Ixodes ricinus density, Borrelia prevalence and the density of infected nymphs along an urban–rural gradient in southern England

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Abstract
Ticks are found across a range of habitats, with woodland being particularly important for high densities and prevalence of Borrelia infection. Assessments of risk in urban woodland can be difficult if there are low densities and small sample sizes for Borrelia prevalence estimates. This study targeted six urban woodlands with established tick populations, as well as six woodlands in peri-urban zones and six woodlands in rural zones in and around the cities of Bath and Southampton, in the South of England. Nymph densities were estimated, and 100 nymphs were tested from each of the 18 woodlands studied. Ixodes ricinus ticks were found in all woodlands surveyed, and overall density of nymphs (DON) per 100 m² was 18.17 in urban woodlands, 26.0 in peri-urban woodlands and 17.67 in rural woodlands. Out of 600 nymphs tested across urban woodlands, 10.3% were infected with Borrelia. The same proportion of nymphs collected in rural woodlands were positive for Borrelia. In peri-urban woodlands, 10.8% of nymphs tested positive. Across both cities combined, density of infected nymphs (DIN) was 2.73 per 100 m² in peri-urban woodland, 1.87 per 100 m² in urban woodland and 1.82 per 100 m² in rural woodland. Overall, DON, Borrelia prevalence and DIN did not differ significantly along an urban–rural gradient. This suggests the risk of Lyme borreliosis transmission could be similar, or perhaps even elevated in urban woodland if there is higher public footfall, subsequent contact with ticks and less awareness of the risks. This is particularly important from a public health perspective, as Borrelia garinii dominated across the gradient and this genospecies is linked to neuroborreliosis.

KEYWORDS
connectivity, Ixodes ricinus, Lyme borreliosis, public health, ticks
Landscape structure and connectivity have been shown to impact *I. ricinus* density and *Borrelia* prevalence, including in urban green space (Heylen, Lasters, et al., 2019; Millins et al., 2018). A wide range of habitats are found across urban, peri-urban and rural areas and each can be made up of varying patches, corridors or barriers which could support ticks and their hosts or indeed hinder their access or survival (Estrada- Peña, 2002). Urbanization gradients have long been used to assess the potential impacts of anthropogenic change on the ecology and distribution of plant and animal species. Often, the highest intensity of urbanization effects will be found in city centres (McDonnell & Hahs, 2008). As the level of urbanization reduces, so do effects such as noise, pollution, artificial light and human disturbances. Urban habitats often have smaller green space patches that are less connected compared to surrounding peri-urban or rural habitat. Although some tick host species may thrive or adapt to urban green spaces, others require larger, better connected green space with less disruption to that found in urban areas. Such fragmentation can alter host communities, vegetation and microclimate (Kilpatrick et al., 2017), which as a result, can impact tick populations.

*Ixodes ricinus* is a generalist species which is found feeding on a wide range of wildlife hosts (Herrmann & Gern, 2015). This provides increased opportunities for involvement with pathogen transmission cycles (Jongejan & Uilenberg, 2004), which is particularly important as *I. ricinus* is also one of the most common tick species found biting humans in Europe (Estrada- Peña & Jongejan, 1999). One important pathogen is *Borrelia burgdorferi* sensu lato (s.l.), the causative agent of Lyme borreliosis. *Borrelia burgdorferi* s.l. is transmitted between *I. ricinus* and several small mammal and bird hosts (Mannelli et al., 2012), often within woodland habitat which provides suitable conditions for tick and wildlife host survival (Gray, 2002). Although deer species are not involved in the transmission of *B. burgdorferi* s.l., they are important for feeding all tick life stages, including adult ticks which are generally found on larger hosts (Gray et al., 2021).

