Mapping human risk of infection with *Borrelia burgdorferi* sensu lato, the agent of Lyme borreliosis, in a periurban forest in France

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ABSTRACT

Lyme borreliosis is a major zoonosis in Europe, with estimates of over 26,000 cases per year in France alone. The etiological agents are spirochete bacteria that belong to the *Borrelia burgdorferi* sensu lato (s. l.) complex and are transmitted by hard ticks among a large range of vertebrate hosts. In Europe, the tick *Ixodes ricinus* is the main vector. In the absence of a vaccine and given the current difficulties to diagnose and treat chronic Lyme syndromes, there is urgent need for prevention. In this context, accurate information on the spatial patterns of risk of exposure to ticks is of prime importance for public health. The objective of our study was to provide a snapshot map of the risk of human infection with *B. burgdorferi* s. l. pathogens in a periurban forest at a high resolution, and to analyze the factors that contribute to variation in this risk. Field monitoring took place over three weeks in May 2011 in the suburban Sénart forest (3,200 ha; southeast of Paris), which receives over 3 million people annually. We sampled ticks over the entire forest area (from 220 forest stands with a total area of 35,200 m^2) and quantified the density of questing nymphs (DON), the prevalence of infection among nymphs (NIP), and the density of infected nymphs (DIN), which is the most important predictor of the human risk of Lyme borreliosis. For each of these response variables, we explored the relative roles of weather (saturation deficit), hosts (abundance indices of ungulates and *Tamias sibiricus*, an introduced rodent species), vegetation and forest cover, superficial soil composition, and the distance to forest roads. In total, 19,546 questing nymphs were collected and the presence of *B. burgdorferi* s. l. was tested in 3,903 nymphs by qPCR. The mean DON was 5.6 nymphs per 10 m^2 (standard deviation = 10.4) with an average NIP of 10.1% (standard deviation = 0.11). The highest DIN was 8.9 infected nymphs per 10 m^2, with a mean of 0.59 (standard deviation = 0.6). Our mapping and modeling revealed a strong heterogeneity of risk within the forest. The highest risk was found in the eastern part of the forest and localized patches in the northwestern part. Lyme borreliosis risk was positively associated with stands of deciduous trees (mainly oaks) and roe deer abundance. Contrary to expectations, DIN actually increased with distance from the point of introduction of *T. sibiricus* (*i.e.*, DIN was higher in areas with potentially lower abundances of *T. sibiricus*). Thus, despite the fact that *T. sibiricus* is an important reservoir host for *B. burgdorferi* s. l., our study found that other explanatory factors played a more important role in determining the density of infected ticks. Precise mapping of the risk of exposure to Lyme borreliosis in a highly visited forest represents an important tool for targeting prevention and control measures, as well as making the general public and local health officials aware of the risks.

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1. Introduction

Lyme borreliosis is a systemic inflammatory disease caused by pathogenic spirochetes of the *Borrelia burgdorferi* sensu lato genospecies complex. These bacteria are transmitted by hard ticks of the genus *Ixodes*, mainly *I. ricinus* in Europe and *I. scapularis* in North America (Piesman and Gern, 2004). Despite major research and economic investment, Lyme borreliosis is still the most prevalent vector-borne disease in temperate ecozones of the Holarctic.

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Recent studies suggest that the annual number of Lyme borreliosis cases in the USA has been underestimated by a factor of ~10 (*i.e.*, 300,000 instead of 30,000 cases/yr) (CDC, 2013, Stricker and Johnson, 2014). Likewise, over 26,000 cases per year are estimated to occur in France (Réseau Sentinelles, 2014) and approximately 65,500 cases are diagnosed in Europe each year (Hubalek, 2009). The true incidence of the disease is probably underestimated, due to uncertainties in symptomatic diagnoses, poor reliability of diagnostic tests, and limited efficiency of treatments (e.g., Stricker and Johnson, 2014). Whatever the real estimate is, the fact that tens of thousands of Lyme borreliosis cases are reported annually in the Northern hemisphere highlights the need for new preventive actions, bearing in mind that no vaccine exists and current diagnosis and treatment of its chronic forms are difficult. In this context, accurate information on the spatial distribution of the risk of exposure to *B. burgdorferi* s. l. infected ticks is of prime importance for public health. With this knowledge, the public could avoid high-risk areas and know to inspect for ticks. This information would also alert the medical community, and potentially lead to more accurate diagnoses of tick-borne diseases (Piesman and Eisen, 2008).

The risk of human exposure to infection with *B. burgdorferi* s. l. is closely linked to the density of nymphal ticks (DON), and directly linked to the density of infected nymphs (DIN) that quest for vertebrate hosts in the environment (Mather et al., 1996; Stafford et al., 1998; Pepin et al., 2012; Diuk-Wasser et al., 2012). Other developmental stages represent a lower risk of transmission: larvae are not infected with the bacteria (Richter et al., 2012; Rollend et al., 2013), and adult ticks are less numerous and easier to detect and remove from the skin. Many studies have estimated DON or DIN in different locations with the aim to compare estimates of DON and DIN between locations, to correlate estimates with the incidence of Lyme borreliosis in humans, or to investigate environmental factors associated with tick abundance (e.g., Beytout et al., 2007; Boyard et al., 2007; Ruiz-Fons et al., 2012; James et al., 2013). Advances in geographic information systems have facilitated the mapping of DON or DIN so that it is now feasible to identify areas of high exposure risk and their associated environmental factors at a large spatial scale (countries or continents, e.g., Estrada-Peña, 2002; Diuk-Wasser et al., 2007, 2012). However, to our knowledge, no study has yet generated a fine-scale risk map of a smaller area, such as a forest, in order to identify localized high-risk areas and the main environmental factors associated with the risk. Such a map would be of great help for local stakeholders in improving Lyme borreliosis prevention, especially for forests receiving many visitors, such as in periurban areas.

