

An efficient microinjection method to generate human anaplasmosis agent
***Anaplasma phagocytophilum*-infected ticks**

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Supplementary information

Supplementary Figure legends

Supplementary Figure 1. MRSP spectradyne measurement of *Ap*-DC. Microfluidics resistive pulse sensing (MRPS) using Spectradyne instrument (nCS1, Spectradyne LLC, USA) was used to determine the size distribution and concentration of isolated *A. phagocytophilum* -DC. Diameter versus transit time is shown. TS-2000 cartridge was used to perform the measurements. The graph shows the excluded (blue dots) and included (red dots) events during the MRPS measurements for *Ap*-DC. The X-axis shows the transit time (μ s), and Y-axis shows the particle diameter (nm).

Supplementary Figure 2. Full images of PCR analysis for actin in mice blood samples. Full image of PCR amplification of mouse beta-actin fragment in blood samples is shown. This data is shown as a cropped image in main Figure 4A.

Supplementary Figure 3. Full images of PCR analysis for *A. phagocytophilum* p44 and mouse beta-actin in murine liver samples. Full image of PCR amplification of *A. phagocytophilum* p44 and mouse beta-actin fragments in liver samples is shown. This data is shown as a cropped image in main Figure 4A.

Supplementary Figure 4. Full images of PCR analysis for *A. phagocytophilum* p44 and mouse beta-actin in murine spleen samples. Full image of PCR amplification of *A. phagocytophilum* p44 and mouse beta-actin fragments in spleen samples is shown. This data is shown as a cropped image in main Figure 4A.

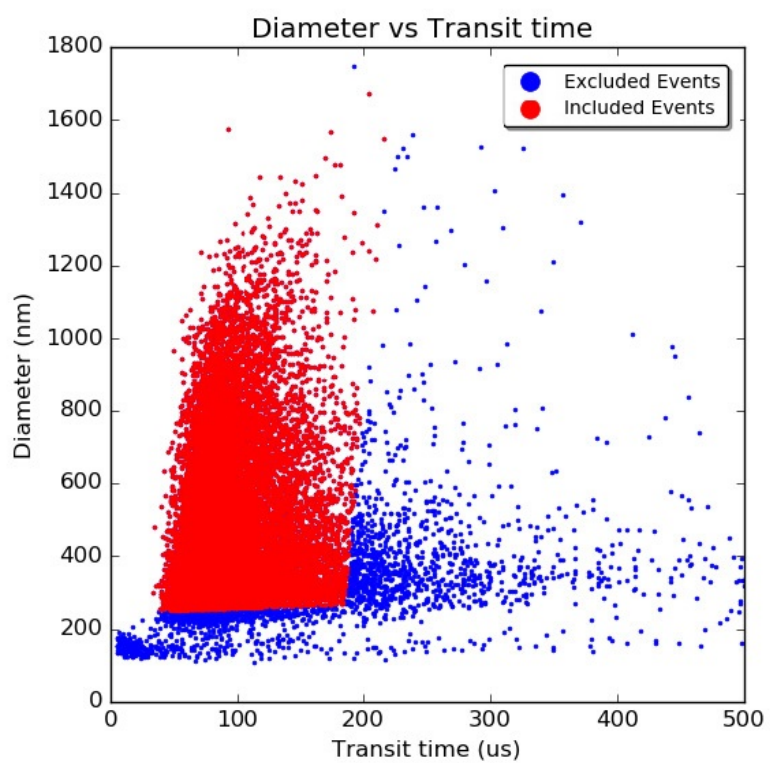
Supplementary Figure 5. Analysis of additional *Ap*-DC-injected nymphal ticks that retain bacteria in its body after feeding on a vertebrate host. Agarose gel electrophoresis image showing PCR amplification of *A. phagocytophilum* p44 gene product from DNA samples generated from *Ap*-DC-injected fed nymphs. Amplification of tick 5.8s rRNA in the same samples was used as sample control. M indicates DNA ladder marker (1kp plus ladder, Invitrogen). PCR amplification of p44 or 5.8s rRNA in three DNA samples generated from uninfected nymphs fed on naive mice (uninfected fed nymphs) and eleven DNA samples from *Ap*-DC-injected nymphs fed on naive mice (*Ap*-DC-injected fed nymphs) is shown. Previously sequenced PCR product (denoted as +) was used as a positive control. NTC indicates no DNA template control.

