SLOVAKIA'S URBAN TICK ECOSYSTEM: EXPLORING HOSTS, PATHOGENS, AND PUBLIC HEALTH IN KOŠICE (EASTERN SLOVAKIA)*

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Hard ticks are important vectors of dangerous pathogens worldwide. They are vectors of a wide range of protozoal, bacterial, and viral pathogens that are significant to humans and animals. In urban habitats, small and medium-sized mammals, birds, companion animals (cats and dogs), and larger animals (deer, roe deer, and wild boars) play a role in maintaining tick populations and as reservoirs of tick-borne pathogens. The abundance of hard ticks was monitored in Kosice Eastern Slovakia in 4 urban localities: 1, Zberne suroviny, 2, Tahanovce sidlisko, 3, Tahanovce kostol, and 4, Hornbach. Ticks were collected by the flagging method with a white cotton flag (1m^2). They were examined using molecular biology methods, reverse line blot hybridization (RLB) for the detection of hosts, and polymerase chain reaction (PCR) for pathogen detection. A total of 216 ticks were collected. Two species were identified: *Ixodes ricinus and Dermacentor reticulatus.* From the 216 questing ticks collected during spring 2022, we found 72 males, 68 females and 34 nymphs, 2 larvae of *I. ricinus* and 11 males and 29 females of *D. reticulatus.* Altogether, 40 D. reticulatus and 40 *I. ricinus* ticks were tested for the presence of tick-borne pathogens by PCR. Prevalence of *Babesia* spp. in *D. reticulatus* was 7,5%; however, in *I. ricinus* ticks, no positive sample was detected. Prevalence of *Rickettsia* spp. was 7,5% in *D. reticulatus* and 10% in *I ricinus*. The most represented host in *D. reticulatus* were birds and *I. ricinus* parasites in small rodents, artiodactyls (*Sus scrofa, Capreolus capreolus*), birds (*Turdus* sp., *Parus* sp.) and *Erinaceus europaeus* on which they fed in the previous developmental stages. Hard ticks of the species *I. ricinus* and D. reticulatus were found in the center of Kosice city. These results indicate a stable tick population in the city. Therefore, birds and small mammals introduce immature ticks into urban areas. In cities, they then complete their developmental cycle to adulthood. This research sends an important public message about the high risk of tick bites and, consequently, of contracting tick-borne diseases.

tick, tick hosts, Slovakia, bloodmeal analysis

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INTRODUCTION

Ticks are an important group of ectoparasites, parasitic on various spectrums of hosts from reptiles to birds and mammals and can transmit pathogenic microorganisms, including protozoa, bacteria and viruses (Medlock et al., 2013; Siuda, 1993). Ticks are responsible for the majority of arthropod-borne diseases in humans and animals in Europe (Boulanger et al., 2019) characterized by the diversity of pathogens they transmit, by their impact on human and animal health, and by their socioeconomic implication especially in countries of the Southern Hemisphere. In Europe, Ixodes is the most important tick due to its wide distribution in the ecosystems and the variety of transmitted pathogens, in particular Borrelia (responsible for Lyme borreliosis.

Humans and pets are at a significantly higher risk of coming into contact with ticks due to the emergence of ticks in urban areas (Rizzoli et al., 2014). Depending on the species, ticks may be observed in highly varied habitats, from the driest to the most humid. The habitat range of ticks includes both natural and urban environments, such as recreational areas or parks, which can ensure the biotic and abiotic requirements for optimal development of the off-host stages

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(Rizzoli et al., 2014). Urban landscapes are supported by green infrastructure, which provides a wide range of ecosystem services, from improving health and well-being to storing carbon and reducing heat (Paude, States, 2023). Urban green infrastructure includes urban forests, open green spaces, and green corridors (e.g., wildflower strips and hedgerows), which can form a network that supports diverse plant species and wildlife movement from surrounding rural habitats. This network can provide temporary or stable habitats for urban biodiversity and facilitate wildlife movement, particularly if nearby home ranges for wildlife exist (Evans et al., 2010; Hansford et al., 2023)and of sufficient case studies. Here, we develop such a framework. This draws upon a wide range of ecological and evolutionary theory and the increasing number of studies of how the markedly divergent conditions in urban and rural areas influence the traits of urban populations and the structure of urban assemblages. We illustrate the importance of this framework by compiling a detailed case study of spatial and temporal variation in the urbanisation of the blackbird Turdus merula. Our framework identifies three separate stages in the urbanisation process: (i. Neglected areas in urban environments pose a risk of tick infestation to the general public (Bellato et al., 2021; Uspensky, 2014)such as city parks or peri-urban forests, across Europe. Land use changes, like the transformation of natural ecosystems into residential or recreational areas, and the restoration of natural areas connectivity for biodiversity purposes, facilitate human contact with these vectors. We evaluated the infestation by Ixodid ticks and their infection by zoonotic agents in two natural reserves (La Mandria and Stupinigi. In the past few years, several studies reported ticks and tick-borne diseases in city parks and urban forests across Europe (Hansford et al., 2023; Kowalec et al., 2017)nymph density and Borrelia species (spp..

