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 infantum and *Leishmania tarentolae* are two sympatric parasites of significant ecological and epidemiological interest in the Mediterranean basin. In this study, we investigated the prevalence of *L. infantum* and *L. tarentolae* in two gecko species (*Tarentola mauritanica* and *Hemidactylus turcicus*) present on Mallorca Island, Spain, using duplex quantitative PCR. A total of 60 geckos were sampled across the island, including 54 *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 high prevalence rates in *T. mauritanica*, with 24/54 (44.44%) testing positive for *L. infantum* and 22/54 (40.74%) for *L. tarentolae.* Coinfection with both parasites was detected in 11/54 individuals (20.37%). In contrast, *H. turcicus* showed a prevalence of 3/6 (50%) for *L. infantum* and 1/6 (16.66%) for *L. tarentolae*, with no coinfections detected, probably due to small sample size. Blood and heart tissues were more likely to harbor *L. infantum* in geckos, while *L. tarentolae* displayed no significant organ-specific predilection. These findings indicate that *T. mauritanica* and *H. turcicus* may act as reservoir hosts for both *L. infantum* and *L. tarentolae*. The shared vectors and overlapping transmission routes (e.g., vector-borne, oral, or gastrointestinal transmission) suggest that these gecko species could contribute to the maintenance of *Leishmania* transmission cycles in the region. The high prevalence rates and frequent coinfections highlight potential ecological interactions between the two parasites. Furthermore, the cross-reactivity of *L. infantum* serological tests with *L. tarentolae* reinforces the need for molecular methods to distinguish between these species. This study underscores the ecological importance of reptiles in *Leishmania* transmission and provides valuable data on the potential role of geckos in endemic areas like Mallorca.

Keywords: *Leishmania infantum*; *Leishmania tarentolae*; *Tarentola mauritanica*; *Hemidactylus turcicus*; Mallorca Island.

Author Summary

Leishmaniosis is a 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 its spread. In this study, centered in wild geckos from Mallorca Island (Spain), we looked for *L. infantum* and *Leishmania tarentolae*, a related parasite mostly found in reptiles. Using quantitative PCR, we found that 44% of *Tarentola mauritanica* geckos carried *L. infantum* and 41% carried *L. tarentolae*. Additionally, we detected coinfection of both parasites in 20% of the geckos. The second gecko species we studied, *Hemidactylus turcicus*, was also infected but at lower rates, and no coinfections were found. These results suggest that geckos may host both parasites, potentially influencing their spread. Since *L. tarentolae* has been detected in dogs, and both parasites are found in the same environments, there could be alternative ways of transmission (e.g., dogs eating infected geckos). The high number of infections raises new questions about the role of reptiles in the disease cycle, the reliability of current diagnostic tests, and the need for further research to understand these connections.

Introduction

In Europe, zoonotic visceral leishmaniosis is caused by *Leishmania infantum*, dogs being its primary reservoir. This disease has a wide distribution throughout the Mediterranean basin (1,2). Apart from *L. infantum* (or in some cases *Leishmania donovani*), no other pathogenic species of *Leishmania* are 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 (5,6). *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 (7–9). 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 (10).

Among Mediterranean reptiles, the most frequently reported *Leishmania* species is *Leishmania tarentolae*. This species, the type species of the subgenus *Sauroleishmania*, is limited to the Old World in association with geckos in Europe, North Africa and the Middle East. *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 (3,11,12). Studies in Italy have detected infection rates of *L. tarentolae* up to 7% in geckos and 16.5% in lacertids (10,13). In Spain, where *Sergentomya minuta* is widely distributed (14) and where canine leishmaniosis shows high prevalence rates, especially in the Balearic Islands (15–17), it is unknown whether one or more *Leishmania* species infect reptiles and which role they may play as *Leishmania* reservoirs or even possibly as alternative infection routes (18).

Only two species of saurids inhabit the island of Mallorca, both of which are geckos: *Tarentola mauritanica* and *Hemidactylus turcicus*. 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 (19). 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. 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 2000 years ago (19).