Supplementary Figure 6. Full images of PCR analysis for *I. scapularis* 5.8S rRNA in DC-injected fed nymphs. Full image of PCR amplification of *I. scapularis* 5.8S rRNA fragment in DC-injected fed tick samples is shown. This data is shown as a cropped image in main Figure 5.

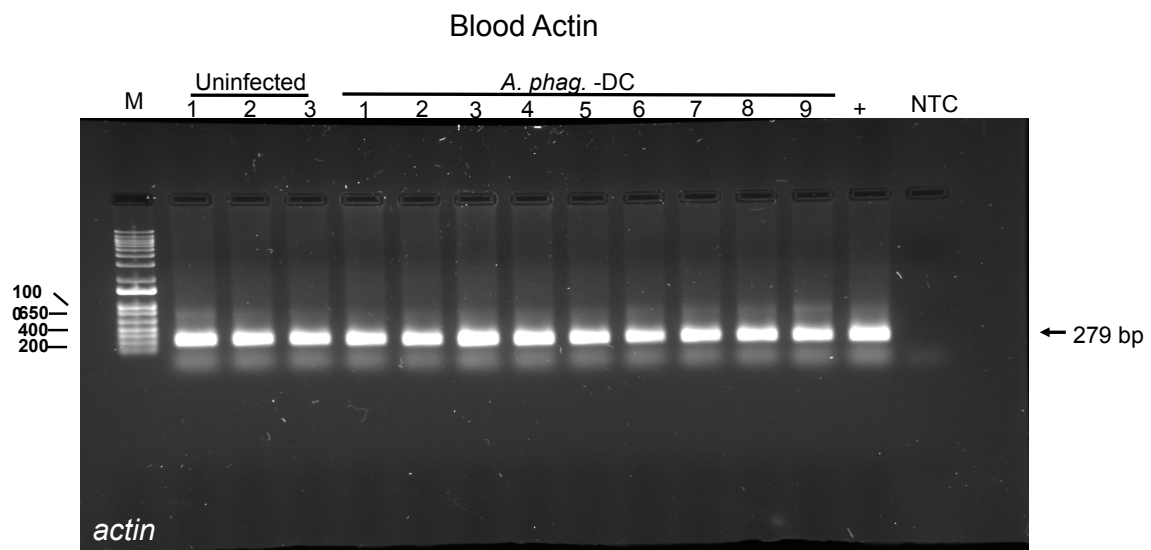
Supplementary Figure 7. Analysis of additional larvae that shows successful acquisition of *A. phagocytophilum* from mice previously infected with *Ap*-DC-injected nymphs. Agarose gel electrophoresis image showing PCR amplification of *A. phagocytophilum* *p44* gene product from DNA samples generated from larvae fed on infected mice (generated by feeding *Ap*-DC-injected nymphs). Amplification of tick 5.8s rRNA in same samples was used as sample control. M indicates DNA ladder marker (1kp plus ladder, Invitrogen). PCR amplification of *p44* or 5.8s rRNA in DNA samples generated from uninfected larvae fed on naive mice (three samples) and DNA samples from larvae fed on mice previously infected with *Ap*-DC-injected nymphs (eleven samples) is shown. Previously sequenced PCR product (denoted as +) was used as a positive control. NTC indicates no DNA template control.

