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Blood meal analysis reveals sources of tick-borne pathogens and differences in host utilization of juvenile *Ixodes ricinus* across urban and sylvatic habitats

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Abstract

Aims: Urban green spaces are locations of maximal human activity, forming areas of enhanced risk for tick-borne disease (TBD) transmission. Being also limited in spatial scale, green spaces form prime targets for control schemes aiming to reduce TBD risk. However, for effective control, the key species maintaining local tick and tick-borne pathogen (TBP) populations must be identified. To determine how patterns of host utilization vary spatially, we utilized blood meal analysis to study the contributions of voles, shrews, squirrels, leporids and cervids towards blood meals and the acquisition of TBPs of juvenile *Ixodes ricinus* in urban and sylvatic areas in Finland.

Methods and Results: A total of 1084 nymphs were collected from the capital city of Finland, Helsinki and from a sylvatic island in southwestern Finland, and subjected to qPCR analysis to identify DNA remnants of the previous host. We found significant differences in host contributions between urban and sylvatic environments. Specifically, squirrels and leporids were more common hosts in urban habitats, whereas cervids and voles were more common in sylvatic habitats. In addition to providing 18.4% of larval blood meals in urban habitats, red squirrels were identified as the source of 28.6% ($n=48$) of *Borrelia afzelii* detections and 58.1% ($n=18$) of *Borrelia burgdorferi* sensu stricto detections, indicating an important role for local enzootic cycles.

Conclusions: Our study highlights that the key hosts maintaining tick and TBP populations may be different in urban and sylvatic habitats. Likewise, hosts generally perceived as important for upkeep may have limited importance in urban environments. Consequently, targeting control schemes based on off-site data of host importance may lead to suboptimal results.

KEYWORDS
blood meal, *Borrelia*, host, *Ixodes ricinus*, tick-borne pathogens, urban green space

1 | INTRODUCTION

Habitat fragmentation and urbanization are driving changes in ecological communities, pressuring more and more species to adapt to urban environments. As a result, species like squirrels, foxes and hares have inhabited urban areas with increasing populations in many countries (Jokimäki et al., 2017; Plumer et al., 2014). Urbanization of forest animals and their close interaction with humans in urban environments also raise the risk of zoonotic diseases in these areas, especially in areas where vectors of pathogens like ticks can be found (Tazerji et al., 2022). Currently, ticks have been found to inhabit urban green spaces and parks across Europe, raising the risk of tick-borne pathogen (TBP) infections in humans and pets (Hansford et al., 2022; Mancini et al., 2014; Tazerji et al., 2022). As the main reservoir and transmission route for TBPs, urbanized host animals such as small mammals and birds play an important role in maintaining the life cycles of ticks and TBPs in urban areas, as well as transferring ticks and TBPs across different habitats (Hansford et al., 2017; Olsén et al., 1995).

Ixodes ricinus (Acari: *Ixodidae*) is one of the most multipotent and common vectors of pathogens in Europe (Rizzoli et al., 2014), and in previous studies, *I. ricinus* has already been found to be present in urban and peri-urban areas in many countries and cities (Grochowska et al., 2020; Hansford et al., 2017; Mancini et al., 2014). They require three separate blood meals during their lifecycle and can use hundreds of different species as hosts (Kahl & Gray, 2023; Sonenshine & Roe, 2013). The most common way for a tick to obtain a pathogen is through a blood meal from a host animal, although some pathogens can be transferred maternally (transovarially) within tick eggs, sexually via infected male ticks, or horizontally from one tick to another while feeding (co-feeding) (Hauck et al., 2020; Hayes et al., 1980; Voordouw, 2015). The most common TBPs in Europe are *Borrelia burgdorferi* sensu lato spirochetes and tick-borne encephalitis virus (TBEV), collectively infecting hundreds of thousands of people every year (ECDC, 2020; Marques et al., 2021). *Borrelia burgdorferi* s.l. remain the most common TBPs, including rodent-associated *Borrelia afzelii*, and bird-associated species *Borrelia garinii* and *Borrelia valaisiana* (Kurtenbach et al., 2002; Strnad et al., 2017). Given that certain tick-borne pathogens are associated with particular host animals (Hanincova et al., 2003; Kurtenbach et al., 2002), and tick larvae and nymphs mainly use smaller host animals than adult ticks (Kahl & Gray, 2023), precise assessment of local enzootic cycles requires us to determine the relative contributions towards maintenance of several host species.

