Detection and Genotyping of *Borrelia burgdorferi* sensu lato by Polymerase Chain Reaction

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**Aim.** To isolate and genotype *Borrelia burgdorferi* genospecies in serum samples of Croatian patients with erythema migrans.

**Methods.** DNA isolates from sera of patients with erythema migrans were analyzed by nested polymerase chain reaction (PCR), amplifying a segment of flagellin gene with primers encompassing the conserved region of the gene. To screen PCR products for heterogeneity, we performed single-stranded conformation polymorphism (SSCP) analysis. The samples showing differences in SSCP patterns were sequenced, and the sequence compared in the GeneBank for sequence homology with known *Borrelia burgdorferi* genospecies. We also constructed phylogenetic tree of all known borrelial sequences.

**Results.** The nested PCR method using specially designed flagellin gene primers, achieved the sensitivity of 10 genome copies (0.01 pg of purified *Borrelia burgdorferi* DNA from culture) by dilution analysis. The assay specificity was confirmed by amplification of a part of the flagellin gene from different bacterial species. The primer pairs successfully amplified only *Borrelia burgdorferi* flagellin gene. The genome of *Borrelia burgdorferi* sensu lato was detected in the sera of all 10 tested patients with erythema migrans. Sequence data and phylogenetic analysis confirmed that all amplified samples belonged to *Borrelia afzelii* genospecies.

**Conclusion.** Phylogenetic tree analysis placed the borrelial isolates together with *Borrelia afzelii* sequences into a single group. This finding was additionally supported by sequence homology analysis, which produced a homology score of 99%. In patients with erythema migrans who come from the northwest Croatia, an endemic area for Lyme borreliosis, *Borrelia afzelii* was the cause of skin manifestations of Lyme borreliosis.

**Key words:** bacterial gene proteins; bacterial typing techniques; biotyping, bacterial; *Borrelia burgdorferi*; Croatia; erythema chronicum migrans; flagellin; gene products, bacterial; polymerase chain reaction; nested PCR