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RESEARCH

# Tick abundance and infection with three zoonotic bacteria are heterogeneous in a Belgian peri-urban forest

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## Abstract

*Ixodes ricinus* is a vector of several pathogens of public health interest. While forests are the primary habitat for *I. ricinus*, its abundance and infection prevalence are expected to vary within forest stands. This study assesses the spatio-temporal variations in tick abundance and infection prevalence with three pathogens in and around a peri-urban forest where human exposure is high. Ticks were sampled multiple times in 2016 and 2018 in multiple locations with a diversity of undergrowth, using the consecutive drags method. Three zoonotic pathogens were screened for, *Borrelia burgdorferi* s.l., *Coxiella burnetii*, and *Francisella tularensis*. The influence of season, type of site and micro-environmental factors on tick abundance were assessed with negative binomial generalized linear mixed-effects models. We collected 1642 nymphs and 181 adult ticks. Ticks were most abundant in the spring, in warmer temperatures, and where undergrowth was higher. Sites with vegetation unaffected by human presence had higher abundance of ticks. Forest undergrowth type and height were significant predictors of the level of tick abundance in a forest. The consecutive drags method is expected to provide more precise estimates of tick abundance, presumably through more varied contacts with foliage. *Borrelia burgdorferi* s.l. prevalence was estimated from pooled ticks at 5.33%, *C. burnetii* was detected in six pools and *F. tularensis* was not detected. *Borrelia afzelii* was the dominant *B. burgdorferi* genospecies. Tick abundance and *B. burgdorferi* s.l. infection prevalence were lower than other estimates in Belgian forests.

**Keywords** Forest · Tick-borne disease risk · *Ixodes ricinus* · Tick-borne pathogens · *Borrelia burgdorferi s.l.*

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**Abbreviations**

| | |
|---|---|
| AIP | Adult infection prevalence |
| DIA | Density of infected adults |
| DIN | Density of infected nymphs |
| DOA | Density of adults |
| DON | Density of nymphs |
| DOT | Density of ticks |
| GLMM | Generalized linear mixed models |
| IF | Sites located deep in the forest, in a dense undergrowth |
| NB | Negative binomial |
| NIP | Nymph infection prevalence |
| OF | Sites located out of the forest |
| RE | Random effect |
| SU | Sampling unit |
| SE | Sampling event |
| TF | Sites located in the forest close to the trails in small undergrowth |
| TIP | Tick infection prevalence |
| ZI | Zero-inflated |

## Introduction

Ticks are important vectors of pathogens in Western Europe (Dantas-Torres et al. 2012; de la Fuente et al. 2008). These pathogens include microorganisms (e.g., bacteria, viruses or protozoa) transmitted through the bite of infected ticks. Lyme borreliosis (LB) is the most widespread tick-borne disease in Western Europe (Gray 1998; Stanek and Reiter 2011). If untreated, an infection may result in skin, neurological, musculoskeletal, or cardiac complications (Stanek and Reiter 2011; Strle and Stanek 2009). This multi-systemic inflammatory disease is caused by spirochetes from the species complex *Borrelia burgdorferi* sensu lato. At least six genospecies from this complex are responsible for human diseases: *Borrelia afzelii, B. garinii*, *B. burgdorferi* sensu stricto, *B. bavariensis, B. spielmanii* and *B. valaisiana* (Stanek and Reiter 2011; Strle and Stanek 2009). In Belgium, the prevalence of *B. burgdorferi* s.l. in questing ticks was found to vary between 8% and 18% (Cochez et al. 2015), depending on undergrowth and landscape connectivity (Heylen et al. 2019), undergrowth (Tack et al. 2013), and year (Ruyts et al. 2017). A full list of tick infection studies in Belgium is found in Supplementary Materials. Infection prevalence in ticks collected from humans was estimated at 13.9% at the national level (Lernout et al. 2019), and the incidence of Lyme borreliosis at 103 per 100 000 inhabitants (Geebelen et al. 2019).

In Western Europe, the main tick vector, *Ixodes ricinus*, also transmits *Francisella tularensis* and *Coxiella burnetii*, the causative agents of respectively tularaemia and Q fever. Tularaemia, which occurs mainly in the Northern Hemisphere, starts with flu-like symptoms but can evolve towards serious clinical manifestations and significant mortality if untreated (WHO 2007). Routes of infection include skin contact with infected animals, ingestion of contaminated water, and arthropod bites (ECDC 2017; WHO 2007). Lagomorphs and rodents are the main reservoir of its causative agent, the gram-negative intracellular bacterium *F. tularensis* (Carvalho et al. 2014). Tularaemia is rare in Belgium, but the number

of cases detected is increasing, similarly to the number of serological tests: 28 cases were detected between 1950 and 2021, of which 25 were reported between 2012 and 2021 with an unknown source of infection for most cases (Litzroth and Mori 2021).

*Coxiella burnetii* is a gamma(γ)-proteobacteria of the Legionella order (Körner et al. 2021; Woldehiwet 2004) that infects a wide range of hosts, including dogs, bovine, deer, rodents, birds and ticks (Cutler et al. 2007; Mori et al. 2017). In humans, it can cause acute or chronic illness, but the diagnosis is challenging because most human infections are subclinical (Anderson et al. 2013; Cutler et al. 2007; Mori et al. 2017). In Belgium, 15 cases of Q fever were reported in 2021 (Litzroth et al. 2021). Infections generally occur through inhalation of airborne particles contaminated with *C. burnetii* in milk, faeces, urine and birth products from infected ruminants (Anderson et al. 2013; Cutler et al. 2007; Duron et al. 2015). *Coxiella burnetii* was found in about 40 tick species (Anderson et al. 2013), including *I. ricinus* (Hildebrandt et al. 2011). Transmission through tick bites has been demonstrated in experimental conditions, but its role is disputed (Duron et al. 2015). *Ixodes ricinus* in particular was rarely found infected in the Netherlands (Sprong et al. 2012) and Switzerland (Pilloux et al. 2019), for example. Tick bites may constitute another route of transmission for human infections, but likely less important than the airborne one (Sprong et al. 2012), which is identified as the most important one (Anderson et al. 2013).

Tick-borne disease (TBD) occurrence depends on the probability of contact between pathogens, ticks, reservoir hosts and susceptible human hosts (Lambin et al. 2010). The life cycle of *I. ricinus* spans over two to six years. *Ixodes ricinus* takes a single blood meal by active stage (larva, nymph, and adult) on a broad range of vertebrates, including mammals, birds, and reptiles (Estrada-Peña and de la Fuente 2014). This generalist species spends extended periods off-host in the environment, for questing, rehydrating, moulting or diapausing. These activities are strongly seasonal (Dobson 2013; Dobson et al. 2011). *Ixodes ricinus* is found in various vegetated environments, but forests are their primary habitats and are often associated with high tick abundance at regional (e.g., Li et al. 2012; Ruiz-Fons et al. 2012; Vanwambeke et al. 2010) and local scales (e.g., Tack et al. 2012; Van Gestel et al. 2021; Vourc'h et al. 2016). Peri-urban forests are of particular interest, for both providing suitable habitats for ticks, that is, having a high level of hazard, and being intensively visited by humans, that is, generating high exposure (Dobson et al. 2011; Ruiz-Fons and Gilbert 2010; Zeimes et al. 2014). In Belgium, 35% of tick bites were reported in forests (https://epistat.sciensano.be/ticks/, last access: 7th of August 2023). Variations in tick abundance and infection in endemic areas are key components of tick-borne disease risk assessment (Horobik et al. 2006; Mysterud et al. 2013).

Tick abundance in forests is often estimated by collecting ticks by flagging or dragging on a delimited area and counting the number of ticks collected (Nyrhilä et al. 2020). Estimates of hazard drawn from ideal tick habitat are sometimes used to understand risk but do not necessarily represent well areas of intense human exposure in forests, as people may avoid bushy areas or be prevented from leaving paths as per forest regulations as often apply in peri-urban forests. Variability in tick density and pathogen prevalence is substantial in a forest, as indicated by Tack et al. (2012) and Van Gestel et al. (2021). In this study, we repetitively collected ticks throughout the period of tick activity. We assessed the spatio-temporal heterogeneity of tick density within and around a peri-urban forest, also accounting for varying degrees of potential human exposure. We hypothesize that tick density and infection prevalence are not homogeneous within a forest and are affected by within-forest

heterogeneity in undergrowth. The prevalences of the three above-mentioned pathogens (*B. burgdorferi* s.l., *F. tularensis* and *C. burnetii*) in ticks were also assessed.

## Material and methods

### Study area

The Bois de Lauzelle is a peri-urban forest of nearly 200 hectares located 30 km south of Brussels (Fig. 1). This peri-urban region has a high and growing population and has a higher incidence of erythema migrans than the Belgian average (Geebelen et al. 2019). The Bois de Lauzelle is bordered by a golf course, the city of Louvain-la-Neuve, and high-speed roads. The forest belongs to and is managed by the University of Louvain (UCLouvain) since 1970. It presents a diversity of local conditions, with an alternation of loamy and sandy-loamy soils and altitudes ranging from 45 to 153 m around the Blanc Ry River. The forest is dominated by deciduous trees, mainly beech (*Fagus sylvatica*) and oak (*Quercus petraea, Quercus ruber*) (Wallonie 2021), and is legally protected by several statutes (e.g., Natura 2000, code BE31006) (EEA 2020). The mean annual temperature and rainfall of the region are 10.6 °C and 849.4 mm / year (Royal Meteorological and Institute 2023).

