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Abstracts of the BalTick25: The Baltic Tick Conference

The main purpose of **BalTick25**, the Baltic Tick Conference, held in Kaunas on 1–3 October 2025, was to promote the exchange of scientific knowledge and advanced practices among experts in acarology, veterinary science, medicine, and ecology with the aim of a deeper understanding of the role of ticks.

At BalTick25, over 50 participants from the Baltic countries, Norway, Sweden, Denmark, the United Kingdom, Poland, Slovakia, and the Czech Republic took the opportunity to exchange ideas, views, and knowledge about ticks in ecosystems, their impact on human and animal health, and the development of effective monitoring, prevention, and control strategies. The most important research results shared at BalTick25 are presented here in 39 abstracts, including three plenary talks, 13 oral presentations, and 23 poster presentations.

We are sincerely grateful to the Lithuanian Academy of Sciences for their editorial support.

Algimantas Paulauskas
Chair of the Local Organising Committee

Plenary presentations

THE ECO-EPIDEMIOLOGY OF *BABESIA* SPP.: TICK INTERACTION

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Babesia spp. are protozoan parasites of great medical and veterinary importance. Ticks are known vectors of *Babesia* spp., although some *Babesia* tick interactions have not been fully elucidated [1]. We investigated the specificity of *Babesia* tick species to indicate the main vectors of important *Babesia* species based on published research papers and molecular data from the GenBank database.

Repeated observations of certain *Babesia* species in specific species and genera of ticks in numerous independent studies, carried out in different areas and years, have been considered eco-epidemiological evidence of established *Babesia*-tick interactions. The best studied species of ticks are *Ixodes ricinus*, *Dermacentor reticulatus*, and *Ixodes scapularis* [2]. Eco-epidemiological studies have confirmed a specific relationship between *Babesia microti* and *Ixodes ricinus*, *Ixodes persulcatus*, and *Ixodes scapularis*, and also between *Babesia canis* and *D. reticulatus*. Additionally, four *Babesia* species (and one genotype), which have different deer species as reservoir hosts, displayed specificity to the *I. ricinus* complex.

Interestingly, pioneering studies on other species and genera of ticks have revealed the existence of likely new *Babesia* species, which need more scientific attention. Finally, we discuss the gaps in knowledge and detection of *Babesia* spp. in feeding ticks and critically evaluate the data on the role of the latter as vectors.

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Keywords: babesiosis, piroplasm, tick specificity

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A ONE-HEALTH PERSPECTIVE ON TICKS AND TICK-BORNE DISEASES: EXPERIENCE ON TRANSMISSION DYNAMICS AND EPIDEMIOLOGY FROM SCANDINAVIA

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In Northern Europe, ticks are the most important vectors for transmitting infections, which may cause deaths and are frequently associated with long-term suffering and high societal costs.

During the recent decades, ticks carrying disease-causing bacteria, viruses, and eukaryotic protozoans have increased in numbers and spread to new areas. Thus, the number of persons and animals affected by tick-borne infections (TBI) are continuously increasing. The reasons for that are complex and include climate change (direct and indirect effects), increased urbanisation, and other human influences on our ecosystems.

The most abundant infection is borreliosis, caused by the Borrelia bacteria, and the most severe one is tick-borne encephalitis (TBE), caused by the TBE virus, both pathogens increasing in number of cases and geographical distribution. If correctly diagnosed, borreliosis can in most cases be cured with correct antibiotic treatment, but no treatment is available for TBE. However, there are efficient vaccines on the market.

It is challenging for health services and authorities to be updated on optimal strategies for prevention, diagnostics, and management of TBI and to keep up with new emerging tick-borne microorganisms. Evidence-based and cost-effective strategies for control of TBI are currently the weakest chain in surveillance.

