* s.s. ($p=0.08$). Furthermore, there was significant agreement between *Leishmania* spp. and/or clinical forms and vectors, although Cohen's kappa coefficients indicated only slight agreement for *L. donovani* s.s. (kappa=0.01), *L. tropica* (kappa=0.15) and *L. major* (kappa=0.15), and fair agreement for *L. infantum* (kappa=0.22), all *Leishmania* spp. combined (kappa=0.27), VL (kappa=0.24), CL (kappa=0.23) and combinations of all *Leishmania* spp. and clinical forms (kappa=0.28) (Table 2).

Table S1[ii] list localities where *Leishmania* species and/or clinical forms were reported but there is no evidence of vector presence either because no vector surveillance has been performed or because the area was surveyed and no vectors were found. Table S2 lists localities where there is no evidence of autochthonous leishmaniasis and vectors are present.

**Table 2. Number of polygons with presence and absence of *Leishmania* spp. and/or clinical forms and presence and absence of confirmed and suspected sand fly vector species, and degree of agreement**

| *Leishmania* species and/or form | Vectors | *L-V- | L-V+ | L+V- | L+V+ | kappa (95% CI) |
|---|---|---|---|---|---|---|
| *L. infantum* | Vectors of *L. infantum* | 186 | 164 | 132 | 293 | 0.22 (0.15-0.29) |
| *L. donovani* s.s. | Vectors of *L. donovani* s.s. | 317 | 448 | 1 | 9 | 0.01 (0.00-0.03) |
| *L. tropica* | Vectors of *L. tropica* | 232 | 169 | 23 | 52 | 0.15 (0.08-0.22) |
| *L. major* | *P. papatasi* | 309 | 253 | 8 | 54 | 0.15 (0.11-0.20) |
| Any *Leishmania* spp. | Any vector | 163 | 167 | 104 | 355 | 0.27 (0.21-0.34) |
| Visceral leishmaniasis | Vectors of *L. infantum* | 243 | 230 | 75 | 227 | 0.24 (0.18-0.3) |
| Cutaneous leishmaniasis (all areas) | Any vector | 225 | 295 | 42 | 227 | 0.23 (0.17-0.28) |
| Any clinical form | Any vector | 200 | 224 | 67 | 298 | 0.28 (0.22-0.34) |
| Cutaneous leishmaniasis in Türkiye, M. East and N. Africa | Any vector | 18 | 39 | 15 | 112 | 0.22 (0.08-0.37) |
| *L. infantum* and visceral leishmaniasis | Vectors of *L. infantum* | 182 | 146 | 136 | 311 | 0.25 (0.18-0.32) |
| Any species and clinical form anywhere | Any vector | 151 | 144 | 116 | 378 | 0.28 (0.21-0.35) |

*Note. *L-:* Leishmania *absent; L+:* Leishmania *present; V-: vectors absent; V+: vectors present; CI: confidence interval.*

[ii] Supplementary tables and figures can be found here: https://www.ecdc.europa.eu/en/publications-data/spatial-relationship-between-presence-and-absence-leishmania-spp

# Relative contribution of *Phlebotomus spp.* vectors to the distribution of *Leishmania spp.* and visceral leishmaniasis

Supplementary Tables S3, S4, S5, S6 and S7[iii] present the results of the logistic regression analysis and Cohen´s kappa coefficients, for all possible bivariable comparisons between vector combinations and *L. infantum* and VL, *L. infantum, L. tropica, L. major* and *L. donovani*, respectively. Table 3 below provides the results of multivariate logistic regression analysis for *L. infantum* and VL, *L. infantum,* and *L. donovani* (all with up to 11 vector species and their combinations), as well as for *L. tropica* (up to two vector species and their combinations) and *L. major* (*P. papatasi* only).

**Table 3. Selected (multivariate) logistic regression models of the relationship between *Leishmania* spp. and/or clinical forms and their sand fly vector species**

| Model outcome variable and selected explanatory sandfly species | Adjusted McFadden pseudo $R^2$ | LRT p-value | kappa |
|---|---|---|---|
| ***L. infantum* and/or visceral leishmaniasis** | | | |
| *P. perniciosus* | 0.070 | NA | 0.244 |
| *P. perniciosus* + *P. tobbi* | 0.091 | 0.000 | 0.325 |
| *P. perniciosus* + *P. tobbi* + *P. alexandri* + *P. kandelakii* | 0.094 | 0.036 | 0.342 |
| ***L. infantum*** | | | |
| *P. perniciosus* | 0.045 | NA | 0.293 |
| *P. perniciosus* + *P. tobbi* | 0.065 | 0.000 | 0.299 |
| ***L. donovani*** | | | |
| *P. major* s.l. | 0.172 | NA | 0.082 |
| *P. major* s.l. + *P. halepensis* | 0.251 | 0.001 | 0.078 |
| ***L. tropica*** | | | |
| *P. sergenti* | 0.055 | NA | 0.191 |
| ***L. major*** | | | |
| *P. papatasi* | 0.098 | NA | 0.150 |