Several methods to identify the previous host of a parasite from bloodmeal remnants have been developed (Borland & Kading, 2021). For quantifying and analysing small fragments of DNA, quantitative PCR is referred to as 'the gold standard' due to its sensitivity and specificity (Borland & Kading, 2021). DNA remnants of a previous host animal can be detected from a tick with qPCR even after 8 months from feeding, and PCR-based methods have previously been used successfully to identify host animals from bloodmeal remnants of mosquitoes and ticks (Borland & Kading, 2021; Collini et al., 2015). On the other hand, the method is limited by the available species-specific primer sets and probes that target the expected species, leaving unsearched and unexpected hosts unidentified (Borland & Kading, 2021). In addition, a long time between feeding and bloodmeal analysis may cause the bloodmeal remnants to deteriorate to such a level that host identification is no longer possible (Borland & Kading, 2021). In any case, as evidenced by the scarcity of publications regarding tick blood meals and the varying methodologies used, no universally functional methods for tick blood meal assays have emerged. Recently, qPCR assays targeting host animal retrotransposons have been developed and successfully used in the United States (Goethert et al., 2021). Recent tests of the protocols for European host species have also yielded promising results, potentially opening the way for more precise assessments of host contributions towards tick population and TBP upkeep (Sormunen et al., 2023). The ability to identify the most important hosts would enable more precise targeting of measures to reduce tick risk, particularly in urban green areas, where risk may be expected to be high due to human activity (Sormunen et al., 2020).

In this research, we study the contributions of voles, shrews, squirrels, leporids and cervids towards blood meals and the acquisition of TBPs of juvenile *I. ricinus* in urban and sylvatic habitats in Finland.

Impacts

- Differences in blood meal and pathogen sources of host-seeking juvenile *Ixodes ricinus* between urban and sylvatic habitats were studied for the first time utilizing blood meal analysis.
- Red squirrels were identified as important maintenance hosts for ticks and *Borrelia* particularly in urban habitats, whereas deer provided nearly one-fifth of larval blood meals in sylvatic habitats.
- Differences in host utilization observed between urban and sylvatic habitats highlight that prior knowledge of locally important hosts is required for effective control of tick-related risk.

2 | MATERIALS AND METHODS

We utilized tick samples collected from vegetation via cloth dragging in August and September 2019 from eight urban study sites in the capital city of Finland, Helsinki (Sormunen et al., 2020), and from six sylvatic transects on Seili Island in southwestern Finland in 2021 (Sormunen et al., 2023). The utilized samples consisted of 1084 *I. ricinus* nymphs, identified to the species level using stereo microscopes and morphological keys (Estrada-Peña et al., 2018).

Only nymphs collected in August and September were used, since blood meal analysis success rate is lower for samples from May to July due to degradation of the blood meal fragments (Sormunen et al., 2023). We screened tick samples for blood meal remnants of squirrels (Mammalia: Sciuridae), leporids (Mammalia: Lagomorpha), shrews (Mammalia: Soricidae), voles (Mammalia: Arvicolinae) and cervids (Mammalia: Cervidae), using qPCR-based protocols. No previous blood meal analyses had been made for samples from Helsinki, whereas for samples from Seili, the contributions of voles, shrews and squirrels had been previously examined (Sormunen et al., 2023). All the samples had also previously been screened for *B. burgdorferi* s.l. (including specific analyses for *B. afzelii*, *B. garinii*, *B. valaisiana* and *B. burgdorferi* sensu stricto), *Rickettsia* spp., *Babesia* spp., *Neoehrlichia mikurensis* and *Anaplasma phagocytophilum* (Sormunen et al., 2020, 2023). A detailed description of sample collection, DNA extractions and sample storage, and qPCR-based TBP screenings are presented in Sormunen et al. (2023) and Sormunen et al. (2020). Blood meal assay protocols are provided in the Tables S1 and S2.

