Presence of Lyme disease agents in the Portuguese ixodofauna: development of real-time PCR assays for the identification of *Borrelia burgdorferi* genospecies

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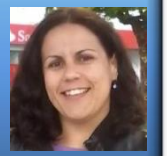

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INTRODUCTION

Lyme Borreliosis (LB) is the most common **arthropod-borne disease** in North America and Europe, and the **main vectors** are **ticks** from *Ixodes* genus. This zoonosis is caused by genetically diverse spirochetes from ***Borrelia burgdorferi* sensu lato** complex (***B.b.*s.l.**), currently comprising **19 genospecies** with diverse geographic distributions, hosts specificity and virulence. In **Portugal**, there are suitable hosts and favorable climatic conditions contributing for the distribution and maintenance of ticks and tick-borne diseases in nature. Since 1989 **several LB cases have been reported**, confirming the circulation of pathogenic strains.

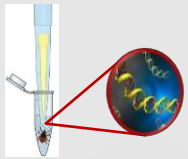
Identification of ***B.b.*s.l.** genospecies is essential to better understand the respective role in pathological involvement on LB manifestations, several PCR- and qPCR-based methods have been developed for the detection of these species.

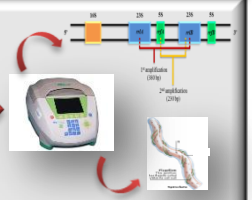
The **aims** of this study were: ***i)*** evaluate the **infection rate of *B.b.*s.l.** in ticks collected in nine sites of Portugal; ***ii)*** **develop a TaqMan® multiplex qPCR** assay targeting the ***flagellin*** gene for the detection and quantification of the most prevalent genospecies of ***B.b.*s.l.**.

MATERIAL AND METHODS

➢ Field work and *B.b.*s.l. DNA analysis

Ticks were collected from animals and vegetation (by flaging/dragging), and identified to species level.

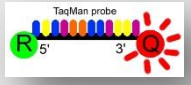




DNA - extract with 20% hydroxide ammonium. ***B.b.*s.l** DNA - screened with two nested-PCR protocols (targets: Intergenic Spacer Region between 5S and 23S rRNA and *flagellin* gene).

➢ qPCR design

Flagellin gene sequences - most prevalent ***B.b.*s.l** species in Europe.



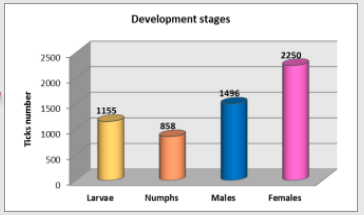
qPCR assays - optimized in a *Rotor-Gene 3000* thermocycler (analytical sensitivity and specificity of assays were evaluated).

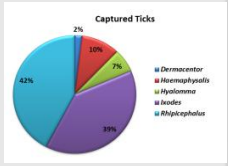
RESULTS

➢ Field work and *B.b.*s.l. DNA analysis

5,759 ticks were collected in nine sites of Portugal

- 2,236 from animals
- 3,523 from vegetation





***Borrelia* DNA found in 2.4% of the analyzed ticks**

➢ qPCR design

Probes targeting the genus ***Borrelia*** and ***B. afzelii***, ***B. lusitaniae*** and ***B. burgdorferi*** s.s. species were evaluated and revealed to be **100% specific** and **highly sensitive**. Currently, these qPCR assays are being **tested in patient's biological samples** and in **tick vectors** collected from animals and vegetation.

CONCLUSIONS

B.b.*s.l. DNA** was found not only in ***I. ricinus, but also **in other tick species**, being **nymphs** the **most infected** immature stage. Although the vector competence of these other tick species hasn't been proven, they can be **considered as potential vectors** with specific distributions and sylvatic cycles, contributing to Lyme agents maintenance in Portugal. Although the preliminary character of our results, the **qPCR assays** underdevelopment are **promising** for the **identification and quantification** of the most prevalent species of ***Borrelia burgdorferi* s.l.** in Europe, contributing to a faster and more efficient diagnosis of Lyme disease.

REFERENCES

- Franke J. *et al.* (2013). *Ticks & Tick-Borne Dis*, 4: 11-25;
- Collares-Pereira M. *et al.* (2004). *J Clin Microbiol*, 42(3):1316-1318;
- Jekings *et al.* (2012). *Exp Appl Acarol*, 58(4):431-9;
- Nolte O. (2012). Open Neurol J. 2012;6:129-39.