



# AperTO - Archivio Istituzionale Open Access dell'Università di Torino

# Monitoring and detection of new endemic foci of canine leishmaniosis in northern continental Italy: An update from a study involving five regions (2018-2019)

This is a pre print version of the following article:

Original Citation:

Availability:

This version is available http://hdl.handle.net/2318/1837063

since 2022-02-01T16:16:16Z

Published version:

DOI:10.1016/j.vprsr.2021.100676

Terms of use:

**Open Access** 

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

# Monitoring and detection of new endemic foci of canine leishmaniosis in northern continental Italy: an update from a study involving five regions (2018-2019)

2 3

4 Luigi Gradoni<sup>a\*</sup>, Ezio Ferroglio<sup>b</sup>, Stefania Zanet<sup>b</sup>, Walter Mignone<sup>c</sup>, Luigi Venco<sup>d</sup>, Gioia Bongiorno<sup>a</sup>,

5 Eleonora Fiorentino<sup>a</sup>, Rudi Cassini<sup>e</sup>, Marika Grillini<sup>e</sup>, Giulia Simonato<sup>e</sup>, Alice Michelutti<sup>f</sup>, Fabrizio

6 Montarsi<sup>f</sup>, Alda Natale<sup>f</sup>, Manuela Gizzarelli<sup>g</sup>, Valentina Foglia Manzillo<sup>g</sup>, Fabrizio Solari Basano<sup>h</sup>,

7 Roberto Nazzari<sup>h</sup>, Ornella Melideo<sup>i</sup>, Diego Gatti<sup>i</sup>, Gaetano Oliva<sup>g</sup>

- 8
- 9
- <sup>a</sup>Unit of Vector-borne Diseases, Istituto superiore di Sanità, Roma, Italy
- <sup>11</sup> <sup>b</sup>Dipartimento di Scienze Veterinarie dell'Università degli Studi di Torino, Grugliasco, Torino, Italy
- <sup>12</sup> <sup>c</sup>Istituto Zooprofilattico sperimentale del Piemonte, Liguria e Valle d'Aosta, Sezione di Imperia, Italy
- 13 <sup>d</sup>Veterinary practitioner, Santa Giuletta, Pavia, Italy
- <sup>14</sup> <sup>e</sup>Dipartimento di Medicina Animale, Produzioni e Salute, Università degli Studi di Padova, Legnaro,
- 15 Padova, Italy
- 16 <sup>f</sup>Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padova, Italy
- <sup>g</sup>Dipartimento di Medicina Veterinaria e Produzioni Animali, Università degli Studi di Napoli
- 18 Federico II, Napoli, Italy
- 19 <sup>h</sup>Arcoblu s.r.l., Milano, Italy
- 20 <sup>i</sup>Elanco Italia S.p.A., Sesto Fiorentino, Firenze, Italy
- 21
- 22 \*Corresponding author.
- 23 *E-mail address*: luigi.gradoni@iss.it (L. Gradoni).
- 24
- 25 Keywords:
- 26 Northern Italy
- 27 Canine leishmaniosis
- 28 Phlebotomine sand flies
- 29

#### 30 ABSTRACT

Canine leishmaniosis (CanL) is an emerging zoonosis caused by Leishmania infantum and transmitted 31 in southern Europe by phlebotomine sand flies of the subgenus *Phlebotomus (Larroussius)*. Endemic 32 foci of CanL have been recorded in northern continental Italy since early 1990s and attributed to the 33 northward expansion of vector populations due to climatic changes in association with 34 travelling/relocated infected dogs from the southern Mediterranean littoral. In this study, further 35 spread of endemic Leishmania foci was monitored during 2018-2019 in five regions (Aosta Valley, 36 Piedmont, Lombardy, Veneto and Friuli-Venezia Giulia), with focus to territories where investigations 37 38 were not performed, or they have been inconclusive. Clinical cases of CanL identified by local veterinary practitioners and confirmed by reference diagnosis centers were regarded as autochthonous 39 if their origin from, or travel to, areas endemic for CanL were excluded in the previous  $\geq 2$  years. 40 Around these index cases, i) serosurveys for L. infantum were carried out where indicated, ii) 41 42 sampling from eligible dogs was intensified by collaborating veterinary practitioners, and iii) suitable sites were investigated for the presence of competent phlebotomine vectors. Fifty-seven municipalities 43 44 whose enzootic status of CanL was unreported before 2018, were identified as endemic. The stability of 27 foci recorded over the past decade, was also confirmed. Competent phlebotomine vectors, 45 mainly Phlebotomus perniciosus, were collected for the first time in 23 municipalities. The newly 46 recorded endemic municipalities appear to be distributed over a west-to-east decreasing gradient: 30 47 in Piedmont, 21 in Lombardy, 4 in Veneto and 2 in Friuli-Venezia Giulia. As regards Veneto, it 48 should be noted that a relatively restricted territory was investigated as several municipalities from 49 other provinces had already been detected as endemic for CanL in the past. Cold climate conditions of 50 the easternmost region of Friuli-Venezia Giulia bordering non-endemic territories of Slovenia, are 51 probably less favorable to L. infantum transmission. 52

