Online Appendix A: Laboratory Analyses

Laboratory analysis protocols

To identify possible exposure to ticks, blood samples collected in ethylenediaminetetraacetic acid (EDTA), sodium heparin, and SST tubes (BD Vacutainer®) were first tested using Lyme immunoblot. Antigen strips were prepared using recombinant proteins derived from U.S. and European species of *Borrelia burgdorferi*. The proteins include those used for scoring by the CDC in addition to the following in-house antigen bands: 18, 23 (OspC), 28, 30, 31 (OspA), 34 (OspB), 39 (BmpA), 41 (FlaB), 45, 58, 66, and 93. Specifically, P23 (OspC) and P31 (OspA) were used as the target antigens in the Lyme immunoblot. Strips were incubated with alkaline phosphatase-conjugated goat anti-human IgG at 1:10,000 dilution and IgM at 1:6000 dilution (KPL Inc.). Proteins were purified using metal affinity chromatography followed by gel filtration. Lastly, 90% purity was established after SDS-PAGE. Purified proteins were sprayed onto a nitrocellulose membrane (Amersham Protran, GE Healthcare Life Science) using a liquid dispenser (BioDot). Alkaline phosphatase-conjugated rabbit antibody to the 39/93 kDa *B. burgdorferi sensu lato* antigens (Strategic Biosciences) diluted in human serum was used as control.20

Polymerase chain reaction (PCR)

Samples collected in the EDTA tubes were mixed with 200 µl of sample processing bugger (SPB-100mM Tris-HCl, pH 7.4, 40 mM EDTA, 5M guanidine thiocyanate, and 1% sarkosyl). Three capture probes (A, B, and C) were tagged with biotin (Operon Technologies/Qiagen). Probe A is 5’-biotin-GCC TTA ATA GCA TGT AAG CAA AAT GTT AGC GAT-3’. Probe B is 5’-biotin-TCC ATC GCT TTT AAT TCC GTA TTC AAG TCT GGT TCC-3’. Lastly, Probe C is 5’-biotin-ATC TGT AAT TGC AGA AAC ACC TTT TGA AT-3’. Specifically, Probes A and B were designed to bind OspA and Probe C to *flagellin* gene sequences. Following incubation, the hybrids were captured using streptavidin (Progema), washed, and separated from the DNA by centrifugation. The purified DNA was amplified using an OspA primer, 5’-CTT-TGT-TTT-TTT-TGC-TTA-CAA-GAA-C-3’, and a second primer, 5’-GCT TTT TTG TTA GGA TCT GAG GTT TCT TT-3’. PCR assays were performed in a Eppendorf Thermocycler 7000 for 50 cycles using denaturation techniques outlined in the mentioned references.21,24,25

Dot blot analysis

The products were detected using two *B. burgdorferi*-specific 5’ digoxigenin-labeled probes (1 and 2) using the Roche hybridization techniques. Probe 1, 5’-TTC TGC AAT TTT AGC ATC TTT GTA TTC AAG TCT GGT TCC-3’, binds the *flagellin* gene, and Probe 2, 5’-GAA AAA CAG CGT TTC AGT AGA TTT GCC TGG AAT GAA-3’, targets the *OspA* gene. The denatured PCR produced was transferred to a membrane using a dot-blot apparatus (Bio-Rad Laboratories, Inc). The product was then hybridized to target PCR products in 50% formamide hybridization buffer overnight, according to the kit manufacturer’s protocols (Roche Molecular Diagnostics). The positive samples were then analyzed by gel electrophoresis on agarose gels in Tris-borate, EDTA pH 8.1 (Bio-Rad Laboratories QA). Different concentrations were then aliquoted and purified and tested by dot-blotting in the PCR assay.19