

1 **Occurrence of canine and human leishmaniosis in the Alto Tâmega**
2 **region (Portugal): 2020-2022**

3
4 Running title: Leishmaniosis in Portugal

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21 **Abstract.** Zoonotic visceral leishmaniosis is a severe neglected zoonotic parasitic disease caused by
22 *Leishmania infantum* with dogs as the major reservoir hosts. Although the European Centre for Disease
23 Prevention and Control (ECDC) reported, in 2022, no human or animal cases in the Alto Tâmega region
24 between 2009-2020, the truth is that there were notified cases. The present study main objective was to
25 determine the occurrence of infection and disease among 217 clinically suspected dogs subjected to serological
26 tests in a Reference Veterinary Clinic in the Alto Tâmega region and among human patients of the Public
27 Health Unit of Alto Tâmega e Barroso actuation area, in northern Portugal. In addition, a questionnaire was
28 presented to 100 of the clinic's clients during the last fortnight of October 2022. The majority of sampled dogs
29 had no defined breed (43.7%), they were mainly males (56.7%) and under 10 years of age (29.5%). Between
30 January 2020 and September 2022, there were 64 seropositive dogs among those subjected to anti-*Leishmania*
31 antibody detection, indicating that canine leishmaniosis (CanL) had 29.5% of occurrence within the sample of
32 the population studied. Among the seropositive dogs, 44.4% were mongrel dogs and 56.52% were males. There
33 were no reported human cases between 2020 and 2022. The majority of the questionnaire respondents believed
34 that leishmaniosis does not affect humans (39.0%) and that is not a public health issue (33.0%) and a
35 considerable part of the sample does not have a position about those questions (33.0% and 42.0%,
36 respectively). Based on the survey sample collected, most people may not associate the disease with a zoonosis
37 and, therefore, there may be a risk of underdiagnosis and underreport, as there is no demand for treatment and
38 medical diagnosis directed towards some suspected cases or because human cutaneous leishmaniosis is not a
39 notifiable disease. An improvement in education of the population in areas of endemicity is needed to reach
40 the objective of minimizing the impact of leishmaniosis, perform a proper diagnosis and treatment, and ensure
41 a safer bond between animals and humans.

42

43 Key words: dog; human; leishmaniosis; public health; veterinary medicine

44

45 **INTRODUCTION**

46

47 *Leishmania* spp. are parasitic protozoa transmitted via infected female sand flies
48 (Phlebotominae) endemic in several countries of the Mediterranean Basin (Mihalca et al.,
49 2019; Cardoso et al., 2021; Maia et al., 2022). As estimated by the World Health
50 Organization (WHO, 2022), leishmaniosis, a severely neglected zoonotic parasitic disease,
51 caused by *Leishmania* spp., is considered to lead to more than 1 million new human cases
52 per year around the world (Azami-Conesa et al., 2020).

53

54 Cutaneous, mucocutaneous and visceral diseases are the clinical forms of human
55 leishmaniosis (Gonzalez-Lombana et al., 2013). Visceral leishmaniasis (VL) caused by
56 parasites of *Leishmania donovani* complex is a severe human disease, being domestic dogs
57 (*Canis familiaris*) the major host of *Leishmania infantum*, one of the species of that complex,

58 and the main reservoir host for zoonotic human infection (Campino and Maia, 2018; Velez
59 and Gállego, 2020). Importation of dogs from endemic areas and climate changes are the
60 key factors to the expansion of CanL (Morales-Yuste et al., 2022). Some studies have related
61 genetics, breed and dog's sex as factors of susceptibility to infection (Campino and Maia,
62 2018). Among surveillance, treatment of infected animals and prophylaxis, early detection
63 is an important step regarding to the disease control, being enzyme-linked immunosorbent
64 assay (ELISA) widely used for laboratory diagnosis, a technique that provides a good
65 sensitivity (86 to 99%) and also specificity (Morales-Yuste et al., 2022).