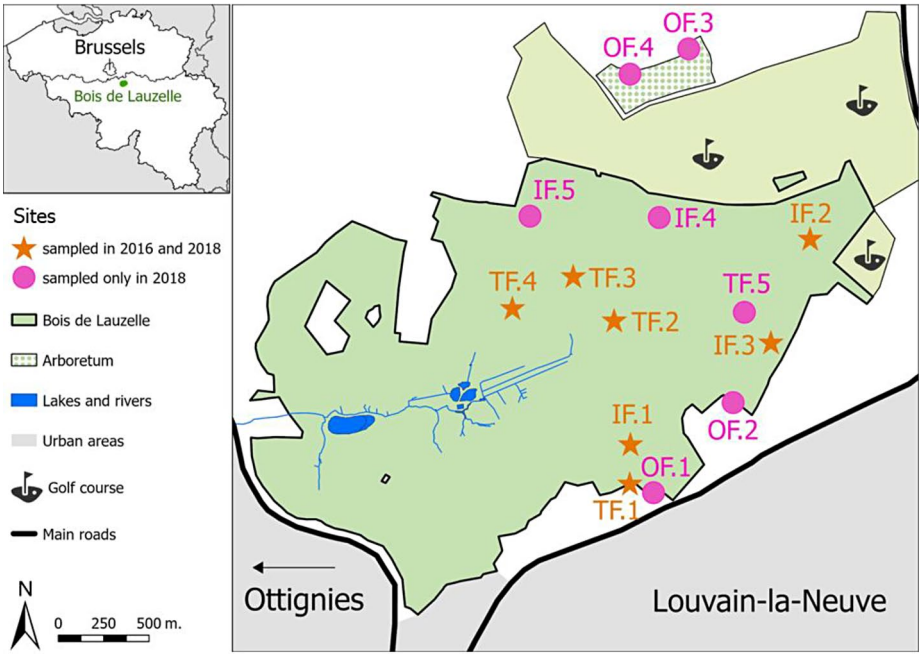


**Fig. 1** Location of the sites in the Bois de Lauzelle. Sites sampled in 2016 and 2018 are represented by orange stars, those only in 2018 by pink circles. IF, TF and OF stand for interior-forest, trail-forest, and outside-forest site types vrespectively

## Tick sampling

Ticks were sampled in seven sites in 2016 and an additional seven sites in 2018 (Fig. 1, orange stars and pink circles). Each site was a delimited area of 10 m², using a 1 m²-white flannel over the leaf litter, woodland scrub, or low vegetation. We included three types of sites. Interior-forest (IF) sites were forested sites with dense undergrowth and generally further from the trails (36 ± 9 m). Trail-forest (TF) sites were forested sites with low or sparse undergrowth generally closer to the trails (17 ± 13 m). Outside-forest (OF) sites were in a vegetated environment outside but close to the forest. A description of the 14 sites is provided in Supplementary files (Figures S1– S14). The categories IF and TF describe the configuration of the sites rather than specifically a typical distance from the trails. While IF sites are typically not frequented by walkers and have vegetation growing more freely, TF can be used by walkers (such as a picnic area, a bench and its vicinity), regardless of the vicinity of the trail. Typically, TF1 (edge of the scouts grounds) and TF3 (a grassy clearing used for picnic) are not directly adjacent to the trail. Further composing the slight overlap between the measured distances is the positional uncertainty of digitised trails.

Ticks were collected between March and November. In 2016, ticks were sampled every two weeks, resulting in 17 sampling events (SE). In 2018, sites were visited every three weeks, resulting in 11 SE. The sampling design was the same as in 2018 (Table 1).

Samplings were performed on dry and non-windy days between 8:00am and 1:00pm. Sites were sampled in a random order during each SE to account for daily fluctuations in humidity and temperature with the time of the day. During each SE, we dragged a flannel on each sites several times consecutively in a rapid sequence. In 2016, we dragged 10 times consecutively, and in 2018 we reduced it to six times based on observations in 2016. Collections by drag were recorded in 2018 but not in 2016. It takes approximately 9 ± 4 min for six drags. The flannel was changed whenever it became dirty or humid and was examined for ticks at the end of each drag. Nymph and adult ticks were counted and collected after each drag, and ticks from the same SU were stored together in a vial with 70% ethanol. Larvae were not systematically sampled.

## Tick identification and preparation

Tick life stage and species were identified under a Leica EZ4 binocular (X35), based on two conventional morphological identification keys (Estrada-Pena et al. 2004; Hillyard 1996). We also used Heylen et al. (2014a) to distinguish between three similar species found in Belgium, *I. ricinus*, *Ixodes frontalis* and *Ixodes arboricola*. Ticks were washed in three consecutive baths with ethanol and sterilized water and crushed individually with a sterilized

**Table 1** summary of the data characteristics for 2016 and 2018

| | 2016 | 2018 |
|---|---|---|
| *Sites sampled* | 7 | 14 |
| *Sampling events (SE)* | 17 | 11 |
| *Sampling units (SU) (sites*events)* | 119 | 154 |
| *Pooling* | Adults and nymphs together | Adults and nymphs separately |
| *Environmental monitoring* | No | Yes |

loop in Eppendorfs containing 200 µl of Dulbecco's modified Eagle's medium (DMEM) cell culture medium. Then, they were grouped in pools of four tick (50 µl/each, for a total volume of 200 µl) unless only a smaller pool was possible. Nymphs and adults collected in 2016 were pooled together. Nymph and adult ticks sampled in 2018 were pooled separately. Pooled ticks were from the same site and season. SE occurring between mid-March and mid-June were classed as spring, between mid-June to mid-September as summer, and between mid-September to the end of October as fall.

### Dna extraction, sequencing, and PCR

DNA was extracted from 100 µl pool medium. The complete methods for DNA extraction and sequencing are described elsewhere (Rousseau et al. 2021). Tick genus (*Ixodes* versus *Dermacentor*) was confirmed, and DNA extraction validated using SYBR Green real-time PCR targeting 5 S and ITS2 genes. Ticks were screened for *B. burgdorferi* s.l., *F. tularensis*, and *C. burnetii* using: (i) two qPCR targeting the Outer Surface Protein A gene (*OspA*) and the *Borrelia* flagellin gene (*Fla*) (method adapted from Kesteman et al. (2010), (ii) TaqMan real-time (Light-Cycler® TaqMan® Master, Roche Diagnostics GmbH, Germany) targeting *Francisella* (ISFtu primers) (Versage et al. 2003)d *tularensis* (Tul4 primers) (Michelet et al. 2014), (iii) TaqMan real-time (Light-Cycler® TaqMan® Master, Roche Diagnostics GmbH, Germany) targeting *C. burnetii* insertion element IS1111 with previously described primers (Mori et al. 2013). The identification of *Borrelia* species was performed by Sanger sequencing on the Genetic Analyzer ABI 3730XL (Applied Biosystems, Invitrogen Life Technologies, Carlsbad, CA, USA), with the BigDye Terminator kit (Applied Biosystems). To achieve more consistent sequencing results, we used the universal M13 tailed primer attached to the locus-specific primers described by Kesteman et al. (2010). The obtained consensus sequences were analysed with the Basic Local Alignment Search Tool (BLAST) for species determination.

### Tick variables

The density of nymphs (DON) and of adults (DOA) were computed as the total number of nymphs and adults sampled in the 10 or six consecutive drags in 2016 and 2018 respectively in each site and at each SE. We also computed drag-specific indices, $DON_i$ and $DOA_i$, the cumulative number of nymphs and adults captured during the first *i* consecutive drags. Nymph (NIP) and adult (AIP) infection prevalences were calculated by maximum likelihood with the PoolTestR R package (McLure et al. 2021). Finally, the densities of infected nymphs (DIN) and adults (DIA) were calculated by multiplying DON and DOA by NIP and AIP respectively. For 2016, as nymphs and adults were not pooled separately, we could only compute tick infection prevalence (TIP), which may overestimate DIN and underestimate DIA as adults had more blood meals and are usually infected at higher rates (Gray 1998).

### Environmental variables

We investigated the effect of environmental predictors on DON measured in 2018. Environmental characteristics were not monitored at individual SE in 2016. *Ixodes ricinus* retreats in the leaf layer for transstadial development and when questing conditions are unfavour-

able. When questing for a bloodmeal, it uses vegetation as a support (Gray 1998; Van Overbeek et al. 2008). For each SU (site at a particular SE), we measured undergrowth height and categorized it as low (<20 cm), medium (20 to 40 cm), and high (>40 cm). Forest edge habitats are suitable for various tick hosts (Allan et al. 2003; Brownstein et al. 2005; Tack et al. 2012). In peri-urban environments, Hansford et al. (2017) found more nymphs at the woodland edge than in other habitats. We measured the distance between each site and the closest forest edge. Guerra et al. (2002) found more *I. scapularis* on sandy and loamy-sand soils, compared to clay and loamy soils. Soil texture was extracted from the digital soil map of Wallonia (SPW 2005).

*Ixodes ricinus* is vulnerable to desiccation, and temperature and relative humidity influence its survival, development rates and activity periods (Brownstein et al. 2005; Diuk-Wasser et al. 2010; Medlock et al. 2013; Ruiz-Fons et al. 2012). We measured temperature and relative humidity over the course of the study period using a HOBO U23 Pro v2 Temperature/Relative Humidity Data Logger placed at 130 cm above the ground close to site TF.3. Temperatures measured at 130 cm and 5 cm in a forest are often correlated (Ruyts et al. 2018). The recording interval was 15 min. Temperatures during sampling events ranged from 5.2 to 25.3 °C and relative humidity from 41.6 to 99.5%. Mean temperatures and relative humidity for the 14 days preceding the samplings ranged from 7.5 to 21.7 °C and from 68.6 to 89.4%.