Considering the biology of these two gecko species and the high prevalence of canine leishmaniosis in Mallorca (15,17), we hypothesized that the gecko populations in Mallorca could be parasitized by both *L. infantum* and *L. tarentolae*. Therefore, the aim of this study was to assess the prevalence *L. infantum* and *L. tarentolae* in gecko species at different locations of Mallorca Island using nucleic acid detection (qPCR), instead of serological testing, as current *L. infantum* serology tests cross-react with *L. tarentolae* (11).

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 (20). 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 (21). Whole blood was immediately obtained via cardiac puncture and stored in a portable refrigerator before being frozen at -20ºC after each capture session. The animals were dissected, and the spleen, heart, lungs, liver, and kidneys were individually collected and split in two pieces, one frozen at -20ºC for DNA extraction and the other kept in 9% saline solution with penicillin, which was refrigerated until culture. For juvenile individuals with smaller body sizes, coelomic organs were not separated but frozen whole. Complete sampling 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**).

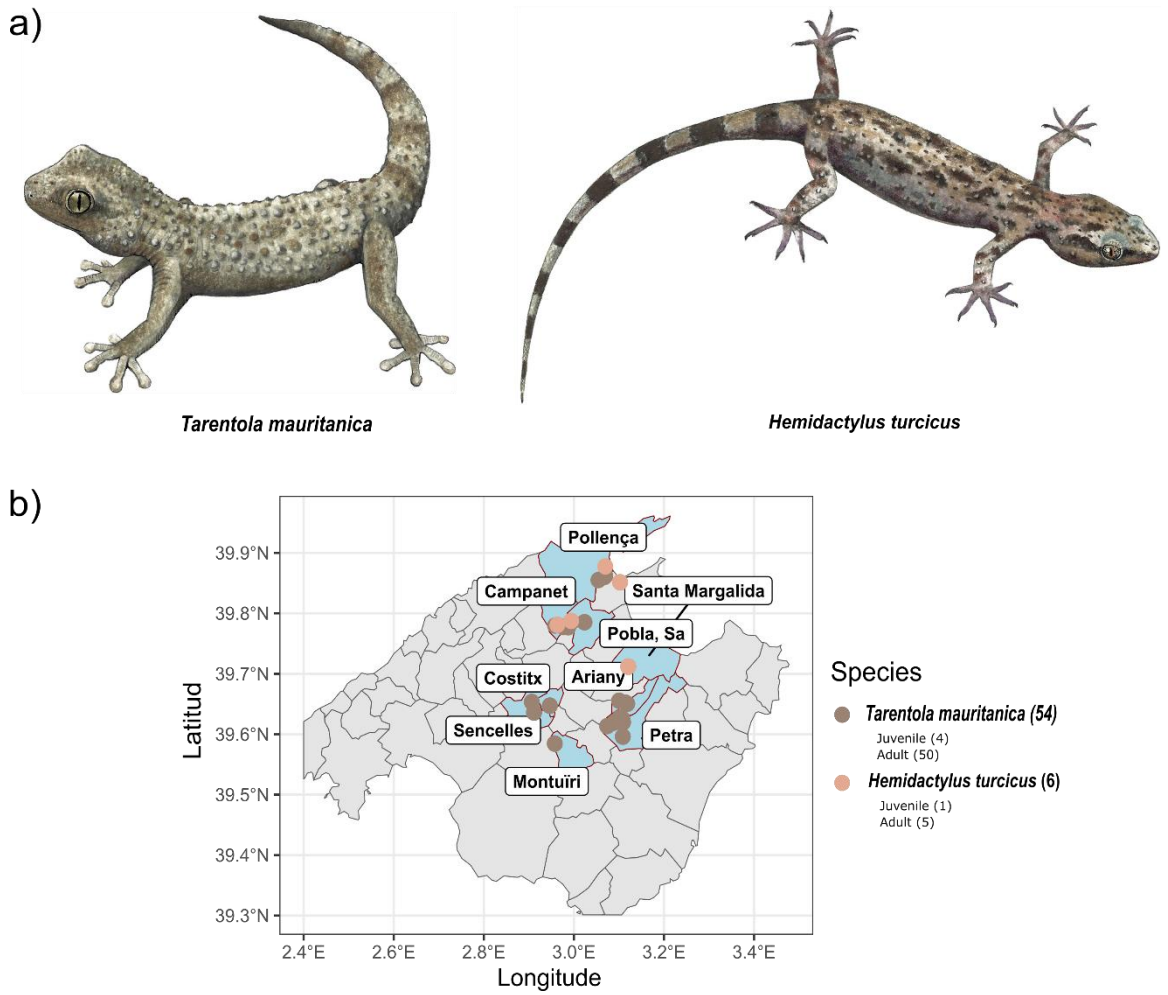

Figure 1. **Sampling distribution of *T. mauritanica* and *H. turcicus* in Mallorca Island in May 2024.** Panel a) Colored illustration of adult *T. mauritanica* and *H. turcicus* (B. Roqué). Panel b) Sampling distribution of *T. mauritanica* (grey) and *H. turcicus* (pink) by place of sampling (municipalities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

Parasite culture and isolation

Upon arrival from Mallorca, samples were initially stored in 9% saline solution with penicillin at 4°C overnight. The following day, the solution was replaced, and the samples were kept under the same conditions for an additional two hours. Only qPCR-positive samples were further processed, washed in sterile saline solution with penicillin, and homogenized using a Potter homogenizer. The homogenized material was inoculated into two NNN culture tubes (1 ml/tube) and a flask containing 5 ml of Schneider medium supplemented with 20% fetal bovine serum and 1–2% sterile human urine. Cultures were incubated at 25–26°C.

