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TICK-BORNE BACTERIAL PATHOGENS IN CZECH URBAN ENVIRONMENTS: FIRST RESULTS OF THE SURVEILLANCE PROJECT

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Abstract Tick-borne diseases (TBD), such as particularly Lyme borreliosis, belong among the most important zoonoses in the Czech Republic. Although TBD infections were traditionally associated with natural habitats especially deciduous and mixed forests, the tick occurrence in urban green areas was recently reported from several Czech urban areas. The project "Tick-borne bacterial diseases in urban environments: where does the real danger lurk" thus aims to study the tick abundance and prevalence of the selected tick-borne pathogens (TBP) in all regions across the Czech Republic. In this project, the three sites in Praha, České Budějovice, and Ostrava are sampled monthly. Also, the urban or suburban areas in the other ten regional capitals are monitored once a year. The first results from 2023 showed significant effect of locality on both tick abundance and bacterial pathogens composition. The tick abundance also varied between the individual transects within the locality. The prevalence of the bacterial TBP in the captured ticks ranged 41-52 %. *Borrelia burgdorferi* s.l. was shown to be major TBP at all localities with the exception of Ostrava, where *Anaplasma phagocytophilum* dominated. Furthermore, other TBPs such as *Rickettsia spp.*, *Neoehrlichia mikurensis* and *B. miyamotoi* were detected in the collected ticks. To generate a comprehensive dataset capable of assessing annual and seasonal variations in both tick populations and TBP prevalence, data collection at the selected urban areas will continue annually through 2026.

Key words: city, vectors, *Borrelia*, *Anaplasma*, *Ricketsia*, *Neoehrlichia*

INTRODUCTION

The Earth's surface is undergoing a critical transformation, with natural landscapes increasingly being replaced by urban and agricultural areas. Urbanization is one of the most significant and widespread forms of human-driven environmental change. According to the United Nations (2024), the global population reached 8 billion in November 2022, with over half residing in urban areas—a proportion expected to rise to 70% by 2050. In response, efforts to enhance biodiversity, ecosystem functioning, and human well-being are increasingly focused on maintaining urban green spaces and improving their ecological connectivity through various management strategies, such as reforestation and the restoration of grasslands and wetlands. Additionally, forest remnants surrounded by buildings and infrastructure have become an integral part of the urban landscape.

Green infrastructure is widely promoted as a multifunctional "nature-based solution," providing numerous benefits, including improved water management, air quality, temperature regulation, noise reduction, recreational opportunities, and biodiversity-related ecosystem services such as pollination (Konijnendijk et al., 2008; Zellner & Massey, 2024; Graffigna et al., 2024). However, despite these advantages, urban green development can also increase the risk of human exposure to animal-associated parasites and pathogens (e.g., Braks et al., 2016; Kybicová et al., 2017; Heylen et al., 2019; Bellato et al., 2021). Therefore, further research into the impact of ecological connectivity and urban wildlife on the risk of zoonotic infections in human populations is essential. Fragmented urban green spaces are home to various vertebrate species, including small ungulates, rabbits, rodents, birds, and lizards, many of which can serve as both tick hosts and reservoirs for tick-borne pathogens (Bellato et al., 2021; Musilová et al., 2022). Hence the diversity and abundance of these vertebrates are lower in urban areas compared to natural ecosystems, their population density and exposure to ticks are often higher in cities (Kowalec et al., 2017).

In Central Europe, tick-borne diseases (TBDs) are among the most significant zoonoses. In 2023, the database of the National Institute of Public Health reported 3268 cases of Lyme disease, three cases of anaplasmosis, and five cases of rickettsiosis in the Czech Republic (ISIN, 2024). While coniferous forests were traditionally considered the primary risk areas for TBDs, findings by Kybicová et al. (2017) suggest that infection risks in urban and suburban environments may be comparable to—or even higher than—those in natural ecosystems. This was later supported by Richtrová et al. (2022), who confirmed a high prevalence of *Borrelia* species in urban habitats.