In this review talk, aspects related to the on-going climate change will be discussed in connection to the One Health perspective. The most abundant and emerging tick-borne pathogens, including the bacteria *Borrelia* spp., *Rickettsia helvetica*, *Anaplasma phagocytophilum*, *Neoehrlichia mikurensis*, the TBE virus, and the eukaryotic protozoans *Babesia* spp., will be brought up. The surveillance of prevalence of different tick-borne pathogens, using different methodological approaches, including blood-feeding ticks from rodents, roe deer, migratory birds and humans will be connected to data obtained from field-collected ticks. In addition, the risk for humans to contract a TBI will be assessed.

Finally, I wish to acknowledge all colleagues and co-workers, mainly at the Division of Inflammation and Infection, Linköping University, Linköping, and at the National Reference Laboratory for Diagnostics of Bacterial Tick-Borne Infections, Jönköping Region, both in Sweden.

Keywords: *Ixodes ricinus*, tick-borne infection, One Health, climate change, epidemiology

TICK-BORNE DISEASES IN DOMESTIC RUMINANTS, WITH SPECIAL FOCUS ON ANAPLASMOSIS

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Tick-borne diseases in production animals, such as anaplasmosis, caused by *Anaplasma marginale*, and babesiosis, caused by *Babesia divergens*, were first described in cattle for more than a hundred years ago. Since that time several other tick-borne pathogens, including bacteria, viruses, and parasites, several of which have a zoonotic potential, have been identified in animals and humans. More than 900 tick species exist worldwide. In Europe, over 50 hard tick species have been identified so far. In our continent, the main tick species causing infection in mammals is *Ixodes ricinus*, a species that is widespread, particularly in the central and northern areas. Besides *I. ricinus*, other tick species such as *Dermacentor marginatus*, *D. reticulatus*, *Haemophysalis punctata*, *Hyalomma marginatum*, and *Rhipicephalus bursa* may also be involved as ruminant pathogen vectors in Europe.

In domestic ruminants, several tick-borne pathogens, belonging to different genera such as Anaplasma, Babesia, Borrelia, Ehrlichia, Theileria, and TBE virus, have been detected. Several of these microorganisms, such as different species of Borrelia, Ehrlichia and TBE-virus, may cause severe infections in humans, but with limited clinics in ruminants.

In Europe, the main tick-borne diseases in domestic ruminants are anaplasmosis and babesiosis. However, on other continents other species are important pathogens, such as *Ehrlichia ruminantium* (causing heartwater), *Theileria lestoquardi* (causing malign theileriosis), *Theileria annulata* (causing tropical theileriosis), and *Theileria parva* (causing East Coast fever/Corridor disease). Theileria species with low pathogenicity have sporadically been reported from our continent. Ticks and hosts may be coinfected with different pathogens.

Several Babesia species occur in ruminants, involving *Babesia ovis*, *B. motasi*, and *B. crassa* in small ruminants and *Babesia divergens*, *B. bigemina*, *B. major*, and *B. bovis* in cattle. *Babesia venatorum* has also been identified in both cattle and sheep in Europe. The most important species occurring in our continent are *B. divergens* in cattle and *B. motasi/ovis* in small ruminants. The main clinical signs related to babesiosis are anaemia and haemoglobinuria, which may cause high mortality. *Babesia divergens* and *B. venatorum* also have a zoonotic potential.

Genus Anaplasma includes pathogens such as *A. capra*, *A. centrale*, *A. ovis*, *A. marginale*, and *A. phagocytophilum*. The most important species for ruminants in Europe are *A. ovis*, which causes anaemia in small ruminants, and especially *A. phagocytophilum*, causing severe infection in ruminants and also disease in species like cats, dogs, and horses. Wild ruminants may become ill, and mortality has been reported in roe deer (*Capreolus capreolus*) and moose (*Alces alces*). The disease in domestic ruminants is named tick-borne fever. *A. phagocytophilum* is the pathogen with the highest zoonotic potential in genus Anaplasma, with more than 40,000 human cases diagnosed so far in the USA.

