



## Research paper

# Variations in seroprevalences of canine leishmaniasis: Could it be a consequence of the population structure?



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## ABSTRACT

Canine leishmaniasis is a parasitic disease caused by *Leishmania infantum* and is transmitted by Phlebotominae vectors. Despite numerous publications on the subject, some essential aspects of the epidemiology are not yet sufficiently clear. We proposed a stochastic model with the aim of identifying some important gaps in the current knowledge of leishmaniasis, such as the frequency of vector infection or a dog's life expectancy depending on their purpose and their health status. We only found that the purpose was a significant factor. Furthermore, we detected relationships among age, gender and habitat with the dogs' purposes that can affect the calculation of the overall seroprevalence of the analysed sample. The development of this model will allow us to discard potential confounding factors as gender, age, purpose or habitat.

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## 1. Introduction

Leishmaniasis is a parasitic disease transmitted by Phlebotominae vectors that mainly affects canines, felines, equines, rodents and humans (Chitimia et al., 2011; Gramiccia, 2011). The geographical distribution of the disease is defined, among other factors, by the vector distribution: *Phlebotomus* spp. in Europe, Africa and Asia and *Lutzomyia* spp. in America (Killick-Kendrick, 1990).

*Leishmania* vectors are holometabolous nematoceran insects with complete metamorphosis (Killick-Kendrick, 1979; Molina, 1994). At least 70 of the 600 known species and subspecies are capable of transmitting this protozoan (Killick-Kendrick, 1990). They have crepuscular activity when there is no wind or rain and the temperature exceeds 18 °C (Killick-Kendrick et al., 1986). Its ideal temperature is approximately 20 °C, but it may be active above 16 °C. Once fed, females return to their natural shelter to rest and filter blood before searching for the place of oviposition.

Dogs are the most affected domestic animal, and *Leishmania infantum* is the aetiological agent of most cases of canine leishmaniasis (CL) in the Mediterranean area. There is huge variability in the estimated prevalence due to different individual and environmental factors that affect the transmission of the disease. Some relevant studies carried out in Spain during the last 30 years have found a

prevalence ranging from 3.9% to 67%, depending on geographical area (Table 1).

These differences may be due to the geographic region or the specific time in which the study was carried out; however, it has been found that there are factors associated with the prevalence of CL such as gender, habitat (indoor/outdoor), breed, age and purpose (Gálvez et al., 2010; Cortes et al., 2012; Miró et al., 2012). When comparing observational surveys, we can observe contradictions, and the prevalence of CL should be analysed from an epidemiological point of view to understand the role of confounding factors.

Leishmaniasis has been classified into several types according to the symptoms the patient develops, although it is common to find simultaneously visceral and cutaneous clinical manifestations. Among domestic animals, dogs are the most affected species and the incubation period ranges from three months to seven years. We should note that there are dogs that develop severe clinical signs in a short time, while others remain asymptomatic and carriers throughout their life.

Epidemiology brings us the most practical information to establish prevention and control measures. Specifically, the use of theoretical models in epidemiology can be a useful tool for disease research where experimentation and field observations are very complex due to the existence of multiple factors related to animal welfare, time, money, etc. (Garner and Hamilton, 2011).

Epidemiological modelling has been used for decades to simulate the spread of the disease in animal and human populations (Anderson and May, 2002). Increasingly, mathematical modelling is

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**Table 1**  
Prevalence surveys of canine leishmaniasis in Spain in the last 30 years.

Area	n	Prev.	Reference
Barcelona	617	28.9%	Botet et al., 1987
Cataluña	1514	31.0%	Portús et al., 1987
Granada	1503	8.84%	Reyes Magaña et al., 1988
Córdoba	540	23.7%	Martínez Cruz et al., 1990
Cáceres	381	14.0%	Gómez Nieto et al., 1992
Granada	615	5.3%	Acedo Sánchez et al., 1996
Mallorca	100	67.0%	Solano-Gallego et al., 2001
Madrid	1803	7.8%	Miró et al., 2007
Las Alpujarras	439	13.0%	Martín-Sánchez et al., 2009
Madrid	1076	8.1%	Gálvez et al., 2010
Orense	101	35.6%	Miró et al., 2012

being used in veterinary research for these purposes (Morris et al., 2002; Keeling et al., 2003; Risk Solutions, 2005; Guitian and Pfeiffer, 2006). With models is possible to explain and predict the pattern of disease outbreaks, simulate them and develop different strategies for management, eradication and disease control (Ögüt and Bishop, 2007).

The reliability of the models has been questioned, as they may fail to take into account the real impact of disease control and the establishment of preventive measures. Despite the criticism, according to de Jong (1995), mathematical modelling allows the study of complex phenomena such as the dynamics of the infectious agent in populations and pathogen-host interaction.

