Leishmania tarentolae and *Leishmania infantum* in geckos from Mallorca Island, Spain

Joan Martí-Carreras[1], Johan Espunyes[2], Laura Carrera-Faja[3], Carlotta Pasetto[3], Maria Magdalena Alcover Amengual[4], Sarah Chavez-Fisa[4], Marina Carrasco[1,5], Xavier Roura[1,6], Olga Francino[1,5], Lluís Ferrer[1,7#]

#Corresponding author: lluis.ferrer@uab.cat

[1]Nano1Health S.L., Edifici EUREKA, Parc de Recerca UAB, 08193 Bellaterra, Spain.

[2]IRTA. Programa de Sanitat Animal. Centre de Recerca en Sanitat Animal. Campus de la Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain.

[3]Wildlife Conservation Medicine Research Group, Departament de Medicina i Cirurgia Animals, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain.

[4]Laboratori de Parasitologia, Departament de Biologia Sanitat i Mediambient, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona, Av. Joan XXIII 27-31, 08028 Barcelona, Spain.

[5]Molecular Genetics Veterinary Service (SVGM), Veterinary School, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain.

[6]Hospital Clínic Veterinari, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain.

[7]Departament de Medicina i Cirurgia Animals, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain.

Abstract

Leishmania tarentolae and *Leishmania infantum* are two sympatric parasites of significant ecological and epidemiological interest in the Mediterranean basin. This study investigated the prevalence of *L. tarentolae* and *L. infantum* in two gecko species (*Tarentola mauritanica* and *Hemidactylus turcicus*) present on Mallorca Island, Spain, using duplex quantitative PCR. A total of 59 geckos were sampled across the island, including 53 *T. mauritanica* and six *H. turcicus*. Tissue and blood samples were screened for both parasites, and generalized linear models were used to assess organ-specific infection patterns. The results revealed the prevalence of *Leishmania* infection in adult *T. mauritanica*, with 10/49 (20.41%) testing positive for *L. tarentolae* and with 1/49 (2.04%) for *L. infantum*. Coinfection with both parasites was detected in 3/49 geckos (6.12%). No positives were identified in *H. turcicus*, probably due to small sample size. Regarding positivity by tissues, coleomic organs were more likely to be positive for *L. tarentolae* in adult *T. mauritanica* than blood, with a slighter positivity in the liver, spleen and lung. Interestingly, *L. infantum* was detected more frequently coinfecting with *L. tarentolae* than as a single parasite. These prevalence rates and the existence of coinfections highlight potential ecological interactions between the two parasites and reptiles. This study provides valuable data on the potential role of geckos in endemic areas like Mallorca.

Keywords: *Leishmania* infection; Leishmaniosis; *Tarentola mauritanica*; *Hemidactylus turcicus*; Balearic Islands.

Author Summary

Leishmaniosis is a zoonotic disease caused by parasites that can infect both animals and humans. In Europe, *Leishmania infantum* is the main species responsible for the disease, with dogs acting as its primary host. However, recent research suggests that other animals, including reptiles, might also play a role in their spread. In this study, centered in wild geckos from Mallorca Island (Spain), we investigated the prevalence of *L. tarentolae* and *L. infantum* in two gecko species (*Tarentola mauritanica* and *Hemidactylus turcicus*). Using quantitative PCR, it was found that 14/49 (28.57%) of adult *Tarentola mauritanica* were positive for *Leishmania* parasites, 13/49 (26.53%) for *L. tarentolae* and 74/49 (8.16%) for *L. infantum*. Additionally, coinfection of both parasites was detected in three geckos (3/49, 6.12%). The second gecko species studied, *Hemidactylus turcicus*, was not positive for *Leishmania*, possible due to a small sample size. These results reinforce previous studies suggesting that geckos may carry both parasites. Since *L. tarentolae* has also been detected in dogs, and both *Leishmania* species are sympatric, it raises questions about the role of reptiles in leishmaniosis cycle and transmission, as well as the reliability of current diagnostic tests in coinfections of closely related parasite species.

Introduction

In Europe, zoonotic visceral leishmaniosis is caused by *Leishmania infantum*, dogs being their primary reservoir. This disease has a wide distribution throughout the Mediterranean basin (1,2). *L. infantum* and *Leishmania donovani* are the dominant pathogenic species of *Leishmania* present in western and central Europe (3). In recent decades, different studies have shown that the epidemiology of leishmaniosis in Europe is much more complex than previously thought, broadening the range of reservoir hosts species (4) or reporting an increase in drug resistance (5–7). *Leishmania infantum* infections have been detected in other species of vertebrates, such as lagomorphs (i.e., rabbits and hares), which play an important role as sylvatic reservoirs in endemic areas of Europe (8–11). More recently, the infection of different Mediterranean reptile species with *L. infantum* has also been demonstrated, although its epidemiological role remains to be elucidated in this case (12).