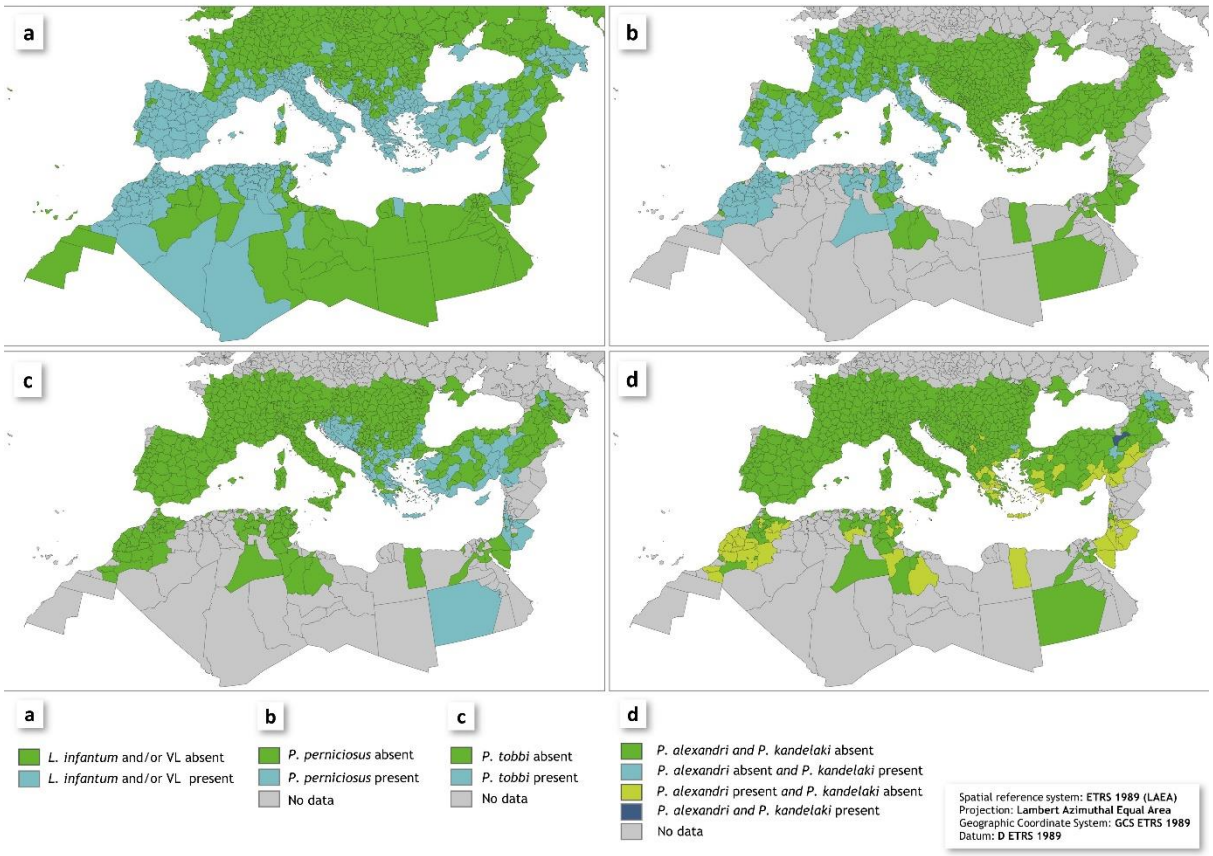
***Note.*** *Models were selected based on highest adjusted McFadden pseudo-R-squared values, and progressively increased the number of sand fly species as explanatory variables until the likelihood ratio test between successive models was no longer significant at alpha=0.05. Models with one or more negative coefficients were excluded. Kappa values are for models combining the total area of selected vectors. LRT: likelihood ration test.*

## *Leishmania infantum* and VL

The adjusted McFadden $R^2$ value was 0.070 for the model which only included *P. perniciosus* and increased to 0.091 when adding *P. tobbi* and to 0.094 when adding both *P. alexandri,* and *P. kandelakii* (Table 3). Adding only *P. alexandri* to a model with *P. perniciosus* + *P. tobbi* yielded a (non-significant) LRT p-value of 0.060. The kappa value of the composite of these four vectors (0.34) was much larger than the kappa value of the composite of all 11 vectors (0.25, see Table 2). The spatial distributions of *Leishmania infantum* and/or visceral leishmaniasis and the identified vectors in the selected models are shown in Figure 2.

[iii] Supplementary tables and figures can be found here: https://www.ecdc.europa.eu/en/publications-data/spatial-relationship-between-presence-and-absence-leishmania-spp