Currently, comprehensive data on tick abundance and pathogen prevalence in urban and suburban parks across the Czech Republic are lacking. The project *"Tick-borne Bacterial Diseases in Urban Environments: Where Does the Real Danger Lurk?"* aims to address this gap by collecting extensive data on tick populations and the prevalence of selected bacterial tick-borne pathogens across all regions of the Czech Republic. The findings will contribute to raising awareness of the health risks associated with urban and semi-urban green spaces and support the implementation of preventive measures within the framework of personal protection.

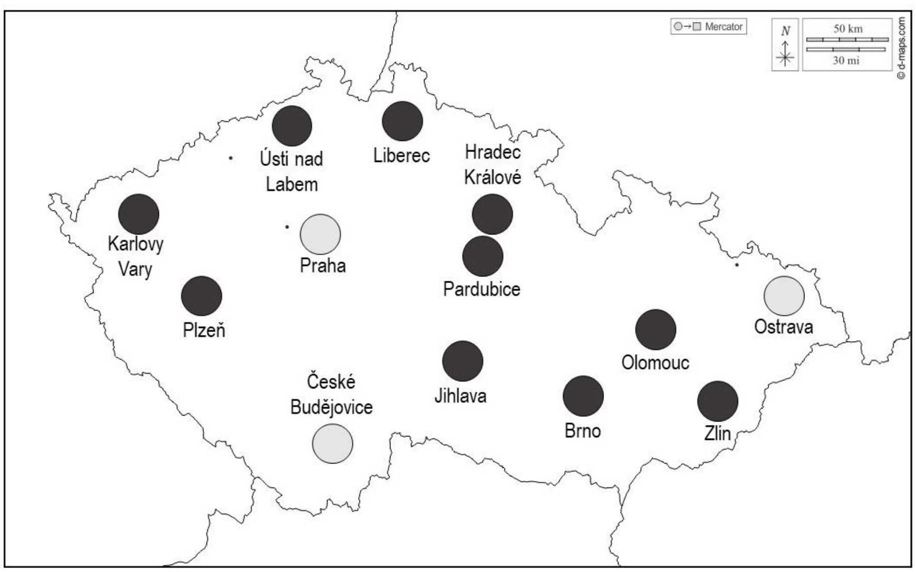

Figure 1 Localities sampled in the Czech Republic. Localities monitored intensively are grey color, black spots show extensively monitored localities.

METHODS

Study sites In 2023, we conducted intensive monitoring (once per month from March/April to December) of six 100 m² patches in three urban and suburban areas: České Budějovice (CB), Praha (PRG), and Ostrava (OVA). Additionally, extensive monitoring (once per year) was carried out during the expected peak of the tick season (May 2023) in parks in ten other regional capitals (see Figures 1 and 3). During extensive monitoring, at least 100 ticks were collected from each locality.

Ticks and sampling Ticks were collected using the flagging method. The collection flag consisted of white cotton flannel cloth sized 1 × 1 m, attached to a wooden pole. The actual collection of ticks was executed by dragging the flag over the vegetation. Captured nymphs and adults were collected to 50mL vials containing 70% ethanol. After sampling termination, the ticks were transported to the laboratory. Species, sex, and life stage were identified using taxonomic keys (Arthur, 1963; Hillyard, 1996) and the ticks were then preserved in 2.0mL Eppendorf vials in 70% ethanol for further studies on tick-borne pathogens. The sampling area (in square meters) at each monitored locality was recorded and tracked using the mobile application Mapy.cz. Temperature and relative humidity were measured approximately 10 cm above the soil at each patch using a thermo-hygrometer (COMET, Comet System, Czech Republic).

DNA extraction and amplification screening Ticks were individually transferred into Bead Tubes Type D (Macherey-Nagel) and homogenized using the MagNA Lyser instrument (Roche). Genomic DNA extraction was performed using the croBEE NA16 Nucleic Acid Extraction System (GeneProof) according to the manufacturer's protocol. DNA samples were stored at -20°C until PCR amplification. Two multiplex qPCR methods were used to screen the ticks for the presence of tick-borne pathogens; PCR1: *Borrelia burgdorferi s.l.* (simultaneous detection of OspA, FlaB gene targets) + *B. myiamotoi* (FlaB) (Heylen et al. 2019); PCR2: *Anaplasma phagocytophilum* (MSP2) (Courtney et al. 2004) + *Neoehrlichia mikurensis* (Jahfari et al. 2012) + Rickettsia spp. (gltA) (Stenos et al. 2005). Real-time PCR was performed with the CFX Real-Time PCR Detection System (Biorad).