Urban environments represent many unique ecological features in the complex communities of pathogens, ticks and hosts. From a public and veterinary health perspective, urban parks and peri-urban recreational areas are typical meeting places for humans (and their pets) and ticks. Ticks act as a bridge for pathogens, connecting reservoir hosts with humans (Földvári et al., 2007) 1,424 ticks were removed from 477 dogs appearing for clinical consultation in veterinary practices and clinics countrywide. Ixodes ricinus and Dermacentor reticulatus were the most common species occurring in most of the studied areas. Females of these two species were selected for molecular analyses. One to twelve specimens were used in each sample for DNA extraction. Polymerase chain reactions were performed with BSLF/BSL-R primers for detecting Borrelia spp. in I. ricinus and with PIRO-A1/PIRO-B primers to amplify Babesia spp. DNA in D. reticulatus. Randomly selected PCR products were sequenced to identify the pathogens' species or subspecies. DNA of Borrelia spp. could be detected in six (5.6%). Small-sized vertebrates such as rodents, shrews, birds and lizards can play a role as tick-borne pathogens reservoirs (Amore et al., 2007; Randolph et al., 1999, Dudek et al., 2016). Several urban and peri-urban green spaces have experienced the anthropogenic introduction of non-native mammal species, such as chipmunks, which contribute to the emergence of tick-borne pathogens (Marchant et al., 2017). Some important tick-maintenance and pathogen reservoir hosts (hedgehogs, squirrels, and songbirds) have no or very few natural enemies within urban environments; thus, their populations might reach significantly higher densities compared to natural ones (Földvári et al., 2007)1,424 ticks were removed from 477 dogs appearing for clinical consultation in veterinary practices and clinics countrywide. Ixodes ricinus and Dermacentor reticulatus were the most common species occurring in most of the studied areas. Females of these two species were selected for molecular analyses. One to twelve specimens were used in each sample for DNA extraction. Polymerase chain reactions were performed with BSLF/BSL-R primers for detecting Borrelia spp. in I. ricinus and with PIRO-A1/PIRO-B primers to amplify Babesia spp. DNA in D. reticulatus. Randomly selected PCR products were sequenced to identify the pathogens' species or subspecies. DNA of Borrelia spp. could be detected in six (5.6%.

This study investigates the range of tick species in urban habitats in the city of Kosice (eastern Slovakia), focusing on areas frequented by humans. Additionally, the collected ticks were tested for the presence of., *Rickettsia* spp. and *Babesia* spp. pathogens transmitted by ticks. The method of blood meal analysis was used to identify reservoir hosts for ticks.

MATERIALS AND METHODS

Model localities

Questing ticks were collected using the flagging method from 4 urban localities in Kosice, Eastern Slovakia (1, Zberne suroviny, 2, Tahanovce sidlisko, 3, Tahanovce kostol, and 4, Hornbach) (Fig. 1). The sites have been selected based on a large green urban area isolated from the surrounding forests, suitable for the survival of ticks and their hosts (e.g., mammals,

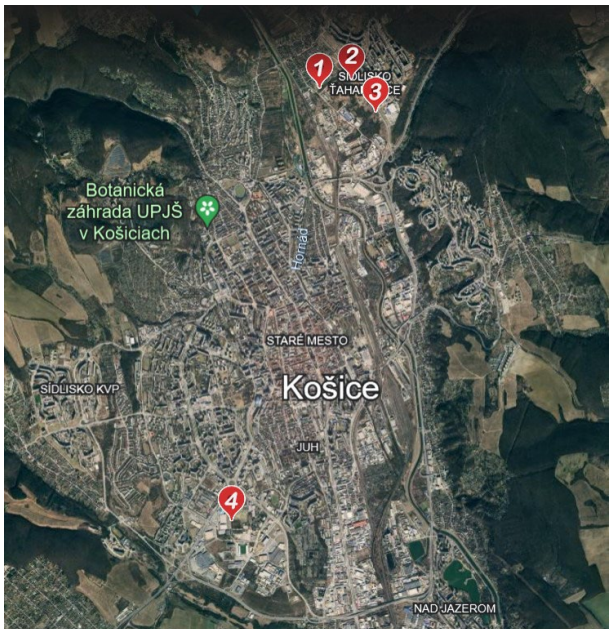


Fig. 1. Selected localities in Kosice (1 – Zberne suroviny , 2 – Tahanovce sidlisko , 3 –Tahanovce kostol, 4 –Hornbach). The areas were chosen as isolated green areas in the city surrounded by people's dwellings, isolated from the adjacent forest through which people walk. On these sites there is a whole spectrum of greenery from grass to shrubs and trees.

birds and lizards). All locations are open to the public year-round. City dwellers often use selected sites as transition points between residential areas or for animal walkers. From a climatological point of view, the long-term temperature trends in the city of Kosice are increasing. From 2013 to 2020, the temperature increased by more than 1.5 °C on average 9 °C. Rainfall for Kosice has a decreasing trend ('METEOROLÓGIA A KLIMATOLÓGIA - KLIMATICKÉ ZMENY - Trend teploty vzduchu na Slovensku od 1881,' n.d.).

