Received: 18 November 2020 | Revised: 8 January 2021 | Accepted: 3 February 2021

DOI: 10.1111/tbed.14025

SHORT COMMUNICATION

Transboundary and Emerging Diseases WILEY

Incidence of *Dirofilaria immitis* and *Leishmania infantum* infections in sheltered dogs from Southern Italy

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Abstract

Leishmania infantum and *Dirofilaria immitis* are among the most important vector-borne pathogens in Europe, affecting animal and human health. In endemic areas, the epidemiology of both infections is conditioned by abundance of vectors and chemoprophylaxis measures. However, knowledge on the incidence of heartworm (HW) and *Leishmania* infections occurring in sympatry is still scant. Thus, this study aimed to evaluate the incidence of both infections in two dog shelters from southern Italy, which represent hotspots for these two diseases. In June and in October 2020, all dogs that previously scored negative for *L. infantum* (n = 111, site 1; n = 70, site 2) and *D. immitis* (n = 58, site 1; n = 61, site 2) in 2019 were tested for the estimation of the incidence of both infections. Anti-*L. infantum* IgG was detected by immunofluorescence antibody test, whereas *D. immitis* infection was diagnosed by modified Knott's test, SNAP 4Dx Plus test and real-time PCR. The overall *D. immitis* and *L. infantum* infection incidence values were both higher in site 2 (i.e. 63.9% and 10%, respectively) than site 1 (i.e. 39.7% and 1.8%, respectively). The dog shelter in site 2 was shown to be more suitable for the development of the mosquito/sand fly populations and, consequently, for the spreading of both parasites representing a potential threat for animal and human health. The high incidence of both infections recorded in this study suggests the need for chemoprophylaxis measures and vector monitoring and control to minimize the risk for animals and humans living in shelters or in their neighbourhoods.

KEYWORDS

Dirofilaria immitis, heartworm disease, incidence, *Leishmania infantum*, leishmaniosis, vectors

1 | INTRODUCTION

Dogs are continuously exposed to vector-borne pathogens (VBPs), being some of them of zoonotic concern. Among these, *Dirofilaria* spp. and *Leishmania* spp. are common VBPs in Europe, causing canine dirofilariosis and leishmaniosis, respectively (Otranto et al., 2009). *Dirofilaria immitis* (Leidy, 1856) and *Dirofilaria repens*, Railliet & Henry, 1911, are mosquito-transmitted nematodes and etiological agents of heartworm disease (HWD) and subcutaneous dirofilariosis (SCD), respectively, with a broad geographical distribution in temperate and tropical regions (Otranto et al., 2009). On the other hand, the most important agent of canine leishmaniosis (CanL) is *Leishmania infantum*, Nicolle, 1908, a sand fly-transmitted protozoan infecting dogs throughout all continents except Oceania (Dantas-Torres et al., 2012). Both pathogens above have a zoonotic potential, with dogs playing a key epidemiological role as a source of infection for other hosts, including humans living in the same endemic areas (Dantas-Torres et al., 2012; Montoya-Alonso et al., 2011). In the Mediterranean basin, *Dirofilaria* larval development occurs in mosquitoes of the genus *Aedes*, *Anopheles* and *Culex* (Eldridge

& Edman, 2000), while *L. infantum* is transmitted by phlebotomine sand fly species (e.g. *Phlebotomus perniciosus*, Newstead, 1911; *Phlebotomus neglectus*, Tonnoir, 1921; *Phlebotomus papatasi*, Scopoli, 1786; *Phlebotomus ariasi*, Tonnoir, 1921; *Phlebotomus perfiliewi*, Parrot, 1930; *Phlebotomus langeroni*, Nitzulescu, 1930; *Phlebotomus tobbi*, Adler, Theodor and Lourie, 1930) (Maroli et al., 2013). The epidemiology of VBP infections in endemic areas is mainly affected by the abundance and composition of the vector population and the chemoprophylactic measures (Dantas-Torres et al., 2019; Panarese, Iatta, Latrofa, et al., 2020). While chemoprophylaxis reduces the prevalence of parasite infections in an endemic area (Mendoza-Roldan et al., 2020), the vectors involved in the transmission of these pathogens are a major driver of the geographical distribution and spreading to previous non-endemic regions (Panarese, Iatta, Latrofa, et al., 2020). Although the prevalence of HW infection in different canine populations has been investigated under different ecological scenarios (Mendoza-Roldan et al., 2020), data on its incidence in shelter animals are scant. The same applies to *L. infantum*, where the incidence of infection has been investigated in a population of farmed dogs from southern Italy (Paradies et al., 2006) and in untreated, control, sheltered animals, in studies aiming to test the efficacy of pyrethroids in different formulations for preventing CanL (Otranto, de Caprariis, et al., 2010; Otranto et al., 2007). Therefore, the overall calculation of the incidence of the pathogen's infection may be useful in planning efficient control programmes. Thus, this study aimed to evaluate the 1-year incidence of *D. immitis* and *L. infantum* infections in sheltered dog populations living in two hotspots for these diseases.

