

CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

Department of Animal Science and Food Processing



Faculty of Tropical
AgriSciences

**Hidden Linkages: Investigating Ixodid Ticks, Tick-Borne
Pathogens, and their Presence in Neglected Ecosystems and
Unconventional Hosts**

PH.D. DISSERTATION THESIS

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Hidden Linkages: Investigating Ixodid Ticks, Tick-Borne Pathogens, and Their Presence in Neglected Ecosystems and Unconventional Hosts

Objectives of thesis

This thesis is driven by the overarching goal of advancing our comprehension of ticks and tick-borne diseases (TTBDs), with a primary focus on the influence of landscape dynamics and the involvement of unconventional host animals. In pursuit of this objective, the study is designed to achieve the following key aims: The first aim involves a meticulous evaluation of the potential risk posed by TTBDs to exotic animals residing in both farm and zoo environments. This investigation aims to shed light on the susceptibility of these unique populations to tick-borne pathogens, thereby contributing to our understanding of the broader ecological implications of TTBDs. The second aim centers on examining the intricate relationship between landscape management practices and the prevalence of ticks. By scrutinizing the impact of human interventions, such as land use changes and fragmentation, on tick populations, the study seeks to unravel the complex interplay between environmental alterations and tick abundance. This aim is crucial for devising effective strategies to mitigate the spread of TTBDs in response to changing landscapes. The third aim involves a comprehensive evaluation, comparison, and proposition of management strategies tailored for the control of ticks and tick-borne diseases. By synthesizing existing knowledge and incorporating novel findings, this aspect of the research aims to contribute practical insights that can inform the development of more effective measures for the prevention and management of TTBDs. These multifaceted aims collectively underscore the commitment of this research to delve into less explored dimensions within the realm of tick ecology. The anticipated outcomes of this study aspire to provide nuanced perspectives, fostering a deeper understanding of TTBDs and paving the way for impactful applications across diverse research domains.

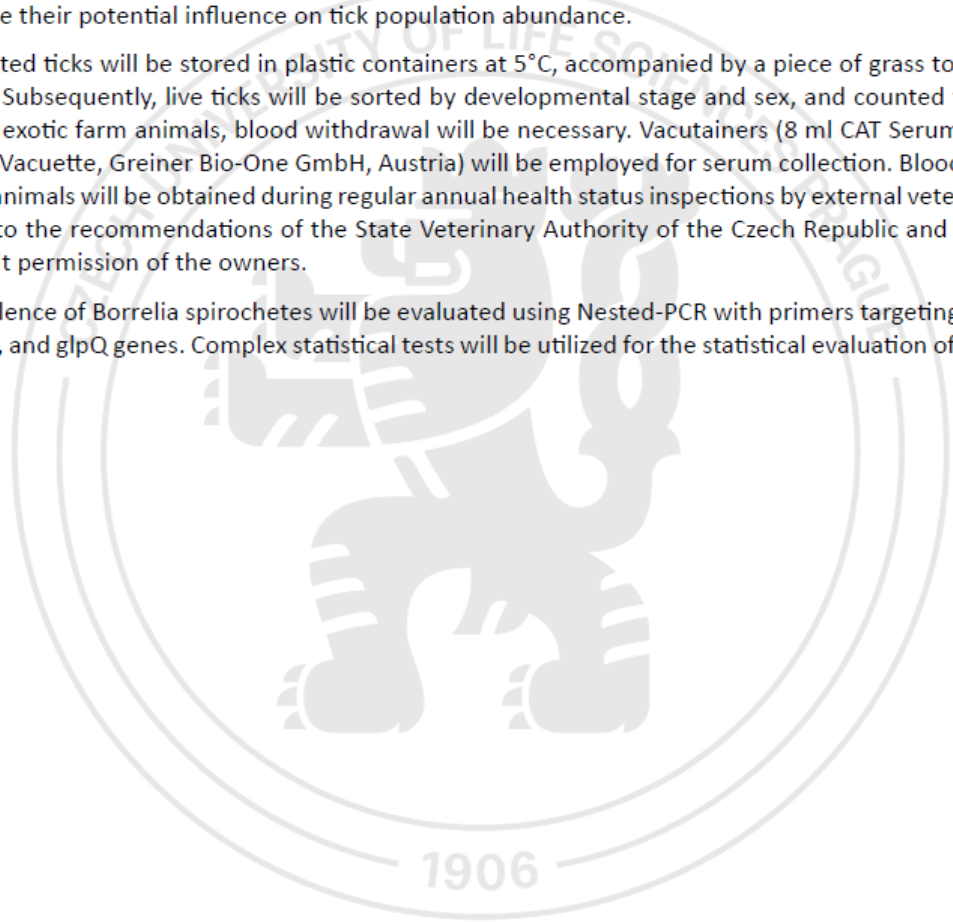
Methodology

There will be multiple methods employed in this research. To estimate the abundance of *Ixodes ricinus* ticks in the areas of interest, we will initiate the process with tick collection, utilizing flagging as our primary collection method. This involves dragging a 0.5 x 0.5 m piece of fabric with a 1 cm hair length over specific transects. Tick activity will be quantified per person-hour of sampling. At the vegetation level, temperature and humidity will be assessed using a hygrometer with an integrated thermometer (LUTRON LM-81HT)

placed on the ground at the level of the vegetation. The objective of these microclimatic measurements is to evaluate their potential influence on tick population abundance.

The collected ticks will be stored in plastic containers at 5°C, accompanied by a piece of grass to maintain moisture. Subsequently, live ticks will be sorted by developmental stage and sex, and counted for evaluation. For exotic farm animals, blood withdrawal will be necessary. Vacutainers (8 ml CAT Serum Sep Clot Activator, Vacuette, Greiner Bio-One GmbH, Austria) will be employed for serum collection. Blood samples from live animals will be obtained during regular annual health status inspections by external veterinarians, adhering to the recommendations of the State Veterinary Authority of the Czech Republic and only with the explicit permission of the owners.

The prevalence of *Borrelia spirochetes* will be evaluated using Nested-PCR with primers targeting flagellin, ospC, p66, and glpQ genes. Complex statistical tests will be utilized for the statistical evaluation of the data.



The proposed extent of the thesis

50-100pp

Keywords

Ixodes ricinus, tick-borne diseases, exotic animals, landscape management, Borrelia spp., tick ecology

Recommended information sources

Daniel E. Sonenshine, Biology of ticks, Volumes 1-3, Oxford University Press

José de la Fuente: Controlling ticks and tick-borne diseases...looking forward. Ticks Tick Borne Dis. 2018 Jul;9(5):1354-1357. doi: 10.1016/j.ttbdis.2018.04.001. Epub 2018 Apr 10.

Nicholas Johnson: Ticks – Biology, Ecology and Diseases, Academic Press, 2023, ISBN: 9780323911481

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Declaration

I, Johana Alaverdyan, hereby declare that I have independently authored this thesis, titled "Hidden Linkages: Investigating Ixodid Ticks, Tick-Borne Pathogens, and Their Presence in Neglected Ecosystems and Unconventional Hosts," with the exception of section 3, "Publications". In this section, all co-authors of the included scientific publications have been duly acknowledged, and their consent for publication as part of this thesis has been obtained. Additionally, I confirm that the thesis comprises original content, and all utilized sources have been appropriately cited and acknowledged in the reference list within the "Thesis references" chapter, adhering to the citation rules of FTA. I further declare that this work has not been submitted for any other degree to this university or any other institution, both within and outside Czechia.

In Prague, 28. 12. 2023

.....

Ing. Johana Alaverdyan

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Abbreviations, Tables, Figures

DEET - N,N-diethyl-meta-toluamide

Hsp20 – Heat shock protein 20

Hsp70 – Heat shock protein 70

IFN- γ – Interferon gamma

LD – Lyme disease

OspA – Outer surface protein A

OspC – Outer surface protein C

Salp15 – Salivary protein 15

SAT – Saliva-activated transmission

SCI - Site of European Community Importance

TBDs – Tick-borne diseases

TBEV – Tick-borne encephalitis virus

TBPs – Tick-borne pathogens

Th1 – T-helper cells 1

Th2 – T-helper cells 2

TTBDs – Ticks and tick-borne diseases

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Abstract

This dissertation explores the intricate world of ticks and tick-borne diseases (TTBDs) by investigating the connections between ticks, their neglected habitats, and unusual hosts. While there is a substantial body of existing knowledge, significant gaps in our understanding of tick-related issues persist. In the context of a changing Earth, including climate change, landscape alterations, and increasing human impact, ticks and tick-borne pathogens are undergoing significant transformations in distribution, genetic diversity, species composition, activity patterns, and resilience. Understanding the impact of landscape use, habitat transformation, and host selection on the prevalence of TTBDs is vital within the complex mosaic of tick-related science. The interplay between ticks, hosts, and habitat is intricate, particularly for exophilic ticks as *Ixodes ricinus*, a key vector for various tick-borne diseases in Europe, including Czechia. To comprehensively evaluate the evolving landscape of tick ecology, a multifaceted, One-Health approach is necessary. This dissertation addresses less explored and understudied issues within the field of TTBDs, offering new perspectives and valuable insights with applications across various research areas. The initial literature review underscores the significance of TTBDs as a crucial concern for zoo-housed animals. However, there is a notable absence of information on TTBDs in exotic farm-kept animals. To address this gap, I conducted a unique empirical study on TTBDs in exotic animal farms in Czechia. The findings revealed that these animals serve as suitable hosts for TTBDs, and notably, large animal pastures are associated with reduced tick activity. This observation was further validated by collecting data in the Milovice natural reserve during rewilding initiatives, where pasture not only contributed to overall biodiversity enhancement but also led to a decrease in tick activity. Conversely, postindustrial landscapes, after years of ecological succession, emerged as an ideal biotope for TTBDs. Drawing on my collected data and insights from the literature, I undertook a comparative analysis of various tick management strategies. Subsequently, I proposed novel approaches to mitigate TTBDs. These recommendations aim to benefit both the general public and specialists involved in addressing this crucial issue. In summary, this dissertation underscores the complexity of TTBDs and the need for a holistic approach to tick-related research. The findings provide valuable insights into tick ecology, tick-ecosystem interactions, and the impacts of rewilding on tick populations. Further research is essential to assess actual risks to both human and animal health, including the effects of tick-borne pathogens on exotic animal species and vector/host competence among such diverse hosts. This research aimed to open new avenues in tick-related studies within the growing field of modern tick research.

Keywords

Ixodes ricinus, tick-borne diseases, exotic animals, landscape management, *Borrelia* spp., tick ecology

1. INTRODUCTION

Ticks, parasitic arthropods of great scientific and social interest, have become a focal point of substantial research efforts in recent years. This surge is evident in the constantly increasing number of published studies, mirroring the emergence of novel problematics and research methodologies concerning tick population biology, ecology, and tick-borne pathogens (TBPs). This rapidly expanding repository of knowledge has unveiled unexplored concerns related to the influence of ticks on human, animal, and environmental health. The embrace of the "One Health" approach has revealed the global threat represented by ticks, underscoring the urgency for inventive strategies to address the challenges posed by ticks and TBPs.

Amidst shifting Earth conditions like climate change, landscape fragmentation, landscape use change, and the burgeoning impact of human activities on the environment, ticks and the ensuing TBPs are undergoing transformations in their distribution (Diuk-Wasser et al. 2020), genetic diversity (Krücken et al. 2021), species composition (Bacon et al. 2022), activity patterns (Ogden et al. 2021), and resilience (Gilbert 2021). In the past four decades, a consistent rise has been observed in the abundance and geographic dispersion of numerous significant tick species. Notably, these ticks have expanded their presence northward and to elevated terrains, thereby increasing their worldwide implications for public and veterinary health (Süss et al. 2008; Léger et al. 2013). This significant ecological reconfiguration within the realm of ticks and tick-borne diseases (TTBDs) has caused substantial financial burdens within both the medical and veterinary domains. Globally, the combined toll of ticks and TBPs culminated in an estimated economic loss ranging from \$22 to 30 billion annually in 2015, solely in the realm of cattle production (Lew-Tabor & Rodriguez Valle 2016). Considering the increase of the cattle population from 1.47 billion in 2015 to an estimated 1.52 billion animals in 2021, as reported by FAOSTAT (FAO 2023), and adjusting for inflationary effects, this figure is poised to escalate substantially in 2023. Another example, in the medical sector, the financial burden associated with Lyme disease (LD) in the United States was appraised with a mean patient cost of approximately \$1,200 (median \$240) and a mean societal cost of approximately \$2,000 (median \$700) during the period spanning 2014 to 2016 (Hook et al. 2022). This translated into an annual aggregate cost for diagnosed LD that swelled to a range as wide as \$345–968 million in the United States only (Hook et al. 2022). These numbers can be expected to increase in the following years if no precautionary measures are taken, and if scientists and governmental bodies do not provide further data and protection strategies.

Conducting research on the subjects covered in this dissertation, such as the impact of landscape utilization and change, along with the exploration of unconventional hosts for ticks, holds significant importance in advancing the overall knowledge base. This knowledge dissemination benefits a wide spectrum of professionals, encompassing infrastructure workers like farmers, forest laborers, woodland managers, veterinarians, medical practitioners, as well as governmental entities. Absence of such insights would compromise

the accuracy and efficacy of forecasts for prospective measures, such as ecological restructuring of landscapes (e.g., rewilding), combatting TTBDs on animal farms or urban areas, and forming of legislative reforms.

The role of landscape fragmentation and habitat transformation in driving the prevalence of TTBDs has been backed by empirical evidence (Piedmonte et al. 2018; Millins et al. 2018; Václavík et al. 2021). Recent transformations in agricultural landscapes hold significant sway in effecting alterations within both vertebrate and invertebrate communities, as noted by Perez et al. in 2016. Landscape properties, including composition and configuration, and biological interactions of fauna and flora, influence the abundance of ticks and their hosts (Fig. 1) (Ehrmann et al. 2017). While many tick species display resilience and adaptability in the face of new ecological pressures, their survival remains hinged upon the availability of suitable hosts (Krasnov et al. 2007; McCoy et al. 2013). The categorization of a host as suitable varies among tick families and species, encompassing both generalist and specialist tendencies within the Ixodida order (McCoy et al. 2013). Continuously, novel host species come to light, and as ticks continue to evolve, new preferences and populations are forged. Such preference changes carry the potential to escalate the infiltration of TTBDs into unexpected areas.

Given that ticks spend the majority of their lifecycle detached from their hosts, their capacity to survive and develop is equally reliant on the environmental conditions they encounter (Perez et al. 2016; Ehrmann et al. 2017). Various habitats exhibit a diverse array of environmental traits, each capable of impacting a tick's capacity for seeking hosts. Numerous publications have highlighted the pivotal functions that humidity and temperature fulfill in shaping the behaviors of ticks as they search for hosts (Schulze et al. 2001; James et al. 2013; Richardson et al. 2022), ticks are therefore more likely to be found in habitats that reduce desiccation risk. For example, if humidity levels drop below 80% the chances of survival diminish in *Ixodes ricinus* ticks and questing periods are shortened, as noted by Gray (1991). Unfed ticks emerge from the sheltered undergrowth and the soil layer, where humidity is higher, and ascend into the higher vegetation layer in search of a potential host (Perret et al. 2000; Schulze et al. 2001). Once on exposed plants, ticks rapidly lose body moisture while awaiting a host. If water loss becomes excessive, ticks must cease their host-seeking and retreat to the soil and leaf layer to rehydrate through sorption (Gray 1984). However, different tick species, such as argasid ticks within the *Ornithodoros erraticus* complex (Diatta et al. 2012) or ixodid ticks like *Ixodes scapularis* (Brunner et al. 2023), are better adapted even to drier environmental conditions. Temperature, in addition to humidity, stands as another important factor influencing tick dynamics. Like most invertebrates, ticks can only remain active above a certain ambient temperature. Furthermore, ticks must continually weigh the energetic costs and mortality risks associated with host-seeking against the likelihood of finding a host. This balance has driven the evolution of diapause patterns, frequently occurring over the winter in temperate areas, within the tick's life cycle, as described by Jensen et al. (1999). However, during the decades dedicated to tick research it has been observed that ticks possess the capability to evade or withstand unfavorable

environmental conditions in nature, and what proves fatal to one stage in their life cycle may not necessarily affect other stages (Brunner et al. 2023).

The interplay between parasites, hosts, and their habitat is especially intricate in the case of exophilic ticks—which actively seek hosts beyond their typical habitat (see Fig.1).

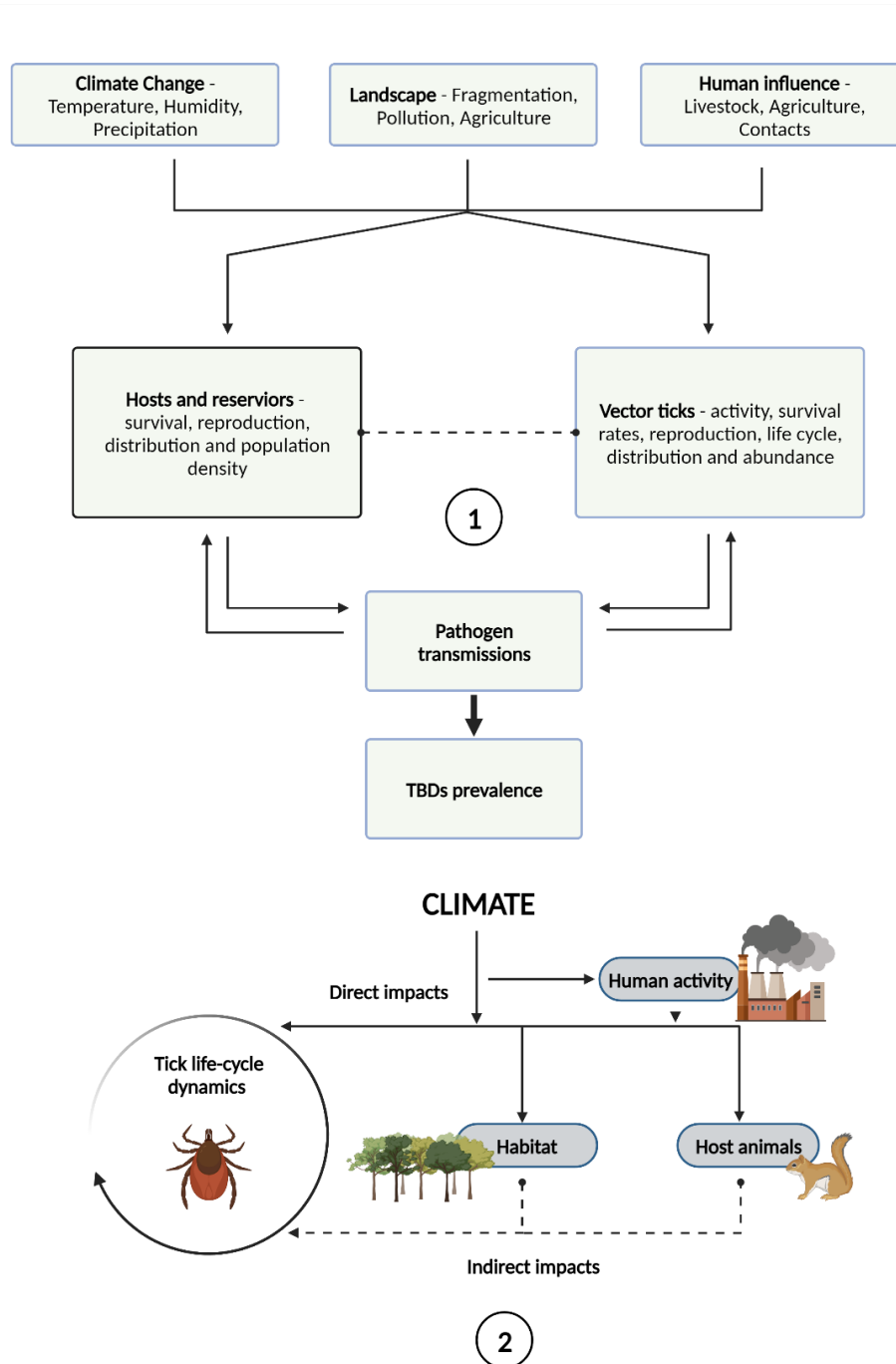


Figure 1, Dynamics of Climate, Landscape, and Human Factors in Ecology of TTBDs: (1) The interplay between shifts in climate, landscape management, and human activities influencing the ecology of ticks and tick-borne diseases. (2) Diagrammatic representation depicting both the direct and indirect effects of climate on the distribution and population densities of ticks.

This intricacy is exemplified by the tick *I. ricinus*, which stands as the most abundant tick species in Europe, including Czechia. This species takes center stage in this dissertation,

presenting itself as a focal point of most of our research. *I. ricinus* is an important vector for various tick-borne diseases (TTBDs) within Europe, including, among others, borreliosis, tick-borne encephalitis, ehrlichiosis, and babesiosis (Heyman et al. 2010). This variety of diseases causes a considerable impact on both human and animal health, underscoring the critical importance of *I. ricinus* ticks as subjects of extensive research. While the intricate ecology of *I. ricinus* has been subject to thorough investigation, the ongoing transformations in land management, climate patterns, and urban expansion have significantly altered the distribution and abundance of these ticks (Kahl & Grey 2023; Janzén et al. 2023). These shifts introduce fresh opportunities and challenges in the regulation of TTBDs dynamics in Europe (Hauck et al. 2020).

For a comprehensive evaluation of the rapidly evolving landscape of tick ecology, multiple factors warrant consideration, and novel perspectives must be studied. Our research has been dedicated to addressing less apparent and understudied issues within this field. These issues deserve attention and recognition since they hold the potential to provide valuable new insights that could have significant applications in various research fields.

1.1 Aims

Considering the aforementioned context, the primary goal of this thesis was to offer enhanced understanding regarding the handling of TTBDs, with particular emphasis on landscape variations and atypical host animals. In pursuit of these objectives, this dissertation endeavors to:

1. Assess the potential risk posed by TTBDs to exotic animals housed in both farm and zoo settings.
2. Assess the relationship between landscape management practices and the abundance of ticks.
3. To evaluate, compare and propose management strategies for the control of ticks and tick-borne diseases.

2. LITERATURE REVIEW

2.1 Tick history

The complex biology of ticks has been extensively studied, resulting in a comprehensive understanding of its characteristics. Ticks are obligatory parasites that exclusively feed on the blood of vertebrate host. The parasitic relationship between ticks and their hosts dates as far back as Paleozoic (Mans et al. 2011, 2016) or Mesozoic era (Beati & Klompen 2019). It is well known that tick-host ties are very strong, and ticks are often dependent on the biology and ecology of their selected hosts. Naturally, ticks developed throughout their long evolution to parasitize on specific wild animals, however eventually some species have adapted to feed on huge variety of animals including domesticated animals introduced to the food chain later in evolutionary development. Over the course of history, an abundance of accounts has recorded ticks and their engagements with domesticated animals. There are records of *Rhipicephalus sanguineus* sensu lato ticks infesting dogs as early as the era of Ancient Egypt (Huchet et al. 2013; Otranto et al. 2014). Aristotle (355 B.C.) described ticks in his *Historia Animalium* as “disgusting parasitic animals” that are “generated from couch grass”. Later Marcus Porcius Cato (circa 200 B.C.) made references to ticks in the context of sheep care, underscoring the role of proper management in reducing tick infestations. Finally, Pliny the Elder (A.D. 77) provided a more comprehensive account of ticks in his *Historia Naturalis*, offering detailed descriptions and highlighting their occurrence among various domestic animals, including dogs, cattle, goats, and sheep (Arthur 1965).

2.2 Tick biology and ecology

Ticks are a ubiquitous, and versatile group with many variabilities between different species. As indicated in the Introduction chapter (pp. 1-4), this work centers on the European castor bean tick, *I. ricinus*. Given its dominance as the most common tick species in Europe, it stands out as the natural and optimal subject for study.

Ticks belong to the class Arachnida, subclass Acari, superorder Parasitiformes and order Ixodida. The Ixodida order is currently divided into five families: three recent families Argasidae, Ixodidae, Nuttalliellidae and two now extinct Deinocrotonidae and Khimairidae (Chitimia-Dobler et al. 2022). Most tick species undergo maturation through several development stages (larva, nymph, adult) (Oliver 1989; Anderson 2002). Ixodid ticks feed once during each developmental stage to trigger molting. In certain species, adult males exhibit stunted feeding organs, hindering their ability to feed. This characteristic is observed in the tick species of interest—*I. ricinus* ticks. (Dantas-Torres & Otranto 2022). Argasid ticks usually feed once only in the larval and nymphal stages but can feed several times in the

adult stages. (Oliver 1989). Some argasid species are able to molt even without feeding or have only rudimentary mouth parts and cannot feed at all in adult stages (Oliver 1989; Santiago et al. 2019). Ticks can survive exceptionally long periods of time without feeding, up to one year in ixodid ticks (Dantas-Torres et al. 2012), and even up to 18 years in argasid ticks like the *Ornithodoros lahorensis* (Hoogstraal 1985). The longest recorded survival time under laboratory conditions was documented in the African tick *Argas brumpti*, which lived for more than 26 years. (Shepherd 2022).

2.3 Reproduction and oviposition

In ticks, reproduction is induced and regulated by specific pheromones of many different types (Roe et al. 2008). Copulation can occur both in the environment, and on the host. Apart from *Argas transversus*, argasid ticks always copulate in the environment (Shepherd 2023). Male ixodid ticks from the *Ixodes* genus reach maturity and produce spermatids before feeding and can mate in the environment or on the host. Female ticks need to feed in order to finish oviposition with the exception of some argasid species capable of autogeny like *Otobius* spp., *Argas persicus*, *Ornithodoros fonsecai* and others (Santiago et al. 2019; Dantas-Torres & Otranto 2022). Spermatophore transfer lasts only few minutes, but ticks can remain in sexual contact for days (Dantas-Torres & Otranto 2022). Some species of ticks are believed to be parthenogenetic like the tick *Amblyomma rotundatum* (Luz et al. 2013). It is hypothesized that parthenogenetic races could form if enough survival pressure is introduced to some ticks as was observed in *Haemaphysalis longicornis* (Chen et al. 2012; Soares et al. 2021). Mating is also one of the regulatory systems influencing tick feeding patterns. Most females will not finish engorging until mated and finish rapid engorgement only after fertilization (Kiszewski et al. 2001). Oviposition follows in several days after final engorgement, but oviposition diapause can follow in unsuitable conditions with temperature being the key factor (Troughton & Levin 2007; Dantas-Torres et al. 2011). After laying a single batch of thousands of eggs the ixodid females die. Unlike ixodid ticks, argasid females can lay several batches of eggs and often display maternal or a brooding behavior (Pienaar et al. 2018). The number of eggs is directly connected to the amount of engorged blood (Troughton & Levin 2007; Dantas-Torres & Otranto 2022).

2.4 Feeding and saliva

The feeding time is varied and can last from several hours to several days or even weeks, depending on the species and development stage (Chmelař et al. 2016; Dantas-Torres & Otranto 2022). The feeding process is complex and is one of the most important processes influencing and incurring the transmission of TBPs (Brossard & Wikel 2004). The feeding process in ticks is not continuous, but rather consists of distinct phases of sucking, salivation,

and resting (Dantas-Torres & Otranto 2022). During the feeding, tick saliva is injected into the bite area alternating with blood meal ingestion by the tick. Since ticks are pool feeders, and ingest all the fluids accumulated in the bite wound most host-tick-pathogen vital interactions are realized during tick feeding (Šimo et al. 2017) (see Figure 2 for details). The reaction of a host to tick attachment and feeding includes natural processes characteristic to any invasion of foreign body on skin and blood stream including vasoconstriction, inflammation, activation of the coagulation cascade, tissue remodeling and forming of hemostatic plug (Kotál et al. 2015) (Fig.2). Tick saliva has evolved to curb all such defenses of the host using complex pharmacological armament which enables the tick to successfully feed even for prolonged periods of time (Liu & Bonnet 2014; Kotál et al. 2015; Chmelař et al. 2016). It has also been demonstrated that tick saliva creates a hospitable environment for TBPs increasing their survivability, propagation and facilitating their transmission (Wikel 1999; Hovius 2009; Kazimirova & Stibraniova 2013) by modulating or disrupting immune response of the host (Wikel 1999; Wikel & Alarcon-Chaidez 2001) and providing matrix for pathogen transmission (Fig.2). We can demonstrate the close feeding-saliva-pathogen interactions on the *Borrelia* spirochetes causing Lyme disease. Initially spirochetes attach passively to the midgut lining of ticks in a state of rest, and they only become active when the tick starts to feed. The activation is induced by upregulation of outer surface protein C (OspC) that starts to replace the dormant-state surface protein OspA (Schwan & Piesman 2000). Once activated, the spirochetes are able to migrate from the midgut into the tick's circulatory system, ultimately targeting the salivary glands. Eventually, they are expelled into the host through the tick's saliva (Schwan & Piesman 2002). Ticks modulate the adaptive immune response by directly affecting B and T lymphocytes through saliva molecules (Fontaine et al. 2011; Kazimirova & Stibraniova 2013; Wikel 2013). Additionally, they induce changes in dendritic and other antigen-presenting cells, as well as soluble mediators of the immune response (Skallová et al. 2008; Mason et al. 2014). Other interferences have been demonstrated like T-helper cells 2 (Th2) polarization of the immune response and diminishing the T-helper cells 1 (Th1) cytokines like IFN- γ (Mejri et al. 2001). Mice infected with *B. burgdorferi* via nymph feeding exhibited a Th-2 cell response, whereas mice injected with a syringe displayed a mixed response involving both Th-2 and Th-1 cells. These differences are probably influenced, at least in part, by the proteins found in tick saliva (Christe et al. 2000). Finally, tick saliva activates the inhibition of coagulation cascade and impacts effectors of innate and adaptive immunity (Guo et al. 2009; Wikel 2013). This implies that the effectiveness of the host's immune response to *Borrelia* spp. infection is shaped, at least in part, by the way the pathogen is influenced by tick saliva. Many other saliva-host-pathogen interactions have been demonstrated, however, to go into more detail about this problematic would be beyond the scope of the dissertation thesis.