Model organisms

Questing ticks were collected from vegetation by the flagging method with a white cotton blanket 1 m^2 in eastern Slovakia (Kosice – 48,42° N, 21,15° E) from March to May 2022. Ticks were kept in polypropylene tubes with 70% ethanol and individually identified to species level using a morphological key (S i u d a , 1993). The ticks were isolated for DNA.

Molecular methods

DNA from ticks was isolated by the DNeasy blood and tissue kit (Qiagen, Hilden, Germany). Each tick was first removed from 70% ethanol and dried. Ticks were put in sterile tubes and cut into small pieces using a sterile scalpel. Then 180 µl ATL buffer (provided in the kit) with proteinase K 20µl was added to each tube and treated in a dry bath (Benchmark) at 56 °C for 2 hours. The subsequent steps were carried out according to the manufacturer´s instructions.

The PCR was performed using oligonucleotide primers D767f (CGATGGTAGCATTAAAAGCT), and D1390r (CTTGCTTTTCAGCAATATCAC), which amplify a 623-bp fragment of the *sca4* gene of *Rickettsia* spp. (95°/30s; 50°/30s; 68°/90s; 40x) (S e k e y o v a et al., 2001). Primers BN (TAGTTTATGGTTAGGACTACG) and BJ (GTCTTGTAATTGGAATGATGG) amplifying part of 18s RNA (450-bp) gene were used for the detection of *Babesia* spp. (94°C/30s; 54 °C/30s; 72 °C 40s; 40x) (Casati et al., 2006). Negative and positive controls were included in each PCR assay. The 50 µl reaction mixture consisted of 35,7 µl molecular water, 10x buffer 5µl, dNTP 0,5 µl, 1,8µl of each primer (final concentration 10pml/l), 0,2µl Taq polymerase and 5 µl template. The DNA amplification was performed in the BIORAD MyCycler thermal cycler. The PCR product was visualized on 1% agarose gels at 5V cm^{-1} stained with 1x Borax solution, and the following visualization was done under blue light.

Host detection

The reverse line blotting (RLB) hybridization was used as a molecular method to identify the bloodmeal sources in the ticks. A fragment of length 145-bp of the vertebrate mitochondrial 12S rDNA was used as a molecular marker to distinguish vertebrate host species. The method includes a single run polymerase chain reaction amplification of the 12S rDNA molecular marker by using primers 12S-6F (CAAACTGGGATTAGATACC), B-12S-9R (5 biotin-AGAACAGGCTCCTCTAG) (Humair et al., 2007), followed by a reverse line blot hybridization assay by using specific oligonucleotide probes. The palette of probes allowed for distinguishing major groups of host vertebrates, e.g., small rodents, artiodactyls, birds, and lizards, and identifying the bloodmeal sources at the genus or species level (Table 1).

Probes contain N-terminal N- (trifluoracetamidohexyl-cyanoethyl,N,N-diisopropyl phosphoramidite [TFA])-C6 amino linker. Biodyne C membrane was activated in 10 ml of 16% EDAC 1-ethyl-3- (3-dimethylamino-propyl) carbodiimide for 10 min at room temperature and put inside a miniblotter after washing with demineralised water. Residual liquid on the membrane was aspirated. Then 150 µl of each probe, which was diluted to a 50 to 1200 pmol/150 ml concentration in 500 mM NaHCO3 (pH 8.4), was filled into the channels of the miniblotter. Then, the membrane was incubated for 10 min at room temperature. Liquids in slots were aspirated after incubation. The membrane was deactivated in 100 mM NaOH for 10 min after removing from the miniblotter at room temperature. Finally, it was washed in 2X SSPE/ 0.1% SDS for 5 min at 60 °C. The hybridization procedure was based on H u m a i r et al. (2007).The PCR product

Table 1. Oligonucleotide sequences of primers and probes used in PCR amplification and RLB assays