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 was used for screening the presence of *L. tarentolae* and *L. infantum* with a discriminative duplex qPCR system targeting the ITS1 region: Fw 5'-GCAGTAAAAAAAAGGCCG-3', *L. infantum* probe 6-FAM-5'-CACGCCGCGTATACAAAAACAC-3'-f1-quencher-MGB and *L. tarentolae* probe VIC-5'-TAACGCACCGGCCTATACAAAAGCA-3'-f1-quencher-MGB (13) and Rw L5.8S-5'-TGATACCACTTATCGCACTT-3' (22). The analysis threshold for qPCR was set at 0.039 for

L. infantum and 0.052 for *L. tarentolae*. A sample was considered positive if the Ct value was ≤40 after revision of the amplification curves. 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.

Data analysis

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 (23). Sample distribution was located with packages mapSpain v0.9.2, tidyverse v2.0.0 (24). Generalized linear models (binomial logit) were conducted to identify which organs were statistically more prone to be positive for *L. infantum* and *L. tarentolae* with packages for logistif v1.26.0 (25) and emmeans v1.10.5 (26). Analysis was only conducted for adult *T. mauritanica* (n = 50) as juvenile *T. mauritanica* or *H. turcicus* did not have enough sample size.

Results

A total of 60 geckos were collected, comprising five juveniles and 55 adults. Of these 60 geckos, 54 individuals were identified as *T. mauritanica* and 6 as *H. turcicus*, as shown in **Figure 1b**. Duplex qPCR was performed for the simultaneous detection of *L. infantum* and *L. tarentolae*. Overall, 39/60 (65.00%) of geckos (both species) were positive for *Leishmania*: 16/60 individuals were positive for *L. infantum*, 12/60 for *L. tarentolae* and 11/60 were coinfected with both species (**Figure 2**). For *L. infantum*, the average Ct of detection was 38.36 with a minimum Ct of 33.80 and maximum Ct of 39.85, meanwhile for *L. tarentolae*, the average Ct value of detection was 36.67 with a minimum Ct of 28.89 and a maximum Ct of

39.99. Only cultures that were derived from qPCR positive organs were maintained. Unfortunately, no *Leishmania* could be isolated due to fungal contamination of samples.

