Leishmania infantum and *Dirofilaria immitis* infections in Italy, 2009-2019: changing distribution patterns

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Research

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Abstract

Background: For long time, canine leishmaniosis (CanL) was considered endemic in the southern, central, and insular regions of Italy, whereas heartworm disease (HW) by Dirofilaria immitis in the northern region and in the swampy Po valley. Following the reports of new foci of both diseases, in this study we update the distribution patterns and occurrence of new foci of CanL and HW discussing the main drivers for the changes in the epidemiology of these two important zoonotic canine vector-borne diseases.

Methods: Based on the statistical analyses of serological assays (n=90,633) on L. infantum exposure and D. immitisinfection performed by reference diagnostic centres in Italy over a ten-year period (2009–2019), the distribution patterns of both pathogens were herein presented along with the occurrence of new foci.

Results: Results highlighted the changing distribution patterns of L. infantum vs D. immitis infection in Italy. CanL is now endemic also in the northern regions and HW has endemic foci in central and southern regions and islands.Significant differences in L. infantum exposure and HW infection prevalence among the study macroareas were detected. The overall prevalence of L. infantum exposure was 28.2% in southern Italy and Islands, 29.6% in central Italy and 21.6% in northern Italy and that of HW of 2.83% in northern Italy, 7.75% in central Italy and 4.97% in southern Italy and islands. HW prevalence significantly varied over years ($\chi 2=108.401$, d.f.=10, $p<0.0001$), gradually increasing from 0.77% in 2009 to 8.47% in 2016-2017.

Conclusions: The new epidemiological scenarios have been discussed according to a range of factors (e.g. environmental modifications, occurrence of competent insect vectors, transportation of infected animals to non-endemic areas, chemoprophylaxis or vector preventative measures), which may affect the current distribution. Overall, results advocate for epidemiological surveillance programs, more focussed preventative and control measures even in areas where few or none cases of both diseases have been diagnosed.

Background

In the last decades, canine vector-borne diseases (CVBDs) have been expanding worldwide due to several factors linked with increase in pet travelling along with owners, relocation of sheltered animals from endemic to previously non-endemic regions, as well as to the modification of the ecology of arthropod vectors and, importantly, environmental modifications [1-3]. Therefore, the spreading of new parasites and pathogens, and related infections, in previously non-endemic geographical areas poses major concerns to veterinary practitioners and, in the case of zoonotic ones, to public health officials [2]. Protozoan *Leishmania infantum* Nicolle and nematode *Dirofilaria immitis* Leidy represent paradigmatic examples of the modification in the distribution of the diseases they cause (i.e., canine leishmaniosis, CanL, and heartworm disease, HW). The expansion of the above diseases has been related to the distribution of their vectors (i.e., for CanL, sandflies of the genus *Phlebotomus* in the Mediterranean region; for HW, several mosquito species, belonging to the genera *Aedes*, *Anopheles* and *Culex*; [4-7]).

In specific geographical contexts, such as Italy, where both CanL and HW have been endemic for long time [8], their ecology and distribution have been studied and new foci were reported by a retrospective analysis focusing on the period from 1990 to 2009 [1]. Indeed, until 1990, CanL was considered endemic in the southern, central, and insular regions of Italy, whereas dirofilariosis in the northern region and in the Po valley [9,10]. Sporadic case reports suggested that the distribution of both CVBDs has been changing, in that, dirofilariosis expanded towards the southern regions and CanL to the northern regions [1]. The spreading of dirofilariosis in previously non-endemic areas has been facilitated by the absence of chemoprophylaxis measures in the canine population, by using macrocyclic lactones (ML), as routinely performed in endemic areas of northern Italy [11]. Indeed, the only species of filarioids historically diagnosed in southern Italy have been *Acanthocheilonema reconditum* and *Dirofilaria repens*, causing less pathogenic subcutaneous filariosis [12,13].

Meanwhile, the perception of clinicians and parasitologists has most likely changed, resulting into an increase of the request for diagnostic tests of *D. immitis* infection in central-southern Italy, as well of *L. infantum* exposure in northern regions [8]. In this scenario, the aim of the present study is to analyse the results of serological assays performed by reference diagnostic centres in Italy over a ten-year period (2009 – 2019), therefore updating the distribution patterns and occurrence of new foci of CanL and HW.

Methods

Databases of serological tests in dogs from Italy

Databases from two diagnostic reference centres (hereafter reported as database A and B) were analysed (total number of serum samples =90,633). Italian macroareas were defined as northern, central and southern/islands macroareas (Fig. 1).