In human leishmaniasis, there are some models that examine the transmission of the parasite, taking into account the interaction between humans, animal populations and vectors (Elmojtaba et al., 2010; Agyingi et al., 2011; Kaabi and Ahmed, 2013). However, we cannot find cross sectional models for humans or animals to estimate prevalence.

If we try to build a model that accurately represents the real world, the result can be so complex that we cannot find all of the parameters contained in it, or we do not have an algorithm efficient enough to perform the computations in a reasonable time; in contrast, if we simplify the model to make it easier to build and test, it may represent a scenario far away from reality. For this reason, it is really important to obtain a balance between simplicity and realism that is often difficult to achieve. The most difficult point is the determination of the functions, and the most important aspect necessary to make it possible to do this successfully is to properly identify the factors that we will consider. Another important factor in model building is the fact that it is often very difficult to have sufficient and detailed data to validate the model (Reeves et al., 2011).

Generally, medical databases are incomplete because they reflect only some of the variables that have been used in model making. Therefore, it is necessary to have an expert who is familiar with the system to be modelled with the aim of bringing the reality needed.

**Table 2**  
Sample distribution taking into account purpose, gender and area.

Purpose	Total		Urban areas		Rural areas	
	% (n)	% (n) females/males	% (n)	% (n) females/males	% (n)	% (n) females/males
Hunter	69.3% (255)	38.0%/62.0% (97/158)	24.2% (15)	46.7%/53.3% (7/8)	78.5% (240)	37.5%/62.5% (90/150)
Companion	9.8% (36)	52.8%/47.2% (19/17)	18.8% (12)	91.7%/8.3% (11/1)	7.8% (24)	33.3%/66.7% (8/16)
Guard	14.4% (53)	47.2%/52.8% (25/28)	18.3% (11)	45.5%/54.5% (5/6)	13.7% (42)	47.6%/52.4% (20/22)
Breeder	6.5% (24)	62.5%/37.5% (15/9)	38.7% (24)	62.5%/37.5% (15/9)	0.0% (0)	– (–)
Total	100.0% (368)	42.4%/57.6% (156/212)	16.8% (62)	61.1%/38.9% (38/24)	83.1% (306)	38.6%/61.4% (118/188)

Taking into account all of these circumstances, we have designed an epidemiological model for the seroprevalence of CL with which to study the role of factors including area, gender, purpose and age.

## 2. Materials and methods

### 2.1. Observational study

To obtain needed parameters for the construction of the model, a cross-sectional study was carried out over 368 dogs during 2008 in different locations of Aragón (a region in northeast of Spain) grouped as urban or rural areas. Data collected from owner volunteers were age, gender, purpose and infection status (determined using a DAT analysis) (Oskam et al., 1996). All samples were collected in different veterinary premises from Aragón (Spain) as part of routine health control, and the owners signed a document of informed consent.

From this survey a sample structure was obtained taking into account purpose, gender and area (Table 2). These three variables were significantly associated among them: gender-purpose ( $p=0.045$ ); gender-area ( $p=0.001$ ) and purpose-area ( $p<0.001$ ). Based on the age of the dogs provided by the owners (although this was not very precise), we define average values stratified by purpose and gender (Table 3).

The association between seroprevalence and residence area, purpose and gender was assessed using the Pearson's Chi-square test, or likelihood ratio test when the Chi-square test was not valid (Daniel, 2000). All statistical analyses were carried out with IBM SPSS 19.0 for Windows, and alpha error was set at 0.05.

### 2.2. Construction of the model

A stochastic model based on binary logistic regression was used because it provides insight into the relationship between a qualitative dependent variable and one or more explanatory independent variables or covariates (de Jong, 1995). This model allows estimating the probability of the outcome of a dichotomic variable using equation 1, taking into account one or more independent variables ( $x_1, x_2, \dots, x_n$ ).

$$p(y) = \frac{e^{\beta_0 + \beta_1 \cdot x_1 + \dots + \beta_n \cdot x_n}}{1 + e^{\beta_0 + \beta_1 \cdot x_1 + \dots + \beta_n \cdot x_n}} \quad (1)$$

Using the coefficients  $\beta$  of the model, it is possible to estimate the odds ratios corresponding to the independent variables.

Once the logistic regression model was defined, an algorithm to generate virtual populations was built using PHP 5.5.19 and MySQL 5.6.21 over the web server XAMPP for Windows 5.5.19.

An iterative procedure was designed to generate a population, and dogs were defined, with their own characteristics, in each iteration. First, a random number was generated to assign a purpose depending on a selected purpose distribution. Then, the gender is assigned by comparison between a new random number and the

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