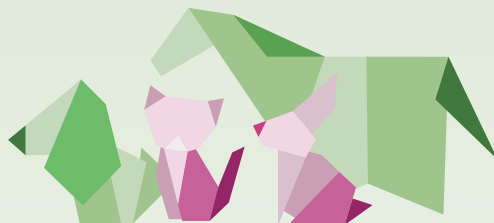# ALIVE

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Serological survey of *Leishmania infantum* in Castilla y León (North-Centre Spain)

Iván Rodríguez-Escolar[1], Patricia Pérez Pérez[1,2], Marta Ruíz Somacarrera[1], Carmen González Días-Cano[1], José Ángel Sánchez Agudo[3], Elena Carretón[4], José Alberto Montoya-Alonso[4], Rodrigo Morchón[1]

[1]*Zoonotic Infections and One Health GIR, Laboratory of Parasitology, Faculty of Pharmacy, University of Salamanca, Campus Miguel Unamuno, Salamanca, Spain.*
[2]*Instituto Universitario de Enfermedades Tropicales y Salud Pública de Canarias, Universidad de La Laguna, San Cristóbal de La Laguna, Spain.*
[3]*Grupo de Investigación en Biodiversidad, Diversidad humana y Biología de la Conservación, Campus Miguel de Unamuno s/n, Universidad de Salamanca, E-37007 Salamanca, Spain.*
[4]*Internal Medicine, Faculty of Veterinary Medicine, Research Institute of Biomedical and Health Sciences (IUIBS), University of Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain.*

*Email address of corresponding author: rmorgar@usal.es

Background

Zoonotic diseases are one of the most important health problems affecting both domestic dogs and humans worldwide. In Europe, many of these diseases are considered emerging diseases [1]. Most of the work describing the situation of these diseases assesses the influence of possible factors contributing to this spread with the intention of more accurate predictive modelling and to make control measures and forms of treatment as optimal as possible [2]. The protozoan *Leishmania infantum*, transmitted by *Phlebotomus* spp., is the cause of canine leishmaniasis; it induces a severe disease with severe clinical signs that, if left untreated, can lead to the death of the animal. In addition, *L. infantum* can cause skin lesions in humans or visceral leishmaniasis, the latter being a severe disease, especially in immunocompromised patients [1]. In Spain, canine leishmaniasis is

one of the most studied diseases affecting domestic dogs. However, epidemiological studies are not complete, and many of the regions studied have not individualized the results by province [1,2,3]. The Autonomous Community of Castilla y León is located in the northwestern quadrant of the Iberian Peninsula (Spain). With an area of 94,224 km², is the largest region in Spain and one of the largest of Europe. The orography of Castilla y León is mainly formed by a plateau with an average altitude of around 800 meters above sea level, surrounded by a belt of mountainous reliefs to the north, east and south, and bordering the west with Portugal. Administratively it is divided into nine provinces (León, Zamora, Salamanca, Valladolid, Palencia, Burgos, Soria, Segovia and Ávila). Therefore, the aim of this study was to deepen the seroprevalence as well as the geographic distribution patterns of *L. infantum* in domestic dogs in the autonomous community of Castilla y León (Spain) by using GIS, to understand the causal relationship between the environmental variables and the prevalence of the infections.

Materials and methods

Dogs' samples: Blood from a total of 1475 domestic dogs from the nine provinces of Castilla y León were collected between 2019 and 2020. Samples were collected from dogs undergoing a medical examination in 44 veterinary clinics and hospitals. The participation of clinics and hospitals was voluntary, and samples were collected throughout the duration of the study. The criteria for inclusion were: a) no previous history of infection, b) not receiving regular chemoprophylaxis for the studied vector-borne diseases, and c) owner consent to participate in the survey. Epidemiological data, such as sex, age at presentation to the clinics and habitat (indoor, outdoor, or mix: at least 1–50% of the time spent outdoors), were recorded.

Sample analysis: Samples were tested for the detection of antibodies against *L. infantum* using Uranotest Leishmania (Uranovet, Barcelona Spain) following the manufacturer's instructions.

Geographic Information System Mapping: A map of the sampling area was constructed using ArcMap v.10.8, including the following layers of relevant environmental information that have been considered to be relevant for the

dynamics of the analyzed organisms and their transmission vectors: climate, potential vegetation, and surface waters and surface and edaphic humidity (rivers, lakes, lagoons, irrigated croplands and parks). The canine samples were geo-referenced by the location of the health centers where the veterinary consultations occurred.

Köppen's climate classification: Castilla y León falls within the continental's Mediterranean climate, presenting long and cold winters, with average temperatures between 3 and 6 °C in January, as well as short, hot summers (average temperatures from 19 to 22 °C). A large part falls within the temperate with dry or temperate summer (Csb) or temperate with a dry season and temperate summer (Cfb) sub-climates. In several areas of the central plateau, the sub-climate is classified as temperate with dry or hot summer (Csa), or cold steppe (BSk). At high altitudes in the mountain areas, the climate present is cold temperate and dry summers (Dsb or Dsc).

Statistical analysis: Chi-square and Fischer exact tests to compare proportions were performed. In all cases, the significance level was established at $p < 0.05$.

Results

The overall seroprevalence of *L. infantum* was 4.61% (68/1475). The provinces with the highest seroprevalences were located in the south. According to climate, positive animals for *L. infantum* were found in BSk, Cfb, Csa and Csb sub-climates. No significant differences were found by sex or age. However, when habitat was assessed, significant differences between outdoor and mixed dogs were observed ($p < 0.05$). From a geospatial point of view, 97.35% of infected animals were located in areas with high edaphic availability of water, either as stagnant water as well as close to forest and groves vegetation, near holm oak, Pyrenean oak groves, "quejigo" oak forest or riparian forest, all of them mainly found in wet locations with a great abundance of irrigated arable land in the surrounding area. In fact, 5.9% (4/68) were infected by *L. infantum*.

Conclusions

The data obtained reveal the influence of the climate, orography and presence of water, which will allow comprehending their evolution in Castilla y León. Given the

risk of infection or exposure to pathogens, as their presence in humans in Spain has been described, a close relationship between veterinarians, physicians and public health administrations under the concept of One Health is needed.

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