Oligonucleotid primer or probe	Nucleotid sequence (5′ - 3′)	Target organisms
Primers		
12S-6F	CAAACTGGGATTAGATACC	Vertebrates
B-12S-9R	5 biotin-AGAACAGGCTCCTCTAG	Vertebrates
Probes		
Small rodent	5 amino-GGCGGTACTTTATATCCAT	*Muroidea (Muridae, Cricetidae)*
Mus	5 amino-TGCTTAGCCATAAACCTAAAT	*Mus musculus*
Apodemus	5amino-TAAACTTAAATAATTTAATAACAAAACTAT	*Apodemus sylvaticus, A. flavicollis*
Ratus norvegicus	5 amino-AACCTTAATAATTAAACCTACAAAAT	*Ratus norvegicus*
Birds	5 amino-TACGAGCACAAACGCTTAA	*Birds*
rus	5 amino-TGAGCGTCCGCCTGA	*Parus major, P. caeruleus*
Turdus Parus	5 amino-TGATGCTCGATATTACCTG	*Turdus merula, T. iliacus, T. philo-melos, T. pilaris, Parus major, P. caeruleus*
Fringilla	5 amino-TGATGCTTACCCCTACTAA	*Fringilla coelebs, F. montifringilla, Pyrrhula pyrrhula*
Sylvia	5 amino-GCTCGATCTTACTGGAG	*Sylvia atricapilla*
Glis	5 amino-AAACCCTTACTAACGCAAC	*Myoxus glis*
Garrulus	5 amino-TTGACACTCTATGCTACCT	*Garrulus glandarius*
Artiodactyl	5 amino-TATTCGCCAGAGTACTAC	*Bovidae, Cervidae, Suidae*
Capreolus	5 amino-CCTAAACACAAGTAATTAATATAACAA	*Capreolus capreolus*
Lepus	5 amino-TTAAACCTAAATAATTTCCTAACAAA	*Lepus europaeus*
Mustela erminea	5 amino-CATAAATAGTTCTAACAACAAAAC	*Mustela erminea*
Erinaceus	5 amino-GACAGTTACTTAACAAAATTGTA	*Erinaceus europaeus*
Sus	5 amino-ACCCAAATAGTTACATAACAAAA	*Sus scrofa*
Vulpes	5 amino-CTATAACAAAACAATTCGCCA	*Vulpes vulpes*
Canid	5′-amino-CCCTAAACATAGATAATTTTACAACAA	*Canis familiaris, Canis latrans*
Lizard	5 amino-GAGAACTACAAGTGAAAAACT	*Lizards*
Felid	5′-amino-CAAAACTATCCGCCAGAGAA	*Felis catus*
Sciurus	5 amino-AACATAGACACTCAATTAACAAG	*Sciurus vulgaris*

was also visualized on 1% agarose gels at 5V cm^{-1} stained with 1x Borax solution. For the positive control, we used DNA isolating from *Mus musculus*. We excluded the steps with chemiluminescent dyes for the visualisation of the results. We used IRDye 680RD Streptavidin supplied as a liquid in buffer containing 10 mM phosphate, 183 mM NaCl, 2.7 nM KCl, pH 7.4 with sodium azide 0.005% (w/v) as a preservative for results visualization on Li-Core Odyssey DLx Imager.

RESULTS

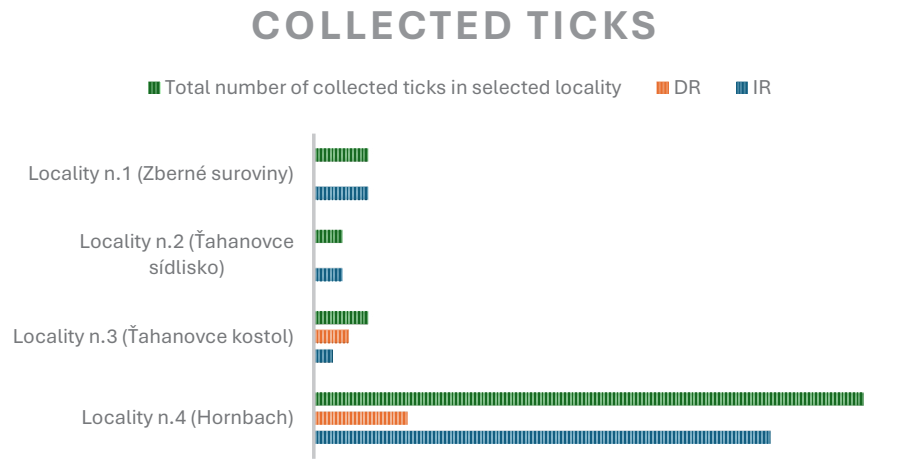
In the first year of the study, 2 species of ticks were identified, *I. ricinus* and *D. reticulatus*, from the 4 localities (1, Zberne suroviny 2, Tahanovce sidlisko, 3, Tahanovce kostol, and 4, Hornbach) (Fig. 1).

A total of 216 were collected. From the 216 questing ticks collected during spring 2022, 72 males, 68 females, 34 nymphs and 2 larvae of *I. ricinus* and 11 males and 29 females of *D. reticulatus* were found (Fig. 2). Individual *I. ricinus* ticks were randomly selected for DNA isolation from each locality (questing larvae were excluded). *I. ricinus* ticks were found in all localities. The *D. reticulatus* ticks were only found in two localities (no. 2, Tahanovce kostol and 4, Hornbach).

