Ph.D. THESIS

Ecology of ticks and tick-borne pathogens in urban environments in Romania

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ABSTRACT

Defined by intricate developmental cycles, ticks are temporary blood-feeding parasites of vertebrate animals, and vectors of a great diversity of pathogens that cause numerous animal and human diseases globally (RIZZOLI ET AL., 2014).

Driven by the currently ongoing intense worldwide urbanization process, a growing body of research started focusing on the presence of tick populations in urban habitats, and the impact of such changes on public health.

The presence of ticks has long been documented among the urban fauna, with a high number of reports namely in suburban areas (USPENSKY, 2014). In recent decades, the global rise in urbanization has significantly altered biodiversity levels and impacted both tick-host assemblages and tick infection rates (ESTRADA-PEÑA AND DE LA FUENTE, 2014). Consequent to the human activities connected to this change, many urban and peri-urban sites throughout Europe are deemed as favourable habitats for human-tick encounters. To increase the urban life quality for the city inhabitants, numerous green spaces such as forests, gardens, parks, cemeteries, and large private properties in peri-urban areas inside many of Europe's cities have been preserved. Such locations provide suitable environmental conditions and sufficient host densities for the development of ticks, respectively the maintenance of TBP foci (GINSEBERG AND FAULDE, 2008; RIZZOLI ET AL., 2014; USPENSKY, 2014).

Approximately 700 hard tick (Ixodidae) species have been described to date. The most relevant genera for public health are *Ixodes*, *Haemaphysalis*, *Dermacentor*, *Rhipicephalus*, *Hyalomma,* and *Amblyomma* (PFÄFFLE ET AL., 2013). *Ixodes ricinus,* Europe's most widespread tick species, the main vector for several zoonotic pathogens (RIZZOLI ET AL., 2014), and the most prevalent tick species reported to bite humans in Romania (BRICIU ET AL., 2014; ANDERSSON ET AL., 2018B), customarily prefers habitats with undergrowth and shrub cover like deciduous forests, which can ensure appropriate developmental conditions. Nevertheless, recently a growing number of studies report the presence of established *I. ricinus* tick populations in urban areas such as public parks and gardens (RIZZOLI ET AL., 2014), sites widely frequented by large numbers of citizens.

Still, not all habitats are favourable for tick development, or the circulation of TBPs. To be suitable for the transmission of TBPs, a certain area has to first meet the fundamental abiotic and biotic requirements of ticks and their hosts. These specific factors mediate the development of tick vectors and associated TBPs, as well as the presence, diversity, and abundance of hosts and their interactions with ticks (PFÄFFLE ET AL., 2013).

Lyme borreliosis (LB), the infection caused by the *B. burgdorferi* s.l. complex is the most common zoonotic disease transmitted by *Ixodes* spp. ticks in Europe and North America (STRNAD ET AL., 2017). Other TBPs vectored by *Ixodes* spp. like bacteria of the order Rickettsiales, or *Bartonella* spp., protozoans of the *Babesia* genus, or the TBEV can cause human infections, and in some cases even severe long-term or permanent sequelae (HAGLUND AND GÜNTHER, 2003).

Given the generalist feeding behaviour of *I. ricinus*, co-infections with several micro-organisms are frequently reported in this tick species (REIS ET AL., 2011). Ticks can acquire various strains of bacteria, parasites, and viruses either from a host with multiple infections, by feeding on subsequent hosts (along with the individual development), or through co-feeding mechanisms (PIESMAN AND HAPP, 2001). Transstadial, or in the case of some TBPs (i.e., *Borrelia* spp., *Rickettsia* spp., and TBEV), transovarial transmission in ticks can also contribute to the ecology of such pathogens (SPRONG ET AL., 2009; RIZZOLI ET AL., 2011; KARBOWIAK AND BIERNAT, 2016). Moreover, the transmission of pathogens from co-infected ticks can alter the severity of clinical disease signs in humans or animals, sometimes causing delays or even diagnostic errors (CUTLER

ET AL., 2020). Therefore, due to the potential impact of co-infections in urban ticks and the likelihood of co-transmission of TBPs it is essential to identify local enzootic cycles, even more so in recreational areas.

A number of 532 human Lyme disease cases were serologically diagnosed in Romania and detailed in the most recent infectious diseases report during 2018 (NCSCC, 2018). Concerning the Rickettsiales order, *A. phagocytophilum* has yet to be described from humans, nonetheless, SFG rickettsiae have been reported from human patients (ŞERBAN ET AL., 2009; ZAHARIA ET AL., 2016). The risk of humans acquiring the TBEV in Romania is still unknown due to a passive surveillance system of the TBEV, and no regular screening available (CHITIMIA-DOBLER ET AL., 2020). Still, variable seroprevalence rates against TBEV in humans and animals across several counties in Romania are described (IONESCU ET AL., 2008; SALAT ET AL., 2017).