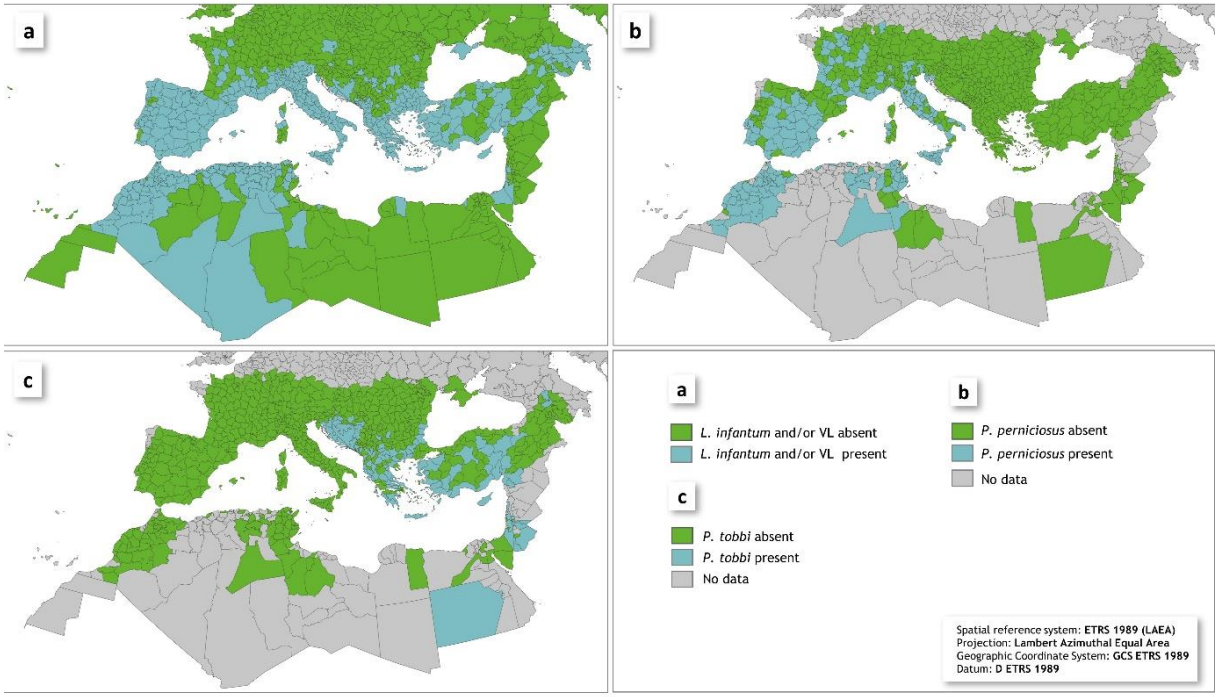
**Figure 2. Spatial distribution of *Leishmania infantum* and/or visceral leishmaniasis (panel a) and *Phlebotomus perniciosus* (panel b), *P. tobbi* (panel c), *P. alexandri* and *P. kandelakii* (panel d) in Europe and neighbouring countries**




## *Leishmania infantum*

In the analysis for *L. infantum* (alone, without those areas where VL was reported in the absence of reports of *L. infantum*), only *P. perniciosus* and *P. tobbi* were selected (Table 3), with a McFadden $R^2$ of 0.065 and a kappa of 0.299 (versus an $R^2$ of 0.091 and kappa of 0.325 in the analysis for *L. infantum* and VL combined). The spatial distributions of *Leishmania infantum* and *P. perniciosus* and *P. tobbi* are shown in Figure 3, with *P. perniciosus* explaining the distribution in the west of the study areas, and *P. tobbi* the distribution in the east.

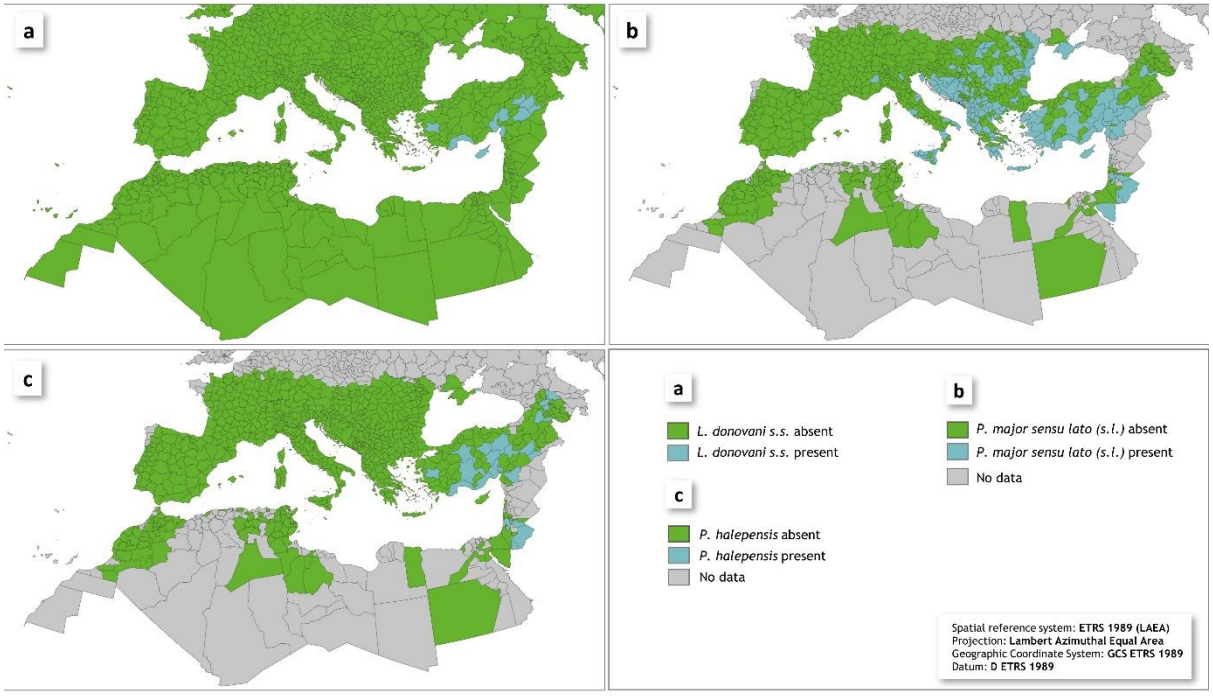
**Figure 3. Spatial distribution of *Leishmania infantum* and/or visceral leishmaniasis (panel a) and *Phlebotomus perniciosus* (panel b), and *P. tobbi* (panel c) in Europe and neighbouring countries**