Altogether, 40 *D. reticulatus* and 40 *I. ricinus* ticks were tested for the presence of tick-borne pathogens by PCR. The DNA of *Babesia* spp. was amplified from 3 ticks during the initial molecular screening, representing a prevalence of 7,5%. However, no positive samples from the 40 *I. retinues* tick for the presence of *Babesia* spp. were found. DNA of *Rickettsia* spp. was amplified from 3 ticks, *D. reticulatus* (7,5% prevalence) and 4 ticks, *I. ricinus*, with a 10% prevalence.

RLB host identification was performed in all PCR-tested tick samples, and host DNA could be detected in 70% of questing *I. ricinus* and 65% of *D. reticulatus*. More than half of the *D. reticulatus* ticks with identified host DNA (57,5%) were identified at the group level. All identified hosts of the *D. reticulatus* adult

ticks were birds at a group level on which the ticks had parasites in previous developmental stages (Fig. 3). Only 4 ticks of *D. reticulatus* were identified at the species level, with probes capturing birds of the species *Turdus* sp. and *Parus* sp. (Fig. 3). Small rodents (32,5%) and Artiodactyls (25%) were identified in *I. ricinus* ticks at the group level. *Erinaceus europaeus* (n=2) and *Sus scrofa* (n=2) were the most frequently detected in questing *I. ricinus* ticks, followed by the *Capreolus capreolus* (n=1), *Turdus* sp. and *Parus* sp. (n=2) (Fig. 4).



	Locality n.4 (Hornbach)	Locality n.3 (Ťahanovce kostol)	Locality n.2 (Ťahanovce sídlisko)	Locality n.1 (Zberné suroviny)
Total number of collected ticks in selected locality	171	17	9	17
DR	29	11	0	0
IR	142	6	9	17

Fig. 2. Graph of the tick species and number of ticks in selected localities in Kosice (Eastern Slovakia). DR – *Dermacentor reticulatus*, IR - *Ixodes ricinus*

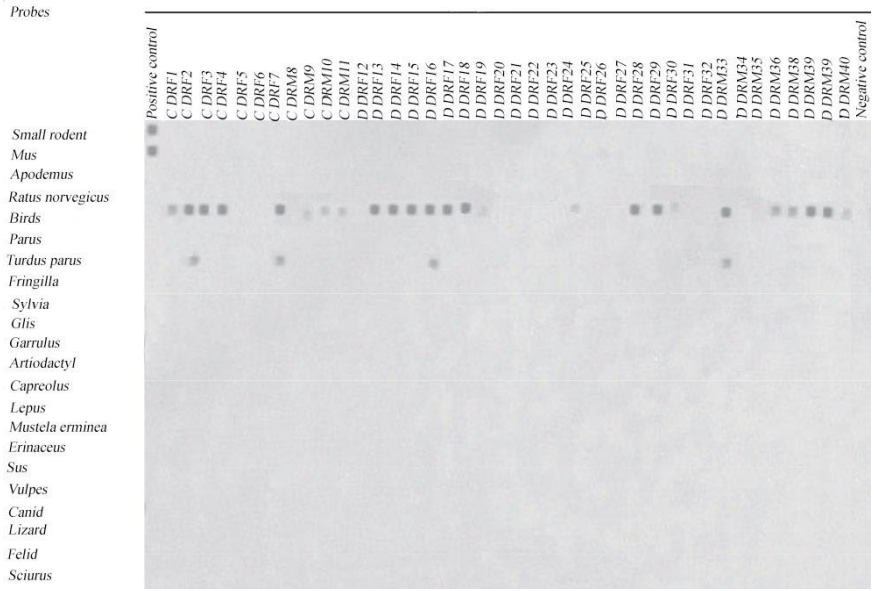


Fig. 3. Biodyne C membrane, visualization of the hosts RLB blood meal analysis hybridization method. On the right are the names of the probes, above are the name of samples in the key (letter C/D: C-Tahanovce kostol, D-Hornbach; DRF – D. reticulatus female, DRM – D. reticulatus male, and numer of ticks). The dots of different intensities represent the chemiluminescent signal of the RLB hybridization.

DISCUSSION

This work describes the occurrence of ticks in urban environments, the prevalence of tick-borne pathogens and the identification of blood meal sources in the *I. ricinus* and *D. reticulatus* ticks.

Questing ticks were collected at the beginning of the tick activity in spring 2022, which indicates that the previous blood meal occurred during the previous tick season and that the method is sensitive enough to detect and identify the source of blood meals that are several months old. A better sensitivity might be expected with the summer or autumnal tick, as observed by P i c h o n et al. (2005despite relatively low detection levels (49.4%, n = 322).

