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Role of the Lizard *Teira dugesii* as a Potential Host for *Ixodes ricinus* Tick-Borne Pathogens

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PCR screening of ticks and tissue samples collected from 151 *Teira dugesii* lizards seems to indicate a potential role of this lizard species in the maintenance and transmission cycle of some *Ixodes ricinus* tick-borne agents, such as *Rickettsia monacensis*, *Rickettsia helvetica*, and *Borrelia lusitaniae*, that are circulating on Madeira Island.

Lizards have been identified as vertebrate hosts of ixodid ticks (3). The existence of an endemic lizard population of *Teira dugesii* species that have become one of the most prevalent vertebrate species on Madeira Island and are known to host immature stages of *Ixodes ricinus* drew our attention. Additionally, on this island, *I. ricinus* has been shown to be infected with different genospecies of *Borrelia* spp., *Rickettsia monacensis*, and *Anaplasma phagocytophilum* (6, 10, 11, 17). A comprehensive investigation was thus undertaken to study the potential role of *T. dugesii* in the maintenance and transmission of *I. ricinus* tick-borne agents.

One hundred fifty-one tissue samples from lizard tails and 211 *I. ricinus* ticks removed from 100 lizards were collected monthly from March 2009 to February 2010 in the Calheta and Campanario areas on Madeira Island. DNA samples were analyzed by PCR for *Rickettsia*, *Borrelia*, and *A. phagocytophilum* using specific primer pairs targeting two genes for each agent, as previously described (2, 4, 9, 16, 19). Additionally, 60 DNA samples extracted from lizard blood prints were tested for *A. phagocytophilum*.

Ticks were present only in lizards captured in Calheta (n = 130) and were mostly found in the forelimb axial region. An average of 2.1 ticks per animal was observed. Nymphs were detected in all studied periods, but larvae were present only from early April to November (Fig. 1).

Lizards and ticks infected with *Borrelia lusitaniae*, *R. monacensis*, *Rickettsia helvetica*, and *A. phagocytophilum* were found only in Calheta, as shown in Table 1. *Rickettsia*-positive amplicons showed 100% sequence identity with *gltA* (341/341 bp) and *ompB* (384/384 bp) of *R. helvetica* (GenBank accession numbers U59723 and HQ232251) and 100% identity with *gltA* (341/341 bp) and *ompB* (384/384 bp) of *R. monacensis* (GenBank accession numbers HM149283 and EU883092), previously detected in Portuguese ticks (6, 12). Two main genetic variants of *B. lusitaniae* were detected in the intergenic spacer (IGS) partial sequence from 5S (*rrf*) to 23S (*rrl*): variant 1, presenting 100% identity to PotiB6, a tick isolate previously reported on Madeira Island (GenBank accession number EU078961) (6), and variant 2, detected in only one tick and showing 100% identity to PoAnB1, a Portuguese isolate from *Apodemus sylvaticus* (GenBank accession number EF647595) (7). *A. phagocytophilum*-positive amplicons showed 100% sequence identity to previously described *rrs* sequences from Portuguese ticks (GenBank accession number EU098006) (16). However, *groESL* gene analysis revealed two main polymorphic positions in the encoding sequence, enabling the identification of three genetic variants: variant A, found in one lizard and eight ticks and showing 100% identity to a North American strain isolated from humans (GenBank accession number U96728), variant B (nucleotide 401, G→A; nucleotide 497, T→C), detected in five ticks, and variant C (nucleotide 401, G→A), found in two ticks and 100% identical to the *A. phagocytophilum* partial sequence detected in an *I. ricinus* species from Madeira Island (GenBank accession number EU004826) (17).

Rickettsia was the most prevalent agent detected in both lizards and ticks, with a higher infection in nymphs. An interesting finding was the presence of *R. monacensis* and *R. helvetica* in lizard tissues because the latter had never been detected on the island. These results suggest not only that disseminated infections can occur in *T. dugesii* but also that this species may be a potential or transitory reservoir infecting ticks. The fact that all the ticks collected from lizards were localized on the lizard forelimb axial area and that sample tissue for PCR detection was removed from the tail seems to indicate that disseminated infection may occur. *R. monacensis* had previously been detected by PCR on Madeira ticks, albeit with a lower prevalence (20%) than that of the present study (41.1%) (6). This may be due to the screening of different areas and the fact that ticks were collected in vegetation and not in lizards. The detection of *B. lusitaniae* in lizard tissues and the high level of infected parasitizing ticks also support the importance of *T. dugesii* in the maintenance of this genospecies on Madeira Island, a finding which is in agreement with those of studies carried out in other lizard species (1, 5, 8, 15). *B. lusitaniae* has also been associated with other reservoirs, such as rodents (*A. sylvaticus*) and migratory birds (7, 14). It is suggested by several authors that bird ticks may be responsible for the geographic dispersion of *B. lusitaniae*, and both rodents and lizards may be responsible for perpetuating this genospecies in enzootic cycles in which they are the major vertebrate hosts for *I. ricinus* (7, 15). As described for *R. monacensis*, a

