

Table S1. PCR settings and primers used for the detection of tick-borne pathogens in tick nucleic acids extracts.

| Target organism | Target gene (amplicon size, bp) | Method | Primer and probe names and 5'–3' sequences | Cycling conditions | Reference |
|----------------------------|---------------------------------------|------------|--|---|-----------|
| TBEV | 3' non-coding region (67 bp) | qRT-PCR | F-TBE1: GGG CGG TTC TTG TTC TCC R-TBE1: ACA CAT CAC CTC CTT GTC AGA CT TBE-probe-W: TGA GCC ACC ATC ACC CAG ACA CA | 48 °C – 5 min, 95 °C – 30 sec, (95 °C – 3 sec, 60 °C – 30 sec) × 45 | [18] |
| | E gene (465 bp) | RT-PCR | E frw1: GTT GTG TGG YTG ACY STG GA E rev1: TCK GAK ACY TCY CTC CAC AC | 65 °C – 5 min, on ice – 1 min, 25 °C – 5 min, 50 °C – 45 min, 70 °C – 15 min | [19] |
| | | nested PCR | 283F1: GAG AYC AGA GTG AYC GAG GCT GG 827R1: AGG TGG TAC TTG GTT CCM TCA AGT | (94 °C – 1 min, 57 °C – 1 min, 72 °C – 2 min) × 35 | [13] |
| | | | 349F2: GTC AAG GCG KCT TGT GAG GCA A 814R2: TTC CMT CAA TGT GYG CCA CAG G | (94 °C – 1 min, 60 °C – 1 min, 72 °C – 120 sec) × 30 | |
| <i>B. burgdorferi</i> s.l. | 5S-23S intergenic spacer (245-256 bp) | nested PCR | NC1: CCT GTT ATC ATT CCG AAC ACA G NC2: TAC TCC ATT CGG TAA TCT TGG G | (94 °C – 30 sec ; 58 °C – 30 sec ; 72 °C – 1 min) × 35 | [21] |
| | | | NC3: CTG CGA GTT CGC GGG AGA NC4: TCC TAG GCA TTC ACC ATA | (94 °C – 30 sec ; 52 °C – 30 sec ; 72 °C – 1 min) × 30 | |
| <i>B. miyamotoi</i> | p66 (532 bp) | nested PCR | M1F: TTC TAT ATT TGG ACA CAT GTC M2R: CAG ATT GTT TAG TTC TAA TCC G | (94 °C – 30 sec , 52 °C – 30 sec , 72 °C – 1 min) × 35 | [20] |
| | | | M3F: CTA AAT TAT TAA ATC CAA AAT CG M4R: GGA AAT GAG TAC CTA CAT ATG | (94 °C – 30 sec , 49 °C – 30 sec , 72 °C – 1 min) × 35 | |
| Anaplasmataceae | 16S rRNA (1350 bp) | nested PCR | Ehr1: GAA CGA ACG CTG GCG GCA AGC Ehr6: GAC CCA ACC TTA AAT GGC TGC | (94 °C – 30 sec , 58 °C – 30 sec , 72 °C – 1 min) × 35 | [10] |
| | | | Ehr7: TAA CAC ATG CAA GTC GAA CG Ehr8: CTT CGA GTT AAG CCA ATT CC | (94 °C – 30 sec , 50 °C – 30 sec , 72 °C – 1 min) × 35 | |
| <i>Rickettsia</i> | <i>gltA</i> (74 bp) | qPCR | CS-F: TCG CAA ATG TTC ACG GTA CTT T CS-R: TCG TGC ATT TCT TTC CAT TGT G | 95 °C – 3min, (95 °C – 10 sec, 60 °C – 60 sec) × 40 | [11] |

| | | | | |
|-------------------------|------------|---|--|------|
| | | CS-P (probe): TGC AAT AGC AAG AAC CGT AGG CTG GAT G | | |
| <i>gltA</i> (667 bp) | nested PCR | glt1: GAT TGC TTT ACT TAC GAC CC glt2: TGC ATT TCT TTC CAT TGT GC | (94°C – 30 sec , 48°C – 30 sec , 72°C – 60 sec) × 35 | [23] |
| | | glt3: TAT AGA CGG TGA TAA AGG AAT C glt4: CAG AAC TAC CGA TTT CTT TAA GC | (94°C – 30 sec , 45.5°C – 30 sec , 72°C – 60 sec) × 30 | |
| <i>sca4</i> (843 bp) | nested PCR | Sc4-1: ATG TCT CTG AAT TAA GCA ATG C Rj2837r: CCT GAT ACT ACC CTT ACA TC | (94°C – 60 sec, 52°C – 60 sec, 72°C – 2 min) × 35; | [20] |
| | | sc4-3: AAT TAT TAG GCT CTG TAT TAA AGA sc4-4: GAA AGG ATA GCA CGA AAA GTA | (94°C – 60 sec, 50°C – 60 sec, 72°C – 2 min) × 30 | |
| <i>ompB</i> (769 bp) | PCR | 120-2788F: AAA CRA TAA TCA AGG TAC TGT 120-3599R: ACY STG GAR AGT GTG GTG AC | (95°C – 30 sec, 51°C – 30 sec, 72°C – 60 sec) × 35 | |

Cycling conditions modifications are indicated bold.

reaction mix contents:

qRT-PCR: final volume of 20 uL, containing Quanta qScript One-Step Fast qRT-PCR kit with low ROX (Quantabio, Beverly, MA, USA), forward and reverse primers at final concentration of 900 nM each, 200 nM of probe and 5 uL of RNA/positive/negative control.

RT-PCR: SuperScript III Reverse Transcriptase kit (ThermoFisher Scientific, USA)

qPCR: final volume of 20 uL, containing Takyon Low ROX Probe qPCR Mastermix (Kaneka Eurogentec, Belgium, EU) with each primer concentration at 900 nM and probe at 200 nM

PCR and nested PCR: final volume of 25 µl, containing 10X DreamTaq PCR buffer (ThermoFisher Scientific, USA), per 0.8 mM of each dNTPs, 0.5 mM of each forward and reverse primers, per 1.5 mM MgCl₂ for outer and 1 mM MgCl₂ for inner reaction and 1 U of DreamTaq DNA Polymerase (ThermoFisher Scientific, USA) and 5 µl of cDNA, DNA or PCR reaction product