A. phagocytophilum may cause immunosuppression, making the animal vulnerable to secondary infections, which may lead to such diseases as arthritis, pyaemia, septicaemia, and pneumonia. High mortality, abortion, reduced fertility, and reduced production may occur. In both cattle and sheep, *A. phagocytophilum* causes persistent infection that may last for months. There exist different variants/strains of *A. phagocytophilum*, with at least four ecotypes occur-

ring in nature, of which ecotypes I and II include variants from domestic ruminants. Animals may simultaneously become infected with several variants, since cross-immunity between genotypes is limited. Cervids may be the reservoir hosts for variants causing disease in domestic ruminants, for instance, red deer (*Cervus elaphus*) may harbour variants causing disease in sheep. However, cervids as reservoir hosts need further exploration. A vaccine against anaplasmosis is not yet available.

Keywords: ruminants, tick-borne infections, Babesia, Anaplasma, review

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Oral presentations

HOST SPECTRUM IMPACT ON THE GENETIC POPULATION STRUCTURE OF TICKS

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Tick species vary in their degree of host specificity. *Hyalomma aegyptium* is a tick species that in its adult stage feeds predominantly on tortoises of the genus *Testudo*. *Dermacentor reticulatus*, on the other hand, has a broad host spectrum. Larvae and nymphs feed on small mammals, while adults prefer medium-sized and large mammals. This study assessed the intraspecific genetic diversity and population structure of these two species as representative models of differing host preferences. A total of 431 *H. aegyptium* specimens from 79 locations in 17 countries and 865 *D. reticulatus* ticks from 65 sites across 21 countries were analysed using species-specific microsatellite markers. Based on the Bayesian analyses values provided by the STRUCTURE software, the population of *H. aegyptium* is composed of two or three genetic clusters, exhibiting an east-to-west distribution in both cases. Admixed individuals are found mainly in the central part of the distribution area. The plots showing population structure from K = 1 to K = 10 indicate that the Maghreb region emerged as the most genetically uniform population segment. Microsatellite analyses of *D. reticulatus* revealed three genetic clusters with a gradient between the eastern and western clusters supplemented by a northern cluster. The vast number of admixed individuals is also recorded throughout the entire distribution. This pattern could be the result of the eastern and western clusters deriving from separate refugia with the northern cluster originating from admixture between them. These findings suggest that host specificity influences genetic stability, with more host-restricted ticks, such as *H. aegyptium*, exhibiting more defined and stable population structures compared to generalist species, like *D. reticulatus*.

Keywords: *Hyalomma aegyptium*, *Dermacentor reticulatus*, population structure, microsatellite loci, tick-host spectrum

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TICK DISTRIBUTION IN DENMARK BY CITIZEN SCIENCE: FLAATINFO.DK

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Ixodes ricinus is the most common tick species in Northern Europe and an important vector of multiple pathogens [1, 2]. Despite its importance, little is known about how demographic factors shape human-tick interactions. Furthermore, the actual geographical distribution is difficult to assess. A citizen science initiative, sharing results quickly is key to keeping the public engaged and active in tick monitoring. To support this, an interactive map was created to display tick bites and sightings reported since august 2021 in Denmark

Analysing over 10.000 user reports and filtering for completeness from the citizen science platform flaatinfo.dk [3], correlations between tick bite occurrence and age and sex were examined. The data revealed clear demographic patterns, with senior citizens reporting disproportionately high numbers of bites, while significant differences in bite outcomes across sexes were also discovered. Rash outcomes following bites differed also between demographic groups, indicating potential behavioural influences on exposure and response. These findings highlight the importance of standardised citizen science reporting for epidemiological monitoring, complementing traditional surveillance methods. Strengthening such platforms across regions could significantly improve early detection of changing tick-human dynamics and support targeted public health strategies, including awareness campaigns and improved access to tick removal resources.