The ecology of ticks and TBDs is a topic that has received a great amount of attention worldwide in the past decades. Global warming, deforestation, wildlife management, and urbanization are some of the main factors that have shifted the focus of attention towards urban habitats, namely assessing the diversity, abundance, seasonal activity patterns of ticks, and associated TBPs in these environments. To date, a considerable number of studies describe the ecology (SONENSHINE AND ROE, 2013) and pathogens vectored by *I. ricinus* worldwide (KEESING ET AL., 2010;RIZZOLI ET AL., 2014; STRNAD ET AL., 2017). Despite a fair amount of reports analysing the diversity and prevalence of TBPs in questing or engorged ticks (collected from humans or animals), and samples collected from humans in Romania, we still lack important and, in some cases, basic information, especially concerning the situation of urban and peri-urban sites. Through our research we aimed to fill these scientific gaps, as follows:

- Evaluate the ecological factors that influence the diversity, abundance, and seasonal activity of questing ticks in urban and peri-urban areas in Romania
- Analyse the influence of macro-and microscale ecological factors have on the distribution of questing ticks in selected urban micro-locations
- Detect the TBPs in urban and peri-urban questing and engorged ticks, and in tissue samples from wildlife fauna
- Determine the TBPs co-infection rates in the abovementioned sample types
- Perform a comparative statistical analysis of infection rates and pathogen diversity in ticks from urban and peri-urban habitats
- Assess the impact of the movement restrictions generated by the COVID-19 pandemic on the recreational behaviour, risk perceptions, and protective practices concerning ticks of the Romanian public by using a web-based questionnaire

The first part of this thesis (1. Introduction), presents summarized information from the literature regarding general aspects of the biology and ecology of hard ticks, together with an overview of the most common TBPs reported in urban environments in Europe, and urban tick-maintenance hosts. The second part (2. Original research), consists of a total of 4 original manuscripts and is divided into two parts. The first part covers the studies regarding the ecology of questing ticks and TBPs (in questing and engorged ticks) in several urban and peri-urban sites in Cluj-Napoca, Romania (three manuscripts), while the second part focuses on the attitudes of the Romanian public towards ticks during the nationwide COVID-19 lockdown (one manuscript). At the end of the thesis, we summarized our conclusions and lastly, we included the references listed from our studies.

In the first chapter of the second part of this thesis (2.1.1), we aimed to collect information regarding the abiotic and biotic factors that shape the distribution, abundance, and seasonality of hard ticks in Cluj-Napoca, the third-most populous city in Romania. Recently, the urbanization rate in Romania has been on a constant rise. Nonetheless, there is still an overall dearth of information regarding the

ecology of tick populations and the risk of acquiring TBDs in Romania's urban recreational areas. Apart from one study in Iaşi county (PAVEL ET AL., 2014), there is no published data available on the ecology of hard ticks in urban environments, the herein study reporting these data for the first time from the north-western region of Romania. In this regard, we selected seven locations from four types of habitats in Cluj-Napoca: parks, gardens, a cemetery, and peri-urban forests that were surveyed for the presence of questing ticks by flagging. Additionally, hedgehogs, birds, and micromammals were also sampled and searched for ticks, using standard methods (i.e. torch-based searches, ornithological mist nets, snap-traps, etc.), and the vegetation was evaluated in the surveyed areas. Ticks were collected from all the urban sites with variations in diversity and abundance, mostly influenced by climate and local availability of suitable hosts. A higher abundance of ticks was seen in urban parks and gardens compared to the peri-urban forests. Since *I. ricinus* was the most abundant tick species detected both in the environment and on the hosts, tick-management policies to control the risk of TBDs in urban premises should be implemented in cities.

The second manuscript (2.1.2) is the logical continuation of the previously mentioned work. Once again, despite the existence of reports from various counties in Romania concerning the TBPs detected in questing or engorged ticks, and samples from animal hosts, little is known about the TBPs diversity, prevalence, or co-infection rates in questing ticks and wildlife hosts in urban and peri-urban sites throughout Romania (RAILEANU ET AL., 2017; 2018). Thus, we randomly selected and isolated DNA from 443 samples collected during the former study, and by using a powerful broad-spectrum high-throughput approach we screened them for the presence of 44 vector-borne pathogens. A total of 15 pathogens were identified to species and 6 to genus level. Seven *Borrelia* spp. species were identified in questing ticks, and five in engorged ticks. Other pathogens of the order Rickettsiales were also present with variable prevalence. Co-infections were frequent both in engorged and questing ticks. Considering the outcome, we highlight the need to establish proper tick-surveillance programs in cities and include co-infections in the management plan of TBDs in Romania.