Among Mediterranean reptiles, the most frequently reported *Leishmania* species is *Leishmania tarentolae*. This species, subgenus *Sauroleishmania*, is limited to the Old World and it is found in association with geckos in southern Europe and North Africa (13,14), and possibly also other areas with endemic Old World gecko populations, such as the Middle East (15). *Leishmania tarentolae* is transmitted by reptile-biting sandflies of the genus *Sergentomya* and is considered sympatric with *L. infantum*. It is regarded as non-pathogenic to mammals, including dogs (3,16,17), yet infection and parasite persistence can occur (18). Studies in Italy have detected infection rates of *L. tarentolae* up to 7% in geckos and 16.5% in lacertids (12,14). In Spain, where *Sergentomya minuta* is widely distributed (19) and where canine leishmaniosis shows high prevalence rates, especially in the Balearic Islands (20–22), it is under research which *Leishmania* species infect reptiles and which role they may play on leishmaniosis epidemiology and *Leishmania* transmission cycles (23).

Only two species of saurids inhabit the island of Mallorca, both of which are geckos: *Tarentola mauritanica* and *Hemidactylus turcicus* (24). There are no native representatives of the *Lacertidae* family, except for a few representatives of *Podarcis pituytensis*, recently introduced from the neighboring island of Eivissa (24). The two gecko species are very abundant throughout the island, with reported densities of over 1200 individuals/km^2, due to the presence of a very suitable biotope and the absence of competitors (24). Both species are anthropophilic, often living in houses and gardens. It is thought that these geckos have been introduced to Mallorca from North Africa (*T. mauritanica*) and the eastern Mediterranean (*H. turcicus*) more than 2,000 years ago (24).

Considering the biology of these two gecko species and the high prevalence of canine leishmaniosis in Mallorca (20,22), the study hypothesis was that the gecko populations in Mallorca could be parasitized by both *L. tarentolae* and *L. infantum*. Therefore, the aim of this study was to assess the prevalence of *L. tarentolae* and *L. infantum* in gecko species at different locations of Mallorca Island using nucleic acid detection (qPCR), instead of serological testing, as current *L. infantum* serology may cross-react with *L. tarentolae* (16).

Material and methods

Field trapping and dissection

Tarentola mauritanica (common wall gecko, *dragó comú*) and *Hemidactylus turcicus* (Mediterranean house gecko, *dragonet* or *dragó rosat*), as illustrated in **Figure 1a**, were captured over five days in May 2024 in the northern and central regions of Mallorca Island (Balearic Islands, Spain), as seen in **Figure 1b**. Geckos were captured by hand and identified to species level using reference keys (25). They were then humanely euthanized in situ using

intramuscular anesthesia consisting of ketamine (20 mg/kg) and medetomidine (0.2 mg/kg), followed by pitching, in accordance with the guidelines for the euthanasia of animals published by the American Veterinary Medical Association (26). The whole blood was immediately obtained via cardiac puncture and stored in a portable refrigerator before being frozen at -20ºC after each capture session. The geckos were dissected, and the spleen, heart, lungs, liver, and kidneys were individually collected and frozen at -20ºC for DNA extraction. For juvenile geckos with smaller body sizes, coelomic organs were not separated but frozen whole. Complete sample information is provided in **Supplementary Table 1**. The capture, euthanasia, and sampling in the present study was conducted following local law compliance on ethical and legal requirements for sampling wild animals (see **Ethical statements**).

