Extraction of DNA and amplification of the rRNA-23S and OspA genes

First, DNA was extracted from nymphs and mice biopsies using the DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The extracted DNA was then quantified using the Cytation 5 machine (Biotek, Winooski, VT, USA). The DNA was stored at -20 °C until PCR testing.

To test for the presence of *Borrelia* spp., we first screened the samples using primers that amplify the *rRNA-23S* gene (Courtney et al. 2004). Positive samples were subsequently tested for the presence of *B. burgdorferi* using primers that amplify the *OspA* gene (Tokarz et al. 2017). The final PCR solutions contained between 10-30 ng/µL of extracted genomic DNA and 10 nM of *23S-rRNA* or *OspA* primers (Integrated DNA Technologies, Coralville, IA, USA). For *Borrelia* spp. screening, PCR was performed using 10X Taq polymerase (Bio Basic, Markham, ON, Canada) in a Proflex PCR System (Life Technologies, Carlsbad, CA, USA). The amplification conditions are described in Table P1. PCR products were electrophoretically resolved in 1 X TE, 2% agarose gels stained with 6X orange DNA loading dye (Thermo scientific, Vilnius, Lithuania). Images were captured using a ChemiDoc MP Imaging System (Bio-Rad Laboratories, Hercules, CA, USA) and positive samples were identified by the presence of a 75 bp band.

Table P1. Amplification conditions of the *23S-rRNA* PCR reaction

Phase		**Temperature (°C)**	**Time**	**Number of cycles**
Polymerase activation		95	2 m	1
Amplification	Denaturation	95	20 s	
	Annealing	60	40 s	35
	Extension	72	8 s	

m = minutes, s = seconds.

To identify *B. burgdorferi*, qPCR was performed using 1X iTaq Universal SYBR Green Supermix (Bio-Rad Laboratories) and OspA primers on the CFX Opus 96 real-time PCR system (Bio-Rad Laboratories). The reaction conditions are listed in Table P2. Fluorescence was measured after each amplification cycle. At the end of the amplification phase of the *OspA* assay, a melting curve was set up from 56 °C to 95 °C with a 0.5 °C increase every 5s to confirm that the amplified DNA was the target gene. A test was confirmed positive when the quantification threshold was reached within 30 amplification cycles and a melting peak was detected at 76 °C. Samples were tested with negative control (sterile water) and positive control (1 pg of a gene fragment from gBlocksTM; Integrated DNA Technologies).

Table P2. Amplification conditions of the *OspA* qPCR reaction

Phase		**Temperature (°C)**	**Time**	**Number of cycles**
Polymerase activation		95	3 m	1
Amplification	Denaturation	95	10 s	
	Annealing	60	10 s	39
	Elongation	56	5 s	

m = minutes; s = seconds

References

Courtney, J. W., L. M. Kostelnik, N. S. Zeidner, and R. F. Massung. 2004. Multiplex real-time PCR for detection of *Anaplasma phagocytophilum* and *Borrelia burgdorferi*. J Clin Microbiol 42: 3164-3168.

Tokarz, R., T. Tagliafierro, D. M. Cucura, I. Rochlin, S. Sameroff, and W. I. Lipkin. 2017. Detection of *Anaplasma phagocytophilum*, *Babesia microti*, *Borrelia burgdorferi*, *Borrelia miyamotoi*, and Powassan virus in ticks by a Multiplex real-time reverse transcription-PCR assay. mSphere 19;2(2):e00151-17.