Cross-sectional survey of canine leishmaniasis in Pantelleria island in Sicily

Fabrizio Vitale[1*], Federica Bruno[1], Antonella Migliazzo[1], Antonella Galante[1], Angela Vullo[1], Raffaele Graziano[1], Salvatore D'Avola[1], Valentina Caputo[2] and Germano Castelli[1]

[1]*Istituto Zooprofilattico Sperimentale della Sicilia, Centro di Referenza Nazionale per le Leishmaniosi, via G. Marinuzzi 3, 90129 Palermo, Italy.*

[2]*Section of Dermatology, Department of Health Promotion, Maternal-Infant, Internal Medicine and Specialization of Excellence "G. D'Alessandro" (PROMISE) University of Palermo, Palermo, Italy.*

[*]*Corresponding author at: Istituto Zooprofilattico Sperimentale della Sicilia, Centro di Referenza Nazionale per le Leishmaniosi, via G. Marinuzzi 3, 90129 Palermo, Italy.*
Tel.: +39 091 6565348, e-mail: fabrizio.vitale@izssicilia.it.

Veterinaria Italiana 2020, **56** (2), 103-107. doi: 10.12834/VetIt.2059.10976.3
Accepted: 02.01.2020 | Available on line: 31.12.2020

Keywords
Leishmania infantum, Pantelleria island, Canine leishmaniasis.

Summary
Dogs are the major reservoir of *Leishmania infantum*, the causative agent of canine visceral and cutaneous human leishmaniasis in the Mediterranean basin. Canine and human leishmaniosis are endemic in Italy, particularly in central and southern regions, including islands. Here we show a preliminary, clinical, serological and molecular study carried out in Pantelleria island during 2017. In this study, we clinically examined 136 dogs for the presence of symptoms compatible with leishmaniasis, determined the titer of anti-*Leishmania* antibodies, and investigated *Leishmania* DNA by real time PCR in blood and/or lymph node of each dog. The prevalence of disease was equal to 27% with 95% CI [21%; 32%], lower than prevalence obtained in the other Sicily islands (Lampedusa, Lipari). We observed that enlarged lymph nodes was more positivitely associated with canine leishmaniasis (CanL) than other clinical signs. The results obtained showed that in an endemic area, such as Sicily, diagnosis of CanL needs to be carried out by including an immunological, molecular clinical approach.

Introduction

In the Mediterranean basin, *Leishmania infantum* is transmitted by dipteran insects of Phlebotomus genus *Phlebotomus (Larroussius)* subgenus, including the main Italian vector *P. perniciosus* (Busani *et al.* 2012). Seroprevalence of canine leishmaniosis (CanL) varies between 10 and 37% (Solano-Gallego *et al.* 2011). Symptomatic and asymptomatic dogs are sources of the parasite and phlebotomine sand flies play an active role in the transmission of *Leishmania* to humans (Molina *et al.* 1994). Seroprevalence in southern Europe has ranged from less than 5% to more than 50% according to the geographical areas. However, the prevalence is significantly higher than seroprevalence and may even exceed 65%, as demonstrated by the detection of specific *Leishmania* cellular immunity (Cabral *et al.* 1998, Cardoso *et al.* 1998) and by the detection of parasite's DNA in seronegative dogs (Solano-Gallego *et al.* 2001, Fernández-Bellon *et al.* 2008). In fact, the majority of dog population is exposed and becomes infected without showing clinical evidence of disease or even antibodies (Baneth *et al.* 2008). Sicily is a region highly endemic for CanL (Brianti *et al.* 2014, Brianti *et al.* 2016) and approximately 47% of the Sicilian population lives in areas at risk for visceral leishmaniasis (Cascio *et al.* 2002), thus making an early diagnosis, coupled with vector control strategies in cat, human and dog populations, necessary. Several studies have also been conducted on Sicilian islands, such as in Lampedusa island (Foglia Manzillo *et al.* 2018). This study showed that more than 50% of dogs tested was sieropositive while in Lipari and Vulcano islands, prevalence was 41.7% and 23.6%, respectively (Otranto *et al.* 2017).

Materials and methods

Study area

The present study was carried out in Pantelleria, a small island (80 km^2) (36°47'27"N 11°59'38"E) during February 2017. This island is located the southwest

of Sicily and 60 km (37 miles) east away from the Tunisian coast. Dogs largely represent the most abundant domestic animals present on the island; less than 1,000 dogs have been estimated.

