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# Rickettsiae in ticks from wild and domestic carnivores of Doñana National Park (Spain) and surrounding area

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The aim of this study was to determine the vector distribution and the prevalence of *Rickettsia* species that circulate among populations of several wild and domestic carnivores in Doñana National Park, a nature reserve located in southern Spain. The presence of *Rickettsia* in hard ticks was evaluated using molecular techniques (PCR amplification and sequencing) over a sample of 430 specimens belonging to eight tick species: *Ixodes* (I.) *ricinus* (Linnaeus, 1789); *I.* (I.) *ventalloi* Gil Collado, 1936; *Pholeoixodes hexagonus* (Leach, 1815); *Hyalomma* (*Euhyalomma*) *lusitanicum* Koch, 1844; *Rhipicephalus* (*Rhipicephalus*) *sanguineus* (Latreille, 1806); *Rh.* (*Rh.*) *turanicus* Pomerantsev, 1940; *Rh.* (*Rh.*) *pusillus* Gil Collado, 1938, and *Rh.* (*Digeneus*) *bursa* Canestrini & Fanzago, 1878. These ticks were parasitising Iberian lynx (*Lynx pardinus*), common genet (*Genetta genetta*), Egyptian mongoose (*Herpestes ichneumon*), Eurasian badger (*Meles meles*) and red fox (*Vulpes vulpes*) studied in the programme for Iberian lynx conservation in Doñana National Park [1]. In addition, samples from free-roaming cats and dogs were included in this study (Table 1). After collection, the ticks were immediately placed in vials with 70% ethanol, properly labelled, and were later identified in the laboratory by species, gender and stage using existing taxonomic keys [2]. DNA was extracted individually or from monospecific lots using the kit Nucleo Spin Tissue (Macherey-Nagel, Düren, Germany) and specific rickettsial sequences were detected by using PCR primers that amplify a portion of *glta*, *ompA* and *ompB* genes, respectively [3,4]. Positive PCR products were sequenced using PCR primers and the GenomeLab DTCS- Quick Start kit (Beckman Coulter Life, Brea, CA, USA) and a CEQ 2000XL capillary DNA sequencer (Beckman Coulter) according to the manufacturer's instructions. Sequences were manually aligned and analysed with Bioedit vers. 7.0.1. and identified using the BLAST feature of GenBank.

Overall, specific rickettsial DNA was detected in 52 (31.7%) of 164 of the examined tick lots by PCR amplifying of at least two rickettsiae-specific fragments. Sequence analysis of amplicons of *glta*, *ompA* and *ompB* genes revealed the presence of three rickettsiae species. *Rickettsia monacensis* and *R. helvetica* were found in *Ixodes ricinus* and *I. ventalloi* (prevalence of 32.3 and 5.9%, respectively) parasitising five species of wild carnivores included in this study (lynx, genet, mongoose, badger and fox). Ticks of the *Rhipicephalus sanguineus* group (*Rh. sanguineus*, *Rh. turanicus* and *Rh. pusillus*) from lynx, mongoose, fox, cat and dog appeared infected with *R. massiliae* (prevalence of 28.3%). None of the *Ph. hexagonus*, *H. lusitanicum* or *Rh. bursa* specimens harboured rickettsiae.

Our study confirms the widespread distribution of *Rickettsia* sp. in ticks infesting carnivores in the Doñana area. The most striking finding of our study was the exclusive detection of three *Rickettsia* species (*R. monacensis*, *R. helvetica* and *R. massiliae*) with a demonstrated zoonotic character. Most of the tick species studied are resting on vegetation, showing a distinct preference for carnivores, ungulates and lagomorphs that coexist in the same habitat. This suggests that, in the natural environment, most rickettsiae detected are circulating between several species of mammals (predators and potential prey) of this well-conserved Mediterranean habitat. Moreover, in entropic conditions, only *R. massiliae* appeared to be infesting dog brown ticks, while *R. conorii*, the causal agent of Mediterranean spotted fever, was absent.

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No conflicts of interest declared.