RESULTS AND DISCUSSION

Abundance, intensively monitored localities As shown in Figure 2, the abundance of questing ticks in the monitored urban areas of PRG and CB was higher than in OVA, with peak activity observed in May and June, respectively. A minor increase in tick activity during autumn was noted in OVA and CB. In PRG, tick abundance slightly increased in August before declining. These findings are largely consistent with those of Daniel et al. (2015), who monitored tick activity in Central Bohemia, Czech Republic between 2001 and 2006. The maximum number of collected ticks was 260 in PRG, 196 in CB, and 69 in OVA. Although the general abundance trend was similar across all study sites, the number of captured ticks varied considerably. This variation may be explained by differences in habitat characteristics. The Krčský les site in PRG is a forest park with minimal human intervention, allowing for natural vegetation and native ecosystems to thrive. In contrast, the study sites in OVA consist of city parks with landscaping and lawns. Stromovka in CB includes both maintained and non-maintained areas. The weather and microclimate effect on tick activity was previously described by for instance Schulz et al. (2014). Since tick collection was conducted across six different patches within each study site, we were also able to observe abundance differences among individual patches (Figure 3b, d, g) and the tick abundance was obviously higher in shady, humid patches overgrown with vegetation or with high layer of litter leaf compared to sunny areas with regularly mowed grass. The comprehensive approach allowed us to assess tick exposure risk in relation to seasonality and location but also within different microhabitats of a single site.

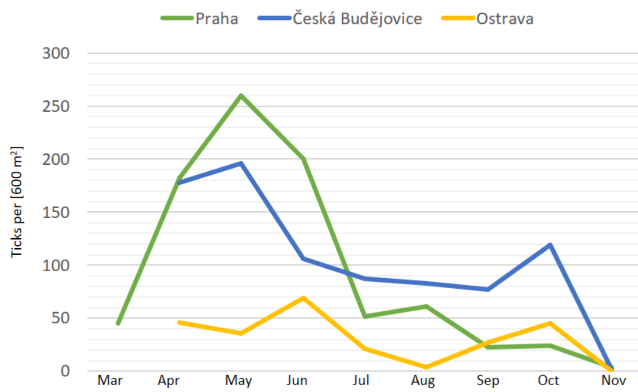

Figure 2. Abundance of questing ticks obtained by flagging at intensiv monitored localities in March-November 2023.