We divided the study sites into five habitat groups with roughly equal sample sizes: sylvatic coniferous forest ($n = 134$), sylvatic deciduous forest (226), urban forest (242), urban green spaces (268) and urban islands (214). Total sample sizes were 360 for sylvatic and 724 for urban areas. Habitat- and area-specific TBP prevalence rates are reported in the Table S4. Sylvatic coniferous and deciduous forests were located on Seili Island and classified based on the dominant tree species present. For coniferous forests, these were Norway spruce (*Picea abies*) and/or Scots pine (*Pinus sylvestris*), for deciduous forests downy birch (*Betula pubescens*), common alder (*Alnus glutinosa*) and/or common hazel (*Corylus avellana*). For urban habitats, classification was based on spatial and land-use traits. Urban forests were two study sites located in the large, roughly uniform forest area of the central park of Helsinki (Hakuninmaa & Laakso). Urban green spaces were areas next to or within human habitation with significant human maintenance and activity, including areas in public parks and the vicinity of an arboretum and a large community garden (Lapinniemi, Meilahti, Kumpula). Finally, study sites in urban islands were small forest patches surrounded by artificial surfaces or water, and thus more isolated than urban forests (Lehtisaari, Lauttasaari, Seurasaari). Corine land cover (CLC), natural difference vegetation index (NDVI) and aerial photographs of the urban study sites are presented in Sormunen et al. (2023).

We used generalized linear models (GLM) with binomial distribution and logit link function to analyse study site-specific differences in the probabilities of *I. ricinus* nymphs having fed on specific hosts. We tested correlations between host utilization, land use and tick densities using Bayesian correlation. We analysed host–pathogen interactions with odds-ratio tests and calculated study site- and host-specific entomological risk indices (densities of infected nymphs, DIN) (Mather et al., 1996). More specific descriptions of used statistical methods are provided in the Appendix S1.

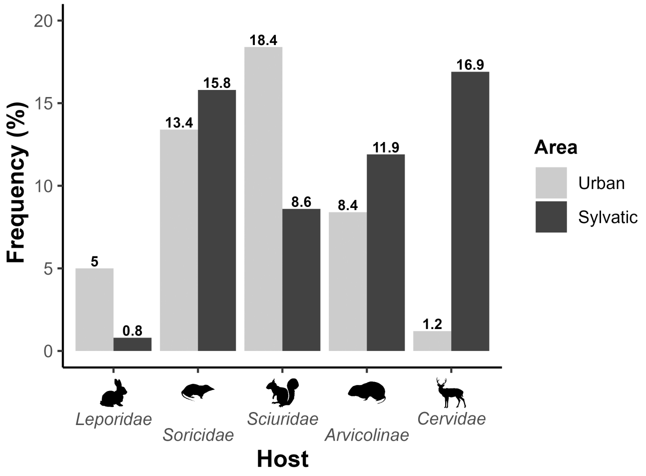

FIGURE 1 Percentage of larval blood meals provided by different host animals in urban and sylvatic areas. The proportions of unidentified hosts were 52.2% in urban and 45.0% in sylvatic areas.

3 | RESULTS

In total, 1084 *I. ricinus* nymphs from urban and sylvatic study areas were analysed for blood meal sources. Out of these, the source of the previous blood meal could be identified for 544 (50.2%), whereas for 540 (49.8%), the previous blood meal source remained unknown. There was noticeable variation in host contributions towards tick bloodmeals between urban and sylvatic areas and habitats (Figures 1 and 2). Squirrels and leporids were generally observed to be more common hosts in urban habitats of Helsinki, whereas voles and cervids were more common hosts in sylvatic forests of Seili, with variation observed in host use also across habitats within main study areas (Figure 2, Table S3). However, no differences between areas were observed for shrews.