## Statistical analyses

Statistical analyses were conducted in R.4.2.1 (Core Team 2022). The dataset has multiple observations from the same locations and thus required a multi-level approach. **S**patial autocorrelation was tested for DON, DOA, AIP, NIP, TIP, DIN, and DIA using Moran's I. Due to the multilevel structure, we performed repeated measures correlation between these indicators with the rmcorr R package (Bakdash and Marusich 2023). This technique deals with repeated, nested samples and has a greater statistical power because no aggregation is necessary (Bakdash and Marusich 2017). The complete R code is available as supplementary material.

First, we used generalized linear mixed models (GLMM) to investigate differences in DON and DOA, with the type of site (IF, TF and OF), season (spring, summer or fall), year (2016 and 2018) and their one-way interaction, with site as a random effect. We used $DON_6$ and $DOA_6$ for 2018, and $DON_{10}$ and $DOA_{10}$ for 2016, as we consider they were the best proxies of true tick abundances. They were tested at SE level (resulting in 273 observations for 2016 and 2018). We had too few observations for NIP, AIP, DIN to adjust a GLMM. To assess overdispersion, we compared Poisson distributions with negative binomial and their zero-inflated versions, using Vuong's non-nested tests (pscl R package, Jackman 2020).

DON is usually estimated with the number of nymphs captured during a single drag. We created six GLMMs exploring the effects of season and type of sites on six measures using the number of nymphs collected using different number of drags for 2018.

Negative binomial mixed models were used to assess the effects of environmental variables on $DON_6$ in 2018, which had a complete environmental record. After standardization of the environmental predictors following Gelman (2008), the complete model included all non-collinear predictors. Collinear predictors were identified using a variance inflation factor<3. We also computed the best-AIC model, based on Akaike's Information Criterion

(AIC). The most parsimonious of models with differences of AIC smaller than|2|, considered equivalent, was chosen. All GLMMs were performed using the glmmTMB R package (Brooks et al. 2017). Residuals were controlled for dispersion, zero-inflation, heteroscedasticity and spatial autocorrelation with the DHARMa R package (Hartig 2022).

Finally, we calculated the diversity of *Borrelia* genospecies with the R vegan package (Oksanen et al. 2020). We used the exponential back-transformation of the Shannon-Wiener Index to compute a biodiversity "effective number of species", to facilitate interpretability, comparison and interpretability (Jost 2006).

## Results

### Tick abundance and tick-borne pathogen prevalence

We collected 1642 *Ixodes* nymphs and 181 adults (Fig. 2 and Figure S15). Of these, 1804 were identified as *I. ricinus*, 17 as *Ixodes frontalis* and three as *Ixodes ventalloi*. 786 nymphs and 81 adults were collected in 2016. Based on the consecutive drags method, we obtained a DON of 6.61 ± 8.39 and a DOA of 0.68 ± 1.11 ticks per 10 m² for 2016. In 2018, 856 nymphs and 100 adults were collected, resulting in DON of 5.56 ± 9.06, and DOA of 0.65 ± 1.44 ticks per 10 m² based on consecutive drags. All sites reached their maximum abundance before July. In 2018, DON was higher in forested (IF and TF sites, 7.06 ± 10.14 nymphs / 10 m²) than in non-forested sites (OF sites, 1.80 ± 3.38). There was no statistical difference in DON and DOA between 2016 and 2018 when estimated on all sites (6.61 and 5.56 for DON and 0.68 and 0.65 for DOA) or across the different types of sites (Table S1).

Nymphs and adults were grouped in 499 pools (221 for 2016 and 278 for 2018). *Borrelia burgdorferi* s.l. was detected in 90 pools (31 for 2016 and 59 for 2018). We found *Borrelia* positive pools in all sites, except TF.3, TF.4, OF.3. The mean TIP was 5.33%, 95%-CI

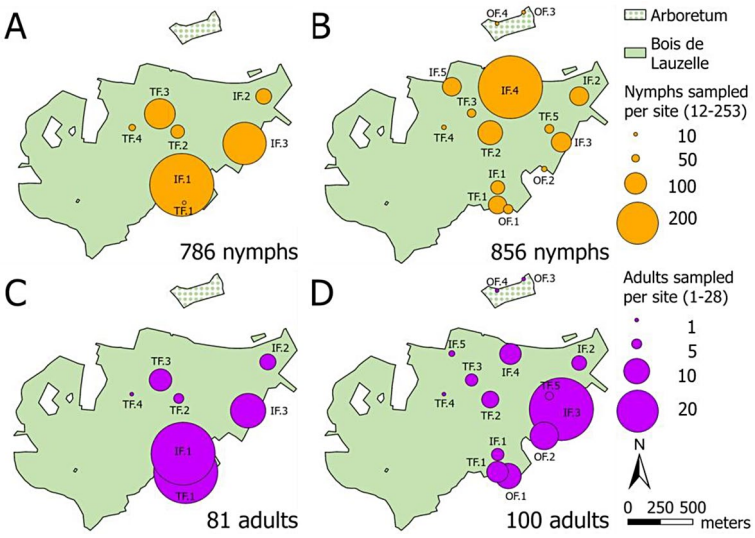


**Fig. 2** Number of nymphs sampled in 2016 (**A**) and 2018 (**B**) and adult ticks sampled in 2016 (**C**) and in 2018 (**D**) by site. IF, TF and OF stand for interior-forest, trail-forest, and outside-forest respectively

**Table 2** Repeated measures correlations between tick indicators for 2016 and 2018. Upper part: coefficient [95% - confidence intervals]. Lower part: significance based on p-values. Degrees of freedom = 258. N.S. stands for not significant. * $p$-value < 0.05, ** $p$-value < 0.01, and *** $p$-value < 0.001. Significant correlations at $p$-value < 0.05 are in bold

| | DON | DOA | TIP | DIN | DIA |
|---|---|---|---|---|---|
| **DON** | - | 0.28 [0.16–0.39] | -0.01 [-0.13–0.11] | 0.63 [0.55–0.70] | -0.02 [-0.14–0.10] |
| **DOA** | *** | - | -0.03 [-0.15–0.10] | 0.24 [0.12–0.35] | 0.45 [0.35–0.55] |
| **TIP** | N.S. | N.S. | - | 0.23 [0.11–0.34] | 0.05 [-0.07–0.17] |
| **DIN** | *** | *** | *** | - | -0.02 [-0.14–0.11] |
| **DIA** | N.S. | *** | N.S. | N.S. | - |

**Table 3** Negative binomial generalized linear mixed model estimates for density of nymphs (DON) and adults (DOA). The estimates and standard errors are expressed as the exponential of the log coefficients of the models. N.S. stands for not significant. * $p$-value < 0.05, ** $p$-value < 0.01, and *** $p$-value < 0.001. Significant estimates at $p$-value < 0.05 are in bold

| | Estimate (standard error) | |
|---|---|---|
| Fixed Effects | DON | DOA |
| *Intercept* | **18.93 (5.01)** *** | 1.01 (0.41) N.S. |
| *Year (baseline = 2016)* | | |
| Year 2018 | 0.95 (0.21) N.S. | 1.38 (0.46) N.S. |
| *Season (baseline = spring)* | | |
| Summer | **0.59 (0.13)** * | 1.01 (0.34) N.S. |
| Fall | **0.13 (0.03)** *** | **0.22 (0.10)** *** |
| *Site type (baseline = IF sites)* | | |
| TF sites | **0.37 (0.11)** *** | 0.55 (0.25) N.S. |
| OF sites | **0.16 (0.06)** *** | 0.57 (0.31) N.S. |
| *Interactions* | | |
| Year 2018 * Season Summer | 0.78 (0.23) N.S. | **0.36 (0.17)** * |
| Year 2018 * Season Fall | 1.01 (0.35) N.S. | 0.92 (0.55) N.S. |
| *Random Effects* | **Estimate (standard deviation)** | |
| **Site** | 0.99 (1.32) | 1.01 (1.55) |

[4.32–6.47], higher in 2018 (6.82%, 95%-CI [5.26–8.63]) than in 2016 (3.77%, 95%-CI [2.61–5.21]), and higher in fall (9.04%, 95%-CI [4.94–14.7]) over summer (5.60%, 95%-CI [3.95–7.62]) over spring (4.64%, 95%-CI [3.42–6.10. In 2018, NIP varied from 0 to 19% (IF.2) and AIP from 0 to 33% (IF.1 and OF.1). DIN varied from 0 to 1.25 infected nymphs per 10 m² in IF.2, and DIA from 0 to 0.20 in IF.3.

Spatial autocorrelation was only detected for DOA in 2016 (p-value = 0.04) (Table S2). We found significant repeated measures correlations between DON and DIN, DOA and DIA and DON and DOA, but not between tick prevalences and densities of ticks and infected ticks (Table 2). The strongest correlations were between DON and DIN (0.63, p-value < 0.0001) and DOA and DIA (0.45, p-value < 0.0001). Repeated correlation measures for individual years are found in Tables S3 and S4.

