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First survey on canine leishmaniasis in a non classical area of the disease in Spain (Lleida, Catalonia) based on a veterinary questionnaire and a cross-sectional study

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ABSTRACT

The Spanish distribution of canine leishmaniasis (CanL) is heterogeneous and very few data are available for the north of the country, including the province of Lleida (Catalonia, Spain). This work describes the results obtained from a questionnaire sent to veterinarians throughout the province of Lleida. The majority of veterinarians (25/32, 78.1%) believed CanL cases were increasing and that the dogs had been infected locally (30/32, 93.8%). Also, a cross-sectional study was performed on the seroprevalence of CanL in kennel dogs, with and without compatible clinical signs, in the county of Pallars Sobirà (Pyrenees of Lleida), where an autochthonous case of CanL had been previously detected. Four serological tests were used (IFAT, ELISA, Western blot, ICF) and dogs that tested positive with at least two immunological methods were considered seropositive and probably infected. 33.1% (48/145) of the dogs were seropositive. The results of a mixed logistic regression model showed that the risk of seropositivity increased with age (OR = 1.35, p-value = 0.002), among dogs living in the southern part of Pallars Sobirà (OR = 6.20, p-value = 0.025) and among dogs whose owners considered their animals to be at risk of leishmaniasis infection (OR = 1.26, p-value = 0.024) and who were unaware of anti-sand fly preventive methods (OR = 11.6, p-value = 0.009). The risk decreased when dogs lived in an urban-periurban habitat (OR = 0.17, p-value = 0.002). The information gathered in the veterinary questionnaires helped us to define the knowledge, perception and awareness of the disease in a naïve region, supporting the hypothesis of an existing CanL focus in Pallars Sobirà, which was confirmed by the seroepidemiological survey. The seroprevalence study carried out on kennel dogs of local origin proved useful for detecting an autochthonous focus of leishmaniasis through the analysis of a small number of animals.

1. Introduction

Changes in global climate, human activities and migration have resulted in the emergence or re-emergence of vector-borne diseases in some parts of the world, including leishmaniasis, the only tropical vector-borne disease that

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remains endemic throughout southern Europe (Dujardin et al., 2008).

Leishmaniasis in the Mediterranean region is due to *Leishmania infantum* and its transmission is mainly by the bite of permissive sand flies. Two proven vectors exist in the Iberian Peninsula, *Phlebotomus ariasi* and *P. perniciosus* (Guilvard et al., 1996; Portús et al., 2007), both present in the province of Lleida (Gállego et al., 1990) where *L. infantum* DNA has been found in *P. ariasi* (Alcover et al., 2012).

The first cases of human and canine leishmaniasis in Spain were published in 1912 and 1913, respectively (Pittaluga, 1912, 1913). The human incidence of the disease decreased at the end of the 1940s, which is attributed to the use of insecticides in the agricultural and domestic environment as well as an antipaludic campaign (Gil Collado, 1977; Botet Fregola and Portús Vinyeta, 1993; Portús et al., 2007). There has been a re-emergence in recent years, mainly in immunosuppressed adults (Alvar et al., 1997). From 1982 until 1996 human leishmaniasis (HL) was considered a notifiable disease in Spain, but its notification is currently mandatory in only 12 of the country's 17 autonomous communities, including Catalonia. From 1982 to 2010, 879 HL cases were officially recorded in Catalonia, 37 of them in Lleida province (updated from Ballart et al., 2012a). Nevertheless, as individual cases are not always officially recorded, the real incidence is not well defined (Gállego, 2004; Portús et al., 2007; Dujardin et al., 2008).

The distribution of canine leishmaniasis (CanL) in Spain is heterogeneous, with the lowest seroprevalence in the North (1.6%) and the greatest in the South (34.6%) (Alvar Ezquerra, 2001; Morillas et al., 1996). Nevertheless, data on CanL distribution is incomplete, as declaration of the disease is not compulsory, with very few data available for the north of the country, including the province of Lleida (Catalonia, NE of Spain) where the first case of CanL has been recently reported (Ballart et al., 2012a).