Keywords: *Ixodes ricinus*, tick bites, Denmark, Lyme disease, population dynamics, epidemiology

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INFECTION OF ALPACAS WITH *ANAPLASMA PHAGOCYTOPHILUM* IN LITHUANIA

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Anaplasma phagocytophilum is an obligate intracellular bacterium and an emerging vector-borne pathogen of zoonotic concern, responsible for granulocytic anaplasmosis in a broad range of mammalian hosts. Its increasing prevalence across Europe is closely linked to the expanding distribution of *Ixodes* tick vectors and ecological shifts driven by climate change [1, 2]. In 2023–2024, blood samples were collected from 28 alpacas showing clinical signs consistent with blood parasite infections across ten districts in Lithuania (Jonava, Trakai, Anykščiai, Jurbarkas, Širvintos, Vilnius, Kazlų Rūda, Šilalė, Klaipėda, and Ukmergė). Molecular testing confirmed *Anaplasma phagocytophilum* in 17 animals. Six alpacas died during the study period. In addition to clinical signs, infected animals showed evidence of anaemia, including reduced haemoglobin and red blood cell counts, as well as morphological changes such as anisocytosis, poikilocytosis, and hypochromia. These haematological findings complement clinical presentation and emphasise the significant impact of blood parasite infections on alpaca health. Our results indicate that such an infection is an important health concern in alpacas in Lithuania, associated with severe anaemia and increased mortality.

Keywords: alpacas, *Anaplasma phagocytophilum*, Lithuania

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LITHUANIAN-NORWEGIAN COLLABORATION IN LONG-TERM INVESTIGATIONS OF TICKS AND TICK-BORNE PATHOGENS

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Tick-borne diseases constitute a major health problem in many parts of the world. In the past three decades, many tick-borne pathogens have emerged, creating new challenges for public and animal health in Europe. The factors that drive the emergence of tick-borne diseases are difficult to identify due to the complexity of the pathogen-vector-host triad. Long-term studies

are important because they may improve our understanding of the ecological factors that shape the dynamics of tick-borne pathogens. In Lithuania, the first of our studies on tick and tick-borne pathogens began in 1995. Analysis based on long-term datasets (2005–2025) of the incidence of vector-borne diseases in humans and animals in Lithuania and Norway demonstrated that exposure to ticks was an important factor influencing the incidences of tick-borne diseases in humans and animals in Lithuania. The geographical and spatial distributions of some European ticks have been changing in the last few decades, and new viral, bacterial, and protozoan tick-borne pathogens have been detected in former non-endemic areas. Climate changes over the recent decades have led to a wider spatial distribution of ticks and to an extension in their periods of activity in Northern Europe. Climatic changes, the significant increase of tourism, and travel of dogs across Europe have caused an increase in the geographical range of canine babesiosis. Currently, the Baltic countries are an endemic area for several vector-borne diseases such as Lyme borreliosis, tick-borne encephalitis, anaplasmosis, babesiosis, bartonellosis, rickettsiosis. The advances in molecular biology during the last two decades and using of molecular diagnostic techniques have allowed researchers to better diagnose, trace, and genetically characterise the ticks and causative agents of important endemic tick-borne diseases and led to the discovery of new emerging vector-borne pathogenic organisms in Lithuania and Norway.

Keywords: Lyme borreliosis, tick-borne pathogens, *Ixodes Ricinus*

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QUANTITATIVE ASSESSMENT OF TICK-BORNE ENCEPHALITIS VIRUS LOADS ACROSS HOSTS AND VECTORS USING DIGITAL PCR

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Tick-borne encephalitis virus (TBEV) is maintained in complex natural cycles involving multiple hosts and vectors ticks. While TBEV detection in field samples is routinely performed using RT-qPCR, quantitative data on viral load across different origins remain scarce [1]. In this study, we analysed 213 TBEV-positive samples originating from ticks, rodents, goats, dogs, horses, and raw goat milk. Viral RNA copy number per microliter was determined using absolute quantification with digital PCR.

Mean viral loads (viral copies/μL) after outlier elimination were: horses 6.68, ticks 3.45, rodents 4.06, goats 3.64, milk 2.80, and dogs 3.89. Statistical comparisons revealed significantly higher viral loads in horse samples compared to ticks ($p = 0.0012$), rodents ($p = 0.0262$), goat milk ($p = 0.0110$), and dogs ($p = 0.0398$). Without outlier removal, the corresponding means were higher overall: horses 31.35, ticks 8.33, rodents 8.23, goats 3.64, milk 3.57, and dogs 3.89.