The presence and/or density of key tick hosts in suitable tick habitat along an urban–rural gradient will have an impact on the density of ticks, the prevalence of *Borrelia* and thus the density of infected ticks. For *I. ricinus* survival in any habitat patch, suitable microclimate conditions are required. Some urban woodland patches provide this, but ticks also need access to a suitable wildlife host for a blood meal (Jeremy S Gray, 2002). Urban tick hosts include deer, particularly roe deer (*Capreolus capreolus*), that are known to frequent urban areas in the UK (The British Deer Society, 2021; The Deer Initiative, 2011), likely introducing, and feeding ticks (Gilbert et al., 2012; Gray et al., 1992, 2021; Gray & Ogden, 2021). Additionally, several bird species are also likely to be important in feeding and perpetuating *Borrelia* transmission cycles in urban areas (Dubska et al., 2009; Heylen, Schmidt, et al., 2019; Kurtenbach et al., 1998).

As well as being linked to Lyme borreliosis transmission during the earliest days of cases being reported (Wood & Lafferty, 2013), woodland habitat is often extensively associated with enzootic hazard (higher densities of infected nymphs; Diuk-Wasser et al., 2021).

This is likely due to woodland habitat supporting a wide range of wildlife hosts that serve as reservoirs for *Borrelia* transmission cycles. Drastic decline in woodland coverage in the UK has resulted in many woodland patches becoming small and fragmented. Reduction in patch area and increased isolation can have a negative impact on biodiversity and can reduce movement of wildlife between patches (Watts et al., 2005). Although reduced biodiversity is not always observed in fragmented woodland patches (Diuk-Wasser et al., 2021), it has, in some cases, been suggested to increase the risk of *Borrelia* transmission (dilution effect; Ehrmann et al., 2018; Kilpatrick et al., 2017). If urban woodland patches have lower biodiversity, they could also harbour ticks with a higher *Borrelia* prevalence. In addition, some bird species that are important Lyme borreliosis reservoirs have adapted to thrive, particularly in peri-urban patches where densities can be higher than surrounding rural habitat (Evans et al., 2009). Deer have also been shown to be more likely to move between patches with greater fragmentation, particularly for resources or in response to threats (Eycott & Watts, 2011; Lovari et al., 2017; Morellet et al., 2013), and have been frequently reported in peri/urban areas. This suggests that urban woodland could indeed have similar *Borrelia* prevalence, or perhaps higher prevalence, compared with surrounding rural woodland.

The aims of this study were to investigate the density of nymphs (DON), *Borrelia* prevalence and the density of infected nymphs (DIN) in urban woodland and surrounding peri-urban and rural woodland habitat. Using known urban woodlands with established tick populations, nymphs were collected across an urban–rural gradient in and around two cities to obtain enough nymphs to estimate and compare *Borrelia* prevalence, DON and DIN. *I. ricinus* density in rural woodland was expected to be higher compared to urban woodlands, as better connectivity and access for wildlife should support higher tick numbers (Diuk-Wasser et al., 2021; Mathews-Martin et al., 2020). Based on data on *Borrelia* prevalence in an urban study in Bath (Hansford, Wheeler, Tschirren, & Medlock, 2022) and from a countrywide study of *Borrelia* prevalence across England and Wales (Cull et al., 2021), prevalence was expected to be similar across the gradient or perhaps elevated in urban woodland, and bird-related genospecies were expected to dominate.

### Impacts

- Ticks infected with *Borrelia* that can cause Lyme borreliosis are found in deciduous woodlands in urban, peri-urban and rural areas in and around cities.
- Nymph density, *Borrelia* prevalence and the density of infected nymphs did not differ in the studied cities along an urban–rural gradient, suggesting risk of Lyme borreliosis could be similar across these zones.
- Additional research is needed to further investigate risk by incorporating datasets on tick bites and Lyme borreliosis transmission.
2 | MATERIALS AND METHODS

2.1 | Site selection

Bath and Southampton in southern England were selected for this study because both have evidence of tick activity and associated cases of Lyme borreliosis (UK Health Security Agency, 2017a, 2017b). To ensure enough nymphs could be collected from urban woodlands for Borrelia testing, three urban green space woodland patches with known tick populations were selected from each city. For comparison, three deciduous, publicly accessible woodlands in the surrounding peri-urban habitat and another three in the wider rural habitat around each city were randomly selected. Peri-urban and rural deciduous woodland was identified using Land Cover Map (Rowland et al., 2017). Woodlands were classed as peri-urban if they were within 1 km of the Office for National Statistics (2015) Major Towns and Cities boundary and rural if they were at least 1 km away from the peri-urban zone but within 5 km of the peri-urban boundary (Figures 1 and 2).