The objective of our study was to map, with a high resolution, the risk of human exposure to infection with *B. burgdorferi* s. l. at the scale of a periurban forest and to study the factors that contribute to variation in this risk. Our study took place in the Sénart forest (near Paris, France), where previous investigations have revealed ticks, pathogens, and reservoir hosts in abundance (Pisanu et al., 2010; Marsot et al., 2011, 2013). This forest is potentially a hotspot for Lyme borreliosis exposure because over 3 million people visit this area each year (Maresca, 2000). Using a standardized protocol, we collected ticks from 220 locations within the forest, analyzed the presence of *B. burgdorferi* s. l., mapped the human risk of infection (*i.e.*, the DIN) and its components (DON and NIP, the nymphal infection prevalence), and studied the influence of environmental factors on the variation in exposure risk.

2. Materials and methods

2.1. Study area, tick sampling, and Borrelia detection

Questing ticks were collected in the Sénart forest (Essonne, France, 48°40′ N, 2°28′ E), located 22 km southeast of Paris, in a temperate climate. This forest (3,200 ha, approximately 9 × 4 km) sits on loamy (56%), sandy (28%), and clay soils (16%). Conifers (*Pinus nigra, P. sylvestris*) cover 6% of the total forest area, which is dominated by deciduous trees. The dominant forest stand is oak-hornbeam (Bissardon et al., 1997), comprised of *Quercus robur, Q. petraea*, and *Carpinus betulus*. Other well-represented tree species are *Tilia cordata* and *Castanea sativa*, with *Betula pendula, Populus tremula, Acer* spp., *Fraxinus excelsior*, and *Prunus avium* locally abundant in patches. The shrub layer consists mainly of *Corylus avellana, Crataegus monogyna*, and *Lonicera periclymenum*, and the herb layer of *Brachypodium sylvaticum, Rubus* gr. *fruticosus*, and *Anemone nemorosa*, with localized patches of *Calluna vulgaris* and *Pteridium aquilinum.* The forest is divided into 197 plots managed by the National Forest Office (ONF). Two ungulates are abundant: the roe deer (*Capreolus capreolus*) and the wild boar (*Sus scrofa*). Annually, about 140 roe deer and hundreds of wild boars are culled by hunting (C. Briou, ONF, pers. com.). The hunting effort covers all parts of the forest, but is less intense in the eastern part due to the scarcity of wild boar, the main target species (C. Briou, ONF, pers. com.).

We collected ticks in 220 of 273 forest stands that were distributed among the 197 managed forest plots. Here, a forest stand is a unit of forest composed of homogeneous vegetation. Each sampled stand was investigated along two parallel transects (of 220 m), for a total of 440 transects. The transects originated from the approximate middle of a stand edge along a forest road, ran perpendicularly from the road into the forest, and were 20-m distant to each other. Each transect was composed of 8 sampling units of 10 m² each (1 m × 10 m) that were separated by a distance of 20 m. In total, 3520 sampling units were investigated for a total surface of 35,200 m².

Questing nymphs of *I. ricinus* were collected in each sampling unit of 10 m² from 9 to 26 May 2011 using the drag sampling method with a 1 m² white cloth (MacLeod, 1932; Vassallo et al., 2000). Ticks were generally collected between 9 and 12 AM or 1 PM and 6 PM. Eleven different individuals participated in the sampling effort for a total of ca 576 person-hours. The number of steps for each collector was calibrated for the 10 m distance and collectors walked at a slow pace. The flags were dragged once over each sampling unit and were changed whenever they became dirty or humid. Collected ticks were immediately stored in 70% ethanol. All ticks of the same stage collected from the same transect were stored in the same vial.

In total, 20% of the nymphs present in each vial were randomly screened for the *B. burgdorferi* s. l. pathogens. Total DNA was extracted from each tick using ammonia (Humair et al., 2007), and the presence of *B. burgdorferi* s. l. was detected in the extracts using quantitative PCR that targeted a fragment of the *flaB* gene. Two primers were modified from Gomez-Diaz et al. (2010): FlaB_outF 5′ CAATATAACCAAATGCACATGTT, and FlaB_inR 5′ ACATTAGCWG-MATAAATATTTACAG. Amplification was performed with the SYBR Green SsoAdvanced Supermix kit on a CFX Touch Detection System (Biorad, Hercules, California, USA) in a 20-μl total reaction volume, which included 10 μl of 2× mix, 2 μl of each primer at 10 μM, 1 μl of H$_2$O, and 5 μl of DNA template solution. Thermal cycling reactions began with an initial denaturation step of 2 min at 98 °C, and continued with 50 cycles that included a 5-s denaturation step at 98 °C and a 30-s annealing/elongation step at 60 °C. Fluorescence was recorded during each cycle.