Ixodes ricinus is a hard tick that transmits a large variety of pathogens of medical and veterinary importance and is an ectoparasite infesting various vertebrates and invertebrates (S t a n k o et al., 2022). *Dermacentor reticulatus* can survive in a wide variety of habitats, providing an opportunity to host and transmit a wide range of pathogens. All stages of *D. reticulatus* are more seasonal compared to *I. ricinus* (F ö l d v á r i et al., 2016). Between the 1950s and 1970s, the distribution range of *D. reticulatus* in Slovakia was found to be limited to river basins (M a č i č k a et al., 1955; N o s e k , 1972). *Dermacentor reticulatus* is now expanding northwards and also into the center of towns (R u b e l et al., 2016). Ticks in the cities are a hazard to humans and animals. Studies of urban gradients have also found mixed results, with some suggesting that higher levels of urbanization are associated with lower tick densities in urban green spaces (B u c z e k et al., 2014). Other studies suggested the opposite information about ticks (K o w a l e c et al., 2017)contributing to Lyme disease agents Borreliella [(Borrelia burgdorferi (sensu lato. Factors such as the availability of local hosts for ticks to feed on and become infected, the specific types of habitats under examination, and the connectivity of these habitats to others are likely contributors to these conflicting results. Despite the fact that birds for *D. reticulatus* ticks are occasional hosts (A k i m o v , N e b o g a t k i n , 2016), we found that preimaginal stages of ticks for *D. reticulatus* feed on birds using the RLB hybridization method. For the first time, the immature stage of *D. reticulatus* was found in birds in the southern region of Ukraine (A k i m o v , N e b o g a t k i n , 2016). The results of host feeding blood meal analysis in *I. ricinus* at the subadult stages show feeding on a wide variety of small mammals, such as small rodents, and artiodactyls; other hosts include blackbirds (*Turdus* sp*., Parus* sp.) and *Erinaceus europaeus*. However, other authors found that *I. ricinus* ticks in urban greens are also parasites on lizards (*Lacerta agilis*) (M a t u s c h k a et al., 1991).

Poor fauna is typical for urban green. Small to medium-sized mammals such as hedgehogs and squirrels which can host all life stages of ticks play an active part in the maintenance of tick-borne pathogens in these areas (D z i e m i a n et al., 2014).

In this paper we wanted to point to the high risk of tick infestation in urban green spaces in Kosice (eastern Slovakia). Our observations suggest that urban greenery represents a closed environment that presents an ideal habitat for ticks of their hosts and a close relationship which depends on the vector density or, more exactly, on the vector to host ratio.

Recommendation section

We recommend repellents not only for those who go into the woods but also for those who walk in the city. For humans, repellents come in the form of sprays or oils. For animals, the most effective are tablets that

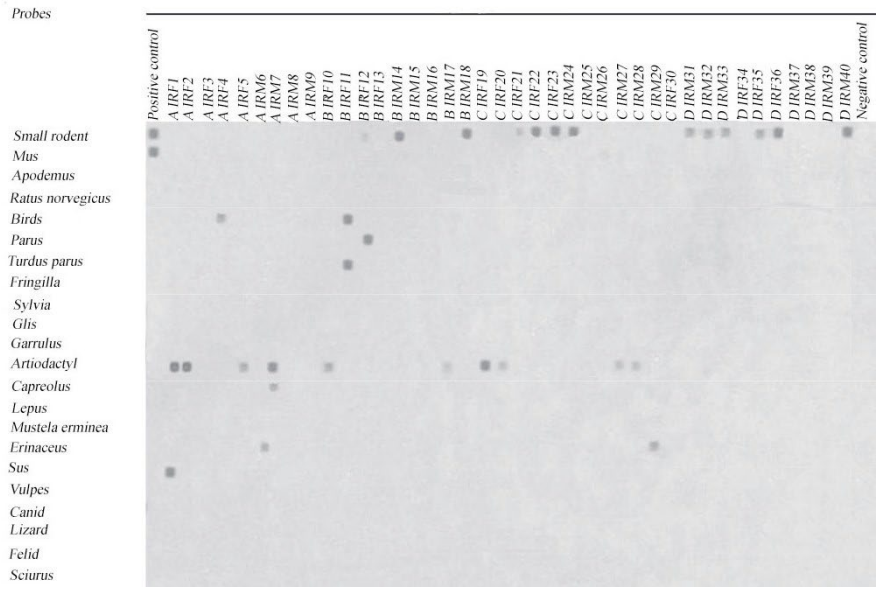


Fig. 4. Biodyne C membrane, visualization of the hosts RLB blood meal analysis hybridization method. On the right are the names of the probes, above above are the name of samples in the key (Letter A,B,C,D: A - Zberne suroviny, B – Tahanovce sidlisko, C-Tahanovce kostol, D-Hornbach, IRF – *I. ricinus* female, IRM – *I.ricinus* male, number of ticks). The dots of different intensities represent the chemiluminescent signal of the RLB hybridization.

release the active ingredient through the skin. There are a number of manufacturers on the market offering different repellents for humans and animals.