2.2 | Data collection

Locations were accessed via public footpaths, as identified using a 1:25,000 Ordnance Survey map. Sites were only visited on dry days during May 2018, within a two-week sampling period (16th–26th). Based on existing Borrelia prevalence studies in England generated by testing questing I. ricinus nymphs (and available during the planning of this study), prevalence was expected to be within the region of 5.1% (3.3% Bettridge et al., 2013; 3.9–18.1% Hansford et al., 2015, 2017; 3.4% Nelson et al., 2015). A sample size of at least 75 nymphs was required to accurately estimate prevalence with a level of 95% confidence with a 5% margin of error (Daniels, 1999). Samples of 100 nymphs were obtained from each of the 18 woodlands surveyed. Upon arrival, a series of 10 x 10 m transects, spaced by at least 10 m, were completed by flagging a 1 x 1 m piece of poly-cotton cloth over the vegetation. Flagging was continued until a sample of 100 nymphs had been obtained. Any captured ticks were placed into 1.5 ml Eppendorf tubes and placed into a −80°C freezer until identification, DNA extraction and Borrelia testing could take place. All ticks were identified to species level using morphological keys (Estrada-Peña et al., 2017; Hillyard, 1996). Nymphs were tested individually to estimate Borrelia prevalence. DNA was extracted using ammonium hydroxide (NH₄OH). DNA extracts were tested for the presence of Borrelia DNA using a pan-Borrelia qPCR targeting the 16S rRNA gene. Samples were prepared using a QIAgility system (QIAGEN) with 10 μl of ABI TaqMan Fast Universal master mix (Applied Biosystems), 1 μl of primer/probe mix (16S forward 5′-AGC CTT TAA AGC TTC GCT TGT AG-3′, 16S reverse 5′-GCC TCC FIGURE 1 Sampling locations in and around the city of Bath. Polygons in the city centre in dark grey are urban, those in lilac at the urban boundary are peri-urban and those outside the hatched area are rural woodlands. Sites randomly selected for surveying are numbered.
CGT AGG AGT CTG G-3', probe 5'-6FAM- CCG GCC TGA GAG GGT GAACGG- BHQ1 3'); 0.18 μl forward primer, 0.18 μl reverse primer, 0.05 μl probe and 0.59 μl H₂O per sample; 4 μl PCR grade H₂O and 5 μl of sample DNA. A QuantoStudio 7 Flex real-time PCR system (Applied Biosystems) was used with a hold step for 20 s at 95°C, followed by 40 cycles of 3 s at 95°C and 20 s and 60°C (Cull et al., 2021).

Positive samples were sequenced to determine genospecies using the 5S-23S rRNA intergenic spacer region. A master mix was prepared (5 μl 10× PCR reaction buffer [MgCl₂], 1 μl 10 mM dNTPs, 1.5 μl 50 mM MgCl₂, 2 μl each primer (5S-23S forward 5′-GAG TTC GCG GGA GAG TAG GTT ATT GCC-3′, 5S-23S reverse 5′-TCA GGG TAC TTA GAT GGT TCA CTT CC-3′), 0.2 μl Platinum Taq DNA polymerase (Invitrogen) and 33.3 μl PCR grade H₂O = 50 μl) and added to 0.2 ml PCR tubes. A thermocycler was used with the following conditions: 5 min at 94°C, followed by 10 cycles of 94°C for 20 s, 70°C for 30 s (lowering by 1°C each cycle) and 72°C for 30 s, then 40 cycles of 94°C for 20 s, 60°C for 30 s and 72°C for 30 s, with a final extension of 72°C for 7 min (Cull et al, 2021). Prepared samples were sent to the UK Health Security Agency Genomic Service and Development Unit (Colindale, London) for Sanger sequencing.