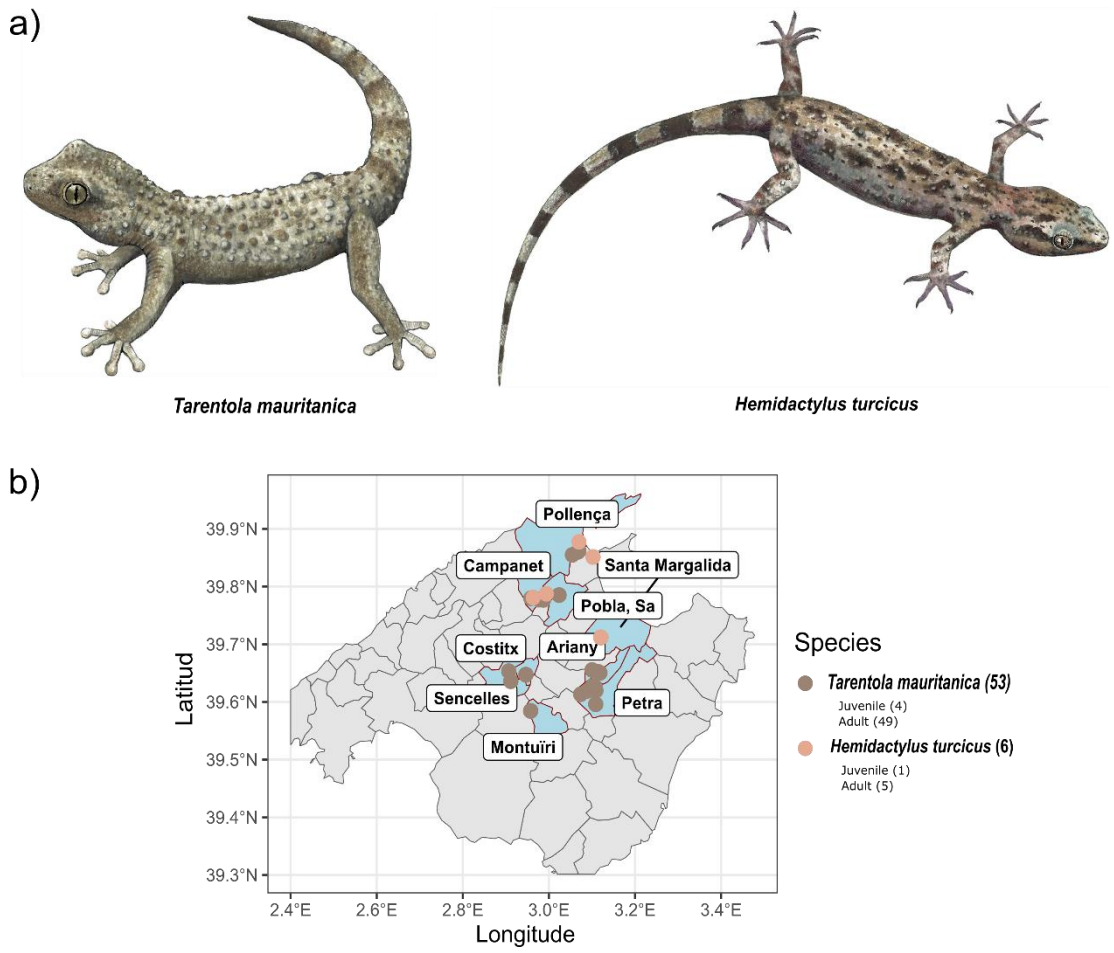

Figure 1. **Sampling distribution of *T. mauritanica* and *H. turcicus* in Mallorca Island in May 2024.** Panel a) Colored illustration of adult *T. mauritanica* and *H. turcicus* by Bruna Roqué. Panel b) Sampling distribution of *T. mauritanica* (grey) and *H. turcicus* (pink) by place of sampling (municipalities of Mallorca) and life stage (juvenile or adult).

DNA extraction and qPCR screening

Tissue and blood samples were extracted on 96-well plates with an extraction robot Chemagic 360 (Perkin Elmer, Waltham, Massachusetts) using two different extraction protocols: Chemagic DNA Tissue10 360 prefilling H96 VD150506 for solid tissue samples and Chemagic DNA Blood 250 prefilling 360 VD180628 for blood samples. Elution was performed in 80 µl. Samples were diluted 1/10 before proceeding to qPCR screening. The TaqMan Fast Advanced Master Mix (Themo Fisher Scientific, Waltham, Massachusetts, USA) was used for screening the presence of *L. tarentolae* and *L. infantum* with a discriminative duplex qPCR system previously described targeting the ITS1 region: Fw 5'-GCAGTAAAAAAAAGGCCG-3', *L. tarentolae* probe 6-FAM-5'-CACGCCGCGTATACAAAAACAC-3'-f1-quencher-MGB and *L. infantum* probe VIC-5'-TAACGCACCGGCCTATACAAAAGCA-3'-f1-quencher-MGB (14) with a different reverse primer Rv L5.8S-5'-TGATACCACTTATCGCACTT-3' (27). Testing was conducted in duplicate with extraction controls for both blood and tissue samples. Positive controls for *L. tarentolae* and *L. infantum* were used. Non-template controls (NTC) were added to each qPCR plate. The analysis threshold for qPCR was set at 0.052 for *L. tarentolae* and 0.039 for *L. infantum*. A sample was considered positive if the Ct value was ≤38.5 for both replicates after revision of the amplification curves. Kinetoplast qPCR: LEISH1 5'- AACTTTTCTGGTCCTCCGGGTAG -3', probe FAM-5'-AAAAATGGGT GCAGAAAT- 3' f1-quencher-MGB and LEISH2 5'-ACCCCCAGTTTCCCGCC-3' was used for confirmation of *L. infantum* (28). Coinfection was considered when a gecko had the

presence of both parasites in the same tissue sample or in different tissue samples from the same gecko. No significant amplification was identified in extraction or NTC controls.

Data analysis

Primers and probes were evaluated using blastn (29) against the NCBI Nucleotide database (June 2024) using the recommended parameters for primer and probe design: expected threshold of 1000, word size of 7 and unmasking low complexity regions (30).

Eds qPCR files were processed with app.thermofisher (https://apps.thermofisher.com/apps/spa/), where amplification curves were manually revised. Data analysis was conducted with R v4.4.1 (31). Sample distribution was located with packages mapSpain v0.9.2, tidyverse v2.0.0 (32). Generalized linear models (binomial logit) were employed to identify which organs were statistically more prone to be positive for *L. tarentolae* and *L. infantum* with packages for logistif v1.26.0 (33) and emmeans v1.10.5 (34). Analysis was only conducted for adult *T. mauritanica* (n = 49) as juvenile *T. mauritanica* or *H. turcicus* did not have enough sample size.