These findings highlight substantial variation in the TBEV copy number depending on sample origin and wide variation within a sample type. Our results further demonstrate the utility of digital PCR for precise viral quantification in diverse biological matrices, which may aid in refining diagnostic approaches and risk assessment in both veterinary and public health contexts.

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Keywords: tick-borne encephalitis virus, TBEV epidemiology, TBEV viral load

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TICK-BORNE PATHOGENS IN URBAN HABITATS OF LITHUANIA: ASSESSING DISEASE RISK IN RECREATIONAL AND RESIDENTIAL AREAS

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Urban green spaces offer many benefits but also pose emerging public health risks, particularly from vector-borne diseases. Lithuania is highly endemic for Lyme borreliosis and tick-borne encephalitis, primarily transmitted by *Ixodes ricinus*, a widespread tick species. While tick-borne pathogen (TBP) transmission has traditionally been associated with natural habitats, factors such as urbanisation, climate change, increased wildlife, and human encroachment into natural areas have heightened the risk in urban environments [1, 2]. This study presents the data on the tick abundance and the prevalence of TBPs in urban green spaces across 22 cities in ten counties of Lithuania [3, 4]. A total of 3,599 *I. ricinus* ticks were collected. Ticks were found in 82.7% of the surveyed sites: in 22 out of 31 urban and in all 21 peri-urban locations. A statistically significant higher tick abundance was observed in peri-urban areas compared to urban areas. Ticks were screened for the presence of *Borrelia* spp., *Anaplasma phagocytophilum*, *Ca.* Neoehrlichia mikurensis, *Rickettsia* spp., and *Babesia* spp. using multiplex real-time PCRs. Overall, 41.6% of ticks were infected with at least one pathogen. Prevalence varied by site: *Borrelia* spp. (3.7–41.5%), *A. phagocytophilum* (0.71–23.53%), *Ca. N. mikurensis* (0.87–16.67%), *Rickettsia* spp. (1.2–25%), and *Babesia* spp. (1.0–5.6%). Six *Borrelia* species, two *Rickettsia* species, and three *Babesia* species were identified. Coinfections with 2–5 pathogens were detected. Our findings demonstrate that urban and peri-urban green spaces support tick populations harbouring diverse, medically important pathogens. This underscores the need to consider urban environments in tick surveillance and public health strategies, especially in popular recreational and residential areas.

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Keywords: tick-borne pathogens, peri-urban, urban areas, Lithuania

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THE FIRST FINDING OF *HYALOMMA RUFIPES* IN POLAND IN 2024: THE PROMISING START OF A CITIZEN SCIENCE PROJECT

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Hyalomma spp. ticks play a crucial role as vectors for the Crimean-Congo haemorrhagic fever virus. *Hyalomma* spp. larvae and nymphs are transported via migratory birds to temperate regions of Europe from Africa, the Middle East, and Mediterranean areas. Recently, the emergence of adult ticks has been documented in numerous countries where they were previously not reported. Using a citizen science project, this study aimed to monitor the potential occurrence of *Hyalomma* spp. ticks in Poland.

A dedicated website was created through which volunteers could submit photos of unusual ticks. Between April and November 2024, more than 500 online submissions containing tick photos were received, 11 of which were identified as *Hyalomma* spp. ticks. Additionally, we received 65 parcels containing ticks, including four *Hyalomma* spp. ticks (of 11 online submissions). Amplification and sequencing of the partial mitochondrial cytochrome c oxidase subunit I gene (*cox 1*) was successfully performed for all received specimens.

Hyalomma spp. ticks were recorded in different regions of Poland, and most records were from Greater Poland and Silesia regions, in western and south-western Poland, respectively. Two of the identified specimens were morphologically and molecularly characterised as *Hyalomma rufipe*s, while another two were identified as *Hyalomma marginatum*.