2 | MATERIALS AND METHODS

2.1 | Study design and enrolled animals

The study was conducted in two dog shelters (i.e. site 1, 40.608705N, 17.994495E, Brindisi; site 2, 40.419326N, 18.165582E, Lecce) of Apulia region, southern Italy. All dogs that scored negative for *L. infantum* ($n = 111$ from site 1 and $n = 70$ site 2) and *D. immitis* ($n = 58$ from site 1 and $n = 61$ site 2) in 2019 were tested for the estimation of the 2020 incidence values (Panarese, Iatta, Latrofa, et al., 2020; Panarese, Iatta, Mendoza-Roldan, et al., 2020). In June and in October 2020, dogs were tested by immunofluorescence antibody test (IFAT) for *L. infantum* and by modified Knott's test, antigen detecting rapid test and real-time PCR (qPCR) for *D. immitis*. All animals included in the study remained untreated with ectoparasiticides/repellents or macrocyclic lactones. Dogs scoring positive for *D. repens* but negative for *D. immitis* ($n = 7$ from site 1 and $n = 4$ from site 2) at the modified Knott's method were included in the examined population. Before any blood sampling, a clinical examination was conducted for each animal. Signalment (e.g. age, sex, breed), clinical signs and previous treatments were recorded in each animal's file along with the individual microchip number. The animals were handled and sampled following the approval of the Ethical Committee of the Department of Veterinary Medicine of the University of Bari, Italy (Prot. Uniba 12/20).

2.2 | Blood sampling and examination

Whole blood was collected into vacuum containers with EDTA (2.5 ml) and into serum collection tubes with clot activator (5 ml) from each dog. The samples were processed by a modified Knott's test for the detection of microfilariae (mfs) as described in Knott (1939). Body length and width of mfs, as well as the morphology of the front end and the tail of five mfs for each sample, were determined using a digitally captured image software LAS V4.5 (Leica Microsystems) and compared with the descriptions given in identification keys (Kelly, 1973). The SNAP® 4Dx® Plus Test (IDEXX Laboratories, Inc.) is a bi-directional flow enzyme-linked immunosorbent assay designed for a point of care (POC) testing and used for the antigen detection of the adult females of *D. immitis*. The test was performed according to the manufacturer's instructions. To assess the exposure of dogs to *L. infantum*, serum samples were analysed by IFAT as described in Otranto, Testini, et al. (2010), for the detection of IgG anti-*L. infantum*. For each test, positive and negative controls were included.

2.3 | Molecular analysis

From an overall of 119 dogs negative for HW infection, genomic DNA was extracted from 100 μl of blood using the QIAamp DNA Minikit (Qiagen, GmbH) and, then, screened by duplex real-time PCR (qPCR) for detection and differentiation of the two species of *Dirofilaria* as described in Latrofa et al. (2012). All DNA samples were tested in duplicate, and positive and negative controls were included in each qPCR run. The specificity of the qPCR assay was established by the melting curve analysis (Latrofa et al., 2012).

2.4 | Data handling and statistical analysis

Data regarding the incidence (i.e. number of new cases of infection in the negative dog population at the start of time interval of 1-year study) of *D. immitis* and *L. infantum* in dogs were recorded in a Microsoft Excel spreadsheet and analysed by Quantitative Parasitology 3.0 software for the subsequent statistical analyses (Rozsa et al., 2000). Odds ratio (OR) values were calculated at a 95% confidence interval (CI). p-values $<.05$ were considered significant.

3 | RESULTS AND DISCUSSION

The 2020 incidence of HW infection, detected by the modified Knott's test in the two dog shelters, was 25.9% (95% CI: 15.2–39.1) and 32.8% (95% CI: 21.9–45.9), in site 1 and 2, respectively, and

TABLE 1 Incidence (95% CI) of *Dirofilara immitis* and *Leishmania infantum* infections in sheltered dogs from site 1 and site 2 detected by modified Knott's test, SNAP 4Dx Plus test, qPCR and IFAT

	D. immitis infection					*L. infantum* exposure		
Sampling site	Knott's test	95% CI	SNAP 4Dx Plus Test	95% CI	qPCR	95% CI	IFAT	95% CI
Site 1	25.9 (15/58)	15.2–39.1	39.7 (23/58)	27.4–52.6	39.7 (23/58)	27.4–52.6	1.8 (2/111)	0.3–6.6
Site 2	32.8 (20/61)	21.9–45.9	60.7 (37/61)	47.5–72.3	63.9 (39/61)	50.8–75.6	10 (7/70)	4.8–19.2

increased up to 39.7% (95% CI: 27.4–52.6; site 1) and 60.7% (95% CI: 47.5–72.3; site 2) using the SNAP 4Dx Plus test. The qPCR conducted on the same blood samples resulted in an incidence of 63.9% (95% CI: 50.8–75.6) in site 2, exceeding the positivity in the serological diagnosis, while confirming the previous incidence value for site 1. All the data above are summarized in Table 1.