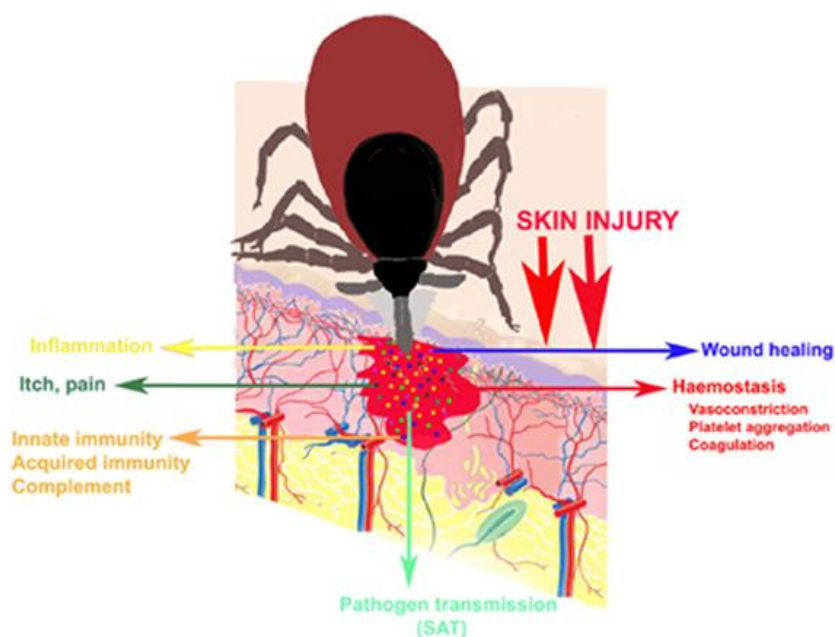


Figure 2: Tick Salivary Modulation of Host Responses and Pathogen Transmission. Tick mouthparts attach via a cement cone, creating a bleeding pool. During their blood meal, ticks release varied active saliva molecules that manipulate host responses (itch, pain, inflammation, immunity). This saliva contains SAT factors facilitating pathogen transmission and infection. Source: Kazimirova & Stibraniova 2013.

The close relationship between ticks and their hosts is further highlighted by the specific patterns of tick drop-off following feeding. The drop-off of ticks, when they are satiated and prepared for molting or oviposition, is intricately linked to the daily rhythms of their hosts (Dantas-Torres 2010). For instance, in the case of *Haemaphysalis leporispalustris* ticks and rabbits, most ticks will detach during the day when rabbits seek refuge in their nests or burrows, which serve as safe habitats for both the ticks and the rabbits. Conversely, tick drop-off during the night, when rabbits are active and outside, is relatively rare (George 1971; Oliver 1989). This example underscores the interconnectedness between ticks and their hosts, shedding light on the complex array of interactions that ticks engage in with their hosts.

2.5 Host selection and specificity

Ticks can be both generalists and specialists in their host selection (Dantas-Torres & Otranto 2022). Generalists can feed on a broad range of various vertebrate species of mammals, amphibians, reptiles, and birds. Specialists on the other hand, feed on selected animal species. Very strict host specificity is apparently uncommon in ticks as is indicated in research comprising 4172 records of hard ticks from Neotropical regions (Nava & Guglielmone 2013) where all tick species were associated with 3 to 20 host animal species. However, host specificity and selection seem to be fairly complex with variability among different

populations of the same species of ticks. For example, *I. ricinus* tick is commonly identified as a host generalist with development and evolution more closely connected to environment than to host biology and ecology (Klompen et al. 1996). However, the formation of the so-called host races has been observed in this and other species of ticks (Magalhães et al. 2007; Kempf et al. 2011) linking tick populations to specific preferred hosts in different areas of occurrence inducing genetic differentiation of studied populations. Some ecological traits of the hosts, like high reproduction rates or low dispersal predispositions for example, would suggest that ticks could favor one host over others at least on a local habitat scale (Kempf et al. 2011). Host preference can change and evolve quickly in hard ticks like *Ixodes uriae* (McCoy et al. 2001; McCoy et al. 2005; Kempf et al. 2009) and even in one-host tick like *Rhipicephalus microplus* (De Meeûs et al. 2010). To further confound the matter of host selection, the habitat ticks live in also influences host selection. For instance, particular tick-repellent compounds discovered in specific plants may play a role in the selection process (Jaenson et al. 2005; Thorsell et al. 2006). Ticks are more likely to avoid areas where such repellent plants thrive even with abundant host presence (Kayaa 2000). Also, more or less alluring host odors could enhance or diminish such habitat predispositions (Douglas et al. 2004; Crooks & Randolph 2006). It is now apparent that host selection by even generalist ticks is not completely random and is closely linked to the area of dispersion of given tick population.

Ticks' remarkable adaptability enables them to parasitize understudied animal species (e.g., non-indigenous zoo animals). Little is known about the prevalence of TBPs in these hosts, their ability to transmit TBPs, and the suitability of exotic hosts for different tick species. Our team has published a comprehensive review on this topic, providing valuable insights for tick and TBP research. The review is provided on pages 32-58 of this thesis.

2.6 Habitats and distribution

Most tick species spend a significant portion of their lifecycle in environments that subject them to considerable selection pressures due to varying abiotic factors (Dantas-Torres et al. 2011). Ticks have evolved a range of strategies to adapt to these conditions. Numerous argasid ticks, along with select *Ixodes* species, have developed a nidicolous life cycle strategy, residing their entire lives within nests, animal shelters, caves, burrows, and even human housing (Guglielmone et al. 2014; Dantas-Torres & Otranto 2022). However, most ticks inhabit a diverse array of ecosystems, spanning from tropical to temperate climates, encompassing steppes, prairies, and even tundra (Guglielmone et al. 2014). Notably, many ixodid ticks display resilience in unexpected arid and hot environments, such as deserts (Brain & Bohrmann 1992).

Traditionally, it is believed that ticks flourish predominantly in primary forests, a notion supported by recent empirical evidence (Lamattina et al. 2018). However, the influx of exotic tick species, facilitated by global changes in the environment caused by the ongoing

climate change (Léger et al. 2013; Lee & Chung 2023), urbanization (Diuk-Wasser et al. 2020), landscape fragmentation (Froeschke et al. 2013) and change of migratory patterns of birds (Butler 2003; Matthysen et al. 2011), has shifted the focus also toward urban, periurban and agricultural areas as equally suitable environments for native and non-native tick species. As temperature and precipitation patterns fluctuate, we are observing concurrent shifts in tick behavior, survival, and maturation (Gilbert 2021; Voyiatzaki et al. 2022). An illustrative case involves the *I. ricinus* tick whose typical active period spans from March to October. However, a noteworthy change was observed when these ticks started questing even in winter, extending from early November to early March (Gray 2008; Gray et al. 2009). The rise in average temperatures has led to escalated metabolic rates, expanded questing durations, and increased opportunities for suitable host encounter, resulting in apparent reductions in the duration of tick development, decreased mortality rates, and heightened rates of host exploitation (Ogden et al. 2004, 2021).

The adaptive ability of ticks to inhabit a broad spectrum of habitats contributes to their global distribution, with certain species spanning multiple countries and even continents. Genera like *Ixodes*, *Haemaphysalis*, and *Amblyomma* are present in all six zoogeographical regions (Afrotropical, Neotropical, Oriental, Australasian, Nearctic and Palearctic), where the *Ixodes* genus boasts the most extensive geographical range. Among its species, 32 (accounting for 13%) are found in multiple zoogeographic regions (Guglielmone et al. 2014). Other genera are restricted to specific regions, such as *Anomalohimalaya* in the Palearctic, *Nuttalliella namaqua* in southern Africa (Mans et al. 2011), or *Nosomma* in the Oriental region (Dantas-Torres & Otranto 2022).

2.7 Prevention and management

Dealing with TTBDs is quite a challenging task, involving various connected factors that need careful attention. Because of the close ecological links between ticks, their hosts, and the environment, it's important to grasp how these variables interact. Our team has taken a close look at this problematic in connection with the widely prevalent *I. ricinus* tick and created an in-depth review article which is presented on pages 12-31 of this thesis.

3. PUBLICATIONS

This dissertation thesis is founded on a compilation of scientific writings, encompassing two scientific review articles, two original research articles, and one original research manuscript currently undergoing revision for publication in a scientific journal. All of the published pieces have been featured in international, peer-reviewed scientific journals with impact factors within the Q1 or Q2 categories of journal rankings in the given field of research. The individual texts have been arranged, both chronologically and thematically, into distinct chapters presented on the following pages, ensuring a coherent presentation.



“The road to tick collection”, Milovice natural reserve (photo by Johana Alaverdyan)

CHAPTER 1 – Management Options for *Ixodes ricinus*-Associated Pathogens: A Review of Prevention Strategies



Looking for ticks in an animal enclosure at Common Eland Research Facilities (photo by Silvie Neradilová)

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Review

Management Options for *Ixodes ricinus*-Associated Pathogens: A Review of Prevention Strategies

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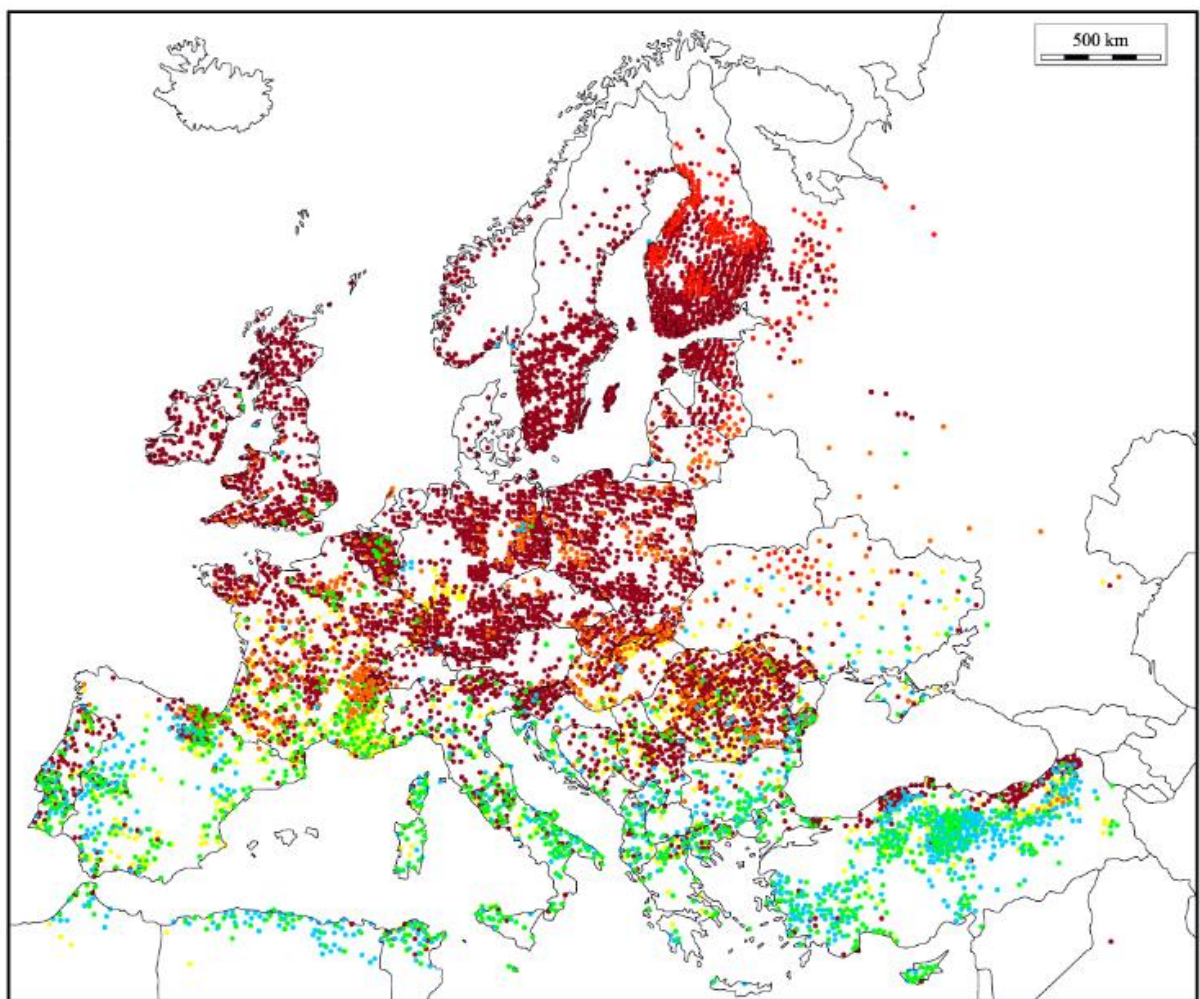


Abstract: Ticks are important human and animal parasites and vectors of many infectious disease agents. Control of tick activity is an effective tool to reduce the risk of contracting tick-transmitted diseases. The castor bean tick (*Ixodes ricinus*) is the most common tick species in Europe. It is also a vector of the causative agents of Lyme borreliosis and tick-borne encephalitis, which are two of the most important arthropod-borne diseases in Europe. In recent years, increases in tick activity and incidence of tick-borne diseases have been observed in many European countries. These increases are linked to many ecological and anthropogenic factors such as landscape management, climate change, animal migration, and increased popularity of outdoor activities or changes in land usage. Tick activity is driven by many biotic and abiotic factors, some of which can be effectively managed to decrease risk of tick bites. In the USA, recommendations for landscape management, tick host control, and tick chemical control are well-defined for the applied purpose of reducing tick presence on private property. In Europe, where fewer studies have assessed tick management strategies, the similarity in ecological factors influencing vector presence suggests that approaches that work in USA may also be applicable. In this article we review key factors driving the tick exposure risk in Europe to select those most conducive to management for decreased tick-associated risk.

Keywords: tick management; tick; *Ixodes ricinus*; tick-borne diseases

1. Ecological Drivers Affecting Tick Activity

Ticks (Ixodida) are the most important vectors of arthropod-borne pathogens in Europe. Out of more than 900 currently described known tick species, approximately 10% are of medical importance to humans [1,2]. In Europe, approximately 70 tick species are established [1]. European tick species are classified within two families and seven genera, namely: *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, and *Rhipicephalus* (all Ixodidae), and *Argas* and *Ornithodoros* (both Argasidae). As a part of the VectoNet project, the European Centre of Disease Prevention and Control (eCDC) monitors seven tick species (*Ixodes ricinus*, *Ixodes persulcatus*, *Dermacentor reticulatus*, *Hyalomma marginatum*, *Rhipicephalus bursa*, *Rhipicephalus sanguineus*, *Ornithodoros* spp.) due to their potential to transmit important human pathogens [3]. Some of tick species, such as *Dermacentor marginatus*, are vectors of important animal diseases [4]. The most important tick vectors in Europe are widely distributed throughout the continent (Figure 1). *Ixodes ricinus* is the most abundant European tick species [1] and therefore, this review will focus on this tick.



- *Ixodes ricinus*
- *Ixodes persulcatus*
- *Dermacentor marginatus*
- *Dermacentor reticulatus*
- *Hyalomma marginatum*
- *Rhipicephalus sanguineus* complex

Figure 1. Ticks in Europe: Distribution of six medically and veterinary important tick species in Europe is depicted. The figure shows geographic locations where important species have been reported (as indicated by colored dots) and was prepared according to data from Estrada-Peña et al. [1].

Ixodes ricinus is a vector for multiple tick-borne diseases common in Europe and as a result has a significant impact on public health. [5]. It is the primary vector for *Borrelia burgdorferi* sensu lato spirochetes, causing Lyme borreliosis, and tick-borne encephalitis virus. Further, many other important tick-borne pathogens (TBPs) such as *Borrelia miyamotoi*, *Rickettsia slovaca*, *Rickettsia helvetica*, *Rickettsia monacensis*, *Anaplasma phagocytophilum*, *Babesia divergens*, *Babesia venatorum*, *Babesia microti*, *Bartonella henselae*, *Coxiella burnetii* and *Francisella tularensis* have been detected in *I. ricinus* and it is either confirmed or likely that this tick species is an important vector for many of these TBPs [6,7]. The active lifecycle of *I. ricinus* includes three developmental stages: larva, nymph, and adult. As a three-host tick, during each of these stages the tick must obtain a single blood meal which allows it to molt and advance to the subsequent stage, or for adult females, to successfully oviposit. Larval and nymphal feeding events offer opportunities for ticks to acquire TBPs from potentially-infected hosts. As most TBPs are either not transmitted transovarially, or have low transovarial transmission efficiency, later developmental stages are generally more likely to carry TBPs [8]. Although *Ixodes ricinus* males may feed on vertebrates to some extent, their role in TBPs transmission is considered minimal. It is however, possible they may influence TBPs ecology via sexual transmission of some tick-borne viruses from infected males to naïve females, as was previously reported in other tick species [9,10].

Ixodes ricinus is present over a broad geographic range, from the Mediterranean coast of north Africa on its southern edge, to the Arctic circle at the northern limit, and from Portugal in the west to Karelia, the Baltic states, and Ukraine in the east, where it shares its biotopes with a closely related tick *I. persulcatus* [1,3]. *Ixodes ricinus* is most frequently observed in lowland habitats, though it has been found on animals at much greater elevations up to 2000 meters above sea level (a.s.l.) in Switzerland [11] (these animals were most likely infested at lower altitudes). Field collection of ticks in Czechia and Austria demonstrated that questing *I. ricinus* of all developmental stages could be found at localities about 1300m a.s.l., though their prevalence rapidly decreased according to increase in altitude [12–15]. In accordance with climate change trends, *I. ricinus* ticks are reported more frequently at higher elevations and new foci are emerging north beyond the previous extent of their range [14,16,17], resulting in an expanded region where people are at risk for tick-borne diseases (TBDs). Taken together, tick activity and prevalence of TBPs are steadily growing in Europe in recent years due to increased activity within long-standing, well established foci, as well as expansion into newly emerging zones [18–22].

Ixodes ricinus is an exophilic tick that actively quests for hosts and consequently, its presence is strongly associated with specific biotope and climatic conditions. Typical habitats where *I. ricinus* are present tend to be lowland, relatively humid biotopes such as unmanaged grasslands, heaths, forest edges, woodlands, and broad-leaf forests with sufficiently dense undergrowth [23]. Key climatic factors influencing tick activity include air and soil temperature, air and soil humidity, and solar radiation [21,24–26]. Additionally, climatic cycles associated with seasonality have a major impact on host-seeking period and diapause. In central Europe, ticks are typically active within the period from March/April to October/November [27], but as they can be active in temperatures close to freezing point, adult ticks can be occasionally found questing during warmer winter days [26]. The lowest relative air humidity limit in which *I. ricinus* has been recorded active is 24% [28]. At these lower limits of tolerable humidity, following brief periods of active questing, relocation to the base of vegetation/soil interface to rehydrate is required for ticks to avoid desiccation [28]. For rehydration, *I. ricinus* requires relative humidity in the microhabitats close to soil to be approximately 85% [29]. In drier conditions *I. ricinus* is not able to survive and this intolerance for low humidity is an important limitation preventing *I. ricinus* from establishing populations in arid regions near the Mediterranean [24]. For this same reason, *I. ricinus* activity in central Europe normally peaks in May/June and in September, which are relatively warm and humid months [21,30,31]. Given that climate, and precipitation in particular are predicted to become more irregular in Europe, it is likely that tick activity will change correspondingly in the future [18].

Another very important ecological determinant for *I. ricinus* is the presence of suitable hosts. *Ixodes ricinus* does not typically aggregate on hosts in large quantities and therefore it does not harm them by blood loss connected with feeding itself like other tick species (e.g., *Amblyomma americanum* or *Rhipicephalus microplus*) can [32]. *Ixodes ricinus* has a very wide range of potential hosts including mammals, birds, and reptiles. Larvae and nymphs feed preferentially on small mammals (rodents, insectivores), birds, and reptiles, while adult females prefer medium size or large mammals (lagomorphs, carnivores, and ungulates). However, the latter set of hosts can also host immature stages [33,34]. Questing ticks were shown to be more frequently present in localities with conspicuous signs of host presence such as wildlife trails and bedding sites, relative to surrounding terrain [35]. Moreover, because *I. ricinus* ticks are ambush predators and consequently, do not actively move long distances, dispersal by animal hosts, especially migratory birds is an important vehicle for establishing new populations of *I. ricinus* [36,37].

The *I. ricinus* life strategy as a host generalist is very important with respect to transmission of TBPs. On one hand, this offers the opportunity to infect many host species. However, not all of these species may be efficient reservoir hosts for pathogens for one of several reasons [38] including transient presence of viremia/bacteremia/parasitemia in the hosts [39,40], low pathogen concentration in host tissues and/or blood, or active host immune response against pathogen [41,42]. Host immunity can even lead to clearance of pathogen not only from the host, but also from the tick itself as in the case of borreliacidal activity of lizard serum [43,44].

2. Anthropogenic Factors Affecting Tick Activity and Abundance of Tick-borne Diseases

While important, environmental factors are not the only factors leading to increased activity of ticks and elevated prevalence of tick-borne diseases. In addition, human alterations to landscapes and changes in human activities that increase exposure to tick inhabited biotopes can amplify TAR.

Several studies have shown that human induced changes in local biotopes that lead to greater abundance of tick hosts also result in increased tick activity and prevalence of TBPs [12,34,45]. Conversely, activities such as forest clearance can lead to decreased tick activity [46]. Effect of grazing on TAR is ambiguous. Grazing can lead to decrease of tick activity as it leads to change in vegetation cover which become more hostile to ticks, but it also provides more potential hosts for tick feeding which could lead to increased TAR [47,48]. The use of insecticides in agriculture can have a significant effect on tick densities. Randolph et al. hypothesized that decrease in agricultural intensity following collapse of Soviet empire in eastern Europe at the beginning of 1990s, was one of the factors leading to increase of tick-borne disease prevalence in these countries [49]. However, this theory could be challenged on the basis that agriculture in eastern European countries has recovered during past 20 years with regard to intensity as well as return to insecticide usage [50,51], yet the incidence of TBDs continues to increase [52].

Epidemiological data also show that incidence of tick-borne diseases increases during periods of economic instability and that low-income economic classes are more vulnerable to this increase [49]. Examples include the prohibitive cost of vaccination against TBEV as well as an increased reliance on activities based in areas where risk of tick exposure is elevated [49]. Furthermore, participation in outdoor leisure activities in high risk areas for tick exposure is one of the key variables determining number of reported tick bites and prevalence of TBPs. It was shown that number of tick bites reports increased during sunny and warm conditions following rainy periods, when more people travel to forested areas for leisure activities, such as hiking, and picking forest berries or mushrooms [53]. Similarly, calendar peaks in nymphal activity and TBEV incidence frequently coincide with peak of mushroom picking period in countries where this activity is a popular leisure activity or a source of occasional income [52,54].

3. Tick Management Actions

Tick-associated risk (TAR), defined here as the potential for exposure to pathogens via tick bite, is heavily influenced by both tick and human activity. Therefore, evidence-based management of these activities can lead to decrease of TAR (see Figure 2 for graphical description of TAR management actions).

To date, the subject of tick management has been studied more extensively in North America than it has been in Europe. Therefore, for certain applications and methods where data from Europe is lacking, the authors of this review are limited to projecting the effect of these measures based on observations in North America. The observed outcomes in North America may not be mirrored in Europe precisely as many conditions (e.g., tick species, host species) are different. In Europe (north of the Alps), *I. ricinus* is the most medically-important tick species as it accounts for vast majority of TBP infections [1]. In North America the *Ixodes* ticks (*I. scapularis* and *I. pacificus*) are also major source of TBP infections, but they are more often accompanied by other ticks from different genera (e.g., *Amblyomma*, *Dermacentor*, *Rhipicephalus*, etc.) which can expand the variety of TBPs present in a given location [55]. Further, Europe and North America belong to different biogeographic zones (Palearctic and Nearctic, respectively). It brings important differences in many aspects such composition of potential host species (e.g., small mammals) as well in vegetation cover. But the minor differences in fauna and flora mentioned earlier, would only create minor differences in TAR.

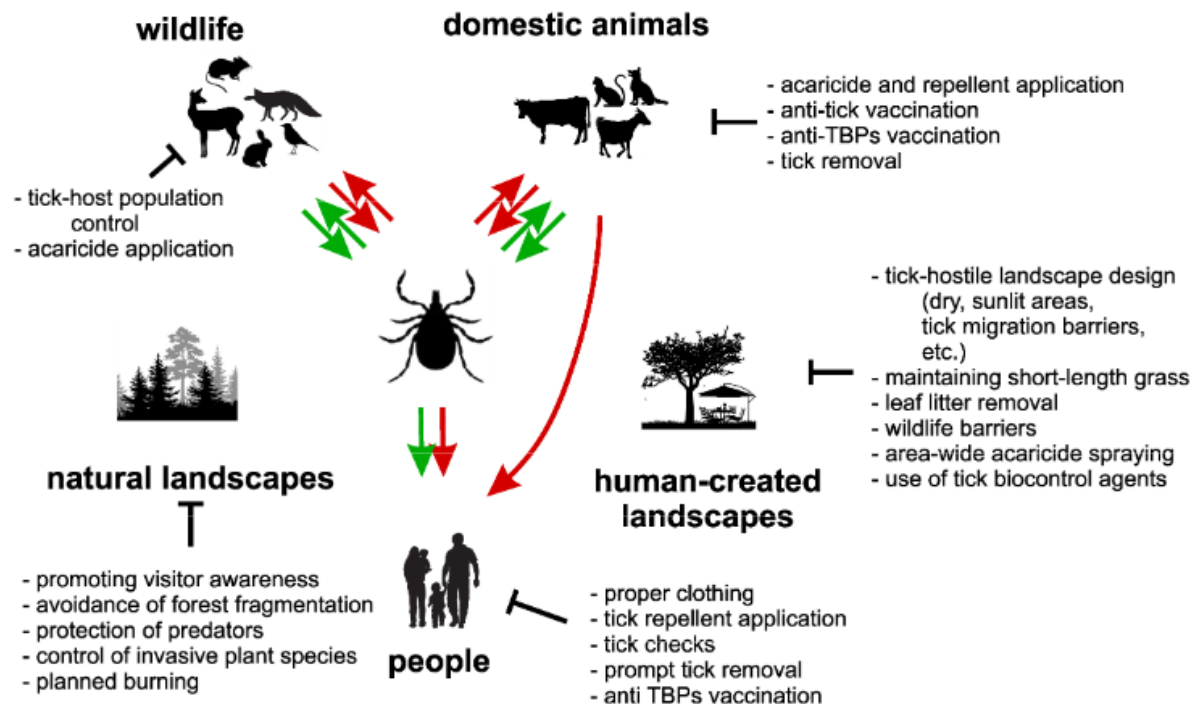


Figure 2. Tick management strategy: Tick activity and risk associated with TBPs can be managed using a broad variety of approaches. These include direct reduction of tick populations using chemical or natural-origin acaricides, tick pathogens (e.g., fungi), or encouraging tick predators. Further, tick activity can be decreased by limiting availability of potential animal hosts (e.g., rodents or ungulates) and by creating tick-hostile environments (dry, sunlit areas lacking leaf litter and other vegetal debris) which limit tick survival and reproduction. Finally, TAR can be decreased by adhering to proper practices (wearing long clothes, using tick repellents, checking body after visiting tick-infested areas and prompt removal of all attached ticks) as well as basic public health (vaccination against TBEV, medical consultation immediately after appearing of any possible marks of infection by a TBP). Green arrows show pathways important in tick life cycle; red arrows show possible avenues of TBP transmission.

3.1. Personal Protective Measures

Personal protection is a specifically targeted, and often, the most practical approach for tick prevention. It includes reducing time spent in tick infested habitats, using the adequate tick protective clothing and repellents, and physically checking oneself for ticks after leaving the tick infested habitats [56].

Ticks are typically not distributed evenly across the landscape and are more often concentrated in habitats with suitable microclimate conditions and higher concentrations of their hosts [57,58]. Therefore, when hiking in natural habitats, it is advisable to avoid shaded places with dense undergrowth and attempt to use areas with open canopies and minimal grass coverage [57,58]. Human use of wildlife trails also increases the likelihood of tick encounter [35]. When hiking through tick-infested localities, one should walk down the middle of the pathway and minimize contact with surrounding vegetation [56].

Wearing light-colored clothes is beneficial as it makes ticks easier to detect and remove before they have the opportunity to attach. Long pants tucked into socks and wearing non-open shoes can reduce contact with ticks [56,59].

Repellents can also dramatically decrease the risk of tick attachment [60,61] and for optimal benefit, proper application of repellents should include consideration for the concentration of active compound and the duration of activity. Modern synthetic anti-tick repellents which can be applied directly on the human skin frequently contain N,N-diethyl-*m*-toluamide (DEET), ethyl butylacetyl-aminopropionate (IR3535) or 1-piperidinecarboxylic acid (picardin), as primary active ingredients. Permethrin-based repellents (3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylic acid, (3-phenoxyphenyl) methyl ester) should be limited to treatment of clothing as the chemical is a skin irritant. The potential for carcinogenic activity has been demonstrated for rats [60,62], but has not confirmed for humans for permethrin [63]. Naturally occurring compounds offer another option and have been used as repellents for many years [64]. Currently, numerous compounds isolated from plant essential oils are being tested and used for their tick repellent activity and they show promising results [65–69]. Moreover, these compounds are considered not to be harmful for humans or ecosystems. Various products are already sold commercially as potential tick repellents, though effectiveness of some of them has yet to be scientifically proven.

Careful inspection of one's entire body after returning from tick infested environment, followed by proper and prompt removal of all found ticks is another important behavior that can help prevent of TBD [56]. Though, some pathogens (e.g., TBEV) are transmitted shortly after tick attachment, others (e.g., *Borrelia burgdorferi sensu stricto* or *babesia apicomplexans*) need up to several days of feeding before being transmitted. Attached ticks should be removed using tweezers or a tool similarly capable of grasping the tick as closely as possible to its mouthparts [70].

3.2. Vaccination, Prophylaxis and Treatment

The only vaccine(s) for TBD currently available for human usage are the vaccine against tick-borne encephalitis virus [71]. A vaccine against Lyme borreliosis approved for human usage was available in USA between 1998 and 2002 but was later withdrawn from the market [72,73]. However, vaccines against Lyme borreliosis are still available for veterinary usage [74,75]. Several research teams are currently working on development of other Lyme borreliosis vaccines, and more broadly, anti-tick vaccines, which could impede tick feeding and interfere with transmission of multiple tick-borne diseases. This approach has previously been applied successfully with BM86 derived vaccine against the cattle tick *Rhipicephalus microplus* [76,77].