CanL is considered an important disease by the World Organization for Animal Health (OIE) from the socio-economical and sanitary points of view. Dogs constitute the main reservoir hosts of the parasite, which represents a risk for humans (Gállego, 2004; Ready, 2010), particularly considering the high number of asymptomatic animals (Alvar et al., 2004; Dujardin et al., 2008; Molina et al., 1994).

The true extent of CanL in many parts of southern Europe is unknown, due to a lack of survey data, its incidence only being inferred indirectly (de Ybáñez et al., 2009). For this reason, a questionnaire was designed by the EDEN project (Emerging Diseases in a changing European eNvironment) to rapidly obtain information from veterinarians about the presence, diagnosis and perception of the disease. This questionnaire was applied in the province of Lleida as has been done in other parts of Europe by EDEN team participants (Morosetti et al., 2009; Farkas et al., 2011; Gálvez et al., 2011).

Described here are the results obtained from the EDEN questionnaire as well as a cross-sectional study on the seroprevalence of CanL in Pallars Sobirà (Pyrenees) where the aforementioned CanL case was detected, with particular focus on the possible factors responsible for the CanL emergence in the area. This is the first survey carried out on dogs in the province of Lleida and was prompted by the possibility of the disease becoming wide-spread and established in the Pyrenees.

2. Materials and methods

2.1. Study area

A questionnaire-based survey on CanL was conducted among clinical veterinarians throughout the province of Lleida (Catalonia, NE of Spain). Lleida (41°36′N and 0°37′E; 12,173 km^2) shares its northern border with Andorra and France and is territorially divided in 13 counties, occupying a great variety of habitats that range from just over sea level to over 3000 m above sea level (m a.s.l.) (Fig. 1). The climate varies from Mediterranean high-mountain in the mountainous areas of the north to continental in the central depression. With a population of 436,402 inhabitants in 2009 (approximately 35.8 inhabitants/km^2) Lleida is the province with the lowest population density of Catalonia (IDESCAT Institut d'Estadística de Catalunya; http://www.idescat.cat). The only locality with more than 20,000 inhabitants is the capital, Lleida city, where 30% of the population lives.

Additionally, a cross-sectional serological study of CanL was carried out in Pallars Sobirà, a county of the province of Lleida located in the Spanish Pyrenees (42°25′N, 1°08′W), with a surface area of 1378 km^2. Its population density is very low (5.6 inhabitants/km^2 in 2009) and concentrated in small villages (IDESCAT Institut d'Estadística de Catalunya; http://www.idescat.cat). The altitude ranges from 600 to 3143 m a.s.l. and the climate is Mediterranean high-mountain, with cold winters and mild summers, except at over 1500 m a.s.l., where it could be considered alpine. The annual mean rainfall varies from 700 mm in the valleys to 1000 mm in the peaks, summer being the wettest season while winter is the driest. The orography of the region and its particular climate notably determine its vegetation, which is characterised by five different floors: basal with mixed oaks (from 600 to 1000 m a.s.l.), mountainous with oaks, beeches, firs and red pines (from 1000 to 1600 m a.s.l.), subalpine with black pine (from 1600 to 2200 m a.s.l.), alpine with pastures (from 2200 to 2800 m a.s.l.) and the mainly rocky snow floor (over 2800 m a.s.l.).

2.2. Veterinarian questionnaire on canine leishmaniasis

In 2009, a total of 41 questionnaires were sent to all the veterinarians with a pet veterinary clinic in the province of Lleida to survey their opinions on trends in CanL prevalence and control measures. The questionnaire was designed by members of the Leishmaniasis sub-project of the EDEN Project (http://www.eden-fp6project.net/emerging_diseases/leishmaniasis), and consisted of two pages of questions on different aspects of the disease in the area, including the approximate number of suspected and confirmed cases observed in the previous year, clinical signs and their frequency of appearance and utility in diagnosis, methods used to confirm CanL cases, etc. (Gálvez et al., 2011).