The complied data was included in the same database to observe significant variations on positive serological results and records of new non-endemic foci. The database A included 64,375 records of dogs collected in a 10-year period (2009–2019) throughout the Italian peninsula, including information regarding breed, sex, age (data not shown). Different serological (Novatec® kit for CanL and Dirocheck Zoetis® for HW) Enzyme Linked Immune Sorbent Assay (ELISA) tests were used. Out of 64,375 records, 78 were excluded due to uncertainty of the origin of the samples.

The second dataset (B) included information of 26,258 of dogs examined from northern Italy (i.e., Emilia Romagna, Lombardia, Trentino-Alto Adige, and Piedmont), central Italy (i.e., Lazio and Tuscany), and mainly from southern Italy and islands (i.e., Basilicata, Calabria, Campania, Apulia, Sicily and Sardinia) in a five-year period (2015 – 2019). This dataset also comprised information regarding breed, sex, age (data not shown), and serological (VetLine® *Leishmania* for CanL, sensitivity and specificity: >98%; and Filarcheck 96® for HW, sensitivity: 97.6%, specificity: 100%) ELISA, and an indirect semi-quantitative immunofluorescence test (MegaFLUO LEISH® for leishmaniosis, sensitivity: 96.9 %, specificity: 98.7 %) with positive or negative results. Out of 26,258 records, 70 were excluded due to uncertainty of the origin of the samples, and other 423 samples from the northern and central regions were not considered for the statistical analysis given that the data was biased by transportation of positive animal from endemic areas.

Statistical analyses

Differences in the prevalence of *L. infantum* exposure and *D. immitis* infection in the studied dogs from northern, central and southern Italy over time were analysed by JMP 9 (SAS) by using weighted generalized linear models (GLZ) with a binomial distribution to test model positive and negative serological outcomes. For each parasite, a GLZ with two fixed factors was used to assess significant differences in *L. infantum* exposure or *D. immitis* infection prevalence among the study macroareas over years: $y = X\beta + \varepsilon$ where y is the vector of the observation [i.e. serological outcome: positive=1, negative=0, X is the incidence matrix, β is the vector of fixed effects (the study macroarea (northern, central or southern Italy and islands) and years)] and ε is the vector of the random residual effects (p=0.05).

Then, a dataset was created for each study macroarea and a GLZ with two fixed factors was used to evaluate significant differences in *L. infantum* exposure or *D. immitis* infection prevalence among the region and years within a given macroarea; the structure of the GLZ was identical to the above described one, with two fixed effects [i.e. the study region and year]. A p-value of 0.05 was used as threshold to assess significant differences among values. To verify that the changing distribution patterns of *L. infantum* and *D. immitis* were not random, a contingency analysis assessing the relationship between the *L. infantum* exposure and *D. immitis* infection prevalence in the various macroareas, regions and study years was also carried out [14].

Serological data were presented in terms of bi-annual and cumulative prevalence; distribution maps of cumulative positive cases for *L. infantum* exposure and *D. immitis* infections were generated using QGIS version 3.4.4-Madeira [15].

Results

Overall, the number of serological tests for *L. infantum* exposure (n=80,309) performed in the three areas of Italy is reported in Table 1, being higher in southern Italy (37.7% of all tests performed) than in central Italy (31%), and northern Italy (24.7%). Conversely, the overall number of tests requested for the diagnosis of *D. immitis* infection throughout Italy (n=10,324) was significantly lower (11.3% of all the requested serological tests) than that for *L. infantum* exposure, with a relative high number in northern Italy (51.7% of all tests performed) and the lowest in southern Italy (Table 1).

Results showed a significant difference in *L. infantum* exposure prevalence among the different study macroareas (χ^2=218.564, *d.f.*=2, p<0.0001), with an overall prevalence of 28.2% in southern Italy and islands, 29.68% in central Italy and 21.62% in northern Italy (Table 1; Fig. 2). The impact of the study year on *L. infantum* exposure prevalence in Italy was also significant (χ^2=559.846, *d.f.*=10, p<0.0001), with values >30% in 2011, 2012, 2014 and 2015. *D. immitis* infection prevalence showed significant differences among the study macroareas (χ^2=114.879, *d.f.*=2, p<0.0001), being of 2.83% in northern Italy, 7.75% in central Italy and 4.97% in southern Italy and islands. On the whole Italian territory, HW prevalence significantly varied over years (χ^2=108.401, *d.f.*=10, p<0.0001), gradually increasing from 0.77% in 2009 to values ranging from 5.19-8.47% in 2016-2017 (Table 1; Fig. 3).