## *Leishmania donovani*

For models with a single vector, the adjusted McFadden $R^2$ was highest with *P. major* s.l. ($R^2$ = 0.172) and adding *P. halepensis* increased the $R^2$ =to 0.251) (Table 3). The kappa value of the composite of these two vectors was only 0.08, but this was higher than the kappa value of the composite of all 11 possible vectors (0.01, Table 2). The spatial distributions of *Leishmania donovani* and *P. major* s.l. and *P. halepensis* are shown in Figure 4, with both vectors being predominantly present in the east of the study area.

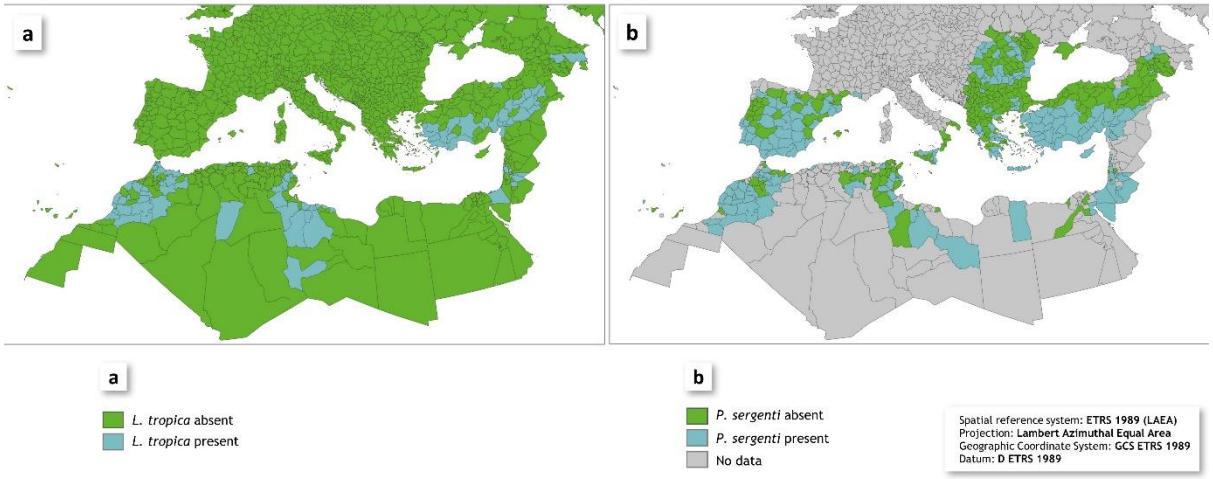
**Figure 4. Spatial distribution of *Leishmania donovani* s.s. (panel a) and *Phlebotomus major* s.l. (panel b) and *P. halepensis* (panel c), in Europe and neighbouring countries**



## *Leishmania tropica*

Among the three possible *L. tropica* models, the highest kappa (0.191) and adjusted McFadden's $R^2$ (0.055) were for the model including only *P. sergenti*. Moreover, inclusion of *P. similis* together with *P. sergenti* led to a significant negative regression coefficient for the former species and lower adjusted McFadden $R^2$ (0.050), indicating no further contribution to the variance explained by *P. sergenti*. The spatial distributions of *Leishmania tropica* and *P. sergenti* are shown in Figure 5.