66

67 Portugal is country where leishmaniosis is endemic, with the Trás-os-Montes e Alto Douro,
68 Lisbon and Algarve geographic regions as the principal foci of the disease (ECDC, 2022).
69 According to the report of notifiable diseases (2013-2016) by the Portuguese Directorate-
70 General for Health (DGS, 2017), 3% of the notified VL human cases were reported from the
71 Alto Tâmega region. Relating to the health statistics in the Transparency Portal from the
72 National Health Service (SNS, 2018; INE, 2020), between 2014 and 2018, 4% of the
73 reported VL cases in Portugal were recorded in the Alto Tâmega area, although the report
74 commissioned by the European Centre for Disease Prevention and Control (ECDC, 2022)
75 concluded that there were no reported cases of human or animal leishmaniosis between 2009
76 and 2020. In this present study, in a Reference Veterinary Clinic (RVC), located in the Alto
77 Tâmega region, just in 2020, the prevalence of CanL cases was about 31% in dogs subject
78 to a serological test. There is a lack of information about the prevalence and incidence of the
79 disease in this Portuguese region, which lies very close to the northern border with Spain.

80

81 The present study main objective is to determine the occurrence of infection and disease
82 among the dogs subjected to serological tests in an RVC in the Alto Tâmega region and
83 among human patients. Another objective was to determine the knowledge on leishmaniosis
84 among a sample of 100 clients of the clinic in the month of October 2022 and to see where
85 the lack of information takes place, in order identify methods of communication to reach a
86 best acquaintance of the population who owns at least one animal in a leishmaniosis
87 endemic region.

88

89 **MATERIAL AND METHODS**

90

91 The present study was carried out in an RVC in the municipality of Chaves, Alto Tâmega,
92 northern Portugal, which has about 13 000 clients. From the baseline of analytical results,
93 with data from January 2020 to mid-September 2022, results from 217 clinically suspected
94 dogs subjected for the first time to analysis for detection of anti-*Leishmania* antibodies, by
95 means of Leiscan® (Ecuphar, Spain), were selected. Leiscan® is a serological ELISA test
96 for the detection antibodies to *Leishmania*, which has a reported sensitivity and specificity
97 of 95.3% and 99.8%, respectively. The collected data included sex, age, breed, qualitative
98 result, the reason of the serum sample (Rz), a quantitative result and the location of origin
99 of the seropositive dogs.

100

101 It should be noted that the submission of animals to this type of diagnosis depends on the
102 clinical condition that may lead to the suspicion of infection, not forgetting the financial
103 availability of the owners or tutors. In general, the animals tested were animals with clinical
104 signs and whose tutors accepted to pay for the analysis. Additionally, a small questionnaire
105 was carried out to about 100 clients of the RVC in the last 15 days of October 2022.

106

107 To obtain epidemiological data on human leishmaniosis in the Alto Tâmega region, the
108 Public Health Unit of Alto Tâmega e Barroso, based in Chaves, was contacted. A descriptive
109 statistical analysis was performed in the Excel Office 2019.

110

111 **RESULTS**

112

113 **Characterization of the canine sample**

114

115 Dogs (n = 217) were subjected to a serological test for the first time to detect anti-
116 *Leishmania* antibodies, between January 2020 and mid-September 2022.

117

118 In terms of sex, there were 43.3% female and 56.7% male dogs (Table 1); 29.5% of the total
119 dogs had less than 10 years old, 18.9% more than 10 years and there was no age data
120 available for the remaining 51.6% (Table 1). Regarding breed, 43.7% had no defined breed,
121 42.8% had a defined breed and there was no information for 13.5% (Table 1).

122

123 In 2020, 67.5% of the tested dogs were negative, 2.6% had a doubtful result, 5.2% were
124 positive, 6.5% were low positive, 11.7% were highly positive and 6.5% were very high

125 positive. In 2021, 66.7% of the tested dogs were negative, 2.8% had a doubtful result, 12.5%
126 were positive, 2.8% were low positive, 12.5% were highly positive and 2.8% were very high
127 positive. In 2022, between January and the mid of September, 69.1% of the tested dogs were
128 negative, 2.9% had a doubtful result, 2.9% were positive, 8.8% were low positive, 8.8%
129 were highly positive and 7.4% were very high positive (Table 1).

130

131 In total, 67.7% of the samples were seronegative (Table 1) and 29.5% were seropositive. In
132 2020, the results of Rz were 0.83 ± 0.98 ; in 2021, 0.87 ± 0.99 , and in 2022, 0.95 ± 1.08 (Table
133 2).