2.2. Response variables (DON, NIP, DIN)

We quantified three response variables that are commonly investigated in studies of Lyme borreliosis risk in the environment: (i) density of nymphal ticks (DON), (ii) nymphal infection prevalence (NIP), and (iii) density of infected nymphs (DIN). DON was

defined as the number of questing *I. ricinus* nymphs per 10 m^2 (the sampling unit). NIP was the proportion of the number of nymphs infected by *B. burgdorferi* s. l. to the number of tested nymphs within a transect. DIN corresponded to the NIP multiplied by the number of questing nymphs collected on the transect, divided by 8 to have the same units as DON, i.e. number of infected nymphs per 10 m^2.

2.3. Explanatory variables

To explain variation in the DON, NIP, or DIN, we considered seven environmental variables that could be divided into three general categories: (i) weather, (ii) vertebrate hosts, and (iii) local environment. The saturation deficit (SD) is a weather variable that influences the questing activity of nymphs (Gray, 2008). The host-related variables included the abundances of roe deer and wild boar and the presence index of Siberian Chipmunks (*Tamias sibiricus*, a small ground squirrel introduced from Korea). Roe deer and wild boar were included because of their known (for roe deer) or suspected (for boar) influence on tick abundance (Ruiz-Fons et al., 2006; James et al., 2013). The Siberian chipmunks were included because of its known contribution to Lyme borreliosis risk (Marsot et al., 2013). The four local environment variables included tree composition, ground cover, soil characteristics, and location of sampling units relative to forest roads. These local environment variables can influence tick presence and activity either via effects on microclimate or on the composition of the host community (Medlock et al., 2008; Halos et al., 2010).

Temperature and humidity were measured with a digital thermo-hygrometer (TFA, Germany) at soil level (in the shade) during tick collection at the beginning of each sampling transect. With these measurements, we calculated the saturation deficit (SD, Fig. A.1) in millimeters of mercury, a measure of the "drying power" of the air: $SD = (1 - RH/100) \times 4.9463\exp(0.0621\,T)$, in which RH is the average daily relative humidity and T is the average daily temperature. Previous studies have found that tick questing is impaired by a high saturation deficit (Perret et al., 2000; Tagliapietra et al., 2001; Herrmann and Gern, 2010). Saturation deficit (in units of mmHg) was divided into three quantiles: [4.4, 9.0], [9.0, 13.4] and [13.4, 22.2].

The influence of wild ungulates was investigated using a proxy of their relative abundances. This proxy was calculated in different parts of the forest based on the number of animals killed in the 2011–2012 hunting season (C. Briou, ONF, pers. com.). These data were missing for some forest stands; thus a spatial interpolation based on weighting by the inverse distance was used (Shepard, 1968). The numbers of animals killed were assigned to one of three categories of low, medium, and high abundance. For roe deer the limits of these abundance categories were: [0–3], [4–7], and [8–14] killed roe deer whereas for boar the categories were: [0–4], [5–10], and [11–30] killed boar. The abundance limits were chosen so that each category contained an equal number of sampling units (Fig. A.3). We used a different method to estimate the influence of Siberian chipmunks. These animals have been sold in European pet shops since the 1960s, and intentionally released into the wild since the 1970s (Chapuis, 2005). Since then, 22 populations of chipmunks in Europe, and 11 in France, have been identified in forests and urban parks, of which the Sénart population is the largest (>10,000 individuals estimated, Chapuis et al., 2009), especially in the western area of the forest where chipmunks were initially introduced (Chapuis, 2005). The eastern and southeastern areas of the forest were only recently colonized (Marmet, 2008), probably due to the presence of the N6 road which created a barrier to dispersal. Because no estimates of abundance exist at the scale of the entire forest area for this species, we calculated the

distance of each sample stand from the estimated point of major introduction (i.e. Chêne d'Antin: 48°40'19.96"N, 2°25'47.61"E; J-L Chapuis personal observations) as a proxy for chipmunk presence. This distance (in km) was divided into three quantiles: [0–3.2], [3.2–5.3], and [5.3–8.5] (Fig. A.4).

Two vegetation variables were used: tree composition and ground cover. Dominant tree species composition was inferred from the descriptions of the main types of forest stands published by the National Forest Office. These descriptions were grouped into three categories: coniferous trees, oaks (*Quercus* spp.), and other deciduous trees (Fig. A.5). Ground cover described the dominant understory vegetation observed in each of the sampling units. Six categories were used (Fig. A.6): (1) bare ground, including moss, (2) dead leaves, including dead branches, (3) herbaceous vegetation, (4) blackberry, (5) fern, and (6) heather. Superficial soil characteristics were inferred at the forest stand level from data provided by the "Bureau de Recherches Géologiques et Minières" and divided into three categories according to the dominant particles in each soil type: sand, clay, or silt (Fig. A.7).