#### 54 1. Introduction

55

Canine leishmaniosis (CanL) is a zoonotic disease of emerging importance caused by the protozoan 56 parasite Leishmania infantum (Kinetoplastida: Trypanosomatidae) and transmitted in the European 57 region by phlebotomine sand flies of the subgenus *Phlebotomus* (*Larroussius*) (Diptera: Psychodidae) 58 (Alten et al., 2016; Gradoni et al., 2017). The disease is traditionally endemic in southern countries, 59 but factors associated with the de novo colonization and establishment of vectors, and increased 60 61 burden of infected canine hosts may contribute to the spread and occurrence of this disease in other 62 areas (Baneth et al., 2008). Expansion of sand flies due to climatic and environmental changes into territories where they were not previously established, was predicted (Medlock et al., 2014) and, 63 actually, permanent vector populations were recorded in regions or countries north of their traditional 64 distribution such as South Tyrol, Austria and Germany as regards central Europe (Morosetti et al., 65 66 2009; Poeppl et al., 2013; Oerther et al., 2020). Travelling with or relocating dogs from Leishmania endemic to non-endemic areas is a phenomenon that needs to be carefully monitored (Wright et al., 67 68 2020), for the risk of a shift in disease transmission from the Mediterranean subregion to the naïve dog populations of continental Europe (Menn et al., 2010). According to a European Expert Panel on 69 Animal Health, regions or countries endemic for CanL should be defined as such based on the 70 demonstration of locally acquired canine infections (autochthonous cases) associated with the 71 presence of competent phlebotomine vectors in the same territory (EFSA AHAW Panel, 2015). Local 72 73 episodes of dog-to-dog *Leishmania* transmission by non-vectorial routes, including sexual, vertical or blood transfusion-borne infections, even though non-occasional (Svobodova et al., 2017; Wright et al., 74 2019), should not be attributable to an endemic status of the area in the absence of the specific vector. 75

In northern continental Italy, the occurrence of autochthonous CanL and human leishmaniasis has 76 been associated with a 30-year northward expansion and increase in density of the phlebotomine 77 vectors Phlebotomus perniciosus and Phlebotomus neglectus (Maroli et al., 2008). Before 1990, there 78 was no convincing evidence for local L. infantum transmission north of Liguria and Emilia-Romagna 79 regions; from early 1990 through mid 2000s, retrospective literature analysis and prospective field 80 81 surveys on CanL and phlebotomine sand flies allowed to identify about 20 disease foci in pre-alpine and Po valley territories of the 5 northern regions of continental Italy included in the present study, 82 83 namely Aosta Valley, Piedmont, Lombardy, Veneto and Friuli-Venezia Giulia, with some 150 autochthonous infected dogs (Ferroglio et al., 2005; Cassini, 2008; Maroli et al., 2008). Starting from 84 85 2010, collection and analysis of CanL diagnosis records available at the public Institutes for Zooprophylaxis and veterinary University Departments was performed in the frame of two European 86 87 projects, namely the ECDC "European Network for Arthropod Vector Surveillance for Human Public 88 Health" (VBornet) and the EU FP7-Health "Biology and control of vector-borne infections in Europe"

- (EDENext). By 2013, 328 municipalities were mapped as endemic for CanL throughout northern Italy
  (Gradoni and Melosi, 2013) (Fig. 1).
- The aim of the present study was to monitor further spread of endemic foci of disease within the 91 aforementioned 5 regions, with particular focus to areas where investigations have not been 92 performed, or previous investigations gave inconclusive results. Therefore the study area did not 93 include pre-apennine territories of Emilia-Romagna and the southern part of Piedmont, for which a 94 diffuse endemic status of human and canine Leishmania infections has definitely emerged over the 95 96 past decade (Biglino et al., 2010; Varani et al., 2013; Franceschini et al., 2016; Ferroglio et al., 2018; Calzolari et al., 2019; Moirano et al., 2020). The Autonomous Province of Bolzano-South Tyrol, 97 98 located in the northernmost territory of the Italian eastern Alps, was recently investigated for the same purpose (Morosetti et al., 2020). 99
- 100

## 101 **2. Methods**

102

# 103 2.1 Organization and study design

104

The study was carried out regionally by the veterinary Departments of Torino and Padova 105 Universities, with the collaboration of private and public veterinarians and laboratories operating in 106 the study areas, under the coordination of Istituto Superiore di Sanità, Roma, and the veterinary 107 Department of the Naples University. The first step was to identify index cases from target territories, 108 consisting of dogs suspected to be infected with L. infantum on clinical ground as reported by local 109 veterinary practitioners. The animals had to be in the age range 6 months -12 years, had to have 110 confirmed diagnosis of CanL by reference diagnosis centers, and the infection had to be acquired 111 locally, i.e. be autochthonous with reasonable confidence; this criterion required accurate individual 112 113 anamnesis which excluded origin from, or travel to known areas endemic for leishmaniosis in the previous 2 years, at least, or preferably more. Where indicated, active L. infantum serosurveys were 114 115 organized around the index cases to test a representative sample of the healthy canine population for the exposure to the parasite. Alternatively or in parallel, samples from both healthy or clinically 116 suspected dogs living in the index case's area were collected through the help of local veterinary 117 practitioners. Suitable sites identified in the territory were also investigated for the presence of 118 phlebotomine sand fly species, with focus on competent L. infantum vector species expected in the 119 area, i.e. *P. perniciosus* and *P. neglectus*. 120

#### 122 *2.2 Study area*

#### 123

It included the largest part of northern Italy, from Piedmont at west to Friuli-Venezia Giulia at east, 124 covering 78,780 km<sup>2</sup> and corresponding to one fourth of the Italian territory, divided into about 3600 125 municipalities with 20.5 million human population. Within the involved regions, a selection of the 126 territories to be investigated was operated by excluding municipalities having geomorphological and 127 environmental characteristics considered unsuitable for the colonization by phlebotomine sand flies. 128 To this aim, a nation-wide GIS database of bio-geographic factors such as aspect, elevation and land 129 130 use associated with the presence of phlebotomine sand flies, was used. Digital Elevation Model and COoRdination of INformation on Environment Land Cover layer were employed to identify 131 conditions suitable for all Italian species of sand flies, both competent and non-competent vectors of 132 L. infantum (Rossi et al., 2007; Bongiorno et al, 2008; Morosetti et al., 2009; Bongiorno et al, 2010; 133 134 Busani et al, 2012; data on file, Istituto Superiore di Sanità).

