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

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ABSTRACT

Canine leishmaniosis (CanL) is an emerging zoonosis caused by *Leishmania infantum* and transmitted in southern Europe by phlebotomine sand flies of the subgenus *Phlebotomus* (*Larroussius*). Endemic foci of CanL have been recorded in northern continental Italy since early 1990s and attributed to the northward expansion of vector populations due to climatic changes in association with travelling/relocated infected dogs from the southern Mediterranean littoral. In this study, further spread of endemic *Leishmania* foci was monitored during 2018-2019 in five regions (Aosta Valley, Piedmont, Lombardy, Veneto and Friuli-Venezia Giulia), with focus to territories where investigations 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 if their origin from, or travel to, areas endemic for CanL were excluded in the previous ≥ 2 years. Around these index cases, i) serosurveys for *L. infantum* were carried out where indicated, ii) 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 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, mainly *Phlebotomus perniciosus*, were collected for the first time in 23 municipalities. The newly recorded endemic municipalities appear to be distributed over a west-to-east decreasing gradient: 30 in Piedmont, 21 in Lombardy, 4 in Veneto and 2 in Friuli-Venezia Giulia. As regards Veneto, it should be noted that a relatively restricted territory was investigated as several municipalities from other provinces had already been detected as endemic for CanL in the past. Cold climate conditions of the easternmost region of Friuli-Venezia Giulia bordering non-endemic territories of Slovenia, are probably less favorable to *L. infantum* transmission.

1. Introduction

Canine leishmaniosis (CanL) is a zoonotic disease of emerging importance caused by the protozoan parasite *Leishmania infantum* (Kinetoplastida: Trypanosomatidae) and transmitted in the European region by phlebotomine sand flies of the subgenus *Phlebotomus* (*Larroussius*) (Diptera: Psychodidae) (Alten et al., 2016; Gradoni et al., 2017). The disease is traditionally endemic in southern countries, but factors associated with the *de novo* colonization and establishment of vectors, and increased burden of infected canine hosts may contribute to the spread and occurrence of this disease in other 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, actually, permanent vector populations were recorded in regions or countries north of their traditional distribution such as South Tyrol, Austria and Germany as regards central Europe (Morosetti et al., 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., 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 Animal Health, regions or countries endemic for CanL should be defined as such based on the demonstration of locally acquired canine infections (autochthonous cases) associated with the presence of competent phlebotomine vectors in the same territory (EFSA AHAW Panel, 2015). Local 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., 2019), should not be attributable to an endemic status of the area in the absence of the specific vector.

In northern continental Italy, the occurrence of autochthonous CanL and human leishmaniasis has been associated with a 30-year northward expansion and increase in density of the phlebotomine vectors *Phlebotomus perniciosus* and *Phlebotomus neglectus* (Maroli et al., 2008). Before 1990, there was no convincing evidence for local *L. infantum* transmission north of Liguria and Emilia-Romagna regions; from early 1990 through mid 2000s, retrospective literature analysis and prospective field 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, 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 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 projects, namely the ECDC "European Network for Arthropod Vector Surveillance for Human Public

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 aforementioned 5 regions, with particular focus to areas where investigations have not been performed, or previous investigations gave inconclusive results. Therefore the study area did not include pre-apennine territories of Emilia-Romagna and the southern part of Piedmont, for which a diffuse endemic status of human and canine *Leishmania* infections has definitely emerged over the 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, located in the northernmost territory of the Italian eastern Alps, was recently investigated for the same purpose (Morosetti et al., 2020).

2. Methods

2.1 Organization and study design

The study was carried out regionally by the veterinary Departments of Torino and Padova Universities, with the collaboration of private and public veterinarians and laboratories operating in the study areas, under the coordination of Istituto Superiore di Sanità, Roma, and the veterinary Department of the Naples University. The first step was to identify index cases from target territories, consisting of dogs suspected to be infected with *L. infantum* on clinical ground as reported by local veterinary practitioners. The animals had to be in the age range 6 months – 12 years, had to have confirmed diagnosis of CanL by reference diagnosis centers, and the infection had to be acquired locally, i.e. be autochthonous with reasonable confidence; this criterion required accurate individual 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 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 suspected dogs living in the index case's area were collected through the help of local veterinary practitioners. Suitable sites identified in the territory were also investigated for the presence of phlebotomine sand fly species, with focus on competent *L. infantum* vector species expected in the area, i.e. *P. perniciosus* and *P. neglectus*.