134

135 Among the seropositive dogs, 43.5% were female and 56.5% were male in 2020, 45.5%
136 female and 54.55% male in 2021 and 30.0% and 70.0% in 2022, respectively (Table 3). In
137 the seropositive group, 44.4% had no defined breeds, 11.1% were Labrador Retrievers,
138 5.5% were German Shepherds, 3.6% were Irish Setters, 3.2% were Pointers. Estrela
139 Mountain dogs, Pitbulls, Brittanies, Siberian Huskies, Chow-Chows, English Setters, St.
140 Bernards, Rottweillers and French Bulldogs were represented by 1.8% each, and 1.6% were
141 Pinschers. For 16.4% of the seropositive dogs there was no information about their breed
142 (Table 4). Among the seropositive dogs, 4.7% had 1 year old, 7.8% 2 years, 7.8% 3 years,
143 12.5% 4 years 12.5% 5 years, 10.9% 6 years, 6.3% 7 years, 1.6% 8 years, 4.7% 9 years,
144 10.9% 10 years, 4.7% 11 years old, 3.1% 12 years, and 1.6% had 13 years old (Figure 1).

145

146 About 90.0% of the seropositive dogs were from the municipality of Chaves, 2.1% from that
147 of Montalegre, 2.1% from Vila Pouca de Aguiar and 6.3% from Valpaços. Within the
148 municipality of Chaves, 18.8% were from the parishes of Santa Cruz, Trindade e Sanjurge,
149 12.5% from Vale da Anta, 10.4% from Santa Maria Maior, 10.4% from Madalena e
150 Samaiões, 6.3% from Vilar de Nantes, 4.2% Vilarelho da Raia, 4.2% from Vidago, Arcossó,
151 Selhariz e Vilarinho das Paranheiras, 4.2% from Ervededo, 4.2% from Curalha, 2.1% from
152 Vilela Seca, 2.1% from Vila Verde da Raia, 2.1% from Travancas e Roriz, 2.1% from Santo
153 Estêvão, 2.1% from Paradela de Monforte, 2.1% from Outeiro Seco and 2.1% from Faiões
154 (Figure 2).

155

156 **Human data, 2020-2022**

157

158 According to the Public Health Unit of Alto Tâmega e Barroso, there were no human VL
159 cases between 2020 and 2022. The last one recorded was in 2017. They were no known
160 cutaneous leishmaniosis cases either, because the latter is not a notifiable disease.

161

162 **Questionnaire**

163

164 During the last fortnight of October 2022, a questionnaire was presented to the RVC. This
165 fortnight, a low season, the number of clients rounded 130. One hundred clients were asked
166 to answer the questionnaire and all of them were receptive. Table 5 represents the
167 characterization of the sample according to gender, age and education.

168

169 Out of the clients, 27.0% responded that leishmaniosis affects humans, 39.0% answered that
170 this disease does not affect humans and 33.0% that they did not know if it affects. In
171 addition, 32.0% believed that leishmaniosis only affects dogs, 42.0% answered that dogs
172 are not the only victims and 24.0% did not know if dogs were the ones affected.
173 Furthermore, 29.0% marked that leishmaniosis is caused by bacteria, 20.0% by viruses,
174 12.0% by protozoa, 2.0% by fungi, 1.0% by nematodes and 24.0% believed that is caused
175 by a different agent.

176

177 Thirty per cent of the answers indicated that leishmaniosis is directly transmitted from
178 animal to animal, 33.0% that it is not and 35.0% did not know. Fifty-six per cent were
179 affirmative when asked if the signs of the disease in dogs were apathy, skin lesions, hair
180 loss, initially around the eyes and ears, weight loss, tearing and abnormal nail growth, 8.0%
181 believed that they were not and 34.0% did not know. Seventeen per cent had had at least
182 one animal with leishmaniosis, 68.0% had not and 11.0% did not know.

183

184 Thirty-two per cent responded that there is a cure to leishmaniosis, 36.0% answered that
185 there is no cure and 31.0% did not know. Twenty-two per cent considered that leishmaniosis
186 is a public health issue, 33.0% that is not a public health problem and 42.0% had no position
187 about that.

188

189 Fifty-three believed that a vaccine can prevent the disease, 18.0% believed not and 26.0%
190 did not know. Forty-seven per cent answered that deworming can prevent it, 23.0%
191 answered that it cannot and 27.0% had no idea.