135

#### 136 2.3 CanL diagnosis and entomological surveys

137

138 Index cases of leishmaniosis were confirmed by the following Italian public reference centers for 139 Leishmania infection diagnosis: Istituto Zooprofilattico sperimentale del Piemonte, Liguria e Valle d'Aosta, Imperia; Dipartimento di Scienze Veterinarie dell'Università degli Studi di Torino, 140 Grugliasco; Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padua; and Istituto 141 Superiore di Sanità, Roma. Serum samples from suspected clinical cases were tested by in-house 142 IFAT at the threshold titer of 1:80 or 1:160 (Gradoni and Gramiccia, 2014), or by in-house Western 143 blotting (Ferroglio et al., 2007) using the same reference strain of L. infantum as the antigen source. 144 For the test of sera collected in surveys on healthy canine populations, considering an expected low 145 parasite exposure in the study area with prevalent subclinical infections, IFAT was performed at the 146 147 serum dilution of 1:40 as threshold titer (Morosetti et al., 2020).

PCR was mainly used as confirmatory technique and performed on DNA extracted from total 148 peripheral blood, bone marrow aspirate samples, or other tissue material where appropriate. Different 149 amplification protocols were used by laboratories: conventional PCR targeting a L. infantum-specific 150 fragment of kDNA (Zanet et al., 2014); nested-PCR targeting a Leishmania specific sequence in a 151 kinetoplastid-specific fragment of the small subunit rRNA gene (Gramiccia et al., 2010); real-time 152 PCR targeting a L. donovani sequence of cytochrome c oxidase subunit II gene (Vascellari et al., 153 2016). Dogs' identification, anamnesis records, clinical data and CanL diagnosis results were recorded 154 155 using Castor Electronic Data Capture database [2019 online version].

Trapping of adult phlebotomine sand flies was performed during the expected density peaks in 156 northern Italian latitudes (i.e. July-August) and mainly carried out using 20×20 cm sticky papers 157 coated with castor oil set in each site for 1-2 nights up to a maximum of 5 nights. CDC miniature light 158 traps (John W. Hock Co., Gainesville, FL, USA; Bioquip Products, Rancho Dominguez, CA, USA) or 159 CDC-CO<sub>2</sub> traps (Byblos, Cantù, Italy) filled with 1 kg dry ice (Signorini et al., 2013) were also used. 160 They were placed overnight outside or inside animal shelters. Sand fly specimens were identified by 161 morphological characteristics to species level according to Theodor (1958), Léger et al. (1983) and 162 Dantas-Torres et al. (2014). 163

- 164
- 165 **3. Results**
- 166

The selection of target territories within the 5 regions was preceded by the GIS identification of municipalities considered to be unsuitable to phlebotomine life cycle, which are shown in blue in the maps of **Fig. 2**, and of municipalities already recorded as endemic for leishmaniosis (n=309 by the end of 2017) shown in red in the same maps. It should be pointed out that a number of the latter municipalities were included in the new investigations, in order to confirm their epidemiological status and provide evidence for the stability of CanL foci recently established in northern Italy.

Altogether, the present study made it possible to identify an additional 57 municipalities whose endemicity status for CanL was unreported before 2018 (shown in green in the maps of **Fig. 2**), which however were differently distributed over the five regions surveyed as detailed in the following sections.

177

#### 178 *3.1 Aosta Valley and Piedmont*

179

Among 16 dogs examined from Aosta Valley and which met the criteria for inclusion, two were found
positive and diagnosed as autochthonous CanL cases. They were both resident in the municipality of
Aosta already recorded as endemic for the disease (Fig. 2A).

Investigations in the Piedmont territory involved 593 dogs which met the criteria for inclusion and were resident in 171 municipalities. Eighty-seven dogs (14.3%) from 45 municipalities were found infected with *L. infantum* by serology and/or PCR (**Table 1**). Of them, 52 dogs were from 15 municipalities already recorded as endemic for CanL, whereas 35 animals were from 30 municipalities - belonging to all seven provinces of Piedmont - for which the endemic condition of leishmaniosis was previously unknown (**Fig. 2**A). Entomological surveys were carried out using sticky traps. The stable presence of the vector *P*. *perniciosus* was confirmed in 14 municipalities already recorded as endemic for CanL, whereas this sand fly species was found for the first time in sites of 13 municipalities newly demonstrated as endemic.

193

#### 194 *3.2. Lombardy*

195

Six index cases of confirmed autochthonous CanL were identified in municipalities belonging to 4 196 provinces (Table 2). Presence of *L. infantum* infection/exposure in animals living in the same areas as 197 index cases and which met inclusion criteria, was mainly assessed in Pavia province. Because this part 198 of the study started late in the warm season 2019, dedicated entomological surveys could not be 199 performed in most cases. Investigations in the Lombardy territory involved 74 candidate dogs besides 200 index cases, which were resident in 34 municipalities. Thirty-five dogs (47.3%) living in 21 201 municipalities whose endemic status was previously unknown, and mainly consisting of animals with 202 203 suspected clinical signs referred by local veterinary practitioners, were found infected with L. infantum by serology and/or PCR (Table 2; Fig. 2B). Presence of P. perniciosus, assessed through the 204 use of sticky traps, could only be investigated and confirmed in 3 of the above municipalities, 205 however this vector species was collected in 3 additional municipalities belonging to Pavia province 206 (Bagnaria, Fortunago and Godiasco), from which no CanL cases were referred by vets. Finally, 207 phlebotomine species other than P. perniciosus were recorded using sticky papers in other sites of 208 Lombardy, indicating the presence of suitable areas for sand flies. The most common species recorded 209 210 was Sergentomyia minuta, collected in the municipalities of San Colombano al Lambro (Milan province), Salvano Palazzago (Bergamo province) and Ponte Nizza (Pavia province). Phlebotomus 211 papatasi was collected in Badia Pavese (Pavia province). 212