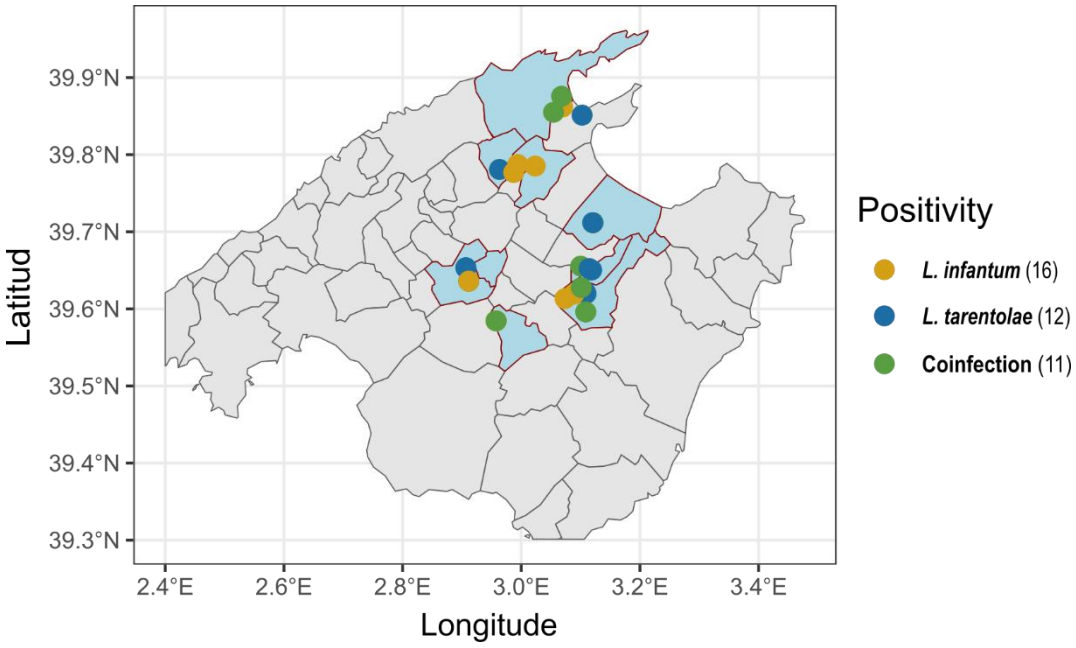

Figure 2. Distribution of *L. infantum* and *L. tarentolae* positive geckos in Mallorca Island in May 2024. Gecko positivity by RT-qPCR against *Leishmania infantum* (yellow), *Leishmania tarentolae* (blue) and coinfection with both parasites (green).

For *Tarentola mauritanica* (n = 54), the overall prevalence of *L. infantum* was 24/54 (44.44%), and for *L. tarentolae*, it was 22/54 (40.74%), with a coinfection rate of 11/54 (20.37%). Eleven adults and two juveniles tested positive for *L. infantum* in blood (6), kidney (3), spleen (3), heart (2) and lung (1). For *L. tarentolae*, 11 adults were positive in liver (7), spleen (6), lung (5), kidney (5), heart (4) and blood (1). There were 11 adults coinfected in heart (8), blood (7), spleen (7), kidney (6), liver (5) and lung (5), as summarized in **Table 1**. Detailed information can be found in **Supplementary Table 2**.

Regarding *H. turcicus* (n = 6), the overall prevalence of *L. infantum* was 3/6 (50.0%) and for *L. tarentolae* of 1/6 (16.66%), with no coinfection detected. Only four adults were positive,

three for *L. infantum* in blood and heart and one for *L. tarentolae* in liver, and the other two adults were negative. (**Supplementary Table 3**).