Supplementary Figure 8. Full images of PCR analysis for *I. scapularis* 5.8S rRNA in larvae fed on mice infected with DC-injected nymphs. Full image of PCR amplification of *I. scapularis* 5.8S rRNA fragment in larvae fed on mice infected with DC-injected nymphs is shown. This data is shown as a cropped image in main Figure 6.

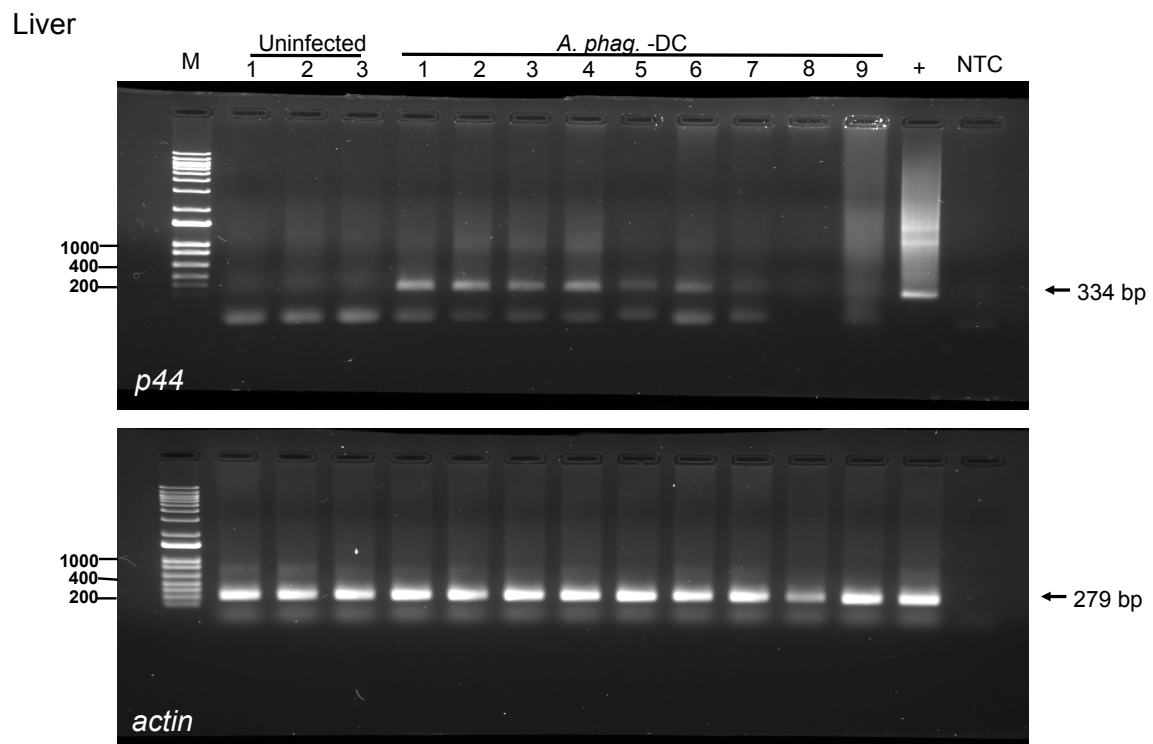
Supplementary Figure 9. Full images of PCR analysis for *I. scapularis* 5.8S rRNA in molted nymphs. Full image of PCR amplification of *I. scapularis* 5.8S rRNA fragment in nymphs molted from larvae fed on mice infected with DC-injected nymphs is shown. This data is shown as a cropped image in main Figure 7.