A detailed analysis over *D. immitis* infection and *L. infantum* exposure prevalence trend over time in the three Italian macroareas was provided by assessing the impact of the study year and region on *D. immitis* infection and *L. infantum* exposure prevalence. In northern Italy *L. infantum* exposure prevalence showed significant differences over the study years (χ^2=286.277, *d.f.*=10, p<0.0001), being highest in 2012 and 2014 (i.e., 33.51% and 27.27%, respectively) (Fig. 2). Notably, significant variations in *L. infantum* exposure prevalence among the various northern Italy regions were recorded (χ^2=190.657, *d.f.*=7, p<0.0001), with the highest values in Piedmont, Trentino Alto Adige, Valle d'Aosta, and Friuli Venezia Giulia (28.93%, 27.59%, 27.40% and 27.17%, respectively). The study year and region significantly impacted *D. immitis* infection prevalence in northern Italy (χ^2=56.954, *d.f.*=10, p<0.0001; χ^2=40.555, *d.f.*=7, p<0.0001, respectively), being highest prevalence rate observed in 2016 (7.20%) (Fig. 3). Highest prevalence values of *D. immitis* infection were retrieved in Val d'Aosta (11.36%), Trentino Alto Adige (7.41%) and Piedmont (6.29%), while the lowest regional prevalence was in Veneto (2.12%).

In central Italy, both the study year and region led to significant differences in *L. infantum* exposure prevalence (χ^2=371.252, *d.f.*=10, p<0.0001; χ^2=609.769, *d.f.*=5, p<0.0001, respectively). It was more than 30% in 2012, 2014, 2015 and 2016 (Fig. 2), with the highest values in Lazio (38.52%), followed by Umbria (35.61%) and Abruzzo (34.10%). *Dirofilaria immitis* infection prevalence was also affected by the study year (χ^2=55.333, *d.f.*=10, p<0.0001), showing values >10% in 2016 (i.e., 12.1%) and 2017 (i.e, 13.32%) (Fig. 3). Significant differences in the prevalence of *D. immitis* infection were noted among central Italy (χ^2=80.975, *d.f.*=4, p<0.0001, respectively) with the highest values recorded in Tuscany (11.48%) and Marche (7.84%).

In southern Italy and islands, *L. infantum* exposure prevalence was significantly different among the study years (χ^2=201.963, *d.f.*=10, p<0.0001), with values ranging from 24.55% (2017) to 50.66% (2011) (Fig. 2). *L. infantum* exposure prevalence in this macroarea also showed significant differences among regions (χ^2=642.949, *d.f.*=6, p<0.0001) with the highest values recorded in Molise (54.26%), Sicily (50.18%) and Sardinia (38.34%). On the other hand, the study region did not play a significant role impacting *D. immitis* infection prevalence, even if a trend was observed (χ^2=10.723, *d.f.*=6, p=0.10, respectively). Indeed, the largest number of analysed samples was from Apulia (n = 1608) followed by Basilicata (n = 80) and Sardinia (n = 70). Considering regions with sample size >40, the highest prevalence rates were from Sardinia and Apulia (10%, and 4.73%, respectively). The effect of the study year was not significant (χ^2=16.145, *d.f.*=10, *p*=0.136) due to the limited number of samples examined in the years 2009-2014 (i.e., n=117), at variance with the larger samplings done in 2015-2019 (i.e., n=1,729).

Contingency results obtained analysing the separate datasets for each study macroarea are given in the Supplementary Online Material Table S1. Overall, observed changing distribution patterns of *L. infantum* exposure and *D. immitis* infection in Italy were not random or due to a biased sampling over the different areas and years (*L. infantum*, macroarea: χ^2=486.62, *d.f.*=2, *p*<0.0001; year: χ^2= 827.903, *d.f.*=10, *p*<0.0001, respectively; *D. immitis*, macroarea: χ^2=104.545, *d.f.*=2, *p*<0.0001; year: χ^2=99.070, *d.f.*=10, *p*<0.0001, respectively). Serological *L. infantum* results had a similar cumulative prevalence throughout the Italian territory (Fig. 2), showing an annual slight decrease in all the regions, from 2015 to 2018. *Leishmania infantum* was widely distributed throughout the Italian peninsula, with a number of positive animals >400 in central (Lazio and Tuscany) and southern (Apulia and Basilicata) regions as well as in both islands (Sardinia and Sicily). In addition provinces with >300 positive samples were in northern regions (i.e., Turin, Piedmont, and Vicenza Veneto) (Table 1; Fig. 4).