The 2020 incidence of *L. infantum* infection was 1.8% (95% CI: 0.3–6.6) in site 1 and 10% (95% CI: 4.8–19.2) in site 2. The dog's exposure to the VBPs in the sampling sites was statistically different in site 2 compare to site 1, for both *D. immitis* ($\chi 2 = 7.02$; $p = .008$) and *L. infantum* ($\chi 2 = 6.11$; $p = .013$).

The 1-year incidence of HW infection herein recorded indicates that this geographical area is one of the most hyperendemic in the Mediterranean regions, therefore representing a high risk of infection for both animals and humans. Indeed, the few data available in literature on HW incidence in owned dogs reported lower values (e.g. 5.2%, Thailand; Boonyapakorn et al., 2008). Although the prevalence of HW infection was not significantly different between the two sampling sites (40.3% in site 1 and 50.3% in site 2; Panarese, Iatta, Latrofa, et al., 2020), the incidence was higher in site 2 than in site 1, indicating the former as more suitable for the perpetuation of HW infection. Similarly, the incidence for *L. infantum* was higher in site 2 (i.e. 10%) than in site 1 (i.e. 1.8%), though the percentage obtained in the latter is close to that reported in previous studies conducted in the same region (i.e. 9.5%, Paradies et al., 2006; 10.6%, Otranto et al., 2007; 7.1%, Otranto, de Caprariis, et al., 2010). Thus, the shelter of site 2 may be a more suitable location for the perpetuation of both VBP infections and should be considered not safe for the dog's health. However, dog shelters may represent a bias in assessing the incidence of any infection by VBPs, due to the non-natural epidemiological context in which many dogs, crowded together, are potentially exposed to high vector populations (Otranto et al., 2017). In fact, dog shelters may offer suitable biotopes for the vector development (i.e. humidity, walls, shadow), hence explaining a higher density than that of a more natural environment. Under the above circumstances, in absence of individual preventative measures (e.g. against vector sand flies and mosquitoes), sheltered dogs are more exposed to arthropod bites and, thus, are at high risk of infection by VBPs (Otranto et al., 2017).

Although a limitation of this study is the lack of entomological surveys in both sampling sites, data collected during the previous years have shown the presence of several mosquito species (e.g. *Aedes caspius*, Pallas, 1771; *Aedes albopictus*, Skuse, 1895; *Anopheles maculipennis*, Meigen, 1818; *Coquillettidia richiardii*, Ficalbi, 1889; *Culex pipiens*, Linnaeus, 1758; *Culiseta annulata*, Schrank, 1776) (Panarese, Iatta, Latrofa, et al., 2020). In particular, the higher density of *Cx. pipiens* and *Ae. caspius* in sites 1 and 2, respectively, could explain the differences in the circulation of *D. immitis* . Indeed, based on vectorial capacity, some mosquito species may be more efficient (e.g. *Aedes* spp.) than others (e.g. *Cx. pipiens*, *Anopheles* spp.) in transmitting the pathogen to the host (Coluzzi & Trabucchi, 1968; McGreevy et al., 1978; Russell & Geary, 1996). Regarding the sand fly population, although no data are available in any of the shelters, the most diffused species reported in the same study region were *Ph. perniciosus*, *Ph. neglectus* and *Ph. papatasi* (Tarallo et al., 2010). However, based on the ecological features of site 1, this seems to be a less suitable environment for their development (i.e. windy area and absence of ravines and dry-stone walls) compared to site 2, where sand flies use to thrive (Maroli et al., 2013). In addition, the low incidence observed for *L. infantum* infection in site 1 (i.e. 1.8%), compared to the high prevalence, suggests that dogs were probably infected by *L. infantum* before their entrance into the shelter.

Data indicate that dogs living in a shelter located in a hyperendemic area for these infections, as well as humans working in shelters or in their neighbourhoods (e.g. animal shelter workers, volunteers) are at high risk of infection. Therefore, veterinarians should be warned of the risk of infection and adopt chemoprophylaxis for reducing the risk of VBP transmission. Importantly, vector control should be constantly advocated in shelters, in order to guarantee animal welfare and public health, given the zoonotic potential of both parasites.

ACKNOWLEDGEMENTS

The authors thank Thomas Matthiew Arme for his support and help during the preparation of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, Rossella Panarese, Roberta Iatta, Domenico Otranto; methodology, Rossella Panarese and Roberta Iatta; formal analysis, Rossella Panarese; data curation, Rossella Panarese and Roberta Iatta; writing—original draft preparation, Rossella Panarese and Roberta Iatta; editing, Rossella Panarese, Roberta Iatta, Frederic Beugnet and Domenico Otranto; Supervision, Roberta Iatta, Domenico Otranto; project administration, Domenico Otranto.

ETHICAL APPROVAL

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to the European Directive 2010/63/EU, following the rules of the ethic committee of the Department of Veterinary Medicine of the University of Bari, Aldo Moro, (Bari, Italy) and the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author.

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How to cite this article: Panarese R, Iatta R, Beugnet F, Otranto D. Incidence of *Dirofilaria immitis* and *Leishmania infantum* infections in sheltered dogs from Southern Italy. *Transbound Emerg Dis*. 2021;00:1–4. https://doi.org/10.1111/tbed.14025