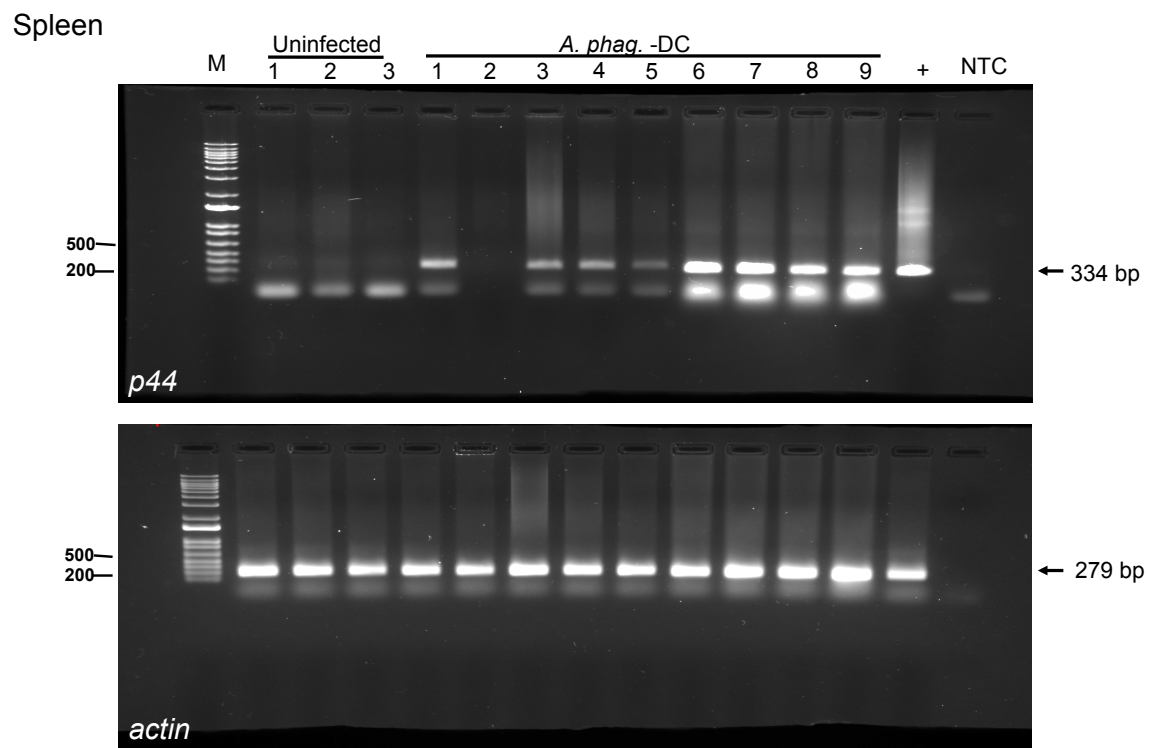
Supplementary Figure 1



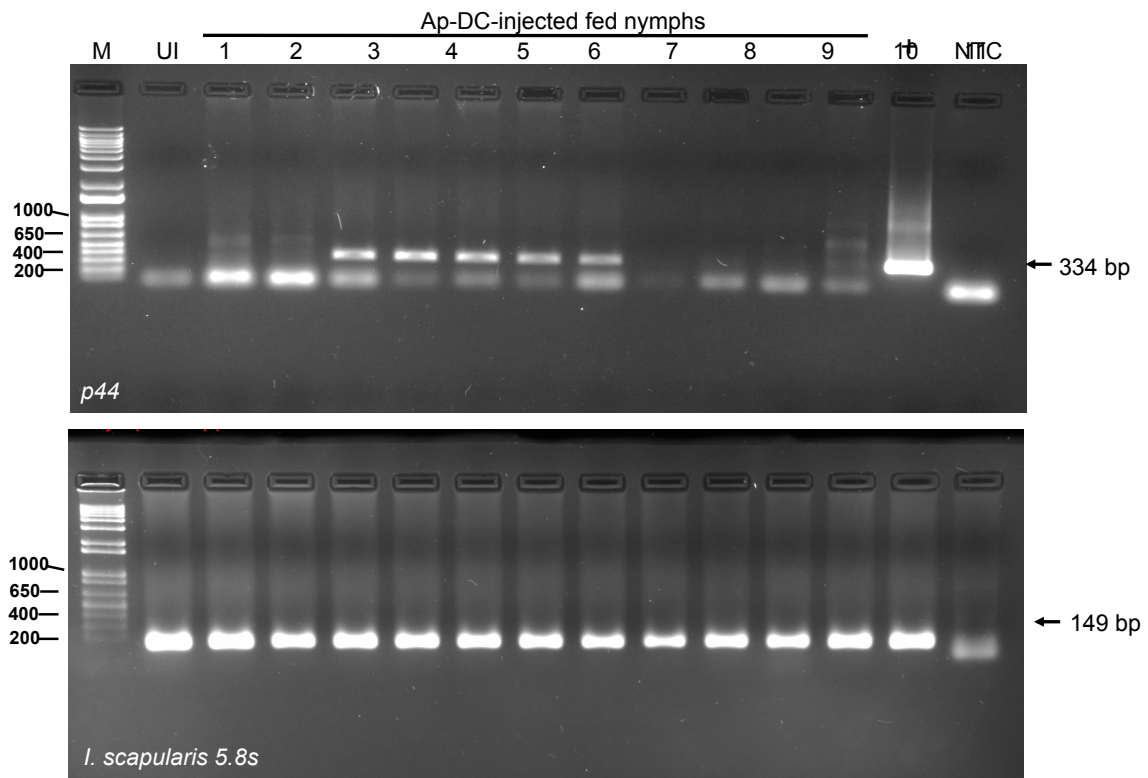
Supplementary Figure 2



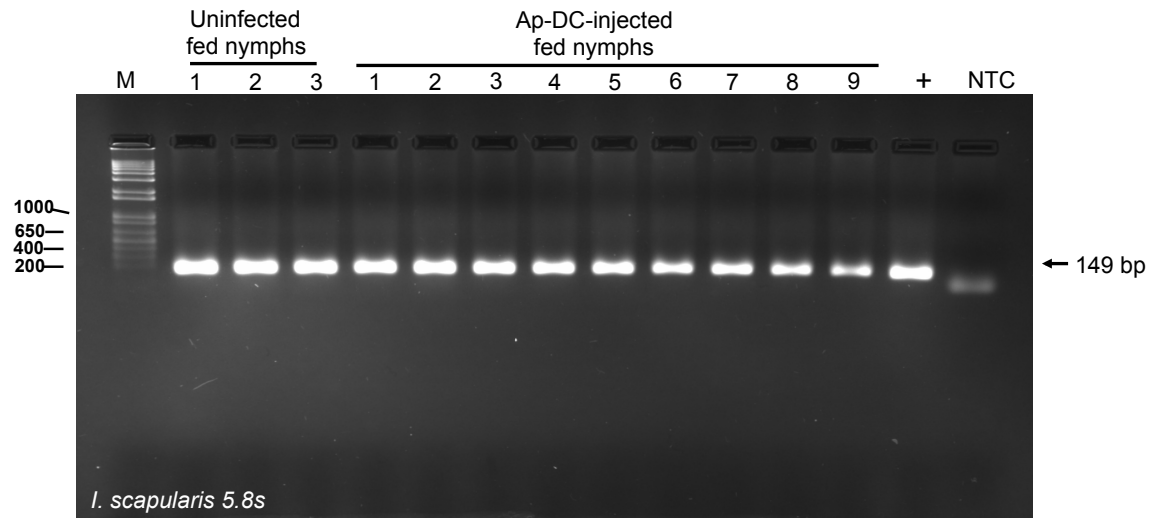
Supplementary Figure 3



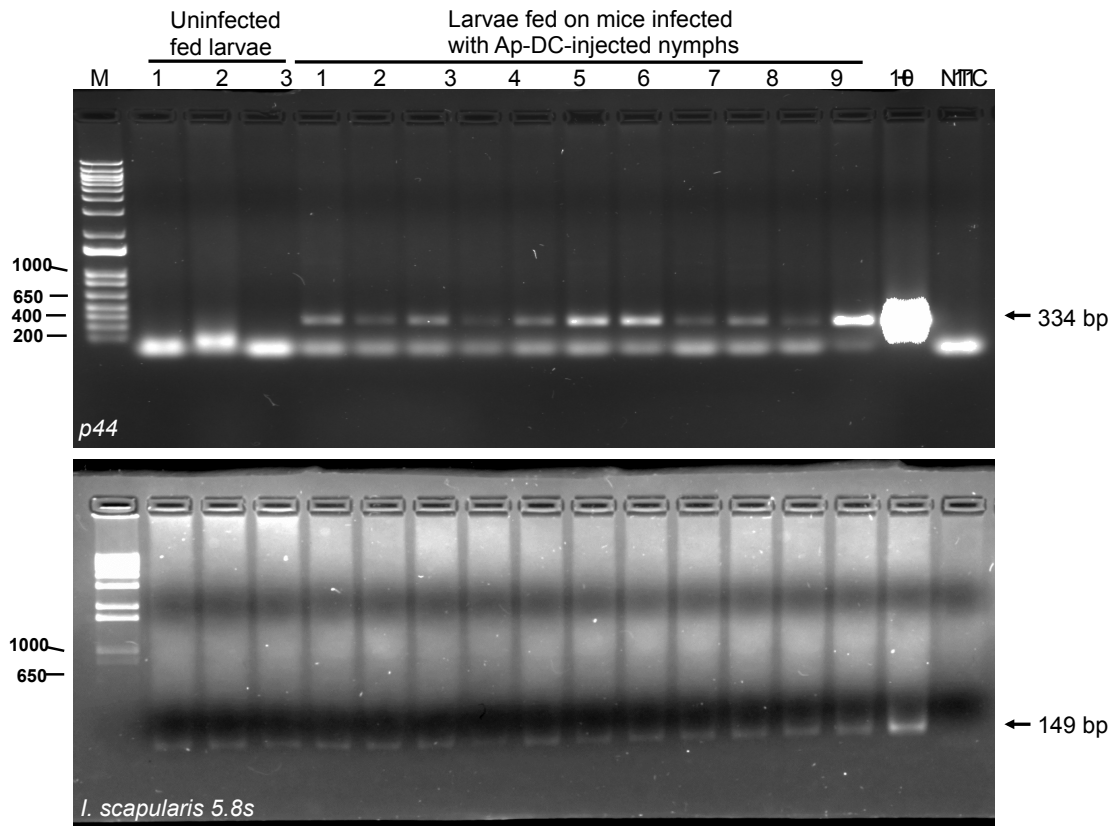
Supplementary Figure 4



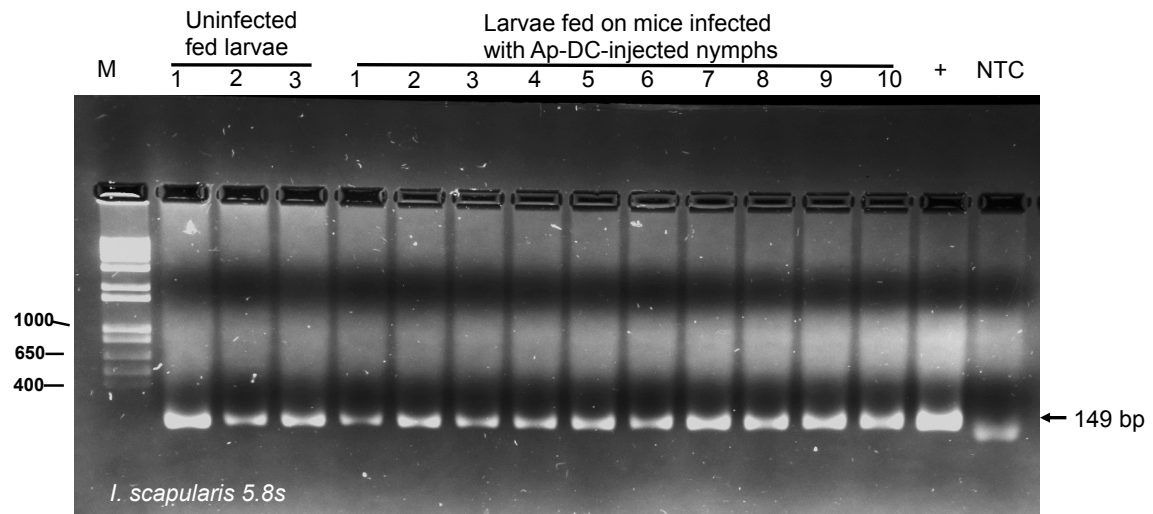