213

### 214 3.3 Veneto and Friuli-Venezia Giulia

215

Index cases of confirmed autochthonous CanL were identified in 4 municipalities belonging to 2 provinces of Veneto region, Vicenza and Treviso (**Table 3**; **Fig. 2**C). Because 5 and 3 cases were from Valdagno and Caltrano municipalities, respectively, active case detection through field surveys were implemented in the two areas with the help of local authorities and the clinical team of the veterinary Department of Naples University. Point seroprevalence values recorded among 206 dogs examined in Valdagno and 89 dogs in Caltrano, were 2.4% and 22.4%, respectively. By including also individual samples sent by veterinary practitioners from these municipalities, and from other territories of the Vicenza province, the seroprevalence of autochthonous CanL in the whole territory was 6.6% among 556 examined dogs. Only 7 dogs from 2 municipalities of Friuli-Venezia Giulia region, Trieste and Staranzano, were recorded as positive for CanL, and 4 of them were considered autochthonous cases (**Table 3**; **Fig. 2**D). The large number of serum samples examined from Trieste allowed to estimate a 2.9% seroprevalence of autochthonous CanL in this municipality.

Entomological surveys were performed in provinces of Vicenza (7 municipalities) and Trieste (5 municipalities), using three types of traps set in 2-3 collecting sites per municipality on average. Municipalities included both, confirmed and newly endemic foci (**Table 4**). CDC-CO<sub>2</sub> traps resulted to be the most efficient, whereas the efficacy of sticky traps in collecting *Phlebotomus* spp was negligible despite 37 *Sergentomyia minuta* specimens were collected by this method in Valdagno. Among a total of 689 *Phlebotomus* spp specimens trapped, the majority was identified as *P. perniciosus* (97.0%), followed by *P. neglectus* (2.9%). A few adults of *P. papatasi* were also recorded.

- 235
- 236
- 237

#### 238 4. Discussion

239

Whereas about twenty investigated municipalities already known to be endemic for leishmaniosis 240 were confirmed to be so, the new endemic territories recorded by this study were not evenly 241 distributed throughout the 5 regions surveyed, as our results showed an evident west-to-east 242 decreasing gradient: 30 newly endemic municipalities detected in Piedmont, 21 in Lombardy, 4 in 243 Veneto and 2 in Friuli-Venezia Giulia. As regards Veneto region, this could be partially explained by 244 the relatively old records of CanL endemicity involving several municipalities of Verona and Padua 245 provinces, which had been actively investigated in the past and hence new investigational territories 246 247 were limited in number (Figure 2C). Cold climate conditions of the easternmost region of Friuli-Venezia Giulia, bordering non-endemic territories of Slovenia, are probably unfavorable to 248 249 widespread L. infantum transmission and CanL.

Among the new endemic records, municipalities in pre-alpine and pre-apennine hilly environments were the most represented, whereas low lands of Po valley characterized by intensive agriculture or wet environments were only sporadically interested. In Lombardy, two territory belts of CanL endemicity can be clearly seen, one consisting of pre-alpine sites at north, the second of pre-apennine sites at southwest (**Figure 2B**). It should be noted that the presence of sand flies, in particular *P*. *perniciosus*, has recently been reported from northeastern plains of Veneto and Friuli Venezia Giulia,

thanks to the intense entomological collections performed over 3 consecutive years in the frame of 256 integrated surveillance of West Nile disease. However, the sand fly population density was confirmed 257 to be extremely low and hence with limited epidemiological significance (Michelutti et al., 2021). In 258 22/57 newly endemic municipalities, competent L. infantum vectors (mainly P. perniciosus) could be 259 demonstrated by means of sticky paper or CDC-trap collections performed during a few nights per 260 season, thereby suggesting an elevated phlebotomine density as also expected by predictive models 261 recently applied in Piedmont, Veneto and Friuli-Venezia Giulia (Signorini et al., 2014; Moirano et al., 262 263 2020).

264 As regards CanL prevalence, Italy was found to rank the highest in median seroprevalence (17.7%) amongst countries of southwest Europe over the 1971-2006 period, as determined by serosurveys 265 266 involving more than 420,000 dogs (Franco et al., 2011). Because of the country's geomorphological features, however, CanL does not exhibit a homogenous diffusion in the Italian territory, being 267 268 typically more prevalent in canine populations of the Tyrrhenian coast, inland territories of southern peninsula, and islands. Early in the northward expansion of the disease, rates of autochthonous 269 270 seropositive dogs from pre-alpine/Po valley territories were found to range 1.8%-2.6% on average (Maroli et al., 2008), in contrast to rates often exceeding 30% in southern territories and islands 271 272 (Franco et al., 2011; Foglia Manzillo et al., 2018). Because the present study did not involve 273 systematic serosurveys in normal canine populations throughout the investigated territory, we could 274 not make in-depth comparisons with prevalences from previous studies. Two point-prevalence values were recorded in municipalities of Vicenza province, Veneto region, and found to differ each other 275 about ten folds (2.4% in Valdagno versus 22.4% in Caltrano) (Table 3). This finding confirms 276 previous reports (Simonato et al., 2020) on the scattered presence in north Italy of newly established 277 foci with intense transmission, where canine seroprevalence may reach values similar to those found 278 in southern Italy. On the other hand, data gathered and added from a number of other sites of Vicenza 279 province, and involving over 500 dogs in total, resulted in 6.6% prevalence of autochthonous CanL 280 281 (Table 3), which seems to be a more reasonable figure to describe the overall situation. For comparison with previous data from the same Veneto region, in 2003-2005 the prevalence of 282 autochthonous L. infantum seropositives from two foci and involving about 1100 examined dogs, was 283 1.7% in Verona province and 1.0% in Treviso province, respectively (Maroli et al., 2008). 284

It is noteworthy that two major Italian private diagnostic centers for CanL have recently reported *L. infantum* seropositivity values as high as 21.6% among 21,545 examined dogs from northern Italian regions over a 10-year period (Mendoza-Roldan et al., 2020). However, this prevalence value is likely to be the result of a biased sampling of animals referred to veterinary clinics (e.g. unhealthy dogs with clinical signs referable to CanL) and simply based on dog's residence without individual anamnesis to
exclude origin from, or travel to, known areas endemic for leishmaniosis.