## Generalized linear mixed models with site type, season and year

For all $DON_i$ indicators, the negative binomial distribution was preferred (Table S2). GLMMs expressed variations of the density of nymphs and of adults (Table 3) by year, season, and type of site. The highest estimation of DON was for spring 2016 and in IF sites:

18.62 nymphs per 10 m². It decreased in summer and in fall and in TF sites and OF sites. There were no differences between the two years and the first-level interaction between year and season was not significant. No pattern was detected in the residuals. The estimated DOA in spring 2016 and in IF sites was 0.99 adults per 10 m². There were no differences between years, seasons and types of sites, except a lower tick density in fall. The first-level interaction between year and summer was also significant. No pattern was detected in the residuals (Dharma residuals plots presented in Figure S.16 for the models with interaction).

### Generalised linear mixed models for different estimates of don

In 2018, 34% of the nymphs (Table S6) and 51% of the adults (Table S7) were collected in the first drag. The lowest rates for both nymphs and adults were in IF sites. GLMM using DON estimated with various numbers of drags in 2018 show that the estimates of DON in spring and in IF-site increased from 4.10 nymphs per 10 m² for $DON_1$ to 16.64 for $DON_6$ (Table 4). As in Table 3, DON decreased from spring to fall and was higher in IF compared to OF and TF-SU. Difference in DON between site type was detected when DON was estimated with the ticks sampled from three consecutive drags or more.

### Generalized mixed model for the environmental predictors of tick density in 2018

When estimating the effects of the environmental variables on 2018-DON estimators, we found an estimate of 20.10 nymphs per 10 m² in sites with high undergrowth, loamy sands and excessive drainage (Table 5). DON was also negatively affected by relative humidity and temperature of the 14 previous days and positively by temperature during the sampling. Undergrowth height was significant and included in the best-AIC model, but no other variables describing sites. Dispersion, zero-inflation, heteroscedasticity, and spatial autocorrelation were not detected in the residuals of these models.

### *Borrelia* genospecies diversity

Six genospecies were identified: *B. afzelii* was dominant (54 isolations), followed by *B. garinii* (24), *B. burgdorferi* s.s. (13) and *B. valaisiana* (5). *Borrelia bavariensis* and *B. spielmanii* were only isolated once, in 2016 (Fig. 3). The effective diversity number for *Borrelia* genospecies was 3.21, higher in 2016 (3.42) compared to 2018 (2.93). It was also higher in forested sites (4.00 for TF and 2.71 for IF) than in non-forested sites (1.51 for OF.). TF.2, TF.1, IF.4, IF.2 and IF.3 presented the highest diversity in the number of genospecies (5, 4, 3, 3 and 3 respectively), and of isolations (14, 17, 16, 13 and 14 respectively) (Table 6). OF.3 had no genospecies isolated while TF.4, TF.5 and OF.2 had only one (*B. spielmanii* for the former and *B. afzelii*, for the last two). *Coxiella burnetii* was detected in six pools, three in 2016: one from TF.1 in spring and two from IF.1 in summer and three in 2018: one from IF.2 in spring and two from IF.4 in spring and summer. Due to the low prevalence, these results were not analysed further. *Francisella tularensis* bacteria were not detected.

**Table 4** Negative binomial generalized linear mixed model estimates for the number of nymphs sampled by consecutive drags in 2018 ($DON_i$, with *i* from 1 to 6). The estimates and standard errors are expressed as the exponent of the log coefficient of the model. N.S. stands for not significant. * *p*-value < 0.05, ** *p*-value < 0.01, and *** *p*-value < 0.001. Significant estimates at *p*-value < 0.05 are in bold

| | $DON_1$ | $DON_2$ | $DON_3$ | $DON_4$ | $DON_5$ | $DON_6$ |
|---|---|---|---|---|---|---|
| Fixed Effects | | | | | | |
| Estimates (standard deviations) | | | | | | |
| **Intercept** | **4.10 (1.49) *** ** | **8.04 (2.45) *** ** | **10.80 (3.24) *** ** | **12.95 (3.97) *** ** | **14.5 (4.21) *** ** | **16.64 (4.82) *** ** |
| **Season– baseline = Spring** | | | | | | |
| Summer | **0.60 (0.14) *** | **0.51 (0.12) ** ** | **0.48 (0.10) *** ** | **0.51 (0.11) ** ** | **0.50 (0.11) ** ** | **0.46 (0.09) *** ** |
| Fall | **0.17 (0.06) *** ** | **0.16 (0.05) *** ** | **0.15 (0.04) *** ** | **0.13 (0.04) *** ** | | **0.13 (0.03) *** ** |
| **SU Type– baseline = IF sites** | | | | | | |
| TF sites | 0.57 (0.28) N.S. | 0.52 (0.20) N.S. | **0.45 (0.18) *** | **0.43 (0.17) *** | **0.41 (0.15) *** | **0.39 (0.15) *** |
| OF sites | **0.21 (0.12) ** ** | **0.20 (0.09) *** ** | **0.18 (0.08) *** ** | **0.17 (0.08) *** ** | **0.17 (0.07) *** ** | **0.17 (0.07) *** ** |
| **Random Effects** | | | | | | |
| | **Estimates (standard deviations)** | | | | | |
| **Sites** | 0.99 (1.56) | 0.99 (1.43) | 0.99 (1.43) | 0.99 (1.44) | 0.99 (1.41) | 0.99 (1.41) |

## Discussion

We investigated the heterogeneity of tick density and infection prevalence in a peri-urban forest. Acarological indicators were heterogeneous within the forest, as previously reported by Vourc'h et al. (2016) in France. The mean densities of questing nymphs in forested sites, estimated at 6.61 and 7.07 nymphs per 10 m² in 2016 and 2018 respectively, were in the upper range of other studies in Belgian forests. Elsewhere, DON was estimated at of 4.05, 6.1 and 6.4 nymphs per 10 m² in 2013, 2018 and 2019 respectively (Ruyts et al. 2016; Van Gestel et al. 2021). However, direct comparison with our estimates is not straightforward, as other studies estimated DON by dragging the vegetation once. DON estimated from a single drag at the Bois de Lauzelle were 2.93 and 2.38 nymphs per 10 m² in 2016 and 2018 respectively.

Dragging is the most common method for tick sampling for its cost-effectiveness and its ease of implementation and replication among field workers (Nyrhilä et al. 2020). However, it tends to underestimate absolute tick abundance, and its efficiency also fluctuates, i.e., with the time of the day or the vegetation sampled (Bord et al. 2014; Boyard et al. 2007). Vegetation modifies the contacts between the drag and questing ticks (Tack et al. 2011). With varying capture probabilities, comparable abundance estimates are difficult to achieve (Mackenzie et al. 2002). We estimated DON based on consecutive drags, performed in fast succession during mornings to collect individuals from a closed population, which gives better proxies of the true abundances (Bord et al. 2014). The use of 10 consecutive drags in 2016 may have resulted in higher DON estimates, but 10 drags may sample a new population as ticks become active. DON and DOA did not significantly differ between our two years of sampling. Variations in DON between site types (IF, TF, OF) was only significant when it was estimated with at least three consecutive drags. Sampling the same transect repeatedly increases the probability of tick/drag contact in complex undergrowth. While vegetation structure, especially when several strata of foliage are present, would always interfere with dragging, we believe the consecutive drags method allows to bring into light differences between sites. We believe our estimate to be reliable and recommend performing DON estimations based on at least three consecutive drags when comparing areas with different vegetation types.

Tick samplings were regularly performed throughout the year to capture the temporal variation of tick density, as recommended by Dobson (2013) and Salomon et al. (2020). This limits the biases of occasional samplings caused by a variety of factors influencing questing, sometimes locally (e.g., passage of hosts before the sampling). In both years, ticks were active during the entire period of sampling, but their abundance was higher in spring over summer, over fall, as observed in other peri-urban areas of Western Europe (e.g., Hansford et al. 2017). Tick density also varied within seasons. Body inspection should therefore be recommended to forest users throughout the season of tick activity.

The density of sampled ticks was influenced by temperature and relative humidity at the time of sampling and the 14 previous days. Temperature and humidity affect desiccation and therefore *I. ricinus* survival and activity (Perret et al. 2000). DON was positively associated to temperature at sampling time but negatively to the 14 previous day average. The influence of temperature is complex and not easily reproducible (Boyard et al. 2011). In Belgium, temperature is likely not a limiting factor for tick survival and persistence, but may affect tick activity (Medlock et al. 2013). DON was negatively influenced by relative

**Table 5** Negative binomial estimates for the generalized linear mixed models of the density of nymphs by the environmental variables. The estimates and standard errors are expressed as the exponential of the log coefficients of the models. The AIC for the null model was 812.9. N.S. stands for not significant. * $p$-value < 0.05, ** $p$-value < 0.01, and *** $p$-value < 0.001

| | Complete model | Best-AIC model |
|---|---|---|
| AIC | 784.2 | 778.7 |
| **Fixed Effects** | **Estimates (standard errors)** | |
| Intercept | 20.10 (13.00) *** | 14.86 (5.98) *** |
| Relative humidity – 14 days | 0.73 (0.16) N.S. | - |
| Temperature – 14 days | 0.34 (0.11) *** | 0.40 (0.11) ** |
| Relative humidity – sampling | 0.56 (0.13) * | 0.50 (0.11) ** |
| Temperature – sampling | 2.05 (0.62) * | 1.91 (0.57) * |
| Undergrowth height (baseline = high) | | |
| Medium | 0.21 (0.08) *** | 0.21 (0.08) *** |
| Low | 0.14 (0.07) *** | 0.14 (0.07) *** |
| Texture (baseline = loam) | | |
| Sand | 1.12 (0.67) N.S. | - |
| Sandy loam | 0.65 (00.38) N.S. | - |
| Drainage (baseline = excessive) | | |
| Favourable | 0.79 (0.46) N.S. | - |
| Distance to forest edge | 0.50 (0.25) N.S. | - |
| **Random Effects** | **Estimates (standard deviations)** | |
| Site | 0.98 (1.72) | 0.98 (1.52) |

humidity at sampling time as observed in other studies (e.g., Hubálek et al. 2006; Kiewra et al. 2014; Li et al. 2012; Schwarz et al. 2009). We recorded temperature and relative humidity at a single forest location, above the litter, not where *I. ricinus* shelter. Results by Boehnke et al. (2017) suggest that on-site measurement may explain differences between sites, however, our results indicate that overall conditions also significantly affect tick activity. This would be relevant for using general weather observation as part of risk assessment.