The highest prevalence of *D. immitis* was registered in the central regions (i.e., Tuscany and Lazio) followed by southern and northern regions with annual variation patterns throughout the observation period (Fig. 3). An overall lower number of samples was positive for *D. immitis* infection compared to *L. infantum* exposure, with >10 positive cases recorded in three provinces from the northern Italy (i.e., Turin, Piedmont; Genova, Liguria; and Belluno, Veneto). In central Italy, the province with the highest number of HW cases (i.e., Florence, Tuscany) was surrounded by provinces with more than 20 positive samples (i.e., Bologna, Emilia Romagna; Arezzo and Prato, Tuscany). The largest number of positive samples in southern Italy was recorded in the Apulia region (i.e., Brindisi and Lecce provinces). No data was available for four provinces of the North (i.e., Biella, Piedmont; Lecco Lombardia; Piacenza, Emilia Romagna; and Verona, Veneto), for two provinces of southern Italy (i.e., Isernia, Molise; Vibo-Valentia, Calabria), and many provinces of the islands (i.e., Nuoro and Oristano, Sardinia; Agrigento, Caltanissetta and Ragusa, Sicily) (Table 1; Fig. 5).

Discussion

Based on the results of this large dataset (n= 89,186) of serological assays presented herein, the distribution patterns of *L. infantum* exposure and *D. immitis* infection have changed over the last ten years' period (2009-2019)., and in comparison with data available from the prior decade (1999-2009) [1]. The seroprevalence of *L. infantum* exposure in northern Italy increased in the examined 10 years' period (2009 to 2019) from 2.1% (1999-2009; [1,16] to 21.6%. The results also indicate that *L. infantum* exposure has spread progressively in the past decade in the northern Italian regions, with an overall prevalence higher than that recorded in previous studies [1,17]. For example, in the province of Bologna (Emilia Romagna) the seroprevalence increased from 6.6% (i.e., 16 out of 245 animals examined in the period 2007-2009; [17] up to 17.16% (i.e., 217 out of 1,264 tests in this study). Overall, *L. infantum* exposure has spread progressively in the past decades from endemic southern towards northern regions, making the whole Italian peninsula endemic for this infection. Under these circumstances, the effect of relocation of infected animals from the South to the North of Italy could not be fully assessed in the study period. Nonetheleess, the northward spread of the main sand fly vectors [i.e., *Phlebotomus perniciosus* (Newstead) and *Phlebotomus neglectus* Tonnoir], which are now established in several provinces of the northern macroarea [16,18,19] supports the evidence of *L. infantum* endemicity and the occurrence of new foci. In addition, clinicians' perception and awareness of the presence of CanL in non-endemic areas seem to be increased in the northern regions, with more than 21,545 tests performed, whereas the South remains the area with the largest number of tests requested (Table 1).

On the other hand, the cumulative prevalence of *D. immitis* infection greatly increased in central (7.7%) and southern Italy and Islands (5%), being higher than in the North (2.8%), which was historically considered the sole endemic area [1,20]. The decreased prevalence of *D. immitis* infection in northern Italy regions could be a consequence of clinicians' awareness of the disease and thus of the continuous usage of chemoprophylactic programs in this area. Nonetheless, resistance to ML has been demonstrated in *D. immitis* populations from this macroarea [21], which may represent a potential issue (not yet proven) for emergence of resistent strains. Moreover, the low number of tests performed for *D. immitis* infection (10.5% of all the requested serological tests) throughout the Italian peninsula also indicates the scant awareness of the occurrence of the disease, especially in southern regions (Table 1). Given the increased prevalence in the Centre, the South and Islands, clinicians should consider the occurrence of HW cases in non-endemic areas.

Furthermore, our analyses showed that the distribution patterns of *L. infantum* and *D. immitis* in Italy are related to a significant relationship between *L. infantum* exposure/*D. immitis* infection prevalence and the geographical provenience (i.e., macroarea) and study years (2009-2019). Conversely, a major hindrance of this study could be that the anamnesis of animals was not available and therefore their travelling history from/to historically endemic areas for both diseases (i.e., northern Italy for HW and southern and central Italy for CanL) [5,22].