Results

A total of 59 geckos were collected, comprising five juveniles and 55 adults. Of these 59 geckos, 53 individuals were identified as *T. mauritanica* and six as *H. turcicus*, as shown in **Figure 1b**. Duplex qPCR was performed for the simultaneous detection of *L. tarentolae* and *L. infantum*. Counting both species, 14/59 (23.73%) geckos were positive for *Leishmania* spp. with 13/59 (22.03%) for *L. tarentolae* and 4/59 (6.77%) for *L. infantum*. Three of the four positives for *L. infantum* were also positive for *L. tarentolae*, thus counting as coinfection cases (**Figure 2**).

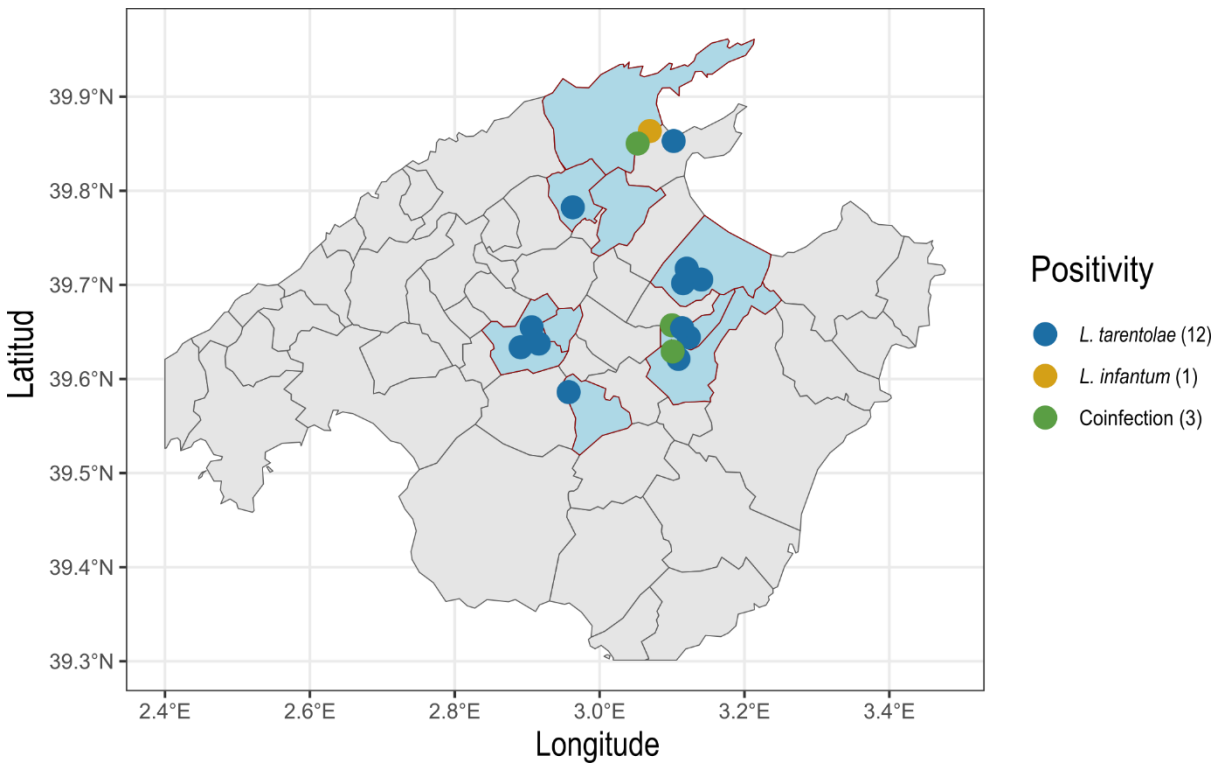

Figure 2. Distribution of *L. tarentolae* and *L. infantum* positive geckos in Mallorca Island in May 2024. Gecko positivity by RT-qPCR against *L. tarentolae* (blue), *L. infantum* (yellow) and coinfection with both parasites (green).

For adult *Tarentola mauritanica* (n = 49), the prevalence of *L. tarentolae* was 13/49 (26.53%) and 4/49 (8.16%) for *L. infantum*, with a coinfection rate of 3/49 (6.12%). In detail, *L. tarentolae* tested positive in 13 adults in liver (6), spleen (6), lung (6), kidney (4), heart (4) and blood (0). Regarding *L. infantum*, four adults tested positive in blood (2), spleen (2), liver (1), lung (1) and heart (1). The three coinfections detected were detected in spleen (2), liver (1), lung (1), heart (1) and blood (1), as summarized in **Table 1**. No juvenile specimens were positive for *Leishmania*. Generalized linear models were used to identify which organs were statistically more prone to be positive for *L. tarentolae* and *L. infantum* (not accounting for coinfection cases, **Table 1**) and provide guidance for their potential use for parasite detection. In adult *T. mauritanica* (n = 49), *L. tarentolae* is more likely to be identified in coelomic organs

(heart, liver, spleen, lung and kidney), when compared to blood (glm, p-value = 0.0266), with a slightly higher positivity in liver, spleen and lung. Regarding *L. infantum*, no organ was significantly more likely to be positive due to its smaller sample size.