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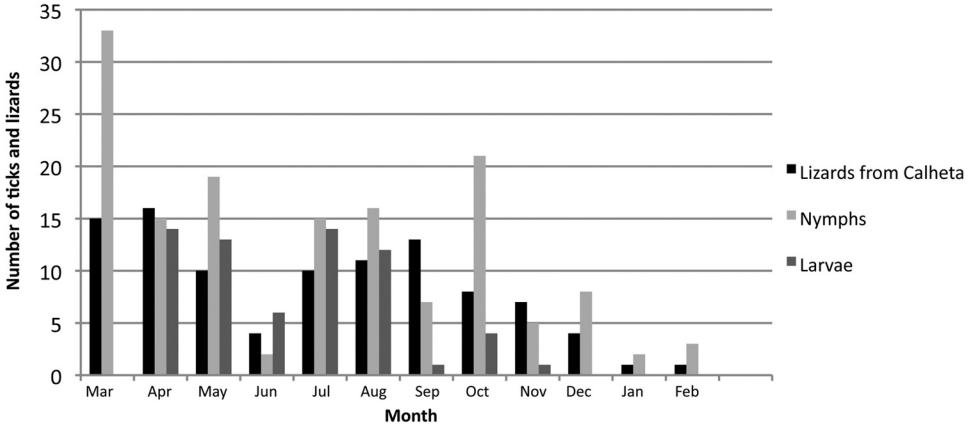

FIG 1 Numbers of lizards and their ticks (nymphs and larvae) collected in Calheta.

higher prevalence (11.8%) of *B. lusitaniae* in ticks from lizards was found in this work than in our previous work (2.7%) on Madeira Island (6). Another important finding in this study is the presence of polymorphisms that reveal the presence of genetic variants of *B. lusitaniae*, which appear to be specifically associated with lizard hosts, in some sequences. Further studies need to address the eventual evolution forces responsible for this genetic diversity and the presence of *B. lusitaniae* subpopulations associated with specific reservoirs. Other *Borrelia* genospecies, such as *Borrelia afzelii*, *Borrelia garinii*, and *Borrelia valaisiana*, that were previously detected in ticks on Madeira Island in the same area were not detected in either ticks attached to lizards or lizard tissues (11). Currently, we are testing the hypothesis that *T. dugesii* might have a borrelicidal effect on some *Borrelia* genospecies other than *B. lusitaniae*.

Regarding *A. phagocytophilum*, the data obtained thus far suggest that Madeira Island lizards are exposed to infected ticks but that they do not seem to be a primary reservoir host for this agent. In fact, the sole *T. dugesii* positive tail snip detected contrasts with the number of studied lizards and with the much higher number of positive parasitizing nymphs found in the same area. Moreover, no DNA was found in the lizard blood prints tested, even with the blood-borne nature of this agent. Since *A. phagocytophilum* is not transovarially transmitted, the absence of *A. phagocytophilum* infection in all attached larvae suggests that lizards were not infectious for ticks and raises the hypothesis that positive nymphs may have resulted from previous infections as larvae when feeding on other reservoir hosts. These results are in accordance with previous findings in an experimental infection study of *A. phagocytophilum* in lizards (13). Studies performed by Matuschka and collaborators (10, 11) suggest that rats are potential reservoir hosts for *Borrelia* on this island. This could also be true for *A. phagocytophilum*. Nevertheless, a preliminary study performed on Madeira Island in rodents did not find *A. phagocytophilum* infection in two of the three species present on the island (18). During the present study, field work was also directed toward the capture of rodents, but unfortunately, no animals were captured, a result which might be related to recent massive campaigns for rodent control in the area.

In conclusion, Madeira Island harbors a large population of *T. dugesii* lizards that seem to be involved in the maintenance and transmission cycle of at least two tick-borne agents. Future experimental studies involving *R. monacensis* maintenance in lizards and its transmission to feeding ticks are crucial to evaluate whether lizards are transitory reservoirs and how long they can sustain the infection. The study of other potential hosts is also

TABLE 1 Prevalence of *Borrelia burgdorferi* sensu lato, *Rickettsia* spp., and *Anaplasma phagocytophilum* infection in lizard tissue samples and ticks collected from lizards

Sample type and stage (*n*)	No. (%) infected with:					
	Rickettsia monacensis	*Rickettsia helvetica*	*R. monacensis* or *R. helvetica*	*Borrelia lusitaniae*	*Anaplasma phagocytophilum*	Coinfection(s) (no. [%])[a]
Ticks						
Larvae (65)	20 (30.8)	0	20 (30.8)	3 (4.6)	0	1 (1.5) Bl + Rm
Nymphs (146)	67 (45.9)	2 (1.4)	69 (47.3)	22 (15.0)	15 (10.3)	10 (6.8) Bl + Rm; 5 (3.4) Ap + Rm
Total (211)	87 (41.2)	2 (1.4)	89 (42.2)	25 (11.8)	15 (7.1)	16 (7.6)
Lizard tissues						
Adults (83)	6 (7.2)		6 (7.2)	3 (3.6)		0
Juveniles (68)	4 (5.9)	2 (1.3)	6 (7.2)	4 (5.9)	1 (0.7)	0
Total (151)	10 (6.6)	2 (1.3)	12 (7.9)	7 (4.6)	1 (0.7)	0
Lizard blood prints						
Total					0	

[a] Bl + Rm, *Borrelia lusitaniae* and *Rickettsia monacensis* coinfection; Ap + Rm, *Anaplasma phagocytophilum* and *Rickettsia monacensis* coinfection.

important to understand the complex interaction between ticks and hosts and pathogens on this island.