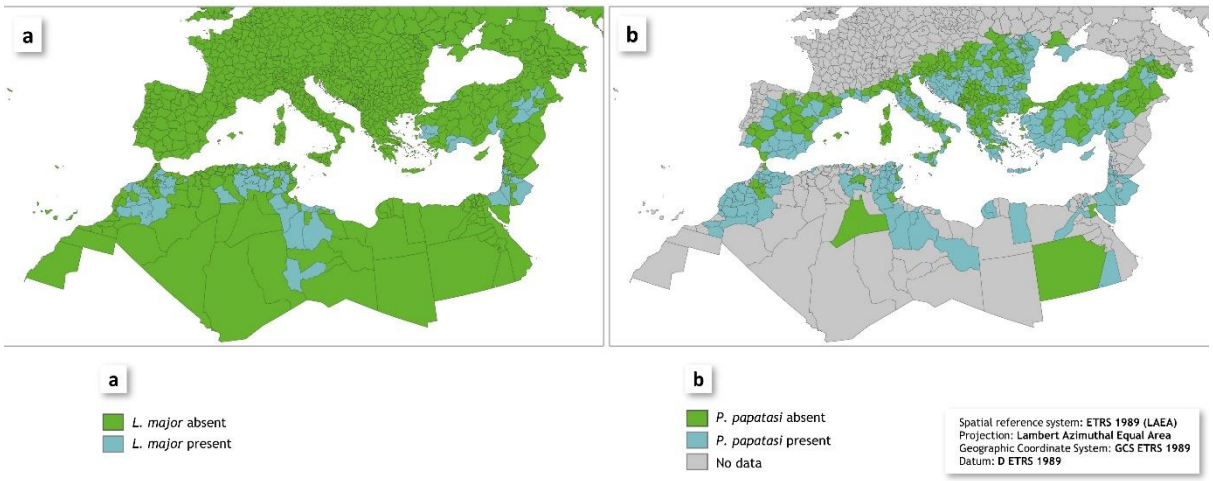
**Figure 5. Spatial distribution of *Leishmania tropica* (panel a) and *Phlebotomus sergenti* (panel b), in Europe and neighbouring countries**




## *Leishmania major*

The kappa and adjusted McFadden $R^2$ values for the model of *L. major* with only *P. papatasi* were 0.191 and 0.098, respectively. The spatial distributions of *Leishmania major* and *P. papatasi* are shown in Figure 6.

**Figure 6. Spatial distribution of *Leishmania major* (panel a) and *Phlebotomus papatasi* (panel b), in Europe and neighbouring countries**



# Discussion

## Frequency distributions and maps of *Leishmania* species, clinical forms and vectors

In the VectorNet area (largely covering the Western Palaearctic region), *Leishmania* species (in autochthonous cases), clinical cases and vectors are confined almost exclusively to countries bordering the Mediterranean Sea and Black Sea, and the large distributional differences between *Leishmania* species are associated with distinct transmission cycles, reservoir host distributions and *Phlebotomus* spp. vector competences. The wide spread of *L. infantum* and VL is for a large part explained by dogs being a ubiquitous primary reservoir of this species throughout the study area and arguably, by the large number of vector species able to transmit the parasite. In contrast, in the VectorNet area, *L. major* and *L. tropica* transmission is restricted to North Africa, the Middle East, Türkiye and Azerbaijan, although their vectors (*P. papatasi* of *L. major* and *P. sergenti* and *P. similis* of *L. tropica*) are found throughout the study area. Zoonotic cycles of these *Leishmania* species rely on rodent species and hyraxes that are not present in Europe. Anthroponotic transmission cycles of *L. tropica* in North Africa, the Middle East and Türkiye and *L. donovani* s.s. in Türkiye are typically associated with poor urban and rural environments where the human-vector contact is high. There is concern about the potential of spread of *L. tropica* and *L. donovani* s.s. in southern European countries where competent vectors are widespread [22].

## Relationship between the presence of *Leishmania* species and clinical forms and vectors

The reasons for the low degree of agreement between the distributions of *Leishmania* species and clinical forms and the corresponding vector distributions may include:

- absence of *Leishmania* spp. infection in vector populations, particularly in periendemic areas such as most of France, southern Germany and Austria for *L. infantum* and throughout Europe for *L. donovani* s.s., *L. tropica* and *L. major*;
- scarce and scattered vector distribution studies and surveillance;
- substantial underreporting of clinical cases of leishmaniasis, particularly CL and canine leishmaniasis [9];
- lack of diagnosis of *L. infantum* infections because many infected dogs and most infected people are asymptomatic and infection in healthy humans may be self-limiting and never be detected [23];
- the possibility of leishmaniasis being diagnosed and reported by reference hospitals from non-endemic areas where patients and samples were referred to, but not marked as imported;
- the use of administrative units in the analysis, which do not necessarily reflect the ecology of the vectors, or hosts;
- imbalances among administrative unit sizes, which could bias the analysis, which is not corrected for spatial autocorrelation.

Further vector surveillance in areas where *Leishmania* species and/or clinical forms were reported but either no sand fly information is available or sand flies were not found in previous studies (Table S1) may allow for a more meaningful analysis in the future. In Europe, many of these areas are situated in the periphery of the wider *L. infantum* endemic area, such as Galicia, Asturias, Cantabria and País Vasco in northern Spain. Considering enhanced leishmaniasis surveillance in areas bordering endemic areas may facilitate detection of parasite introduction via movement of infected people and animals. The risk of *L. infantum* spreading into these areas following the introduction of infected dogs is considered high [24]. This risk can be mitigated by testing dogs before importation and the use of insecticides on dogs during travelling and after return or importation into a non-endemic area [24].

## Relative contribution of *Phlebotomus spp.* vectors to the distribution of *Leishmania spp.* and visceral leishmaniasis

Better spatial agreement is observed with *L. infantum* and VL and the combination of a limited number of vector species rather than when all eleven vector species are considered together. This was expected as some vectors are present in areas where there are no autochthonous leishmaniasis cases. An example of this are large parts of central Europe where *P. mascittii* is present (albeit with a low density) but leishmaniasis is not endemic. Given that a small number of vector species appear to predict *L. infantum* distribution better than all confirmed and suspected eleven species together, we could deduce that greater vector diversity is not necessarily associated with more efficient parasite transmission. It may also support the idea that vectorial competence varies between species and this deserves further investigation.