*B. burgdorferi* infection prevalence in *I. ricinus* ticks was estimated here at 5.3%, 95%-CI [4.3–6.5], lower than prevalences in questing ticks in Belgium: 17.8% (Heylen et al. 2019), 15.6% (Ruyts et al. 2016), 12% (Kesteman et al. 2010) and 9.1% (Tack et al. 2012). The low prevalence *B. burgdorferi* found at the Bois de Lauzelle may also relate to the lack of connections for wildlife to other forests, or to the presence of specific hosts and host composition (Ruiz-Fons et al. 2012). To analyse the effects of hosts composition on tick abundance, *B. burgdorferi* prevalence and genospecies composition, further studies should consider including methods like live-trapping for small mammals or camera for large-sized mammals (Pérez et al. 2012; Ruyts et al. 2018).

We screened ticks in pools, a conventional method for arthropod vector screening. This method presents several challenges: the exact number of infected ticks in a positive pool cannot be determined and there may be a dilution effect for pools with high number of ticks (Fracasso et al. 2023). We grouped ticks in pools of four ticks, by site, season, and stage (in 2018). We estimated prevalence with the maximum-likelihood estimate of pooled prevalence, which is less influenced by pool size and infection rate of ticks than the pool positiv-

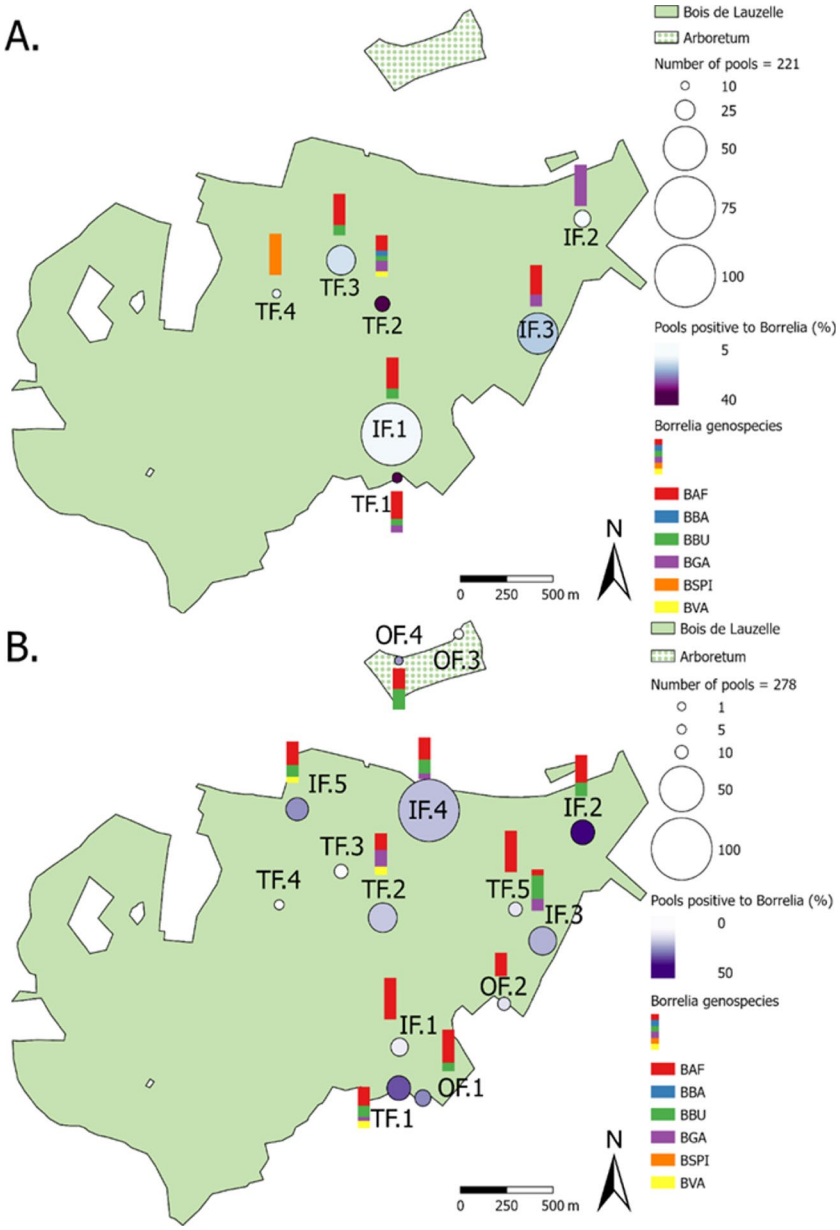


**Fig. 3** *Borrelia burgdorferi* s.l. infections in pooled ticks and genospecies composition by site in (**A**) 2016 and (**B**) 2018. BAF stands for *B. afzelii*, BBA for *B. bavariensis*, BGA for *B. garinii*, BSPI for *B. spielmanii* and BVAL for *B. valaisiana*

ity rate and minimum infection rate methods (Fracasso et al. 2023). This method produces robust estimates and confidence intervals of *B. burgdorferi* infection prevalence.

Tick infection prevalence with *B. burgdorferi* was variable, but the small number of pools and the broad confidence intervals prevented us from analysing the differences in

**Table 6** *Borrelia burgdorferi* s.l. genospecies diversity by site. BOR stands for *Borrelia burgdorferi* s.l., BAF for *B. afzelii*, BBA for *B. bavariensis*, BGA for *B. garinii*, BSPI for *B. spielmanii* and BVAL for *B. valaisiana*

| Sites (pools) | BOR effective number | BOR species richness | BOR species abundance | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | BOR | BAF | BBA | BBU | BGA | BSPI | BVAL |
| **TF** | **2.71** | **2.71** | **54** | **32** | **0** | **14** | **7** | **0** | **1** |
| TF.1 (39) | 3.26 | 4 | 17 | 9 | 0 | 4 | 2 | 0 | 2 |
| TF.2 (50) | 4.11 | 5 | 13 | 5 | 1 | 1 | 4 | 0 | 2 |
| TF.3 (46) | 1.75 | 2 | 4 | 3 | 0 | 1 | 0 | 0 | 0 |
| TF.4 (19) | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| TF.5 (11) | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| **IF** | **4** | **6** | **35** | **18** | **1** | **6** | **6** | **0** | **4** |
| IF.1 (85) | 1.65 | 2 | 5 | 4 | 0 | 1 | 0 | 0 | 0 |
| IF.2 (46) | 2.36 | 3 | 13 | 8 | 0 | 4 | 1 | 0 | 0 |
| IF.3 (76) | 2.94 | 3 | 14 | 6 | 0 | 4 | 4 | 0 | 0 |
| IF.4 (71) | 2.58 | 3 | 16 | 9 | 0 | 5 | 2 | 0 | 0 |
| IF.5 (22) | 1.57 | 2 | 6 | 5 | 0 | 0 | 0 | 0 | 1 |
| **OF** | **1.51** | **2** | **7** | **6** | **0** | **1** | **0** | **0** | **0** |
| OF.1 (14) | 1 | 1 | 4 | 4 | 0 | 0 | 0 | 0 | 0 |
| OF.2 (10) | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| OF.3 (6) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| OF.4 (4) | 2 | 2 | 2 | 1 | 0 | 1 | 0 | 0 | 0 |
| **Total** | **3.21** | **5** | **97** | **56** | **1** | **21** | **13** | **1** | **5** |

space and time. *Borrelia burgdorferi* infection prevalence was also found to be highly variable in Belgium and the Netherlands, ranging from 0 to 2% to 20–25%, over short distances (Hartemink et al. 2021; Kesteman et al. 2010). Tick infection prevalence should therefore not be estimated from a single location in a forest on a single timeframe. Hartemink et al. (2021) and Ruyts et al. (2017) did not find inter-annual variation in NIP and considered that DIN is mostly determined by DON. Forests present a diversity of micro-environments influencing tick density and infection prevalence. We found a weak correlation between DON and TIP (0.23), which is common in endemic areas (Randolph 2001), and has been reported elsewhere (James et al. 2013; Jouda et al. 2004; Ruyts et al. 2017; Vourc'h et al. 2016).

*Borrelia afzelii* was the dominant genospecies, followed by *B. burgdorferi* s.s. *and B. garinii*, which was consistent with other studies in Belgium. A recent meta-analysis of *Borrelia* prevalence in *I. ricinus* questing ticks in Western Europe also identified a composition of 46.6% for *B. afzelii*, 23.8% for *B. garinii*, 11.4% for *B. valaisiana*, 10.2% for *B. burgdorferi* s.s., and *B. bavariensis*, *B. spielmanii* rarely detected (Strnad et al. 2017). The effective number of *Borrelia* species, calculated as the exponential back-transformation of the Shannon Index, was higher in the forest, especially in sites with open areas and under-developed undergrowth. *Borrelia bavariensis* and *B. spielmanii*, identified once in this study, are rare genospecies in Belgium. *Borrelia afzelii* and *B. spielmanii* are commonly associated with small mammals, while *B. garinii* and *B. valaisiana* are hosted by sea birds and songbirds. *B. burgdorferi s.s.* is generalist (Comstedt et al. 2006; Gray 1998; Hanincova et al. 2003; Heylen et al. 2014b; Kurtenbach et al. 2002; Pedersen et al. 2020). The dominance of *B. afzelii* over *B. garinii* may suggest that rodents (e.g., *Apodemus sylvaticus*) are the most important feeding hosts for larval ticks in the Bois de Lauzelle.