In addition, we tested two alternative hypothesis regarding the effect of the proximity of forest roads on the DON. First, proximity to a forest road may increase tick density because the resulting increase in sunlight leads to increased habitat diversity for hosts (ungulates, small rodents and birds). Alternatively, proximity to a forest road could decrease nymphal density because such open areas are avoided by ungulates (Ward et al., 2004). On each transect, sampling units were numbered in ascending order from the forest road (rank 1) to the interior of the forest (rank 8). We tested the influence of the location of the sampling unit by comparing those close to the forest roads (i.e. less than 50 m distant, sampling units 1 and 2) to all other units grouped together.

For analyses of DON, the explanatory variables were defined at the level of the sampling unit. For the analyses of NIP and DIN, the explanatory variables were defined at the scale of the transects. The transect-level explanatory variables included the vegetation that covered the highest number of sampling units per transect as well as mean temperature and humidity per transect.

2.4. Statistical analysis

The response variables (DON, NIP, and DIN) were modelled using a generalized linear model (GLM) approach (McCullagh and Nelder, 1989) with a log link function. The NIP was modeled using the number of infected nymphs per transect and the logarithm of the number of tested nymphs as an offset term, which is equivalent to test the proportion of infected nymphs (McCullagh and Nelder, 1989). Two types of distribution were tested: the Poisson distribution and the negative binomial distribution. The negative binomial distribution is expected to give a better fit than the Poisson distribution when counts are aggregated in space (or time) and overdispersion occurs (Wilson and Grenfell, 1997). For each response variable, we compared the two types of distribution with models that included all explanatory variables (full model). We choose the distribution that gave the lower ratio between the residual deviance and the residual degrees of freedom (McCullagh and Nelder, 1989). For DON, NIP and DIN, the best error distribution was the negative binomial distribution with an associated overdispersion ratio (residual deviance/residual degrees of freedom) of 1.10, 1.06 and 1.02, respectively. The influence of each explanatory variable was tested using the log-likelihood ratio tests that compared the full model to a reduced (or simplified) model that contained all the explanatory variables except the one under consideration. A chi-squared test was used to assess the *P*-value

Table 1
Results of the negative binomial models for density of nymphs (DON), prevalence of infection by *Borrelia* (NIP), and density of infected nymphs (DIN) in the Sénart forest (2011). For each explanatory variable, rate parameters are displayed with 95% confidence intervals in brackets. Rate parameters measure how much the level of the tested variable would increase (if rate parameter >1) or decrease (if rate parameter <1) the risk in comparison to a reference level. The symbols '*', '**', and '***' indicate significant explanatory variables at *P*-value <0.05, *P*-value <0.01, and *P*-value <0.001, respectively.

Explanatory variables	Levels	Density of nymphs (DON)	Prevalence of infection by *Borrelia* (NIP)	Density of infected nymphs (DIN)
Autocorrelation	–	**1.069 (1.067–1.071)*****	–	–
Saturation deficit (mm)	[4.4–9.0]	1	1	1
	[9.1–13.4]	1.06 (0.96–1.17)	1.00 (0.77–1.29)	1.05 (0.74–1.48)
	[13.5–22.2]	0.92 (0.83–1.01)	1.03 (0.78–1.34)	0.93 (0.65–1.34)
Deer abundance index (number)	[0–3]	1	1	1
	[4–7]	1.09 (0.97–1.22)	1.41 (1.04–1.91)*	**1.92 (1.29–2.86)****
	[8–14]	**1.29 (1.13–1.46)*****	1.11 (0.79–1.55)	1.25 (0.78–2.00)
Boar abundance index (number)	[0–4]	1	1	1
	[5–10]	0.94 (0.84–1.04)	0.92 (0.67–1.25)	0.95 (0.64–1.41)
	[11–30]	**0.58 (0.52–0.65)*****	0.99 (0.71–1.38)	0.57 (0.36–0.89)*
Distance from chipmunk invasion (km)	[0–3.2]	1	1	1
	[3.2–5.3]	0.88 (0.79–0.98)*	1.16 (0.85–1.57)	1.23 (0.84–1.80)
	[5.3–8.5]	1.14 (1.00–1.03)	**1.81 (1.29–2.54)*****	**3.80 (2.48–5.82)*****
Vegetation	Ground	1	1	1
	Fern	1.05 (0.75–1.46)	0.83 (0.28–2.51)	0.96 (0.19–4.72)
	Heather	0.55 (0.37–0.83)**	0.52 (0.12–2.35)	0.23 (0.03–1.66)
	Herbaceous	1.26 (0.95–1.66)	0.83 (0.35–1.99)	1.07 (0.28–4.06)
	Shrubs	1.29 (0.93–1.77)	0.81 (0.26–2.59)	0.75 (0.14–3.97)
	Dead leaves	1.13 (0.86–1.49)	0.71 (0.30–1.69)	0.79 (0.21–2.99)
Forest cover	Coniferous	1	1	1
	Oak	**1.38 (1.16–1.63)*****	1.73 (0.98–3.06)	**2.62 (1.32–5.19)****
	Other deciduous	0.89 (0.70–1.13)	**3.76 (1.87–7.54)*****	**3.92 (1.63–9.42)****
Superficial soil	Sand	1	1	1
	Clay	**1.21 (1.06–1.38)****	0.64 (0.46–0.90)*	0.78 (0.50–1.21)
	Silt	**1.34 (1.21–1.48)*****	**0.69 (0.54–0.90)****	0.83 (0.59–1.17)
Order of sampling units relative to forest road	t1–t2	1	–	–
	t3–t8	**1.33 (1.22–1.46)*****		

of this ratio. The parameter estimate for each explanatory variable is the rate parameter (RP), which measures how much the level of the tested explanatory variable would increase (if RP > 1) or decrease (if RP < 1) the risk in comparison to a reference level.

