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**Título: Molecular Identification of two Tick-borne Relapsing Fever-like *Borrelia sp.* in hard ticks from Portugal**

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**Introduction:** Tick-borne pathogens are believed to be responsible for over 100,000 cases of human disease. Ticks are only second to mosquitoes as worldwide vectors of agents causing human diseases, but the most important vectors of pathogens in animals. Tick-borne diseases are emerging due to expanding tick populations, global warming and increased contact between humans, animals and ticks, imposing high risks to public health.

*Borrelia* genus comprises three phylogenetic groups: Lyme disease (LD) *Borrelia*, which belong to *Borrelia burgdoferi* sensu lato (*B.b.s.l.*) complex; Relapsing fever (RF) *Borrelia*; and Reptile-associated (REP) *Borrelia*. LD and REP are transmitted by hard ixodid ticks, while most RF are transmitted by soft argasid ticks. Lately several RF species like *B. theileri*, *B. miyamotoi*, and *B. lonestari* have adapted to hard ticks, such as *Rhipicephalus*, *Ixodes* or *Amblyomma*, that can act as vectors.

**Objectives:** Ascertain the infection rate of the Portuguese ixodofauna with focus on the detection and identification of *Borrelia* spp..

**Methods:** Ticks collected from eight Portuguese districts (Braga, Vila Real, Aveiro, Santarém, Lisboa, Setúbal, Évora and Faro) were identified to species level, and their DNA extracted by alkaline hydrolysis. Four genes were targeted for amplification by PCR: intergenic spacer 5S-23S, *flaB*, 16S rDNA and *glpQ*, followed by phylogenetically analysis of the obtained amplicon sequence.

**Results:** A total of 3988 questing ticks were collected, and five genera were identified: *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, and *Rhipicephalus*. *B.b.s.l.* DNA was detected in 96 (3,3%) samples out of 2915 ticks analyzed, including 11<sup>+</sup>/82 larvae (13%),

53<sup>+</sup>/1008 nymphs (5%) and 32<sup>+</sup>/1087 adults (3%). The predominant genospecies were *B. lusitaniae* and *B. garinii*, followed by *B. burgdorferi* sensu stricto, *B. valaisiana* and *B. afzelii*. Moreover, eight sequences, were identified as been part of the RFB group, clustering with *B. lonestari*, *B. theileri*, and unknown *Borrelia* sp. reported in Japan and Brazil.

**Conclusions:** Besides the presence of LD agents in the Portuguese ixodofauna, putative and uncharacterized RF-like *Borrelia* associated to hard ticks (*R. sanguineus* and *H. punctata*) were identified. As the potential risk of these spirochetes to human health is still unknown, further studies are necessary, including culture isolation, to better understand and classify this new species and to assess its distribution across Portugal.