2.3 | Statistical analysis

All statistical analyses were carried out in R version 4.1.1 (R Core Development Team, 2019). The effects of city and zone on DON, nymph infection prevalence (NIP) and DIN were investigated using generalized linear mixed models (GLMM). Significance of fixed effects was determined by comparing two nested models, with and without the factor of interest, using likelihood ratio tests (lmtest; Zeileis & Hothorn, 2002). Violation of model assumptions (overdispersion, zero-inflation) were validated using the DHARMa package (Hartig, 2019). Statistically significant terms (p < 0.05) are highlighted. 95% confidence intervals (CI) are presented and were calculated using the DescTools package (Signorell, 2021).

2.4 | Questing nymph density (DON)

A GLMM with a negative binomial error (used due to overdispersion) and log link function was used to investigate DON. The number of nymphs collected per 10 m² was used as the response variable and city and zone were included as fixed effects. Survey site was included as a random effect, to account for the non-independence of observations from the same site (Harrison, 2014). Only nymphs were...
### TABLE 1
Ticks collected during 2018 in and around the cities of Bath and Southampton, showing the area (m²) sampled in each zone, the total collected, mean density per 100 m² and 95% confidence intervals of each life stage. ♂ = male, ♀ = female, N = nymph.

<table>
<thead>
<tr>
<th>City</th>
<th>Zone</th>
<th>Total area (m²)</th>
<th>♂: Total; mean per 100 m² (95% CI)</th>
<th>♀: Total; mean per 100 m² (95% CI)</th>
<th>N: Total; mean per 100 m² (95% CI)</th>
<th>Total ticks (incl. Larvae): Total; mean per 100 m² excl. Larvae (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bath</td>
<td>Urban</td>
<td>300</td>
<td>0; 0 (0)</td>
<td>1; 0.33 (0–1.02)</td>
<td>47; 15.67 (8.97–22.37)</td>
<td>48; 16.0 (9.03–22.98)</td>
</tr>
<tr>
<td></td>
<td>Peri-urban</td>
<td>300</td>
<td>3; 1 (0–2.14)</td>
<td>1; 0.33 (0–1.02)</td>
<td>42; 14.0 (5.03–22.97)</td>
<td>46; 15.33 (5.79–24.88)</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>300</td>
<td>0; 0 (0)</td>
<td>1; 0.33 (0–1.02)</td>
<td>38; 12.67 (5.81–19.53)</td>
<td>48; 13.0 (6.98–25.02)</td>
</tr>
<tr>
<td>Southampton</td>
<td>Urban</td>
<td>300</td>
<td>2; 0.67 (0–1.61)</td>
<td>2; 0.67 (0–2.03)</td>
<td>62; 20.67 (13.40–27.93)</td>
<td>66; 22.0 (14.25–29.75)</td>
</tr>
<tr>
<td></td>
<td>Peri-urban</td>
<td>300</td>
<td>11; 3.67 (0–9.17)</td>
<td>16; 5.33 (0.36–10.31)</td>
<td>114; 38.0 (21.96–54.04)</td>
<td>141; 47.0 (27.58–66.42)</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>300</td>
<td>1; 0.33 (0–1.02)</td>
<td>1; 0.33 (0–1.02)</td>
<td>68; 22.67 (12.86–32.47)</td>
<td>70; 23.33 (13.32–33.35)</td>
</tr>
<tr>
<td>Study total</td>
<td></td>
<td>1800</td>
<td>17; 0.94 (0.02–1.87)</td>
<td>22; 1.22 (0.33–2.11)</td>
<td>371; 20.61 (16.60–24.63)</td>
<td>419; 22.78 (18.62–27.94)</td>
</tr>
</tbody>
</table>

Note: Note that the 48 total ticks for rural woodland in Bath includes 9 larvae, and the total overall also includes 9 larvae.

### TABLE 2
Ticks tested for Borrelia from the cities of Bath and Southampton, grouped overall by zone, with the total nymphs tested, total positive and 95% confidence intervals (CI).