192

193 **DISCUSSION**

194

195 The majority of the sampled dogs had no determined breed (43.7%), they were males
196 (56.7%) and under 10 years of age (29.5%). Between January 2020 and end of September
197 2022, there were 64 seropositive among dogs subjected to anti-*Leishmania* antibody
198 detection for the first time, indicating that CanL had 29.5% of occurrence within the sample
199 of the studied population. Different studies have shown that more than half of the
200 seropositive animals were apparently healthy (Solano-Gallego et al., 2001; Campino et al.,
201 2022; Miró et al., 2007). This circumstance seems to have influenced the obtained results,
202 since the analyses in our study were carried out only in symptomatic animals under tutors
203 informed consent. Tutor's willingness to submit the animal to the test may be a limitation.

204

205 Among the seropositive dogs, 44.4% were mongrel dogs and 56.5% males. Age does not
206 seem to explain the variability of results, and there was no association between age and
207 infection. Sex had no correlation with the results either. This might be explained by the fact
208 that tutors use to be more careless with mongrel animals. In Portugal, especially in the
209 North, a rural lifestyle is traditional, dogs are common backyard animals and the majority
210 of them receive limited veterinary care (Mihalca et al., 2019). Regarding dogs with no
211 defined breed, they may be even more exposed to infection, because tutors tend to care more
212 about pure breeds, giving them access to indoors and better veterinary treatments, among
213 other benefits. The higher exposure of mongrels raises the probability of contact with the
214 vectors, and less or late veterinary care.

215

216 In other studies, the prevalence of *L. infantum* infection has been associated with the
217 animal's age, maybe because older animals have been exposed for longer times to
218 phlebotomine sand flies (Mihalca et al., 2019). Some studies defend sex as factor that
219 increases the risk of infection, while others find no link between sex and infection. One of
220 the reasons that can explain the higher prevalence of seropositive male dogs is the fact that
221 male dogs are preferred for guarding and hunting activities (Mihalca et al., 2019).

222

223 There was no declared human case between 2020 and 2022 in the studied geographical area.
224 The last case was diagnosed in 2017 and, as explained by the health delegate, it was a
225 complex process to determine that the human patient was really affected by VL. One of the

226 problems of this notifiable disease is that that there is a lack of information about
227 leishmaniosis among the population and also among health professionals. There is also a
228 high possibility of mistake in diagnosing this disease, as clinical signs can be the same as
229 those of several other diseases. Furthermore, this couple of years were those of the COVID-
230 19 pandemic period and it could have limited patient searching for healthcare.

231

232 As we observe by the sample of clients that responded to the questionnaire, there is a lack
233 of information about the zoonotic potential of leishmaniosis. The majority of the
234 respondents, clients with at least one animal, believed that the disease does not affect
235 humans and that it is not a public health issue. Development in terms of population's
236 education is needed, specially by veterinarians and firstly having a good of communication
237 with their clients. The population living in endemic areas must be aware about the risks,
238 clinical signs, basic biological cycle to prevent risk behaviours and to strengthen the
239 necessity of safe bonds between tutors and their animals, through caring for animals.

240

241 **CONCLUSION**

242

243 Leishmaniosis is a public health issue, but it is poorly known even among people in endemic
244 areas. One of the risk factors for the disease in an endemic region is the lack of knowledge
245 about it. Thus, it is necessary to improve the basic notions about this disease, in order to
246 mitigate cases in animals and humans, by investing in education for prevention and
247 prophylaxis. Based on results of the present study, we can hypothesize that people's tradition
248 and mentality associated with animal ownership may influence the occurrence of the
249 disease. Endemic places where there are more animals sheltered in the backyard, which
250 usually belong to breeds that are less respected in the perception of the population and with
251 which there is less concern to take care of in relation to pure breeds. The latter are more
252 expensive and seen as almost luxury items that need more care and attention, so the former
253 may have higher occurrence levels. On the other hand, based on the survey sample collected,
254 most people may not associate the disease with a zoonosis and, therefore, there may be a
255 risk of underdiagnosis and underreport, as there is no demand for treatment and medical
256 diagnosis directed towards some suspected cases or because in the case of cutaneous
257 leishmaniosis it is not a notifiable disease. It would be interesting in a future study in an
258 endemic region to assess how many of the animals diagnosed with leishmaniosis were

259 previously subjected to prophylactic measures and how their owners were alert to the
260 disease before knowing the diagnosis.