Among the eleven confirmed and suspected *L. infantum* vectors considered in the study, only eight fulfil conventional criteria for vector incrimination, including *P. ariasi*, *P. balcanicus*, *P. kandelakii*, *P. langeroni*, *P. neglectus* (*P. major s.l.*), *P. perfiliewi*, *P. perniciosus* and *P. tobbi* [8]. The selected species explaining the spatial distribution of *L. infantum* and VL included suspected vector *P. alexandri* and confirmed vectors *P. perniciosus*, *P. tobbi* and *P. kandelakii*. Most of the explained variation was accounted for by *P. perniciosus* and *P. tobbi*. *Phlebotomus perniciosus* is a widely dispersed species and the predominant vector in Western Europe and the western countries of North Africa [25], where *L. infantum* and VL cases were reported from many territorial units, and *P. tobbi* is amply distributed in areas in the Balkans, Greece, Middle East and Türkiye where also many *L. infantum* and VL are reported. The strong association between *P. tobbi* and *L. infantum* is particularly important because it shares its geographical distribution with other suspected and confirmed vector species particularly, *P. major s.l.* and *P. perfiliewi* [26,27], which may suggest a greater vectorial capacity compared to them. These latter two species are also reported from *L. infantum* endemic areas where there are other selected vectors including *P. perniciosus* [28,29] and *P. kandelakii* [30], as well as in many non-endemic areas in the Balkans thus limiting its predictive value for *L. infantum* distribution. *P. kandelakii* is restricted to the Southern Caucasus and some areas in Türkiye and in spite of its narrow distribution, 54% of its records were in areas where the main two vectors, *P. perniciosus* and *P. tobbi* were not reported. *Phlebotomus ariasi* reports are also relatively scarce but it is an important *L. infantum* vector in in colder climates in Western Europe where *P. perniciosus* is not present such as the Pyrenees and the Cevennes Mountain range in France [31,32]. However, in contrast to *P. kandelakii*, only 19% of *P. ariasi*´s records are from areas where neither *P. perniciosus* or *P. tobbi* were reported. *Phlebotomus alexandri* is the species with the widest longitudinal dispersion in the study area, common in Northern Africa, Middle East, Türkiye and Greece, and reported in Serbia and Kosovo[iv], but it is not an abundant species. The vectorial capacity of this species was demonstrated for *L. donovani* s.s. experimentally [33] and it is considered a probable vector of *L. infantum* in Iran [34] and Iraq [35].

*Phlebotomus balcanicus* is a comparatively infrequent species and although it is an important vector in the Southern Caucasus together with *P. kandelakii* [30,36], most reports are from Romania where very few cases of leishmaniasis are reported, explaining its negative regression coefficient. *Phlebotomus langeroni* is considered a proven vector of *L. infantum* [37] yet only two records where available for this species, from Spain and Morocco, respectively, where *P. perniciosus* was also reported.

Similarly, *P. similis* was not associated to *L. tropica* since it was reported from the same areas where *P. sergenti* and *L. tropica* are found. The vectorial role of *P. similis* has not been proven although it was considered responsible for the transmission of *L. tropica* in some areas in Greece [38]. No species other than *P. papatasi* is incriminated in the transmission of *L. major* and there was a strong statistical relationship between the distribution of the vector and the parasite species, albeit a low spatial agreement for the reasons stated above.

Finally, the close agreement and strong statistical association between *L. donovani* s.s. and *P. halepensis*, a suspected vector reported in the Middle East, Türkiye and southern Caucasus, is worth noting. The role of *P. halepensis* in transmitting *L. donovani* has not been confirmed and deserves further investigation.

Greater insight into the relationship of the spatial distributions of vectors and leishmaniasis requires a better understanding of the distributions themselves and should ideally consider vector density. New information may be obtained by engaging in further surveillance and/or developing predictive vector distribution models based on environmental variables. The study area may then be categorised and the relationship between *L. infantum* and *Phlebotomus* spp. analysed, using ecological rather than administrative territorial units.

# Conclusions and potential implications

The spatial correlation between leishmaniasis and vectors in Europe and neighbouring countries was low, especially because of vectors present beyond the *Leishmania* spp. geographical limits, and the limited availability of information on vector distribution in many *Leishmania* spp. endemic areas. Accurate information on (seasonal) presence/absence of vectors is necessary to understand *Leishmania* spp. transmission cycles and assess the risk of leishmaniasis and future changes in parasite and vector distributions. Vector surveillance in areas where there is no information and the parasite is present, can help establish which vectors are contributing to the transmission. Considering *Leishmania* and leishmaniasis surveillance in non-endemic areas where vectors are reported may facilitate detection of parasite introduction via movement of infected people and animals. The study also highlights the need for some extra scrutiny for vector species selected in the statistical models as potentially more important species than the ones that were not selected. This information could be very useful for improving predictions of *Leishmania* spp. distribution and incidence, and towards the development of an Early Warning System for sand fly bone diseases in Europe and neighbouring countries.