Search for indisputable autochthonous cases of *L. infantum* infection proved difficult and labor intensive, because of the accurate and selective anamnesis required for dogs' inclusion and the diffusion of sites already endemic in northern Italy. Several animals did not meet study criteria because travelled, even shortly, to endemic areas of leishmaniosis. These not only consisted typically of southern Mediterranean sites - e.g. most often visited during summer holidays or being frequent origin of rehomed dogs - but also neighboring municipalities within the same region or other northern territories during the sand fly activity period.

In conclusion, regions of northern continental Italy currently include a number of territories endemic for leishmaniosis which should now be regarded as "traditionally endemic", as well as a number of territories which appear less suitable for persisting and sustained transmission of *L. infantum* leading to widespread CanL. Different climatic and environmental conditions are most likely the origin of these observations, however continuous monitoring appears necessary because of the rapid changes in such conditions.

304 305

#### 306 **References**

Alten, B., Maia, C., Afonso , M.O., Campino, L., Jiménez, M., González, E., Molina, R., Bañuls, A.L., 308 Prudhomme, J., Vergnes, B., Toty, C., Cassan, C., Rahola, N., Thierry, M., Sereno, D., 309 Bongiorno, G., Bianchi, R., Khoury, C., Tsirigotakis, N., Dokianakis, E., Antoniou, M., 310 Christodoulou, V., Mazeris, A., Karakus, M., Ozbel, Y., Arserim, S.K., Kasap, O.E., Gunay, F., 311 Oguz, G., Kaynas, S., Tsertsvadze, N., Tskhvaradze, L., Giorgobiani, E., Gramiccia, M., Volf, 312 P., Gradoni, L., 2016. Seasonal dynamics of phlebotomine sand fly species proven vectors of 313 314 Mediterranean leishmaniasis caused by Leishmania infantum. PLoS Negl. Trop. Dis. 10, e0004458. 315

<sup>Baneth, G., Koutinas, A.F., Solano-Gallego, L., Bourdeau, P., Ferrer, L., 2008. Canine leishmaniosis new concepts and insights on an expanding zoonosis: part one. Trends Parasitol. 24, 324-330.</sup> 

Biglino, A., Bolla, C., Concialdi, E., Trisciuoglio, A., Romano, A., Ferroglio, E., 2010. Asymptomatic
 *Leishmania infantum* infection in an area of northwestern Italy (Piedmont region) where such
 infections are traditionally non-endemic. J. Clin. Microbiol. 48, 131–136.

- Bongiorno, G., Gramiccia, M., Morosetti, G., Maroli, M., Gradoni, L., 2010. Geomorphology and
   environmental factors associated with recent sand flies spread in northern Italian regions.
   Parassitologia 52, 162.
- Bongiorno, G., Scortichini, M.G., Gradoni, L., Gramiccia, M., Maroli, M., 2008. Environmental and
   climatological factors as determinants of the distribution of two *Leishmania* vectors,
   *Phlebotomus perniciosus* and *Phlebotomus perfiliewi*, in the Apennine mountains of central
   Italy. Parassitologia 50, 100.
- Busani, L., Mughini Gras, L., Romi, R., Boccolini, D., Severini, F., Bongiorno, G., Khoury, C.,
  Bianchi, R., Gradoni, L., Capelli, G., 2012. Mosquitoes, sand flies and ticks: bibliographical
  atlas of species of medical importance in Italy (1985-2009). Roma: Istituto Superiore di Sanità,
  Rapporti ISTISAN 12/22, 1-105 (in Italian).
- Calzolari, M., Carra, E., Rugna, G., Bonilauri, P., Bergamini, F., Bellini, R., Varani, S., Dottori, M.,
   2019. Isolation and molecular typing of *Leishmania infantum* from *Phlebotomus perfiliewi* in a
   re-emerging focus of leishmaniasis, Northeastern Italy. Microorganisms 7, 644.
- Cassini, R., 2008. Aspetti epidemiologici e rischi zoonosici delle malattie trasmesse da vettori:
   Babesiosi e Leishmaniosi in Italia Nord-Orientale. PhD thesis, University of Padova.
   (http://paduaresearch.cab.unipd.it/794/1/Tesi Dottorato Cassini.pdf).
- Dantas-Torres, F., Tarallo, V.D., Otranto, D., 2014. Morphological keys for the identification of
   Italian phlebotomine sand flies (Diptera: Psychodidae: Phlebotominae). Parasit. Vectors 7, 479.
- Franceschini, E., Puzzolante, C., Menozzi, M., Rossi, L., Bedini, A., Orlando, G., Gennari, W.,
  Meacci, M., Rugna, G., Carra, E., Codeluppi, M., Mussini, C., 2016. Clinical and
  microbiological characteristics of visceral leishmaniasis outbreak in a northern Italian
  nonendemic area: a retrospective observational study. Biomed Res. Int. 2016, 6481028.
- EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), 2015. Scientific Opinion on
  canine leishmaniosis. EFSA J. 13, 4075.
- Ferroglio, E, Maroli, M, Gastaldo, L, Mignone, W, Rossi, L., 2005. Canine Leishmaniasis, Italy.
  Emerg. Infect. Dis. 11, 1618–1620.
- Ferroglio, E., Battisti, E., Zanet, S., Bolla, C., Concialdi, E., Trisciuoglio, A., Khalili, S., Biglino, A.,
  2018. Epidemiological evaluation of *Leishmania infantum* zoonotic transmission risk in the
  recently established endemic area of Northwestern Italy. Zoonoses Public Health 65, 675-682.
- Ferroglio, E., Centaro, E., Mignone, W., Trisciuoglio, A., 2007. Evaluation of an ELISA rapid device
   for the serological diagnosis of *Leishmania infantum* infection in dog as compared with
   immunofluorescence assay and Western blot. Vet. Parasitol. 144, 162–166.