Sampling

The population study comprised 136 stray and domestic dogs. The dogs were selected based on the owners' willingness to have their pet included in the survey. Dogs were of different sex, breed and age. Clinical assessment was performed and all data was recorded including signaling, anamnestic history, repellents used and clinical examination. Finally, a dermatologic examination for ectoparasites and changes compatible with canine leishmaniosis (e.g., alopecic, nodular, ulcerative, crusty, or scaly dermatitis) was conducted. For each dog a blood sample was performed, while from 102/136 dogs needle aspirates of lymph nodes were collected. All samples (serum, whole blood, lymph node aspirate) were sent to the National Reference Centre for Leishmaniosis (C.Re.Na.L.).

Anti-Leishmania antibody detection by IFAT

The collected serum samples were tested by IFAT for the *L. infantum* antibodies (Bio Merieux Spa, Florence, Italy).

DNA extraction and real time PCR assays

Total DNA was extracted from EDTA blood and lymph node aspirate using an E.Z.N.A Tissue DNA kit (Omega biotech VWR, Norcross, GA, USA) following the manufacturer's instructions. The real-time polymerase chain reaction (RT-PCR) was carried out in a LightCycler® 96 (Roche Life Science) using 1 × TaqMan Universal Master Mix (Applied Biosystems, Monza, Italy) and performed as previously described (Vitale *et al.* 2004).

Table I. *Variables considered in the analysis.*

Variable	Description and coding
IFAT	Serological test result (0 = negative; 1 = positive)
Dog owner	0 = Domestic dog; 1 = Kennel
Sex	0 = Male; 1 = Female
Age	≤ 5 years; > 5 years
Dog breed	0 = Purebreed dog; 1 = mixed-breed dog
Repellent tools	0 = never/ occasionally; 1 = Regularly
Cohabitation with other dogs	0 = No; 1 = 1 or more
Slimming	0 = No; 1 = Yes
Skin signs	0 = No; 1 = Yes
Enlarged lymph nodes	0 = No; 1 = Yes
Ocular signs	0 = No; 1 = Yes

Statistical analysis

A descriptive analysis of the data was performed according to 11 variables that have been collected to evaluate possible associations with the presence of *L. infantum* in dogs. Data is showed in Table I. Variables were analyzed in the logistic regression model using the STATA 9.2 software (StataCorp LP, College Station, Texas).

Results

Characteristics of the dog study population in Pantelleria island were summarized in Table II: 63.24% were owners dog (36.76% owned by the kennel); 58.96% of the dogs examined were males, of mixed breed (70.58%); 58.65% of the dogs in the sample were aged less than or equal to 5 years, while 71.97% of the dogs lived with one or more dogs and 89.71% underwent regular repellent tools (collars, spot-on and spray formulations). The results of the evaluation of typical clinical signs of CanL were expressed in percentages (%) and are shown in Table III. Clinically, 30/136 (25.73%) of the animals showed at least one

Table II. *Characteristics of the canine population in Pantelleria and variables considered in the study, absolute frequencies and percentage values.*

	Absolute frequency	%
Dog		
Dog owners	86	63.24
Kennel	50	36.76
Sex		
Male	79	58.96
Female	55	41.04
Breed		
Purebred dog	40	29.42
Mixed breed	96	70.58
Age		
≤ 5 years	78	58.65
> 5 years	55	41.35
Repellent tools		
Never/ occasionally	14	10.29
Regularly	122	89.71
Cohabitation with other dogs		
Yes	95	71.97
No	37	28.03

Veterinaria Italiana 2020, **56** (2), 103-107. doi: 10.12834/VetIt.2059.10976.3

Table III. *Clinical signs in absolute frequencies and percentage values observed in dog.*

	Absolute frequency	%
Clinical signs		
Yes	30	25.73
No	106	74.27
Weight loss		
Yes	9	6.62
No	127	93.38
Cutaneous signs		
Yes	16	11.85
No	119	88.05
Enlarged lymph nodes		
Yes	22	16.18
No	114	83.82
Ocular signs		
Yes	5	3.73
No	126	96.27
Cohabitation with other dogs		
Yes	95	71.97
No	37	28.03

Table IV. *Coefficient and OR for logistic regression on IFAT and age, enlarged lymph nodes.*

Variables	Coefficient	P-value	OR	95% CI for OR	
				Lower	Upper
Age	0.981	0.017	2.67	1.192	5.961
Enlarged lymph nodes	1.357	0.017	3.89	1.277	11.825

CI = Confidence interval; OR = Odds ratio.

