## Supplementary data

**Table S1**: Summary of the primers and probes used for *Borrelia burgdorferi* sensu lato complex, *A. phagocytophilum* and *Borrelia miyamotoi*

| Pathogens | Primers (5'->3') | Probe sequence | Protocol/Reference |
|---|---|---|---|
| ***Borrelia burgdorferi* sensu lato complex**<br><br>**Targeted gene:**<br><br>***Flagellin - flaB*** | **FlaBbsl 2a** **TCTTGGACITTAAGAGTTCA**<br><br>**FlaBbsl 4c** **GAATTAACTCCACCTTGAGIAGG** | **Mix 2/3 probe 1 and 1/3 probe 2:**<br><br>**Probe 1:** CAA GAT GAA GC [6FAM]/[TAMRA] - Sigma<br>and<br>**Probe 2:** CAA GAT GAG GC [6FAM]/[TAMRA] - Sigma | **1 cycle**: Denaturation: 95 ℃, 10 min<br><br>**55 cycles**: Hybridation / elongation: 95 ℃ - 10s then 53 ℃ – 45s<br><br>**1 cycle**: cooling 40 ℃ - 30s |
| ***Anaplasma phagocytophilum***<br><br>**Targeted gene:**<br>***msp2 – p44*** | **F-Anap** **TGTAGCTATGGAAGGCAGTGTTG**<br><br>**R-Anap** **GCGCTCGTAACCAATCTCAAG** | **CGGTATTGGTGGTGCCAGGGTT GA**<br>[VIC] / [TAMRA]<br>Applied biosystems/Life technologies | **(Koebel et al., 2012)** |
| ***Borrelia miyamotoi***<br><br>**Targeted gene:**<br>**16S-rDNA** | **F-relfev sens GTA AAA TAC CAC RGC TCA ACT GTG with R = A and G**<br><br>**R-relfev anti-sens1 GCT TCG GTA CTA ACC TYT CGA with Y = C and T**<br>**R-relfevpersica anti-sens2**<br>**GTT AGC TTC GGT ACT AAT CTC TCA** | **TATGCTRGAAACTGCATGACTA GAGTCTGATAGG**<br><br>[6FAM]/ [BHQ1] Sigma | **(Hovius et al. 2013)** |

FAM: 6-carboxyfluorescein (reporter), TAMRA: 6-carboxytetramethylrhodamine (quencher), VIC: 2′-chloro-7′phenyl-1,4-dichloro-6-carboxy-fluorescein, BHQ1: black hole quencher1 (quencher). LNA® (Locked nucleic acid) bases are underlined.

**Table S2**. Internal fluorescently labelled probes for *B. burgdorferi* sl species detection and identification by the Melting temperature (Tm) Plus/minus standard deviation (SD).

| Probe | Technology used | Species specificity | Nucleotide sequence from 5' to 3' | Fluorescent dyes | Nucleotide location from initiation codon of B31 strain | Tm +/- SD Melting temperature |
|---|---|---|---|---|---|---|
| *FlaBbss* 3'Fluo<br>*FlaBbss* 5'LC Red | LightCycler® Probes | *B. burgdorferi* sensu stricto | GAG GGA GCT CAA ACT GCT CAG GCT GC<br>CGG TTC AAG AGG GTG TTC AAC AGG | 3' fluorescein<br>5' LC Red 705 | 784 - 809<br>812 - 835 | 67.4 +/- 0.3 |
| *FlaBg* 3'Fluo<br>*FlaBg* 5'LC Red | LightCycler® Probes | *B. garinii/B. bavariensis* | AGC AAA TCA AGA TGA (inosine)GC GAT TGC TGT<br>ATA TTT ATG CTG C(inosine)A ATG TTG CAA ATC T | 3' fluorescein<br>5' LC Red 640 | 717- 743<br>746- 773 | 60.0 +/- 0.2 |
| *FlaBaf* 3'Fluo<br>*FlaBaf* 5'LC Red | LightCycler® Probes | *B. afzelii* | AAA TCT TTT TGC TGG TGA GGG AGC T<br>AAG CTG CTC AGG CTG CAC CTG TTC A | 3' fluorescein<br>5' LC Red 705 | 768 - 792<br>794 - 818 | 65.1 +/- 0.4 |
| *FlaBval* 3'Fluo<br>*FlaBval* 5'LC Red | LightCycler® Probes | *B. valaisiana* | AAT CTG TTT TCT GGT G<br>AGC TCA A(inosine)C TGC TC | 3' fluorescein<br>5' LC Red 640 | 769 - 784<br>789- 802 | 55.0 +/- 0.4 |
| *FlaBlus* 3'Fluo<br>*FlaBlus* 5'LC Red | LightCycler® Probes | *B. lusitaniae* | AGA CTG CAC CTG CTC AAG AAG GTG TT<br>AAC AAG AAG GAG CTC AAC AAC CAG CGC | 3' fluorescein<br>5' LC Red 705 | 804 - 828<br>830 - 856 | 67.3 +/- 0.4 |

TAMRA, 6-carboxytetramethylrhodamine (quencher). FAM: 6-carboxyfluorescein (reporter). LNA® (Locked nucleic acid) bases are underlined.