For *L. tarentolae*, the average Ct of detection was 34.02 with a minimum Ct of 28.69 and maximum Ct of 38.42, meanwhile for *L. infantum*, the average Ct value of detection was 36.44 with a minimum Ct of 34.55 and a maximum Ct of 38.33. Primer and probe in silico analysis showed that TaqMan-MGB probes were highly specific against their targets. The TaqMan-MGB Probe Lt had 100% identity and 100% coverage against *L. tarentolae* (only taxon that matched). The TaqMan-MGB Probe Li had a 100% identity and 100% coverage against *L. donovani-infantum* complex. The complete specificity of TaqMan-MGB probes ensures the appropriate signal for each species. No cross hits were identified between probes, although primers align against several *Leishmania* species (*L. tarentolae*, *L. infantum*, *L. donovani*, *L. amazonensis*, *L.major*, *L. tropica* and *L. mexicana*) with a 100% identity and 100% sequence coverage. Additionally, positive samples for *L. infantum* Li TaqMan-MGB probe were also tested with a different qPCR assay targeting the kinetoplast for confirmation (24).

Table 1. Prevalence of *L. tarentolae* and *L. infantum* by qPCR in all coelomic organs and blood in *T. mauritanica*. Detection is expressed as Ct value for positives, and Neg. for negative samples for coinfections. For each positive organ and gecko, the average Ct value is provided for *L. tarentolae* and *L. infantum*. For coinfections the average Ct is first given for *L. infantum* and after double bar to *L. tarentolae*.

L. infantum							
GeckoID	**Heart**	**Liver**	**Spleen**	**Lung**	**Kidney**	**Blood**	**Juvenile**
Dra24032						38.18	
L. tarentolae							
GeckoID	**Heart**	**Liver**	**Spleen**	**Lung**	**Kidney**	**Blood**	**Juvenile**
Dra24001			35.87				
Dra24011	33.06	34.05	34.02	34.39	37.51		
Dra24015	38.42						
Dra24017			37.84				
Dra24018		34.54		32.15	35.17		
Dra24022		34.13	36.49	32.53			
Dra24030	36.84	33.83		33.96	35.2		
Dra24036	37.49	37.74		36.02			
Dra24037		35.49	36.46	34.63	37.41		
Dra24038			37.68				
Coinfection							
GeckoID	**Heart**	**Liver**	**Spleen**	**Lung**	**Kidney**	**Blood**	**Juvenile**
Dra24020	Neg // 30.92	36.67 // 30.09		Neg // 30.92	Neg // 34.55	38.09 // 33.33	
Dra24029	35.38 // 29.65	Neg // 29.97	33.93 // 28.69	34.55 // 28.17	Neg // 30.40	Neg // 34.00	
Dra24033	Neg // 32.64	Neg // 31.42	38.30 // 35.94	Neg // 31.52	Neg // 33.61		

Discussion

The Balearic Islands, including Mallorca, are endemic for *L. infantum*, the primary causative agent of leishmaniosis in humans and companion animals (20–22). In humans, the average annual incidence of clinical leishmaniosis ranges from 0.7 to 3.5 cases per 100,000 inhabitants (22). However, prevalence studies reveal that up to 5.9% of human blood donors harbor *L. infantum* DNA, indicating that most infections are asymptomatic (21). Similarly, *L. infantum* prevalence is high in dogs, with positivity ranging from 56% to 67% depending on dog population (free-living vs. kennel) or testing method (serology vs. PCR) (20,35). In cats, while data specific to the Balearic Islands is limited, the seroprevalence of *L. infantum* across Spain is approximately 15%, comparable to the Mediterranean average of 17.3% (36). It is worth noting that serological testing of *L. infantum* may be overreporting the incidence of infection, as current serological tests may not discriminate between *L. infantum* (16) and *L. tarentolae*, believed to be non-pathogenic but able to infect and persist in mammals (3,16–18).