Table V. *Detection and accurate parasite quantification of the qPCR in lymph nodes aspirates.*

Lymph node aspirates qPCR	Negative IFAT titre (< 1:40)	Threshold IFAT titre (1:40 and 1:80)	Positive IFAT titre (≥ 1:160)	Total
Negative	27	24	22	73
1-10 *Leishmania*/ml	0	2	4	6
10-100 *Leishmania*/ml	0	3	2	5
100-1,000 *Leishmania*/ml	1	4	2	7
> 1,000 *Leishmania*/ml	0	2	9	11
				102

typical clinical sign of CanL: 6.62% of dogs showed weight loss, 11.85% cutaneous signs, 3.73% ocular signs while 16.18% of examined dogs had enlarged lymph nodes 30.88% of the dogs examined (42/136) were positive at IFAT with titers ranging from 1:160 to 1:5,120 and 51 of 136 exhibiting an antibody titer from 1:40 to 1:80. Table IV shows the results obtained in the logistic regression after applying the stepwise backward variable selection method. The results obtained from the analysis showed that the age and the enlarged lymph nodes were positively associated with serum-positivity to IFAT, ie dogs over 5 years of age had a probability of being positive with *L. infantum* 3 times higher compared to older dogs less than or equal to 5 years; while dogs with enlarged lymph nodes were 4 times more likely to be seropositive to *L. infantum* than dogs with no enlarged lymph nodes.

Real time PCR assay (qPCR) was carried out from 136 blood and 102 lymph node samples, providing useful information to support the tissue of choice for diagnosis of CanL. Among qPCR 73/102 negative dogs 27 samples showed negative IFAT titre (< 1:40), 24 displayed a threshold titre (from 1:40 to 1:80) and 22 dogs were positive (≥ 1:160) (Table V). Twenty-nine dogs (28.43%) tested positive for *L. infantum* DNA by qPCR from lymph node matrices with different parasite load.

Only one dog tested qPCR positive (0.73%) in blood showing higher parasite load (> 1000 Leishmania/mL) than lymph node. Out of 29 dogs qPCR positive, 13 showed no symptoms of leishmaniasis (weight loss, skin and ocular signs and enlarged lymph nodes); the remaining 16 dogs showed one or more symptoms mentioned above. The prevalence of the infection was 27% (95% CI, 21%-32%).

Discussion

CanL constitutes a considerable veterinary challenge, as well as an important public health problem. Pantelleria island (Sicily) is featured by optimal conditions to study a well-defined population of animals and pathogens. The prevalence of *Leishmania* infection, estimated from the model, was 27% (95% CI, 21%-32%). This prevalence was lower than Lipari island (41.7%) but similar to the Vulcano island (23.6%) (Otranto *et al.* 2017), while seroprevalence (30.88%) was lower than Lampedusa island seroprevalence (54%) (Foglia Manzillo *et al.* 2018). This variability in canine seroprevalence in Sicilian islands could be due to differences in disease surveillance and, importantly, for the presence of a kennel in Pantelleria which surely promotes stray dog population control. This finding, however, could be also related to the differences of the population under examination. Repellent tools (collars, spot-on and spray formulations) performed on 90% of the dog study population had surely a protective effect. Repellent

tools remains the most effective antivectorial method to control *L. infantum*. Furthermore, the presence of seropositivity of dogs older than 5 years (30.88%), was positively associated with *Leishmania* infection. During investigation, cutaneous and ocular signs and weight loss observed were related to *Leishmania* disease in dogs, since percentages of association obtained are very low. Only enlarged lymph nodes were positively associated with Leishmania infection, even if only 16,18 % of dogs presented this clinical sign (Table IV). Early CanL diagnosis was crucial to identify infectious dogs through several diagnostic tests, but the correct interpretations of these were of great importance to make an accurate diagnosis of the disease. IFAT test 'gold standard' technique for mass screening dogs, could however generate false positive results due to both serological cross-reactivity with other pathogens and low sensitivity in asymptomatic dogs. The use of qPCR for the assessment of parasite load could be more informative. However, as only one dog showed parasite's DNA in blood, this study also suggests that blood matrices is inadequate for qPCR, since hematogenous dissemination occurs only rarely. On the other hand, these results indicated that lymph nodes were the matrix of choice for molecular detection of CanL in dogs (Martínez *et al.* 2011, Moreira *et al.* 2007). Overall, the combination of serology, direct detecton of DNA and clinical examination should be performed in order to reach a correct diagnosis. The absence of reports of clinical case of human cutaneous/visceral leishmaniasis infection in Pantelleria island during the period of our study could be related to asymptomatic *Leishmania* infections in immunocompetent hosts without clinically evident disease.

Acknowledgements

This research was granted by Ministry of Health RC IZS SI 01/2017.

References

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.

Brianti E., Gaglio G., Napoli E., Falsone L., Prudente C., Solari-Basano F., Latrofa M.S., Tarallo V.D., Dantas-Torres F., Capelli G., Stanneck D., Giannetto S. & Otranto D. 2014. Efficacy of a slow-release imidacloprid (10%)/flumethrin (4.5%) collar for the prevention of canine leishmaniosis. *Parasit Vectors*, **7**, 327.