The idea behind the third manuscript (2.1.3) was also motivated by the results obtained in our first study. We now knew that urban sites were capable of sustaining established tick populations even in the absence of large animal hosts. Nonetheless, the micro-and macroecological features that cause ticks to favour one microsite over another one from the same location are still largely unexplored. The Campus of the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca (USAMV Campus) ranked second concerning the number of ticks collected in the previous tick-surveillance campaign and considering the overall intensive human activity this location receives we deemed it as a valuable site for further investigations. Therefore, we selected three distinct areas inside USAMV Campus where we collected ticks by flagging, recorded micro-and macro habitat characteristics such as climatic variables (termo hygrometers), fauna activity (snap-traps and trail cameras), and vegetation composition (visual estimation). The results of the flagging campaigns showcased *I. ricinus* as the dominant tick species in all three areas. There were also significant differences among the three locations regarding the number of ticks collected, which appeared to be influenced mostly by climatic variables: average temperature and saturation deficit, and the frequency of fauna visits. Nevertheless, despite no significant differences recorded for the vegetation structures or the local abundance of rodents, the impact of these variables on the distribution of ticks in certain microhabitats should not be overlooked. Further long-term investigations are required for a better understanding of how environmental factors impact questing tick densities in urban microhabitats in a constantly changing climate context.

The second part of the original research (2.2.1) explores the attitudes of the Romanian public toward ticks in a time full of changes and uncertainty faced by humanity, respectively during the nationwide

COVID-19 lockdown. Many countries implemented early quarantine measures as a means to control the spreading of the virus (RUBIN AND WESSELY, 2020). Nationwide lockdown programs have challenged the everyday life and well-being of the human population, with people worldwide experiencing various psychosocial sufferings. Gaining insight into the risk perceptions and the knowledge evolution of the public to emerging or changing health risks is vital for the improvement of public health strategies. Despite nationwide reports of the presence of ticks throughout various environments, the attitudes of the Romanian public regarding ticks and TBDs are largely unknown. Thus we aimed to assess the impact of the movement restrictions generated by the COVID-19 pandemic on the recreational behaviour, risk perceptions, and protective practices concerning ticks of the Romanian society. By using a web-based questionnaire we concluded that even though the majority of respondents spent less time outdoors, they reported finding ticks on themselves or their dogs more frequently. Changes in the preferences for recreational locations, rates of protective practices usage, amount of time spent in specific areas, or tick seasonal activity, might have contributed to this outcome. Most respondents felt like the risk of acquiring a tick or a TBD did not change or decreased during the lockdown. It is possible that the risk of encountering ticks or acquiring a TBD might have been deemed as less important or of least concern by most respondents. Concerning risk groups, men of all ages, senior citizens, and rural inhabitants should be targeted by the relevant Romanian authorities when promoting local or nationwide tick awareness campaigns. Also, greater efforts are needed to encourage the use of prevention behaviours, that were generally even less used during the lockdown.

General conclusions and recommendations

The main aim of this Ph.D. thesis was to investigate the ecology of ticks and TBPs in urban environments in Romania. With our investigations, we managed to bring new information concerning the prospective risks people are facing in habitats mainly used for recreational purposes. We concluded that ticks can, and have established populations in city parks and gardens, while many of the assessed ticks have also proven to be carriers of single, or multiple strains of zoonotic pathogens. Moreover, in the current climatic context, considering all contributing factors globally (socio-demographic, ecological factors), the behaviour of ticks is also adapting to changes, meaning that if the ecological factors are suitable at a macro and microhabitat scale, ticks can use a variety of green spaces, even highly fragmented locations with intensive human activity, lack of large animal maintenance hosts, and periodic vegetation management. Thus, apart from the indisputable benefits of increasing urban green space and promoting its usage in developing cities, city residents should be mindful about these areas also serving as a habitat for urban wildlife and their associated ticks. It is the author's personal belief that the outcomes of the studies performed in Cluj-Napoca concerning the eco-epidemiology of ticks in urban spaces could also be largely extrapolated for other cities in Romania. Greater efforts are required by the Romanian health authorities in the promotion of tick awareness campaigns through various channels not just social media, with a special emphasis on the importance of using tick prevention methods.

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