We tested the deviance residuals for autocorrelation using Moran's *I*-test (McCullagh and Nelder, 1989). If the test was significant, an autocovariate term was added to the model (Dormann, 2007) to remove the autocorrelation and improve the fit. As spatial autocorrelation was detected in the residuals of the regression model for DON (*P*-values <0.001), the autocorrelation term was calculated for each sampling unit as the mean number of nymphs from the previous and the next sampling unit along the transect. For NIP and DIN, the autocorrelation term was calculated as an inverse-distance-weighted average of the response values within a defined neighborhood (Dormann et al., 2007) and was not significant (*P*-value = 0.556 for NIP, *P*-value = 0.909 for DIN).

Observations, predictions, and residuals were interpolated using the inverse-distance-weighted method in order to generate a continuous map (Shepard, 1968). The first parameter of the interpolation is the exponent that controls the decay of the influence of observations based on the distance from the interpolated points. The second is a smoothing parameter that limits excessive influences of the nearest observation points. Both parameters were estimated using a cross-validation method and the root mean square error as a maximization criterion (Tomczak, 1998). Finally, we tested the correlation between NIP and DON using a Spearman test *R* in order to check whether DON could be a good predictor of NIP at the scale of a forest. All the analyses were conducted with R (R-Core-Team, 2013) and QGis software (QGIS-Development-Team, 2009).

3. Results

3.1. Effect of explanatory variables on the density of questing nymphs (DON)

In total, we surveyed 3,520 sampling units of 10 m² and 440 transects. We collected 19,546 questing nymphs of *I. ricinus* from 413 transects (27 transects had no nymphs). The number of nymphs per sampling unit of 10 m² ranged from 0 (24% of the sampling units) to 215, with a mean of 5.6 (standard deviation = 10.4) and a median of 3 nymphs per 10 m². High values of DON were observed in the eastern part of the forest (Fig. A.8).

All the explanatory variables except saturation deficit significantly explained a significant amount of the variation in DON (Table 1). High abundances of roe deer and boar were associated with a higher (*P*-value <0.001) and a lower (*P*-value <0.001) density of questing ticks, respectively. Distances categorized as "medium" from the chipmunk point of introduction were weakly associated with lower DON compared to the shorter distances. DON was significantly lower in sites where heather was present compared to those with only bare ground, and significantly higher in stands of oaks compared to those of conifers. DON was higher on clay and silt than on sandy soils. DON was also significantly higher in sampling units farther from forest roads compared to those that were close.

3.2. Effect of explanatory variables on the nymphal infection prevalence (NIP)

In total, 3,903 nymphs from the 413 transects in which ticks were found were screened for *B. burgdorferi* s. l. pathogens. Among these, 394 (10.1%, standard deviation = 0.11) of the nymphs were
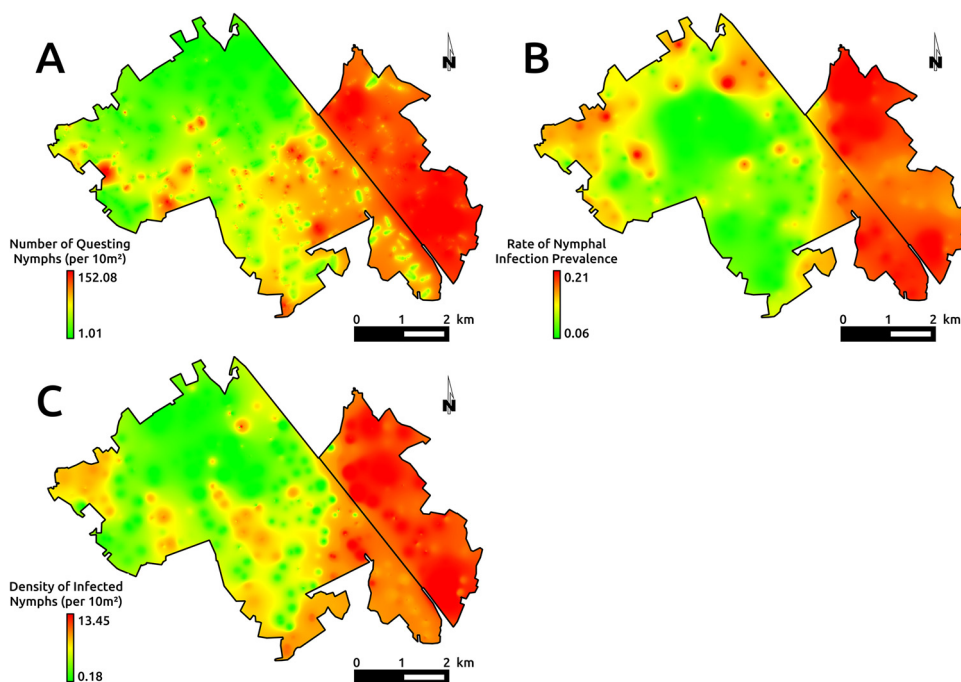
Fig. 1. Map of the estimated (a) density of questing nymphs (DON), (b) nymphal infection prevalence (NIP), and (c) density of infected nymphs (DIN).

found to be infected. High values of NIP were observed in the north-western and in the eastern parts of the forest (Fig. A.10).

