Taking aim at *Borrelia burgdorferi* s.l. complex: A new molecular tool for *B. garinii*, *B. afzelii* and *B. burgdorferi* s.s. detection in *Ixodes ricinus*

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Background

Lyme disease (LD), caused by spirochetes ascribed to the ***Borrelia burgdorferi* sensu lato complex**, is the most common human **tick-borne disease** in Europe. Due to the highly inter- and intra-genospecies **genetic variability**, a reference standard for species-specific detection in ticks and vertebrates **is still lacking**. The species-specific identification of the spirochetes is pivotal for both diagnostics and epidemiological studies.

Aim of the work

This work aims at finding **reliable species-specific targets** for the development of PCR protocols for the identification, without further sequencing, of the **three most common LD agents in Italy**: *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* and *Borrelia afzelii*.

Material and Methods

PCA for *B. burgdorferi* s.l. complex genomes clustering

OrthoANI analyses to assess genomes similarity

DAPC analysis to determine the most significative orthologs for each species

Species-specific primers designed on unique regions for each *B. burgdorferi* s.l. genospecies

In silico and *in vitro* PCR tests

Results

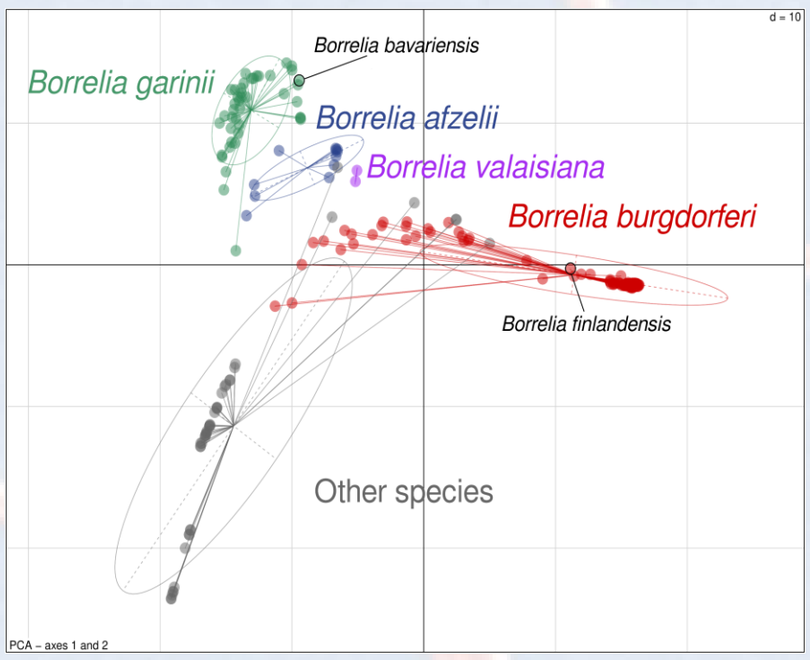

Fig. 1 PCA (Principal Component Analysis) plot (based on presence-absence matrix) of the orthologs. *B. bavariensis* and *B. finlandiensis* genomes were re-assigned at species level following OrthoANI analysis.

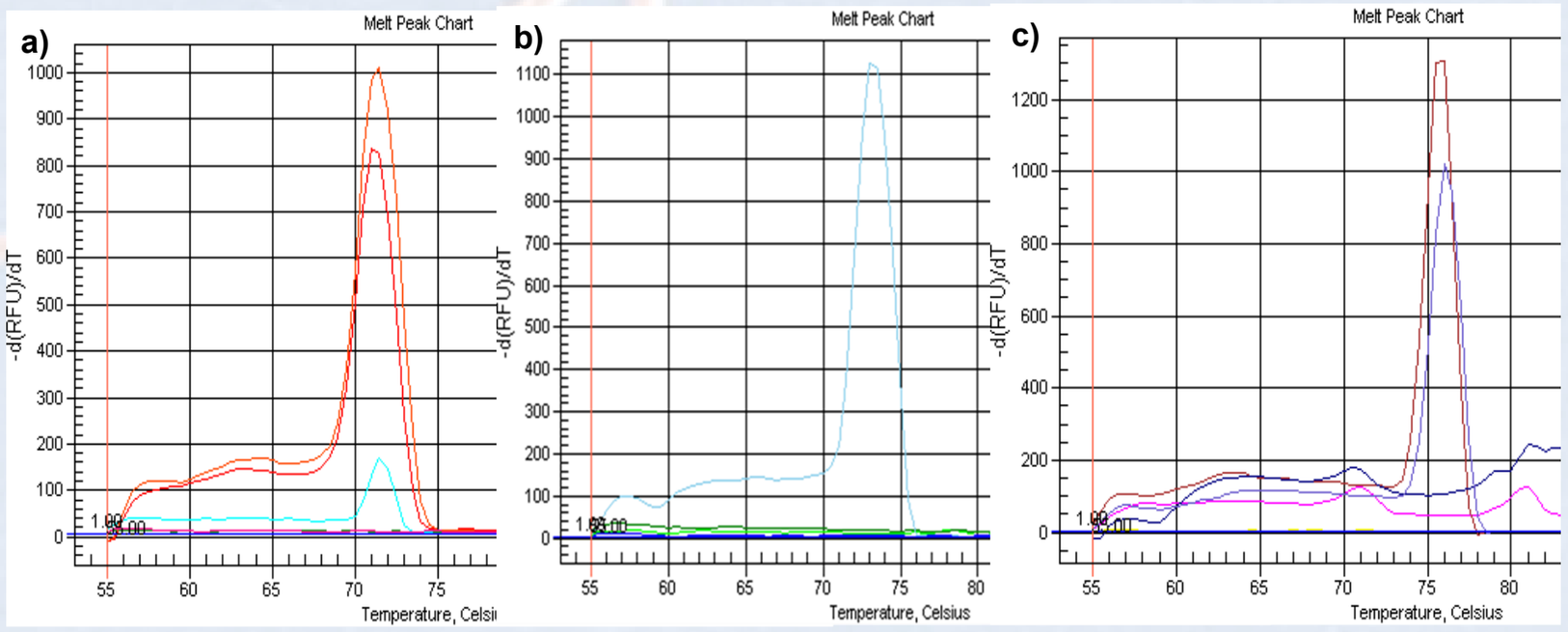

Fig. 2 Melting curve plots obtained for each species-specific real time PCR amplification using primers designed on unique *B. garinii* (a), *B. burgdorferii* s.s. (b), *B. afzelii* (c) orthologs. Each protocol was tested on the DNA of the three species and each PCR fragment shows a specific melting temperature (a=71 °C, b=73 °C, c=76 °C). The molecular approach was subsequently validated on field collected ticks.

Conclusions

The proposed approach **allows species-specific detection** of ***B. burgdorferi* s.s.**, ***B. afzelii*** and ***B. garinii*** in tick samples. The protocols can be used in both conventional and real time PCRs, also leading to further development of multiplex PCR assays. The proposed protocols are sensitive, sensible and also effective in detecting *B. burgdorferi* s.l. **co-infections** in tick hosts, suggesting their application in epidemiological studies and diagnostics.

REFERENCES: Mysterud et al., 2019 *Proc Biol Scl*. 286: 20190759; Yang et al., 2012 *Diagn Microbiol Infect Dis*. 73:80-83; Page et al., 2015 *Bioinformatics*. 31:3691-3; Lee et al., 2016 *Int J Syst Evol Microbiol*. 66:1100-3; Jombart et al., 2010 *BMC Genet*. 11:94; Perini et al., 2020 *Sci Rep*. 10:1307.