For *B. burgdorferi*, it has been demonstrated that a single 200 mg dose of doxycycline within 72 hours after a bite by infected tick can prevent onset of Lyme disease [78,79]. Doxycycline treatment can be in this case offered as an evidence-based treatment option as ticks can be commercially tested for presence of *Borrelia* and results may be available within 24 hours from submission. Among the antibiotics effective against tick-transmitted bacterial infections, doxycycline is the primary option for treatment of Lyme borreliosis and anaplasmosis and is most effective if used during the early stages of infection) [80,81]. Tick-borne piroplasmida are sensitive to quinine derivatives [80,82]. Currently there is no anti-viral drug to cure the infections caused by tick-borne viruses, and therefore treatment in these cases is limited to supportive care. However, several compounds have been proven to inhibit TBEV replication in mice in laboratory experiments [83] and may progress to viable treatment options in the future.

3.3. Tick Host Management

As obligate parasites, ticks are dependent on the presence of appropriate hosts. The same is true for TBPs, which in general, are enzootically maintained. Therefore, suitable management of tick hosts can lead to decrease of both ticks and TBPs.

Mathematical modeling and in field experiments done in the USA have shown that abundance of the North American tick *Ixodes scapularis*, a close relative of *I. ricinus*, is strongly associated with density of deer [84]. A decrease in numbers of deer was consistently associated with a decrease in tick population in following years [85,86]. Field studies in Europe yielded similar results for *I. ricinus* [87,88]. Nevertheless, these studies still need further confirmation [89]. Because population control of large ungulates by hunting is not feasible, it would be interesting to test whether the presence of ungulate predators (e.g., wolves or lynxes) might have an impact on tick activity [90].

Small vertebrates, especially rodents, are important reservoirs of most TBPs, which makes them potential targets for prophylactic approaches. In one such application of this strategy, placing of acaricide containing rodent bait boxes led to decrease in Lyme borreliosis prevalence USA [86]. Similarly, providing the rodents with acaricide-treated cotton for nest lining could lead to decrease in TBPs in treated areas [91]. *Ixodes ricinus* abundance also depends on rodent abundance. Increased activity of rodent predators led to a decrease in tick abundance and a decrease in prevalence of TBP-infected ticks. [92]. Nevertheless, as *I. ricinus* immature stages are not exclusively dependent on small mammals, the potential effect of bait boxes on their populations has yet to be determined at broad scale. Further, deer can be protected from tick-infestation by acaricide application using special "4-Poster" feeding racks that apply acaricide to the head and neck regions as the deer access the feed. One study reported that these devices were associated with a rapid decrease in tick activity in areas studied. [93]. Nevertheless, widespread use of "4-Posters" is complicated because of many factors which include the high price for installation and maintenance, unknown effects on deer and other animals, and potentially, the human health impacts of long term exposure to acaricides contained in the "4-Posters" [94].

In regions with low numbers of wild ungulates but high numbers of livestock, livestock can play a role of the host on which adult tick females feed and it can also serve as reservoir of TBPs [95]. Contrary to livestock, companion animals such as cats and dogs do not play a significant role in ecology of *I. ricinus* or *I. ricinus*-borne diseases. These are usually just occasional hosts for *I. ricinus* and dead-end hosts for *I. ricinus*-borne pathogens. Nevertheless, companion animals also suffer from TBPs. For example, infection of dogs by *Borrelia* spirochetes can lead to arthritis [96].

Apart from those measures which are used also for humans, several additional approaches can be used to decrease TAR for non-human animals. This includes both the anti-tick BM-86-based vaccine working against *Rhipicephalus* spp. but not *Ixodes ricinus* [97] and a vaccine to prevent Lyme disease in canines [98]. Additionally, various acaricides can be applied directly on animal skin, though most require multiple applications over the course of a tick season [99,100].

Finally, it is important to mention possible direct transmission of some TBP from infected animals to humans by contaminated animal product. The best-known example in Europe is transmission of TBEV from small ruminants to humans by unpasteurized milk, leading to a massive outbreak in Rožňava (Slovakia) in 1951 that resulted in the infection of an estimated 660 persons [101]. Direct transmission between infected animals and humans has also been proven for other TBPs such as Omsk hemorrhagic fever virus [102]. Infection with TBPs can also occur through blood transfusions [103,104].

3.4. Landscape Management

Because *I. ricinus* presence is limited by highly specific habitat requirements, carefully planned landscape management can easily lead to significant decrease of TAR. Such landscaping actions should be preferentially targeted to localities with high frequency of human outdoor activities during tick activity season such as city parks, recreational venues, sporting areas, campsites, and other outdoor areas.

Tick management can be challenging in protected environments such as nature reserves, where efforts are made to minimize impact of human activities in these areas. In these instances, the focus should be on informing and promoting visitor awareness of TARs in order to successfully influence behavioral patterns (see previous sections) [56]. Additionally, minimally-invasive actions such as cutting grass short, or leaf litter removal can be performed at minimum, around the visitor centers and the most frequent hiking paths [56]. Planned burning of some localities as a part of fire management has also proven to be effective for management of ticks on other continents [105,106] though data from Europe are absent and planned burns are not currently allowed in most European countries. Furthermore, legislation intended to decrease TAR can be implemented. For example, this might include restrictions designed to prevent forest fragmentation, which can lead create favorable habitat for preferred hosts of certain tick species, including *I. ricinus* [107–109]. Additionally, for certain areas, livestock grazing might offer a cost-effective compromise between productive land use and limiting TAR. Intensive grazing by domestic animals has been shown to decrease TAR in multiple studies [47,48].

In human-altered landscapes such as public parks, stronger measures can be employed relative to natural landscapes. Residential lawns were shown to be poor environments for *I. scapularis* [110], and the reduction of tree canopy cover, thereby increasing sunlight penetration and decreasing humidity, can lead to further reduction in tick activity and survival. Migration of ticks to the maintained areas can be decreased by installing > 1 m wide wood chip, mulch, sand or gravel border between lawn and tick infested areas [111]. Using spaces of this kind that are unwelcoming to ticks for leisure activities such as barbecues etc. could decrease TAR. Likewise, whenever possible, children's playgrounds should be placed in these tick-hostile zones.

Tick management can provide benefits not only in natural and suburban settings, but also in urban areas [112]. For example, city parks have been shown to support high tick densities as well as high prevalence of tick-borne pathogens [113]. Additionally, synanthropic animals have been shown to be in frequent contact with TBPs and could thereby potentially serve as reservoirs of these diseases in urban settings where ticks are also present [114]. Nothing is known about tick activity on brownfields and other abandoned postindustrial localities, which are also part of urban landscape.

As most of tick bites in Europe are reported to occur in publicly accessible areas, which typically account for larger areas than privately owned lands, the primary responsibility to employ adequate anti-tick landscape management lies with local municipalities or districts. Therefore, it is paramount that informed citizens take an active role lobbying their local government agencies and national public health authorities to invest resources toward decreasing TAR. Sufficient financial support is often necessary as these efforts are typically costly and require long-term sustained action to produce measurable results [115]. Fortunately, some of these actions minimizing TAR are being successfully employed in Europe, such as increased percentage of TBEV vaccinated citizens [116], while other solutions, such as increase in the percentage of grazed land are achieved as a part of other landscape management goals [117].

3.5. Acaricides

Acaricides (pesticides that kill ticks and mites), are widely used for tick management and are a particularly effective tool for livestock producers to limit *Rhipicephalus microplus* infestation of cattle [118,119]. Acaricides are also an effective option for managing *I. scapularis* ticks [120]. Nevertheless, several tick species from genus *Rhipicephalus* have already developed resistance against many acaricides [121,122].

The most commonly used acaricides currently are chlorinated hydrocarbons (e.g., lindane), organophosphorus compounds (e.g., coumaphos), carbamates (e.g., carbaryl), formamidines (e.g., amitraz), pyrethroids (e.g., permethrin, flumethrin), formamidines (e.g., amitraz), macrocyclic lactones (e.g., ivermectin), phenylpyrazoles (e.g., fipronil), insect growth regulators (e.g., fluzaron), and isoxazolines (e.g., afoxalaner, fluralaner, sarolaner). These compounds differ with regard to route of application, length of protection, efficiency, specificity, ability of ticks to reach resistance and many other parameters [119,123]. There are yet additional acaricidal options beyond those listed here, which we will not include given both the broad scope of this review, and existence of reviews that cover the subject in greater depth [62,124].

Theoretically, acaricides can be used in two different ways. They can be applied either topically on potential tick hosts, an application that is typical for protection of livestock and companion animals [125]. Alternatively, acaricidal formulations can be sprayed broadly in a liquid formulation, where an area, rather than a host is being treated. This type of application has been demonstrated to be very effective for controlling tick populations on private properties in the USA [120]. Nevertheless, effective acaricidal broad-spraying requires that specific conditions are met including appropriate temporal period of application, favorable weather conditions during application, and typically, use of several rounds of re-application. Therefore, it is often prohibitively expensive in many instances and may only be feasible for smaller areas. Moreover, many acaricides are toxic for insects, beneficial mites, and/or aquatic organisms. In Europe, reports of decreased insect abundance and diversity at both the individual, and the species levels [126]. Therefore, the authors of this review feel that proper acaricide broad-spraying should be recommended only on very limited scale and in absence of other effective options. Currently, novel eco-friendly compounds isolated from natural plant sources with acaricidal activity are being tested with promising results [119,127,128]. Nevertheless, these substances may also impact non-target species, such as ecologically-beneficial arachnids and should be evaluated for such outcomes prior to large-scale application.

3.6. Biological Agents

Several biological agents have been described that could potentially be used to manage tick populations. A minute parasitic wasp, *Ixodiphagus hookeri*, is a geographically widespread tick parasite. The adult females of this wasp lay their eggs into the ticks of many genera (*Ornithodoros*, *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes* and *Rhipicephalus*). Unfortunately, field studies done in New England, where *I. hookeri* was artificially introduced, showed that its presence does not have a significant impact on local tick populations [129].

Ticks are also known to be parasitized by several nematode species [130]. In nature, nematodes from family Mermethidae were found parasitizing ticks [131]. Under laboratory conditions nematodes from families Steinernematidae and Heterorhabditidae showed robust ability to kill *Rhipicephalus* ticks [130].

Perhaps the most promising pathogens of ticks are two fungal species, *Metarhizium anisopliae* and *Beauveria bassiana* [132]. They showed not only a strong capability to kill ticks in the laboratory and field studies, but also to negatively impact tick populations by reducing the reproductive efficacy of survivors [133]. Several strains of them are currently approved for area-wide use in management of ticks [134].

Ticks are also preyed upon by many soil-dwelling arthropods such as predatory mites, spiders, and ants [135]. Engorged ticks of all stages are especially prone to predation [135]. According to anecdotal observations, fowls such as chickens, guinea-fowls, and turkeys are able to reduce local tick populations. The best described tick predators are African oxpeckers, which specialize on various exoparasites of large mammals [136]. One study showed that oxpecker presence was negatively correlated with tick abundance [137]. Additionally, some species of rodents and insectivores are reported to actively feed on ticks [138]. Paradoxically, these animals also serve as very important tick hosts, so their presence could both limit and increase tick density.

Biological control is used for management of mosquitoes and mosquito-borne pathogens and may provide guidance for tick-related applications. This includes two strategies which are very promising in management of mosquito associated risks, yet understudied in TAR management research. First, the impact of midgut microbiome on mosquito vector competence has been studied extensively [139–141]. Several laboratory and field experiments showed that release of *Wolbachia*-infected mosquitoes can reduce prevalence of mosquito-borne diseases such as Zika, dengue, and chikungunya [142]. *Wolbachia* have also been identified in *A. americanum*, yet their effect on vector capacity of this tick is not known [143]. Likewise, other symbionts have been described for a range of tick species and several have been shown to affect pathogen transmission, including a *Rickettsia* endosymbiont of *Dermacentor andersoni* which can facilitate competitive exclusion of a pathogenic species (*R. rickettsia*), and a *Coxiella* endosymbiont of *A. americanum* that may impair transmission of a pathogen that causes ehrlichiosis (*Ehrlichia chaffeensis*) [144,145]. Yet with a few exceptions, the interactions between most microbes and ticks are poorly understood and in general, our understanding of the relationship between tick microbiome and vector capacity is in its early stages [146].

Secondly, genetically modified mosquitoes, especially those bearing mutations causing male sterility or female lethality are widely and successfully utilized in field trials to manage mosquito populations [147]. While this strategy is also a priority for tick-control, ongoing research is still in developmental stages and practical application is therefore unlikely to be an option available for tick management in the short-term.

4. Tick Management in the USA in Comparison to Europe

As in Europe, TAR is a significant public health concern in the USA. However, there are several important differences potentially influencing TAR which influence how it should be managed. In Europe, most exposures to TBPs occur during outdoor activities in public areas. Whereas in high incidence parts of the northeastern USA where suburban residential properties are frequently located in wooded, tick-infested areas, most infections are acquired peridomestically, on private properties [148,149]. In the latter instance, it can be assumed that individuals have greater opportunity to actively manage the environments where they are most likely to be exposed, whereas persons in Europe may have less influence over conditions in settings where they may be exposed, and thereby, greater reliance is placed on the role of local government.

Secondly, in the USA, it is relatively uncommon for livestock to graze in forested environments where *I. scapularis* ticks are present. However, in Europe, recent changes such as more intensive livestock production, and related land usage practices such as grazing in outfield (minimally managed) pastures can lead to increased interaction between *I. ricinus*, livestock and humans [150,151]. Balancing economic interests with public health creates an added degree of complexity these locations.

On the other hand, many ecological drivers affecting tick activity and associated prevalence of TBPs are very similar in Europe and the USA. Therefore, it can be expected that Europe could benefit from adopting approaches that are successfully employed in the USA to improve on current management of TAR. Perhaps the principle lesson to be learned from practices in the USA may be the opportunity to improve deficits in the overall population's awareness about TAR, which would lead to better individual prevention as well as to emergence of new markets focused on management of TAR. In the USA, evidence-based, state-issued advisories for the public and healthcare professionals, related to decreasing TAR are available and typically easily accessible [111,152]. These can be useful tools for land owners to plan and manage their properties according to prescribed recommendations, often with the effect of creating markets for landscape architects, tick testing biotechnological companies, facility management companies and others. Currently, these types of recommendations are not available in most European countries and it is probable that an increase in awareness of strategies to limit TAR could lead to improved practices and a reduction in TBD.

5. Conclusions

Ticks and TBPs pose a significant threat to humans and domestic animals in Europe. Current climatic and social changes have been linked to increases in TAR in areas where ticks have traditionally present, as well in new areas where novel foci are emerging.

The most abundant tick in Europe is *I. ricinus*, and its presence is closely tied to biotope suitability, which includes sufficient humidity, vegetation cover, and the availability of animal hosts. TAR is connected not only with tick abundance but also with human activities in tick infested habitats, many of which can be managed to decrease tick-associated risk.

Proper personal protective practices, beginning with appropriate clothing and use of tick repellents when entering tick infested habitats, as well as personal checks after leaving these areas are basic fundamental behaviors for avoiding tick bites. These measures can be applied not only for humans, but also for domestic animals. Management of wildlife hosts for ticks is also a potentially effective approach, which can include encouraging the presence natural predators of these tick hosts, therein limiting availability of hosts, and ultimately, reducing tick populations and prevalence of TBPs. Further, outdoor public spaces with high human activity during tick season (city parks, recreational spaces, nature preserves, etc.) should be properly managed to decrease TAR. This includes regular grass cutting or grazing, and removal of residual organic debris (cut and flattened grass, fallen leaves, etc.) Because most tick bites in Europe are associated with activities in public outdoor localities, management for TAR should be organized by local municipalities or districts. In cases when simple mechanical measures are not sufficient to decrease TAR, chemical treatment by acaricides can be employed, while carefully accounting for the potential toxicity of these chemicals and adhering to environmental regulations that exist to minimize potential risk for local ecosystems. Use of biological agents, which are much less harmful to other parts of local biotopes, is an alternative to chemical acaricides for controlling tick populations, but even these approaches can have a negative effect on beneficial, non-target arthropod species. Information about the impacts of predators on tick abundance in total, is very sparse and more research is needed [153]. Therefore, further research is needed to develop, test, and release new natural acaricides and improve their specificity. Manipulation of the tick microbiome and genetic modification to reduce vectorial capacity are two additional promising, yet currently under-developed approaches that may eventually be realized.

While there is currently no perfect single solution, TAR can be reduced using an integrated approach to tick management This type of approach has been shown to be very useful at reducing mosquito-borne infections, with the caveat that such programs require close management and long-term maintenance [87,154,155].

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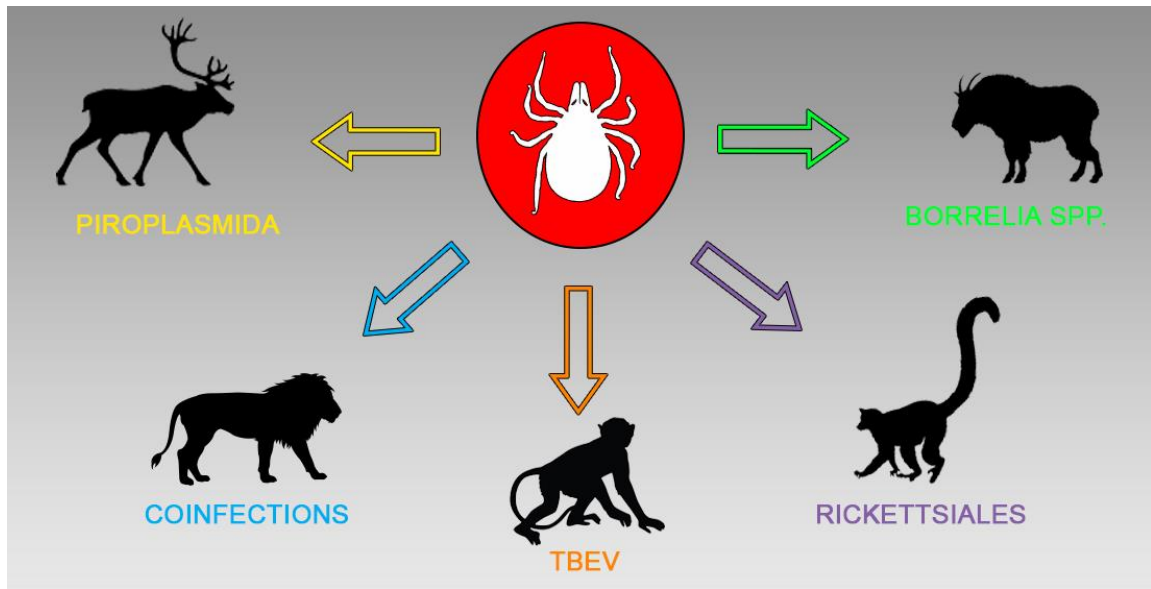
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CHAPTER 2 – Role of Zoo-Housed Animals in the Ecology of Ticks and Tick-Borne Pathogens – A Review



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Review

Role of Zoo-Housed Animals in the Ecology of Ticks and Tick-Borne Pathogens—A Review

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Abstract: Ticks are ubiquitous ectoparasites, feeding on representatives of all classes of terrestrial vertebrates and transmitting numerous pathogens of high human and veterinary medical importance. Exotic animals kept in zoological gardens, ranches, wildlife parks or farms may play an important role in the ecology of ticks and tick-borne pathogens (TBPs), as they may serve as hosts for local tick species. Moreover, they can develop diseases of varying severity after being infected by TBPs, and theoretically, can thus serve as reservoirs, thereby further propagating TBPs in local ecosystems. The definite role of these animals in the tick–host–pathogen network remains poorly investigated. This review provides a summary of the information currently available regarding ticks and TBPs in connection to captive local and exotic wildlife, with an emphasis on zoo-housed species.

Keywords: Ixodidae; ectoparasites; tick-borne diseases; tick hosts; zoo animals; exotic species; wildlife parks

1. Introduction

Ticks (Acari: Ixodidae) are arthropod ectoparasites, distributed worldwide. They are strictly hematophagous and feed on numerous terrestrial vertebrate species, including mammals, reptiles, birds and amphibians [1]. Studies suggest that, on a local scale, host selection of ticks and other ectoparasites is connected mainly with the ecological habitat they occupy [2–4]. Even though ticks are highly adaptable and able to colonize various habitats, they are usually recognized (mainly among the public) as parasites typically found in rural or forest areas. This notion is contradicted by several recent studies which showed that ticks are also frequently observed in urban and peri-urban habitats [5–8]. Typical urban areas inhabited by ticks include recreational areas, parks and cemeteries [9–11]. The increasing rate of urbanization worldwide facilitates the creation of ecotones which are ideal for the emergence of hotspots of tick-borne pathogens (TBPs) that might infect free-living, domesticated and possibly even zoo-housed animal species, potentially also endangering the urban human population [12–14]. Zoological gardens (zoos) are popular urban recreational areas with a semiforested or park-like character. The semi-natural, fragmented environment characteristic for zoos is created to host various animal species with different habitat requirements. This is a factor that positively influences the life cycle of ticks and other ectoparasites [15–18]. That is why zoos are nowadays recognized as potential TBPs refugia [19–22]. Animal species kept in such refugia can therefore potentially serve as tick and TBP reservoirs, allowing further propagation of TBPs within their local ecosystems.

Indeed, several indigenous tick species have been reported in the areas of zoos, wildlife parks or farms worldwide. In the United States of America (USA) and Canada, *Ixodes pacificus* [23], *Ixodes scapularis* [24–26], *Amblyomma americanum*, *Rhipicephalus sanguineus* and *Dermacentor variabilis* [25,27,28] have been reported to exist in such captive exotic animal facilities. In Southern America, Brazilian zoo-animal infection cases have been connected to the following tick species of the *Amblyomma* and *Rhipicephalus* genera: *A. dubitatum*, *A. calcaratum*, *A. aureolatum*, *A. sculptum* or *R. sanguineus* in Southeastern regions of Brazil [21]. More *Amblyomma* species were collected from animals kept in zoos located in Northern and Northwestern Brazil: *A. dissimile*, *A. variatum*, *A. geayi*, *A. longirostre*, *A. goeldii*, *A. humerale*, *A. naponense* or *A. nodosum* [29,30]. In Europe, *Ixodes ricinus* is the most common tick found in zoos and wildlife parks or farms [20,31–34]. Nonindigenous tick species have been reported to feed on zoo animals, for example, the Asian tick, *Amblyomma javanense*, has been found on zoo-kept Asian water monitor (*Varanus salvator*) in South Carolina, USA [25].

All tick species belonging to the tick genera mentioned above (*Amblyomma*, *Dermacentor*, *Ixodes* and *Rhipicephalus*) develop in the three-host life cycle. The three-host life cycle is characteristic in its variability of host selection for each tick developmental stage (larvae, nymph and adult) [12]. Generally, the selection of natural hosts depends strongly on the development stage, in part due to different questing strategies connected to the position of the ticks on vegetation (how high each development stage can climb) [35]. Ideal hosts for tick larvae include small rodents like mice (for example *Peromyscus* spp. [36], *Apodemus* spp. [37]) or voles (for example *Myodes* spp. and *Microtus* spp. [37]), reptiles (like *Bothrops* spp. or *Dispas* spp. in Brazil [38]) and birds (for example migratory species like *Anthus trivialis* in Europe [39] or *Melospiza melodia* in the USA [40]). Such hosts are also suitable for nymphs. Both nymphs and larvae can also be found on larger animals like sheep, goats or other medium-sized animals [41,42]. Adult

ticks frequently feed on larger animals, e.g., species of the Cervidae, Bovidae or Suidae families [35,42]. With each blood meal, ticks can acquire or spread various TBPs either by horizontal (stage-to-stage) transmission, vertical (female-to-egg) transmission or by co-feeding (nonsystemic) transmission [12,43,44]. Natural foci with the potential for emergence of TBPs represent a danger that is supported further by the ability of ticks and TBPs to adapt to host and habitat change [3,4,45,46].

2. Tick-Borne Pathogens in Zoo-Housed Animals

Infections caused by numerous TBPs have been reported in exotic (and local) animals under captive care in zoos, ranches, private farms and other similar facilities in many parts of the world (Figure 1). The various tick species that are able to transmit pathogens and are found in such facilities generally have well-studied vector capacity and competence for pathogens of medical and veterinary importance. Such key information can provide us with information regarding the risk of zoo-housed or urban-dwelling animals contracting tick-borne infections in a given geographic region.

The tick species that belong to *I. ricinus* complex, which are predominant in Eurasian zoos and wildlife farms, i.e., *I. ricinus* and *Ixodes persulcatus*, are the primary vectors of Rickettsiales like *Anaplasma phagocytophilum* [47], tick-borne encephalitis virus (TBEV) [20,48,49], *Bartonella* spp., *Francisella tularensis*, multiple *Borrelia* spp. [20,49,50] and *Babesia* spp. [49,51]. The ticks commonly found in North American and Canadian zoos or ranches, i.e., *I. scapularis* and *I. pacificus*, are also recognized vectors of dangerous pathogens. Both *I. scapularis* and *I. pacificus* are known to transmit spirochetes from *Borrelia burgdorferi* sensu lato complex and *Borrelia myamotoi* [50], *Babesia microti*, *A. phagocytophilum*, *Ehrlichia muris*-like sp. or deer tick virus [52]. *A. americanum* and *D. variabilis* ticks are known vectors of *Cytauxzoon felis* [53]. *A. americanum* is also known vector of *Ehrlichia chaffeensis* [54], *Ehrlichia ewingii* [55], *Rickettsia amblyommii* and *Borrelia lonestari* [56]. *D. variabilis* transmits *Rickettsia rickettsii* - causative agent of Rocky Mountain spotted fever and other Rickettsiales [57]. *R. sanguineus*, found in Southern and Northern American zoos, were confirmed to transmit *Anaplasma platys*, *Hepatozoon canis*, *Cercopithifilaria* spp. [58–60], *Ehrlichia canis*, *Rickettsia massiliae*, *Rickettsia conorii* and *R. rickettsii* [59,60]. The majority of tick species found on animals housed in zoos and botanical gardens of Southern America, Brazil in particular, belong to the genus *Amblyomma*. In the Northern regions of Brazil *A. geayi*, *A. varium*, *A. longirostre* have been confirmed as vectors of *Rickettsia amblyommatis* [29,61–64]. *A. varium*, *A. nodosum* and *A. humerale* are able to transmit *Rickettsia bellii* [29,62–65]. *A. dissimile* was confirmed to carry *Rickettsia* sp. of the colombianensi strain [29,66], *A. nodosum* is also able to carry *Rickettsia parkeri*-like agent [29,65]. Further studies confirmed the presence of *A. sculptum* and *A. aureolatum* the main vectors of *R. rickettsii* (Brazilian spotted fever) in Southern regions of Brazil [21,67]. Other released results revealed the ability of *A. calcaratum* to vector the NOD strain of *Rickettsia* sp. [68] while *A. dubitatum* was confirmed to transmit several *Rickettsia* sp. [69] (see Figure 1 for an overview of the various tick species and their natural geographical distribution).

These findings reveal the heightened risk for captive wildlife animals to be infected with the aforementioned pathogens. The risk of infection, however, is influenced by a large spectrum of factors including the reservoir capacity of the infected animal species or the presence of natural reservoir hosts of selected TBPs (for example, i.e. *Peromyscus leucopus*) that are able to thrive in urban environment [70]. The clinical manifestation of tick-borne diseases (TBDs) depends on the infected animal species; they can be hidden and non-specific, which leads to underestimates of the epizootiology and pathology of many TBDs and their related issues among captive wildlife species. However, there are also reports of infections of tick-borne pathogens which have led to serious diseases and even fatalities, as will be discussed in this review.

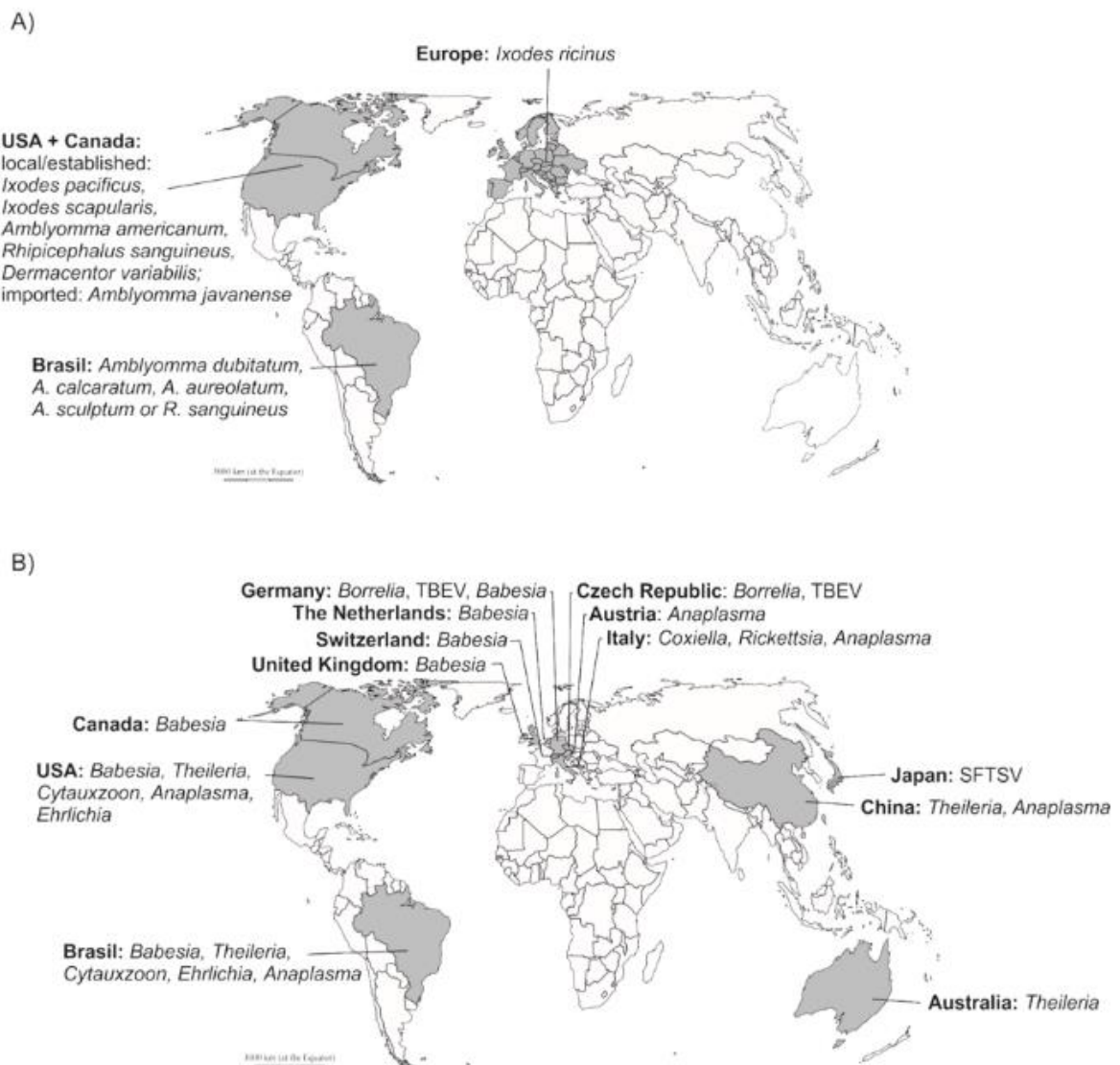


Figure 1. Ticks and tick-borne pathogens reported from zoo-housed animals: Ticks (A) or tick-borne pathogens (B) feeding on/detected in zoo-housed animals were found in all countries where this kind of research was performed. It indicates that zoo-housed animals may serve as hosts and reservoirs for local/established but also imported ticks and tick-borne pathogens. Nevertheless, lack of wider data and their anecdotal nature does not allow us to make definitive presumptions. Further research is needed to help us in understanding of the role of zoo-housed animals in tick biology. TBEV—tick-borne encephalitis virus. SFTSV—severe fever and thrombocytopenia syndrome phlebovirus

3. Tick-Borne Encephalitis Virus (TBEV)

The TBEV can infect a wide range of mammals [71]. In humans, nonhuman primates, dogs and some rodent species, it can cause serious, and sometimes fatal, meningoencephalitis [72–74]. In ungulates, TBEV usually causes a subclinical infection, but the virus can be excreted into the milk of viremic individuals [75]. In rodents and insectivores, TBEV infection leads to long viremia without symptoms; this makes such species suitable reservoirs for the virus [71,76,77].