- Foglia Manzillo, V., Gizzarelli, M., Vitale, F., Montagnaro, S., Torina, A., Sotera, S., Oliva, G., 2018.
  Serological and entomological survey of canine leishmaniasis in Lampedusa island, Italy. BMC
  Vet. Res. 14, 286.
- Franco, A.O., Davies, C.R., Mylne, A., Dedet, J.P., Gállego, M., Ballart, C., Gramiccia, M., Gradoni,
  L., Molina, R., Gálvez, R., Morillas-Márquez, F., Barón-López, S., Pires, C.A., Afonso, M.O.,
  Ready, P.D., Cox, J., 2011. Predicting the distribution of canine leishmaniasis in western Europe
  based on environmental variables. Parasitology 138, 1878-1891.
- Gradoni, L., Melosi, M., 2013. National mapping of leishmaniosis risk areas. In: Canine leishmaniosis
   and other vector-borne diseases: our current state of knowledge. Proc. Int. SCIVAC Congr., Pisa
   (Italy) 8-10 March 2013.
- Gradoni, L., López-Vélez, R., Mokni, M., 2017. Manual on case management and surveillance of the
   leishmaniases in the WHO European Region. World Health Organization Regional Office for
   Europe, Copenhagen.
- Gradoni, L., Gramiccia, M., 2014. Leishmaniosis. In: Manual of Diagnostic Tests and Vaccines for
   Terrestrial Animals (Mammals, Birds and Bees). Office International des Epizooties, Paris.
   <a href="http://www.oie.int/manual-of-diagnostic-tests-and-vaccines-forterrestrial-animals/">http://www.oie.int/manual-of-diagnostic-tests-and-vaccines-forterrestrial-animals/</a>.
- Gramiccia, M., Di Muccio, T., Fiorentino, E., Scalone, A., Bongiorno, G., Cappiello, S., Paparcone,
   R., Foglia Manzillo, V., Maroli, M., Gradoni, L., Oliva, G., 2010. Longitudinal study on the
   detection of canine *Leishmania* infections by conjunctival swab analysis and correlation with
   entomological parameters. Vet. Parasitol. 171, 223–228.
- Léger, N., Pesson, B., Madulo-Leblond, G., Abonnenc, E., 1983. Sur la différenciation des femelles
  du sous-genre Laroussius Nitzulescu, 1931 (Diptera-Phlebotomidae) de la région
  méditerranéenne. Ann. Parasitol. Hum. Comp. 58, 611–623.
- Maroli, M., Rossi, L., Baldelli, R., Capelli, G., Ferroglio, E., Genchi, C., Gramiccia, M., Mortarino,
  M., Pietrobelli, M., Gradoni, L., 2008. The northward spread of leishmaniasis in Italy: evidence
  from retrospective and ongoing studies on the canine reservoir and phlebotomine vectors. Trop.
  Med. Int. Health 13, 256-264.
- Medlock, J., Hansford, K., Van Bortel, W., Zeller, H., Alten, B., 2014. A summary of the evidence for
  the change in European distribution of phlebotomine sand flies (Diptera: Psychodidae) of public
  health importance. J. Vector Ecol. 39, 72-77.
- Mendoza-Roldan, J., Benelli, G., Panarese, R., Iatta, R., Furlanello, T., Beugnet, F., Zatelli, A.,
  Otranto, D., 2020. *Leishmania infantum* and *Dirofilaria immitis* infections in Italy, 2009-2019:
  changing distribution patterns. Parasit Vectors 13, 193.

- Menn, B., Lorentz, S., Naucke, T.J., 2010. Imported and travelling dogs as carriers of canine vectorborne pathogens in Germany. Parasit. Vectors 3, 34.
- Michelutti, A., Toniolo, F., Bertola, M., Grillini, M., Simonato, G., Ravagnan, S., Montarsi, F., 2021.
  Occurrence of Phlebotomine sand flies (Diptera: Psychodidae) in the northeastern plain of Italy.
  Parasit Vectors. 14, 164.
- Moirano, G., Zanet, S., Giorgi, E., Battisti, E., Falzoi, S., Acquaotta, F., Fratianni, S., Richiardi, L.,
  Ferroglio, E., Maule, M., 2020. Integrating environmental, entomological, animal, and human
  data to model the *Leishmania infantum* transmission risk in a newly endemic area in Northern
  Italy. One Health, 10, 100159.
- Morosetti, G., Toson, M., Trevisiol, K., Idrizi, I., Natale, A., Lucchese, L., Michelutti, A., Ceschi, P.,
  Lorenzi, G., Piffer, C., Fiorentino, E., Bongiorno, G., Gradoni, L., 2020. Canine leishmaniosis
  in the Italian northeastern Alps: A survey to assess serological prevalence in dogs and
  distribution of phlebotomine sand flies in the Autonomous Province of Bolzano South Tyrol,
  Italy. Vet. Parasitol. Reg. Stud. Reports 21, 100432.
- Morosetti, G., Bongiorno, G., Beran, B., Scalone, A., Moser, J., Gramiccia, M., Gradoni, L., Maroli,
  M., 2009. Risk assessment for canine leishmaniasis spreading in the north of Italy. Geospat.
  Health 4, 115-127.
- 404 Oerther, S., Jöst, H., Heitmann, A., Lühken, R., Krüger, A., Steinhausen, I., Brinker, C., Lorentz, S.,
  405 Marx, M., Schmidt-Chanasit, J., Naucke, T., Becker, N., 2020. Phlebotomine sand flies in
  406 southwest Germany: an update with records in new locations. Parasit Vectors 13, 173.
- 407 Poeppl, W., Obwaller, A., Weiler, M., Burgmann, H., Mooseder, G., Lorentz, S., Rauchenwald, F.,
  408 Aspöck, H., Walochnik, J., Naucke, T., 2013. Emergence of sand flies (Phlebotominae) in
  409 Austria, a Central European Country. Parasitol Res. 112, 4231-4237.
- Rossi, E., Rinaldi, L., Musella, V., Veneziano, V., Carbone, S., Gradoni, L., Cringoli, G., Maroli, M.,
  2007. Mapping the main *Leishmania* phlebotomine vector in the endemic focus of the Mt.
  Vesuvius in southern Italy. Geospat. Health 2, 191-198.
- Signorini, M., Cassini, R., Drigo, M., Frangipane di Regalbono, A., Pietrobelli, M., Montarsi, F.,
  Stensgaard, A.S., 2014. Ecological niche model of *Phlebotomus perniciosus*, the main vector of
  canine leishmaniasis in north-eastern Italy. Geospat. Health 9, 193-201.
- Signorini, M., Drigo, M., Marcer, F., Frangipane di Regalbono, A., Gasparini, G., Montarsi, F.,
  Pietrobelli, M., Cassini, R., 2013. Comparative field study to evaluate the performance of three
  different traps for collecting sand flies in northeastern Italy. J. Vector Ecol. 38, 374-378.

