**Supplemental material**

*Comparison of B. miyamotoi spirochete load for horizontally infected nymphal ticks fed as larvae on SCID mice and naturally transovarially infected nymphal ticks*

We compared *purB* copy numbers, (determined as described in section 2.4), of nymphs infected horizontally via feeding on infectious SCID mice as larvae (section 2.3) and naturally transovarially *B. miyamotoi*-infected *I. scapularis* nymphs. Naturally transovarially infected ticks included (i) F1 generation Minnesota nymphs, with the F0 female fed on a non-infected New Zealand white rabbit (Charles River Laboratories) and the F1 generation larvae fed on non-infected CD-1 mice (Breuner et al., 2018); and (ii) F2 generation Connecticut nymphs, fed on non-infected New Zealand white rabbits as F0 and F1 females, and on non-infected CD-1 mice as F1 generation larvae and nymphs, and F2 generation larvae (Breuner et al., 2017). F2 Connecticut ticks were stored in methanol at -80 °C until DNA extraction and qPCR was performed, as described in section 2.4.

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| source | (n) | positive (%) | median | range |
| MN -17 | 25 | 22 (88.0) | 301,400 | 411 - 811,300 |
| CT - 15 | 15 | 12 (80.0) | 147,700 | 18 - 389,900 |

**Table.** Infection prevalence and median *purB* copy numbers for two groups of lab-reared nymphs that acquired infections transovarially, originating from field-collected females. Median copies per nymph were 3.1 and 1.5 times that of nymphs infected using SCID mice