However, the clustering of positive samples in some spots of *L. infantum* exposure [e.g., the provinces of Turin (Piedmont) and Vicenza (Veneto), northern Italy; Fig. 3] and of *D. immitis* infection [e.g., in Brindisi and Lecce (Apulia), southern Italy; Fig 4] is consistent with previous reports. Endemic foci of CanL in the same provinces of Veneto [23] and Piedmont [24] confirmed the above mentioned epidemiological picture with a competent vector of *L. infantum* (i.e., *P. perniciosus*) captured in entomological surveys performed in that area [19]. In addition, other studies reported cases of CanL and human visceral leishmaniasis diagnosed in the same area, along with their vectors, *P. perniciosus* and *P. neglectus* [25].

The southward changing pattern of HW has been detailed in previous studies [26-29]. A recent questionnaire study pointed out Brindisi and Lecce, from Apulia region, to be areas with more than 20 cases of HW caused by *D. immitis* [30]. The low prevalence of HW in Sicily and Sardinia could be explained by the small number of test requested or no tests performed in many provinces from these islands. Indeed, earlier studies illustrated the islands have a large number of diagnosed HW [30] as they are suitable for *Dirofilaria* spp. to thrive [13]. Specifically, Sardinia has the environmental, climatic and human activities (e.g., tourism with animal transportation) that could allow these nematodes to spread [5, 30]. Finally, central Italy showed a distribution pattern on which both pathogens are highly prevalent. In this macroarea, the prevalence of *L. infantum* exposure was higher (29.6%) of that indicated in previous surveys (up to 10%) [31,32]. Hence, central Italian regions are an important pathway for the spreading of CanL from southern to northern.

Conclusions

The large number of data analized strongly supports the above figure of distribution of *L. infantum* and *D. immitis*. Overall, results highlighted the changing distribution patterns of CanL *vs* HW in Italy over a 10-year period (2009-2019) in which CanL is in fact now endemic in the northern Italy regions, and HW has endemic foci in the central and southern Italy and in islands. Moreover, given the number of test requested, the overall clinicians' awareness in northern Italy is increasing for CanL and diminishing for HW. The presented epidemiological picture suggests that veterinarians and public health officials should be aware that these pathogens are distributed throughout the country, thus epidemiological surveillance, preventative and control measures should be carried out to protect dogs from CanL and HW and reduce the risk of infection in humans.

Abbreviations

CVBDs: canine vector-borne diseases; CanL: canine leishmaniosis; HW: heartworm disease; CI: confidence interval.

Declarations

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

All authors declare that they have no competing interests.

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Authors’ contributions

JAMR and DO conceived the study. JAMR, GB perform the data curation, methods and anaylises of data. JAMR, GB, DO wrote the first draft of the manuscript. TF, FB, AZ, DO, RI, RP reviewed the manuscript. All authors read and approved the final manuscript.

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Table

Table 1. Number and percentage (N/%) of serological tests positive for canine leishmaniosis and hearth worm disease in three main areas of Italy.

	North N (%)	Centre N (%)	South and Islands N (%)
Leishmania infantum	(Tot=21,545)	(Tot=26,128)	(Tot=32,610)
	4,664 (21.6)	7,801 (29.6)	9,208 (28.2)
Dirofilaria immitis	(Tot=5,335)	(Tot =3,119)	(Tot=1,866)
	151 (2.8)	243 (7.5)	93 (4.9)

Figures

Figure 1

Italy. Three main areas with their respective administrative regions.