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

Coinfection						
Gecko ID	**Heart**	**Liver**	**Spleen**	**Lung**	**Kidney**	**Blood**
Dra24015	Neg. // 37.27	Neg.	Neg. // 39.08	Neg.	Neg.	38.43 // Neg.
Dra24018	Neg. // 39.57	Neg. // 34.91	Neg. // 38.89	Neg. // 31.83	Neg. // 36.52	37.44 // Neg.
Dra24020	Neg. // 31.73	38.03 // 30.85	Neg. // 37.47	Neg. // 30.34	Neg. // 35.55	38.86 // 34.62
Dra24029	35.98 // 30.53	Neg. // 30.98	33.80 // 29.31	34.42 // 28.89	Neg. // 31.52	Neg. // 35.59
Dra24033	Neg. // 34.06	Neg. // 32.20	38.65 // 36.85	Neg. // 32.10	Neg. // 34.67	38.94 // Neg.
Dra24034	Neg.	Neg.	Neg.	Neg.	39.42 // 39.97	Neg.
Dra24038	Neg.	Neg.	Neg. // 38.53	Neg.	Neg.	38.92 // Neg.
Dra24047	Neg. // 38.67	Neg. // 39.83	Neg.	Neg.	38.64 // Neg.	Neg.
Dra24050	Neg. // 39.64	38.90 // Neg.	Neg.	Neg.	Neg.	Neg.
Dra24051	Neg. // 38.87	Neg.	Neg.	38.52 // Neg.	Neg.	39.71 // Neg.
Dra24052	Neg.	Neg.	38.07 // 39.06	Neg.	Neg.	Neg.

Generalized linear models were used to identify which organs were statistically more prone to be positive for *L. infantum* and *L. tarentolae* (not accounting for coinfection cases, **Table 2**) and provide guidance for their potential use for parasite detection. In adult *T. mauritanica*, *L. infantum* was significantly more likely to be detected in blood compared to visceral organs (glm, p-value = 0.009), except for the heart, which was also likely to be positive during screening (glm, p-value = 1.17e-6). Pairwise comparison of blood positivity with other organs revealed the following: blood was 9.087% more likely to be positive than liver (glm, p-value = 0.0379, significant) and 13.26% more likely to be positive than lungs (glm, p-value = 0.0552, not significant trend). Spleen, heart and kidney comparisons were deemed not significant (glm, p-value = 0.2742, 0.0951 and 0.1658, respectively). Regarding *L. tarentolae*, no organ was significantly more likely to be positive (glm, p-value > 0.3).

Table 2. Organ positivity of *L. infantum* and *L. tarentolae* in *T. mauritanica* and *H. turcicus* sampled in Mallorca in May 2024.

Tarentola mauritanica							
Species	**Heart**	**Liver**	**Spleen**	**Lung**	**Kidney**	**Blood**	**Juvenile**
L. infantum	3/50 (6.00%)	2/50 (4.00%)	6/50 (12.00%)	3/50 (6.00%)	5/50 (10.00%)	12/50 (24.00%)	2/4 (50.00%)
L. tarentolae	12/50 (24.00%)	12/50 (24.00%)	13/50 (26.00%)	9/50 (18.00%)	10/50 (20.00%)	3/50 (6.00%)	0/4 (0.00%)
Hemidactylus turcicus							
Species	**Heart**	**Liver**	**Spleen**	**Lung**	**Kidney**	**Blood**	**Juvenile**
L. infantum	1/5 (20.00%)	0/5 (0.00%)	0/5 (0.00%)	0/5 (0.00%)	0/5 (0.00%)	3/5 (60.00%)	0/1 (0.00%)
L. tarentolae	0/5 (0.00%)	1/5 (20.00%)	0/5 (0.00%)	0/5 (0.00%)	0/5 (0.00%)	0/5 (0.00%)	0/1 (0.00%)

Discussion

This study represents one of the most comprehensive assessments of *L. infantum* and *L. tarentolae* prevalence in wild gecko populations on Mallorca Island, investigating whether these geckos can serve as hosts for both *Leishmania* species. A high prevalence of both *Leishmania* species was detected in the wild gecko population of the island (**Figure 1b**). *T. mauritanica* exhibited notable prevalences of *L. infantum* and *L. tarentolae,* with several coinfection cases observed in this species. Although the sample size for *H. turcicus* was smaller, *L. infantum* prevalence was comparable, while *L. tarentolae* prevalence was lower. No coinfection cases were detected in *H. turcicus*.