Regarding *L. tarentolae*, its prevalence in Spain is less well-documented, as it is primarily associated with reptiles. *Leishmania tarentolae* or *L. tarentolae*-like parasites have been reported in *Sergentomyia minuta* in continental Spain (37,38). This study represents one of the most comprehensive assessments of *L. tarentolae* and *L. infantum* prevalence in wild gecko populations on Mallorca Island, investigating whether these geckos can serve as hosts for both *Leishmania* species. *T. mauritanica* exhibited notable prevalences of *L. tarentolae*, with some coinfection cases with *L. infantum* observed in this species. Regarding *H. turcicus*, no positive samples were detected, possibly due to a small sample size. In similar studies performed in the south of Italy, Latrofa *et al.*, 2021, reported no cases of *L. tarentolae* or *L. infantum* in *T. mauritanica*, albeit with only three geckos (14). Two studies from Mendoza-Roldán *et al.*, reported the prevalence of *L. tarentolae* was 8.47% in 2021 and 7.69% in 2022, meanwhile the

prevalence of *L. infantum* was of 1.7% and 7.69%, respectively (39,40). Regarding *H. turcicus*, only one gecko was positive for *L. infantum* (12). In this study, adult *T. mauritanica* showed a prevalence of 13/49 (26.53%) for *L. tarentolae* and 4/49 (8.16%) for *L. infantum*. When compared to those prior studies (12,39,40), the prevalence of *L. tarentolae* in *T. mauritanica* is higher in Mallorca Island than in the south of Italy respectively (39,40), both endemic regions of canine leishmaniosis. Interestingly, the prevalence of *L. infantum* is of similar magnitude in both areas. More importantly, this is the first study to detect coinfection with *L. tarentolae* and *L. infantum* in adult *T. mauritanica*, showing that such scenarios occur in wild populations. Such differences of *L. tarentolae* between studies may be accounted for (i) a high density of geckos on the island, which may facilitate parasite transmission; (ii) a high density and distribution of the sandfly vector, which contributes to the spread of *Leishmania*; and (iii) a high prevalence of *Leishmania* infection in dogs, and potentially other mammals inhabiting the island. To the authors knowledge, this is the first study in Spain to examine the prevalence of *L. tarentolae* in reptile species, with findings showing a high prevalence of *L. tarentolae* in adult *T. mauritanica* on Mallorca. The presence of coinfections (3/49) of *L. tarentolae* and *L. infantum* suggests that individual geckos can harbor multiple *Leishmania* species simultaneously, albeit research is still needed in vitro and in vivo regarding its replication and spread.

These findings highlight the potential role of reptiles in the local ecology of *Leishmania* species. While dogs are established reservoirs of *L. infantum* and asymptomatic human and feline infections are common, the high prevalence of *L. tarentolae* and the presence of *L. infantum* in geckos underscores the need to investigate their ecological and epidemiological significance. Further research on additional sensible host species could enhance understanding of *Leishmania* transmission (23), maintenance and alternative hosts (4), contributing to improved strategies for managing human and animal leishmaniosis.

Conclusion

This study aimed to examine the prevalence of *L. tarentolae* and *L. infantum* in the wild gecko population, as possible reservoir host, on Mallorca Island. The findings contribute to identifying the presence of sympatric *Leishmania* parasites in wildlife that cohabit with dogs and humans in isolated endemic regions. Results show a higher prevalence of *L. tarentolae* when compared to data from mainland Italy (south). Interestingly, this is the first study to detect *L. tarentolae* and *L. infantum* coinfections in *T. mauritanica*, showing that such scenario is possible in wild populations. Further research is required to contextualize these prevalence data alongside those in humans and companion animals. Such research opens the question whether *Leishmania* infection in geckos may contribute to *Leishmania* infection in humans and other mammals or remains confined to reptilian hosts.

Acknowledgments

The authors wish to acknowledge the technical assistance of Dr. Anna Mercadé in test automation and the group of Prof. Domenico Otranto for their supply of a positive control sample of *L. tarentolae* for the qPCR testing. The authors would like to acknowledge the natural history illustrator Bruna Roqué (www.brunaroque.com) for her contribution with the illustrations of *T. mauritanica* and *H. turcicus*.

Funding

JMC was supported by PTQ2022-012391 and MC was supported by PRPDIN-2021-011839, both funded by MCIN/AEI/10.13039/501100011033. MC was also supported by the Industrial Doctorate Plan from the Departament de Recerca i Universitats de la Generalitat de Catalunya (AGAUR, 2023 DI 00019).

Conflict of interest

Nano1Health SL is a for-profit organization.

Ethical statement

The capture, euthanasia, and sampling of wild geckos in this study were authorized by the government of the Balearic Island (permit CEP 21/2023).

Bibliography

1. Otranto D, Dantas-Torres F. The prevention of canine leishmaniasis and its impact on public health. Trends Parasitol. 2013 Jul;29(7):339–45.

2. Martín-Sánchez J, Rodríguez-Granger J, Morillas-Márquez F, Merino-Espinosa G, Sampedro A, Aliaga L, et al. Leishmaniasis due to *Leishmania infantum* : Integration of human, animal and environmental data through a One Health approach. Transbound Emerg Dis. 2020 Nov 29;67(6):2423–34.

3. Akhoundi M, Kuhls K, Cannet A, Votýpka J, Marty P, Delaunay P, et al. A Historical Overview of the Classification, Evolution, and Dispersion of Leishmania Parasites and Sandflies. PLoS Negl Trop Dis. 2016 Mar 3;10(3):e0004349.

4. Taddei R, Bregoli A, Galletti G, Carra E, Fiorentini L, Fontana MC, et al. Wildlife Hosts of Leishmania infantum in a Re-Emerging Focus of Human Leishmaniasis, in Emilia-Romagna, Northeast Italy. Pathogens. 2022 Nov 7;11(11):1308.