- Simonato, G., Marchiori, E., Marcer, F., Ravagnan, S., Danesi, P., Montarsi, F., Bononi, C., Capelli,
  G., Pietrobelli, M., Cassini, R., 2020. Canine leishmaniosis control through the promotion of
  preventive measures appropriately adopted by citizens. J. Parasitol. Res. 2020; 2020, 8837367.
- 422 Svobodova, V., Svoboda, M., Friedlaenderova, L., Drahotsky, P., Bohacova, E., Baneth, G., 2017.
  423 Canine leishmaniosis in three consecutive generations of dogs in Czech Republic. Vet. Parasitol.
  424 237, 122-124.
- Theodor, O., 1958. Psychodidae-Phlebotominae. In: Die Fliegen der Palearktischen Region, 9c.
  Schweiterbart'sche Verlagsbuchhandlung, Stuttgart (D), pp. 1–55.
- Varani, S., Cagarelli, R., Melchionda, F., Attard, L., Salvadori, C., Finarelli, A.C., Gentilomi, G.A.,
  Tigani, R., Rangoni, R., Todeschini, R., Scalone, A., Di, Muccio, T., Gramiccia, M., Gradoni,
  L., Viale, P., Landini, M.P., 2013. Ongoing outbreak of visceral leishmaniasis in Bologna
  Province, Italy, November 2012 to May 2013. Euro Surveill.18, 20530.
- Vascellari, M., Ravagnan, S., Carminato, A., Cazzin, S., Carli, E., Da Rold, G., Lucchese, L., Natale,
  A., Otranto, D., Capelli, G., 2016. Exposure to vector-borne pathogens in candidate blood donor
  and free-roaming dogs of northeast Italy. Parasit Vectors 9, 369.
- Wright, I., Baker, S., 2019. Leishmaniosis in a dog with no history of travel outside the UK. Vet. Rec.
  184, 387-388.
- Wright, I., Jongejan, F., Marcondes, M., Peregrine, A., Baneth, G., Bourdeau, P., Bowman, D.D.,
  Breitschwerdt, E.B., Capelli, G., Cardoso, L., Dantas-Torres, F., Day, M.J., Dobler, G., Ferrer,
- 438 L., Gradoni, L., Irwin, P., Kempf, V.A.J., Kohn, B., Krämer, F., Lappin, M., Madder, M.,
- 439 Maggi, R.G., Maia, C., Miró, G., Naucke, T., Oliva, G., Otranto, D., Pennisi, M.G., Penzhorn,
- 440 B.L., Pfeffer, M., Roura, X., Sainz, A., Shin, S., Solano-Gallego, L., Straubinger, R.K., Tasker,
- S., Traub, R., Little, S., 2020. Parasites and vector-borne diseases disseminated by rehomed
  dogs. Parasit. Vectors 13: 546.
- Zanet, S., Sposimo, P., Trisciuoglio, A., Giannini, F., Strumia, F., Ferroglio, E., 2014. Epidemiology
  of *Leishmania infantum*, *Toxoplasma gondii*, and *Neospora caninum* in *Rattus rattus* in absence
  of domestic reservoir and definitive hosts. Vet. Parasitol. 199, 247–249.
- 446

Table 1. Resident dogs found infected with *Leishmania infantum* by serology and/or PCR in
municipalities of Piedmont, and results of entomological surveys targeting the presence of the sand fly *Phlebotomus pernicious*. ND: no entomological survey was performed

Drawings and	No. of	No. of		P. perniciosus
<i>municipality</i>	INO. OI		Endemicity status	presence
municipanty	lested dogs	positive		(yes/no)
Turin				
Angrogna	3	2	Newly demonstrated	Yes
Bibiana	9	1	Newly demonstrated	ND
Cafasse	17	5	Confirmed	Yes
Carmagnola	2	1	Newly demonstrated	ND
Cavour	2	1	Newly demonstrated	No
Cuorgnè	5	4	Confirmed	Yes
Favria	2	2	Confirmed	ND
Feletto	3	1	Newly demonstrated	Yes
Foglizzo	1	1	Newly demonstrated	ND
Givoletto	19	4	Confirmed	Yes
Grugliasco	2	1	Newly demonstrated	No
La Cassa	6	2	Confirmed	Yes
Leini	1	1	Confirmed	Yes
Locana	1	1	Confirmed	Yes
Mathi	2	1	Confirmed	Yes
None	2	1	Newly demonstrated	No
Ozegna	1	1	Confirmed	Yes
Rivoli	4	2	Confirmed	Yes
San Gillio	14	4	Confirmed	Yes
Scalenghe	5	1	Newly demonstrated	No
Turin	4	1	Confirmed	Yes
Val della Torre	44	16	Confirmed	Yes
Vallo Torinese	3	1	Confirmed	Yes
Valperga	1	1	Confirmed	Yes
Cuneo				
Barge	2	1	Newly demonstrated	Yes