Squirrels contributed 15.5 ± 2.2 (95% CL) % of all larval blood meals. Overall, the contribution of squirrels towards bloodmeals was higher in urban than in sylvatic areas (GLM: $F_{1,1064} = 14.69$, $p < 0.001$). Squirrels had the single highest habitat-specific contribution out of all tested animal groups, with the probability of a nymph having fed on a squirrel being 0.22 (0.17, 0.28; 95% CI) in urban islands. The probability of having fed on a squirrel was lower in urban forests than in urban green spaces and islands, whereas no differences could be discerned between urban and sylvatic forests (Table S3). Ticks having fed on squirrels were somewhat more likely to become infected with *B. burgdorferi* sensu lato (Odds ratio, OR = 2.45, 95% CI [1.73, 3.48]), more specifically with *B. burgdorferi* sensu stricto (OR = 7.94 [3.78, 16.7]) and *B. afzelii* (OR = 2.7 [1.83, 3.99]). Out of 31 detections of *B. burgdorferi* s.s., 18 (58.1%) were from squirrels, with the remaining 13 coming from unidentified sources. It was estimated that the contribution of squirrels towards the local density of infected nymphs (DIN; nymphs infected with Borrelia) was 0.3 *Borrelia*-infected nymphs per 100 m^2 in urban habitats and 1.1 in sylvatic habitats.

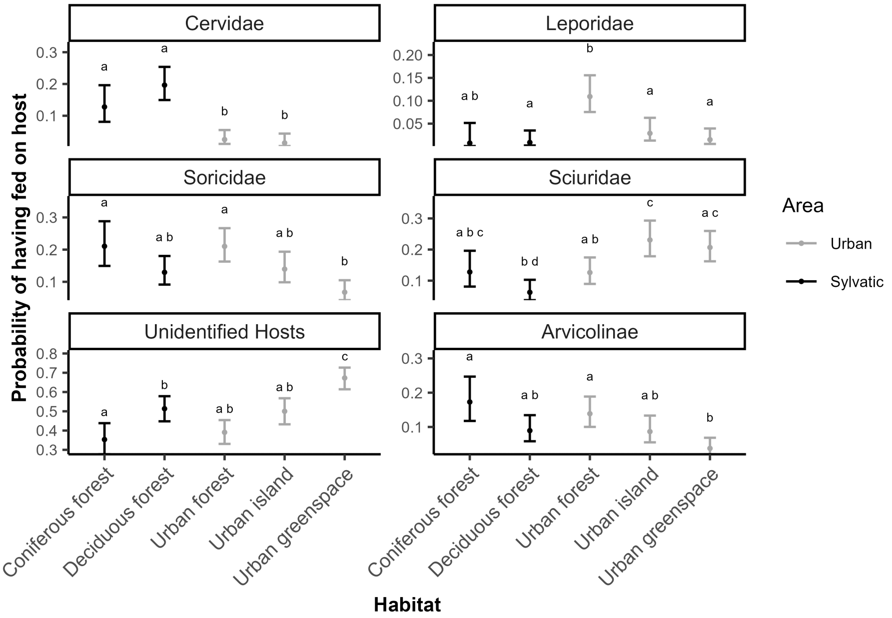

FIGURE 2 Probabilities of larvae having fed on specific host animals across different habitats (from GLMs with a 95% confidence interval). Different letters indicate statistically significant ($p < 0.05$) differences in the contribution of the host animal species between habitats.

Shrews contributed 14.2 ± 2.1% of larval blood meals. Shrews' contribution to bloodmeals did not differ significantly between urban and sylvatic areas (GLM: $F_{1,\,1064} = 2.72$, $p = 0.1$). They had the second highest habitat-specific contributions, with the probability of a nymph having fed on a shrew being 0.21 (0.14, 0.28) in sylvatic coniferous forests. The probability of a nymph having fed on a shrew was lower in urban green spaces than in sylvatic coniferous forests or urban forests (Table S3). Ticks having fed on shrews were less likely to become infected with *B. garinii* (OR = 0.06 [0.004, 0.99]). The contribution of shrews towards local DIN was 0.1 in urban areas and 1.3 in sylvatic areas.