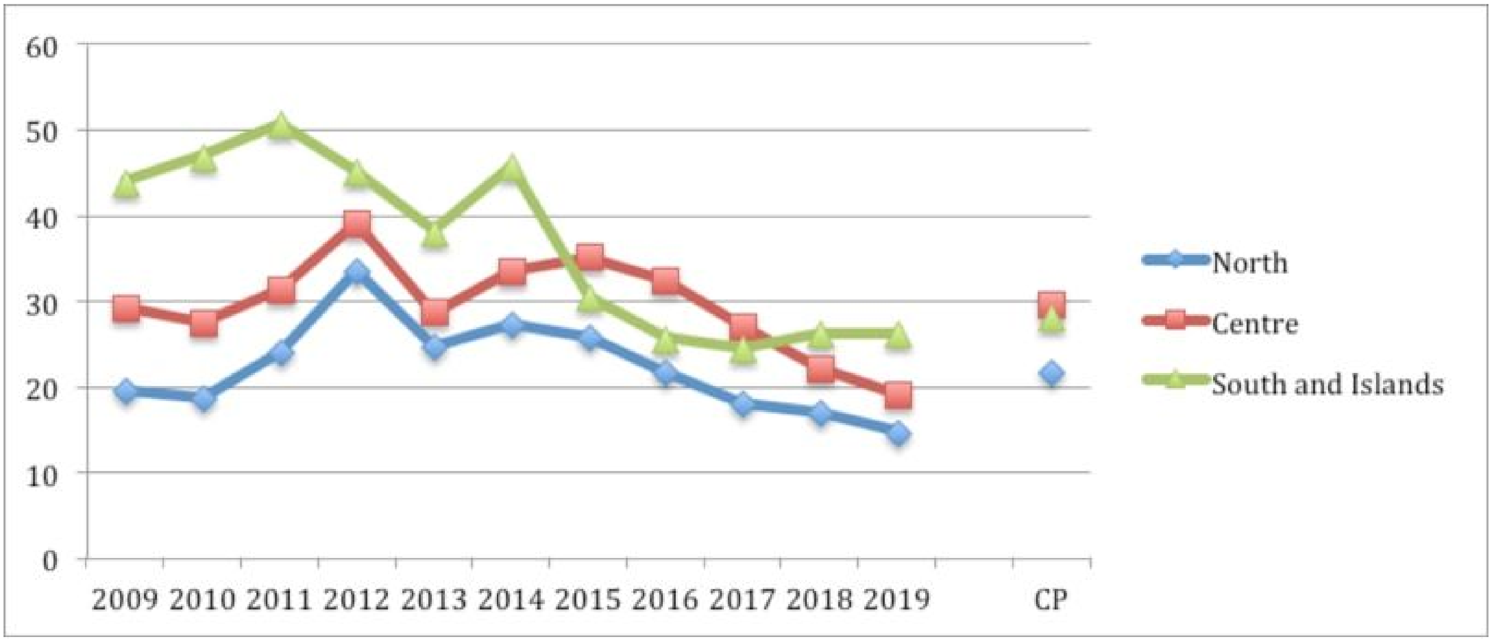

Figure 2

Pattern of mean annual seroprevalence of canine leishmaniosis in the three main regions of Italy. The cumulative prevalence (CP) is also reported.