<table>
<thead>
<tr>
<th>City</th>
<th>Zone</th>
<th>Borrelia positive N/tested; % (95% CI)</th>
<th>Number of nymphs infected with different genospecies, % and 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ba</td>
<td>Bg</td>
</tr>
<tr>
<td>Bath</td>
<td>Urban</td>
<td>27/300, 9.0% (6.0–12.8)</td>
<td>1; 3.7% (0.1–18.9)</td>
</tr>
<tr>
<td></td>
<td>Peri-urban</td>
<td>28/300, 9.3% (6.3–13.2)</td>
<td>0; 0 (0)</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>37/300, 12.3% (8.8–16.6)</td>
<td>2; 5.4% (0.7–18.2)</td>
</tr>
<tr>
<td>Southampton</td>
<td>Urban</td>
<td>35/300, 11.7% (8.3–15.9)</td>
<td>2; 5.7% (0.7–19.2)</td>
</tr>
<tr>
<td></td>
<td>Peri-urban</td>
<td>37/300, 12.3% (8.8–16.6)</td>
<td>7; 18.9% (8.0–35.2)</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>25/300, 8.3% (5.5–12.1)</td>
<td>2; 8.0% (1.0–26.0)</td>
</tr>
<tr>
<td>Study total</td>
<td></td>
<td>189/1800, 10.5% (9.1–12.0)</td>
<td>14; 7.4% (4.1–12.1)</td>
</tr>
</tbody>
</table>

Abbreviations: Ba, *Borrelia afzelii*; Bg, *Borrelia garinii*; Bv, *Borrelia valaisiana*; Ut, untyped.
used for density analysis, as they have the most significant impact on human health (Kilpatrick et al., 2017) and low numbers of adults were collected.

2.5 | Nymph infection prevalence (NIP) and the density of infected nymphs (DIN)

A GLMM with a binomial error and logit link function was used to investigate Borrelia infection in individual nymphs in relation to city and zone. Survey site was included as a random effect. A GLMM with a Poisson error and log link function was used to investigate the influence of city and zone on DIN (one estimated DIN per woodland per zone).

3 | RESULTS

3.1 | Summary

For the transects completed to estimate DON, 419 I. ricinus ticks were collected from 180×10 m² transects across urban, peri-urban and rural woodlands in and around the cities of Bath and Southampton. This was made up of 17 (4.2%) males, 22 (5.2%) females, 371 (88.5%) nymphs and 9 (2.1%) larvae (Table 1). Testing the nymphs already collected, as well as the additional nymphs collected to improve samples sizes, Borrelia-infected nymphs were detected in all woodlands surveyed, with 10.5% (n = 189; 95% CI 9.1–12.0) of 1800 testing positive overall (Table 2). Across both cities combined, DIN was 2.73 per 100 m² (95% CI 2.21–3.54) in peri-urban woodland, 1.87 per 100 m² (95% CI 1.45–2.38) in urban woodland and 1.82 per 100 m² (95% CI 1.41–2.31) in rural woodland.

3.2 | Density of nymphs

City (χ² (1) = 2.62, p = 0.105) and zone (χ² (2) = 0.65, p = 0.724) were not significant predictors of DON in the model. Overall, DON per 100 m² was 18.17 (95% CI 13.33–23.01) in urban woodlands, 26.0 (95% CI 16.56–35.44) in peri-urban woodlands and 17.67 (95% CI 11.72–23.61) in rural woodlands (Figure 3). Overall, DON along the urban–rural gradient in woodland in and around Southampton was 27.11 (95% CI 20.46–33.77) per 100 m². In Bath, overall, DON along the gradient was 14.11 (95% CI 9.90–18.32) per 100 m².

3.3 | Borrelia prevalence and genospecies

City (χ² (1) = 0.10, p = 0.756) and zone (χ² (2) = 0.09, p = 0.957) were not significant predictors of Borrelia prevalence. Borrelia prevalence in woodland in and around the city of Bath was 10.2% (n = 92/900; 95% CI 8.3–12.4). In Southampton, 10.8% (n = 95/900; 95% CI 8.8–13.0) of nymphs collected from all woodland zones were positive. The proportion of nymphs found infected from each zone was also similar, with a mean prevalence of 10.3% in both urban and rural woodland (n = 62/600 each, 95% CI 8.0–13.1) and a mean prevalence of 10.8% in peri-urban woodlands (n = 65/600, 95% CI 8.5–13.6). The mean prevalence at the site level ranged from 2% to 16% for all woodlands sampled, 2%–16% in urban, 7%–14% in peri-urban and 3%–15% in rural areas (Appendix S1).