When compared to prior studies (10,27,28), the prevalence of *L. infantum* and *L. tarentolae* in *T. mauritanica* is higher in Mallorca Island than in the south of Italy, both endemic regions of canine leishmaniosis. Interestingly, this is the first study to detect coinfection with *L. infantum* and *L. tarentolae* in *T. mauritanica*, showing that such scenarios occur in wild populations. In detail, Latrofa *et al.*, 2021, reported no cases of *L. infantum* or *L. tarentolae* in *T. mauritanica*, albeit with a very small sampling size (3 geckos). Two studies from Mendoza-Roldán *et al.*, reported a prevalence of *L. infantum* of 7.14% in 2021 and 1.69% in 2022. Similarly, the prevalence *L. tarentolae* was 7.14% and 8.47%, respectively. Regarding *H. turcicus*, only one gecko was positive for *L. infantum* (10).

In this study, *T. mauritanica* showed a prevalence of 24/54 (44.44%) for *L. infatum* and 22/54 (40.74%) for *L. tarentolae*. Such remarkable differences between studies may be accounted for (i) high prevalence of *L. infantum* infection in dogs, and potentially other mammals inhabiting the island; (ii) a high density of geckos on the island, which may facilitate parasite transmission; and (iii) a high density and distribution of the sandfly vector, which contributes to the spread of *Leishmania*. Another possibility is methodological differences between studies.

In our study, sample positivity was defined with a Ct threshold of Ct 40 (given manual acceptance of amplification curves), whereas other studies established a Ct threshold of 38. Additionally, different reverse primers were used (Schönian et al., 2003 vs. Latrofa et al., 2021). While potential Ct differences introduced by primer selection are likely negligible in the context of a positivity testing, operational variations, such as differences in elution volume during extraction or a 10x dilution during sample preparation for qPCR, can introduce similar or even greater variability in Ct values.

The Balearic Islands, including Mallorca, are endemic for *L. infantum*, the primary causative agent of leishmaniosis in humans and animals (15–17). This study highlights a significant prevalence of *L. infantum* in *T. mauritanica* (44.44%) in Mallorca, suggesting that geckos may act as reservoir hosts. In humans, the average annual incidence of clinical leishmaniosis ranges from 0.7 to 3.5 cases per 100,000 inhabitants (17), However, prevalence studies reveal that up to 5.9% of human blood donors harbor *L. infantum* DNA, indicating that most infections are asymptomatic (16). 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 (serological vs PCR) (15,29). 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% (30). It is worth noting that serological testing of *L. infantum* may be overreporting the incidence of leishmaniosis, as current tests do not discriminate between *L. infantum* and *L. tarentoale* (11), the later which is believed to be non-pathogenic in mammals (3,11,12).

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 (31,32). To the authors knowledge, this is the first study in Spain to examine the prevalence of *L. tarentolae* in non-vector species, with

findings showing a 40.74% prevalence of *L. tarentolae* in *T. mauritanica* on Mallorca. The coinfection rate of *L. infantum* and *L. tarentolae* in geckos, at 20.37%, also suggests that individual geckos can harbor multiple *Leishmania* species simultaneously.

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 both *L. infantum* and *L. tarentolae* in geckos underscores the need to investigate their ecological and epidemiological significance. Further research on additional reservoir host species could significantly enhance our understanding of *Leishmania* transmission (18), maintenance, emergence of drug resistance (33), and alternative reservoir hosts (4), contributing to improved strategies for managing human and veterinary leishmaniosis.

Conclusion

This study aimed to examine the prevalence of *L. infantum* and *L. tarentolae* in the wild gecko population, as possible reservoir host, on Mallorca Island. The findings contribute to understand the possible transmission between species and overall parasite load in isolated endemic regions. Results show a higher prevalence of both parasites when compared to data from southern Italy. Interestingly, this is the first study to detect *L. infantum* and *L. tarentolae* coinfection 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. This will help determine whether *Leishmania* infection in geckos contributes 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. 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.

8. 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.

9. 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.

10. 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.

11. 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.

12. 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.

13. 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.

14. 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.

15. 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.

16. 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.

17. 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.

18. 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.

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

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

21. 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

22. 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.

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

24. 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

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

26. 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

27. 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).

28. 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).

29. 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.

30. 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.

31. 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.

32. 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.

33. 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.