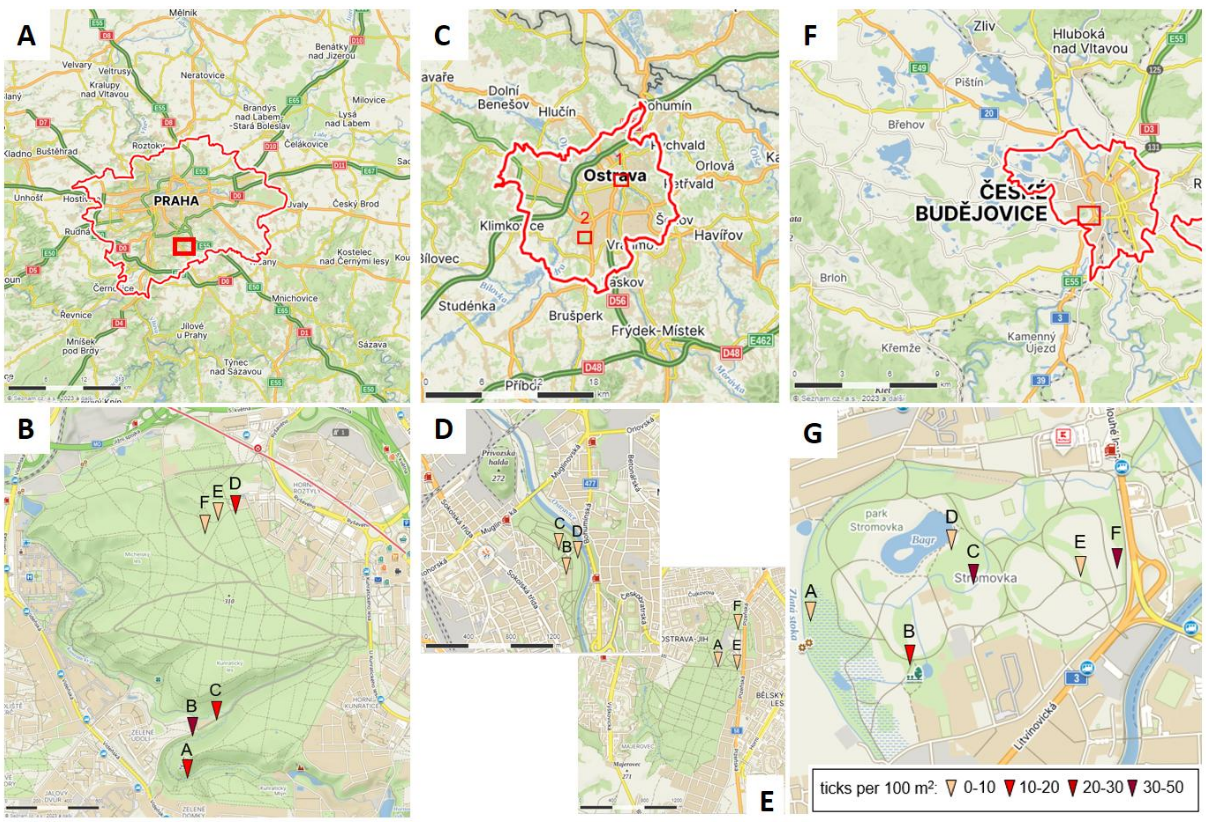

Figure 3a: The intensively monitored localities (a, c, f) and the abundance of questing ticks collected from the individual patches (b, d, g) by flagging.

Abundance, extensively monitored localities As shown in Table 1, tick abundance in the extensively studied localities varied considerably. The highest abundance, 71.9 ticks per 100 m², was recorded in Pardubice, Polabiny. In contrast, the lowest abundance was observed in city parks in Ústí nad Labem (0.1 ticks per 100 m²), Jihlava, and Karlovy Vary (1 tick per 100 m²). Similar to the intensively studied sites, these differences can largely be attributed to habitat characteristics. Areas with intensive maintenance, such as manicured lawns, flowerbeds, and scattered single trees, either lacked ticks entirely or had very low abundance. In these locations, ticks were predominantly found in shaded areas, bushes at park edges, or non-maintained sections of parks. Although tick collection was conducted within a three-week period during the peak season (mid-May to mid-June), we cannot entirely rule out the influence of weather conditions on tick activity at the time of flagging. During sampling, temperatures ranged from 12.7 to 25.1°C, and relative humidity varied between 22% and 66%. Extensive monitoring of these localities is planned for the next three years of the project. The tick abundance observed in urban parks is comparable to the findings of Vacek et al. (2024), who reported an average density of 12.1 ticks per 100 m² in Czech forests. Their study also noted that tick abundance was highest in coniferous forests and along forest edges.

Table 1. The abundance of questing ticks collected in the extensively monitored parks in the Czech Republic using flagging.