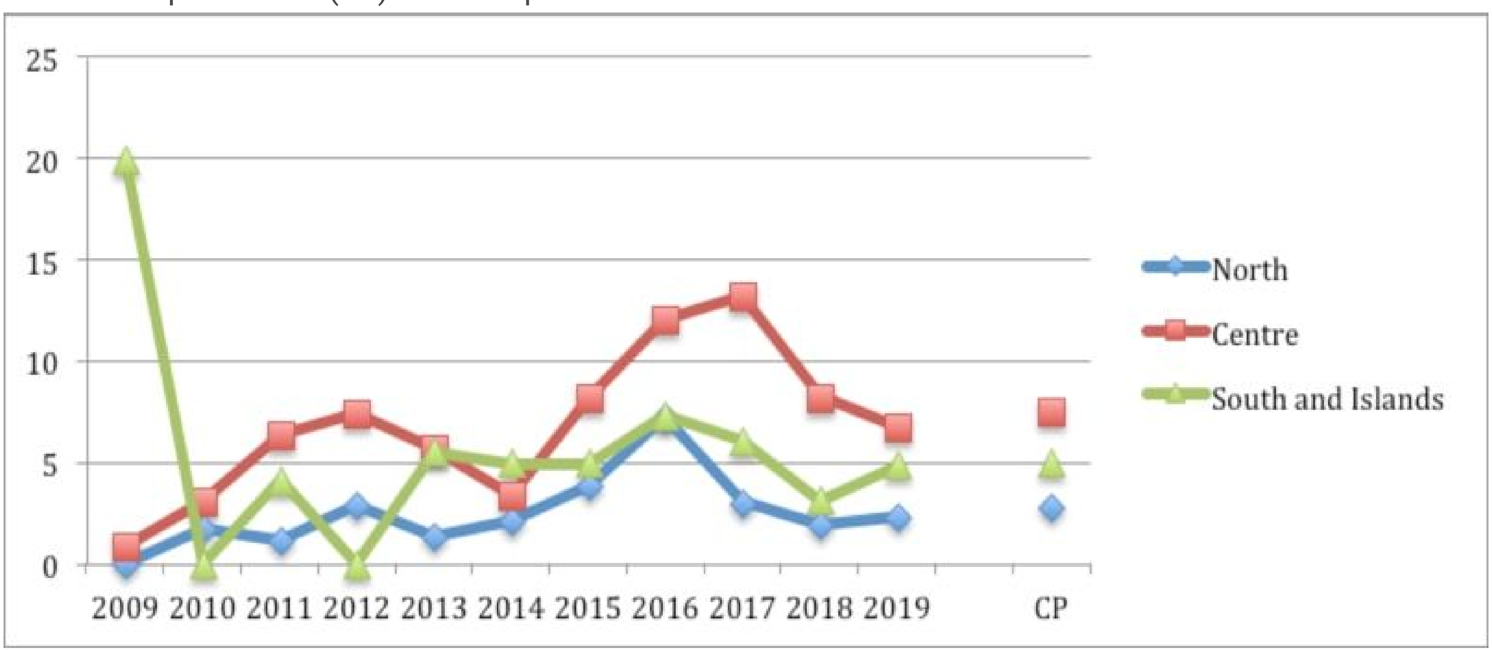

Figure 3

Pattern of mean annual seroprevalence for Dirofilaria immitis in the three main regions of Italy. The cumulative prevalence (CP) is also reported.

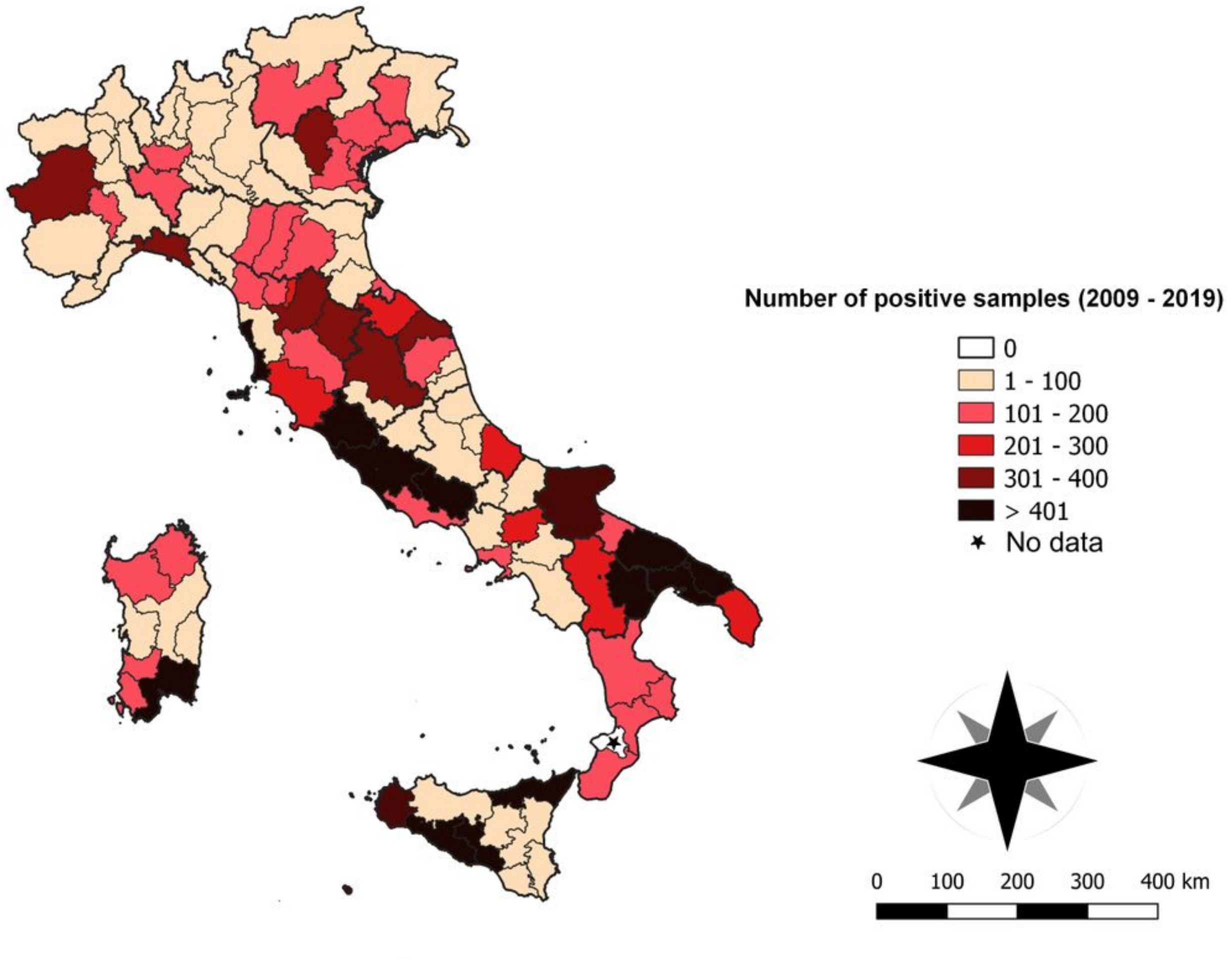

Figure 4

Distribution map with number of cases (2009 – 2019) per province of CanL in Italy.