Supplementary Figure 5



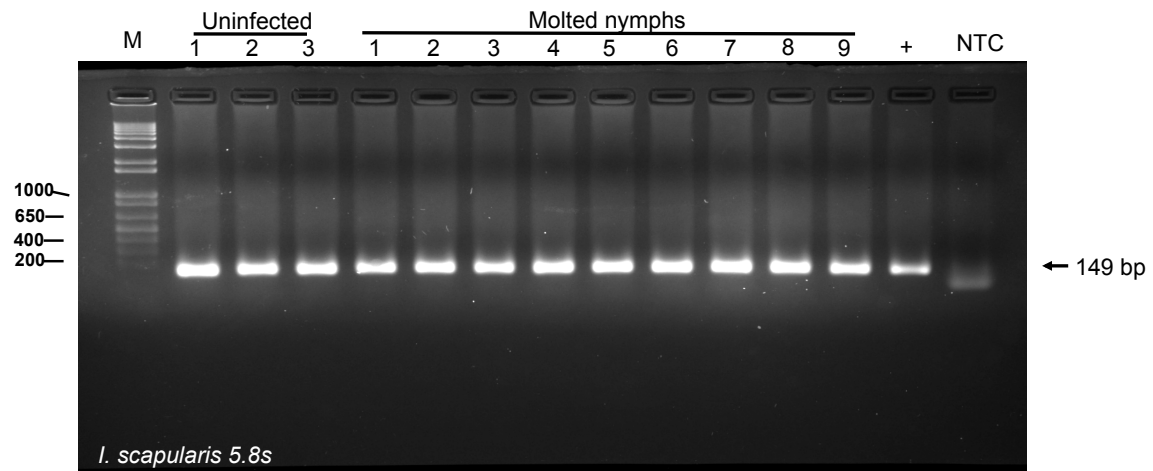
Supplementary Figure 6



Supplementary Figure 7



Supplementary Figure 8



Supplementary Figure 9

Table S1. List of oligonucleotides used in this study.

Primer (5'-3')	Gene, purpose
CCAGCGTTTAGCAAGATAAGAG	<i>Anaplasma</i> p44, qpcr, pcr
GCCCAGTAACAACATCATAAGC	<i>Anaplasma</i> p44, qpcr, pcr
CCCTAGGCACCAGGGTGTGA	mice <i>actin</i> , qpcr, pcr
GGGGTGTGTAAGGTCTCAAACA	mice <i>actin</i> , qpcr, pcr
TCTAAGCGGTGGATCACTCGGT	tick 5.8s, qpcr, pcr
GACCCTCACACAGACGAAGCCA	tick 5.8s, qpcr, pcr
GCGGTTCATAAAGGAGTGGA	<i>Anaplasma dnaA</i> , qpcr (GenBank acc. no. LANT01000003)
AAACTTCCTGCGCCTCTGTA	<i>Anaplasma dnaA</i> , qpcr (GenBank acc. no. LANT01000003)
GATTGGCGCGTTTGTCTTGGA	<i>Anaplasma dnaK</i> , qpcr (GenBank acc. no. LANT01000002)
CAAAGCTGCTGCTGTAGGTTCA	<i>Anaplasma dnaK</i> , qpcr (GenBank acc. no. LANT01000002)