In 2006, a fatal case of TBEV infection was described in a female Barbary macaque (*Macaca sylvanus*) kept within the monkey enclosure of a zoo situated in southern Germany [78]. The monkey suffered staggering paresis of the hindlegs, incoordination and intermittent opisthotonos, before entering a coma four days after the onset of these symptoms. The comatose monkey was subsequently euthanized, and a post mortem necropsy, polymerase chain reaction (PCR) tests and histological tests confirmed an infection with TBEV. Even though this was the first described case of a natural TBEV infection in macaques, it was very similar to experimental infections of macaques used as model organisms for TBEV pathogenesis [78,79]. Later, serological tests were conducted on the remaining 283 macaques living within the same enclosure; among them, six (2.1%) were seropositive for anti-TBEV antibodies [72]. Anti-TBEV antibodies were also detected in sheep on the neighboring pastures, with a seroprevalence of 9% [72]. Similar cases could be prevented in the future, as macaques (and probably other primates) are likely to develop anti-TBEV immunity after vaccination with TBEV vaccines designed for human-use [80].

On the other hand, tests for anti-TBEV antibodies among other zoo animals were mostly negative, according to previous Czech zoological research results [20]. In this research, only two seropositive samples were recorded out of 133 tested serum samples from 69 animal species: one from a markhor (*Capra falconeri*) and one from a reindeer (*Rangifer tarandus*), as confirmed by both enzyme-linked immunosorbent assay and a neutralization test.

4. Lyme Borreliosis Spirochetes

Lyme borreliosis (LB) spirochetes can cause systemic disease in humans, nonhuman primates, carnivores, ungulates and some rodent species [81–83], causing pathological changes in the skin, joints, heart and central nervous system [84,85]. However, clinical symptoms of LB in different animal species are variable [86,87]. They are influenced by, among other factors, the species of the *Borrelia* species and strain [88–90], as well as the host animal species and its breed. Different symptoms can be observed between horses [91,92], dogs [92–94] and natural hosts, like the white-footed mouse (*P. leucopus*) [90]. However, in many individuals, *Borrelia* infection symptoms are nonspecific, and asymptomatic infections are common in seropositive animals with lower antibody titers [87,91,94].

The prevalence of *Borrelia* among zoo animals has been investigated in Germany and the Czech Republic [19,20]. High numbers of *Borrelia*-infected individuals, or individuals having anti-*Borrelia* antibodies, were found in both studies. In the Czech Republic, DNA from spirochetes of the *B. burgdorferi* sensu lato complex was detected in a significant number of the tested vertebrate serum samples (69 positive cases, out of 133 tested samples – 51.8% affected). Those species with the highest number of positive samples were the Barbary sheep (*Ammotragus lervia*) with five positive samples (total sample size: n = 6),

Grant's zebra (*Eguus quagga boehmi*) also with five (n = 6), Hartmann's mountain zebra (*Equus zebra hartmannae*) with four positive samples (n = 5), Grey wolves (*Canis lupus*) with four positives (n = 4) and Addax (*Addax nasomaculatus*) with five positive samples (n = 5) (Table 1; [20]). In Germany, sera from 1487 zoo animals were tested for the presence of anti-*Borrelia* antibodies. One hundred fifty-four samples (10.4%) were positive, while 168 samples (11.3%) produced borderline results. The highest number of positive samples was observed in Przewalski horses (*Equus przewalskii*), with 22 positives out of 98 tested animals, lions (*Panthera leo*), where 11 out of the 49 tested lions were positive, and forest buffalo (*Syncerus caffer nanus*), where four out of nine were positive ([19]; Table 1). Considering these studies [19,20,22], it is obvious that several animal species are susceptible to *Borrelia* infection. Among these are also the domestic goat (*Capra aegagrus f. hircus*), Barbary sheep (*A. lervia*), markhor (*C. falconeri*), mountain goat (*Oreamnos americanus*) and llama (*Lama guanicoe*) (Table 1). However, in some cases, the results of these studies varied. For example, in the German study, significant numbers of positive sera samples were found in domestic cattle (*Bos primigenius f. taurus*) and impala (*Aepyceros melampus*) [19]; however, in the Czech study, the sera of these animal species were negative [20]. On the other hand, the opposite was true for African wild dogs (*Lycaon pictus*) within the two zoos [19,20].

Table 1. The prevalence of *Borrelia* specific antibodies in exotic zoo animals in Czech Republic and Germany together with observed borrelicidal effect of zoo animal sera.

Group	Family	Animal Species	Borrelia Seroprevalence		Borrelicidal Effect				
			DE	CZ	<i>Borrelia burgdorferi</i> s.s.	<i>Borrelia garinii</i>	<i>Borrelia afzelii</i>		
Odd-toed ungulates	Equidae	<i>Equus africanus f. asinus</i>	13/1 (8%)	2/2 (100%)	weak to moderate	moderate to strong	moderate to strong		
		<i>Equus africanus somaliensis</i>	10/1 (10%)	1/1 (100%)	*	-	-		
		<i>Equus ferus caballus</i>	5/2 (40%)	3/3 (100%)	-	-	-		
		<i>Equus ferus ferus</i>	-	5/5 (100%)	-	-	-		
		<i>Equus grevyi</i>	18/1 (6%)	-	-	-	-		
		<i>Equus hemionus kulan</i>	12/2 (17%)	-	-	-	-		
		<i>Equus przewalskii</i>	98/22 (22%)	-	-	-	-		
		<i>Equus quagga</i>	33/9 (27%)	8/7 (88%)	weak	strong	Strong		
		<i>Equus zebra</i>	25/1 (4%)	5/4 (80%)	weak	strong	Strong		
		Tapiridae	<i>Tapirus terrestris</i>	10/2 (20%)	-	-	-	-	
		Rhinocerotidae	<i>Ceratotherium simum</i>	3/2 (67%)	-	-	-	-	
			<i>Diceros bicornis</i>	-	7/0 (0%)	-	-	-	
		Even-toed ungulates	Cervidae	<i>Alces alces alces</i>	13/2 (15%)	1/1 (100%)	-	-	-
				<i>Cervus albirostris</i>	10/1 (10%)	-	-	-	-
<i>Cervus canadensis</i>	-			1/1 (100%)	-	-	-		
<i>Cervus elaphus bactrianus</i>	11/0 (0%)			-	-	-	-		
<i>Cervus elaphus hippelaphus</i>	37/0 (0%)			-	-	-	-		
<i>Cervus eldi thamin</i>	10/1 (10%)			-	-	-	-		
<i>Cervus nippon pseudaxis</i>	20/0 (0%)			-	-	-	-		
<i>Cervus timorensis</i>	3/1 (33%)			-	-	-	-		
<i>Dama dama dama</i>	20/0 (0%)			-	-	-	-		
<i>Elaphurus davidianus</i>	14/0 (0%)			-	-	-	-		
<i>Moschus moschiferus</i>	4/3 (75%)			-	-	-	-		
<i>Rangifer tarandus</i>	13/1 (8%)			1/1 (100%)	-	-	-		
<i>Capreolus pygmaeus</i>	14/1 (7%)			-	-	-	-		
<i>Capreolus bedfordianus</i>	48/3 (6%)			1/1 (100%)	-	-	-		
Camelidae	<i>Lama vicugna</i>	5/1 (20%)	-	-	-	-			
Suidae	<i>Phacochoerus africanus</i>	-	1/0 (0%)	weak	weak	Weak			
	<i>Addax nasomaculatus</i>	-	5/5 (100%)	-	-	-			
Bovidae	<i>Aepyceros melampus</i>	6/1 (17%)	3/0 (0%)	strong	strong	weak to strong			
	<i>Ammelaphus imberbis</i>	-	2/2 (100%)	-	-	-			
	<i>Ammotragus lervia</i>	19/1 (5%)	6/5 (83%)	moderate	weak	Moderate			
	<i>Antidorcas marsupialis</i>	-	1/0 (0%)	-	-	-			

<i>Antelope cervicapra</i>	16/1 (6%)	-	-	-	-	-
<i>Bison bison</i>	14/2 (14%)	-	-	-	-	-
<i>Bison bonasus</i>	17/0 (0%)	-	-	-	-	-
<i>Bos gaurus</i>	8/1 (13%)	-	-	-	-	-
<i>Bos gaurus f. frontalis</i>	4/1 (25%)	-	-	-	-	-
<i>Bos javanicus</i>	23/2 (9%)	-	-	-	-	-
<i>Bos primigenius f. Taurus</i>	21/2 (10%)	2/0 (0%)	weak	strong	strong	Moderate
<i>Boselaphus tragocamelus</i>	7/2 (29%)	-	-	-	-	-
<i>Bubalus arnee f. bubalis</i>	9/2 (22%)	-	-	-	-	-
<i>Budorcas taxicolor</i>	11/3 (27%)	-	-	-	-	-
<i>Capra aegagrus cretica</i>	9/1 (11%)	-	-	-	-	-
<i>Capra aegagrus f. hircus</i>	17/4 (24%)	5/5 (100%)	-	-	-	-
<i>Capra caucasica</i>	-	1/1 (100%)	-	-	-	-
<i>Capra falconeri heptneri</i>	12/5 (42%)	1/1 (100%)	-	-	-	-
<i>Capra ibex nubiana</i>	6/2 (33%)	-	-	-	-	-
<i>Cephalophus natalensis</i>	1/1 (100%)	-	-	-	-	-
<i>Connochaetes gnou</i>	-	3/1 (33%)	weak	strong	strong	Weak
<i>Dama tiscus pygargus philippi</i>	-	1/1 (100%)	-	-	-	-
<i>Eudorcas thomsonii</i>	-	2/2 (100%)	-	-	-	-
<i>Gazella dama</i>	9/1 (11%)	-	-	-	-	-
<i>Hemitragus jemlahicus</i>	10/0 (0%)	-	-	-	-	-
<i>Hippotragus equinus</i>	-	1/1 (100%)	-	-	-	-
<i>Hippotragus niger</i>	-	4/3 (75%)	moderate	strong	strong	Strong
<i>Kobus ellipsiprymnus</i>	11/1 (9%)	1/0 (0%)	moderate	moderate to strong	moderate to strong	moderate to strong
<i>Kobus leche</i>	-	1/1 (100%)	-	-	-	-
<i>Naemorhedus caudatus</i>	-	2/0 (0%)	weak	moderate to strong	moderate to strong	Moderate
<i>Nanger dama</i>	-	5/3 (60%)	weak to moderate	moderate	moderate	Moderate
<i>Oreamnos americanus</i>	20/9 (45%)	2/2 (100%)	-	-	-	-
<i>Oryx gazella dammah</i>	10/0 (0%)	5/3 (60%)	moderate	strong	strong	weak to strong
<i>Oryx gazella gazella</i>	10/0 (0%)	2/2 (100%)	-	-	-	-
<i>Ovibos moschatus</i>	11/8 (72%)	-	-	-	-	-
<i>Ovis ammon f. aries</i>	83/8 (10%)	5/3 (60%)	moderate	strong	strong	Moderate
<i>Ovis ammon musimon</i>	18/3 (17%)	-	-	-	-	-
<i>Ovis dalli</i>	3/1 (33%)	-	-	-	-	-
<i>Ovis nivicola</i>	1/1 (100%)	-	-	-	-	-
<i>Pseudis nayaur</i>	11/0 (0%)	-	-	-	-	-
<i>Rehinca rehinca</i>	14/0 (0%)	1/0 (0%)	strong	strong	strong	Weak
<i>Saiga tatarica</i>	31/1 (3%)	-	-	-	-	-
<i>Syncerus caffer caffer</i>	17/2 (12%)	1/0 (0%)	weak	weak	weak	Weak

	<i>Syncerus caffer nanus</i>	9/4 (44%)	-	-	-	-	-
	<i>Tragelaphus angasi</i>	-	2/1 (50%)	weak	weak	weak	Weak
	<i>Tragelaphus streptoceros</i>	10/0 (0%)	2/2 (100%)	-	-	-	-
Giraffidae	<i>Giraffa c. reticulata</i>	-	1/0 (0%)	moderate	strong	strong	Strong
	<i>Giraffa c. rothschildi</i>	-	2/0 (0%)	moderate	strong	strong	Strong
	<i>Acinonyx jubatus</i>	-	1/0 (0%)	weak	weak	weak	Weak
	<i>Crocuta crocuta</i>	-	1/1 (100%)	-	-	-	-
	<i>Felis lybica</i>	4/1 (25%)	-	-	-	-	-
	<i>Felis serval</i>	3/1 (33%)	1/0 (0%)	weak	weak	weak	Weak
	<i>Lynx rufus</i>	2/1 (50%)	-	-	-	-	-
Felidae	<i>Panthera leo</i>	49/11 (22%)	1/0 (0%)	weak	weak	weak	Weak
	<i>Panthera leo persica</i>	-	1/0 (0%)	weak	weak	moderate	Weak
	<i>Panthera onca</i>	15/1 (7%)	-	-	-	-	-
	<i>Panthera pardus</i>	59/8 (14%)	-	-	-	-	-
	<i>Panthera pardus orientalis</i>	-	1/0 (0%)	weak	weak	weak	Weak
	<i>Panthera tigris</i>	98/2 (2%)	-	-	-	-	-
	<i>Puma concolor</i>	12/0 (0%)	-	-	-	-	
Ursidae	<i>Ursus arctos arctos</i>	11/0 (0%)	-	-	-	-	-
	<i>Ursus maritimus</i>	12/0 (0%)	-	-	-	-	-
	<i>Ursus thibetanus</i>	6/1 (17%)	-	-	-	-	-
	<i>Canis lupus</i>	-	4/4 (100%)	-	-	-	-
Canidae	<i>Canis mesomelas</i>	-	1/1 (100%)	-	-	-	-
	<i>Lycodon pictus</i>	14/0 (0%)	2/1 (50%)	weak	weak	weak	Weak
	<i>Zalophus californianus</i>	1/1 (100%)	-	-	-	-	-
Primates	<i>Cercopithecidae</i>	-	1/0 (0%)	-	-	-	-
	<i>Hyllobates lar</i>	-	1/1 (100%)	-	-	-	-
Birds	<i>Phoenicopteridae</i>	-	1/1 (100%)	weak	weak	weak	Strong
	<i>Struthionidae</i>	-	2/0 (0%)	weak	weak	weak	Strong
Reptiles	<i>Testudinidae</i>	-	1/0 (0%)	strong	strong	strong	Strong
	<i>Crocodylidae</i>	-	3/0 (0%)	weak	weak	weak	Weak
	<i>Pythonidae</i>	-	1/0 (0%)	strong	strong	strong	Strong

DE – Germany, CZ – Czech Republic, * hyphens in the table represent unavailable data in given research.

The serum complement of some animal species has a borreliacidal effect, which not only protects these animals from spirochete infection, but also purges *Borrelia* from infected ticks feeding on these animals [22,95,96]. This has a strong impact on the ecology of LB spirochetes within ecosystems where such animals are present.

In research conducted by Ticha et al. [22], serum samples from zoo animals were tested for possible borreliacidal effects on three species of spirochetes from the *B. burgdorferi* sensu lato complex (*B. burgdorferi* sensu stricto (s.s.), *Borrelia garinii* and *Borrelia afzelii*). From the 135 tested serum samples from various zoo animals, 78 demonstrated some borreliacidal effect towards at least one of the tested *Borrelia* spp. The strongest borreliacidal effect was observed in the sera from the Burmese python (*Python bivittatus*), European rabbit (*Oryctolagus cuniculus*), radiated tortoise (*Astrochelys radiata*) and impala (*A. melampus*) (Table 1). Of all of the tested sera, only some showed borreliacidal effects toward all three tested *Borrelia* spp., as showed in Table 1. Most samples possessed selected resistance (resistance only towards one or two of the tested *Borrelia* types) or were sensitive to the studied *Borrelia* species. Sera from most of the carnivores, even-toed ungulates, rodents and some reptiles, showed only weak borreliacidal effects on the tested spirochetes.

The absence of a borreliacidal effect in the sera of some zoo animals could be an indication of their permissiveness to *Borrelia* infections, suggesting that these animals can theoretically serve as *Borrelia* reservoirs. On the other hand, animals whose sera have strong borreliacidal effects should be resistant to *Borrelia* infection. Unfortunately, no tests were conducted to assess whether these animals could also resolve *Borrelia* in the infected ticks feeding on them.

5. *Babesia*, *Theileria* and *Cytauxzoon* Piroplasmida

Babesia species are often observed in captive or semicaptive cervids and bovids [24,34,97–99], but they have also been found in other captive animal species. These protozoan parasites can complete their life cycle within multiple tick species, including *I. scapularis* [98,99], *I. ricinus* [97,100], *Dermacentor albipictus* [101], *A. americanum* [24] and *I. pacificus* [99]. Babesiosis has a range of typical symptoms, like hemolytic anemia, jaundice, fever, shaking and hemoglobinuria [102]. However, an asymptomatic disease course is also possible, especially in animals with a well-developed immunity [98].

Cases of acute babesiosis in nonindigenous cervids were reported in 2009 and 2012 in Germany [34]. In response, a nation-wide project was conducted in 2013, where samples were collected from 16 zoos located across the country [34]. This survey resulted in the detection of *Babesia capreoli*, *Babesia divergens*, *Babesia venatorum*, *Theileria* spp. and one unidentifiable *Babesia* sp., in captive reindeer (*R. tarandus*). Of the 123 tested reindeer samples, 29 were positive (23.6%), and 12 of the 16 facilities harbored at least one reindeer that tested positive for *Babesia* spp. [34].

Other babesiosis outbreaks were recorded in the Netherlands in 2011 and 2015 [97,103]. In 2011, a captive-bred forest reindeer calf died due to an acute *B. venatorum* infection [103]. In 2015, five out of 14 reindeer kept in the Ouweland Zoo tested positive for *B. capreoli*, either through PCR testing or blood smears. Of the five positive animals, two died, one was euthanized and another animal died without testing positive for *Babesia* spp. All the mortalities were among young calves, born in the year of the outbreak, or in 2014. The surviving individuals with positive test results were adult females with no clinical signs of disease [97]. In Switzerland, a case report was published in 2019 identifying a young, captive reindeer calf with severe babesiosis infection clinical signs as positive for *B. venatorum* [32]. In Great Britain, seven fatal cases of babesiosis were confirmed in captive reindeer between the years 1997 and 1998, and *B. divergens* was identified as the probable causative species [104].

Canada and USA have also reported several acute and subclinical cases of babesiosis. Two fatal cases were reported in Canada in 2012, involving *Babesia* spp. isolated from captive-bred adult wapiti (*Cervus canadensis*) [105]. Between the years 2013 and 2016, nine fatal babesiosis cases were detected in Canadian zoo-kept adult reindeer and wapiti [24]. A higher number of positive cases were observed in Canada between the years 2016 to 2018 in zoo, or farm-kept cervids, like wapiti and red deer (*Cervus elaphus*) [98]. In the USA, fatal babesiosis infections were described very early in captive-bred cervids, including in caribou (*Rangifer tarandus caribou*) at the Minnesota Zoo [106] and the North American elk (*Cervus elaphus canadiensis*) kept on a farm in Texas [101]. Other severe American cases of babesiosis were observed in 2003 (semicaptive, adult North American elk; [107]) and in 2005 (adult captive reindeer from New York zoo; [108]). The study from New York zoo also identified three asymptomatic hosts of *B. odocoilei*: Yak (*Bos grunniens*), muntjac (*Muntiacus reevesi*) and markhor (*C. falconeri*) [108].

All the aforementioned Canadian and American studies related the positive samples to *Babesia odocoilei*. This *Babesia* species seems to be predominant in Canadian and North American captive cervids [24,98,105,107]. Considering these cases, it can be reasonably assumed that babesiosis is yet another global, tick-borne related threat to captive cervids.

However, babesiosis infection is not exclusive to cervids and bovids; research conducted in several Brazilian zoos showed the prevalence of babesiosis in zoo felids, canids and a genet (*Genetta tigrina*). Most animals were seropositive for *Babesia canis*, but some (*Oncifelis colocolo* and the genet) were positive for a *Babesia* sp. with close similarity to *Babesia leo*, according to DNA testing [109]. In addition, free-roaming domestic cats in Brazil often stray into zoo areas and are therefore considered potential carriers of babesiosis. Both *Babesia* sp. (*Babesia vogeli*) and *Theileria* sp. were confirmed in some of the tested cat samples in the same area as these Brazilian zoo animals [110]. In Kansas, USA, an unknown *Babesia* spp. was observed in maned wolves (*Chrysocyon brachyurus*) in 2012 (first occurrence) and again in 2019. Both cases had severe clinical symptoms, and one case (2012) was fatal, even after treatment [111,112].

Piroplasms of the genera *Theileria* and *Cytauxzoon* are also dangerous protozoan parasites. *Theileria* have been observed in many tick species, including *Amblyomma* spp., *Haemaphysalis* spp., *Rhipicephalus* spp. and *Hyalomma* spp. [113], whereas *Cytauxzoon* has

been found in *Dermacentor* spp. [113–115]. *Theileria* species are variable in their pathogenesis and lifecycles; there are the so-called “transforming” species (*T. annulata*, *T. parva*, *T. lestoquardi*, *T. taurotragi* etc.) and the “nontransforming” species (*T. orientalis*, *T. mutans*, *T. cervi* and *T. velifera*) [113,116,117]. The transforming *Theileria* species have the ability to influence host leucocytes, causing them to enable unlimited proliferation of infected cells [116], resulting in symptoms such as polyphagia followed by anorexia, nasal discharge, fever, anemia, febrile generalized lymphadenopathy and hemorrhaging on the mucous membranes of the buccal cavity and conjunctiva [113,118]. Such an infection may be fatal. The nontransforming species lack the ability to cause proliferation, resulting mostly in benign infections with mild symptoms [116]. These symptoms can become chronic, causing anemia or persistent subclinical infections [119]. *Theileria* infections vary in terms of symptoms, depending on the infected animal species and the *Theileria* species. Besides from the free-roaming cats of Brazil [110], an unknown *Theileria* spp. was detected in Missouri, USA, in an adult male captive reindeer (*R. tarandus* [120]). Infections of South American tapirs (*Tapirus terrestris*) with *Theileria equi* were confirmed in zoo and botanical gardens located in Northern parts of Brazil [121]. *Theileria bicornis* was detected in samples of captive white rhinoceros (*Ceratotherium simum*) and black rhinoceros (*Diceros bicornis*) in Australian zoo [117]. A *Theileria* spp. was also found in the blood sample of one captive reindeer (*R. tarandus*) kept in a German zoo [34].

Cytauxzoon felis is a parasite of felids, of both wild and domestic origin. This parasite has been observed on several occasions in samples from zoo felids reared in Brazil, i.e., in ocelots (*Leopardus pardalis*) [114,122], lions (*P. leo*) [115], pumas (*Puma concolor*) and jaguars (*Panthera onca*) [114]. In Florida, USA, a white tiger (*Panthera tigris*) housed in a private breeding facility was also reported as positive for *C. felis* [123]. Cytauxzoonosis infection can be asymptomatic [114], but also fatal [115,123]. The disease has two phases: erythrocytic and macrophagic [124]. The erythrocytic phase is usually connected to anemia, while the macrophagic phase is marked by systemic circulatory obstructions, caused by schizont macrophages, and presents clinical signs such as anorexia, depression, dehydration, fever, icterus and dyspnea [124,125].

6. Rickettsiales

The bacteria of the order Rickettsiales cause a variety of diseases of veterinary and medical importance, including bovine anaplasmosis, human ehrlichiosis, Rocky Mountain spotted fever and scrub typhus [126]. Within the order Rickettsiales, the genera *Rickettsia*, *Ehrlichia* and *Anaplasma* are dependent on tick vectors like *A. americanum*, *R. sanguineus*, *D. variabilis*, *Ixodes* spp., *Haemaphysalis* spp., *Hyalomma* spp. and *Aponomma* spp. [23,26,28,31,127,128]. Various, and often nonspecific, clinical symptoms are associated with Rickettsiales infections in animals (anorexia, depression, dehydration, fever, lethargy, lymphadenopathy and ataxia) [23,26,28,129]. Acute infections with bacteria from the Anaplasmataceae family (*Anaplasma*, *Ehrlichia*) can be detected using blood smears, showing a characteristic “morulae” (mulberry-shaped microcolonies) located in the host cell cytoplasm [26,28,128].

In Europe, several reports have confirmed positive cases for Rickettsiales. *A. phagocytophilum* has been found in blood samples of captive reindeer (*R. tarandus*) kept in German zoos [33]. Furthermore, an asymptomatic lion (*P. leo*) was positive for an infection with *Rickettsia* sp. and *A. phagocytophilum* in Italy [130]. Acute anaplasmosis (*A. phagocytophilum*) was observed in captive timber wolves (*Canis lupus occidentalis*) in Austria [31].

In the USA, several cases were also reported for anaplasmosis (*A. phagocytophilum*), in four captive Przewalski's horses (*E. przewalskii*) from Virginia [26]. *E. chaffeensis* was found in five ring-tailed lemurs (*Lemur catta*) and one ruffed lemur (*Varecia variegata rubra*) in the Duke Lemur Center in North Carolina (USA; [28]). *A. phagocytophilum* (under the old nomenclature of *Ehrlichia equi* in the case report) was confirmed in llama (*Lama glama*) from California, USA [23] and lastly, canine ehrlichiosis was noted in Florida, USA, in wolves, dogs and wolf-dog crosses [27].

Substantial research from Brazilian zoos showed that *Ehrlichia canis* was found in the following captive felids: jaguars (*P. onca*), ocelots (*L. pardalis*), jaguarundi (*Puma yagouaroundi*) and little spotted cats (*Leopardus tigrinus*). In this research, antibodies were found in four felids: two jaguarundi, one little spotted cat and one margay (*Leopardus wiedii*; [131]). Another study from Brazil confirmed that antibodies for *E. canis* existed in captive ocelots [122]. Further studies from André et al. [132] confirmed *Ehrlichia* spp. in captive canids, including European wolves (*C. lupus*), bush dogs (*Speothos venaticus*) and crab-eating foxes (*Cerdocyon thous*). Pumas (*P. concolor*), little spotted cats (*L. tigrinus*), ocelots (*L. pardalis*), jaguarundis (*P. yagouaroundi*), tigers (*P. tigris*) and lions (*P. leo*) also tested positive for *Ehrlichia* spp. Furthermore, *Anaplasma* spp. was confirmed in bush dogs and little spotted cats [132]. Three free-roaming cats surrounding the Brazilian zoo also tested positive for *Anaplasma* spp., which is closely related to *A. phagocytophilum* [110], showing that local animals can be a source of tick-borne pathogens that are then transferred to zoo-kept animals.

7. Coinfections with Multiple and Less Common Pathogens

In a report of Zhang et al. [133], novel *Theileria* spp., together with *A. phagocytophilum* and *Anaplasma bovis*, were found in the post mortem dissection of a one-year old South African giraffe (*Giraffa camelopardalis giraffa*), which was kept in Zhengzhou Zoo, China. The animal died suddenly, one day after the onset of severe clinical symptoms [133]. Another coinfection was observed in a lion (*P. leo*) in the Fasano Safari park in Italy. The animal tested positive for *Coxiella burnetii*, *Rickettsia* sp. and *A. phagocytophilum* [130]. In 2017, a rare emerging tick-borne virus causing severe fever and thrombocytopenia syndrome phlebovirus (SFTSV) was identified in two fatal cases in cheetah, infected in Hiroshima City Asa Zoological Park, Japan [134].

Regarding the aforementioned TBP in zoo-housed and captive animals, Table 2 summarizes the prevalence, country of origin, animal species and collected tick species (excluding *Borrelia* spp. since these are discussed extensively in Table 1)

Table 2. Ticks and tick-borne diseases detected in animals living in zoos and zoo-like establishments.