Across both cities, Borrelia garinii accounted for 41.8% (n = 79/189) of the positive samples. Borrelia valaisiana made up 18.5% (n = 35/189) of positive samples. Borrelia afzelii was found in just 7.4% (n = 14/189) of positive samples. High-quality sequence data could not be obtained for the remaining samples (n = 61) and these were reported as untyped. All three genospecies were found in urban, peri-urban and rural woodland (Table 2; Figure 4). Borrelia garinii dominated in all zones in both cities and overall proportions were similar for each zone. The proportion of B. afzelii was slightly elevated in peri-urban woodland in Southampton (Figure 4).

3.4 | Density of infected nymphs

City (χ² (1) = 0.09, p = 0.755) and zone (χ² (2) = 0.08, p = 0.960) were not significant predictors of DIN. Mean DIN in woodlands in and around the city of Southampton was 2.93 per 100 m² (95% CI 2.39–3.52) and 1.44 per 100 m² (95% CI 1.17–1.75) in Bath. Mean DIN in urban woodland was 1.41 per 100 m² (95% CI 0.94–2.01) in Bath and 2.12 per 100 m² (95% CI 1.72–3.29) in Southampton. In peri-urban woodland, mean DIN was 1.30 per 100 m² (95% CI 0.88–1.85) in Bath and 4.67 per 100 m² (95% CI 3.34–6.31) in Southampton.
FIGURE 4 Genospecies proportion in urban, peri-urban and rural zones in and around the cities of Bath and Southampton, in nymphs collected in 2018.

(Figure 4). DIN in rural woodland around Bath was 1.6 per 100 m$^2$ (95% CI 1.11–2.10). In Southampton, it was 1.88 per 100 m$^2$ (95% CI 1.25–2.74).

4 | DISCUSSION

4.1 | Summary of findings

This study is the first in England to assess Lyme borreliosis risk indicators in highly suitable woodland habitat across an urban–rural gradient. On average, over 10% of nymphs were found infected with *Borrelia* in each of the three zones studied, with approximately two infected nymphs in every 100 m$^2$. Lyme borreliosis risk indicators such as DON, NIP and DIN did not differ significantly between cities or along an urban–rural gradient. This could suggest that the composition of wildlife important for introducing, feeding, and infecting ticks could be similar across the gradient of both cities. This is supported by the dominance of *B. garinii* across the study landscapes, which highlights the key role avian hosts are likely playing along an urban–rural gradient in the UK (Hansford, Wheeler, Tschirren, & Medlock, 2022; Medlock et al., 2022). Other studies have found differences in Lyme borreliosis risk indicators between urban and rural areas, including a European-wide study of land cover classes which found lowest nymph densities in urban areas (Rosà et al., 2018). Nymph density was lower in an urban deer park in London compared to rural Dartmoor in Devon, England (Dobson et al., 2011), in and around the city of Lyon where the highest densities were found in rural woodland, and also in peri-urban woodland compared to urban parks where ticks were rarely found (Mathews-Martin et al., 2020). The lack of significant difference in urban, peri-urban and rural habitat in Bath and Southampton, however, is likely due to the inclusion of high-density woodland habitat from urban areas, and not from other habitat types which have been shown to be significantly less suitable for ticks (Hansford, Wheeler, Tschirren, & Medlock, 2022). This suggests that, for some cities, woodland habitat is of primary importance, regardless of where it is situated along an urban–rural gradient.