Voles contributed 9.6 ± 1.8% of larval blood meals. Voles' contribution to blood meals was lower in urban than sylvatic areas (GLM: $F_{1,\,1064} = 5.76$, $p = 0.017$). Voles were more common blood meal sources in urban forests and sylvatic coniferous forests than in urban green spaces, with the highest habitat-specific contribution being 0.18 (0.11, 0.24) in sylvatic coniferous forests (Table S3). Ticks having fed on voles were more likely to become infected with *B. afzelii* (OR = 2.43 [1.53, 3.86]) and *N. mikurensis* (OR = 9.4 [4.54, 19.4]). The contribution of voles towards local DIN was 0.1 in urban and 1.4 in sylvatic habitats.

Cervids contributed 6.5 ± 1.5% of larval blood meals. Cervids were more common blood meal sources in sylvatic Seili island than in urban Helsinki (GLM: $F_{1,\,799} = 34.23$, $p < 0.001$), with the highest habitat-specific contribution in sylvatic deciduous forests (0.2 [0.14, 0.25]). There was strong evidence in favour of a positive correlation between the proportion of ticks having fed on cervids and the density of tick larvae (correlation = 0.72, $BF_{10} = 15.2$). In urban habitats, there was also very strong evidence in favour of a positive correlation between the proportion of ticks having fed on cervids and uniform forest area at study sites (correlation 0.94, $BF_{10} = 53.7$). Ticks having fed on cervids were less likely to become infected with Borrelia (OR = 0.18 [0.063, 0.48]), and more specifically with *B. afzelii* (OR = 0.04 [0.002, 0.57]). Contrastingly, they were more likely to become infected with *A. phagocytophilum* (OR = 13.3 [4.34, 40.7]). The contribution of cervids towards local DIN was 0 in urban and 0.4 in sylvatic habitats.

Finally, leporids contributed 3.6 ± 1.1% of larval blood meals. They were more common hosts in the urban area (GLM: $F_{1,1064} = 5.45$, $p = 0.02$), with the highest habitat-specific contribution being 0.11 (0.07, 0.15) in urban forests. Ticks fed on leporids were more likely to become infected with *A. phagocytophilum* (OR = 5 [1.1, 23.4]), and less likely to become infected with *Borrelia* (OR = 0.08 [0.01, 0.57]). The only positive detection of *Borrelia* from a tick having fed on a leporid showed a 99.7% similarity to *B. finlandensis*, with only 1/372 bp difference (GenBank Accession: MK604301). The contribution of leporids towards local DIN was 0 in urban and 0.1 in sylvatic habitats.

Overall, for 49.8% of ticks, the source of the previous blood meal could not be identified. This could be either due to the host being an animal not tested (e.g. birds or mice) or due to aspects affecting the success of the qPCR assays, for example, old age of the tick and high degradation of blood meal remnants. Blood meal analyses were least successful in urban green spaces, where the probability of remaining unidentified was 0.68 (0.62, 0.73) (Figure 2). Ticks having fed on unidentified hosts were more likely to become infected with bird-associated *B. garinii* (OR = 7 [2.95, 16.7]) and *B. valaisiana* (OR = 20.5 [2.7, 153]). Contrastingly, they were less likely to become infected with rodent-/shrew-associated *B. afzelii* (OR = 0.52 [0.37, 0.73]) and *N. mikurensis* (OR = 0.32 [0.14, 0.72]). Unidentified hosts had the highest contributions towards local DIN, with 0.6 Borrelia-infected ticks per 100 m^2 produced in urban and 3.9 in sylvatic habitats.

The source of *Borrelia* could be identified in 51.9% of detections, with squirrels being the most common sources (25.2% of all detections), apart from unidentified sources (48.1%) (Table 1). More specifically, the source could be identified for 63.1% of *B. afzelii* detections, and 58.1% of *B. burgdorferi* s.s. detections, 13% of *B. garinii* detections and 4.8% of *B. valaisiana* detections. Regarding identified

TABLE 1 *Borrelia burgdorferi* sensu lato positive samples per host group.