*Francisella tularensis* and *Francisella*-like bacteria were not detected in this study. Their prevalence in ticks is usually low. In the Sénart Forest (France), *F. tularensis* was found in one out of 69 ticks (1.45%) in 2008, and not detected between 2009 and 2014 (Paul et al. 2016). *Coxiella burnetii* was detected in three pools in 2016 and three in 2018 and its prevalence in questing *I. ricinus* is also usually low, 0.2% in a study from the Netherlands (Sprong et al. 2012). The prevalence of these pathogenic agents in ticks is currently not well known in Belgium. The public health significance of pathogens, present at very low prevalence, is difficult to assess with the number of pools tested in this study. Other tick-borne pathogens would warrant investigation in Belgium as well, such as *Anaplasma phagocytophilum*, *Babesia* spp., *Borrelia miyamotoi*, *Neoehrlichia mikurensis* and *Rickettsia helvetica* (Lernout et al. 2019).

## Conclusion

We detected ticks in all locations sampled in and around a periurban forest. DON was assessed with the consecutive dragging method, which offers a representative and comparable estimate of tick abundance in areas with different undergrowth. DON was higher within than outside the forest and heterogeneous within the forest. Forest undergrowth type and height are good indicators of the level of tick abundance in a forest, but other factors such as host abundance likely also affects abundace. Tick abundance variability was associated to two types of factors: (i) the micro-environment of the sampling site providing suitable habitats for ticks and their hosts; (ii) the sampling method and weather at the time of sampling that influence tick capture efficiency and tick activity respectively. *Borrelia* infected ticks were foundeverywhere, but at relatively low prevalence compared to other studies in Belgian forests. Six different *Borrelia* species were identified, with a typical composition for Western European forests. *Coxiella burnetii* was present but rare, and *F. tularensis* was not detected. Further understanding of tick abundance variability and its determinants may help identify high-hazard areas and, in heavily visited forests, assess the feasibility of managing vegetation to limit tick abundance in the most visited areas.

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## Declarations

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**Consent for publication** Not applicable.

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## References


Allan BF, Keesing F, Ostfeld RS (2003) Effect of forest fragmentation on Lyme Disease risk. Conserv Biol 17:267–272. https://doi.org/10.1046/j.1523-1739.2003.01260.x

Anderson A, Bijlmer H, Fournier P-E, Graves S, Hartzell J, Kersh GJ, Limonard G, Marrie TJ, Massung RF, McQuiston JH, Nicholson WL, Paddock CD, Sexton DJ (2013) Diagnosis and management of Q fever—United States, 2013: recommendations from CDC and the Q fever Working Group. Recommendations and Reports, Morbidity and Mortality Weekly Report

Bakdash JZ, Marusich LR (2017) Repeated measures correlation. Front Psychol 8:1–13. https://doi.org/10.3389/fpsyg.2017.00456

Bakdash JZ, Marusich LR (2023) rmcorr: Repeated Measures Correlation

Boehnke D, Gebhardt R, Petney T, Norra S (2017) On the complexity of measuring forests microclimate and interpreting its relevance in habitat ecology: the example of *Ixodes ricinus* ticks. Parasites Vectors 10:1–14. https://doi.org/10.1186/s13071-017-2498-5

Bord S, Druilhet P, Gasqui P, Abrial D, Vourc'h G (2014) Bayesian estimation of abundance based on removal sampling under weak assumption of closed population with catchability depending on environmental conditions. Application to tick abundance. Ecol Modell 274:72–79. https://doi.org/10.1016/j.ecolmodel.2013.12.004

Boyard C, Barnouin J, Gasqui P, Vourc'h G (2007) Local environmental factors characterizing *Ixodes ricinus* nymph abundance in grazed permanent pastures for cattle. Parasitology 134:987–994. https://doi.org/10.1017/S0031182007002351

Boyard C, Barnouin J, Bord S, Gasqui P, Vourc'h G (2011) Reproducibility of local environmental factors for the abundance of questing *Ixodes ricinus* nymphs on pastures. Ticks Tick Borne Dis 2:104–110. https://doi.org/10.1016/j.ttbdis.2011.02.001

Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Maechler M, Bolker BM (2017) glmmTMB balances speed and flexibility among packages for zero-inflated generalized Linear mixed modeling. R J 9:378–400

Brownstein JS, Skelly DK, Holford TR, Fish D (2005) Forest fragmentation predicts local scale heterogeneity of Lyme disease risk. Oecologia 146:469–475. https://doi.org/10.1007/s00442-005-0251-9

Carvalho CL, Lopes de Carvalho I, Zé-Zé L, Núncio MS, Duarte EL (2014) Tularaemia: a challenging zoonosis. Comp Immunol Microbiol Infect Dis 37:85–96. https://doi.org/10.1016/j.cimid.2014.01.002

Cochez C, Heyman P, Heylen D, Fonville M, Hengeveld P, Takken W, Simons L, Sprong H (2015) The presence of *Borrelia miyamotoi*, a relapsing fever spirochaete, in questing *Ixodes ricinus* in Belgium and in the Netherlands. Zoonoses Public Health 62:331–333. https://doi.org/10.1111/zph.12154

Comstedt P, Bergström S, Olsen B, Garpmo U, Marjavaara L, Mejlon H, Barbour AG, Bunikis J (2006) Migratory passerine birds as reservoirs of Lyme borreliosis in Europe. Emerg Infect Dis 12:1307

Core Team R (2022) R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing

Cutler SJ, Bouzid M, Cutler RR (2007) Q Fever J Infect 54:313–318. https://doi.org/10.1016/j.jinf.2006.10.048

Dantas-Torres F, Chomel BB, Otranto D (2012) Ticks and tick-borne diseases: a One Health perspective. Trends Parasitol 28:437–446. https://doi.org/10.1016/j.pt.2012.07.003

de la Fuente J, Estrada-Pena A, Venzal JM, Kocan KM, Sonenshine DE (2008) Overview: ticks as vectors of pathogens that cause disease in humans and animals. Front Biosci 13:6938–6946. https://doi.org/10.2741/3200

Diuk-Wasser MA, Vourc G, Cislo P, Hoen AG, Melton F, Hamer SA, Rowland M, Cortinas R, Hickling GJ, Tsao JI, Barbour AG, Kitron U, Piesman J, Fish D (2010) Field and climate-based model for predicting the density of host-seeking nymphal *Ixodes scapularis*, an important vector of tick-borne disease agents in the eastern United States. Glob Ecol Biogeogr 19:504–514. https://doi.org/10.1111/j.1466-8238.2010.00526.x

Dobson ADM (2013) Ticks in the wrong boxes: assessing error in blanket-drag studies due to occasional sampling. Parasites Vectors 6:344. https://doi.org/10.1186/1756-3305-6-344

Dobson ADM, Taylor JL, Randolph SE (2011) Tick (*Ixodes ricinus*) abundance and seasonality at recreational sites in the UK: hazards in relation to fine-scale habitat types revealed by complementary sampling methods. Ticks Tick Borne Dis 2:67–74. https://doi.org/10.1016/j.ttbdis.2011.03.002

Duron O, Sidi-Boumedine K, Rousset E, Moutailler S, Jourdain E (2015) The importance of ticks in Q fever transmission: what has (and has not) been demonstrated? Trends Parasitol 31:536–552. https://doi.org/10.1016/j.pt.2015.06.014

ECDC (2017) Tularaemia factsheet [WWW Document]. URL https://www.ecdc.europa.eu/en/tularaemia/facts

EEA (2020) European Environment Agency - Site BE31006C0: Vallée de la Dyle à Ottignies [WWW Document]. Nat. 2000, Stand. Data Form./ URL https://natura2000.eea.europa.eu/Natura2000/SDF.aspx?site=BE31006C0

Estrada-Pena A, Bouattour A, Camicas JL, Walker AR (2004) Ticks of domestic animals in the Mediterranean Region: a guide to identification of species. Zaragoza

Estrada-Peña A, de la Fuente J (2014) The ecology of ticks and epidemiology of tick-borne viral diseases. Antiviral Res 108:104–128. https://doi.org/10.1016/j.antiviral.2014.05.016

Fracasso G, Grillini M, Grassi L, Gradoni F, da Rold G, Bertola M (2023) Effective methods of estimation of pathogen prevalence in pooled ticks. Pathogens 45:1–9. https://doi.org/10.3390/pathogens12040557

Geebelen L, Van Cauteren D, Devleesschauwer B, Moreels S, Tersago K, Van Oyen H, Speybroeck N, Lernout T (2019) Combining primary care surveillance and a meta-analysis to estimate the incidence of the clinical manifestations of Lyme borreliosis in Belgium, 2015–2017. Ticks Tick Borne Dis 10:598–605. https://doi.org/10.1016/j.ttbdis.2018.12.007

Gelman A (2008) Scaling regression inputs by dividing by two standard deviations. Stat Med 27:2865–2873. https://doi.org/10.1002/sim