[iv] This designation is without prejudice to positions on status, and is in line with UNSCR 1244 and the ICJ Opinion on the Kosovo Declaration of Independence.

# References


1. European Centre for Disease Prevention and Control (ECDC) Surveillance, prevention and control of leishmaniases in the European Union and its neighbouring countries. Stockholm: ECDC, 2022.
2. Lukes J, Mauricio IL, Schoenian G, Dujardin J-C, Soteriadou K, Dedet J-P, et al. Evolutionary and geographical history of the *Leishmania donovani* complex with a revision of current taxonomy. PNAS. 2007 May 29;104(22):9375-80.
3. World Health Organization (WHO). Manual on case management and surveillance of the leishmaniais in the WHO European region. Copenhagen, Denmark: WHO Regional Office for Europe; 2017.
4. McDowell MA, Rafati S, Ramalho-Ortigao M, Ben Salah A. Leishmaniasis: Middle East and North Africa research and development priorities. Plos Negl Trop Dis. 2011 Jul;5(7).
5. Özbilgin A, Harman M, Karakuş M, Bart A, Töz S, Kurt Ö, et al. Leishmaniasis in Turkey: visceral and cutaneous leishmaniasis caused by *Leishmania donovani* in Turkey. Acta Tropica. 2017;173:90-6.
6. Antoniou M, Haralambous C, Mazeris A, Pratlong F, Dedet J-P, Soteriadou K. *Leishmania donovani* leishmaniasis in Cyprus. Lancet Inf Dis. 2008 Jan;8(1):6-7.
7. Kuhls K, Moskalenko O, Sukiasyan A, Manukyan D, Melik-Andreasyan G, Atshemyan L, et al. Microsatellite based molecular epidemiology of *Leishmania infantum* from re-emerging foci of visceral leishmaniasis in Armenia and pilot risk assessment by ecological niche modeling. Plos Neg Trop Dis. 2021 Apr;15(4).
8. Alten B, Maia C, Afonso MO, Campino L, Jiménez M, González E, et al. Seasonal dynamics of *Phlebotomine* sand fly species proven vectors of Mediterranean leishmaniasis caused by *Leishmania infantum*. PLoS Negl Trop Dis. 2016;10(2):e4458.
9. Gradoni L. The leishmaniases of the Mediterranean region. Curr Trop Med Reports. 2017;4(1):21-6.
10. Kasap OE, Votypka J, Alten B. The distribution of the *Phlebotomus major* complex (Diptera: Psychodidae) in Turkey. Acta Tropica. 2013 Sep;127(3):204-11.
11. Volf P, Myskova J. Sand flies and leishmania: specific versus permissive vectors. Trend Parasitol. 2007 Mar;23(3):91-2.
12. Aoun K, Bouratbine A. Cutaneous leishmaniasis in North Africa: a review. Parasite. 2014 Mar;21:14.
13. Bacaer N, Guernaoui S. The epidemic threshold of vector-borne diseases with seasonality. J Math Biol. 2006 Sep;53(3):421-36.
14. Colacicco-Mayhugh MG, Masuoka PM, Grieco JP. Ecological niche model of *Phlebotomus alexandri* and *P. papatasi* (Diptera: Psychodidae) in the Middle East. Int J Health Geograph. 2010 Jan 21;9:2.
15. Galvez R, Descalzo MA, Guerrero I, Miro G, Molina R. Mapping the current distribution and predicted spread of the leishmaniosis sand fly vector in the Madrid region (Spain) based on environmental variables and expected climate change. Vector-Borne Zoon Dis. 2011 Jul;11(7):799-806.
16. Fischer D, Moeller P, Thomas SM, Naucke TJ, Beierkuhnlein C. Combining climatic projections and dispersal ability: a method for estimating the responses of sandfly vector species to climate change. Plos Negl Trop Dis. 2011 Nov;5(11):e1407.
17. Alten B, Versteirt V, Van Bortel W, Zeller H, Wint W, Alexander N. VBORNET gap analysis: sand fly vector – distribution models utilised to identify areas of potential species –distribution in areas lacking records. Open Health Data; 2016. p. e5.
18. Meneguzzi VC, Dos Santos CB, Leite GR, Fux B, Falqueto A. Environmental niche modelling of phlebotomine sand flies and cutaneous leishmaniasis identifies *Lutzomyia intermedia* as the main vector species in Southeastern Brazil. PLoS ONE. 2016;11(10):e0164580.
19. Erguler K, Pontiki I, Zittis G, Proestos Y, Christodoulou V, Tsirigotakis N, et al. A climate-driven and field data-assimilated population dynamics model of sand flies. Scientific Reports. 2019;9(1):2469
20. Kleinbaum D, Klein M. Logistic regression: a self-learning text. Statistics for Biology and Health ed. 2010. ISBN 978-1-4419-1741-6
21. Dohoo I, Martin W, Stryhn H. Veterinary Epidemiologic Research. 2009. ISBN 0-919013-41-4
22. Fotakis EA, Giantsis IA, Avgerinou A, Kourtidis S, Agathaggelidou E, Kapoula C, et al. Identification of leishmania species in naturally infected sand flies from refugee camps, Greece. Emerg Inf Dis. 2019;25(2):361-4.
23. Molina R, Jiménez M, García-Martínez J, San Martín JV, Carrillo E, Sánchez C, et al. Role of asymptomatic and symptomatic humans as reservoirs of visceral leishmaniasis in a Mediterranean context. PLoS Neg Trop Dis. 2020;14(4):e0008253.
24. European Food Safety Authority (EFSA). Scientific Opinion on canine leishmaniosis. EFSA Journal. 2015;13 (4):4075.
25. European Centre for Disease Prevention and Control (ECDC). Phlebotomine sand flies - Factsheet for experts: Stockholm: ECDC; 2020. Available from: https://www.ecdc.europa.eu/en/disease-vectors/facts/phlebotomine-sand-flies.
26. Svobodova M, Alten B, Zidkova L, Dvorak V, Hlavackova J, Myskova J, et al. Cutaneous leishmaniasis caused by *Leishmania infantum* transmitted by *Phlebotomus tobbi*. Int J Parasitol. 2009 Jan 15;39(2):251-6.