Four explanatory variables significantly influenced NIP (Table 1). First, the highest prevalence of *Borrelia* in nymphs tended to be linked to a "medium" abundance of roe deer. The NIP also increased with distance from the chipmunk point of introduction, and was more strongly associated with "other deciduous" forest stands than with coniferous stands. Unlike DON, however, NIP was significantly lower on clay and silt than on sandy soils.

3.3. Effect of explanatory variables on the density of infected nymphs (DIN)

DIN ranged between 0 and 8.9 infected nymphs per $10\,m^2$, with a mean of 0.59 (standard deviation = 0.6 nymphs per $10\,m^2$). As for DON and NIP, the maximum DIN values were observed in eastern parts of the forest (Fig. A.10).

DIN was influenced by the abundance of roe deer and boar, the distance to chipmunk point of introduction, and forest stand cover (Table 1). A "medium" number of roe deer was significantly associated with the highest DIN, while the highest number of boar was associated with the lowest DIN. As for NIP, DIN increased with distance from the chipmunk point of introduction. Finally, forest stands with oak or "other deciduous" trees were associated with higher DIN values compared to coniferous stands.

3.4. Maps

GLM predictions of DON, NIP, and DIN were mapped (Fig. 1). Visually, the patterns of predicted values for all three response variables were very close to the observed data (Fig. A.8, S10, S12,S13). The highest values for DON, NIP, and DIN were in the southern and eastern parts of the forest, and in a few localized patches in the west. High NIP and DIN values were also seen in the northwest-ern area of the forest, and in a few localized patches. Residuals lay within the range of [−1.96; 1.96], which means that the modeling strategy is relevant and, spatially speaking, were evenly distributed throughout the study area (Fig. A.9, S11, S13).

No correlation was found between DON and NIP (*P*-value = 0.16, *R* = 0.07, *n* = 413) at the level of transect (Fig. A.14).

4. Discussion

Precise mapping of the risk of exposure to Lyme borreliosis in a highly visited forest represents an important tool for efforts to target prevention and control measures, and to raise awareness in the general public and among health decision makers. Our study is the first to investigate the risk of exposure to Lyme borreliosis across a forest area with such a high degree of spatial resolution. We focused our sampling efforts at the level of a landscape unit that corresponded to an entire forested area embedded in a densely urbanized region. Our study sampled over 19,000 nymphs during the period of maximal tick activity in the spring, of which 3,903 were tested for *B. burgdorferi* s. l. pathogens. Very few studies have reported sampling 10,000 or more questing nymphs (but see Tagliapietra et al., 2001; Bingsohn et al., 2013; James et al., 2013). These previous studies in Italy, Scotland, and Germany focused on a much larger spatial scale, but therefore had a lower local resolution. The detection of *B. burgdorferi* s. l. in over 3,000 questing nymphs has been achieved in a larger number of studies (e.g., Diuk-Wasser et al., 2010; Bingsohn et al., 2013; Coipan et al., 2013; Kalmar et al., 2013), but again, these have focused on many localized but separate geographical replicates within different countries. The present mapping of the risk of exposure to Lyme borreliosis (DIN) in the environment and the components of this risk (DON and NIP) revealed a strong heterogeneity of the risk within the forest. The highest risk was found in the eastern part of the forest in stands dominated by oaks or other deciduous trees, in which roe deer were found in medium abundance. Contrary to expectations, increased distance to the point of introduction of Siberian chipmunks was associated with higher, rather than lower, values of DIN. As found in other studies, there was no correlation between DON and NIP (Jouda et al., 2004; Ferquel et al., 2006; James et al., 2013).

Compared to elsewhere in Europe, the mean DON found in Sénart (5.6 nymphs per $10\,m^2$) is close to the upper boundary of previously reported values (e.g., means ranging from 2 to 5.5,

Jouda et al., 2004; Coipan et al., 2013; James et al., 2013). Local densities of more than 30 nymphs per 10 m^2 have been found in different studies, for example in the Alsace region, which indicates the high endemicity of Lyme borreliosis in France (Ferquel et al., 2006). The mean NIP found here (10%) is the range of NIP values found in Europe (mean of 10%, Rauter and Hartung, 2005), although mean NIPs higher than 15% have been reported, for instance, in Switzerland (Jouda et al., 2004) and in Alsace (Ferquel et al., 2006). Altogether, the mean DIN observed in this study (0.59 infected nymphs per 10 m^2) indicates that the Sénart forest poses a substantial risk of Lyme borreliosis to visitors.