5. Leprohon P, Fernandez-Prada C, Gazanion É, Monte-Neto R, Ouellette M. Drug resistance analysis by next generation sequencing in Leishmania. Int J Parasitol Drugs Drug Resist. 2015 Apr;5(1):26–35.

6. Ponte-Sucre A, Gamarro F, Dujardin JC, Barrett MP, López-Vélez R, García-Hernández R, et al. Drug resistance and treatment failure in leishmaniasis: A 21st century challenge. PLoS Negl Trop Dis. 2017 Dec 14;11(12):e0006052.

7. Martí-Carreras J, Carrasco M, Gómez-Ponce M, Noguera-Julián M, Fisa R, Riera C, et al. Identification of Leishmania infantum Epidemiology, Drug Resistance and Pathogenicity Biomarkers with Nanopore Sequencing. Microorganisms. 2022 Nov 14;10(11):2256.

8. Molina R, Jiménez MI, Cruz I, Iriso A, Martín-Martín I, Sevillano O, et al. The hare (Lepus granatensis) as potential sylvatic reservoir of Leishmania infantum in Spain. Vet Parasitol. 2012 Nov;190(1–2):268–71.

9. Tsokana CN, Sokos C, Giannakopoulos A, Mamuris Z, Birtsas P, Papaspyropoulos K, et al. First evidence of Leishmania infection in European brown hare (Lepus europaeus) in Greece: GIS

analysis and phylogenetic position within the Leishmania spp. Parasitol Res. 2016 Jan 19;115(1):313–21.

10. Martín-Sánchez J, Torres-Medina N, Morillas-Márquez F, Corpas-López V, Díaz-Sáez V. Role of wild rabbits as reservoirs of leishmaniasis in a non-epidemic Mediterranean hot spot in Spain. Acta Trop. 2021 Oct;222:106036.

11. Barbero-Moyano J, Gonzálvez M, Bravo-Barriga D, García-Bocanegra I, López-López P, Rivero-Juárez A, et al. Epidemiological landscape in a Mediterranean hotspot of human leishmaniosis in Spain under a One Health approach. Pathog Glob Health. 2025 Mar 23;1–12.

12. Mendoza-Roldan JA, Latrofa MS, Tarallo VD, Manoj RRS, Bezerra-Santos MA, Annoscia G, et al. Leishmania spp. in Squamata reptiles from the Mediterranean basin. Transbound Emerg Dis. 2022 Sep 1;69(5):2856–66.

13. Wallbanks KR, Maazoun R, Canning EU, Rioux JA. The identity of *Leishmania tarentolae* Wenyon 1921. Parasitology. 1985 Feb 6;90(1):67–78.

14. Latrofa MS, Mendoza-Roldan JA, Manoj RRS, Pombi M, Dantas-Torres F, Otranto D. A duplex real-time PCR assay for the detection and differentiation of leishmania infantum and leishmania tarentolae in vectors and potential reservoir hosts. Entomologia Generalis. 2021 Sep 1;41(5):543–51.

15. Rato C, Carranza S, Perera A, Carretero MA, Harris DJ. Conflicting patterns of nucleotide diversity between mtDNA and nDNA in the Moorish gecko, Tarentola mauritanica. Mol Phylogenet Evol. 2010 Sep;56(3):962–71.

16. Iatta R, Carbonara M, Morea A, Trerotoli P, Benelli G, Nachum-Biala Y, et al. Assessment of the diagnostic performance of serological tests in areas where Leishmania infantum and Leishmania tarentolae occur in sympatry. Parasit Vectors. 2023 Oct 8;16(1):352.

17. Mendoza-Roldan JA, Varotto-Boccazzi I, Louzada-Flores VN, Evans A, Cheikhi IB, Carbonara M, et al. Saurian-associated Leishmania tarentolae in dogs: Infectivity and immunogenicity evaluation in the canine model. PLoS Pathog. 2024 Oct 9;20(10):e1012598.

18. Louzada-Flores VN, Latrofa MS, Lucente MS, Dambrós BP, Mendoza-Roldan JA, Varotto-Boccazzi I, et al. Intracellular persistence of Leishmania tarentolae in primary canine macrophage cells. Acta Trop. 2023 Jul;243:106935.

19. Bravo-Barriga D, Ruiz-Arrondo I, Peña RE, Lucientes J, Delacour-Estrella S. Phlebotomine sand flies (Diptera, Psychodidae) from Spain: an updated checklist and extended distributions. Zookeys. 2022 Jun 17;1106:81–99.

20. Cabezón O, Millán J, Gomis M, Dubey JP, Ferroglio E, Almería S. Kennel dogs as sentinels of Leishmania infantum, Toxoplasma gondii, and Neospora caninum in Majorca Island, Spain. Parasitol Res. 2010 Nov 6;107(6):1505–8.

