

## Seroepidemiological study of *Leishmania infantum* in equids in different European countries

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### Background

Leishmaniosis is a vector-borne disease caused by protozoa of the genus *Leishmania* (Kinetoplastida: Trypanosomatidae), which is transmitted by infected sand flies. In Europe, *Leishmania infantum* is the main species circulating, being dogs the most relevant domestic reservoir. However, infection by this parasite has been detected in a wide range of domestic and wild species [2]. Equids can be infected by *L. infantum* and develop clinical symptoms, mainly cutaneous leishmaniosis [3]. Since equids are an important food source for sand flies, these species could act as potential reservoirs for this protozoa. In Europe, several cases of equine leishmaniosis have been reported in endemic areas for *L. infantum*, such as countries from the Mediterranean basin (e.g., Spain and Portugal), and even in non-endemic countries (e.g., Germany and Switzerland) [4]. However, serosurvey studies evaluating the exposure to *L. infantum* in equids are still very scarce in Europe. Here, we aimed to determine the seroprevalence of *L. infantum* and to identify risk factors associated with exposure to this parasite in equids in several European countries.

### Materials and methods

Between 2011 and 2023, blood samples from 1364 equines were collected in four European countries: Spain (n = 561) Italy (n = 395), Ireland (n = 212) and the United Kingdom (n = 196). Two equid species, horses (n = 1005) and donkeys (n = 240) and two equid hybrids mules/hinnies (n = 119), were sampled. Serum samples were tested by indirect immunofluorescence (IFAT) for anti-*L. infantum* antibodies using goat anti-horse IgG conjugate [5]. The cut-off was established at the 1:50 dilution, as reported elsewhere [4]. A Generalized Linear Mixed Model (GLMM) was performed with R statistical analysis program to evaluate potential risk factors associated with *L. infantum* exposure in equids.

### Results

The overall seroprevalence of *L. infantum* was 9.8% (133/1364; 95%IC: 8.2-11.3). By country, a significantly higher seroprevalence was detected in Italy (17.7%; 70/395) compared to Spain (11.2%; 63/561), while no positive animals were found in Ireland and the UK. In those countries where *L. infantum* circulation was found (Spain and Italy), the "species" was identified as a risk factor associated with *L. infantum* exposure. Significantly higher seropositivity was detected in donkeys (28.4%;  $p < 0.001$ , OR = 6.0) and mules/hinnies (22.0%;  $p < 0.001$ , OR = 3.3) compared to horses (6.6%).

### Conclusions

This is the first large-scale serosurvey on *L. infantum* comprising horses, donkeys, and mules/hinnies in Europe. The seroprevalence values detected indicate moderate and heterogeneous circulation of *L. infantum* in equids from southern European countries. Here, donkeys and mules/hinnies showed a higher risk of exposure to this zoonotic parasite compared to horses, probably due to their mainly extensive management conditions that favour contact with *L. infantum* vectors. The seroprevalence found in equids from Mediterranean countries indicates the need to establish control measures in this European area, where cases of leishmaniosis in equids may be going undetected, which could involve a risk to animal and public health.

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