

Abstract

Lyme borreliosis is a multisystemic disease affecting skin, joints, heart and central nervous system. The disease is caused by spirochetes of *Borrelia burgdorferi* sensu lato complex. These bacteria are spread by ticks of *Ixodes* genus. In 2016 there were almost 4,000 newly infected individuals reported in the Czech Republic. Contemporary serological diagnostics of Lyme borreliosis is not sensitive nor specific enough and does not even correlate with the pathology of the disease in the early or late phases. For the correct diagnosis of the disease it is necessary to detect the pathogen and its genotype.

For this reason we had aimed at two goals. Through the digital droplet PCR (ddPCR) method we detected *Borrelia*-specific DNA and its genotype. The detection limit of borrelial DNA was set on gDNA samples isolated from the tick. Detection threshold for the initial amount of 1 ng of tick gDNA is at the range of 10^{-17} g of specific borrelial DNA. *Borrelia* spp. coinfection was detected in 5 out of 12 tested samples. The most frequent type was *B. garinii* which was detected in 5 samples. On the basis of published sequences for virulent factors we have designed specific primers in conserved regions of the genes flanking their variable segments to be PCR amplified. Gene variability will be monitored through sequencing.

An alternative to the current clinical diagnostics of Lyme borreliosis is a degranulation test which is already used for other pathogen detection. That is the reason why we focused on testing the cellular degranulation caused by borrelial virulent factors. Optimization of positive control for the cell activation could not be reached which is the reason why we did not get any data for evaluation.

Key words: Lyme borreliosis, borelial coinfection, virulent factors, digital droplet PCR, detection limit, gene conservation, gene variability, cell degranulation