Pathogen	Animal Species	Tick Species Found	Prevalence (Positive/Tested)	Country	Reference	
TBEV	Barbary macaque (<i>Macaca sylvanus</i>)	<i>Ixodes ricinus</i>	8/284 (2.8%)	Germany	[72,78]	
	Markhor (<i>Capra falconeri</i>)	<i>I. ricinus</i>	1/1 ab* (100%)	Czech Republic	[20]	
	Reindeer (<i>Rangifer tarandus</i>)	<i>I. ricinus</i>	1/1 ab (100%)	Czech Republic	[20]	
	Ocelot (<i>Leopardus pardalis</i>)	N/A	26/43 ab (60.5%)	Brazil	[109]	
	Little-spotted cat (<i>Leopardus tigrinus</i>)	N/A	9/38 ab (23.7%)	Brazil	[109]	
	Margay (<i>Leopardus wiedii</i>)	N/A	2/4 ab (50%)	Brazil	[109]	
	Pampas cat (<i>Oncifelis colocolo</i>)	N/A	3/5 ab (60%)	Brazil	[109]	
	Jaguar (<i>Panthera onca</i>)	N/A	6/13 ab (46.1%)	Brazil	[109]	
	Puma (<i>Puma concolor</i>)	N/A	2/18 ab (11.1%)	Brazil	[109]	
	Jaguarundi (<i>Puma yagouaroundi</i>)	N/A	6/25 ab (24%)	Brazil	[109]	
<i>Babesia spp.</i>	Crab-eating fox (<i>Cerdocyon thous</i>)	N/A	2/39 ab (5.1%)	Brazil	[109]	
	Bush dog (<i>Speothos venaticus</i>)	N/A	8/27 ab (29.6%)	Brazil	[109]	
	Maned wolf (<i>Chrysocyon brachyurus</i>)	N/A	2/2 (100%)	USA	[111,112]	
	Reindeer (<i>R. tarandus</i>)	N/A	1/1 (100%)	USA	[106]	
	Wapiti (<i>Cervus canadensis</i>)	N/A	2/30 (6.7%)	Canada	[98,105]	
	Reindeer (<i>R. tarandus</i>)	speculated <i>Ixodes scapularis</i>	12/12 (100%)	Canada, USA	[24,108]	
	Red deer (<i>Cervus elaphus</i>)	N/A	4/144 (2.8%)	Canada, USA	[98,101,107]	
	Markhor (<i>C. falconeri</i>)	speculated <i>I. scapularis</i>	4/6 (66.7%)	USA	[108]	
	Yak (<i>Bos grunniens</i>)	speculated <i>I. scapularis</i>	1/2 (50%)	USA	[108]	
	Muntjac (<i>Muntiacus reevesi</i>)	speculated <i>I. scapularis</i>	1/2 (50%)	USA	[108]	
<i>Babesia venatorum</i>	Reindeer (<i>R. tarandus</i>)	<i>I. ricinus</i>	21/141 (14.9%)	Germany, Netherlands, Switzerland	[32,34,103]	
	Reindeer (<i>R. tarandus</i>)	<i>I. ricinus</i>	7/137 (5.1%)	Germany, Netherlands	[34,97]	
	Reindeer (<i>R. tarandus</i>)	<i>I. ricinus</i>	7/154 (4.5%)	Germany, Great Britain	[34,104]	
	Reindeer (<i>R. tarandus</i>)	<i>I. ricinus</i>	4/123 (3.3%)	Germany	[34]	
	Reindeer (<i>R. tarandus</i>)	<i>I. ricinus</i>	2/123 (1.6%)	Germany	[34]	
	Genet (<i>Genetta tigrina</i>)	N/A	1/2 (50%)	Brazil	[109]	
	Reindeer (<i>R. tarandus</i>)	N/A	1/1 (100%)	USA	[120]	
	Reindeer (<i>R. tarandus</i>)	<i>I. ricinus</i>	1/123 (0.8%)	Germany	[34]	
	Tapir (<i>Tapirus terrestris</i>)	N/A	11/19 (57.9%)	Brazil	[121]	
	White rhinoceros (<i>Ceratotherium simum</i>)	N/A	2/2 (100%)	Australia	[117]	
<i>Theileria equi</i>	Black rhinoceros (<i>Diceros bicornis</i>)	N/A	1/7 (14.3%)	Australia	[117]	
	Ocelot (<i>L. pardalis</i>)	N/A	7/138 (5%)	Brazil	[114,122]	
	Puma (<i>P. concolor</i>)	N/A	2/9 (22.2%)	Brazil	[114]	
	Jaguar (<i>Panthera onca</i>)	N/A	1/9 (11.1%)	Brazil	[114]	
	<i>Theileria bicornis</i>	White rhinoceros (<i>Ceratotherium simum</i>)	N/A	2/2 (100%)	Australia	[117]
		Black rhinoceros (<i>Diceros bicornis</i>)	N/A	1/7 (14.3%)	Australia	[117]
		Ocelot (<i>L. pardalis</i>)	N/A	7/138 (5%)	Brazil	[114,122]
		Puma (<i>P. concolor</i>)	N/A	2/9 (22.2%)	Brazil	[114]
		Jaguar (<i>Panthera onca</i>)	N/A	1/9 (11.1%)	Brazil	[114]
		<i>Cytauxzoon felis</i>	White rhinoceros (<i>Ceratotherium simum</i>)	N/A	2/2 (100%)	Australia
Black rhinoceros (<i>Diceros bicornis</i>)			N/A	1/7 (14.3%)	Australia	[117]
Ocelot (<i>L. pardalis</i>)			N/A	7/138 (5%)	Brazil	[114,122]
Puma (<i>P. concolor</i>)			N/A	2/9 (22.2%)	Brazil	[114]
Jaguar (<i>Panthera onca</i>)			N/A	1/9 (11.1%)	Brazil	[114]

	Lion (<i>Panthera leo</i>)	<i>Amblyomma cajennense</i>	1/1 (100%)	Brazil	[115]
	Tiger (<i>Panthera tigris</i>)	<i>Amblyomma americanum</i>	1/1 (100%)	USA	[123]
	Reindeer (<i>R. tarandus</i>)	<i>I. ricinus</i>	17/123 (13.8%)	Germany	[33]
	Przewalski's horse (<i>Equus przewalskii</i>)	unspecified Ixodid ticks	4/4 (100%)	USA	[26]
	Lion (<i>P. leo</i>)	N/A	1/10 (10%)	Italy	[130]
	Timber wolf (<i>Canis lupus occidentalis</i>)	<i>I. ricinus</i>	1/1 (100%)	Austria	[31]
<i>Anaplasma phagocytophilum</i>	Llama (<i>Lama glama</i>)	<i>Ixodes pacificus</i>	1/1 (100%)	USA	[23]
	Little-spotted cat (<i>L. tigrinus</i>)	N/A	4/25 (16%)	Brazil	[132]
	Bush dog (<i>Speothos venaticus</i>)	N/A	1/27 (3.7%)	Brazil	[132]
	Jaguar (<i>P. onca</i>)	N/A	2/9 (2.2%)	Brazil	[131]
	Ocelot (<i>L. pardalis</i>)	N/A	3/30 (10%)	Brazil	[122,132]
	Jaguarundi (<i>P. yagouaroundi</i>)	N/A	5/25 ab (20%)	Brazil	[131,132]
	Little-spotted cat (<i>L. tigrinus</i>)	N/A	5/39 ab (12.8%)	Brazil	[131,132]
	Margay (<i>Leopardus wiedii</i>)	N/A	1/1 ab (100%)	Brazil	[131]
	Puma (<i>P. concolor</i>)	N/A	3/17 (17.6%)	Brazil	[131,132]
<i>Ehrlichia canis</i>	Pampas cat (<i>L. colocolo</i>)	N/A	1/3 (33.3%)	Brazil	[131]
	Lion (<i>P. leo</i>)	N/A	2/12 (16.7%)	Brazil	[132]
	Crab-eating fox (<i>C. thous</i>)	N/A	3/39 (7.7%)	Brazil	[132]
	Bush dog (<i>S. venaticus</i>)	N/A	5/27 (18.5%)	Brazil	[132]
	Timber wolf (<i>Canis lupus</i>)	<i>Rhipicephalus sanguineus</i>	13/17 (76.5%)	USA	[27]
	Ring-tailed lemur (<i>Lemur catta</i>)	<i>A. americanum</i>	7/9 (77.8%)	USA	[28]
	Ruffed lemur (<i>Varecia variegata rubra</i>)	<i>A. americanum</i>	1/10 (10%)	USA	[28]
	Little-spotted cat (<i>L. tigrinus</i>)	N/A	3/25 (12%)	Brazil	[132]
	Ocelot (<i>L. pardalis</i>)	N/A	2/15 (13.3%)	Brazil	[132]
	Puma (<i>P. concolor</i>)	N/A	2/8 (25%)	Brazil	[132]
	Tiger (<i>P. tigris</i>)	N/A	2/8 (25%)	Brazil	[132]
	Jaguarundi (<i>P. yagouaroundi</i>)	N/A	1/19 (5.3%)	Brazil	[132]
	Lion (<i>P. leo</i>)	N/A	1/12 (8.3%)	Brazil	[132]
	European wolf (<i>C. lupus</i>)	N/A	1/3 (33.3%)	Brazil	[132]
	Crab-eating fox (<i>C. thous</i>)	N/A	2/39 (5.1%)	Brazil	[132]
	Lion (<i>P. leo</i>)	N/A	2/10 (20%)	Italy	[130]
<i>Rickettsia</i> spp.					
<i>Theileria</i> spp., <i>A. phagocytophilum</i> and <i>A. bovis</i>	South African giraffe (<i>Giraffa camelopardalis giraffa</i>)	N/A	1/1 (100%)	China	[133]
<i>Coxiella burnetii</i> and <i>A. phagocytophilum</i>	Lion (<i>P. leo</i>)	N/A	1/1 (100%)	Italy	[130]
SFTSV	Cheetah (<i>Acinonyx jubatus</i>)	unspecified Ixodid tick	2/2 (100%)	Japan	[134]

specific data. ab*: antibodies positive; without ab: PCR positive; N/A: No ticks found on the positive animals.

8. Conclusions and Recommendations

All of the aforementioned studies confirm the significant threat of ticks and tick-borne diseases to wild animals housed in zoos, wildlife parks or farms. Such zoo and zoo-like areas have been identified as being suitable for tick vectors and reservoir hosts of TBPs. The pathogens found in zoo-housed animals included viruses (TBEV, SFTSV), bacteria (*Borrelia*, *Anaplasma*, *Ehrlichia*, *Rickettsia* spp.) and protozoal parasites (*Babesia*, *Cytauxzoon* and *Theileria* spp.). It was confirmed that infection of the tick vectors with some of these pathogens, for example, *Borrelia* spp., TBEV, *Anaplasma* spp. and *Babesia* spp., increases the tick mobility, cold resistance, desiccation resistance and overall chance of survival [135]. There are other known tick-borne threats that are yet to be observed in zoo-housed animals, like the filariid nematode species *Cercopithifilaria* spp. and *Acanthocheilonema* spp. These parasites are frequently associated with dogs [136–140] and occasionally with wild-living animals [141]. They can be transmitted by various tick species, i.e., *Haemaphysalis flava*, *Haemaphysalis japonica* [141], *A. americanum* [142], *I. scapularis* [143,144] and *R. sanquineus* [145,146]. Focused sampling should be conducted to determine the potential spread of these parasites in zoos and other similar establishments.

Clinical manifestations of infections with the TBPs in captive animals can vary from unapparent to serious and even life threatening [147]. It is clear that captive animals have variable sensitivities to the studied pathogens; however, it is not clear if zoo and farm-housed animals play a significant role as tick hosts and TBP reservoirs in their ecosystems. In the case of TBPs, most of them are probably incidental dead-end hosts, as they would not produce sufficient bacteremia/viremia for the infection of other ticks (although this question remains to be answered definitively). Figure 2 provides a summary of the amount of samples collected and tested across the several orders of zoo-housed animals (with connection to TBPs). More abundant sampling (Artiodactyla, Carnivora,) provides results that can be used to evaluate the role of these animal orders in the ecology of several TBPs. Data on Primates and Preissodactyla are insufficient to draw any wider conclusions in terms of overall TBP transmission, and they usually provide information about the incidence of only one pathogen (case reports).

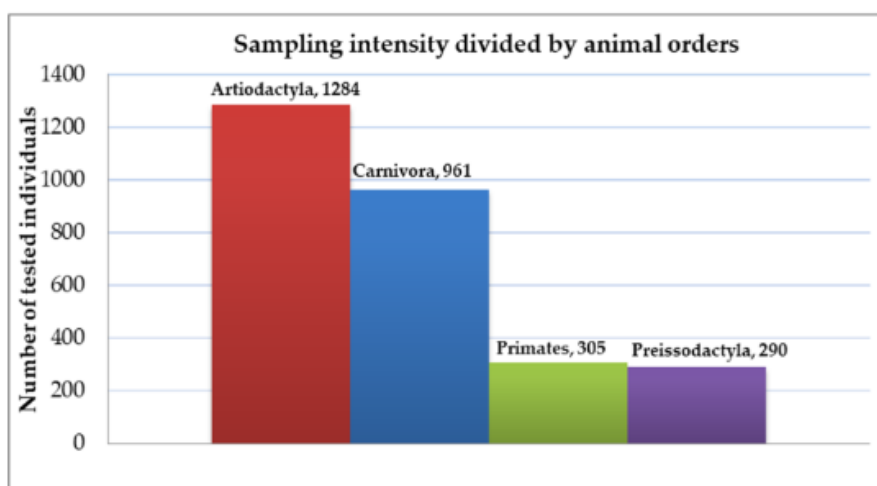


Figure 2. Differences in sampling for TBPs in zoo-housed animals (divided by orders). From this histogram, we can note the lack of testing in the Preissodactyla and Primates order. The orders Struthioniformes (2 samples), Phoenicopteriformes (1 sample), Testudines (1 sample), Squamata (1 sample) and Crocodylia (3 samples) have been tested only for *Borrelia* antibodies and in such small numbers that it would not provide any graphical value in the histogram. The role of these orders in the overall TBPs ecology is unclear; information is isolated only on the one tested pathogen. Some

orders of animals, which are potentially threatened by TBPs and ticks, are omitted completely. For example: Chiroptera, Dermoptera, Edentata, Insectivora, Lagomorpha, Marsupialia, Proboscidea and Pholidata which all have the potential to carry ticks and therefore contract TBDs. Species from these orders are often kept in zoos in outdoor or semi-outdoor enclosures and the contact with ticks can occur. This suggests further need for the increase in research of the omitted or lacking animal orders.

Some of the pathogens (TBEV, *Borrelia* spp., *A. phagocytophilum*, *E. cheffeensis*, *C. burnetii*) and tick species (*A. americanum*, *A. sculptum*, *I. ricinus*, *I. scapularis*, *D. variabilis*) detected in zoos or zoo-like areas represent a notable threat to the health of humans that live nearby. Since zoos are places with high densities of humans, exotic animals, domestic animals and wildlife opportunists, they create ideal hotspots for the spread of TBPs, ticks and other ectoparasites [18]. The importance of surveillance and research of tick vectors and TBPs that exist in close proximity to human habitats is supported by the fact that the annual number of visitors to zoos is more than 700 million worldwide [148]. The already available evidence of tick-borne pathogens infecting zoo-housed animals should raise awareness of scientists, zookeepers, veterinarians, medical doctors and other specialists.

Another risk for zoo and other captive animals is free-roaming domestic cats that often stray into zoo or farm grounds. These cats are commonly infested with local ticks, and are hosts to various vector-borne infections [110,149–151]. They can thus potentially serve as one of the sources that increase the numbers of infected ticks in the areas that they commonly occupy. As a preventative measure, the activity of free-roaming domestic cats should be monitored and minimized in establishments where exotic animals are kept. Advanced preventative techniques in the forms of various vaccines are also available for the prevention of tick-borne infections in some animal species. In addition to the existing TBEV vaccine approved for human use, which was shown to be efficient for other primates [80], there is a borrelia vaccine approved for use in dogs [152]. Recently, this vaccine was tested on horses [153], and it could be expected that it may trigger protection in other animals too, at the very least, in canids. Furthermore, vaccines against bovid ticks from the genus *Rhipicephalus* were developed for use in cattle [154], and since the vaccine works in sheep as well, it can be expected that it may protect other ruminant species [154]. Also, landscape management with respect to tick-associated risks can help lower the prevalence of ticks, and subsequently, of TBPs, thus enhancing any other preventative measures taken [155].

In conclusion, ticks and TBPs present a challenge for a wide range of zoo, veterinary and public health experts. However, due to the poor understanding of the role of zoo animals in the biology of ticks and TBPs, further research in this area is clearly urgently required.

9. Other Potentially Tick-Borne Threats to Zoo-Housed and Captive Animals

Some pathogens are less specialized and spread through a wider range of vectors, e.g., vertebrates, mites, lice, mosquitoes and, of course, ticks. Even though some pathogens are less studied, they still represent a threat to both animal and human health.

Bacteria of the order Chlamydiales have been connected to Ixodid ticks for some time [156–159]. The most intensively studied is the Chlamydiaceae family. Other families are included in the order, but they are usually summarized under the term *Chlamydia*-like organisms (CLOs). These bacterial pathogens are causative agents of wide range of human and animal (some zoonotic) diseases [160]. Tick-borne CLO transmissions have been observed in humans [156], while various species of animals have been confirmed to harbor chlamydial agents, but without the direct connection to ticks. Among vertebrates, several species of bats (free-living and captive) have been found to be positive for a wide range of CLOs [161]. *Chlamydophila psittaci* has been found in the eyes of various livestock [162]. *Chlamydophila abortus* and *Chlamydophila pecorum* has been detected in a water buffalo (*Bubalus bubalis*) [163]. *Chlamydia felis* infection has been confirmed in cats and dogs [164], while *Chlamydiaceae* has been detected in domestic pigs (*Sus scrofa* f. *domestica*) [165]. These studies suggest the possibility of infection for both humans and captive/domestic animals living in their close vicinity.

Another potentially tick-borne pathogen that causes health problems is the bacteria *F. tularensis*. This pathogen can be transmitted through various sources: aerosol droplets, infected animal carcasses, contaminated food (alimentary transmission) or the bite of an infected arthropod [166,167]. *F. tularensis* can be transmitted by all tick life stages and horizontal transmission has been confirmed [167]. There have been positive cases of tularemia infection in animals in several zoological gardens. A fatal case in a Bornean orangutan (*Pongo pygmaeus*) was reported at Topeka Zoo, Kansas in 2003 [25], which was directly connected to tick bite. Several other zoos in North America have confirmed *F. tularensis* infections in other animal species: golden-lion tamarins (*Leontopithecus rosalia*), red-handed tamarin (*Saguinus midas*) [25], squirrel monkeys (genus *Saimiri*) [168], black and white-ruffed lemurs (*Varecia variegata*), ring-tailed lemur (*L. catta*), white handed gibbon (*Hylobates lar*) and greater spotnose guenon (*Cercopithecus nictitans*) [169]. *F. tularensis* infections have also been observed in animals in German zoos (in a wide range of animal species) [170]. Human and animal (tamarins and a talapoin monkey (*Miopithecus talapoin*)) cases have also been reported in Canada [171]. However, none of these studies provided any link to tick or other ectoparasite bites, so it remains unclear whether the connection exists. Nonetheless, it is still evident that zoo-housed animals and humans are threatened by this pathogen.

Bacteria of the genus *Bartonella* are known to cause various diseases, for example, the cat scratch disease in humans [172]. *Bartonella* spp. has been connected to several tick species [172–175]. Domestic cats are known reservoirs of *Bartonella* spp., e.g., *B. henselae*, *B. clarridgeiae* and *B. koehlerae* [176,177]. Samples from free-roaming domestic cats located in zoo areas in Brazil have been found to be positive for *Bartonella* spp. [110]. This could lead to spillover of this pathogen to the zoo tick population, even though the described infestation was most likely flea-borne [110]. Recently, tick-borne *Bartonella* spp. cases have been observed in dromedary camels (*Camelus dromedarius*) infected with *B. henselae* [178], domesticated yaks (*Bos grunniens*) [179] and in livestock animals like cattle [180,181], goats [181,182] and horses [182]. Some of these species, like dromedary camels or yaks, are often kept in zoos, so this information may be useful for the prevention of this potentially tick-borne disease.

There are other widely known pathogens that are yet to be fully established as potentially tick-borne, e.g., the parasite *Toxoplasma gondii*. Even though this parasite is not usually associated with ticks, some studies have proved the ability of ticks to transmit it [183,184]. In conclusion, it should be noted even pathogens which are less commonly attributed to ticks and captive animals have the potential to cause serious damage.

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CHAPTER 3 - *Borrelia* spirochetes in European exotic farm animals



Flagging in llama paddock at Camel Farm Záhostice in Southern Bohemia (photo by Jiří Černý)

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Borrelia spirochetes in European exotic farm animals

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Ticks transmit a broad spectrum of pathogens, threatening both animal and human health. Tick survival and proliferation are strongly dependent on host selection and suitability. The hard tick *Ixodes ricinus*, which is widespread throughout most of Europe, is a host generalist capable of feeding on many different vertebrate species. Pasture-kept exotic farm animals may be at a high risk for tick and tick-borne pathogens infestations but research characterizing this is currently lacking. This study focused on the detection of *Borrelia* spirochetes (including *Borrelia miyamotoi*) in exotic farm animals. Using nested-PCR with *Borrelia*-specific primers, 121 serum samples from 54 exotic farm animals of several species bred in four different farms in Bohemia and Moravia (Czechia) were tested. Positive samples were sequenced for the identification of *Borrelia* species. The prevalence of *Borrelia* DNA in the samples ranged from 13 to 67%, depending on the sampling site. The sequencing results confirmed the DNA presence of multiple spirochete species from the *Borrelia burgdorferi* sensu lato complex. Only one sample from an ostrich (*Struthio camelus*) was found to be positive for *Borrelia miyamotoi*. The results show that exotic farm animals can serve as hosts for hard ticks and can be infected by *Borrelia* spirochetes, transmitted by hard ticks. Therefore, these animals could play a relevant role in maintaining *Borrelia* spirochetes in nature.

KEYWORDS

tick, tick-borne pathogens, *Ixodes ricinus*, tick hosts, *Borrelia burgdorferi* sensu lato, *Borrelia miyamotoi*

Introduction

Ticks are important hematophagous ectoparasites and vectors of several pathogens affecting both animal and human health. The presence of suitable vertebrate hosts is one of the driving forces determining tick survival in a region and subsequent circulation of tick-borne pathogens (1). From the perspective of host-preference, ticks can be characterized as either host generalists or specialists (2). Most tick species of veterinary and medical interest are host generalists, with the potential for transmitting tick-borne pathogens (TBPs) to a wide range of hosts. Availability and diversity of suitable hosts

is closely linked to tick prevalence, so generalists have the “upper hand” in establishing stable populations in much larger areas compared to highly specialized tick species (1). The correct assessment and knowledge of tick-host interactions and TBP prevalence in host populations is vital for understanding the ecoepidemiology of tick-borne diseases and the population dynamics of ticks.

The present research study was conducted in Czechia, where the hard tick *Ixodes ricinus* is the predominant tick species. *I. ricinus* is widespread across Europe and is acknowledged as a typical generalist capable of feeding on as many as 300 different vertebrate species (3, 4). The selection of hosts by ticks, however, is not completely random (5). The factors influencing host selection are numerous and include: tick development stage, size, the geographical range of host and its health state, season, temperature and even infections of the tick with selected pathogens that can modulate tick behavior (6–9). The formation of host-specific races has also been confirmed in some *I. ricinus* populations (7). The tick-host relationship of *I. ricinus* is rather complex and new influencing factors are continually being discovered.

Apart from the primary hosts of *I. ricinus*, like red deer (*Cervus elaphus*), wild boars (*Sus scrofa*), yellow-necked mice (*Apodemus flavicollis*), European hares (*Lepus europaeus*), voles, some incidental host species have been studied in recent years. Animals originating outside of the natural *I. ricinus* geographical range (i.e., animals that are non-indigenous or exotic to Europe, typically kept in zoos) are also prone to *I. ricinus* infestation and can harbor some of the common TBPs within the region (10–13). These findings suggest that, due to the high adaptability of *I. ricinus* to different hosts, all animals kept within the endemic zone of *I. ricinus* (even house pets or accompanying animals) should be considered as potential sources of TBPs. It is still unclear as to whether exotic animals are competent reservoirs and can serve as a source of infection for uninfected tick vectors. Ticha et al. (13) confirmed the sensitivity or resistance of tested *Borrelia* spirochetes to serum complements of several zoo animals, suggesting a tolerance to *Borrelia* infection in at least some exotic species and a potential reservoir competence of these animals. Studies conducted on animals that are exotic to Europe have thus far only focused on zoological gardens, and to the best of our knowledge, no studies concerning exotic species kept on private farms have been published to date. Exotic animals are farmed for a variety of reasons, including production, for example milk (buffalo species), fiber (llama species), or meat (ostrich, buffalo, antelope species). Hobby farms and private zoo parks are also common in Czechia, maintaining animals like camels, llamas, donkeys, ostriches, and others (Table 1). These private establishments provide much closer animal-human-nature contact compared to zoological gardens. Furthermore, these establishments are often located in rural or even forested landscapes, unlike zoological gardens which are more often located in urban or peri-urban areas.

These localities make exotic animal farms and private zoos ideal hotspots for tick-exotic host interactions, providing an increased likelihood of TBP circulation.

Materials and methods

Serum sample collection

A total of 121 blood samples were collected from 54 exotic farm animals of different species (Table 1). Vacutainers (8 ml CAT Serum Sep Clot Activator, Vacuette, Greiner Bio-One GmbH, Austria) were used for serum collection. The blood samples from live animals were obtained during regular annual health status inspection by external veterinarians according to the recommendations of the State veterinary authority of the Czech Republic. Ostrich samples were obtained after slaughter of the animals. Moreover, samples from captive-bred common eland (*Taurotragus oryx*) were collected at the Czech University of Life Sciences Research farm at Lány, Central Bohemia, in the Czech Republic which is accredited for experiments on animals (Permit: 63479_2016-MZE-17214). Specific protocol of approval was obtained by the Institutional Animal Care and Use Committee of the Czech University of Life Sciences, Prague (clearance no. CZU 20/19). The institutions and animal owners agreed to participate in this study and provided written informed consent. After blood withdrawal, the samples were delivered to the laboratory, and serum was separated by centrifugation (10 min at 1,000 rpm) at room temperature. The serum was then refrigerated at 4°C (if used the next day) or frozen at –20°C until analysis.

The animals sampled included: 25 common elands (*Taurotragus oryx*), 6 common ostriches (*Struthio camelus*), 8 water buffaloes (*Bubalus bubalis*), 1 dromedary camel (*Camelus dromedarius*), 10 Bactrian camels (*Camelus bactrianus*), and 4 llamas (*Lama glama*) (Table 1). The location and husbandry practices of the selected exotic animal farms were variable. The Common Eland Research Facilities (CERF) of the Czech University of Life Sciences Prague (CZU) and the buffalo milk and meat farm are both located in Central Bohemia, and had similar husbandry conditions, with short grass grazing in outdoor paddocks and the provision of supplementary feeding of a complete ration in adjacent stables. The buffalo farm is located on the edge of a forested area, while the eland farm is surrounded by agricultural fields and an asphalt road. The hobby camel farm in Southern Bohemia, and the ostrich meat farm in Moravia, had more extensive outdoor paddocks with both short and long grass cover, and supplementary feed provided in their stables. The camel farm was also situated close to the edge of a forest. The animals were free to roam in and out of their stables during most of the year, except for aggressive males or sick animals which had their own smaller paddocks.

TABLE 1 The results of *Borrelia* testing and tick collections on selected exotic animal farms in Czechia.

Farm	Ticks on pasture	Ticks around the farm	Prevalence of <i>Borrelia</i> in animals	Animal (ID)	Age*	Sex	Positive/ Negative	<i>Borrelia</i> species found	Overall prevalence on farm
Hobby camel farm, south Bohemia	1 nymph	11 (5 females, 5 males, 1 nymph)	Llamas; tested: 4, positive: 2, 50%	Llama 1	115 months	F	Positive	<i>Borrelia burgdorferi</i>	38%
				Llama 2	127 months	M	Positive	<i>Borrelia garinii</i>	
				Llama 3	19 months	F	Negative	/	
				Llama 4	19 months	F	Negative	/	
				Bactrian camel 1	91 months	F	Positive	<i>B. ss</i>	
				Bactrian camel 2	127 months	F	Positive	<i>B. ss</i>	
				Bactrian camel 3	79 months	F	Positive	<i>Borrelia afzelii</i>	
				Bactrian camel 4	139 months	M	Negative	/	
				Bactrian camel 5	115 months	F	Negative	/	
				Bactrian camel 6	175 months	M	Negative	/	
Experimental antelope farm, central Bohemia	1 nymph	0	Dromedary camel; Tested: 1, Positive 1, 100% Elands; Tested: 25, Positive: 11, 44%	Bactrian camel 7	55 months	F	Negative	/	44%
				Bactrian camel 8	79 months	M	Negative	/	
				Bactrian camel 9	67 months	F	Negative	/	
				Bactrian camel 10	283 months	F	Negative	/	
				Dromedary camel	55 months	M	Positive	<i>B. ss</i>	
				Eland antelope 249	17 months	F	Positive	<i>B. ss</i>	
				Eland antelope 251	17 months	M	Positive	<i>B. ss</i>	
				Eland antelope 253	17 months	F	Positive	<i>B. afzelii</i>	
				Eland antelope 255	17 months	F	Positive	<i>B. garinii</i> + <i>B. bisetii</i>	
				Eland antelope 258	16 months	M	Positive	<i>B. ss</i> + <i>B. garinii</i> + <i>B. bisetii</i>	
Hobby camel farm, south Bohemia	1 nymph	11 (5 females, 5 males, 1 nymph)	Llamas; tested: 4, positive: 2, 50%	Eland antelope 259	16 months	M	Positive	<i>Borrelia americana</i>	38%
				Eland antelope 261	16 months	F	Positive	<i>B. afzelii</i>	
				Eland antelope 267	17 months	M	Positive	<i>B. ss</i>	
				Eland antelope 268	17 months	F	Positive	<i>B. afzelii</i>	
				Eland antelope 269	16 months	M	Positive	<i>B. afzelii</i>	
				Eland antelope 269	16 months	M	Positive	<i>B. afzelii</i>	

(Continued)

TABLE 1 (Continued)

Farm	Ticks on pasture	Ticks around the farm	Prevalence of <i>Borrelia</i> in animals	Animal (ID)	Age*	Sex	Positive/ Negative	<i>Borrelia</i> species found	Overall prevalence on farm					
Milk and meat Buffalo farm, central Bohemia	2 males + 1 female feeding on animal	11 (3 females, 6 males, 2 nymph)	Buffaloes; Tested: 8, Positive: 1, 13%	Eland antelope 272	15 months	M	Positive	<i>B. ss + B. biszti</i>	13%					
				Eland antelope 248	17 months	F	Negative	/						
				Eland antelope 250	17 months	F	Negative	/						
				Eland antelope 252	17 months	F	Negative	/						
				Eland antelope 254	17 months	M	Negative	/						
				Eland antelope 257	16 months	M	Negative	/						
				Eland antelope 260	16 months	M	Negative	/						
				Eland antelope 262	16 months	F	Negative	/						
				Eland antelope 266	8 months	M	Negative	/						
				Eland antelope 271	3 months	M	Negative	/						
				Eland antelope 273	2 months	F	Negative	/						
				Eland antelope 231	20 months	F	Negative	/						
				Eland antelope 207	32 months	F	Negative	/						
				Eland antelope 219	29 months	F	Negative	/						
				Eland antelope A	19 months	F	Negative	/						
				Buffalo 19	43 months	F	Negative	/						
				Meat ostrich farm, Moravia	N/A	N/A	Ostriches; Tested: 6, Positive: 4, 67%	Buffalo 3		115 months	M	Negative	/	67%
								Buffalo 4		91 months	F	Negative	/	
								Buffalo 11		67 months	F	Negative	/	
Buffalo 21	31 months	F	Negative					/						
Buffalo 13	67 months	F	Negative					/						
Buffalo 14	67 months	F	Negative					/						
Buffalo 18	55 months	F	Positive					<i>B. burgdorferi sensu lato</i>						
Ostrich 1	14 months	M	Positive					<i>B. garinii</i>						
Ostrich 2	16 months	F	Positive					<i>B. garinii</i>						
Ostrich 3	16 months	M	Positive					<i>B. burgdorferi sensu lato</i>						
Ostrich 8	13 months	M	Negative	/										
Ostrich 6	16 months	M	Negative	/										
Ostrich 4	12 months	M	Positive	<i>Borrelia miyamotoi</i>										

* in 2020.