**Table 1.** Capture data (ticks and host) from Doñana National Parks and surrounding area

| Host | Iberian lynx | | | | | | | Cat | | | | Genet | | | | Egyptian mongoose | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Area | P. N. Doñana | | | | | | | P. N. Doñana | | | | P. N. Doñana | | | | P. N. Doñana | | | | |
| Host number | 32 | | | | | | | 6 | | | | 5 | | | | 18 | | | | |
| Tick species | Pos./ass. | *R. hel.* | *R. mon* | *R. mas.* | M | F | N | Pos./ass. | *R. mas.* | M | F | Pos./ass. | *R. mon.* | M | F | Pos./ass. | *R. mon.* | *R. mas.* | M | F |
| *Ixodes ricinus* | 1 (2) | | 1 | | | 2 | | | | | | | | | | 2 (7) | 2 | | | 1 |
| *Ixodes ventalloi* | 4 (10) | 1 | 3 | | 1 | 9 | | | | | | 1 (1) | 1 | 1 | 1 | 0 (2) | | | | 2 |
| *Pholeoixodes hexagonus* | | | | | | | | | | | | | | | | 0 (3) | | | | 3 |
| *Hyalomma lusitanicum* | 0 (1) | | | | 1 | | | | | | | | | | | | | | | |
| *Rhipicephalus sanguineus* | | | | | | 1 | | | | | | | | | | | | | | |
| *Rhipicephalus turanicus* | 1 (8) | | | 1 | 5 | 10 | 4 | 2 (6) | 2 | 6 | 7 | | | | | 3 (3) | | 3 | 4 | 4 |
| *Rhipicephalus pusillus* | 0 (8) | | | | 24 | 8 | | 1 (1) | 1 | 4 | 1 | 0 (5) | | 3 | 5 | 0 (2) | | | 1 | 2 |
| *Rhipicephalus bursa* | | | | | | | | | | | | | | | | | | | | |
| Total tick number | | | | | 31 | 30 | 4 | | | 10 | 8 | | | 4 | 6 | | | | 5 | 12 |

| Host | Badger | | | | | Red fox | | | | | | Dog | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Area | P. N. Doñana | | | | | P. N. Doñana | | | | | | P. N. Doñana | | | Surrounding area | | | | | | | |
| Host number | 4 | | | | | 17 | | | | | | 1 | | | 46 | | | | | Total ticks | | | |
| Tick species | Pos./ass. | *R. mon.* | M | F | N | Pos./ass. | *R. hel.* | *R. mon.* | *R. mas.* | M | F | Pos./ass. | M | H | Pos./ass. | *R. mas.* | M | H | N | M | F | N | Total |
| *Ixodes ricinus* | 2 (3) | 2 | 1 | 1 | 2 | 1 (3) | | 1 | | 1 | 2 | | | | | | | | | 2 | 6 | 8 | 16 |
| *Ixodes ventalloi* | | | | | | 2 (3) | 1 | 1 | | | 3 | | | | | | | | | 2 | 15 | 1 | 18 |
| *Pholeoixodes hexagonus* | | | | | | | | | | | | | | | | | | | | 0 | 3 | 0 | 3 |
| *Hyalomma lusitanicum* | | | | | | | | | | | | | | | | | | | | 1 | 0 | 0 | 1 |
| *Rhipicephalus sanguineus* | | | | | | | | | | | | | | | 28 (81) | 28 | 106 | 125 | 31 | 106 | 126 | 31 | 263 |
| *Rhipicephalus turanicus* | | | | | | 4 (10) | | | 4 | 28 | 5 | 0 (1) | 3 | | | | | | | 46 | 26 | 4 | 76 |
| *Rhipicephalus pusillus* | 0 (1) | | | 1 | | 0 (1) | | | | | 2 | | | | | | | | | 32 | 19 | 0 | 51 |
| *Rhipicephalus bursa* | | | | | | | | | | | | | | | | | | | | 2 | 0 | 0 | 2 |
| Total tick number | | | 1 | 2 | 2 | | | | | 29 | 12 | | 3 | | | | 106 | 125 | 31 | 191 | 195 | 44 | 430 |

Pos./ass., positive vs. assayed; M, male; F, female; N, nymph; *R. hel.*, *R. helvetica*; *R. mas.*, *R. massiliae*; *R. mon.*, *R. monacensis*.

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