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 Trist-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 TGT 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-CTT-TGC-TTA-CAA-GAA-C-3', and a second primer, 5'-GCT TTT TTG TTA GGA TCT GAG GGT 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 TGG AGC TAA ATA TAA GCT TGG AT-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]