Most collections were conducted once per farm during the active *I. ricinus* season at the time of scheduled routine veterinary health inspections. Eland blood sampling was conducted over 3 consecutive months at the CZU Common Eland Research Facilities during summer, from July to September 2020 (78 samples in total from 25 individual animals). All sampled elands were juveniles (3–17 months old) born on the farm.

Live ticks collection

Live ticks were collected on each farm using the flagging method (14) on both the pasture areas and the areas immediately surrounding the farms (Table 1). Only *I. ricinus* ticks were collected; no other tick species were identified during the collections. Flagging was conducted in unified timeframes of 1 h per pasture and 1 h per surrounding area. The collected live ticks were sorted according to their developmental stage and sex, and then stored live at 4°C for further testing. Live tick collection directly from the live animals was mostly impossible due to the handling methods of the animals (crushes with solid sides). However, when possible, live ticks were collected.

Nested polymerase chain reaction (nested-PCR)

Ticks were homogenized using a Tissue Lyser (Qiagen). DNA extraction from the biological samples was performed using a DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's protocol.

All samples were screened using PCR for the presence of DNA from the *Borrelia burgdorferi* s.l. complex, including the species *Borrelia burgdorferi* sensu stricto, *Borrelia garinii*, *Borrelia afzelii*, *Borrelia americana*, and *Borrelia bissetti*. The DNA presence of the relapsing fever spirochete *Borrelia miyamotoi* was also tested. The presence of *Borrelia burgdorferi* s.l. was examined by PCR amplification of partial *ospC* and *flagellin* genes using gene-specific primers. In the case of low-quality sequences, some samples were further re-examined through the amplification of partial *p66* gene. All PCR reactions were conducted in two steps (nested PCR).

Whole EDTA blood could have been used for *Borrelia* detection, but the results would have been unreliable. Since spirochetes causing Lyme borreliosis are not intracellular pathogens, the presence of inhibitory non-target DNA in blood cells would bias the PCR test results toward false negatives. Removal of red blood cells resulted in a reduction of the amount of non-target DNA in the sample, yielding more accurate results. The reliability of the protocol chosen has been shown in numerous previous studies (13, 15–17) and is optimized for

utilizing animal and human serum, resulting in an increased sensitivity of detection for LB spirochetes. Since serum was easily obtainable through routine veterinary examination of the animals and was one of the least invasive but still clinically valid sample types, it was evaluated as the best available option for the detection of *Borrelia* DNA in these animals.

The fragment of the *ospC* gene was amplified by spacer/nested PCR using previously described primers and reaction conditions (18). The first round was conducted using primers targeting a 617 bp long fragment (Table 2); the conditions of both reactions were the same (except the annealing temperature, which was 50 and 52 °C for the first and the second round of PCR, respectively): 30 cycles at 95 °C for 30 s, 50/52 °C for 30 s, and 72 °C for 30 s.

Primers described by Wills et al. (19) were used to amplify a 447 bp long fragment of the *flagellin* gene (Table 2). The cycling conditions of both reactions were the same (except the annealing temperature that was 55 and 58 °C for the first and the second round of PCR, respectively): 30 cycles at 95 °C for 30 s, 55/58 °C for 30 s, and 72 °C for 30 s.

A 684 bp long fragment of the *B. burgdorferi p66* gene was amplified by nested PCR using primers according to Bunikis et al. (18) (Table 2). PCR conditions were the same for both rounds of PCR: 95°C for 5 min followed by 30 cycles of 95°C for 30 s; 50°C for 30 s; and 72°C for 1 min.

The presence of *B. miyamotoi* was also tested by nested PCR for the glycerophosphodiester phosphodiesterase (*glpQ*) *B. miyamotoi* gene, using the protocol described earlier (20) and producing 480 or 461 bp long fragments (Table 2) under the following amplification conditions: 30 cycles at 95 °C for 30 s, 52 °C for 30 s, and 72 °C for 30 s for the first round of PCR, and 25 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s for the second round of PCR.

In all cases, a reaction mix without a DNA template was used as a negative control, and purified DNA from *Borrelia* cultures was used as a positive control, containing a mix of *B. burgdorferi* s.l. complex species. A positive control for *B. miyamotoi* was obtained by the isolation of DNA from infected ticks or by using the cloned *glpQ* gene in a vector plasmid. The PCR reactions were carried out in a final volume of 20 µl, using 2× HotStarTaqPlus Master Mix (Qiagen). Amplicons were visualized by electrophoresis on a 1.5 % agarose gel (1× TAE, pH 8.0) stained with either SYBR[®] Gold DNA gel stain (Invitrogen) or EtBr (Ethidium bromide). DNA extraction steps, PCR, and post-amplification analyses were performed in separated areas of the laboratory with all necessary precautions taken against contamination.

Polymerase chain reaction products of the expected sizes were excised from agarose gels, purified using Centrifugal Filter Units (ULTRAFREE DNA Extraction from Agarose, Millipore) and sequenced in both directions, using the same primers as those used for the PCR. Sequence analysis was performed by SeqMe (SeqMe s.r.o., Czech Republic) and the sequences were

TABLE 2 Selected primers used for *Borrelia* detection.

Target gene	Specification	Primer sequence	Source
Flagellin gene	Out Fw	5'-GCATCACTTTCAGGGTCTCA-3'	(19)
Flagellin gene	Out Rv	5'-TGGGGAACCTTGATTAGCCTG-3'	(19)
Flagellin gene	In Fw	5'-CTTTAAGAGTTCATGTTGGAG-3'	(19)
Flagellin gene	In Rv	5'-TCATTGCCATTGCAGATTGT-3'	(19)
ospC gene	Out Fw	5'-ATGAAAAAGAATACATTAAGTGC-3'	(18)
ospC gene	Out Rv	5'-ATTAATCTTATAATATTGATTTTAAATTAAGG-3'	(18)
ospC gene	In Fw	5'-TATTAATGACTTTATTTTATTTATATCT-3'	(18)
ospC gene	In Rv	5'-TTGATTTTAAATTAAGGTTTFTTTGG-3'	(18)
p66	Out Fw	5'-GATTTTCTATATTTGGACACAT-3'	(18)
p66	Out Rv	5'-TGTAATCTTATTAGTTTTTCAAG-3'	(18)
p66	In Fw	5'-CAAAAAAGAAACACCCCTCAGATCC-3'	(18)
p66	In Rv	5'-CCTGTTTTTAAATAAATTTTGTAGCATC-3'	(18)
glpQ*	Out Fw	5'-ATGGGTTCAAACAAAAAGTCACC-3'	(20)
glpQ*	Out Rv	5'-CCAGGGTCCAATTCATCAGAATATT-3'	(20)
glpQ*	In Fw	5'-ATGGGTTCAAACAAAAAGTCACC-3'	(20)
glpQ*	In Rv	5'-GATGTCCTTACCTTGTTGTTTATGCCA-3'	(20)

*Targeting *Borrelia miyamotoi*.

compared to those available in the GenBank™ dataset by Basic Local Alignment Tool (BLAST) analysis.

Results

The prevalence of *Borrelia* spp. infection was different according to the farm. Of the 54 animals tested, 22 were found positive for one or more *Borrelia* species (Table 1). The overall prevalence of *Borrelia* infection was therefore 41%. *Borrelia burgdorferi* sensu stricto was most common and was found in 9 of the 22 positive animals. Among other detected *Borrelia* species were *Borrelia garinii*, *Borrelia afzelii*, *Borrelia bissetti*, *Borrelia americana*, *Borrelia burgdorferi* sensu lato, and *Borrelia miyamotoi* (Table 1). The collection times and sample sizes differ between each farm; therefore, the presented order of prevalence is chosen primarily to provide a better overview of the results and does not indicate susceptibility of a given animal species to *Borrelia* infections.

The highest prevalence 67%, with the 95% confidence interval (CI) from 47 to 86%, *Borrelia* prevalence, was reported for the ostrich samples, where 4 of the 6 ostriches sampled tested positive for *Borrelia* spp., including one which was positive for *B. miyamotoi*. The detection of *B. miyamotoi* in this ostrich was unique and no other sample in this study tested positive for *B. miyamotoi*.

The second-highest prevalence (44%, CI 34–54%) was observed in the common eland located in Central Bohemia. Of the 25 animals sampled, 11 were positive for the presence of DNA from the *B. burgdorferi* sensu lato spirochetes, including

TABLE 3 Results of *Borrelia* testing in the eland antelope during a 3-month sampling period.

Animal ID	July 2020	August 2020	September 2020
249	Positive	Positive	Positive
251	Negative	N/A*	Positive
253	Positive	Positive	Positive
255	Positive	Positive	Positive
258	Positive	Positive	Positive
259	Negative	Negative	Positive
261	Positive	Negative	Positive
267	Negative	Negative	Positive
268	Positive	Positive	Negative
269	Positive	Positive	Positive
272	Positive	Positive	Positive

*Animal could not be tested.

several co-infections with multiple *Borrelia* species (Table 1). Of the positive animals herein found, most were repeatedly positive (55%, CI 40–70%) during the entirety of the collection period (Table 3). Animals no. 259 and 267 were negative at the start of the monitoring period but were infected in September 2020. Animal no. 268 was negative from September 2020, possibly suppressing the preceding *Borrelia* proliferation. Animal no. 261 seemingly suppressed the infestation in August 2020, but a positive result was found again in September 2020, suggesting a relapse or novel reinfection (Table 3). The unusual result for animal no. 261 might have also been caused

by the faulty isolation or degradation of DNA from the serum sample collected in August. A statistical analysis of the influence of *Borrelia* infection on the eland body condition scores and average daily weight gains during the study period showed no statistically significant effect on these parameters in connection to either ongoing or acquired infection. Only one tick nymph was collected from the eland paddocks, despite repeated flaggings during different periods of tick activity. No feeding ticks were observed on the animals, but the leg and abdominal regions were not thoroughly inspected due to the handling method used (restraint box/squeeze with solid sides).

The third-highest prevalence (40%, CI 27–53%) was found at a camel hobby farm located in Southern Bohemia. Two llamas, three Bactrian camels, and one dromedary camel tested positive, from the fifteen animals tested. No clinical symptoms of borreliosis were observed in any of these animals and they were generally in good health. Live ticks were found in both the pasture (one) and surrounding areas (eleven), but none were found on the animals themselves (Table 1).

Finally, the lowest prevalence (13%, CI 1–24%) was observed at the buffalo milk and meat farm in Central Bohemia, where only one out of eight animals tested positive for *Borrelia*. Three ticks were found in the pasture, of which one was found feeding on a buffalo. Eleven ticks were found in the surrounding areas.

Two Generalized Linear Models with binary response tested the effect of age (in months), sex, and farm or region (one in each model) on the occurrence of *Borrelia* infection. None of the factors were significant, so these effects were disregarded.

Discussion

The tick-host-pathogen network is a complicated, multifactorial biological system, as each component has specific characteristics that potentially influence one another. This complicates the research thereof and has resulted in many influencing factors being currently unknown. The relationships between ticks, pathogens, and their hosts have been studied intensively in household pets, livestock, wildlife, and humans (21–24). Animals kept in zoos in different countries and climates have also been studied, although less thoroughly (10–13). Farm-kept exotic animals have not been considered up until now, in this otherwise extensive research field. Exotic, non-indigenous animal farms can represent one more piece of the puzzle regarding the biology of ticks and TBPs, because they are introducing a new, atypical, and evolutionarily unadapted host into the European tick-host-pathogen network. Since this study has confirmed that such animals can carry some European *Borrelia* species, and

can be infested with ticks, it is suggested that this topic be furtherly investigated.

In their natural habitats, the exotic animal species tested in the present study show varied susceptibility or immunity to tick bites or TBPs. In Africa, eland and other wildlife species developed alongside local tick species for thousands of years, providing them with increased immunity to tick infestations (25, 26). On the other hand, common eland show larger tick infestation numbers in comparison to other wild African large mammal species, possibly suggesting that this antelope is preferred by ticks as a potential host (26, 27). African wild ungulate species are often blamed for the increase of tick numbers in mixed livestock-wildlife farming enterprises; however, no clear evidence of correlation between the presence of wildlife species and tick numbers has been documented (26, 28). In Indian water buffaloes, close relatives of European milk buffaloes, the tick prevalence observed decreased with age and differed between the sexes, suggesting that adult females are less suitable hosts for ticks (29, 30). In dromedary camels (*Camelus dromedarius*), the highly specialized tick, *Hyalomma dromedarii*, is of the biggest concern in the Middle East (31); however, these camels show susceptibility to other tick species as well (32). Since dromedaries are extinct in the wild and are bred solely in captivity, it is difficult to assess their overall resistance to ticks and TBPs, since they inhabit such diverse climates and encounter different tick species. In Bactrian camels (*Camelus bactrianus*), even less information is available about their susceptibility to ticks. Several studies show that these camels harbor some of the well-known TBPs, including *Borrelia* spp (33, 34). Llamas (*Lama glama*) were experimentally confirmed to have the ability to host *Boophilus microplus* ticks (35), and tick paralysis was observed on llamas in the United States and Australia (36); however, there is limited knowledge about their tick resistance or susceptibility in the wild populations.

The lack of interest in farm-kept exotic animals in Europe is surprising for several reasons. First and foremost, animals kept on such establishments are often bred for meat and/or milk production for human consumption. This might pose a threat to human health if no appropriate tick and TBPs prevention strategies are implemented. It is known that tick-borne encephalitis virus (TBEV) is one of the possible alimentary infections from the wide variety of tick-borne infections. On several occasions, TBEV has been transmitted in unpasteurized milk and cheese from goats, cows, and other farm animals (37–40). This specific example might pose a risk for food-borne transmission, especially on smaller, extensive buffalo farms, where the animals are often kept in enclosures without any tick control and in proximity of ideal tick habitats. In this study, a tick was found feeding on a milk-producing buffalo, and a positive case of *Borrelia* infection was also confirmed in one of the animals; thus, it is safe to assume that these

animals come into direct contact with the vectors of TBEV and theoretically could serve as potential infection sources. However, there are still many important gaps in knowledge about buffalo susceptibility and reservoir competence for TBPs, and thus more research needs to be conducted to support this hypothesis.

Secondly, exotic farm-kept animals could participate in the tick-host-pathogen system to some extent. Creation of natural foci of various TBPs in the proximity of exotic animal farms is possible, when we consider the combination of a constant abundance of hosts and good ecological conditions that provide the ideal environment for tick breeding (41, 42). Since the present study focuses solely on the prevalence of *Borrelia* in the selected animals, it is impossible to assess if all TBPs would show the same pattern of prevalence in exotic farm animals, which is one of the limiting factors of this research. Moreover, the reservoir competence, probably the most important factor of TBP host research, remains to be confirmed in these species as well. Efficient reservoir hosts can be characterized by many factors: they are abundant in tick-preferred habitats and can host many vector ticks; pathogens can survive and multiply in the reservoir host for a prolonged period of time or even for a lifetime, and they do not develop a resistance to tick bites even after repeated feedings (13, 43, 44). Since small-scale farms are often located in tick-suitable areas, the possibility of exotic animal species being suitable reservoir hosts is heightened, and should be examined further.

Finally, the current results from the eland research facilities presented another controversial topic. The eland facility is located in an area where the conditions for tick survival were sub-optimal, and only one tick was collected at the location despite repeated attempts during the study period. These results, combined with the high prevalence of *Borrelia* spp., raise an important question about the origin of the infection. Several answers could be offered regarding this discrepancy: one is the hypothesis of congenital or sexual transmission of the pathogen. In the case of *Borrelia*, this mode of transmission has been suggested due to the close evolutionary relationship with *Treponema pallidum*, a bacterium causing syphilis in humans (45, 46). The biological profile similarities of the two bacteria still raise suggestions that the process of congenital or sexual transmission, closely paired with teratogenicity, could be similar (47) even though this has largely been disproven in more recent research (48–50). A great deal of controversy surrounds this topic, as several studies confirm the congenital or sexual transmission of *borrelia* in both humans and animals (51–55), but some studies could not find any supporting evidence for the vertical transmission of *borrelia*, even in controlled laboratory animal studies (56, 57). The obvious absence of live ticks on the farm contrasting with the high prevalence of *Borrelia* infections could suggest

the susceptibility of the eland to vertical transmission of the pathogen. This hypothesis is further supported by the fact that all animals sampled were young calves, 3–17 months old, born and raised on the farm, and thus possible contact with tick vectors is reduced compared to the adults as some of the parents had different places of birth and were imported from other facilities, where they could have had contact with infected ticks. Transmission of *Borrelia* through nursing is improbable, as some human studies suggest (51, 58). Another feasible explanation of this phenomenon might be a bit more straightforward, as many synanthropic animal species like feral cats (*Felis catus*) can access the facilities. The local feral cat population could be infested with ticks, since no anti-acaricidal treatment is usually provided, which could lead to the occasional introduction of non-attached ticks from these feral cats to the eland.

This brief research has shown that exotic farm animals in Europe are susceptible to *I. ricinus* and *Borrelia* spirochetes infection. Our findings suggest that these animals might play a role in the tick-host-pathogen network. We hope that the obtained results will prompt further research focusing on other important TBPs and exotic animal species that were not involved in this research.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

The animal study was reviewed and approved by Czech University of Life Science Animal Welfare and Clearance Committee, Kamýcká 129, 165 00 Praha 6-Suchbát. Written informed consent for participation was not obtained from the owners because informed consent was obtained verbally when the animal owners were consulted about the research. All animal handling (blood withdrawal) was conducted during routine veterinary inspections by veterinarians known by the animal owners.

Author contributions

JH, JC, and LG: research conceptualization and design. JH, JC, MG, AM, FC, JI, TN, and RK: data collection. JH, NR, JC, FC, MG, and RK: analysis and interpretation of results. JH: manuscript draft preparation. FC: statistical analysis. JC,

NR, and LG: supervision. TN: language corrections. All authors contributed to the article and approved the submitted version.

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CHAPTER 4 - Apparent Link Between Naturalistic Grazing and *Ixodes ricinus* Tick Abundance in Rewilding Sites: A Pilot Investigation



Grazed pasture area in the Milovice protected area (photo by Johana Alaverdyan)

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Apparent Link Between Naturalistic Grazing and *Ixodes ricinus* Tick Abundance in Rewilding Sites: A Pilot Investigation

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Abstract

Since 2015, the former military training area in Milovice, Czechia, designated as a Site of European Community Importance (SCI) and later classified as a National Nature Monument, has served as the site for an ongoing trophic rewilding project introducing large semi-wild ungulates for naturalistic grazing. The objective of this pilot study was to investigate the effects of naturalistic grazing on tick abundance and provide empirical data on this topic for the first time. The research was conducted from 2019 to 2021, utilizing flagging as the tick collection method and employing a Generalized Estimating Equations (GEE) model with negative binomial regression to investigate the potential differences in tick abundance between grazed and ungrazed areas. The model was also used to assess the seasonality (months), temperature, and humidity on tick abundance. A total of 586 live ticks were collected, with 20% found in grazed areas and 80% in ungrazed areas. The analysis revealed that landscape management type significantly affected tick abundance, with ungrazed areas showing higher abundance ($p < 0.001$) compared to grazed areas. However, there was no significant difference in tick abundance observed in grazed areas. The rewilding efforts in the

Milovice Reserve, particularly the introduction of large ungulates for natural grazing, apparently have a significant impact on tick abundance and distribution. These findings contribute to our understanding of tick ecology and population dynamics in rewilded areas and highlight the importance of considering factors such as landscape management regime, seasonality, and environmental conditions in tick management strategies and conservation efforts in natural areas.

Key words

ecto-parasites, landscape management, large ungulates, seasonality, ecosystem

Introduction

The *Ixodes ricinus* tick is a major vector of tick-borne diseases (TBDs), including borreliosis, tick-borne encephalitis, ehrlichiosis, and babesiosis, in Europe. These diseases have a significant impact on both animal and human health, making *I. ricinus* ticks an important research target. The prevalence of TBDs presents a significant ecosystem disservice and directly affects public and wildlife health in Europe (Dunn, 2010). The complex ecology of *I. ricinus* has been extensively studied, but ongoing changes in land management, climate, and urbanization have played an important role in altering the distribution and abundance of ticks (Zajac et al., 2021; Janzén et al., 2023). These changes offer new opportunities and challenges to tick populations regulating their dynamics (Hauck et al., 2020). To accurately assess the ongoing evolution of tick ecology, many aspects need to be considered and new perspectives identified.

Landscape properties, including composition and configuration, and biological interactions of fauna and flora, influence the abundance of ticks and their hosts (Ehrmann et al., 2017). Since ticks are dependent on suitable hosts for development and survival, their ecological preferences are inevitably linked (McCoy et al., 2013). Habitat characteristics, such as plant composition, morphology, vertical structure, and coverage, exert selection pressure on both host visitation and tick activity (Tack et al., 2012).

Large herbivores, particularly grazers, play an important role in the trophic chain and can significantly influence ecosystem structure and functioning (Johnson and Cushman, 2007). Such animals are known as "ecosystem engineers" because of their ability to create and maintain habitats suitable for their proliferation (Wilby et al., 2001). In recent years, reintroductions of extirpated large mammalian species have become increasingly popular worldwide (Johnson and Cushman, 2007). This rewilding trend represents a different approach to ecosystem management, offering new, cost-effective options to recreate and maintain biodiverse environments with functional trophic interactions without intensive human intervention (Dvorský et al., 2022). Although there is considerable research activity in this area (Millins et al., 2017), to our knowledge, no empirical data have been published to

date on the abundance of ticks or prevalence of tick-borne diseases in connection to rewilding efforts.

In 2015, the large herbivore trophic rewilding program in the Czechia introduced Exmoor ponies (*Equus caballus*), European bison (*Bos bonasus bonasus*), and Tauros cattle (*Bos taurus*) to a former military area in Milovice, located in central Bohemia. The grazed area has been gradually expanded over the years from 145 ha in 2015 to 355 ha in 2021. The local fauna and flora flourished under naturalistic grazing, increasing the species richness and functional diversity of plant communities, along with the incidence of Red List species (Dvorský et al., 2022). Such effective grazing may partially hamper bush encroachment and could have a positive effect on the control of ticks and tick-borne pathogens (TBPs). However, it should be noted that this management approach may have unintended consequences, such as an increase in the number of potential hosts for questing ticks. This could potentially lead to an increase in tick abundance, as has been observed in other studies focusing on conservation efforts and landscape management (Millins et al., 2017; Myrsetrud et al., 2013). We conducted a small-scale pilot study to examine the impact of large herbivore naturalistic grazing on tick abundance and to assess the potential implications for both the ecosystem and human population.

Materials and Methods

2.1 Study site:

After the recission of the Soviet army in 1991, the Milovice military area covering 12.5 km² was designated as a Site of European Community Importance (SCI) Milovice-Mladá and later classified as a National Nature Monument Mladá. The protected area remained mostly unmanaged between 1991 and 2015, except for occasional off-road vehicle disturbances. On a significant part of the site, the once-diverse vegetation mosaic was homogenized by succession, with competitively strong grasses, such as *Calamagrostis epigejos*, *Brachypodium* spp., *Bromus erectus*, and *Arrhenatherum elatius*, ruderal herbs, and shrubs, including *Crataegus monogyna*, *Prunus cerasifera*, *Prunus spinosa*, and *Rosa canina* (Dvorský et al., 2022).

Since 2015, two separate parts of the reserve, Milovice in the south and Traviný in the north, have been selected for a rewilding project. These areas are now naturally grazed by large semi-wild ungulates, including horses (Exmoor ponies, *Equus caballus*), cattle (back-bred aurochs, Tauros breed, *Bos taurus*), and European bison (*Bos bonasus bonasus*). The presence of these large herbivores has led to an increase in the functional diversity of plant communities. Naturalistic grazing has promoted a vegetation change from species poor tallgrass to species rich, taxonomically, and structurally diverse grassland typical of European forest-steppe ecoregion (Dvorský et al., 2022). Small to medium-sized wildlife can freely

enter and exit the pastures through the wildlife passes in the electric fence. Various common wildlife species are abundant, including predators such as golden jackal (*Canis aureus*, resident, breeding), red fox (*Vulpes vulpes*), badger (*Meles meles*) (Jirků et al., 2018), and herbivore or omnivore species such as roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*), wild boar (*Sus scrofa*), European hare (*Lepus europaeus*), various species of rodents (*Apodemus*, *Myodes*, *Microtus*, *Sorex*, *Mus*, *Micromys* spp.), reptiles (e.g. sand lizard, *Lacerta agilis*) and many other vertebrates, all of which are potential tick hosts.

2.2 Tick collection:

To estimate the abundance of *I. ricinus* ticks in the southern part of the Milovice Reserve, we employed flagging as a collection method, using a 0.5 x 0.5 m piece of cotton fabric with 1cm hair. The flag was employed at a 150 m long and 5 m wide (750 m²) transects (as suggested in Eisen et al., 2019) at two selected sampling sites from March to September between 2019 and 2021. To prevent overcollection bias, only 2-4 collections were performed each month during the designated collection periods. Flags were inspected at intervals of 30 seconds, equating to roughly every 15 meters. Collections were carried out for a period of one hour each. Flagging activities commenced when boundary temperatures reached 8 – 10 °C consistently for a minimum of 5 consecutive days, alongside relative humidity levels slightly below or surpassing 42%, following the criteria outlined by Hubálek et al. (2004). Collections were suspended during periods of windy weather or anticipated heavy rainfall. However, wind speed was not monitored during these observations. As large ungulates are free to roam the reserve, grazed transects were not sampled if the large ungulates were present in the vicinity. Due to this fact, tick activity was measured per person-hour of sampling, rather than normalized to the area of sampling. To minimize potential bias from tick movement, the two sites were separated by a 300 m buffer, with one site being actively grazed and the other ungrazed but neighboring the grazed area. The two selected transects had the following starting and ending points: grazed 50.2341503N, 14.8843336E - 50.2331414N, 14.8829389E, and ungrazed 50.2364631N, 14.8822306E - 50.2377119N, 14.8810506E. The grazed and ungrazed areas showed variances in both grass composition and length, alongside noticeable soil disturbance in pastured areas. However, notwithstanding these distinctions, the bush and tree cover remained largely alike.

The southern grazed area “Milovice” (106 ha) was sampled systematically during 2019-2021, while the northern pasture “Traviny” was sampled on a single occasion in 2020 (Table S1) (ungrazed transect: 50.2895681N, 14.8641875E-50.2895200N, 14.8628089E; grazed transect: 50.2877411N, 14.8695464E-50.2874394N, 14.8705981E).

At the start of each collection period temperature and humidity were assessed at the vegetation level using a hygrometer with an inbuilt thermometer (LUTRON LM-81HT) that was placed on the ground at level with vegetation and left undisturbed for a duration of 1

minute to acclimate. The objective of these microclimatic measurements was to evaluate the potential influence on tick population abundance.

The collected ticks were then stored in plastic containers at 5°C with a piece of grass to provide moisture. Live ticks were then sorted by developmental stage and sex using the key by Otranto et al. (2017), and their abundance was counted for evaluation. Ticks of all developmental stages were included in the abundance evaluation.

2.3 Statistical analysis:

We used a generalized estimating equation (GEE) model with a negative binomial regression approach to assess the impact of landscape, season, temperature, and humidity on the abundance of *I. ricinus* ticks. The GEE model was selected to account for the correlation within the data due to repeated measurements taken at the same locations over time, making it suitable for analyzing data with repeated measures and accommodating the presence of zero values in the dataset. To address any potential overdispersion in the distribution of questing ticks among dragging sites, we utilized a negative binomial error in the models. The models considered total tick counts as the dependent variable, incorporating ticks from all life stages captured by flagging. Adjustments were made for the clustering of observations by sampling site. The independent variables included landscape management (grazed vs. ungrazed), collection month (March, April, May, June, and September), temperature, and humidity. The GEE model assumed a log link function and a Poisson distribution for the tick abundance data. To account for any potential heteroscedasticity in the data, robust standard errors were employed. Additionally, an exchangeable correlation structure was applied to capture the correlation among repeated measurements.

To explore the relationship between continuous predictors and the outcome, we categorized the predictors into seasons and years and conducted graphical evaluations. All statistical computations were performed using R Studio (R Core Team, 2021), and the GEE model fitting was implemented using the `geepack` package (Halekoh et al., 2006). Graphical representations were created using GraphPad Prism 9.0.0 (GraphPad Software, Boston, Massachusetts USA).

Results

During the collection periods, we collected a total of 586 live ticks, consisting of 163 adult females, 132 adult males, 288 nymphs, and 3 larvae. Among these, 118 ticks (20 %) were found in grazed areas, comprising 46 nymphs, 43 adult females, 27 adult males, and 2 larvae, while 468 ticks (80 %) were found in ungrazed areas, consisting of 242 nymphs, 120 adult females, 105 adult males, and 1 larva (Table S1). During the single collection in the "Traviny" section of the reserve, we found a total of 28 ticks: 21 (75%) on the ungrazed transect and 7 (25%) on the grazed transect. The ticks on the ungrazed transect were comprised of 10

females, 7 males, and 4 nymphs, while the ticks on the grazed transect consisted of 1 female and 6 larvae (Table S1).