4.1. Ungulate abundance index

The abundance index of roe deer was significantly correlated with DON, NIP, and DIN. As expected, higher numbers of these ungulates increased the density of *I. ricinus* nymphs. This result is in accordance with many studies that have found a positive association between questing nymph abundance and indices of roe deer abundance (e.g., Matuschka et al., 1993; Tälleklint and Jaenson, 1997; Jensen and Frandsen, 2000; Kurtenbach et al., 2002; Ruiz-Fons and Gilbert, 2010; James et al., 2013). Indeed, cervids act as the major reproductive hosts for *I. ricinus* adult ticks (Gray, 1998; Kiffner et al., 2010). However, high NIP and DIN values were highest for the "medium" abundance of roe deer. That medium roe deer abundances had a stronger effect on NIP and DIN than high roe deer abundances may be due to the fact that roe deer are non-competent hosts for *B. burgdorferi* s. l. pathogens (Bhide et al., 2005; Jaenson, and Tälleklint, 1992). Therefore, while a large deer population promotes the abundance of ticks, it also dilutes the overall infection rate of Lyme borreliosis agents by diverting vectors from feeding on reservoir hosts (Rosef et al., 2009). In contrast, some studies have found no correlation between *Borrelia* prevalence and deer abundance (Pichon et al., 1999; Jensen et al., 2000).

To date, the role of wild boars as a host for *I. ricinus* has received limited attention across Europe (Ruiz-Fons et al., 2006), although this host species is known to be a reservoir for another tick-borne bacterium, *Anaplasma phagocytophilum* (Michalik et al., 2012; Nahayo et al., 2014). Here, the high abundance index of wild boar in Sénart was associated with low DON and DIN. Where wild boars are most abundant in Sénart, roe deer are less abundant, in connection with the hunting management plans for these species (C. Briou ONF, pers. com.). The negative effect of wild boar abundance on DON and DIN therefore support the hypothesis that wild boars are poor hosts for *I. ricinus* in the completion of its reproductive cycle. Alternatively, the ground foraging behavior of wild boar could greatly disturb the development and/or activity of the immature tick stages.

4.2. Distance to chipmunk introduction

Contrary to our expectations, chipmunk presence index (measured as the inverse of the distance from the chipmunk point of introduction) had a negative effect on the NIP and DIN. This result was surprising because *T. sibiricus* is rare at the limits of its expanding range in Sénart Forest (Dozières, 2008), and we had therefore hypothesized that the low chipmunk presence would be related to lower DIN or NIP. In a previous study at La Faisanderie (an enclosure of 12 ha inside the Sénart forest), where chipmunks are in higher densities, this species was found to contribute more to the production of infected questing nymphs than the bank voles (Marsot et al., 2013). Chipmunks made larger contribution to Lyme borreliosis risk because they were more commonly infected with *Borrelia* pathogens and because they had higher tick infestations than native rodents such as *Myodes glareolus* and *Apodemus sylvaticus* (Pisanu et al., 2010; Marsot et al., 2011). Our present study shows that,

when considering the whole forest, other factors are more important in driving the risk of Lyme borreliosis. More specifically, at the study site of La Faisanderie where chipmunks were monitored, the abundance of roe deer was approximately 3 to 13 times higher than in the rest of the forest (J.-L. Chapuis, personal comments). Such a high deer density may have resulted in an overestimation of the ability of chipmunks to contribute to Lyme borreliosis risk. Alternatively, it is possible that *T. sibiricus* contributes more to Lyme borreliosis at the front of invasion range than at the center, as suggested by invasion studies (Llewellyn et al., 2012; White and Perkins, 2012; Millins et al., 2015). However, this hypothesis is not likely because the low density of *T. sibiricus* on the invasion front would not be sufficient to produce a high number of infected nymphs (Phillips et al., 2010). Finally, our findings could be driven by the *Borrelia* genospecies that infected ticks (Kurtenbach et al., 1998; Piesman and Gern, 2004). Indeed, if there were a high proportion of genospecies associated with birds that infected ticks, no relationship with chipmunks should be expected. Analyses that identify *Borrelia* genospecies would address this issue.

4.3. Ground vegetation

Our results regarding ground vegetation indicated that only heather was associated with lower nymph density. Vegetation plays an important role in generating a microclimate and providing the conditions for retaining humidity. Vegetation also influences the population density of small vertebrates that host ticks (Fernandez et al., 1994). In this study, the majority of the sampling units were located in vegetation that provides important moisture (sites with ferns, herbs, shrubs, or dead leaves) and good habitats for small vertebrates. In contrast, heather grows in acidic and rather dry environments. Our results could also be influenced by changes in the efficiency of drag sampling that are linked to the structure of the ground vegetation (Dobson et al., 2011). Recently, Bord et al. (2014) showed that sampling efficiency was higher in sites with dead leaves than in those with shrubs, thus affecting estimates of nymphal densities. Similarly, heather-dominated plant communities may reduce the efficacy of drag sampling. Instead, as seen in the map of forest cover (Fig. A.5), the majority of the forest is composed of deciduous hardwood. In accordance with other studies (e.g., Guerra et al., 2002; Bettridge et al., 2013; James et al., 2013), coniferous stands in Sénart were associated with lower DON, NIP, and DIN than those stands covered with oaks or other deciduous trees. The influence of forest tree composition on the response variables studied here likely derives from its effect on tick hosts (e.g., Fernandez et al., 1994), as old coniferous stands represent poor habitat for roe deer (Gill et al., 1996).