**Table S3**: Summary of the primers used for *Rickettsia* identification by PCR

| Pathogens | Primer/Nucleotide sequence (5'->3') | Probe sequence | Protocols | References |
|---|---|---|---|---|
| ***Rickettsia* spp**<br><br>**Targeted gene: *gltA*** | **Rspp-F : GAGAGAAAATTATATCCAAATGTTGAT**<br><br>**Rspp-R : AGGGTCTTCGTGCATTTCTT** | **CATTGTGCCATCCAGCCTAC GGT**<br>[6FAM]/ [BHQ1] Sigma | **1 cycle:** Denaturation: 50 ℃- 2min and 95 ℃- 10min<br><br>**50 cycles:**Hybridation/elongation: 95 ℃- 15sec and 60 ℃- 1min<br><br>**1cycle**: cooling 40 ℃-30sec | **(Labruna et al., 2004)** |
| ***Rickettsia* spp.**<br><br>**Targeted gene : *rompB*** | **First PCR**<br><br>**Rc.rompB.4362p: GTCAGCGTTACTTCTTCGATGC**<br>**Rc.rompB.4,836n: CCGTACTCCATCTTAGCATCAG** | | **1 cycle**: Denaturation : 98 ℃- 30sec<br><br>**35 cycles** : Denaturation :98 ℃-15sec<br>Hybridation : 54 ℃-15sec<br>Elongation: 72 ℃ - 30s<br><br>**1cycle** :Elongation: 72 ℃-5min +4 ℃ :∞ | **(Choi et al., 2005)** |
| | **Nested PCR**<br><br>**Rc.rompB.4,496p: CCAATGGCAGGACTTAGCTACT**<br>**Rc.rompB.4,762n: AGGCTGGCTGATACACGGAGTAA** | | **1 cycle** : Denaturation: 98 ℃- 30sec<br><br>**35 cycles** :Denaturation: 98 ℃-15sec<br>Hybridation: 56 ℃-15sec<br>Elongation: 72 ℃ - 30s<br><br>**1cycle**: Elongation: 72 ℃-5min +4 ℃ :∞ | **(Choi et al., 2005)** |

**Table S4**

**(A) Total number of *Ixodes ricinus* nymphs collected on each site**

| Site | Golf | Citadelle | | Heyritz | | Orangerie | | Niedermunster | Herrenwald |
|---|---|---|---|---|---|---|---|---|---|
| Year | 2019 | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 | 2019 | 2019 |
| March | ND | ND | ND | ND | ND | ND | ND | 14 | 58 |
| April | 0 | 3 | 0 | 0 | 2 | 9 | 12 | 99 | 197 |
| May | 13 | 1 | 0 | 2 | 0 | 11 | 0 | 183 | 326 |
| June | 9 | 1 | 0 | 0 | 0 | 3 | 0 | 145 | 91 |

**(B) Number of *Ixodes ricinus* nymphs infected with different *Borrelia* species**

| Site | Golf | Citadelle | | Heyritz | | Orangerie | | Niedermunster | Herrenwald |
|---|---|---|---|---|---|---|---|---|---|
| Year | 2019 | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 | 2019 | 2019 |
| March | ND | ND | ND | ND | ND | ND | ND | 1 | 15 (13 Baf + 2Blusit) |
| April | 0 | 1 (Bval) | 0 | 0 | 0 | 2 (Bbss+Baf) | 0 | 6 (3 Bgar + 3 Bval) | 10 (8 baf + 2 Blusit) |
| May | 1 (Bval) | 0 | 0 | 0 | 0 | 0 | 0 | 2 (Baf + Bval) | 7 (5 Baf + 2 Blusit) |
| June | 2 (Baf) | 0 | 0 | 0 | 0 | 0 | 0 | 5 (4 Baf + 1 Bval) | 8 (5 Baf + 2 Blusit + 1 Bval) |

**(C) Number of *Ixodes ricinus* nymphs infected with *Borrelia miyamotoi***

| Site | Golf | Citadelle | | Heyritz | | Orangerie | | Niedermunster | Herrenwald |
|---|---|---|---|---|---|---|---|---|---|
| Year | 2019 | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 | 2019 | 2019 |
| March | ND | ND | ND | ND | ND | ND | ND | 0 | 1 |
| April | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| May | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| June | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |

**(D) Number of *Ixodes ricinus* nymphs infected with *Anaplasma phagocytophilum***

| Site | Golf | Citadelle | | Heyritz | | Orangerie | | Niedermunster | Herrenwald |
|---|---|---|---|---|---|---|---|---|---|
| Year | 2019 | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 | 2019 | 2019 |
| March | ND | ND | ND | ND | ND | ND | ND | 1 | 0 |
| April | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| May | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| June | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 |

**Table S5:** Detailed data concerning the DON, NIP and DIN of the different sites for *Ixodes ricinus*

| Site | Density in *Ixodes* nymph (DON) / 100 m$^2$ [CI 95 %] | | Nymphal infection prevalence (NIP) Whatever the microorganism [CI 95 %] | | Density in *Ixodes* infected nymph (DIN) /100 m$^2$ whatever the microorganism [CI 95 %] | |
|---|---|---|---|---|---|---|
| | **2018** | **2019** | **2018** | **2019** | **2018** | **2019** |
| **Golf** | - | **2.44** [-3.07-7.96] | - | **0.16** [-0.25-0.58] | - | **0.56** [-0.7093-1.82] |
| **Citadelle** | **0.56** [-0.40-1.51] | 0 | **0.11** [-0.37-0.59] | 0 | 0.11 [-0.38-0.59] | 0 |
| **Heyritz** | **0.22** [-0.73-1.18] | **0.22** [-0.73-1.18] | 0 | 0 | 0 | 0 |
| **Orangerie** | **2.56** [-0.90-6.00] | **1.33** [-4.4-7.10] | **0.07** [-0.24-0.39] | 0 | 0.22 [-0.73-1.18] | 0 |
| **Niedermunster** | - | **36.75** [-1.85-75.35] | - | **0.08** [-0.008-0.160] | - | **1.83** [0.18-3.48] |
| **Herrenwald** | - | **56.33** [-10.67;123.3] | - | **0.12** [-0.08-0.32] | - | **3.67** [1.829-5.504] |