The statistical analysis demonstrated that landscape management had a significant effect on tick abundance ($\beta = 18.205$, $p < 0.001$) (Table 1). Specifically, ungrazed areas exhibited significantly higher tick abundance compared to grazed areas ($\beta = 1.41475$, $SE = 0.33158$). However, when comparing tick abundance within grazed areas, we did not observe a statistically significant difference ($\beta = 0.27600$, $SE = 0.37089$). Tick abundance varied across different months, with positive coefficients for the season variables indicating higher tick abundance in certain months, although not all effects were statistically significant. The β values associated with April and May were positive, with a significant peak in April ($p = 0.0145$) for both grazed and ungrazed transects (Table 1, Figure 1). Conversely, a significant negative association was found between tick abundance and the month of September ($\beta = -0.72506$, $p = 0.011$), indicating lower tick abundance during September compared to the other months. However, no significant associations were found for the months of March and June. In contrast, micro-climatic variables such as temperature ($\beta = 0.034$, $p = 0.853$) and humidity ($\beta = 0.740$, $p = 0.390$) did not exhibit a significant impact on tick abundance compared to the landscape management regime and season variables (Table 1).

Discussion and conclusions

Our findings suggest a strong correlation between tick abundance and landscape management regime, and habitat changes induced by naturalistic grazing of large ungulates. We observed a pattern of overall reduced tick numbers in grazed sites. The ungrazed fallow sites left without human intervention showed high tick abundance. The Milovice reserve, which employs a specific type of land management that balances grazing pressure to prevent overgrazing while averting bush and tallgrass encroachment, has increased overall biodiversity in both plant and animal species (Dvorský et al., 2022; Jirků et al., 2018; Konvička et al., 2021). Although such development potentially enhances the suitability of the habitat for tick survival by provision of diverse and abundant vertebrate host communities, our observations suggest otherwise.

Ticks can introduce tick-borne pathogens (TBPs) to ecosystems, and their abundance can be one of the driving factors of TBP prevalence. Tick-host-pathogen relationships are known to be complex and influenced by several factors. For example, the dilution effect predicts reduced infection prevalence in habitats with high species variability (Keesing et al., 2006; LoGiudice et al., 2003). Furthermore, the host's competence to serve as a suitable feeding target and reservoir of tick-borne pathogens (TBPs) is also an essential factor (Keesing et al., 2009). Additionally, ticks may preferentially select hosts based on their competence as a feeding target (Randolph and Dobson, 2012). Habitat characteristics are equally important for the dispersal and prevalence of TBPs (Ehrmann et al., 2017), and it was proposed for habitats to be specified as either diluting or amplifying (Ehrmann et al., 2018).

Our research evaluated solely the abundance of ticks on each landscape management regime, leading to a limited number of conclusions. We observed high numbers of adult ticks on both grazed and ungrazed areas of the Milovice reserve, with peak abundance between April and May of 2020 (Figure 1). Tick collections yielded similar numbers of nymphs and adults. This might be explained by the high abundance of hosts suitable for adult and nymphal stages of *I. ricinus* like hares, hedgehogs, deer, and megafauna inhabiting the Milovice reserve (cattle, horse, bison). As wildlife movement remains unaffected by fencing of pastures and wild animals are able to roam in and out, host induced migration of adult ticks from adjacent areas is obviously occurring. The low quantity of collected tick larvae could be attributed to potential human error during the collection and selection of flags. Given their small size, it is possible that some larvae were overlooked and not accounted for in the final evaluation.

As suggested by the presented results, large ungulate grazing apparently greatly decreases the tick abundance at sampling area. This could be caused by significant change in vegetation structure, its floristic composition and physical turf disruption resulting from natural grazing. Complementary increase of shortgrass and decrease of tallgrass vegetation directly facilitated by large ungulates might be of utmost importance. The significant decrease in surface coverage of dense tallgrass vegetation dominated by graminoids and increase of shortgrass vegetation with high proportion of forbs typically associated with grazed sites (Dvorský et al., 2022) necessarily results in limited availability of microhabitat features essential for survival and successful life cycle completion in *I. ricinus*. These include i) lack of preferential questing sites high above ground on emergent graminoids (Gassner et al., 2011) and ii) scarcity of shady humid microclimatic refugia below the live and dead graminoid biomass used by ticks during hot dry weather (Gern et al., 2008). These grazing-associated vegetation characteristics might well explain the negative correlation between tick abundance and grazing observed in this study. It's worth noting that tick collection bias may have influenced our results. There's ongoing discussion about the suitability of flagging for certain habitats and the variability in flagging efficiency among different transects (Dantas-Torres et al., 2013). To address this potential bias, we followed identical collection protocols on both transects, as recommended by Eisen et al. (2019). Although ungrazed transects presented challenges due to increased grass growth, the plain-like grassland habitat remained accessible. Our statistical analysis suggests that weather conditions, i.e., temperature and humidity have a low overall influence on tick abundance. Instead, landscape management regime (grazed vs. fallow) and habitat properties (shortgrass vs. tallgrass vegetation) are the more important factors in explaining the local variation of tick abundance. These results correlate with the larger study of Ehrmann et al., (2017).

Through small-scale sampling, we have gathered empirical data that establish a baseline for the impact of man-induced trophic rewilding processes on tick abundance. Given that rewilding is a relatively new conservation approach with a somewhat flexible definition, it's important to clarify that in our research, we adhered to the latest definition of the term as outlined in Ledger et al. (2022). According to this definition, rewilding involves regenerating a

human-disturbed or degraded ecosystem with the goal of increasing its autonomy over time. Trophic rewilding, within this context, specifically targets the stimulation of ecological processes through trophic cascades and the introduction of large animals. Our research connects changes in landscape and habitat due to grazing and ecosystem engineering activities of large grazers with the abundance of ticks. While the amount of data collected during this pilot study is limited and represents only a small fraction of the potential data available regarding ecosystems undergoing rewilding, empirical data from ongoing rewilding projects are scarce. In particular, data on ticks and TBDs in the context of naturalistic grazing schemes are virtually lacking. Therefore, our findings have significant implications that could potentially stimulate further research on the association between rewilding efforts and the threat of tick-borne diseases. More detailed research is needed to fully understand the implications of decreased tick abundance on the prevalence of TBPs and the capacity of animal species involved in rewilding projects to act as reservoirs. It is crucial to further investigate this relationship to promote effective rewilding strategies that prioritize both ecosystem restoration and public health.

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Conflict of interest statement

All authors declare that they have no conflicts of interest.

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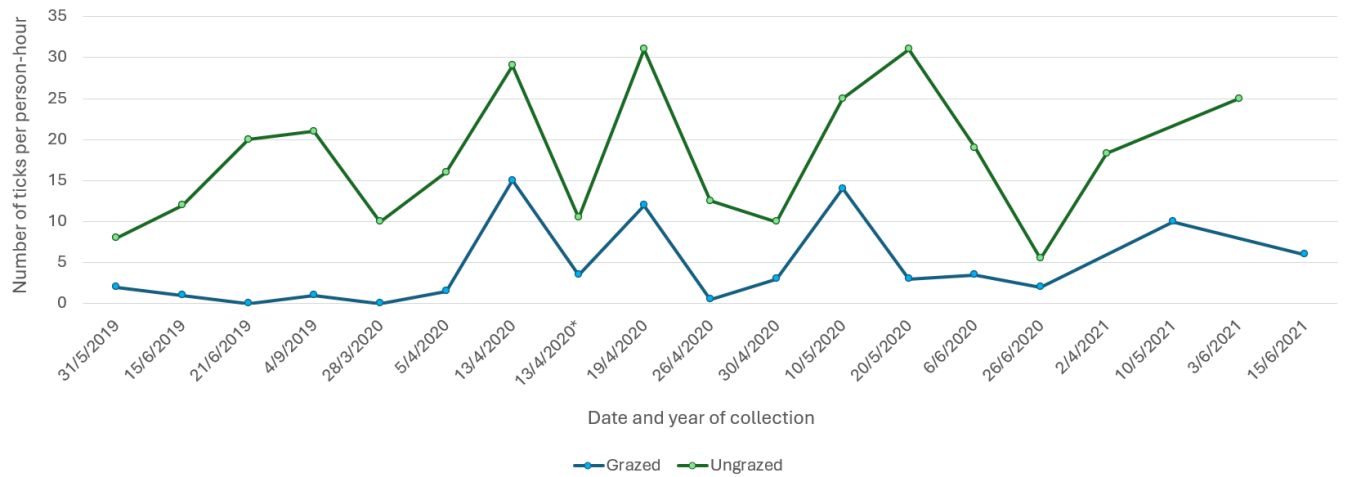
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Figures

Figure 1: Effects of landscape management regime on *Ixodes ricinus* abundance during collection period



* Area of Traviny, neighboring pasture to Milovice

Tables

Table 1: Results of GEE analysis.

Coefficients:	Estimate (β)	Std.err. (SE)	Wald	Pr(> W)
(Intercept)	1.08752	2.2563	0.232	0.6298
Landscape (Grazed)	0.27600	0.37089	0.554	0.4568
Landscape (Ungrazed)	1.41475	0.33158	18.205	0.0000
Month (March)	-0.62454	0.35940	3.020	0.0823
Month (April)	0.34567	0.78920	3.020	0.0145
Month (May)	0.32629	0.63920	0.261	0.6097
Month (June)	-0.89033	0.73383	1.472	0.2250
Month (September)	-0.72506	0.28508	6.469	0.0110
Temperature (°C)	0.01531	0.08275	0.034	0.8533
Humidity (%)	0.01555	0.01808	0.740	0.3897

Supplementary materials

Table S1: Detailed collection data

Transect	Collection date	Landscape*	Total ticks	♀	♂	Nymphs	Larvae	Temperature (°C)	Humidity (%)	Person/hour	Person-hour n. ticks
50.2341503N, 14.8843336E - 50.2331414N, 14.8829389E	31/5/2019	G	2	0	0	2	0	20	63	1p/1h	2
50.2364631N, 14.8822306E - 50.2377119N, 14.8810506E	31/5/2019	U	8	2	3	3	0	20	63	1p/1h	8
50.2364631N, 14.8822306E - 50.2377119N, 14.8810506E	15/6/2019	U	12	3	1	8	0	26	70	1p/1h	12
50.2341503N, 14.8843336E - 50.2331414N, 14.8829389E	15/6/2019	G	1	0	0	1	0	26	70	1p/1h	1
50.2364631N, 14.8822306E - 50.2377119N, 14.8810506E	21/6/2019	U	20	4	2	14	0	24.1	59.1	1p/1h	20
50.2341503N, 14.8843336E - 50.2331414N, 14.8829389E	21/6/2019	G	0	0	0	0	0	24.1	59.1	1p/1h	0
50.2364631N, 14.8822306E - 50.2377119N, 14.8810506E	4/9/2019	U	21	2	2	17	0	19.8	51	1p/1h	21
50.2341503N, 14.8843336E - 50.2331414N, 14.8829389E	4/9/2019	G	1	0	1	0	0	19.8	51	1p/1h	1
50.2364631N, 14.8822306E - 50.2377119N, 14.8810506E	28/3/2020	U	20	4	9	7	0	19.2	39	2p/1h	10
50.2341503N, 14.8843336E - 50.2331414N, 14.8829389E	28/3/2020	G	0	0	0	0	0	19.2	39	2p/1h	0
50.2341503N, 14.8843336E - 50.2331414N, 14.8829389E	5/4/2020	G	3	2	0	1	0	21.5	30	2p/1h	1.5
50.2364631N, 14.8822306E - 50.2377119N, 14.8810506E	5/4/2020	U	32	4	2	26	0	21.5	30	2p/1h	16
50.2341503N, 14.8843336E - 50.2331414N, 14.8829389E	13/4/2020	G	30	1	5	24	0	18	60	2p/1h	15
50.2364631N, 14.8822306E - 50.2377119N, 14.8810506E	13/4/2020	U	58	14	7	37	0	18	60	2p/1h	29
50.2895681N, 14.8641875E - 50.2895200N, 14.8628089E	13/4/2020	U "Traviny"	21	10	7	4	0	18	60	2p/1h	10.5
50.2877411N, 14.8695464E - 50.2874394N, 14.8705981E	13/4/2020	G "Traviny"	7	1	0	6	0	18	60	2p/1h	3.5
50.2341503N, 14.8843336E - 50.2331414N, 14.8829389E	19/4/2020	G	12	4	3	5	0	20	48	1p/1h	12
50.2364631N, 14.8822306E - 50.2377119N, 14.8810506E	19/4/2020	U	31	4	5	22	0	20	48	1p/1h	31
50.2364631N, 14.8822306E - 50.2377119N, 14.8810506E	26/4/2020	U	25	9	9	7	0	22	37	2p/1h	12.5
50.2341503N, 14.8843336E - 50.2331414N, 14.8829389E	26/4/2020	G	1	0	1	0	0	22	37	2p/1h	0.5
50.2364631N, 14.8822306E - 50.2377119N, 14.8810506E	30/4/2020	U	10	3	6	1	0	19.6	44.8	1p/1h	10
50.2341503N, 14.8843336E - 50.2331414N, 14.8829389E	30/4/2020	G	3	2	1	0	0	19.6	44.8	1p/1h	3
50.2364631N, 14.8822306E - 50.2377119N, 14.8810506E	10/5/2020	U	50	17	20	13	0	27	55	2p/1h	25
50.2341503N, 14.8843336E - 50.2331414N, 14.8829389E	10/5/2020	G	28	9	14	5	0	27	55	2p/1h	14
50.2364631N, 14.8822306E - 50.2377119N, 14.8810506E	20/5/2020	U	31	11	14	6	0	23.4	65	1p/1h	31
50.2341503N, 14.8843336E - 50.2331414N, 14.8829389E	20/5/2020	G	3	2	0	1	0	23.4	65	1p/1h	3
50.2341503N, 14.8843336E - 50.2331414N, 14.8829389E	6/6/2020	G	7	3	2	0	2	21.4	73	2p/1h	3.5
50.2364631N, 14.8822306E - 50.2377119N, 14.8810506E	6/6/2020	U	38	18	2	17	1	21.4	73	2p/1h	19
50.2364631N, 14.8822306E - 50.2377119N, 14.8810506E	26/6/2020	U	11	5	5	1	0	29.5	56	2p/1h	5.5
50.2341503N, 14.8843336E - 50.2331414N, 14.8829389E	26/6/2020	G	4	3	0	1	0	29.5	56	2p/1h	2
50.2364631N, 14.8822306E - 50.2377119N, 14.8810506E	2/4/2021	U	55	7	11	37	0	16.7	42	3p/h	18.3
50.2341503N, 14.8843336E - 50.2331414N, 14.8829389E	10/5/2021	G	10	10	0	0	0	23	61	1p/1h	10
50.2364631N, 14.8822306E - 50.2377119N, 14.8810506E	3/6/2021	U	25	3	0	22	0	24.1	58.2	1p/1h	25
50.2341503N, 14.8843336E - 50.2331414N, 14.8829389E	15/6/2021	G	6	6	0	0	0	26.2	55	1p/1h	6

CHAPTER 5 - Postindustrial Landscapes Are Neglected Localities That May Play an Important Role in the Urban Ecology of Ticks and Tick-Borne Diseases – A Pilot Study



Construction waste deposit area in Prague-Sedlec (photo by Anna Dvořáková)

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Brief Report

Postindustrial Landscapes Are Neglected Localities That May Play an Important Role in the Urban Ecology of Ticks and Tick-Borne Diseases—A Pilot Study

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Abstract: Background: Numerous recent studies have shown that ticks and tick-borne pathogens pose a considerable threat in urban areas, such as parks, playgrounds, zoos, cemeteries, etc. Abandoned postindustrial localities, and other types of vague terrain, are other examples of urban wilderness areas that have been absolutely neglected in respect to ticks and tick-borne pathogens thus far, even though they provide ideal biotopes for ticks. Methods: The abundance of ticks and prevalence of *Borrelia burgdorferi* sensu lato spirochetes were compared between a city park and an adjacent abandoned construction waste disposal site in Prague, Czechia from June to October 2021. Results: The results showed that ticks and borrelia spirochetes are present at the city park as well as at the abandoned construction waste disposal site, although in lower numbers. Discussion: According to the best of our knowledge, this is the first report describing the presence of ticks and tick-borne pathogens in an urban postindustrial landscape. More detailed studies are needed to uncover the role of these localities in the ecology of ticks and ecoepidemiology of tick-borne diseases in urban areas.

Keywords: ticks; postindustrial landscapes; vague terrain; urban landscapes; *Ixodes ricinus*; *Borrelia burgdorferi* sensu lato



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1. Introduction

Ticks are important hematophagous parasites and vectors of numerous pathogens of veterinary and medical importance. Ticks and tick-borne pathogens are understood to be a problem connected with rural and natural areas, but numerous recent studies have shown that urban green areas may also provide suitable ecosystems for ticks, tick-borne pathogens, and their hosts/reservoirs [1–3]. These findings clearly demonstrate that urban localities, such as parks, playgrounds, zoos, and cemeteries, play an important role in the ecoepidemiology of ticks and tick-borne diseases [4–7]. Moreover, in these areas, ticks come in frequent contact with humans and their companion animals, which increases the risk of tick-borne pathogen transmission.

On the other hand, risks connected with ticks and tick-borne diseases in urban ecosystems can be substantially decreased by employing proper management strategies within these localities [8]. Regular mowing and removal of organic debris (old grass, leaf litters), the creation of tick hostile ecosystems (light, dry, and sunny areas), control of tick-hosts, as well as area-wide use of acaricides or tick pathogens and parasitoids are just several examples of potential approaches for management of ticks and tick-associated risks in such areas [8]. Nevertheless, usage of these tick-management strategies is expensive, and therefore there are numerous localities within most cities that are undermanaged or not managed at all.

Good examples of such localities are different types of vague terrain, which are places without any clear owners such as abandoned postindustrial landscapes and “spaces within spaces”—localities without any clearly defined public role, usually existing between other

urban areas. These localities are typically transient in time, largely existing only for a few years or decades [9]. However, even a short period can provide enough time for the advanced succession and development of “urban jungle” ecosystems in these localities [10]. These localities can be also sources of many social problems, as they may attract homeless people and drug users, along with increased local criminality. On the other hand, they may also be used by local citizens to walk their pets, for community gardening, and many other beneficial purposes [9].

Despite the fact that these localities apparently offer an ideal ecosystem for ticks and tick-borne pathogens, there are no data about their role in the ecology of ticks and coepidemiology of tick-borne diseases, according to our best knowledge. The goals of this research were (i) to provide the first rigorous data about the presence of ticks and the prevalence of tick-borne pathogens in these localities and (ii) to attract the attention of tick-biologists, urban architects, local authorities, and other relevant entities to these localities, which may lead to the improvement of the current situation.

2. Materials and Methods

The study was conducted from June to October 2021, in two adjacent localities at the northwestern edge of Prague, Czechia: the city park Sedlecký sad (GPS: 50.127463, 14.390946), which is connected to large green areas within the Vltava River valley, outside the city of Prague, and a neighboring abandoned construction waste disposal site (50.130237, 14.388196) (Figure 1). Ticks were collected by flagging using approximately 1 × 1 m large flag made from white cotton. Flagging was performed on both localities several times during each month. The flagged localities within the boundaries of studied areas as well as our movement during flagging was completely random during each tick collection to prevent possible “overhunting” of ticks. Time spent at the locality and number of people who were flagging for ticks differed among the individual “tick hunts”. Therefore, the numbers of collected ticks were normalized according to the person-hours of collection. The presence of tick hosts at both localities was estimated using direct observation, camera traps, and observation of animal residential signs (Figures S1–S10). Vegetation changes at both research sites during the tick season were photo-documented, and the most frequent species were determined using Google Lens (Figures S1–S10). *Borrelia* were detected in the collected tick samples using PCR amplifying a fragment of the *flaB* gene, a marker of *Borrelia burgdoferi* sensu lato (s.l.) infection [11]. PCR results were visualized using agarose gel electrophoresis. Amplicons corresponding to the expected size were cut from the gel, extracted, and sent for sequencing to Biocev, Charles University in Prague. Obtained sequences were compared against sequences of known borrelia species in GenBank. Statistical analyses were performed in Statistica 12 (Tibco). Factorial ANOVA was used for comparison of average values of the collected ticks, depending on the location and month. An X2 test was used to compare prevalence of borrelia between the localities.

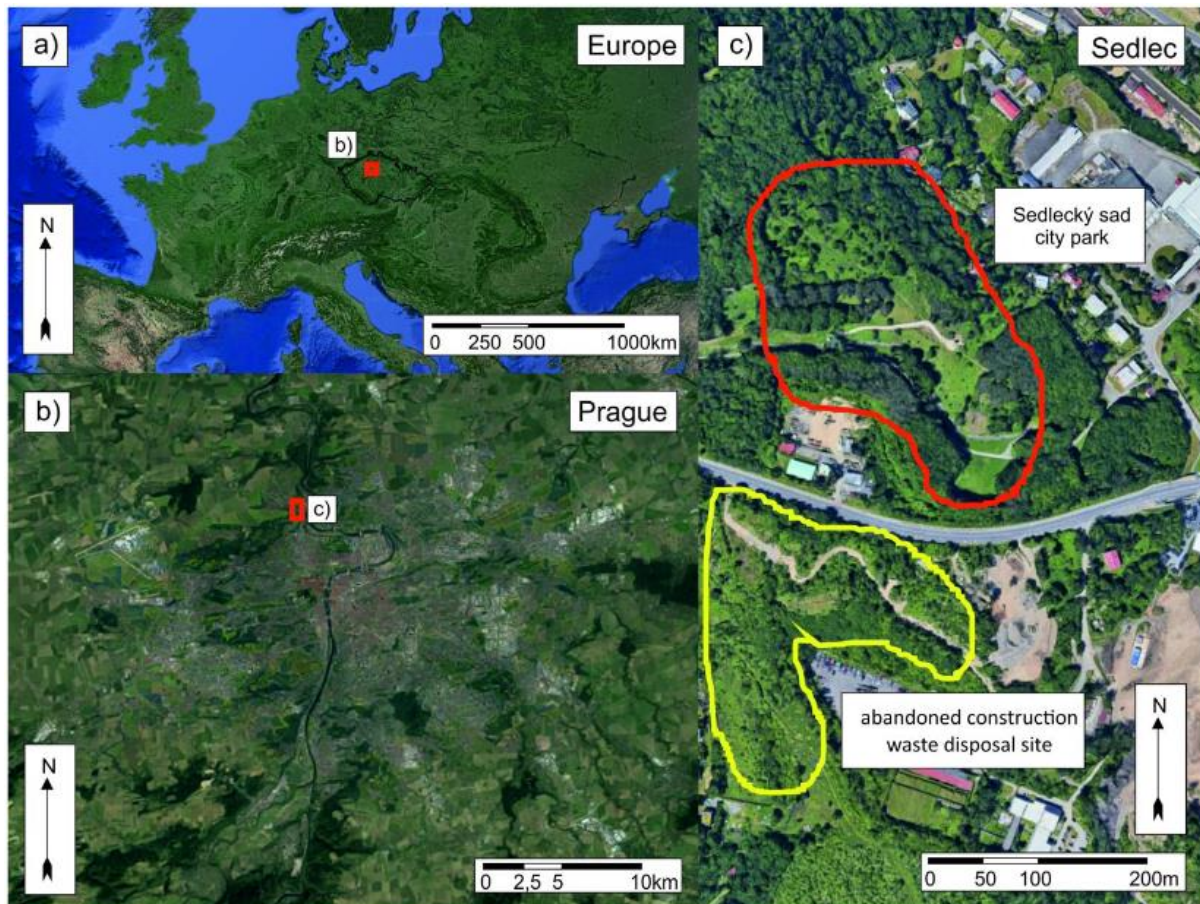


Figure 1. Study site: Location of the study site within the geographical context of (a) Europe, (b) Prague, and (c) the Sedlec neighborhood. The city park Sedlecký sad and the abandoned construction waste disposal site are adjacent areas separated only by Kamýčká street, which has a low traffic intensity, especially during the night, and therefore should pose almost no barrier to animal migration. The approximate flagged areas in the city park, Sedlecký sad, and the abandoned construction waste disposal site are marked with red and yellow lines, respectively.

3. Results

3.1. The Abandoned Disposal of Nontoxic Construction Waste Material Shows Advanced Level of Biological Succession

The construction waste disposal site was used between 2001 and 2011 for the disposal of nontoxic construction waste material, such as rubble, grit, and sand. Since 2011, the locality was abandoned and left for “natural” succession. Therefore, ten years later, in 2021, the whole locality was covered with dense vegetation consisting of various species of grasses, small bushes, and young trees. Representatives of numerous animal species were observed at the abandoned construction waste disposal site including roe deer (*Capreolus capreolus*), red fox (*Vulpes vulpes*), pine marten (*Martes martes*), feral cat (*Felis catus*), ring-necked pheasant (*Phasianus colchicus*), and various songbirds, e.g., Eurasian magpie (*Pica pica*) and Eurasian jay (*Garrulus glandarius*). A similar composition of plant and animal species was observed in the unmanaged parts of the city park Suchdolský sad, but in the city park, there were also areas of twice-a-year mowed meadows, old fruit trees, small groves, and an old oak–beech forest. The animal richness was greater in the city park than at the construction waste disposal site, both in terms of the number of individuals as well as the number of

species. In the city park, there was also higher activity of humans and companion animals (see Figures S1–S10 for photo documentation from both biotopes).

3.2. Ticks Are Present at the Abandoned Disposal of Nontoxic Construction Waste Material Site, despite Being in Lower Abundance Compared to the Adjacent City Park

In total, 250 ticks were collected (92 from the abandoned construction waste disposal site and 158 from the city park—see Table 1 and Figure 2 for details). All collected ticks were morphologically determined as representatives of various developmental stages of the castor bean tick (*Ixodes ricinus*). Statistical analysis showed that a significantly higher number of ticks were present in the city park ($p = 0.008622$). This result can be explained by advanced, but not fully completed, succession at the abandoned construction waste disposal site and overall lower biological richness at that locality. Statistical analysis also showed a significant difference in number of ticks collected in each month ($p = 0.000234$).

Table 1. Ticks collected.

Locality	Month	Date	Flag. per. [h]	Pers.	Flag. [pers-h]	F	M	N	L	T	FS	MS	NS	LS	TS
The abandoned construction waste disposal site	June	02.06.2021	0.5	3	1.5	5	4	21	1	31	3.33	2.67	14	0.67	20.67
		07.06.2021	1	2	2	3	5	17	0	25	1.5	2.5	8.5	0	12.5
		14.06.2021	1	1	1	3	2	15	0	20	3	2	15	0	20
		29.06.2021	1	2	2	0	3	1	0	4	0	1.5	0.5	0	2
	July	03.07.2021	1	1	1	1	0	1	0	2	1	0	1	0	2
		24.07.2021	1	1	1	1	0	0	0	1	1	0	0	0	1
		29.07.2021	1	1	1	0	1	0	0	1	0	1	0	0	1
	August	09.08.2021	1	1	1	1	0	0	0	1	1	0	0	0	1
		23.08.2021	1	1	1	0	0	0	0	0	0	0	0	0	0
	September	06.09.2021	1	1	1	2	0	0	0	2	2	0	0	0	2
		14.09.2021	1	1	1	0	0	0	0	0	0	0	0	0	0
		26.09.2021	1	2	2	0	0	1	0	1	0	0	0.5	0	0.5
	October	04.10.2021	1	1	1	2	0	0	0	2	2	0	0	0	2
		11.10.2021	1	1	1	0	1	0	0	1	0	1	0	0	1
18.10.2021		1	1	1	1	0	0	0	1	1	0	0	0	1	
Subtotal for the abandoned construction waste disposal site					18.5	19	16	56	1	92	1.02	0.86	3.03	0.05	4.97
Sedlecký sad city park	June	02.06.2021	-	-	-	-	-	-	-	-	-	-	-	-	-
		07.06.2021	1	2	2	4	9	16	1	30	2	4.5	8	0.5	15
		14.06.2021	1	1	2	4	1	26	1	32	4	1	26	1	32
		29.06.2021	1	2	1	9	6	9	0	24	4.5	3	4.5	0	12
	July	03.07.2021	1	1	2	1	0	10	0	11	1	0	10	0	11
		24.07.2021	1	1	1	0	0	9	0	9	0	0	9	0	9
		29.07.2021	1	1	1	2	5	7	6	20	2	5	7	6	20
	August	09.08.2021	1	1	1	6	2	6	1	15	6	2	6	1	15
		23.08.2021	1	1	1	0	1	0	9	10	0	1	0	9	10
	September	06.09.2021	1	1	1	0	1	0	0	1	0	1	0	0	1
		14.09.2021	1	1	1	0	2	0	0	2	0	2	0	0	2
		26.09.2021	1	2	1	0	0	2	0	2	0	0	1	0	1
	October	04.10.2021	1	1	2	0	0	0	0	0	0	0	0	0	0
		11.10.2021	1	1	1	1	0	0	0	1	1	0	0	0	1
18.10.2021		1	1	1	0	1	0	0	1	0	1	0	0	1	
Subtotal for the city park					18	27	28	85	18	158	1.5	1.56	4.72	1	8.78

Flag. per. [h]—flagging period, Pers.—number of persons flagging, Flag. [pers-h]—flagging intensity [person-hours], F—females, M—males, N—nymphs, L—larvae, T—total ticks, FS—females-standardized [ticks per hour], MS—males-standardized [ticks per hour], NS—nymphs-standardized [ticks per hour], LS/larvae-standardized [ticks per hour], TS—total ticks-standardized [ticks per hour].