Municipality	**Study site**	**t [°C]**	**RH [%]**	**Type of locality**	**ticks total**	**ticks/ 100 m^2**
Karlovy Vary	Rope center Sv. Linhart	22.5	22	forest park	165	8.8
	Ohře meander	23.4	28	city park	6	1.0
Plzeň	Borský	21.7	31	city park	118	3.5
Ústín n. Labem	Open air-cinema, City park	25.1	30	city parks	19	0.1
	Větruše	23.9	31	forest park	120	17.6
Liberec	Lidové sady	20.5	49	forest park	199	25.8
Jihlava	Park legionářů	22.4	38	city park	10	1.0
	Velký Heulos	22.2	40	forest park	132	4.7
Hradec Králové	Šimek gardens	12.7	64	city park	148	37.3
Pardubice	Polabiny	15.2	53	forest park	174	71.9
Brno	Lužánky	22.2	40	city park	122	2.8
Olomouc	Čech gardens	18.0	65	city park	49	6.1
	Smetana gardens	19.0	60	city park	35	8.8
	Bezručovy gardens	19.0	57	city park	33	8.3
Zlín	Zlín castle	20.0	66	city park	114	14.3
	Gahurův prospekt	19.0	66	city park	11	2.8

Pathogens, intensively monitored *Borrelia burgdorferi* s.l. was the dominant bacterial pathogen in all monitored sites, except for patch B in Ostrava (OVA), where *A. phagocytophilum* (prevalence of 32.6%) was the most prevalent tick-borne pathogen detected (see Table 2). The prevalence of *B. burgdorferi s.l.* varied across patches, ranging from 8.7% to 60.0% of the collected ticks, which may be explained by local factors, particularly the presence/density of potential tick hosts and reservoirs, as well as habitat conditions. Although the total composition of pathogens varied between study sites and patches, the difference in overall prevalence between the localities was minimal, with less than a one percent difference between the highest and lowest prevalences (CB: 42.2%, OVA: 42.4%, and PRG: 43.1%). In CB, the total prevalence of *B. burgdorferi* (30.3%) was higher than in PRG (24.4%) and OVA (18.5%). In contrast, *Rickettsia* and *Anaplasma* were more abundant in ticks from OVA and PRG compared to those from CB.

The data from CB and PRG are in line with those reported by Richtrová et al. (2022), who found 28.1% of ticks in Prague's urban green areas to be positive for *B. burgdorferi s.l.* Similarly, Balážová et al. (2024) reported a prevalence of *B. burgdorferi s.l.* of 27.1% and *B. miyamotoi* of 2.1% in urban areas across Czech Republic. The lower prevalence of *Borrelia* reported in South Bohemia (12.1%) by Hönig et al. (2015) is noteworthy. However, their study did not focus exclusively on urban or suburban areas, suggesting that the prevalence of tick-borne pathogens in wild habitats may be lower than in urban areas due to limited exposure of hosts to questing ticks.

The high prevalence of *A. phagocytophilum* observed in OVA urban parks is unusual for the Czech Republic. In contrast, Kybicová et al. (2017) found only 4.4% of ticks tested positive for *A. phagocytophilum* in various habitats in Czech Republic. To further verify these findings and observe trends, selected parks in CB, PRG, and OVA will continue to be monitored monthly in the following years, allowing for a comprehensive analysis of the influence of seasonality, locality and climate on both tick activity and the prevalence of tick-borne pathogens.

Pathogens, extensively monitored localities In the extensively monitored localities, *Borrelia burgdorferi s.l.* was the most prevalent bacterial pathogen across all monitored urban areas, except in Pardubice, where *A. phagocytophilum* was more prominent (17.2% > 13.8%). The prevalence of *B. burgdorferi s.l.* ranged from 13.8% to 31.0% (see Table 3). As previously mentioned above in the text, this range of *Borrelia* prevalence is consistent with reports from other studies from the Czech Republic (Kybicová et al., 2017; Richtrová et al., 2022; Balážová et al., 2024). The highest overall prevalence of tick-borne bacterial pathogens was recorded in Olomouc, where 61% of the collected ticks were infected. This high prevalence was attributed to both *Anaplasma* (21.0%) and *B. burgdorferi s.l.* (31.0%) pathogens. Interestingly, Olomouc region was reported as the most affected region in terms of Lyme disease cases in 2018-2019 (Orlíková et al., 2022). The lowest prevalence (32.4%) was found in Ústí nad Labem. Data presented are based on a single tick sampling, so further research is necessary to expand on the data from monitored urban parks, to confirm the observed trends, and understand the factors influencing the distribution of tick-borne pathogens in urban areas.