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REFERENCES

1. **Amore G, et al.** 2007. Borrelia lusitaniae in immature Ixodes ricinus (Acari: Ixodidae) feeding on common wall lizards in Tuscany, Central Italy. J. Med. Entomol. **44**:303–307.
2. **Choi YJ, et al.** 2005. Spotted fever group and typhus group rickettsioses in humans, South Korea. Emerg. Infect. Dis. **11**:237–244.
3. **Dantas-Torres F, et al.** 2008. Ticks infesting amphibians and reptiles in Pernambuco, Northeastern Brazil. Rev. Bras. Parasitol. Vet. **17**:218–221.
4. **de Sousa R, et al.** 2005. The presence of eschars, but not greater severity, in Portuguese patients infected with Israeli spotted fever strain of Rickettsia conorii. Ann. N. Y. Acad. Sci. **1063**:197–202.
5. **Dsouli N, et al.** 2006. Reservoir role of lizard *Psammodromus algirus* in transmission cycle of *Borrelia burgdorferi* sensu lato (*Spirochaetaceae*) in Tunisia. J. Med. Entomol. **43**:737–742.
6. **Lopes de Carvalho I, et al.** 2008. Detection of *Borrelia lusitaniae*, *Rickettsia* sp. IRS3, *Rickettsia monacensis*, and *Anaplasma phagocytophilum* in *I. ricinus* collected in Madeira Island, Portugal. Vector Borne Zoonotic Dis. **8**:575–579.
7. **Lopes de Carvalho I, et al.** 2010. Molecular characterization of a new isolate of *Borrelia lusitaniae* derived from *Apodemus sylvaticus* in Portugal. Vector Borne Zoonotic Dis. **10**:531–534.
8. **Majlathova V, Majlath I, Derdakova M, Vichova B, Pet'ko B.** 2006. *Borrelia lusitaniae* and green lizards (*Lacerta viridis*), Karst Region, Slovakia. Emerg. Infect. Dis. **12**:1895–1901.
9. **Massung RF, et al.** 1998. Nested PCR assay for detection of granulocytic ehrlichiae. J. Clin. Microbiol. **36**:1090–1095.
10. **Matuschka FR, et al.** 1994. Transmission of the agent of Lyme disease on a subtropical island. Trop. Med. Parasitol. **45**:39–44.
11. **Matuschka FR, Klug B, Schinkel TW, Spielman A, Richter D.** 1998. Diversity of European Lyme disease spirochetes at the southern margin of their range. Appl. Environ. Microbiol. **64**:1980–1982.
12. **Milhano N, et al.** 2010. Coinfections of *Rickettsia slovaca* and *Rickettsia helvetica* with *Borrelia lusitaniae* in ticks collected in a Safari Park, Portugal. Ticks Tick Borne Dis. **1**:172–177.
13. **Nieto NC, Foley JE, Bettaso J, Lane RS.** 2009. Reptile infection with *Anaplasma phagocytophilum*, the causative agent of granulocytic anaplasmosis. J. Parasitol. **95**:1165–1170.
14. **Poupon MA, et al.** 2006. Prevalence of *Borrelia burgdorferi* sensu lato in ticks collected from migratory birds in Switzerland. Appl. Environ. Microbiol. **72**:976–979.
15. **Richter D, Matuschka FR.** 2006. Perpetuation of the Lyme disease spirochete *Borrelia lusitaniae* by lizards. Appl. Environ. Microbiol. **72**:4627–4632.
16. **Rijpkema S, Molkenboer MJ, Schouls LM, Jongejan F, Schellekens JF.** 1995. Simultaneous detection and genotyping of three genomic groups of *Borrelia burgdorferi* sensu lato in Dutch *Ixodes ricinus* ticks by characterization of the amplified intergenic spacer region between 5S and 23S rRNA genes. J. Clin. Microbiol. **33**:3091–3095.
17. **Santos AS, Santos-Silva MM, Sousa R, Bacellar F, Dumler JS.** 2009. PCR-based survey of Anaplasma phagocytophilum in Portuguese ticks (Acari: Ixodidae). Vector Borne Zoonotic Dis. **9**:33–40.
18. **Santos AS, et al.** 2009. Detection of antibodies against Anaplasma phagocytophilum in Algerian mice (Mus spretus), Portugal. Vector Borne Zoonotic Dis. **9**:663–669.
19. **Sumner JW, Nicholson WL, Massung RF.** 1997. PCR amplification and comparison of nucleotide sequences from the *groESL* heat shock operon of *Erlichia* species. J. Clin. Microbiol. **35**:2087–2092.