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Research paper

Feline and canine leishmaniosis and other vector-borne diseases in the Aeolian Islands: Pathogen and vector circulation in a confined environment

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ABSTRACT

Vector-borne diseases (VBDs) are prevalently investigated in dogs. Studies on feline VBDs are scant, though feline leishmaniosis (FeL) is increasingly recognised as a disease of cats in endemic areas. Comprehensive investigations on the distribution of VBDs in populations of cats and dogs living in relatively small geographical areas, such as islands, are currently lacking. In this study the prevalence of *Leishmania infantum* and other VBD pathogens was assessed in cohorts of cats and dogs living in the Aeolian Islands.

Autochthonous animals (330 cats and 263 dogs) of different age and sex were sampled. Blood and conjunctival samples were collected from cats and dogs for serological and molecular testing. Eighty-five (25.8%) cats were positive for *L. infantum*, 13 (3.9%) for *Bartonella* spp. and 1 (0.3%) for *Hepatozoon felis*. One-hundred and ten dogs (41.8%) were positive for *L. infantum* and three (1.1%) for *Hepatozoon canis*. The incidence of *L. infantum* infection in cats positive after one season of exposure to sand fly was 14.7%. *Leishmania infantum* prevalence and year incidence were higher in dogs than in cats ($p = 0.0001$ and $p = 0.0003$, respectively). Thirty-four cats (10.3%) scored positive for ticks (mean intensity rate of infestation, 2.03 ± 1.4), which were identified to the species level as *Ixodes ventalloi* and *Rhipicephalus pusillus*. Conversely, *Rhipicephalus sanguineus* sensu lato (s.l.) was the only species identified in dogs (10.6%). A larger prevalence of infestation by *Ctenocephalides felis* was recorded in cats (n = 91; 27.6%) than in dogs (n = 33; 12.5%) ($p = 0.0001$). In addition, one female *Nosopsyllus fasciatus* (syn. *Ceratophyllus fasciatus*) and one male *Spilopsyllus cuniculi* were also identified in flea-infected cats. VBDs are endemic in the Aeolian Islands being *L. infantum* the most prevalent vector-borne pathogen circulating between cats and dogs. The overall seroprevalence of FeL herein recorded is higher than that assessed, only by IFAT, in populations of cats in Greece and in Spain. Because *L. infantum* and VBDs are more commonly associated with dogs, the recognition of cats as hosts of different vector-borne pathogens is of paramount importance towards a better management of these diseases in both animals and humans.

1. Introduction

Leishmaniosis and other vector-borne diseases (VBDs) are prevalent in dog populations worldwide being of increasing concern for their zoonotic potential (Otranto et al., 2009a; Otranto et al., 2009b). Conversely, epidemiology of feline VBDs (FeVBDs) are much less investigated resulting in scant data available (Otranto

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and Dantas-Torres, 2010; Pennisi et al., 2015a). This lack of scientific data on FeVBDs apparently contrasts with the large number of cats living with families in Europe and in the US (about 90 and 66 million, respectively), and with the outdoor life-style of many of them (in Otranto, 2015). Though cats are exposed to a number of arthropods such as fleas, ticks and sand flies, and to the pathogens they may transmit (Maroli et al., 2007; Maia et al., 2010; Pennisi et al., 2013), their habits and behaviour (e.g. grooming) seem to minimize the risk for arthropod infection compared to dogs, resulting in scant information on the role of some ectoparasites, such as ticks, as vectors of pathogens to cats (Day, 2016). Thus, cats may be less susceptible than dogs to several pathogens, including vector-borne pathogens (VBPs) (Day, 2016). Over the last decade, studies on FeVBDs increased worldwide, especially those on feline leishmaniosis (FeL) (Pennisi et al., 2015a). Nonetheless, the infection by *Leishmania infantum* is more commonly associated with dogs, which are regarded as the main domestic reservoir of this protozoan. Sand flies, the natural vectors of *L. infantum*, may take their blood meals on cats (Maroli et al., 2009; Sales et al., 2015) and become infected after feeding on naturally infected cats (Maroli et al., 2007). Nevertheless, clinical diagnosis of FeL in endemic areas is not common, probably because of the subclinical infection occurring in most of the infected cats, or, merely because veterinary practitioners do not usually consider this disease in the list of differential diagnosis of their feline patients. The occurrence of FeL and other FeVBDs in cats has been reported in countries around the Mediterranean basin (Tabar et al., 2008; Solano-Gallego and Baneth, 2011; Vilhena et al., 2013; Silaghi et al., 2014), with prevalence generally well below those recorded in dogs (Poli et al., 2002; Cardoso et al., 2010). In addition, the large variability in prevalence data of FeL observed in cat populations (i.e. from 0.7 to 68.5%) has been attributed to the different sensitivity of diagnostic techniques employed and to the cut-off values set for the indirect immunofluorescence antibody test (IFAT) (Pennisi et al., 2015a). In addition, serological and molecular tests have been seldom combined for the diagnosis of FeL in the same animal population and often in a low number of cats (e.g. in Greece Chatzis et al. (2014a), Chatzis et al. (2014b); in Italy Pennisi et al. (2012); in Spain Ayllon et al. (2008), Sherry et al. (2011)), therefore limiting the overall information on the actual prevalence of the infection in cats.