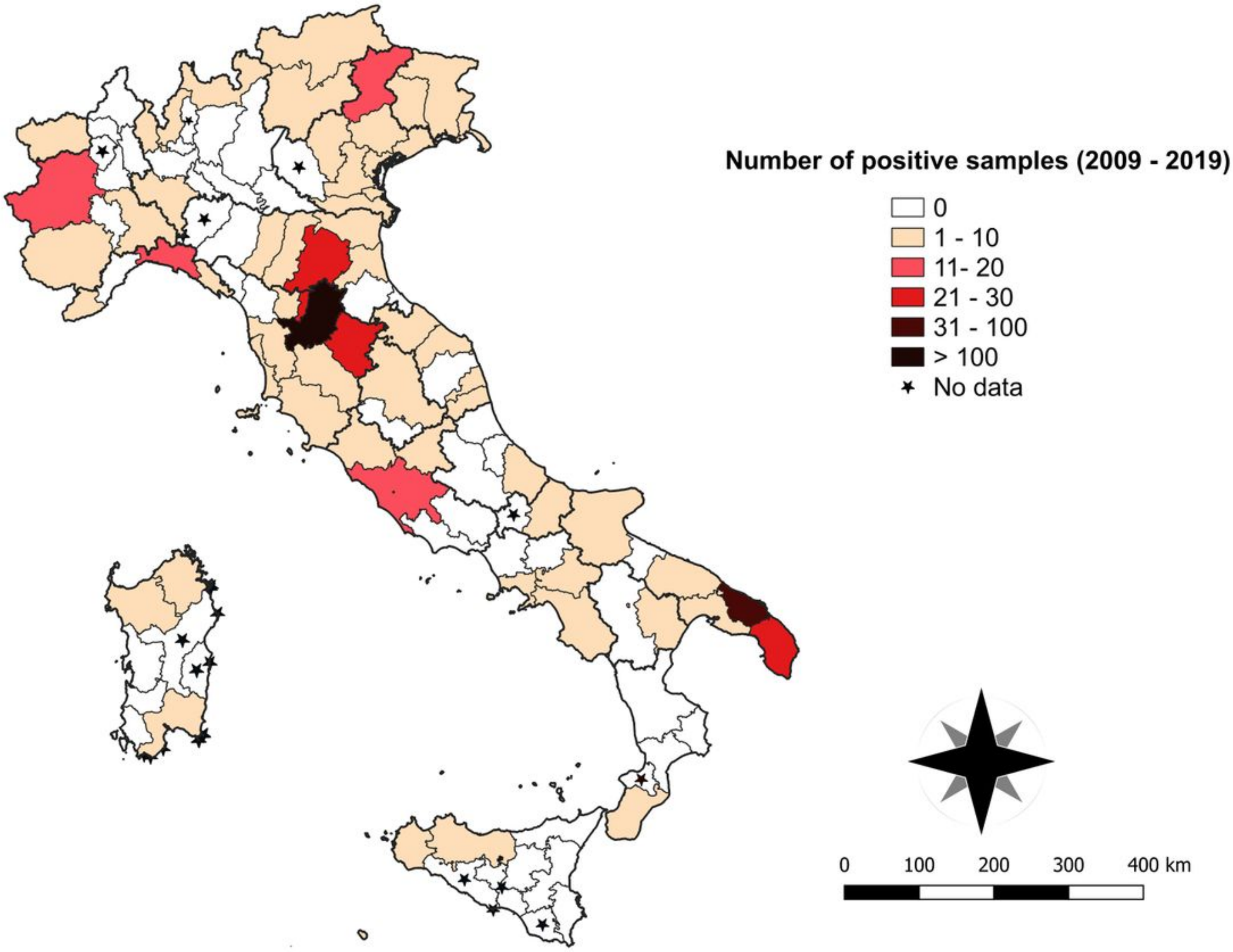

Figure 5

Distribution map with number of cases (2009 – 2019) per province of HW in Italy.

Supplementary Files

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