The citizen science project enabled the confirmation of occurrence of adult *Hyalomma* spp. ticks in Poland, identifying a new hazard for human and animal health.

Keywords: *Hyalomma rufipes*, *Hyalomma marginatum*, Poland, citizen science

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MOLECULAR DETECTION OF PATHOGENS IN BAT-ASSOCIATED *ARGAS VESPERTILIONIS* TICKS

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Argas vespertilionis is a widely distributed, bat-associated soft tick predominantly inhabiting roosts, with occasional human parasitism raising concerns about zoonotic transmission [1]. While its vector potential is documented elsewhere, data from the Baltic region remain scarce. This study aimed to identify soft ticks collected from bats in Lithuania and to screen them for

pathogens using molecular methods. A total of 20 bats and two bat boxes were inspected for soft ticks. Ticks were identified morphologically using identification keys [2], with molecular confirmation of species identity. DNA was extracted individually from each tick with 2.5% ammonium hydroxide. For pathogen screening, DNA from larval ticks was pooled by host (≤6 larvae per pool); nymphs and the single adult were tested individually. Samples were screened by qPCR for *Babesia*, *Bartonella*, *Rickettsia*, and *Borrelia*. qPCR-positive samples underwent conventional and/or nested PCR and Sanger sequencing for phylogenetic analysis. We collected 182 ticks, morphologically identified as *Argas vespertilionis* (larvae, nymphs, and one adult female); molecular assays corroborated this identification. In total, 42 larval pools and separate nymph/adult samples were tested. qPCR suggested the presence of *Bartonella* in 2/182 (minimum infection rate, MIR 1.10%), *Borrelia* in 12/182 (6.59%), *Rickettsia* in 30/182 (16.48%), and *Babesia* in 8/182 (4.40%). Sequencing indicated that *Borrelia*-positive amplicons clustered closest to *Borrelia crocidurae* (relapsing fever group), while *Rickettsia* sequences grouped within the spotted fever group. One tick was confirmed *Babesia canis* – positive by 18S rRNA sequencing. *Bartonella gltA* sequences obtained from *A. vespertilionis* were 100% identical to sequences previously reported from bat fleas in Lithuania and from *A. vespertilionis* in the Netherlands. These findings show that *A. vespertilionis* ticks in Lithuania harbour diverse bacterial and protozoan agents, underscoring the need for continued surveillance and assessment of zoonotic and veterinary risks.

Keywords: *Argas vespertilionis*, bats, soft ticks, Borrelia, Bartonella, Rickettsia, Babesia

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DETECTION AND GENETIC DIVERSITY OF TICK-BORNE ENCEPHALITIS VIRUS IN LITHUANIA

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Tick-borne encephalitis (TBE) is an important zoonotic infection caused by the tick-borne encephalitis virus (TBEV), which circulates in natural foci involving ticks and small mammals. Identifying and characterising TBEV strains in vectors and reservoir hosts is crucial for understanding the ecology and epidemiology of the virus and public health risks. This study investigated the prevalence of TBEV in Ixodidae ticks and small rodents in Lithuania, as well as the distribution of viral subtypes and strains. In total, 9,067 ticks (grouped into 969 pools) and 175 rodents were examined. Viral RNA was detected, and phylogenetic relationships were analysed using molecular methods, including RT-PCR and sequencing. TBEV was identified in 34 tick pools, corresponding to a minimum infection rate of 0.37%. The findings confirmed the role of *Ixodes ricinus* and *Dermacentor reticulatus*, two common tick species in Lithuania, in TBEV transmission. The virus was present across all developmental stages of *I. ricinus*, with infection rates highest in adults (0.6%) compared to nymphs (0.25%) and larvae (0.26%). Among the five rodent species tested, TBEV was detected exclusively in yellow-necked mice (*Apodemus*

flavicollis), highlighting their role as the main reservoir host in the region. The results also demonstrated the presence of diverse European TBEV genotypes in Lithuania, with distribution patterns linked to geographic areas. These findings provide new insights into the ecology of TBEV, identify the key vector and reservoir species in Lithuania, and emphasise the importance of continued surveillance to reduce the risk of human outbreaks.