4.2 | *Borrelia* prevalence and genospecies

Overall *Borrelia* prevalence in nymphs (10.5%) was higher than several other studies in England, which reported between 3.1% and 7.9% prevalence (Cull et al., 2021; Hansford et al., 2015, 2021; Hansford, Wheeler, Tschirren, & Medlock, 2022; Layzell et al., 2018). It was similar, however, to prevalence reported in nymphs in Exmoor (9.9%; Hansford et al., 2015) but lower than reported in peri-urban habitat in Salisbury (18.1%; Hansford et al., 2017). All three zones had woodlands with high prevalence, reaching 16% in urban, 14% in peri-urban and 15% in rural woodland. The lack of significant difference in prevalence across an urban–rural gradient has been reported elsewhere in Europe. *Borrelia* prevalence was similar in urban (27.0%), peri-urban (22.9%) and rural (22.9%) areas in Prague, Stupno and Králický Sněžník in the Czech Republic (Kybicová et al., 2017). Similarly in Poland, significant differences in *Borrelia* prevalence were not detected across a gradient in Lower Silesia (11.9% minimum infection rate in urban, 11.1% in peri-urban and 11.6% in rural; Kiewra et al., 2014) or between a rural area in Białowieża and an urban area in Warsaw (3.0% minimum infection rate and 3.2% respectively; Kowalec et al., 2017). In the Netherlands, *Borrelia* prevalence was detected...
at similar levels in urban (6.8%) and rural forest (8.1%) from different regions (Wieilinga et al., 2006).

Others, however, have reported differences in *Borrelia* prevalence between urban, peri-urban and rural areas. In Turku, Finland, variation in prevalence has been reported between rural (4.0%), peri-urban (0%) and urban areas (0%; Mäkinen et al., 2003). In and around the same city, significantly higher prevalence was reported in ticks collected in rural habitat compared to urban areas (Klemola et al., 2019). In contrast, higher *Borrelia* prevalence was detected in ticks in suburban woodland near Warsaw, Poland (23.5%) compared with urban green space (4.4%–4.8%) and in a rural area (3.4%; Strykiewicz et al., 2012). Elsewhere, significantly fewer infected ticks were found in rural areas in Bavaria, Germany (13.6%), compared with urban locations (24.5%; Răileanu et al., 2021), and prevalence was positively associated with the level of urbanization in a study in Luxembourg (Reye et al., 2010).

As reported in previous studies in England, *B. garinii* continues to be the most commonly detected genospecies (Cull et al., 2021; Hansford et al., 2015; Layzell et al., 2018). An overwhelming majority of studies incorporating urban and rural study locations elsewhere in Europe report a dominance of *B. afzelii* (Borşan et al., 2021; Hansford, Wheeler, Tschirren, & Medlock, 2022; Kiewra et al., 2014; Mäkinen et al., 2003; Răileanu et al., 2021; Wieilinga et al., 2006). To better understand what is driving *Borrelia* genospecies dominance, studies incorporating key tick and *Borrelia* host data are needed.

### 4.3 Density of infected nymphs

DIN was higher in Southampton compared to Bath, and in peri-urban woodland compared with urban or rural woodland but differences were not significant. A particularly high DIN was reported in a peri-urban woodland in Southampton, where on average, 10.7 nymphs were found infected per 100 m². This is comparable to some rural woodland sites and actually higher than many locations sampled as part of a recently published study in England (Medlock et al., 2022). Lack of difference in DIN across an urban–rural gradient suggests that risk along this gradient could be similar. This is in contrast to a study of land cover classes across Europe which reported finding a significantly higher DIN in natural habitat (Rosà et al., 2018) and a study in Antwerp which showed DIN decreased with increased urbanization (Heylen, Lasters, et al., 2019). Lack of significant difference in DIN along an urban–rural gradient in the cities of Bath and Southampton are likely due to sampling only highly suitable urban woodland habitats. Indeed, if less suitable urban green spaces had been included, a reduction would likely have been observed in urban areas (Diuk-Wasser et al., 2021), however, the aim of this study was to compare the highest risk habitats.

This study provides evidence that in these study cities, some urban woodlands have comparable DON, *Borrelia* prevalence and DIN with surrounding peri-urban and rural woodland habitat. Although no significant differences were found between zones, the increased importance of urban green space for recreation may mean the risk of human-tick exposure is higher. Risk may be further exacerbated if tick awareness is lower in urban areas, and tick encounters are unexpected (Bayles et al., 2013). It also suggests that tick wildlife host composition may be similar between the zones sampled, and that bird species or perhaps squirrels are driving *Borrelia* prevalence due to the dominance of *B. garinii*. This genospecies can present a significant public health risk, being linked to neuroborreliosis in humans (Russell et al., 2018; Stanek et al., 2012).