Host	*N*	*Borrelia* spp.	*Borrelia garinii*	*Borrelia afzelii*	*Borrelia valaisiana*	*Borrelia burgdorferi* s.s.	Unidentified *Borrelia*	Host-specific *Borrelia* prevalence (%)
Leporidae	39	1 (0.4)					1[a] (8.3)	2.6
Soricidae	154	31 (11.9)		28 (16.7)			3 (25.0)	20.1
Sciuridae	164	66 (25.3)	3 (6.5)	48 (28.6)		17 (56.7)	4 (33.3)	40.2
Arvicolinae	104	33 (12.6)	1 (2.2)	30 (17.9)			1 (8.3)	31.7
Cervidae	70	4 (1.5)	2 (4.3)		1 (4.8)		1 (8.3)	5.7
Unidentified hosts	540	126 (48.3)	40 (87.0)	62 (36.9)	20 (95.2)	13 (43.3)	2 (16.7)	23.3
Total	1071	261	46	168	21	30	12	

Note: Percentage of all detections given in brackets. Samples with two identified hosts ($n = 13$) were not included, since the source of the pathogen is uncertain.

[a] Identified as *Borrelia finlandensis* with Sanger sequencing.

sources, squirrels were the most common sources for both *B. afzelii* (28.6% of all detections) and *B. burgdorferi* s.s. (58.1%). *Neoehrlichia mikurensis* was detected from ticks having fed on voles (45.5% of all detections), shrews (18.2%) and cervids (9.1%) (Table 2). *Rickettsia* spp. was detected from ticks that had fed on every screened host but were most common in ticks that had fed on squirrels (18.8% of detections) or shrews (15.9%). There was substantial evidence for negative correlations between the local prevalence of *Rickettsia* spp. and nymph (correlation = −0.6, $BF_{10} = 3.6$) and larvae (correlation = −0.66, $BF_{10} = 9.1$) densities. Finally, there was extreme evidence for a positive correlation between *B. garinii* prevalence and the proportion of forests at urban study sites (correlation = 0.95, $BF_{10} = 100.4$).

4 | DISCUSSION

Our study suggests that the relative contributions of different host animals towards the bloodmeals of *I. ricinus* larvae differ between urban and sylvatic environments. This seems to concern also species that are considered universally important for the upkeep of tick populations, such as voles. Consequently, actions to control tick risk targeting specific hosts of perceived importance may produce suboptimal results, if the assessments of importance of hosts have been made in different environments.

Particularly, the increased significance of red squirrels in maintaining tick and TBP populations in highly urbanized areas of Helsinki seems apparent. In previous studies, *I. ricinus* nymphs have been observed on squirrels more often than larvae (Humair & Gern, 1998; Pisanu et al., 2014), whereas by utilizing blood meal analysis, we discovered that squirrels had provided roughly a fifth of all successful larval blood meals in urban study sites. Based on the previous findings, an even higher proportion of urban nymphs may be receiving blood meals from these animals. Given the scarcity of medium to large host animals in urban environments, the synanthropic nature of squirrels (Jokimäki et al., 2017) and their ability to feed both larvae and nymphs, they are likely to be greatly significant for tick population upkeep in urbanized areas. Furthermore, red squirrels were observed to be common sources of *B. burgdorferi* s.l. In particular, we found support for the suggested association between red squirrels and *B. burgdorferi* s.s. (Humair & Gern, 1998; Pisanu et al., 2014). In previous studies, the occurrence of *B. burgdorferi* s.s. in squirrels has been examined by collecting and analysing ticks from squirrels (Humair & Gern, 1998; Pisanu et al., 2014), but no simultaneous assessments regarding other possible local reservoirs were made. In our study, we observed that over half (58.1%) of the observed *B. burgdorferi* s.s. infections were obtained from squirrels, whereas no detections were made from ticks that had fed on voles, shrews, leporids, or cervids in the same study sites. This is strong evidence of the host-specificity of this Borrelia species (Humair & Gern, 1998; Pisanu et al., 2014). The remaining detections of *B. burgdorferi* s.s. were from unidentified hosts, which may contain species that

TABLE 2 Samples positive for other screened tick-borne pathogens, by host group.