2.2 Study area

It included the largest part of northern Italy, from Piedmont at west to Friuli-Venezia Giulia at east, covering 78,780 km^2 and corresponding to one fourth of the Italian territory, divided into about 3600 municipalities with 20.5 million human population. Within the involved regions, a selection of the territories to be investigated was operated by excluding municipalities having geomorphological and environmental characteristics considered unsuitable for the colonization by phlebotomine sand flies. To this aim, a nation-wide GIS database of bio-geographic factors such as aspect, elevation and land 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 conditions suitable for all Italian species of sand flies, both competent and non-competent vectors of *L. infantum* (Rossi et al., 2007; Bongiorno et al, 2008; Morosetti et al., 2009; Bongiorno et al, 2010; Busani et al, 2012; data on file, Istituto Superiore di Sanità).

2.3 CanL diagnosis and entomological surveys

Index cases of leishmaniosis were confirmed by the following Italian public reference centers for *Leishmania* infection diagnosis: Istituto Zooprofilattico sperimentale del Piemonte, Liguria e Valle d'Aosta, Imperia; Dipartimento di Scienze Veterinarie dell'Università degli Studi di Torino, Grugliasco; Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padua; and Istituto Superiore di Sanità, Roma. Serum samples from suspected clinical cases were tested by in-house IFAT at the threshold titer of 1:80 or 1:160 (Gradoni and Gramiccia, 2014), or by in-house Western blotting (Ferroglio et al., 2007) using the same reference strain of *L. infantum* as the antigen source. For the test of sera collected in surveys on healthy canine populations, considering an expected low parasite exposure in the study area with prevalent subclinical infections, IFAT was performed at the 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 peripheral blood, bone marrow aspirate samples, or other tissue material where appropriate. Different amplification protocols were used by laboratories: conventional PCR targeting a *L. infantum*-specific fragment of kDNA (Zanet et al., 2014); nested-PCR targeting a *Leishmania* specific sequence in a kinetoplastid-specific fragment of the small subunit rRNA gene (Gramiccia et al., 2010); real-time PCR targeting a *L. donovani* sequence of cytochrome c oxidase subunit II gene (Vascellari et al., 2016). Dogs' identification, anamnesis records, clinical data and CanL diagnosis results were recorded using Castor Electronic Data Capture database [2019 online version].

Trapping of adult phlebotomine sand flies was performed during the expected density peaks in northern Italian latitudes (i.e. July-August) and mainly carried out using 20×20 cm sticky papers coated with castor oil set in each site for 1-2 nights up to a maximum of 5 nights. CDC miniature light traps (John W. Hock Co., Gainesville, FL, USA; Bioquip Products, Rancho Dominguez, CA, USA) or CDC-CO_2 traps (Byblos, Cantù, Italy) filled with 1 kg dry ice (Signorini et al., 2013) were also used. They were placed overnight outside or inside animal shelters. Sand fly specimens were identified by morphological characteristics to species level according to Theodor (1958), Léger et al. (1983) and Dantas-Torres et al. (2014).

3. Results

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.

3.1 Aosta Valley and Piedmont

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. 2**A).

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.

3.2. Lombardy

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

3.3 Veneto and Friuli-Venezia Giulia

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_2 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.

4. Discussion

Whereas about twenty investigated municipalities already known to be endemic for leishmaniosis were confirmed to be so, the new endemic territories recorded by this study were not evenly distributed throughout the 5 regions surveyed, as our results showed an evident west-to-east decreasing gradient: 30 newly endemic municipalities detected in Piedmont, 21 in Lombardy, 4 in Veneto and 2 in Friuli-Venezia Giulia. As regards Veneto region, this could be partially explained by the relatively old records of CanL endemicity involving several municipalities of Verona and Padua provinces, which had been actively investigated in the past and hence new investigational territories 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 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 integrated surveillance of West Nile disease. However, the sand fly population density was confirmed to be extremely low and hence with limited epidemiological significance (Michelutti et al., 2021). In 22/57 newly endemic municipalities, competent *L. infantum* vectors (mainly *P. perniciosus*) could be demonstrated by means of sticky paper or CDC-trap collections performed during a few nights per season, thereby suggesting an elevated phlebotomine density as also expected by predictive models recently applied in Piedmont, Veneto and Friuli-Venezia Giulia (Signorini et al., 2014; Moirano et al., 2020).