21. Alcover MM, Rocamora V, Ribas A, Fisa R, Riera C. Underestimation of Human Cutaneous Leishmaniasis Caused by Leishmania infantum in an Endemic Area of the Mediterranean Basin (Balearic Islands). Microorganisms. 2023 Jan 4;11(1):126.

22. Alcover MM, Ballart C, Serra T, Portús M, Gállego M. Current status of Leishmaniosis in the Balearic Islands . In: Muñoz-Torrero D, Domínguez À, Manresa À, editors. Recent Advances in Pharmaceutical Sciences. VI. Kerala, India: Research Signpost; 2016. p. 189–211.

23. Reimann MM, Torres-Santos EC, Souza CSF de, Andrade-Neto V V., Jansen AM, Brazil RP, et al. Oral and Intragastric: New Routes of Infection by Leishmania braziliensis and Leishmania infantum? Pathogens. 2022 Jun 16;11(6):688.

24. Mayol Serra J. Rèptils i amfibis de les Balears. Palma de Mallorca: Moll Nova Editorial; 2003.

25. Speybroek J, Beukema W, Bok B, Van Der Voort J. Field guide to the amphibians and reptiles of Britain and Europe. Bloomsbury Publishing; 2016.

26. Leary S, Underwood W, Anthony R, Cartner S, Grandin T, Greenacre C, et al. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition [Internet]. 2020 [cited 2024 Mar 1]. Available from: https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf

27. Schönian G, Nasereddin A, Dinse N, Schweynoch C, Schallig HDFH, Presber W, et al. PCR diagnosis and characterization of Leishmania in local and imported clinical samples. In: Diagnostic Microbiology and Infectious Disease. Elsevier Inc.; 2003. p. 349–58.

28. Francino O, Altet L, Sánchez-Robert E, Rodriguez A, Solano-Gallego L, Alberola J, et al. Advantages of real-time PCR assay for diagnosis and monitoring of canine leishmaniosis. Vet Parasitol. 2006 Apr;137(3–4):214–21.

29. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, et al. BLAST plus: architecture and applications. BMC Bioinformatics. 2009;10(421):1.

30. Clore A. https://eu.idtdna.com/pages/education/decoded/article/tips-for-using-blast-to-locate-pcr-primers. 2011. Using BLAST to find primers—Tips from IDT.

31. R Core Team. R: A Language and Environment for Statistical Computing [Internet]. Vienna, Austria; 2019. Available from: https://www.r-project.org/

32. Wickham H, Averick M, Bryan J, Chang W, McGowan L, François R, et al. Welcome to the Tidyverse. J Open Source Softw [Internet]. 2019 Nov 21;4(43):1686. Available from: https://joss.theoj.org/papers/10.21105/joss.01686

33. Heinze G, Ploner M, Jiricka L, Steiner G. logistf: Firth's Bias-Reduced Logistic Regression. CRAN; 2003.

34. Lenth R V. emmeans: Estimated Marginal Means, aka Least-Squares Means [Internet]. CRAN: Contributed Packages. GitHub; 2017. Available from: https://CRAN.R-project.org/package=emmeans

35. Solano-Gallego L, Morell P, Arboix M, Alberola J, Ferrer L. Prevalence of *Leishmania infantum* Infection in Dogs Living in an Area of Canine Leishmaniasis Endemicity Using PCR on Several Tissues and Serology. J Clin Microbiol. 2001 Feb;39(2):560–3.

36. Carbonara M, Iatta R, Miró G, Montoya A, Benelli G, Mendoza-Roldan JA, et al. Feline leishmaniosis in the Mediterranean Basin: a multicenter study. Parasit Vectors. 2024 Aug 19;17(1):346.

37. Bravo-Barriga D, Parreira R, Maia C, Blanco-Ciudad J, Afonso MO, Frontera E, et al. First molecular detection of Leishmania tarentolae-like DNA in Sergentomyia minuta in Spain. Parasitol Res. 2016 Mar 22;115(3):1339–44.

38. González E, Molina R, Aldea I, Iriso A, Tello A, Jiménez M. *Leishmania* sp. detection and blood-feeding behaviour of S *ergentomyia minuta* collected in the human leishmaniasis focus of

southwestern Madrid, Spain (2012–2017). Transbound Emerg Dis. 2020 May 8;67(3):1393–400.

39. Mendoza-Roldan JA, Latrofa MS, Iatta R, R. S. Manoj R, Panarese R, Annoscia G, et al. Detection of Leishmania tarentolae in lizards, sand flies and dogs in southern Italy, where Leishmania infantum is endemic: hindrances and opportunities. Parasit Vectors. 2021 Dec 1;14(1).

40. Mendoza-Roldan JA, Zatelli A, Latrofa MS, Iatta R, Bezerra-Santos MA, Annoscia G, et al. Leishmania (Sauroleishmania) tarentolae isolation and sympatric occurrence with Leishmania (Leishmania) infantum in geckoes, dogs and sand flies. PLoS Negl Trop Dis. 2022 Aug 9;16(8).