Keywords: ticks, tick-borne encephalitis, *Ixodes ricinus*, *Dermacentor reticulatus*

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FAST-TRACK TBE IMMUNITY: RAPID PROTECTION VIA ACCELERATED VACCINATION SCHEDULES

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Roughly half of travellers seek pre-travel care fewer than four weeks before departure, and 16–26% present within seven days, underscoring the value of vaccination schedules that induce protection rapidly. Encepur is an inactivated K23-strain tick-borne encephalitis (TBE) vaccine with more than three decades of use in Europe. Current adult and paediatric formulations have been widely deployed since 2001, with over 75 million doses administered and a favourable safety profile characterised mainly by short-lived local and systemic reactions. Real-world effectiveness is high: approximately 96% in Germany (matched case-control), around 91% in Switzerland after at least three doses, and consistently high across age groups in the Czech Republic. What differentiates Encepur is the Express schedule (0–7–21 days), which completes primary immunisation in just 21 days, achieves seroconversion approximately 14 days after the second dose, and transitions to a booster at 12–18 months (then every five years until age 49 and every three years thereafter). This design compresses the time to protection, which is crucial for late starters before the tick season or last-minute travellers, and simplifies logistics that may improve series completion. Analogous evidence from hepatitis B vaccination programmes shows that accelerated schedules improve completion rates without sacrificing immunogenicity. For example, in adults, a 0–1–2-month schedule achieved higher third-dose completion compared with 0–1–6 months (89.5% vs 84.5%). Furthermore, the combined hepatitis A/B vaccine uses an approved 0–7–21-day primary series with a 12-month booster to ensure rapid protection in travellers.

Keywords: tick-borne encephalitis, vaccine, accelerated schedule, Encepur, travellers

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ISOLATION OF TICK-BORNE ENCEPHALITIS VIRUS FROM NATURALLY INFECTED DOGS

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Tick-borne encephalitis virus (TBEV) is an emerging concern in canine health [1–2], yet its active circulation in dogs remains insufficiently documented. In a cross-sectional study of 473 dogs from central Lithuania (2020–2021), we confirmed acute infections by PCR, serology, and virus isolation in cell culture.

TBEV RNA was detected in 18.6% of canine samples, and antibodies were present in 21.6%. Importantly, we succeeded in isolating infectious TBEV from seven serum samples and one cerebrospinal fluid specimen. Replication was demonstrated in Neuro-2a, Vero, and Marc-145 cells, with subsequent confirmation by sequencing. These results provide direct evidence that viraemic dogs carry transmissible virus and can serve as a source for successful cultivation.

In parallel, attached ticks collected from the same dogs carried TBEV RNA in 34.2% of suspensions. After three blind passages in cell culture, positivity rose to 56–61%, highlighting that viral replication in ticks often requires amplification to reach detectable levels. Nevertheless, no consistent concordance was found between a dog's infection status and TBEV detection in its ticks.

The study demonstrates that virus isolation from naturally infected dogs and their ticks is feasible and reliable, confirming the role of dogs as suitable sentinel hosts for TBEV. Cultivation in multiple cell lines expands opportunities for experimental studies and strain characterisation. Our findings underline the clinical and epidemiological importance of canine TBEV infections and provide valuable resources for further virological and pathogenesis research.