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 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 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 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 (Franco et al., 2011; Foglia Manzillo et al., 2018). Because the present study did not involve systematic serosurveys in normal canine populations throughout the investigated territory, we could 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 about ten folds (2.4% in Valdagno versus 22.4% in Caltrano) (**Table 3**). This finding confirms previous reports (Simonato et al., 2020) on the scattered presence in north Italy of newly established foci with intense transmission, where canine seroprevalence may reach values similar to those found in southern Italy. On the other hand, data gathered and added from a number of other sites of Vicenza province, and involving over 500 dogs in total, resulted in 6.6% prevalence of autochthonous CanL (**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 autochthonous *L. infantum* seropositives from two foci and involving about 1100 examined dogs, was 1.7% in Verona province and 1.0% in Treviso province, respectively (Maroli et al., 2008).

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.

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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

Province and municipality	No. of tested dogs	No. of positive	Endemicity status	*P. perniciosus* presence (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.

Province and municipality	No. of tested dogs	No. of positive	*P. perniciosus* 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

(*) 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 Giulia). ND: no entomological survey performed.

Province 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

(*) including index case(s)

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

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

Province and municipality	No. of sand fly specimens per trap type			Endemicity status
	Sticky traps	CDC light traps	CDC-CO_2 traps	
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

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

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

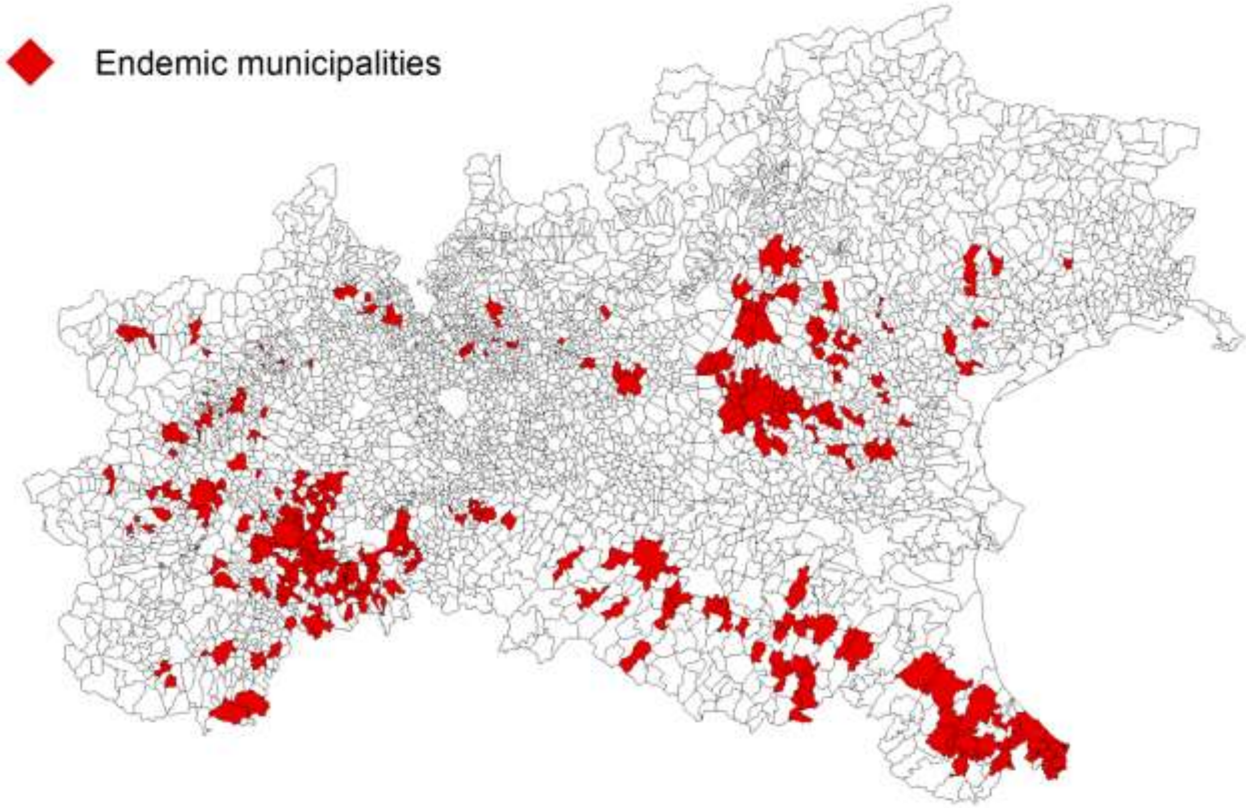
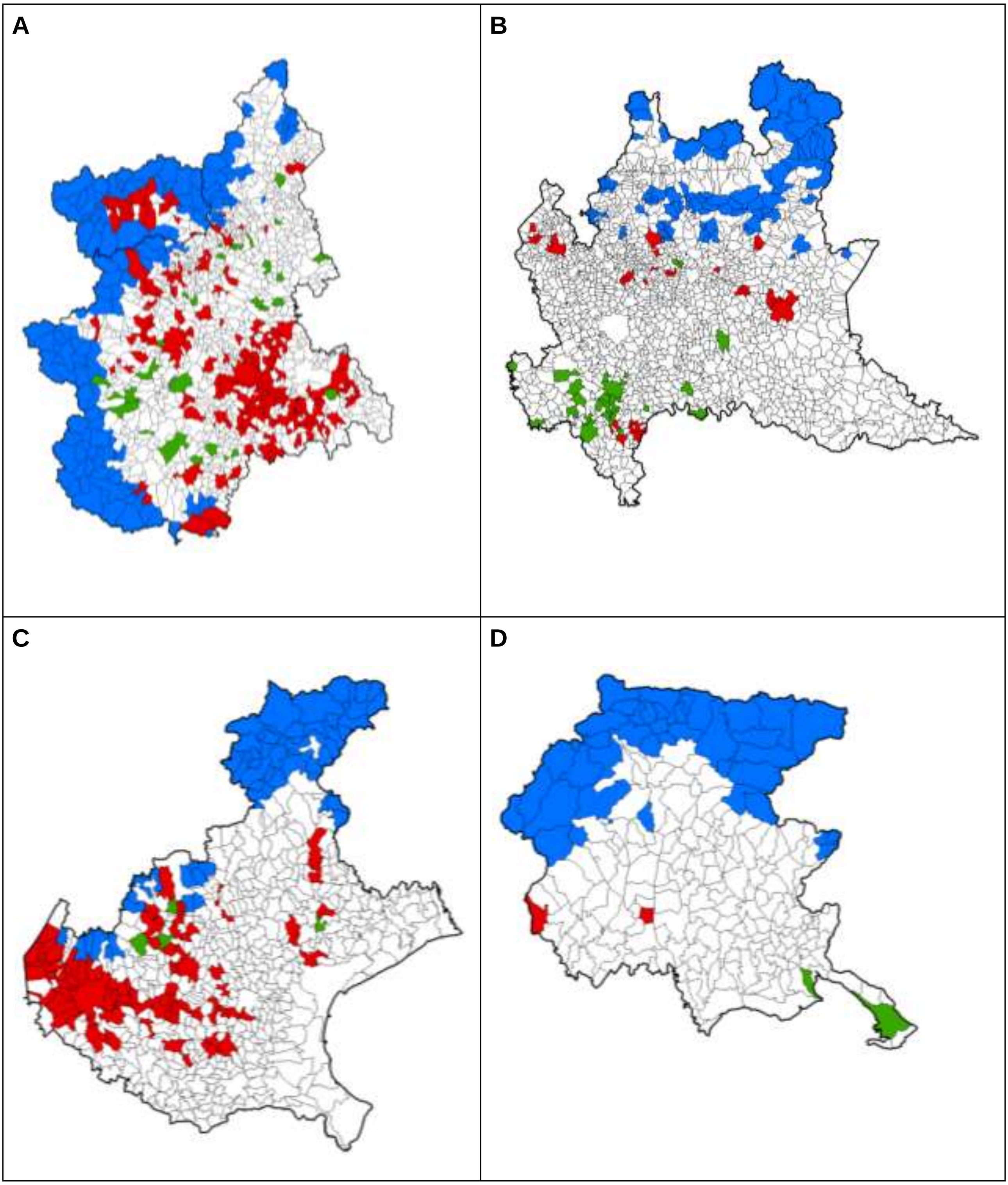


Fig. 2. Maps showing the five investigated northern regions of Italy with municipal boundaries. Municipalities with geomorphological and environmental characteristics considered unsuitable for the colonization of sand flies are shown in blue. Municipalities known already to be endemic for leishmaniosis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


A
B
C
D