4.4. Soil characteristics

Soil characteristics influence tick abundance through their impact on soil moisture, which is mediated by the capacity of the soil to retain water and by the microclimate created by vegetation. Previous studies have found that tick abundance or presence was favored by superficial geological features associated with soil with high water capacity (Jensen et al., 2000). Our results confirm the findings that sandy soils are poor substrates for *I. ricinus* ticks (Medlock et al., 2008). However, our findings that clay and silt layers are linked with lower prevalence of *Borrelia* and lower values of DIN have not been documented before. Prior to this, studies that have analyzed the effects of soil type/characteristics or superficial geology on the prevalence of *Borrelia* have found no significant relationships (Jensen et al., 2000). However, it has been suggested that the presence of different fungi in the different soil types might affect the survival of ticks (Greengarten et al., 2011).

4.5. Distance from forest road

We found higher DON farther away from the forest road, which suggests that tick density is higher where disturbance is lower for their hosts. This could be particularly true for roe deer, which avoid forest trails and roads with high human and domestic animal activities, preferring to rest in undisturbed areas (Ward et al., 2004).

4.6. Saturation deficit

The saturation deficit had no effect on DON, NIP, and DIN, contrary to what we expected based on the existing literature (Perret et al., 2000; Tagliapietra et al., 2001; Herrmann and Gern, 2010; Morán Cadenas et al., 2007). This could be because (i) ticks have adapted to the range of SD described in our study (Gilbert et al., 2014), (ii) detrimental SD are mitigated by the local vegetation, which provided an appropriate microclimate that was not well measured here (SD was measured at the beginning of each transect), or (iii) the detrimental SD did not persist enough to cause an detectable effect on tick abundance.

The questing activity of *I. ricinus* is a temporal process with strong seasonal variation (Dobson et al., 2011; Dobson, 2013). Our analysis of tick populations represented a "snapshot" survey. This approach does not take into account temporal variation in the response variables. It is possible that the relationship between the response variables and time differ between locations. However, we think this variation has a minimal impact on our results for two reasons. First, we made sure that the observed spatial variation of tick abundance would not be confounded with a temporal variation that might occur within the sampling period. To do so, we sampled stands scattered throughout the forest every day. Second, our study was planned at the expected peak window of nymphal activity, which is consistent across habitats. Indeed, monthly sampling efforts conducted from 2005 to 2014 at La Faisanderie (Chapuis et al unpublished, Pisanu et al., 2010) demonstrated a maximum of questing nymphs in May or June and showed that tick abundance in favorable habitats remained higher than in less favorable habitats throughout time.

To further explore the factors that underlie the patterns of spatial variation in infection prevalence, an investigation of the genetic diversity of *Borrelia* could be conducted. High-throughput sequencing of *Borrelia* could yield information on the hosts that maintain transmission cycles (Jacquot et al., 2014). For example, such information could indicate whether genotypes that are associated with chipmunks (Jacquot et al., 2014) are more frequent in areas where *T. sibiricus* is more likely present. Estimates of genetic diversity could also be used to determine the relative contributions of different host species to the risk of Lyme borreliosis (Brisson et al., 2008). Furthermore, genetic approaches could simultaneously help the study of other tick-borne pathogens such as *A. phagocytophilum* and *Babesia* spp. as well as others that remain to be discovered (Vayssier-Taussat et al., 2013).

The ecological factors that explained the risk of transmission of *Borrelia* infected nymhs are likely to occur in other forests in Europe with the same vegetation type and climate. The strategy we used in the present study could be applied to other forests to provide a precise description of the spatial variation of the risk of Lyme borreliosis transmission. To rely on one-shot sampling, good knowledge on the period of the peak of tick activity is necessary. Otherwise, sampling should be repeated 2 or 3 times during the tick activity period. To indicate the highest risk of Lyme borreliosis within a forest, one should combine the risk of exposure in a given area, measured here, with the public popularity of that area. The high-risk areas would be those where the highest risk of exposure is coupled with a high frequency of use. A preliminary study of Sénart forest use, performed on 187 visits, examined only the western part of the forest (Méha et al., 2012). However, the eastern part probably receives more visitors, as it is closer to towns with large populations. Thus, further studies would be important to determine the spatial use of the forest.

5. Conclusion

Altogether, we found a high risk of *Borrelia* transmission in the eastern part of the Sénart forest as well as in localized patches in the northwestern part. The risk was mainly linked to the presence of deciduous trees and to medium-to-high abundances of roe deer. Given the high average risk in the forest and the existence of risk hotspots, information should be provided to the public at the different parking areas and at the highest-risk locations, such as the upper northwestern and the eastern parts of the forest. In addition, information targeted towards medical doctors could be distributed in the area. Our map of the density of questing nymphs shows the underlying risk of transmission of a wide diversity of tick-borne pathogens. This map could further be used as a baseline for future studies of the impact of forest or roe deer management practices on the risk of exposure to *Borrelia*-infected *I. ricinus* nymphs (e.g. Stafford et al., 2003).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:10.1016/j.ttbdis.2016.02.008.

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