Comprehensive investigations on the distribution of FeL and other VBDs in populations of cats living in confined environments, such as in small islands, are currently lacking. Under the above circumstances, the Aeolian Islands (Sicily), representing an environment isolated by the sea for definition, are featured by optimal conditions to study a well-defined population of animals, vectors and pathogens. Overall, Sicily is a region highly endemic for canine leishmaniosis (Brianti et al., 2014; Brianti et al., 2016) with an average of 31.5 notified cases/year in humans from 1987 to 1995 (Cascio et al., 1997).

Therefore, the aim of this study was to assess the prevalence of *L. infantum* infection (by molecular and serological techniques) in populations of cats living in the Aeolian Islands and to compare results with those of dogs from the same islands. A comprehensive analysis of the association among infection by *L. infantum* and other VBPs, anamnestic data and risk factors have also been provided in order to gain more information on the occurrence of these little known infections in cats.

2. Materials and methods

2.1. Ethical statement

This study was conducted in accordance with the principles of Good Clinical Practice (VICH GL9 GCP, 2000). For each animal included in the study the owner signed an informed consent form. The design and the experimental procedures used in this study were authorized by the Italian Ministry of Health (DGSA no. 0006088; 10/03/2015).

2.2. Study site

The study was carried out from January 2015 to June 2016, in Lipari and Vulcano, two of the main islands of the Aeolian archipelago, so named for the demigod of the winds Aeolus. For their beauty and nature, the Aeolian Islands (surface area of 114.7 km^2 in the Tyrrhenian Sea, province of Messina, Sicily, Italy, 38°32′N, 14°54′E) have a strong tourism vocation, with up to 260,000 visitors annually that increase the autochthonous population of nearly 15,000 inhabitants. Lipari has an area of 37 km^2 and is characterized by costal cliffs fronted by rocks, and the profile of the island is dominated by large central building of Monte S. Angelo (499 m a.s.l.). Lipari alternates very different landscapes with the western area being characterized by dry grass prairies with abundant presence of dwarf palm (*Chamaerops humilis*) and spring flowering of many species of orchids. In the highest part of the island prevails the Mediterranean maquis, featured by arbutus, heather, ash and aquiline ferns plants. Vulcano, with an area of 21 km^2, is located very close to Lipari; the two islands are indeed separated by a strait. The highest points of Vulcano are Monte Aria and Monte Saraceno (501 m a.s.l. each). Vulcano is mainly covered with thick bush, among which prevails genista (*Genista tyrrhena*) and cytisus (*Cytisus aeolicus*), two plants peculiar of the Aeolian archipelago. The climate of the Aeolian Islands is temperate, typical of the central Mediterranean area. The average temperatures vary from 10° C during winter to 27° C in summer, and are mitigated by marine breeze (source: Servizio Meteorologico Aeronautica Militare). Aeolian territory hosts a diverse fauna including bird species and wild mammals such as the Garden dormhouse (*Eliomys quercinus*, subspecies *liparensis*) and the widespread European wild rabbit (*Oryctolagus cuniculus*). The occurrence of the infection by *L. infantum* and other VBDs has been reported in the Aeolian archipelago in some symptomatic dogs and cats (Pennisi et al., 2015b; Persichetti et al., 2016).

2.3. Animal populations, sampling procedures and pathogens investigated

All animals sampled in this study (330 cats and 263 dogs) were referred to the only veterinary clinic in the Aeolian archipelago (Ambulatorio Veterinario Santa Lucia, Lipari), owned by one of the authors (LG), and were selected based on the owners' willingness to have their pet included in the survey. Cats and dogs were mostly housed in Lipari and Vulcano islands and in Salina, Filicudi, Stromboli, Alicudi, and Panarea, in descending order, of different age, sex and living outdoor or having constant outdoor access. Data about age, sex, breed, and antiparasitic treatments were collected. Systemic signs (e.g. loss of weight, fever, pale or icteric mucous membranes, peripheral lymphadenomegaly, hepatomegaly, splenomegaly, bleeding), as well as skin (e.g. ulcers, papules, nodules, crusts, haemorrhagic blisters, scales, alopecia/hypotrichosis) and ocular disorders (e.g. blepharitis, conjunctivitis, keratitis, uveitis or panophthalmitis) suggestive of VBDs were recorded in each animal's file along with data on the presence of ticks and fleas.

Dogs and cats were examined for the presence of ticks and fleas by thumb counting. For each dog, the number of ectoparasites and/or developmental stages of ticks detected were recorded in a separate form, ticks were counted and the infestation categorized on the basis of their number into the following four classes: low ($\leq$10); medium (10 > x $\leq$ 20); high (20 > x $\leq$ 30); very high (>30).