Table 2. Bacterial pathogen prevalence in ticks in the intensively monitored parks.

		Patch A (n=86)	Patch B (n=111)	Patch C (n=77)	Patch D (n=56)	Patch E (n=27)	Patch F (n=28)	total (n=385)
Praha (PRG)	*Borrelia*	27.9	20.7	19.5	26.8	29.6	32.1	24.4
	B. miyam	4.7	2.7	2.6	1.8	3.7	0.0	2.9
	Anaplasma	3.5	1.8	0.0	17.9	11.1	7.1	5.2
	Neoehrlichia	2.3	2.7	6.5	8.9	7.4	7.1	4.9
	Rickettsia	7.0	5.4	5.2	3.6	11.1	3.6	5.7
	Prevalence	45.3	33.3	33.8	58.9	63.0	50.0	43.1
České Budějovice (CB)		Patch A (n=86)	Patch B (n=111)	Patch C (n=77)	Patch D (n=5)	Patch E (n=27)	Patch F (n=28)	total (n=385)
	Borrelia	27.7	26.7	30.7	60.0	31.4	31.9	30.3
	B. miyam	0.0	3.3	2.0	0.0	0.0	3.1	2.2
	Anaplasma	0.0	1.2	2.6	0.0	0.0	8.5	3.6
	Neoehrlichia	3.1	2.3	1.3	0.0	0.0	1.3	1.6
	Rickettsia	6.2	0.0	3.9	0.0	5.7	6.5	4.5
	Prevalence	36.9	33.5	40.5	60.0	37.1	51.3	42.2
Ostrava (OVA)		Patch A (n=17)	Patch B (n=46)	Patch C (n=31)	Patch D (n=28)	Patch E (n=56)	Patch F (n=58)	total (n=243)
	Borrelia	29.4	8.7	12.9	21.4	16.1	24.1	18.5
	B. Miyama	0.0	2.2	0.0	0.0	3.6	3.4	2.1
	Anaplasma	11.8	32.6	12.9	3.6	5.4	12.1	13.2
	Neoehrlichia	5.9	0.0	0.0	0.0	3.6	3.4	2.1
	Rickettsia	11.8	4.3	6.5	10.7	5.4	6.9	6.6
	Prevalence	58.8	47.8	32.3	35.7	33.9	50.0	42.4

Table 3 Bacterial pathogen in ticks in the extensively monitored parks.

	Karlovy Vary	Plzeň	Ústí nad Labem	Liberec	Jihlava	Hradec Králové	Pardubice	Brno	Olomouc	Zlín
B. burgdorferi s.l.	23.7	27.0	22.2	31.5	30.2	25.9	13.8	26.3	31.0	31.2
B. miyamotoi	4.2	3.6	1.9	3.9	0.8	1.9	0.9	2.0	1.0	4.0
Anaplasma	0.8	3.6	0.9	1.6	9.3	12.0	17.2	9.1	21.0	12.8
Neoehrlichia	2.5	2.7	0.0	0.0	1.6	0.9	5.2	0.0	0.0	4.8
Rickettsia	12.7	9.0	7.4	9.4	8.5	3.7	3.4	6.1	8.0	5.6
total	44.1	45.9	32.4	46.5	50.4	44.4	40.5	43.4	61.0	58.4

CONCLUSIONS

In urban areas that are not intensively maintained, the presence of ticks is likely, particularly in shady areas, bushes, and overgrown vegetation, which are considered high-risk zones. Our preliminary results from 2023, the first year of the project "Tick-borne bacterial diseases in urban environments: where does the real danger lurk", indicated that between 32.4% and 61.0% of questing ticks in urban parks across the Czech Republic were infected with at least one bacterial pathogen, primarily *Borrelia burgdorferi s.l.* and *Anaplasma phagocytophilum*. These findings underscore the importance of intensive surveillance in urban areas, the need for heightened awareness regarding tick-borne disease prevention, and the critical role of personal protection in mitigating risk.

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