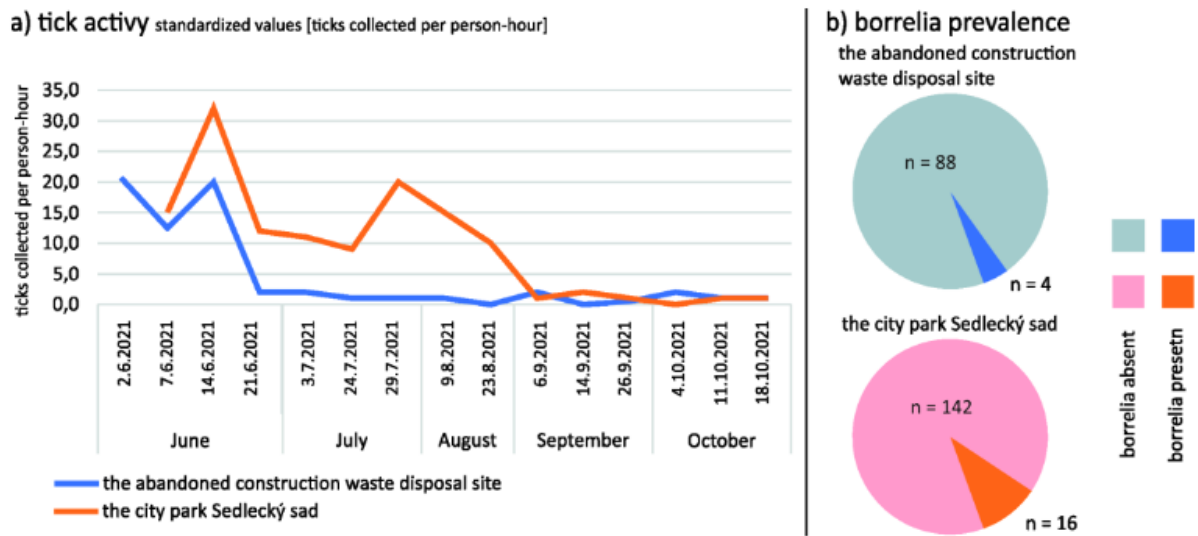


Figure 2. Tick activity and prevalence of borrelia spirochetes: (a) Numbers of ticks collected per person-hour in the city park, Sedlecký sad, (red line) and the abandoned construction waste disposal site (blue line) are indicated, during the tick season in 2021. (b) Prevalence of borrelia spirochetes within the collected tick specimens at both studied localities.

3.3. Borrelia-Infected Ticks Are Present at the Abandoned Disposal of Nontoxic Construction Waste Material Site, despite Lower Prevalence Compared to the Adjacent City Park

In total, 20 tick samples were positive for *Borrelia burgdorferi* s.l. (4 ticks from the abandoned construction waste disposal site and 16 from the city park; see Figure S11 for an example of a positive electrophoresis gel with adequate controls). The overall borrelia prevalence at the locality was 8%. A numerically greater borrelia prevalence was observed among the ticks collected from the city park (10.12%) than from the abandoned construction waste disposal site (4.34%), but this difference was not statistically significant ($p = 0.10433$). The entomological risk index (ERI) [12], calculated from the prevalence of borrelia positive ticks and overall abundance of questing ticks, was estimated to be 0.89 borrelia-infected ticks per hour for the city park and 0.21 borrelia-infected ticks per hour for the abandoned construction waste disposal site. The difference between ERI from both localities was not statistically significant ($p = 0.130570$).

Four species from the *Borrelia burgdorferi* s. l. complex were identified among the positive samples. The most prevalent species were *Borrelia burgdorferi* sensu stricto (10 samples) followed by *Borrelia afzelii* (6 samples), *Borrelia garinii* (2 samples), and *Borrelia lusitaniae* (2 samples) (see Table 2 for details).

Table 2. Detected borrelia species within the *Borrelia burgdorferi* sensu lato complex.

	<i>Borrelia burgdorferi</i> Sensu Stricto		<i>Borrelia afzelii</i>		<i>Borrelia lusitaniae</i>		<i>Borrelia garinii</i>		Subtotal Disposal Site	Subtotal City Park	Total
	Disposal Site	City Park	Disposal Site	City Park	Disposal Site	City Park	Disposal Site	City Park			
Females	1	2	1	1	0	0	0	0	2	3	5
Males	0	0	1	1	0	2	0	0	1	3	4
Nymphs	1	6	0	2	0	0	0	2	1	10	11
Larvae	0	0	0	0	0	0	0	0	0	0	0
Subtotal	2	8	2	4	0	2	0	2			
Total	10		6		2		2				20

4. Discussion

Our results showed that ticks and borrelia spirochetes are present in the city park as well as in the adjacent abandoned construction waste disposal site, but tick numbers and overall prevalence of borrelia spirochetes were lower at the abandoned construction waste disposal site although not always significantly. We detected four species from the *Borrelia burgdorferi* s. l. complex, namely *Borrelia burgdorferi* sensu stricto, *Borrelia afzelii*, *Borrelia garinii*, and *Borrelia lusitaniae* (ordered according to prevalence at the study sites). This sequence is surprising, as in Czechia the highest prevalence is usually found for *B. garinii* and *B. afzelii*, followed by *B. burgdorferi* s.s. The observed discrepancy is most likely caused by the low number of positive samples. According to our best knowledge, this is the first research focused on tick abundance and tick-borne pathogen presence in urban postindustrial landscapes, which are otherwise neglected from a research perspective. Nevertheless, as shown in this study, they may pose an important but missing piece in the puzzle of our understanding of tick ecology and tick-borne disease ecoepidemiology in urban areas. The only similar research, performed on spoil banks resulting from brown coal mining in northern Bohemia, showed that ticks need about 20 years to colonize these localities and that this process can be accelerated by recultivation [13].

As mentioned earlier, many different people use postindustrial landscapes and other types of vague terrain [9], and therefore they can be infested by local ticks and subsequently infected by endemic tick-borne pathogens. Homeless people are probably the group with the highest risk, as they often camp in these localities and frequently suffer poor medical and social care. They usually also suffer from numerous chronic diseases (both infectious and noninfectious) including infection by borrelia spirochetes and other vector-borne diseases [14,15]. Homeless people are also at a higher risk of being infested by ticks and other ectoparasites, although lice and louse-borne diseases usually present higher problems than ticks and tick-borne diseases for homeless people [15,16]. As homeless people usually do not travel long distances, there is a high probability that the vast majority of the tick-borne diseases among them are caused by urban ticks, including those living in postindustrial vague terrain. A similar situation can be expected among other users of this type of landscape and among the animals spending at least part of their time there.

Therefore, higher public awareness is needed to improve the current situation. Local citizens, the at-risk population, as well as local authorities and policy makers should be informed about tick-associated risk in urban areas, including postindustrial landscape and other types of vague terrain. This study should attract more tick-focused researchers to study postindustrial localities, which should provide the relevant data to local authorities to improve public health issues connected with vague terrains.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/pathogens12050648/s1>, Figures S1–S10: PPhoto documentation of the study sites, and Figure S11: Example of an electrophoresis gel with a positive result and adequate controls.

Author Contributions: Conceptualization: J.Č.; tick collection and laboratory analyses: A.D. and J.A.; statistical analyses: A.K.; writing—original draft preparation, review, and editing: all authors. All authors have read and agreed to the published version of the manuscript.

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Supplementary materials



Figure 51. Construction waste material of the deposit. Dvořáková 2021.



Figure 52. Waste material found in the deposit (a) toilet bowl, (b) old bricks. Dvořáková 2021.



Figure S3. Traces of wildlife animals found in the deposit (a) *Felis catus*, (b) *Capreolus capreolus*. Dvořáková 2021



Figure S4. Habitat signs found in the deposit (a) feces, (b) gnawed ear of *Zea mays*. Dvořáková 2021.



Figure 55. Wildlife animals' feces found in the deposit (a) *Vulpes vulpes*, (b) *Capreolus capreolus*. Dvořáková 2021



Figure 56. Male *Capreolus capreolus* and females *Phasianus colchicus*. Czech hunting community 2021.



Figure S7. *Martes martes*. Czech hunting community 2021.



Figure S8. *Pica pica* and female *Phasianus colchicus*. Czech hunting community 2021.



Figure S9. *Vulpes vulpes*. Czech hunting community 2021.



Figure S10. *Felis catus*. Czech hunting community 2021.

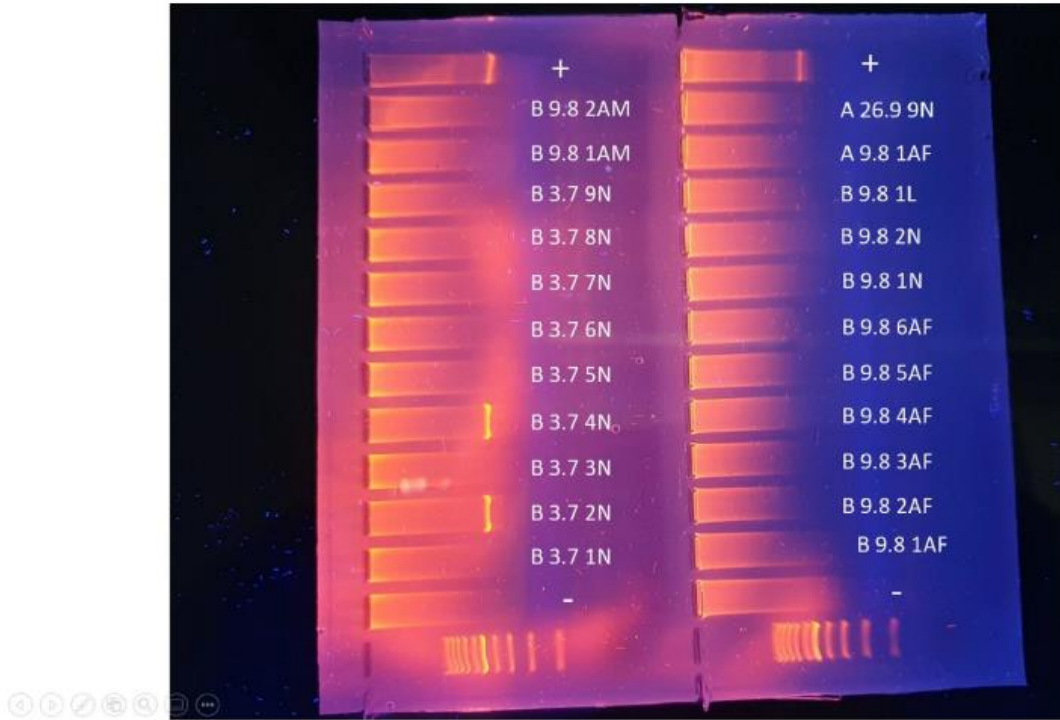


Figure S11. Example of an electrophoresis gel with a positive result and adequate controls.

4. DISCUSSION

This dissertation aimed to explore and delve into novel and understudied subjects that interconnect ticks, their living environment, and the hosts essential for their survival. The obtained data distinctly indicated that gaps persist in the otherwise extensive knowledge of tick-related issues. The ever-increasing complexity of TTBDs ecoepidemiology is further complicated by new variables such as unpredictable climate change, expanding human and agricultural animal populations, and their influence on ecosystems and environments.

The escalating climate change our planet is undergoing appears to be one of the driving forces that accelerate significant alterations in tick distribution, viability, and subsequently, the prevalence TBPs in regions previously unaffected by this threat (Sonenshine 2018; Voyiatzaki et al. 2022; Nuttall 2022; Lee & Chung 2023). Zoonotic diseases carried by hard ticks and primarily associated with wildlife transmission could experience a more pronounced influence from climate change and a relatively lesser influence from human activities compared to other vector-borne diseases like malaria or dengue (Ogden et al. 2014, 2021). Through influencing a wide variety of ecological aspects (host abundance and species, precipitation, habitat change etc.) climatic change can present both advantages and disadvantages for tick populations, introducing new evolutionary pressures with outcomes that remain difficult to predict in the long term. It is theorized that tick species that exhibit a generalist behavior will display greater resilience in the face of alterations. Consequently, this phenomenon could potentially lead to a decrease in biodiversity within tropical areas, while concurrently fostering an upswing in diversity within temperate regions, driven by the shifting geographical ranges of these adaptable tropical species (Davey et al. 2013; Kortsch et al. 2015). Some researchers believe that with decrease in the variety of host animals, the risk of TBPs spread increases (Salkeld et al. 2013; Wood et al. 2014). On the other hand, some believe that having more diverse host populations can be beneficial due to the so called 'dilution effect'. This effect results in a reduction in pathogen transmission due to the increase in ecological community diversity, as outlined by Keesing (2006) and LoGiudice et al. (2003). However, how much the diversity of host animals actually affects the spread of tick-borne diseases is a big topic of debate among experts (Randolph & Dobson 2012; Ostfeld 2013; Levi et al. 2016). The final outcome is of course still unclear and continues to evolve, as far as we know the final outcome could go either way in following years (Ogden & Tsao 2009). The dynamics of climate change, leading to shifts in temperature and humidity, can prompt host animals to explore broader geographical areas for refuge and resources. This unintentional movement can transport ticks to regions previously not identified as viable habitats. Consequently, it becomes imperative to assess all accessible natural and semi-natural environments as potential tick habitats. This evaluation should encompass both the current scenario and the evolving tick ecology in the future.

With changing circumstances any evolutionary advantage is now crucial for the survival of each tick population (Dantas-Torres 2015). Ticks spend the majority of their life

off-host, thereby being susceptible to potential perils including freezing, heat stress, and desiccation (Thomas et al. 2020). In connection to this, ticks have evolved mechanisms to evade suboptimal conditions, often by adopting sheltered positions underneath leaf litter or in soil, or by upholding physiological equilibrium when confronted with these challenges, frequently through rehydration processes (Gray et al. 2021). Comprehensive, long-term study by Brunner et al. (2023) revealed that in semi-natural conditions (artificial tick enclosures in natural environment without external control) tick survivability differed greatly even when subjected to identical treatment protocols and environment state. This divergence was particularly prominent in larvae and nymphs, but adults showed some variability as well. This variability baffled the authors and suggested that there are other factors “in play” apart from the measured air temperature, relative humidity, soil temperature, and soil humidity. The authors discuss the influences of entomopathogenic fungi or predatory arthropod communities (Burtis et al. 2019), but no clear answer presents itself. This research nicely illustrates the great variability of tick survival and further confirms that tick ecology might be even more complicated than previously thought and more variables need to be considered.

As a fascinating variable affecting tick survivability, we can also examine the influence of TBPs on their tick vectors. It is known that pathogens possess the ability to influence the characteristics of both their hosts and vectors, thereby optimizing their own reproductive success. Within the realm of modifiable phenotypic traits, the most intriguing subset involves the manipulation of vector behavior leading to increased transmission of pathogens. This manipulation can be accomplished through diverse mechanisms, like the increase of the number of vector-host interactions, disabling vector reproduction to provide nutrients to the pathogen itself, or boost of vector longevity (Hurd 2003). Changes in tick behavior or viability were observed in many studies and encompassed many TBPs like *Anaplasma* spp., tick-borne encephalitis virus (TBEV) and most prominently borrelia spirochetes. Pilot study by Lefcort and Durden (1996) showed that *Ixodes scapularis* ticks infected by *Borrelia burgdorferi*, exhibited an augmentation in their phototactic response and an enhanced attraction toward vertical surfaces, in contrast to their non-infected counterparts. These modifications in behavior appear to have an adaptive significance, potentially facilitating the transmission of borrelia. This could be attributed to the fact that the heightened phototaxis and increased affinity for vertical surfaces could elevate the probability of interactions between the tick vector and a potential reservoir host, thus promoting more effective transmission of the pathogen. Subsequently, some conflicting studies have emerged, linking borrelia infection to decreased mobility in diverse ixodid tick species (Alekseev et al. 2000). Other borrelia studies observed even more alterations in tick behavior like different temperature preferences of infected ticks (Alekseev & Dubinina 2000), higher surfaces seeking (Lane et al. 2007) or even preference to attach to flagging scientists suggesting infection induced increase in host finding (Faulde & Robbins 2008). Furthermore, physiological changes manifest among ticks carrying borrelia, encompassing alterations beyond behavioral aspects. For example, the invasion of *I. scapularis* salivary glands by *B. burgdorferi* involves the

utilization of a mechanism where a tick salivary protein known as *salp15* is exploited. This protein forms a binding interaction with the spirochetes, affording them protection against elimination mediated by host antibodies. Consequently, this molecular interplay enhances the efficacy of infection within mice (Ramamoorthi et al. 2005). Other advantages connected to infection of ticks by borrelia like desiccation protection (Herrmann & Gern 2010), higher energy (fat) reserves (Herrmann et al. 2013) or boost in overwintering survival (Nabbout et al. 2023) have also been confirmed. Other pathogens like anaplasma can boost expression of supporting genes influencing vector fitness and pathogen transmission (Cabezas-Cruz et al. 2016, 2017), increase synthesis of heat shock proteins *hsp70* and *hsp20* (Villar et al. 2010), preventing desiccation (Busby et al. 2012) or even stimulate the production of an antifreeze glycoprotein, which safeguards ticks from cold temperatures (Neelakanta et al. 2010). Finally, TBEV is hypothesized to increase mobility, questing height and host-seeking activity or boost tolerance to N,N-diethyl-meta-toluamide (DEET) (Süss et al. 2004; Belova et al. 2012). Pathogens from other genera such as *Bartonella*, *Babesia*, or *Rickettsia* have received comparatively less comprehensive investigation regarding their impact on tick vectors (Benelli 2020). It is evident that interactions between tick vectors and pathogens not only exist but also exert significant influence, further complicating the study of TTBDs.

Given the multifactorial nature of TTBDs ecology, it is imperative to integrate additional considerations that may reveal previously undiscovered insights. Our team's comprehensive review paper on the interactions between zoo-housed animals and TTBDs (Hrnková et al. 2021) exposed a knowledge gap, particularly concerning exotic animals. This gap extends beyond zoo environments to private care facilities, including farms. Given that numerous farms in Czechia housing exotic animals emphasize large herbivore species (Lojda 2011), the necessity to appraise their influence on TTBDs became evident. In general, large herbivores, especially grazers, occupy an integral position in the trophic hierarchy, exerting substantial influence on the structure and operation of ecosystems (Johnson & Cushman 2007). These animals are often referred to as "ecosystem engineers" due to their capacity to construct and uphold habitats conducive to their own expansion (Wilby et al. 2001). Based on the tick collections in our investigation pertaining to *Borrelia* spp. in exotic animal farms (Hrnková et al. 2022), it becomes evident that the existence of large grazing ungulates notably diminishes tick populations within paddocks. This decline could be attributed to notable changes in vegetation structure, its botanical composition, and physical ground disruption resulting from grazing. The simultaneous increase in shortgrass and decrease in tallgrass vegetation, directly influenced by the activities of large ungulates, carries substantial significance. The significant reduction in the abundance of dense tallgrass vegetation, primarily composed of grass-like plants, alongside the emergence of shortgrass vegetation containing a substantial proportion of broad-leaved plants commonly observed in grazed environments (Dvorský et al., 2022), restricts the availability of essential microhabitat elements necessary for the survival and proliferation of *I. ricinus* ticks. For example, the absence of elevated vegetation for ticks to quest on above the ground is crucial, as noted by Gassner et al. (2011). Additionally, there is a scarcity of shaded and humid microclimatic

shelters beneath live and dead grass biomass, which ticks rely on during hot and dry weather, as highlighted by Gern et al. (2008). The characteristics of the vegetation resulting from grazing activities help explain the inverse relationship between tick abundance and grazing observed in our data. Landscape management (grazed versus fallow) and habitat features (shortgrass versus tallgrass vegetation) emerge as key factors in clarifying the localized variations in tick populations. Our study conducted in Milovice supports this hypothesis that there exists a clear correlation between landscape properties and tick abundance. Although we did not directly study the host population, it is widely recognized that the presence of suitable habitats for primary host species significantly influences tick population dynamics (Spickett 1994; Fahrig et al. 2011; Ehrmann et al. 2017). The overall diversification and thriving of the flora, as observed in the Milovice rewilding area (Dvorský et al. 2022), are known to foster an increase in the richness of animal species (Wolters et al. 2006; Lewinsohn & Roslin 2008; Castagneyrol & Jactel 2012). This pattern suggests a robust correlation with tick populations, indicating that as the diversity and abundance of hosts increase, there is a likelihood of a parallel rise in tick populations. Nevertheless, our observations revealed distinct differences between pastured and ungrazed areas, where there were no apparent restrictions to the movement of potential hosts apart from the locations of wildlife passes. This contrast could suggest that the landscape and habitat characteristics have a more substantial influence than the mere abundance of hosts. Alternatively, it is plausible that hosts were more densely concentrated in ungrazed areas. However, to finalize this hypothesis, more data regarding local host populations would need to be collected.

In our study dedicated to postindustrial landscapes (Dvořáková et al. 2023), we studied processes opposite to those leading to decrease in tick abundance caused by unsuitable habitats. Here, the interplay of ecological succession alongside the encroachment of bush and tall-grass vegetation enhances the suitability of the "*terrain vague*" (a term borrowed from French) (Gandy 2016) for *I. ricinus* ticks. Numerous studies (Ejrnæs et al. 2003; Kuiters & Slim 2003; Munford et al. 2020; Hutniczak et al. 2022) have documented the successful recolonization of wild plant species in abandoned or semi-abandoned areas previously utilized by humans (e.g., abandoned mines, railroads, sandy fields, old agricultural areas). According to the collected data, the levels of macronutrients such as carbon (C), phosphorus (P), and nitrate-nitrogen (N-NO₃), as well as the abundance of organic matter (litter), silt content, and pH, emerge as critical factors influencing plant encroachment and species composition on these abandoned landscapes (Munford et al. 2020; Hutniczak et al. 2022). Even the construction waste deposited at disposal sites, composed of inert materials including clay, earth, concrete, rubble, and bitumen (Yuan et al. 2021), doesn't necessarily impede plant growth; some plant species are adept at recycling such materials (Ulubeyli et al. 2017). As ecosystems gradually establish themselves after abandonment and tick hosts migrate to reestablish viable populations on such areas (Daniel & Černý 1990; Martínez-Abraín 2019), new opportunities for ticks arise, concurrently presenting novel challenges to the general public.

5. CONCLUSIONS

In conclusion, the undeniable fact is that the extensive research encompassing TTBDs is laden with even greater complexity than initially thought. During the data collection phase for this thesis, the first step involved conducting a systematic review of existing literature concerning the interactions between zoo-housed animals and TTBDs, as well as the identification and assessment of current recognized and endorsed optimal management strategies. The investigation revealed deficiencies in knowledge, especially concerning exotic animals. This observation served as the foundation for subsequent research that addressed this issue on farms housing exotic animal species in Czechia. Upon the collection of data regarding the abundance of ticks and *Borrelia* spp. within farms housing exotic large ungulates, a concurrent discovery emerged. There was a conspicuous absence of all tick stages in pastured areas directly influenced by these animals. This influence was attributed to the animals' grazing behavior and the disturbance of soil resulting from their movements. This discovery prompted an examination of the ecosystem engineering capacities of large ungulates. The Milovice protected area, where a rewilding program is ongoing, served as an ideal study site for this investigation. The hypothesis that tick populations would decrease in pastured areas was substantiated by empirical data from Milovice. These findings provided valuable insights into the impact of rewilding and semi-controlled pasture landscape management on tick abundance. The final research investigation, centered on the less-explored landscapes of the '*terrain vague*,' further substantiated the tick-landscape patterns observed in Milovice. The swiftly changing environment, propelling the expansion of ticks into previously uncharted territories, coupled with the evolutionary advantages TBPs bestow upon their tick vectors, may potentially herald a problematic future. Our team's fresh dataset has introduced innovative viewpoints in this domain. We have presented empirical evidence of *I. ricinus* tick encroachment into unexpected areas undergoing dynamic ecological successions. Additionally, we have established the presence of *Borrelia* spp. in exotic farm animals within Czechia for the first time. We have also provided evidence of the significant impact of large ungulate ecosystem engineers on grazed landscapes affecting tick abundance solely through their presence. The impact of landscape type and management has been affirmed across all our publications. It is obvious that a holistic approach is essential for evaluating all variables within the intricate tick-related science, as exemplified in our review concerning TTBDs management. A comprehensive assessment of risks to both human and animal health necessitates further research. This includes evaluating the effect of borrelia infections on exotic animal species, assessing vector/host competence among exotic and reintroduced animals, and conducting prolonged observations in more "*terrain vague*" areas to yield statistically robust findings. We're aiming for our team's efforts to shed light on new paths in tick-related research and to be a solid part of the growing field of modern tick studies.

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7. CURRICULUM VITAE

EDUCATION

September 2018 – ongoing

Ongoing Ph.D. studies • Czech University of Life Sciences (CULS) • Department of Animal Science and Food Processing • Prague, Czechia

June 2018

Finished Master studies acquiring the Ing. title • CULS • Department of Animal Science and Food Processing • Prague, Czechia

RESEARCH PRACTICE

May 2023 – ongoing

Junior researcher • National Radiation Protection Institute • Prague

Optimized and implemented advanced radiation exposure measurement methods based on cytogenetic analysis, including dicentric chromosome assays, gamma-H2AX, and micronuclei assays. Conducting research to optimize and establish gene expression assays for biodosimetry applications.

May 2022 – March 2023

Researcher and wildlife genetics specialist • Forezní DNA servis s.r.o. • Prague

Conducted research within a forensic genetic laboratory, focusing on the development and testing of novel PCR and fragmentation analysis assays. These assays were designed for the precise species and individual identification of endangered animals. Additionally, engaged in secondary laboratory tasks involving human genealogy and paternity tests, utilizing fragmentation analysis and sequencing techniques.

July 2015 – April 2022

Study research • Laboratory of Molecular Biology CULS • Prague

From 2015 to the completion of my master's and doctoral research, I worked in the molecular laboratory of the Czech University of Life Sciences in Prague. I utilized nested, endpoint, and real-time PCR methods, conducted gel electrophoresis, and performed

DNA/RNA isolation and sequencing. I held operational responsibility for the laboratory, ensuring the perfect purity of samples and the safety of both the laboratory environment and human health during the handling of hazardous materials and pathogens.

June 2018 - January 2020

Laboratory technician • Institute of Molecular Genetics • Czech Academy of Sciences • Prague

Engaged in the study of genetically modified zebrafish (*Danio rerio*), including the care and maintenance of multiple genetic lineages through breeding. Engaged in fundamental laboratory techniques, such as PCR, microscopy, and electrophoresis. Provided valuable support for research endeavors focused on blood cell differentiation.

October 2017 – February 2018

Laboratory intern • State Veterinary Institute • Prague

Engaged in a voluntary internship at the State Veterinary Institute in Prague, with a primary focus on the molecular biology laboratory. Conducted DNA/RNA isolation from diverse sample sources, including tissues, blood, and food. Demonstrated practical expertise in Real-time PCR and Sequencer applications, utilizing GenBank and other relevant software. Proficiently worked with pathogens of bacterial and viral origins during the internship.

PROJECTS

January 2019 – January 2022

Spread of ticks and tick-borne diseases: new and non-negligible risks to domestic animals, livestock and humans • National Agency for Agricultural Research • QK1920258

Conducted research on the prevalence of ticks (*Ixodes ricinus*) and the associated transmission of pathogens. Collaborated with the Biological Center (Czech Academy of Sciences) in České Budějovice, the Veterinary and Pharmaceutical University of Brno, and the Veterinary Research Institute in Brno through a 3-year research project facilitated by the Czech University of Life Sciences (CULS). Published scientific articles and organized a 2021 Workshop, adapted to an online format due to the challenges posed by Covid-19, to engage a broader audience.

December 2020 – April 2022

Humanitarian response to the COVID - 19 pandemic (Ethiopia, Zambia) • Mobile molecular laboratory • Czech Development Agency • ADRA • CULS

Collaborated with the Czech University of Life Sciences on a startup initiative named CZU mobiLAB. This project, conducted in partnership with the non-profit organization ADRA and

the Czech Development Agency, focuses on enhancing the diagnosis of SARS-CoV-2 and other pathogens in remote regions of developing countries, presently in Ethiopia and Zambia.

Our team designed a mobile laboratory, a pivotal element in the initiative, which can be conveniently transported using a pick-up or off-road vehicle. This solution enables diagnostics even in challenging conditions, featuring a spacious tent with an insulating layer, along with air conditioning and heating capabilities. As the co-manager of the laboratory, my role involved overseeing the quality and functionality of molecular diagnostic methods and devices.

January 2021 – January 2022

Project dealing with early detection of dangerous influenza mutations • provisional name GECON • Army of the Czech Republic • CULS

Engaged in a collaborative effort between the Military Health Institute of the Military Health Agency of the Army of the Czech Republic and the Czech University of Life Sciences, focusing on the GECON startup. This project centers on the proactive detection of influenza mutations from databases aggregating sequences of individual influenza strains, particularly utilizing GenBank.

Within this project, I served as an expert advisor and assistant, overseeing the tracking of significant mutations. Collaborating closely with other team members, I contributed to the development and effective operation of the diagnostic program, ensuring its accuracy and functionality.

May 2023 – ongoing

Komplex metod biologické a fyzikální retrospektivní dozimetrie pro radiační mimořádné události • Ministry of the Interior • VK01020052

The current international political situation is drawing attention to the issues of the threat of misuse of ionizing radiation sources, including possible attacks on nuclear power plants and use of nuclear weapons. In the field of nuclear safety and radiation protection, it is necessary to take into account scenarios involving a large number of irradiated persons who were not equipped with conventional dosimeters. The existing system of already established methods of physical retrospective dosimetry needs to be supplemented by methods of biological dosimetry not yet implemented, and to verify their mutual compatibility and complementarity. The increase in capacity consists in the use of advanced (semi)automated biomedical technologies and interlaboratory cooperation.

PUBLICATIONS

Dvořáková A, Klímová A, ALAVERDYAN J, Černý J. 2023. Postindustrial Landscapes Are Neglected Localities That May Play an Important Role in the Urban Ecology of Ticks and Tick-Borne Diseases—A Pilot Study. *Pathogens* **12**:648

HRNKOVÁ J, Golovchenko M, Musa AS, Needham T, Italiya J, Ceacero F, et al. *Borrelia* spirochetes in European exotic farm animals. *Frontiers in Veterinary Science* 2022;**9**, doi: <https://doi.org/10.3389/fvets.2022.996015>

HRNKOVÁ, J.; Schneiderová, I.; Golovchenko, M.; Grubhoffer, L.; Rudenko, N.; Černý, J. Role of Zoo-Housed Animals in the Ecology of Ticks and Tick-Borne Pathogens—A Review. *Pathogens* 2021, **10**, 210, doi:10.3390/pathogens10020210.

Černý, J.; Lynn, G.; HRNKOVÁ, J.; Golovchenko, M.; Rudenko, N.; Grubhoffer, L. Management Options for *Ixodes Ricinus*-Associated Pathogens: A Review of Prevention Strategies. *International Journal of Environmental Research and Public Health* 2020, **17**, 1830, doi:10.3390/ijerph17061830