CONCLUSIONS

We confirm the occurrence of the ticks *D. reticulatus* and *I ricinus* in the urban environment of Kosice. The main hosts of *D. reticulatus* in immature stages were birds, and for *I. ricinus* was a group of small rodents, artiodactyls and birds. Therefore, immature ticks are introduced into urban areas by birds and mammals. In cities, they then complete their developmental cycle to adulthood and are ready to seek other hosts. We have also confirmed the presence of the pathogens *Babesia* spp. and *Rickettsia* spp., which pose a risk to residents and their animals during walking. This research sends an important public message that these urban walking areas are hotspots of disease risk. Therefore, protective measures for both humans and pets with anti-ectoparasitics should be used.

AUTHOR CONTRIBUTIONS:

Zuzana Cellengová: investigation, writing – original draft, Blažena Hajdová, methodology, investigation, editing, supervision, Branislav Peťko: editing, validation.

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CONFLICTS OF INTEREST:

The authors declare no conflict of interest.

REFERENCES

Akimov, I.A., Nebogatkin, I.V., 2016. Ixodid ticks (Acari, Ixodidae) in urban landscapes. A review. Vestn. Zool. 50, 155–162.

Amore, G., Tomassone, L., Grego, E., Ragagli, C., Bertolotti, L., Nebbia, P., Rosati, S., Mannelli, A., 2007. Borrelia lusitaniae in Immature *Ixodes ricinus* (Acari: Ixodidae) feeding on common wall lizards in Tuscany, central Italy. J. Med. Entomol. 44, 303–307. https://doi.org/10.1093/jmedent/44.2.303

Bellato, A., Pintore, M.D., Catelan, D., Pautasso, A., Torina, A., Rizzo, F., Mandola, M.L., Mannelli, A., Casalone, C., Tomassone, L., 2021. Risk of tick-borne zoonoses in urban green areas: A case study from Turin, northwestern Italy. Urban For. Urban Green. 64, 127297. https://doi.org/10.1016/j.ufug.2021.127297

Boulanger, N., Boyer, P., Talagrand-Reboul, E., Hansmann, Y., 2019. Ticks and tick-borne diseases. Médecine Mal. Infect., Maladie de Lyme 49, 87–97. https://doi.org/10.1016/j.medmal.2019.01.007

Buczek, A., Ciura, D., Bartosik, K., Zając, Z., Kulisz, J., 2014. Threat of attacks of *Ixodes ricinus* ticks (Ixodida: Ixodidae) and Lyme borreliosis within urban heat islands in south-western Poland. Parasit. Vectors 7, 562. https://doi.org/10.1186/s13071-014-0562-y

Casati, S., Sager, H., Gern, L., Piffaretti, J.-C., 2006. Presence of potentially pathogenic *Babesia* sp. for human in *Ixodes ricinus* in Switzerland. Ann. Agric. Environ. Med. 13, 65–70.

Dudek, K., Skórka, P., Sajkowska, Z.A., Ekner-Grzyb, A., Dudek, M., Tryjanowski, P., 2016. Distribution pattern and number of ticks on lizards. Ticks and Tick-borne Diseases 7, 172–179. https://doi.org/10.1016/j.ttbdis.2015.10.014

Dziemian, S., Michalik, J., Pi Łacińska, B., Bialik, S., Sikora, B., Zwolak, R., 2014. Infestation of urban populations of the Northern white-breasted hedgehog, *Erinaceus roumanicus*, by *Ixodes* spp. ticks in Poland. Med. Vet. Entomol. 28, 465–469. https://doi.org/10.1111/mve.12065

Evans, K.L., Hatchwell, B.J., Parnell, M., Gaston, K.J., 2010. A conceptual framework for the colonisation of urban areas: the blackbird *Turdus merula* as a case study. Biol. Rev. 85, 643–667. https://doi.org/10.1111/j.1469-185X.2010.00121.x

Földvári, G., Márialigeti, M., Solymosi, N., Lukács, Z., Majoros, G., Kósa, J.P., Farkas, R., 2007. Hard Ticks Infesting Dogs in Hungary and their Infection with *Babesia* and *Borrelia* Species. Parasitol. Res. 101, 25–34. https://doi.org/10.1007/s00436-007-0608-6

Földvári, G., Široký, P., Szekeres, S., Majoros, G., Sprong, H., 2016. *Dermacentor reticulatus*: a vector on the rise. Parasit. Vectors 9, 314. https://doi.org/10.1186/s13071-016-1599-x

Hansford, K.M., Gillingham, E.L., Vaux, A.G.C., Cull, B., McGinley, L., Catton, M., Wheeler, B.W., Tschirren, B., Medlock, J.M., 2023. Impact of green space connectivity on urban tick presence, density and *Borrelia* infected ticks in different habitats and seasons in three cities in southern England. Ticks Tick-Borne Dis. 14, 102103. https://doi.org/10.1016/j.ttbdis.2022.102103