Gray JS (1998) The ecology of ticks transmitting Lyme borreliosis. Exp Appl Acarol 22:249–258. https://doi.org/10.1023/A:1006070416135

Guerra M, Walker E, Jones C, Paskewitz S, Cortinas MR, Stancil A, Beck L, Bobo M, Kitron U (2002) Predicting the risk of Lyme Disease: Habitat Suitability for *Ixodes scapularis* in the North Central United States. Emerg Infect Dis 8:289–297. https://doi.org/10.3201/eid0803.010166

Hanincova K, Schäfer SM, Etti S, Sewell HS, Taragelová V, Ziak D, Labuda M, Kurtenbach K (2003) Association of *Borrelia afzelii* with rodents in Europe. Parasitology 126:11–20. https://doi.org/10.1017/S0031182002002548

Hansford KM, Fonville M, Gillingham EL, Coipan EC, Pietzsch ME, Krawczyk AI, Vaux AGC, Cull B, Sprong H, Medlock JM (2017) Ticks and *Borrelia* in urban and peri-urban green space habitats in a city in southern England. Ticks Tick Borne Dis 8:353–361. https://doi.org/10.1016/j.ttbdis.2016.12.009

Hartemink N, van Vliet AJH, Gort G, Gassner F, Jacobs F, Fonville M, Takken W, Sprong H (2021) Seasonal patterns and spatial variation of *Borrelia burgdorferi* (Sensu Lato) infections in *Ixodes ricinus* in the Netherlands. Parasites Vectors 14:1–16. https://doi.org/10.1186/s13071-021-04607-7

Hartig F (2022) DHARMa: residual Diagnostics for Hierarchical. Multi-Level / Mixed) Regression Models

Heylen D, De Coninck E, Jansen F, Madder M (2014a) Differential diagnosis of three common *Ixodes* spp. ticks infesting songbirds of Western Europe: *Ixodes arboricola*, *I. frontalis* and *I. ricinus*. Ticks Tick. Borne. Dis. 5, 693–700. https://doi.org/10.1016/j.ttbdis.2014.05.006

Heylen D, Matthysen E, Fonville M, Sprong H (2014b) Songbirds as general transmitters but selective amplifiers of *Borrelia burgdorferi* sensu lato genotypes in *Ixodes Rinicus* ticks. Environ Microbiol 16:2859–2868. https://doi.org/10.1111/1462-2920.12304

Heylen D, Lasters R, Adriaensen F, Fonville M, Sprong H, Matthysen E (2019) Ticks and tick-borne diseases in the city: role of landscape connectivity and green space characteristics in a metropolitan area. Sci Total Environ 670:941–949. https://doi.org/10.1016/j.scitotenv.2019.03.235

Hildebrandt A, Straube E, Neubauer H, Schmoock G (2011) Coxiella burnetii and coinfections in Ixodes ricinus ticks in Central Germany. Vector-Borne Zoonotic Dis 11:1205–1207. https://doi.org/10.1089/vbz.2010.0180

Hillyard PD (1996) Ticks of North-West Europe, The Natura. ed. London

Horobik V, Keesing F, Ostfeld RS (2006) Abundance and Borrelia burgdorferi-infection prevalence of nymphal *Ixodes scapularis* ticks along forest-field edges. EcoHealth 3:262–268. https://doi.org/10.1007/s10393-006-0065-1

Hubálek Z, Halouzka J, Juřicová Z, Šikutová S, Rudolf I (2006) Effect of forest clearing on the abundance of *Ixodes ricinus* ticks and the prevalence of *Borrelia burgdorferi* s.l. Med Vet Entomol 20:166–172. https://doi.org/10.1111/j.1365-2915.2006.00615.x

Jackman S, {pscl} (2020) Classes and Methods for {R} Developed in the Political Science Computational Laboratory

James MC, Bowman AS, Forbes KJ, Lewis F, McLeod JE, Gilbert L (2013) Environmental determinants of *Ixodes ricinus* ticks and the incidence of *Borrelia burgdorferi* Sensu Lato, the agent of Lyme borreliosis. Scotl Parasitol 140:237–246. https://doi.org/10.1017/S003118201200145X

Jost L (2006) Entropy Divers Oikos 113:363–375

Jouda F, Perret J-L, Gern L (2004) Density of questing *Ixodes ricinus* nymphs and adults infected by *Borrelia burgdorferi* Sensu Lato in Switzerland: Spatio-temporal pattern at a regional scale. Vector Borne Zoonotic Dis 4:23–32

Kesteman T, Rossi C, Bastien P, Brouillard J, Avesani V, Olive N, Martin P, Delmée M (2010) Prevalence and genetic heterogeneity of *Borrelia burgdorferi* sensu lato in *Ixodes* ticks in Belgium. Acta Clin Belg 65:319–322. https://doi.org/10.1179/acb.2010.069

Kiewra D, Kryza M, Szymanowski M (2014) Influence of selected meteorological variables on the questing activity of *Ixodes ricinus* ticks in Lower Silesia. SW Pol J Vector Ecol 39:138–145. https://doi.org/10.1111/j.1948-7134.2014.12080.x

Körner S, Makert GR, Ulbert S, Pfeffer M (2021) The prevalence of *Coxiella burnetii* in hard ticks in Europe and their role in Q fever transmission revisited - a systematic review. Front Vet Sci 8:1–16. https://doi.org/10.3389/fvets.2021.655715

Kurtenbach K, De Michelis S, Etti S, Schäfer SM, Sewell HS, Brade V, Kraiczy P (2002) Host association of *Borrelia burgdorferi* sensu lato - the key role of host complement. Trends Microbiol 10:74–79. https://doi.org/10.1016/S0966-842X(01)02298-3

Lambin EF, Tran A, Vanwambeke SO, Linard C, Soti V (2010) Pathogenic landscapes: interactions between land, people, disease vectors, and their animal hosts. Int J Health Geogr 9:54. https://doi.org/10.1186/1476-072X-9-54

Lernout T, De Regge N, Tersago K, Fonville M, Suin V, Sprong H (2019) Prevalence of pathogens in ticks collected from humans through citizen science in Belgium. Parasites Vectors 12:1–11. https://doi.org/10.1186/s13071-019-3806-z

Li S, Heyman P, Cochez C, Simons L, Vanwambeke SO (2012) A multi-level analysis of the relationship between environmental factors and questing *Ixodes ricinus* dynamics in Belgium. Parasit Vectors 5:1–11. https://doi.org/10.1186/1756-3305-5-149

Litzroth AA, Mori M (2021) Surveillance épidémiologique de la tularémie. *Francisella tularensis*–2021. Sciensano report

Litzroth AA, Stefani G, Esbroeck M, Van, Mori M (2021) Surveillance épidémiologique de la fièvre Q. Coxiella burnetii–2021

Mackenzie DI, Kendall WL, Ecology S, Sep N (2002) How should detection probability be incorporated into estimates of relative abundance? Ecology 83:2387–2393

McLure A, O'Neill B, Mayfield H, Lau C, McPherson B (2021) PoolTestR: an R package for estimating prevalence and regression modelling for molecular xenomonitoring and other applications with pooled samples. Environ Model Softw 145:105158. https://doi.org/10.1016/j.envsoft.2021.105158

Medlock JM, Hansford KM, Bormane A, Derdakova M, Estrada-Peña A, George J-C, Golovljova I, Jaenson TGT, Jensen J-K, Jensen PM, Kazimirova M, Oteo Ja, Papa A, Pfister K, Plantard O, Randolph SE, Rizzoli A, Santos-Silva MM, Sprong H, Vial L, Hendrickx G, Zeller H, Van Bortel W (2013) Driving forces for changes in geographical distribution of *Ixodes ricinus* ticks in Europe. Parasit Vectors 6:1–11. https://doi.org/10.1186/1756-3305-6-1

Michelet L, Delannoy S, Devillers E, Umhang G, Aspan A, Juremalm M, Chirico J, van der Wal FJ, Sprong H, Pihl B, Klitgaard TP, Bødker K, Fach R, Moutailler P, S (2014) High-throughput screening of tick-borne pathogens in Europe. Front Cell Infect Microbiol 4:1–13. https://doi.org/10.3389/fcimb.2014.00103

Mori M, Boarbi S, Michel P, Bakinahe R, Rits K, Wattiau P, Fretin D (2013) In vitro and in vivo infectious potential of *Coxiella burnetii*: a study on Belgian livestock isolates. PLoS ONE 8:1–9. https://doi.org/10.1371/journal.pone.0067622

Mori M, Mertens K, Cutler SJ, Santos AS (2017) Critical aspects for detection of *Coxiella burnetii*, in: Vector-Borne and Zoonotic Diseases. Mary Ann Liebert, Inc. 140 Huguenot Street, 3rd Floor New Rochelle, NY 10801 USA, pp. 33–41

Mysterud A, Easterday WR, Qviller L, Viljugrein H, Ytrehus B (2013) Spatial and seasonal variation in the prevalence of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* Sensu Lato in questing *Ixodes ricinus* ticks in Norway. Parasites Vectors 6:1–8. https://doi.org/10.1186/1756-3305-6-187

Nyrhilä S, Sormunen JJ, Mäkelä S, Sippola E, Vesterinen EJ, Klemola T (2020) One out of ten: low sampling efficiency of cloth dragging challenges abundance estimates of questing ticks. Exp Appl Acarol. https://doi.org/10.1007/s10493-020-00564-5

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2020) vegan: Community Ecology Package