There have likely been shifts in tick distribution into urban areas across Europe in more recent years. Some studies repeating sampling in the same locations have only recently found ticks (Klemola et al., 2019) in areas where previous surveys did not detect activity (Mäkinen et al., 2003). Some studies also suggest a potentially increasing prevalence of *Borrelia* in recent years compared with previous surveys, in both urban and rural areas. Additionally, a shift in genospecies dominance has also been reported (Kybícová et al., 2017). This is the first study in England to investigate Lyme borreliosis risk indicators along an urban–rural gradient. It provides a baseline of data upon which to build a longer-term dataset to begin to assess potential changes in DON, NIP or DIN over time, as well as a sampling strategy that could be applied to other cities.

Whilst this study provides further evidence of risk of Lyme borreliosis transmission in urban woodland and suggests similar risk to surrounding peri-urban and rural woodland, further studies are now needed to assess how this translates to tick bites and subsequent transmission to humans across this gradient (Kilpatrick et al., 2017). Further studies that incorporate landscape connectivity metrics along urban–rural gradients could help to investigate the effects of landscape structure and connectivity, as shown previously in several studies (Estrada-Peña, 2002; Heylen, Lasters, et al., 2019; Heylen, Schmidt, et al., 2019). Further studies assessing host communities (deer, small mammals and birds) along an urban–rural gradient and the impact on various risk indicators are needed to better understand *Borrelia* transmission dynamics (Borşan et al., 2020; Borşan et al., 2021). Such studies should incorporate the impacts of habitat fragmentation on tick hosts and *Borrelia* transmission cycles (Diuk-Wasser et al., 2021), which is not only important for understanding current risk but also future risk as the landscape and host communities continue to change as part of woodland restoration and rewilding activities. Finally, studies could incorporate sampling over several years to capture temporal fluctuations in nymph density, *Borrelia* prevalence or DIN along gradients (Medlock et al., 2022) along with other important tick-borne pathogens such as *Anaplasma phagocytophilum* (Overzier et al., 2012), *Babesia* spp. (Welc-Falcič et al., 2014) or *Rickettsia* spp. (Szekeres et al., 2015) to gain a better understanding of risk.

### 5 CONCLUSIONS

This study provides the first assessment of nymph density, *Borrelia* prevalence and the density of infected nymphs along an urban–rural gradient in England. The lack of significant differences for any of
these Lyme borreliosis risk indicators suggests that tick and host reservoirs may be introducing, feeding, and infecting ticks across this gradient. The results suggest that risk of exposure to infected ticks could be the same, no matter where the woodland is located. In fact, risk could be elevated in urban green space, if visitor numbers are higher and levels of awareness are lower (Bayles et al., 2013). Finally, this study provides further evidence that B. garinii is the most common genospecies circulating across the landscapes in the study locations, and this has public health implications (Dryden, Saeed, Ogborn & Swales, 2014; Lovett, Evans, O’connell & Gutowski, 2008; Stanek et al., 2012).

ACKNOWLEDGEMENTS

We thank both reviewers for their useful comments on the manuscript. JMM and KMH were partly funded by the National Institute for Health Research Health Protection Research Unit (NIHR HPRU) in Environmental Change and Health at the London School of Hygiene & Tropical Medicine in partnership with UK Health Security Agency (formerly Public Health England), and in collaboration with the University of Exeter, University College London, and the Met Office; and JMM was partly funded by the NIHR HPRU in Emerging Infections and Zoonoses at the University of Liverpool in partnership with UKHSA and Liverpool School of Tropical Medicine. The views expressed are those of the authors and not necessarily those of the National Health Service, the NIHR, the Department of Health or UKHSA.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supplementary file.

ETHICS STATEMENT

No ethical approval was required as this study does not involve any animal subjects.

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UK Health Security Agency. (2017a). Tick surveillance scheme data. Available upon request from tick@uksha.gov.uk. Available at: https://www.gov.uk/guidance/tick-surveillance-scheme


**SUPPORTING INFORMATION**

Additional supporting information can be found online in the Supporting Information section at the end of this article.