Humair, P.-F., Douet, V., Cadenas, F.M., Schouls, L.M., Pol, I.V.D., Gern, L., 2007. Molecular Identification of Bloodmeal Source in *Ixodes ricinus* Ticks Using 12S rDNA As a Genetic Marker. J. Med. Entomol. 44, 869–880. https://doi.org/10.1093/jmedent/44.5.869

Kowalec, M., Szewczyk, T., Welc-Falęciak, R., Siński, E., Karbowiak, G., Bajer, A., 2017. Ticks and the city - Are there any differences between city parks and natural forests in terms of tick abundance and prevalence of spirochaetes? Parasit. Vectors 10. https://doi.org/10.1186/s13071-017-2391-2

Mačička, O., Rosický, B., Černý, V., 1955. Poznámky k bionómii, vývoju, zdravotníckemu a hospodárskemu významu pijáka stepného (*Dermacentor marginatus*) v strednej Európe. Práce II Sekc. Slov. Akadémie Vied Séria Biol. 1, 1–43.

Marchant, A., Le Coupanec, A., Joly, C., Perthame, E., Sertour, N., Garnier, M., Godard, V., Ferquel, E., Choumet, V., 2017. Infection of *Ixodes ricinus* by *Borrelia burgdorferi* sensu lato in peri-urban forests of France. PLoS ONE 12. https://doi.org/10.1371/journal.pone.0183543

Matuschka, F.R., Fischer, P., Musgrave, K., Richter, D., Spielman, A., 1991. Hosts on which nymphal I*xodes ricinus* most abundantly feed. Am. J. Trop. Med. Hyg. 44, 100–107. https://doi.org/10.4269/ajtmh.1991.44.100

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

METEOROLÓGIA A KLIMATOLÓGIA - KLIMATICKÉ ZMENY - Trend teploty vzduchu na Slovensku od 1881 [WWW Document], n.d. URL https://milanlapin.estranky.sk/clanky/trend-teploty-vzduchu-na-slovensku-od-1881.html (accessed 11.14.23).

Nosek, J., 1972. The ecology and public health importance of *Dermacentor marginatus* and D. reticulatus ticks in Central Europe. Folia Parasitol. (Praha) 19, 93–102.

Paudel, S., States, S.L., 2023. Urban green spaces and sustainability: Exploring the ecosystem services and disservices of grassy lawns versus floral meadows. Urban For. Urban Green. 84, 127932. https://doi.org/10.1016/j.ufug.2023.127932

Pichon, B., Rogers, M., Egan, D., Gray, J., 2005. Blood-meal analysis for the identification of reservoir hosts of tick-borne pathogens in Ireland. Vector Borne Zoonotic Dis. Larchmt. N 5, 172–180. https://doi.org/10.1089/vbz.2005.5.172

Randolph, S.E., Miklisová, D., Lysy, J., Rogers, D.J., Labuda, M., 1999. Incidence from coincidence: patterns of tick infestations on rodents facilitate transmission of tick-borne encephalitis virus. Parasitology 118 (Pt 2), 177–186.

Rizzoli, A., Silaghi, C., Obiegala, A., Rudolf, I., Hubálek, Z., Földvári, G., Plantard, O., Vayssier-Taussat, M., Bonnet, S., Špitalská, E., Kazimírová, M., 2014. Ixodes ricinus and Its Transmitted Pathogens in Urban and Peri-Urban Areas in Europe: New Hazards and Relevance for Public Health. Front. Public Health 2. https://doi.org/10.3389/fpubh.2014.00251

Rubel, F., Brugger, K., Pfeffer, M., Chitimia-Dobler, L., Didyk, Y.M., Leverenz, S., Dautel, H., Kahl, O., 2016. Geographical distribution of *Dermacentor marginatus* and Dermacentor reticulatus in Europe. Ticks Tick-Borne Dis. 7, 224–233. https://doi.org/10.1016/j.ttbdis.2015.10.015

Sekeyova, Z., Roux, V., Raoult, D., 2001. Phylogeny of *Rickettsia s*pp. inferred by comparing sequences of "gene D", which encodes an intracytoplasmic protein. Int. J. Syst. Evol. Microbiol. 51, 1353–1360. https://doi.org/10.1099/00207713-51-4-1353

Siuda, K., 1993. Kleszcze Polski (Acari: Ixodida).: Systematyka i rozmieszczenie, Kleszcze Polski : (Acari: Ixodida) / Krzysztof Siuda. Polskie Towarzystwo Parazytologiczne.

Stanko, M., Derdáková, M., Špitalská, E., Kazimírová, M., 2022. Ticks and their epidemiological role in Slovakia: from the past till present. Biologia (Bratisl.) 77, 1575–1610. https://doi.org/10.1007/s11756-021-00845-3

Uspensky, I., 2014. Tick pests and vectors (Acari: Ixodoidea) in European towns: Introduction, persistence and management. Ticks Tick-Borne Dis. 5, 41–47. https://doi.org/10.1016/j.ttbdis.2013.07.011

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