Paul REL, Cote M, Le Naour E, Bonnet SI (2016) Environmental factors influencing tick densities over seven years in a French suburban forest. Parasit Vectors 9:309. https://doi.org/10.1186/s13071-016-1591-5

Pedersen BN, Jenkins A, Kjelland V (2020) Tick-borne pathogens in *Ixodes ricinus* ticks collected from migratory birds in southern Norway. PLoS ONE 15:1–13. https://doi.org/10.1371/journal.pone.0230579

Pérez D, Kneubühler Y, Rais O, Gern L (2012) Seasonality of *Ixodes ricinus* ticks on vegetation and on rodents and *Borrelia burgdorferi* sensu lato genospecies diversity in two lyme borreliosis-endemic areas in Switzerland. Vector-Borne Zoonotic Dis 12:633–644. https://doi.org/10.1089/vbz.2011.0763

Perret J-L, Guigoz E, Rais O, Gern L (2000) Influence of saturation deficit and temperature on *Ixodes ricinus* tick. Parasitol Res 86:554–557. https://doi.org/10.1007/s004360000209

Pilloux L, Baumgartner A, Jaton K, Lienhard R, Ackermann-Gäumann R, Beuret C, Greub G (2019) Prevalence of Anaplasma phagocytophilum and Coxiella burnetii in Ixodes ricinus ticks in Switzerland: an underestimated epidemiologic risk. New Microbes New Infect 27:22–26. https://doi.org/10.1016/j.nmni.2018.08.017

Randolph SE (2001) The shifting landscape of tick-borne zoonoses: tick-borne encephalitis and Lyme borreliosis in Europe. Philos Trans R Soc Lond B Biol Sci 356:1045–1056

Rousseau R, Vanwambeke SO, Boland C, Mori M (2021) The isolation of culturable bacteria in *Ixodes ricinus* ticks of a Belgian peri-urban forest uncovers opportunistic bacteria potentially important for public health. Int J Environ Res Public Health 18:1–14

Royal Meteorological, Institute (2023) Le climat dans votre commune [WWW Document]. Clim. la Belgique. URL https://www.meteo.be/fr/climat/climat-de-la-belgique/climat-dans-votre-commune

Ruiz-Fons F, Gilbert L (2010) The role of deer as vehicles to move ticks, *Ixodes ricinus*, between contrasting habitats. Int J Parasitol 40:1013–1020. https://doi.org/10.1016/j.ijpara.2010.02.006

Ruiz-Fons F, Fernández-de-Mera IG, Acevedo P, Gortázar C, de la Fuente J (2012) Factors driving the abundance of *Ixodes ricinus* ticks and the prevalence of zoonotic *I. ricinus*-borne pathogens in natural foci. Appl Environ Microbiol 78:2669–2676. https://doi.org/10.1128/AEM.06564-11

Ruyts SC, Ampoorter E, Coipan EC, Baeten L, Heylen D, Sprong H, Matthysen E, Verheyen K (2016) Diversifying forest communities may change Lyme disease risk: Extra dimension to the dilution effect in Europe. Parasitology 143:1310–1319. https://doi.org/10.1017/S0031182016000688

Ruyts SC, Tack W, Ampoorter E, Coipan EC, Matthysen E, Heylen D, Sprong H, Verheyen K (2017) Year-to-year variation in the density of *Ixodes ricinus* ticks and the prevalence of the rodent-associated human pathogens *Borrelia afzelii* and *B. Miyamotoi* in different forest types. Ticks Tick Borne Dis 9:141–145. https://doi.org/10.1016/j.ttbdis.2017.08.008

Ruyts SC, Landuyt D, Ampoorter E, Heylen D, Ehrmann S, Coipan EC, Matthysen E, Sprong H, Verheyen K (2018) Low probability of a dilution effect for Lyme borreliosis in Belgian forests. Ticks Tick Borne Dis 9:1143–1152. https://doi.org/10.1016/j.ttbdis.2018.04.016

Salomon J, Hamer SA, Swei A (2020) A beginner's guide to collecting questing hard ticks (acari: ixodidae): a standardized tick dragging protocol. J Insect Sci 20:1–8. https://doi.org/10.1093/jisesa/ieaa073

Schwarz A, Maier Wa, Kistemann T, Kampen H (2009) Analysis of the distribution of the tick *Ixodes ricinus* L. (Acari: Ixodidae) in a nature reserve of western Germany using Geographic Information systems. Int J Hyg Environ Health 212:87–96. https://doi.org/10.1016/j.ijheh.2007.12.001

Sprong H, Tijsse-Klasen E, Langelaar M, De Bruin A, Fonville M, Gassner F, Takken W, Van Wieren S, Nijhof A, Jongejan F, Maassen CBM, Scholte EJ, Hovius JW, Emil Hovius K, Špitalská E, Van Duynhoven YT (2012) Prevalence of *Coxiella burnetii* in ticks after a large outbreak of Q fever. Zoonoses Public Health 59:69–75. https://doi.org/10.1111/j.1863-2378.2011.01421.x

SPW (2005) Services Publics de Wallonie - Carte des Principaux Types de Sols de Wallonie– 1/250000 [WWW Document]. Serv. Publics Wallonie. URL https://geoportail.wallonie.be/catalogue/64bbc088-367c-485c-bd7c-d2d08baedf9d.html (accessed 5.29.21)

Stanek G, Reiter M (2011) The expanding Lyme Borrelia complex-clinical significance of genomic species? Clin. Microbiol Infect 17:487–493. https://doi.org/10.1111/j.1469-0691.2011.03492.x

Strle F, Stanek G (2009) Clinical manifestations and diagnosis of Lyme borreliosis. In: Lipsker, Dan, Jaulhac B (ed) Lyme Borreliosis- Biological and clinical aspects. Karger, Basel, pp 51–110

Strnad M, Hönig V, Ružek D, Grubhoffer L, Rego ROM (2017) Europe-wide meta-analysis of *Borrelia burgdorferi* sensu lato prevalence in questing *Ixodes ricinus* ticks. Appl Environ Microbiol 83:1–16. https://doi.org/10.1128/AEM.00609-17

Tack W, Madder M, de Frenne P, Vanhellemont M, Gruwez R, Verheyen K (2011) The effects of sampling method and vegetation type on the estimated abundance of *Ixodes ricinus* ticks in forests. Exp Appl Acarol 54:285–292. https://doi.org/10.1007/s10493-011-9444-6

Tack W, Madder M, Baeten L, Vanhellemont M, Gruwez R, Verheyen K (2012) Local habitat and landscape affect *Ixodes ricinus* tick abundances in forests on poor, sandy soils. Ecol Manage 265:30–36. https://doi.org/10.1016/j.foreco.2011.10.028

Tack W, Madder M, Baeten L, Vanhellemont M, Verheyen K (2013) Shrub clearing adversely affects the abundance of *Ixodes ricinus* ticks. Exp Appl Acarol 60:411–420. https://doi.org/10.1007/s10493-013-9655-0

Van Gestel M, Verheyen K, Matthysen E, Heylen D (2021) Danger on the track? Tick densities near recreation infrastructures in forests. Urban Urban Green 59:126994. https://doi.org/10.1016/j.ufug.2021.126994

Van Overbeek L, Gassner F, Van Der Plas CL, Kastelein P, Nunes-Da Rocha U, Takken W (2008) Diversity of *Ixodes ricinus* tick-associated bacterial communities from different forests. FEMS Microbiol Ecol 66:72–84. https://doi.org/10.1111/j.1574-6941.2008.00468.x

Vanwambeke SO, Sumilo D, Bormane A, Lambin EF, Randolph SE (2010) Landscape predictors of tick-borne encephalitis in Latvia: land cover, land use, and land ownership. Vector-borne Zoonotic Diseaes 10:497–506. https://doi.org/10.1089/vbz.2009.0116

Versage JL, Severin DDM, Chu MC, Petersen JM (2003) Development of a Multitarget Real-Time TaqMan PCR assay for enhanced detection of *Francisella tularensis* in complex specimens. J Clin Microbiol 41:5492–5499. https://doi.org/10.1128/JCM.41.12.5492-5499.2003

Vourc'h G, Abrial D, Bord S, Jacquot M, Masséglia S, Poux V, Pisanu B, Bailly X, Chapuis JL (2016) Mapping human risk of infection with *Borrelia burgdorferi* Sensu Lato, the agent of Lyme borreliosis, in a peri-urban forest in France. Ticks Tick Borne Dis 7:644–652. https://doi.org/10.1016/j.ttbdis.2016.02.008

Wallonie (2021) Site de Grand Intérêt Biologique (SGIB)–219: Bois de Lauzelle. Site de Grand Intérêt Biologique (SGIB) [WWW Document]. La biodiversité en Wallonie. URL http://biodiversite.wallonie.be/fr/219-bois-de-lauzelle.html?IDD=251659334&IDC=1881 (accessed 4.30.21)

WHO (2007) World Health Organization guidelines on tularaemia: epidemic and pandemic alert and response [WWW Document]. URL http://www.who.int/csr/resources/publications/WHO_CDS_EPR_2007_7.pdf

Woldehiwet Z (2004) Q fever (coxiellosis): epidemiology and pathogenesis. Res Vet Sci 77:93–100. https://doi.org/10.1016/j.rvsc.2003.09.001

Zeimes CB, Olsson GE, Hjertqvist M, Vanwambeke SO (2014) Shaping zoonosis risk: landscape ecology vs. landscape attractiveness for people, the case of tick-borne encephalitis in Sweden. Parasit Vectors 7:370. https://doi.org/10.1186/1756-3305-7-370