Host	N	*Neoehrlichia mikurensis*	*Rickettsia* spp.	*Babesia* spp.	*Anaplasma phagocytophilum*
Leporidae	39		3 (4.3)		2 (15.4)
Soricidae	154	6 (18.2)	11 (15.9)	2 (25)	
Sciuridae	164		13 (18.8)	1 (12.5)	1 (7.7)
Arvicolinae	104	15 (45.5)	5 (7.2)		
Cervidae	70	3 (9.1)	2 (2.9)	1 (12.5)	6 (46.2)
Unidentified hosts	540	9 (27.3)	35 (50.7)	4 (50)	4 (30.8)
Total	1071	33	69	8	13

Note: Percentage of all detections given in brackets. Samples with two identified hosts ($n=13$) were not included since the source of the pathogen is uncertain.

were not analysed (most notably mice and birds) or ticks that had fed on squirrels (or other analysed hosts), but the degradation of the blood meal remnants prevented successful identification.

Along with squirrels, leporids were observed to be more common hosts in urban than sylvatic habitats. In southern Finland, brown hares (*Lepus europaeus*), mountain hares (*Lepus timidus*) and wild rabbits (*Oryctolagus cuniculus*) are present. However, only dwindling populations of mountain hares are present on the eastern and northern fringes of Helsinki. Likewise, while both species of hare likely inhabit Seili, observations during several years of fieldwork and 2 years of camera trapping at study sites are of brown hares (unpublished own data). Brown hares and wild rabbits can be considered synanthropic and are commonly seen in urbanized areas, where they likely benefit from increased warmth in the winter due to the urban heat island effect (Kim, 1992), alternative food sources, and/or scarcity of large predators (Eötvös et al., 2018; Gering & Blair, 1999). However, they still prefer forested areas for shelter, which may explain their highest blood meal contribution in urban forests (Tapper & Barnes, 1986). While they are not abundant in the study areas of the current study, mountain hares have been previously observed to be good hosts for both ticks and tick-borne pathogens, being able to upkeep tick and *B. burgdorferi* s.l. populations alone (Jaenson & Talleklint, 1996). It is therefore interesting that only one nymph that had fed on a leporid carried *B. burgdorferi* s.l. It may be that the locally abundant species are not as suitable reservoirs as mountain hares. Finally, it is worth noting that the only detection of *B. burgdorferi* s.l. from a tick that fed on a leporid most closely resembled *B. finlandensis* (GenBank Accession number: MK604301), a genospecies relatively recently delineated from *B. burgdorferi* s.s. (Casjens et al., 2011). This was also the only detection of the pathogen in this study.

Starkly contrasting rabbits and squirrels, cervids were more common hosts in sylvatic habitats than urban ones. Three species of cervids occur in and around the study areas, white-tailed deer (*Odocoileus virginianus*), roe deer (*Capreolus capreolus*) and elk (*Alces alces*). However, elks are very rare visitors in both study areas, whereas both species of deer are abundant within or in their vicinity. In addition to being more common in sylvatic forests, we observed a positive correlation between the size of the largest uniform forest at urban study sites and the probability of a tick nymph having fed as a larva on a cervid. The occurrence of deer in Helsinki is mostly restricted to larger forests of the central park, whereas they are rare in well-maintained and/or smaller and highly trafficked urban green spaces. Overall, we observed evidence for a positive correlation between local larvae density and the proportion of ticks having fed on cervids—this may well be due to many replete adult ticks dropping off from cervids, leading to high numbers of larvae in areas where they roam. Indeed, as cervids have most commonly been seen as important hosts for adults and nymphs, it is interesting to note that a high proportion of larvae had fed on cervids in sylvatic habitats—at one study site up to 37.2% of all larvae. Consequently, cervids may be able to offer blood meals to all tick life stages, possibly maintaining tick populations alone. However, a recent study of tick densities around deer feeding stations in Southwest Finland, one of the highest deer density areas in Finland, found very few ticks and no larvae, indicating that high deer densities alone may not be sufficient for tick population upkeep if other conditions are unfavourable (Kokkonen, 2022).