Brianti E., Napoli E., Gaglio G., Falsone L., Giannetto S., Solari Basano F., Nazzari R., Latrofa M.S., Annoscia G., Tarallo V.D., Stanneck D., Dantas-Torres F. & Otranto D. 2016. Field evaluation of two different treatment approaches and their ability to control fleas and prevent canine leishmaniosis in a highly endemic area. *PLoS Negl Trop Dis*, **10** (9), e0004987.

Busani L., Gras L.M., Romi R., Boccolini D., Severini F. & Bongiorno G. 2012. Zanzare, flebotomi e zecche: atlante bibliografico delle specie d'interesse sanitario in Italia (1985-2009). Rapporti ISTISAN 12/22. Roma, Istituto Superiore di Sanità.

Cabral M., O'Grady J.E., Gomes S., Sousa J.C., Thompson H. & Alexander J. 1998. The immunology of canine leishmaniosis: strong evidence for a developing disease spectrum from asymptomatic dogs. *Vet Parasit*, **76**, 173-180.

Cardoso L., Neto F., Sousa J.C., Rodrigues M. & Cabral M. 1998. Use of a leishmanin skin test in the detection of canine *Leishmania*-specific cellular immunity. *Vet Parasitol*, **79**, 213-220.

Cascio A., Colomba C., Antinori S., Orobello M., Paterson D. & Titone L. 2002. Pediatric visceral leishmaniasis in Western Sicily, Italy: a retrospective analysis of 111 cases. *Eur J Clin Microbiol Infect Dis*, **21** (4), 277-282.

Fernández-Bellon H., Solano-Gallego L., Rodríguez-Cortés A., Ferrer L., Gallego M., Alberola J. & Ramis A. 2008. Little evidence of seasonal variation of natural infection by *Leishmania infantum* in dogs in Spain. *Vet Parasitol*, **155**, 32-36.

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** (1), 286.

Gramiccia M., Scalone A., Di Muccio T., Orsini S., Fiorentino E. & Gradoni L. 2013. The burden of visceral leishmaniasis in Italy from 1982 to 2012: a retrospective analysis of the multi-annual epidemic that occurred from 1989 to 2009. *Euro Surveillance*, **18**, 20535.

Martínez V., Quilez J., Sanchez A., Roura X., Francino O. & Altet L. 2011. Canine leishmaniasis: the key points for qPCR result interpretation. *Parasit Vectors*, **4**, 57.

Molina R., Amela C. & Nieto J. 1994. Infectivity of dogs naturally infected with *Leishmania infantum* to colonized *Phlebotomus perniciosus*. *Trans R Soc Trop Med Hyg*, **88**, 491-493.

Moreira M.A.B., Luvizotto M.C.R., Garcia J.F., Corbett C.E.P. & Laurenti M.D. 2007. Comparison of parasitological, immunological and molecular methods for the diagnosis of leishmaniasis in dogs with different clinical signs. *Vet Parasitol*, **145**, 245-252.

Otranto D., Napoli E., Latrofa M.S., Annoscia G., Tarallo V.D., Greco G., Lorusso E., Gulotta L., Falsone L., Basano F.S., Pennisi M.G., Deuster K., Capelli G., Dantas-Torres F. & Brianti E. 2017. Feline and canine leishmaniosis and other vector-borne diseases in the Aeolian Islands: pathogen and vector circulation in a confined environment. V*et Parasitol*, **15** (236), 144-151.

Solano-Gallego L., Miró G, Koutinas A., Cardoso L., Pennisi M.G., Ferrer L., Bourdeau P., Oliva G. & Baneth G. 2011. LeishVet guidelines for the practical management of canine leishmaniosis. *Parasit Vectors*, **4**, 86.

Solano-Gallego L., Morell P., Arboix M., Alberola J. & Ferrer L. 2001. Prevalence of *Leishmania infantum* infection in dogs living in an area of canine leishmaniasis endemicity using PCR on several tissues and serology. *J Clin Microbiol*, **39**, 560-556.

Vitale F., Reale S., Vitale M., Petrotta E., Torina A. & Caracappa S. 2004. TaqMan-based detection of *Leishmania infantum* DNA using canine samples. *Ann N Y Acad Sci*, **1026**, 139-143.

World Organization for Animal Health (OIE). 2018. Chapter. 3.1.11 – Leishmaniosis (most recent updates adopted in 2014). *In* Manual of diagnostic tests and vaccines for terrestrial animals, OIE, Paris. https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.01.11_LEISHMANIOSIS.pdf.