Carrù	5	1	Newly demonstrated	Yes
Dogliani	1	1	Newly demonstrated	ND
Fossano	1	1	Newly demonstrated	Yes
Gorzegno	10	1	Newly demonstrated	Yes
Manta	2	1	Newly demonstrated	Yes
San Benedetto Belbo	3	1	Newly demonstrated	ND
Vercelli				
Asigliano Vercellese	7	1	Newly demonstrated	ND
Ronsecco	1	1	Newly demonstrated	ND
Tricerro	1	1	Newly demonstrated	ND
Villarboit	28	7	Newly demonstrated	ND
Villata	2	1	Newly demonstrated	ND
Biella				
Camburzano	1	1	Newly demonstrated	Yes
Candelo	2	1	Newly demonstrated	Yes
Gaglianico	5	1	Newly demonstrated	Yes
Lessona	2	1	Newly demonstrated	ND
Zubiena	7	3	Newly demonstrated	Yes
Novara				
Cavallirio	3	1	Newly demonstrated	Yes
Galliate	3	2	Newly demonstrated	ND
Verbano-Cusio-Ossola				
Omegna	4	3	Newly demonstrated	Yes
Alessandria				
Pozzolo Formigaro	1	1	Newly demonstrated	ND

Table 2. Resident dogs found infected with *Leishmania infantum* by serology and/or PCR in
Lombardy municipalities with previously unknown *Leishmania* endemic status and newly
demonstrated in this study. All tested dogs met criteria for inclusion. ND: no entomological survey
performed.

457

Province and municipality	No. of tested	No. of	P. perniciosus
	dogs	positive	presence
Pavia			
Bressana Bottarone	4	3	ND
Cava Manara	6	4	ND
Ceranova	1	1	ND
Certosa di Pavia	1	1	ND
Cervesina	1	1	Yes
Dorno	3	3	ND
Frascarolo	9	2	ND
Gambolò	2	1	ND
Garlasco	4	1	ND
Mornico Losana	4	3	ND
Palestro	1	1	ND
Pavia	4	2	ND
Pinarolo Po		1 (*)	ND
Robecco Pavese		1 (*)	ND
San Martino Siccomario	1	1	ND
San Zenone al Po	1	1	ND
Sannazzaro de' Burgondi	3	2	ND
Sant'Alessio con Vialone	1	1	ND
Sommo	3	1	ND
Travacò Siccomario	2	2	ND
Verrua Po	2	2	ND
Voghera	1	1	ND
Lodi			ND
Casalpusterlengo	1	1	ND
San Rocco al Porto		1(*)	ND

Bergamo

	Almenno San Bartolomeo	1(*)	Yes	
	Almenno San Salvatore	1(*)	Yes	
	Cremona			
	Soncino	1(*)	ND	
458	(*) index case			—

Table 3. Resident dogs found infected with *Leishmania infantum* by serology and/or PCR in
 municipalities of the provinces of Vicenza (Veneto region), Gorizia and Trieste (Friuli-Venezia)

<i>Province</i> and municipality	No. of tested dogs	No. of positive (%)	Endemicity status	P. perniciosus presence
Vicenza				
Caltrano	99	20 (*)	Newly demonstrated	Yes
Malo	39	1	Newly demonstrated	Yes
Monteviale	24	2	Confirmed	ND
Sarego	16	2	Confirmed	ND
Valdagno	367	10 (*)	Newly demonstrated	Yes
Val Liona (**)	5	1	Confirmed	Yes
Vicenza	6	1	Confirmed	ND
Total	556	37 (6.6%)		
Treviso				
Carbonera		1 (*)	Newly demonstrated	ND
Gorizia				
Staranzano	1	1	Newly demonstrated	ND
Trieste				
Trieste	103	3 (2.9%) (*)	Newly demonstrated	Yes

462 Giulia). ND: no entomological survey performed.

463 (\*) including index case(s)

464 (\*\*) Up to 2017 it was made up of 2 municipalities, Grancona and Germano dei Berici, both already
465 recorded as endemic for CanL

<i>Province</i> and municipality	No. of sa	Endemicity status		
	Sticky traps	CDC light traps	CDC-CO <sub>2</sub> traps	Endemicity status
Vicenza				
Brendola	0	10	195	Confirmed
Caltrano	0	1	45	Newly demonstrated
Lonigo	0	6	50	Confirmed
Malo	0	7	1	Newly demonstrated
Valdagno	37	0	1	Newly demonstrated
Val Liona (*)	0	15	344	Confirmed
Trieste				
Trieste	0	2	12	Newly demonstrated

467 Table 4. Municipalities of Vicenza and Trieste provinces positive for phlebotomine sand flies468

469 (\*) see footnote of Table 3 regarding this municipality.

- 471 Fig. 1. Distribution of municipalities reporting autochthonous cases of canine leishmaniosis associated
  472 with *Leishmania infantum* transmission by phlebotomine vectors north of the Apennine mountains,
  473 Italy, 2013.



477 Fig. 2. Maps showing the five investigated northern regions of Italy with municipal boundaries. 478 Municipalities with geomorphological and environmental characteristics considered unsuitable for the 479 colonization of sand flies are shown in blue. Municipalities known already to be endemic for 480 leishmaniosis by the end of 2017 are shown in red, and those newly identified as endemic in the 481 present study are shown in green. A: Aosta Valley and Piedmont; B: Lombardy; C: Veneto; D: Friuli-482 Venezia Giulia