The trends observed for voles and shrews were similar. Shrews appeared to be more common blood meal sources than voles in urbanized areas, despite voles commonly being highlighted as main hosts for juvenile ticks. This observation was also previously made within the sylvatic habitats of Seili (Sormunen et al., 2023). Regarding differences in contribution in urban and sylvatic areas, voles were observed to be more common hosts in sylvatic than urban areas, whereas no significant differences could be observed for shrews. However, differences between forested habitats, either sylvatic or urban, were small for both animal groups. The only clear trend was that the probability of larvae feeding on a vole or shrew was lower in urban green spaces than in forested habitats. While these species are commonly present in urbanized areas, urban green spaces form the most well-maintained and popular areas for recreation within a city. As such, there are fewer shelters available, potentially less food due to landscape management, and more harassment by humans and pets, which may lead to fewer voles or shrews inhabiting these areas. The movements of humans and pets in the area may also influence the movement patterns of the animals, which may lead to changes in tick contact rates (Jędrzejewski et al., 1993; Korpimaki et al., 1995). Regarding TBPs, the role of shrews in the upkeep of *B. burgdorferi* s.l. appeared to equal that of voles. Taking into account their higher contribution towards larvae blood meals and equally significant role in *B. burgdorferi* s.l. upkeep, it is possible that shrews have been somewhat neglected as important hosts for both ticks and TBPs, as has been suggested in previous studies (Bown et al., 2011; Mysterud et al., 2015). Finally, nymphs that had fed on voles were commonly infected by *B. afzelii* and *N. mikurensis*, supporting previously observed host–pathogen associations (Andersson et al., 2013; Andersson & Råberg, 2011; Kurtenbach et al., 2002).

These five animal groups appeared to have provided the previous blood meal for roughly half of the questing nymphs collected, which is quite a remarkable proportion of blood meals, given that *I. ricinus* can feed on numerous species. Regarding the remaining half—even though we only used nymphs collected in August and September to minimize this risk—it is quite possible that some of the nymphs had not obtained their previous blood meal recently. Longer times between blood meals and analysis give the blood meal remnants more time to deteriorate, eventually rendering blood meal identification impossible (Goethert et al., 2021). As such, we consider the observed numbers minimum estimates—the real proportion of blood meals provided by these animals may be even higher. Unfortunately, we have not found working blood meal analysis methodology for several important host animal species/groups, most notably birds and mice. As such, their contributions towards blood meals and acquisition of TBPs could not be analysed. Consequently, the sources for most detections of *Borrelia* species linked to bird reservoirs (*B. garinii* and *B. valaisiana*) were left unidentified. However, the presence of both of these *Borrelia* species is a strong indication of the presence of bird hosts in both study areas. Likewise, the sources of many detections of *B. afzelii* and *B. burgdorferi* s. s. remained unidentified—these may be from, for example, mice (mainly yellow-necked mouse-, *Apodemus flavicollis*). In any case, the unidentified hosts produced the most *Borrelia*-infected nymphs. While these unidentified hosts may contain members from the studied groups as discussed, this result nevertheless highlights that further development of methodology is required for us to be able to reliably identify the most important maintenance hosts in different environments.

In conclusion, our study has revealed significant differences in the contributions of different host animals towards tick and TBP upkeep in urbanized and sylvatic habitats. The presence of increasing numbers of infected ticks in urban green areas leads to a higher risk of tick-borne diseases, as human activity is particularly high in these areas. While removing ticks from—or even reducing the numbers of ticks in—nature on a wide scale is not feasible with currently available methods, methods such as specifically targeted bait boxes (Dolan et al., 2017; Schulze et al., 2017) working on a smaller scale could be applied to urban green areas, which are often spatially restricted and particularly commonly visited by humans. However, for such methods to produce significant results, the most important hosts maintaining ticks and/or TBPs in these areas need to be determined. Based on our results, methods targeting hosts commonly perceived to be important for tick and TBP upkeep, without on-site information about locally important hosts, may prove to be inefficient for controlling risk.

AUTHOR CONTRIBUTIONS

Jani Jukka Sormunen contributed to experimental design, laboratory work, supervision of laboratory work, data analysis, writing and editing, as well as the acquisition of funding. Jesse Mänttäri was involved in laboratory work, graphics and writing and editing. Eero Juhani Vesterinen contributed to writing and editing, along with the acquisition of funding. Tero Klemola focused on data analysis, writing and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

An ethics statement is not applicable as this study was not classified as a human or animal experiment. Tick samples utilized in this study were field-collected from non-protected areas.

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SUPPORTING INFORMATION

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