261

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263

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267

268 **References**

269

270 Azami-Conesa I, Martínez-Díaz RA, González F, Gómez-Muñoz MT (2020) First detection
271 of *Leishmania infantum* in common urban bats *Pipistrellus pipistrellus* in Europe. Res Vet
272 Sci 132:172-176.

273

274 Campino, LM (2002). Canine reservoirs and leishmaniasis: epidemiology and disease. In
275 *Leishmania* (pp. 45-57). Springer, Boston, MA.

276

277 Campino L, Maia C (2018) The role of reservoirs: canine leishmaniasis. In: Drug Resistance
278 in *Leishmania* Parasites – Consequences, Molecular Mechanism and Possible Treatments.
279 2nd ed, Springer Verlag, Vienna: pp 45-64.

280

281 Cardoso L, Schallig H, Persichetti MF, Pennisi M G (2021) New epidemiological aspects
282 of animal leishmaniosis in Europe: the role of vertebrate hosts other than dogs. Pathogens
283 10:307.

284

285 DGS (2017) Doenças de declaração obrigatória 2013-2016. Volume II – Regiões. Direção-
286 Geral da Saúde. Directorate-General for Health, Lisbon.

287

288 ECDC (2022) Surveillance, prevention and control of leishmaniasis in the European Union
289 and its neighbouring countries. European Centre for Disease Prevention and Control,
290 Stockholm.

291

292 Gonzalez-Lombana C, Gimblet C, Bacellar O, Oliveira WW, Passos S, Carvalho LP,
293 Goldschmidt M, Carvalho EM, Scott P (2013) IL-17 mediates immunopathology in the
294 absence of IL-10 following *Leishmania* major infection. PLOS Pathog 9:e1003243.
295

296 INE (2020) Estatísticas da Saúde – 2018. Instituto Nacional de Estatística. Statistics
297 Portugal, Lisbon.
298

299 Maia C, Fraga DBM, Cristóvão J, Borja LS, da Silva Solcà M, Campino L, Veras PST,
300 Gonçalves L (2022) *Leishmania* exposure in dogs from two endemic countries from New
301 and Old Worlds (Brazil and Portugal): evaluation of three serological tests using Bayesian
302 Latent Class Models. Parasit Vectors 15:202.
303

304 Miró, G, Montoya, A, Mateo, M, Alonso, A, García, S, García, A, ... & Molina, R (2007).
305 A leishmaniosis surveillance system among stray dogs in the region of Madrid: ten years of
306 serodiagnosis (1996–2006). Parasitology research, 101(2), 253-257.
307

308 Morales-Yuste M, Martín-Sánchez J, Corpas-Lopez V (2022) Canine leishmaniasis: Update
309 on epidemiology, diagnosis, treatment, and prevention. Vet Sci 9:387.
310

311 Mihalca AD, Cazan CD, Sulesco T, Dumitrache MO (2019) A historical review on vector
312 distribution and epidemiology of human and animal leishmanioses in Eastern Europe. Res
313 Vet Sci 123:185-191.
314

315 SNS (2018). Doenças de declaração obrigatória. Serviço Nacional de Saúde.
316 <https://transparencia.sns.gov.pt/explore/dataset/doencas-de-declaracao-obrigatoria/?flg=pt>
317 [accessed 01 November 2022].
318

319 Solano-Gallego L, Morell P, Arboix M, Alberola J, Ferrer L (2001) Prevalence of
320 *Leishmania infantum* infection in dogs living in an area of canine leishmaniasis endemicity
321 using PCR on several tissues and serology. J Clin Microbiol 39:560-563.
322

323 Velez R, Gállego M (2020) Commercially approved vaccines for canine leishmaniosis: a
324 review of available data on their safety and efficacy. Trop Med Int Health 25:540-557.
325

326 WHO (2022) Leishmaniasis. World Health Organization. [https://www.who.int/news-](https://www.who.int/news-room/fact-sheets/detail/leishmaniasis)
327 [room/fact-sheets/detail/leishmaniasis](https://www.who.int/news-room/fact-sheets/detail/leishmaniasis) [accessed 01 November 2022].
328