27. Tsirigotakis N, Pavlou C, Christodoulou V, Dokianakis E, Kourouniotis C, Alten B, et al. Phlebotomine sand flies (Diptera: Psychodidae) in the Greek Aegean islands: ecological approaches. Parasites & Vectors. 2018;11(1):97.

28. Maroli M, Khoury C, Bianchi R, Ferroglio E, Natale A. Recent findings of *Phlebotomus neglectus* Tonnoir, 1921 in Italy and its western limit of distribution. Parassitologia. 2002 2002-Jun;44(1-2):103-9.

29. 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 Internat Health. 2008 Feb;13(2):256-64.

30. Giorgobiani EaLPGaBGaDNaJRCaTLaKKaKS. Incrimination of *Phlebotomus kandelakii* and *Phlebotomus balcanicus* as vectors of *Leishmania infantum* in Tbilisi, Georgia. PLoS Negl Trop Dis. 2012;6(4):e1609.

31. Ballart C, Baron S, Alcover MM, Portus M, Gallego M. Distribution of phlebotomine sand flies (Diptera: Psychodidae) in Andorra: First finding of *P. perniciosus* and wide distribution of *P. ariasi*. Acta Tropica. 2012 Apr;122(1):155-9.

32. Prudhomme J, De Meeûs T, Toty C, Cassan C, Rahola N, Vergnes B, et al. Altitude and hillside orientation shapes the population structure of the *Leishmania infantum* vector *Phlebotomus ariasi*. Scientific Reports. 2020;10(1):14443.

33. Guan LR, Xu YX, Li BS, Dong J. The role of *Phlebotomus alexandri* (Sinton, 1928) in the transmission of Kala-Azar. Bull World Health Org. 1986 1986;64(1):107-12.

34. Azizi K, Rassi Y, Javadian E, Motazedian MH, Rafizadeh S, Ershadi MRY, et al. *Phlebotomus* (*Paraphlebotomus*) *alexandri*: a probable vector of *Leishmania infantum* in Iran. Ann Trop Med Parasit. 2006 Jan;100(1):63-8.

35. Coleman RE, Burkett DA, Sherwood V, Caci J, Spradling S, Jennings BT, et al. Impact of phlebotomine sand flies on US military operations at Tallil Air Base, Iraq: 2. Temporal and geographic distribution of sand flies. J Med Entomol. 2007 Jan;44(1):29-41.

36. Strelkova MV, Ponirovsky EN, Morozov EN, Zhirenkina EN, Razakov SA, Kovalenko DA, et al. A narrative review of visceral leishmaniasis in Armenia, Azerbaijan, Georgia, Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan, the Crimean Peninsula and Southern Russia. Parasites & Vectors. 2015;8(1):330.

37. Sáez VD, Morillas-Márquez F, Merino-Espinosa G, Corpas-López V, Morales-Yuste M, Pesson B, et al. *Phlebotomus langeroni* Nitzulescu (Diptera, Psychodidae) a new vector for *Leishmania infantum* in Europe. Parasitol Res. 2018;117(4):1105-13.

38. Christodoulou V, Antoniou M, Ntais P, Messaritakis I, Ivovic V, Dedet J-P, et al. Re-emergence of visceral and cutaneous leishmaniasis in the Greek island of Crete. Vector-Borne Zoon Dis. 2012 Mar;12(3):214-22.

PDF TQ-03-23-074-EN-N

**European Centre for Disease Prevention and Control (ECDC)**

Gustav III:s Boulevard 40, 16973 Solna, Sweden

Tel. +46 858601000
Fax +46 858601001
www.ecdc.europa.eu

An agency of the European Union
www.europa.eu

Subscribe to our publications
www.ecdc.europa.eu/en/publications

Contact us
publications@ecdc.europa.eu

Follow us on Twitter
@ECDC_EU

Like our Facebook page
www.facebook.com/ECDC.EU

PDF ISBN 978-92-9498-617-7