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Keywords: TBEV in dogs, TBEV isolation, TBEV

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COMPREHENSIVE ASSESSMENT OF TICK-BORNE PATHOGENS IN URBAN AREAS USING A MULTIPLEX PCR-BASED APPROACH

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A total of 285 nucleic acid (NA) samples extracted from 226 *Ixodes ricinus*, 42 *Dermacentor reticulatus*, and 14 dog blood samples were screened for tick-borne pathogens using the Seegene (Korea) Novaplex™ Tick-Borne Disease Expanded Assay (RUO). Ticks were collected from three different locations in Lithuania: Verkiai Park (Vilnius), and two riverbank sites in Ukmergė and Vilnius. Dog blood samples were obtained from a veterinary clinic in Ukmergė. Total DNA/RNA was extracted using the Qiagen Mini Kit, following the manufacturer's protocol. Real-time PCR amplification and target detection were performed using the Bio-Rad CFX96 Dx system (USA). Data analysis was conducted with Seegene Viewer RT real-time PCR software (Version 3). In *D. reticulatus* tick samples, specific targets of *Rickettsia* spp. and *Borrelia burgdorferi* sensu lato complex were detected. In *I. ricinus* tick samples, amplification revealed the presence of specific targets for *Rickettsia* spp., *Borrelia miyamotoi*, *B. burgdorferi* sensu lato complex, and *Babesia* spp. Infection with one or more tick-borne pathogens was detected in 53% of *I. ricinus* females, 36.5% of males, and 47.8% of nymphs. The detection of *Babesia divergens*, *Babesia microti*, and *Babesia venatorum* further indicates that individuals visiting public parks may be at risk of exposure to these pathogens. *Rickettsia* spp. was identified in *I. ricinus* larvae, suggesting the possibility of transovarial transmission or the possible presence of the parasitic wasp *Ixodiphagus hookeri*. The Seegene Novaplex™ Tick-borne Disease Expanded Assay (RUO) proved to be a valuable tool for detecting tick-borne pathogens in tick samples. High sensitivity and specificity make this kit suitable not only for research applications but also for clinical use, providing clinicians and patients with fast, reliable, and accurate diagnostic results.

Keywords: *Ixodes ricinus*, *Dermacentor reticulatus*, *Rickettsia* spp., *Borrelia burgdorferi* sensu lato, *Babesia* spp.

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A NOVEL APPROACH PREVENTING THE SPREAD OF SPIROCHAETES IN HUMAN SKIN MODELS (GENOSKIN)

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Lyme borreliosis is the most common TBD in Europe, affecting 650,000–850,000 people annually [1]. Currently, no methods exist for disinfecting the site of a tick bite to prevent infection and spread of bacteria. We evaluated the effectiveness of photoinactivation using a live human skin model (Genoskin) *in vitro*. Skin biopsies were intradermally inoculated with 10 × 5–10 × 7 *Borrelia afzelii* followed by photoinactivation at certain time intervals. To evaluate skin infection, we set bacteria cultures from model medium (evidence of spirochaete migration) and from biopsies, after seven days of incubation. Histopathological examination of infected, treated and control biopsies were performed to assess changes caused by infection and/or treatment. Molecular methods were also used for confirmation of infection.

Results:

1) human skin models could be effectively infected with *B. afzelii*,

2) photoinactivation effectively eradicated spirochaetes in treated samples, with no positive cultures in treated groups.

In untreated controls, however, the presence of *B. afzelii* was confirmed through both culture and PCR. Histopathological examination of infected, untreated samples showed preserved tissue structure, with no substantial infection-induced damage. These findings suggest that photoinactivation may serve as a promising, non-invasive method to disinfect tick bite sites.

Acknowledgements: the study was funded by UW IDUB New Ideas 2B No. IDUB-622-317/2022 (AB).

Keywords: *Borrelia*, Lyme disease, human skin, photoinactivation

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Poster presentations

MOLECULAR IDENTIFICATION OF *RICKETTSI*A SPP. IN TICKS FROM URBAN AREAS

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Tick-borne pathogens pose a significant threat to human and animal health, yet there is a notable gap in knowledge regarding their prevalence in urban areas. This study aimed to identify and characterise tick-borne pathogens in urban environments in Vilnius, Lithuania. DNA samples of *Ixodes ricinus* and *Dermacentor reticulatus* ticks were screened for the presence of pathogens using Polymerase Chain Reaction (PCR) with specific primers, with a primary focus on *Rickettsia* species. Preliminary results revealed the presence of *Rickettsia* spp. DNA in several samples, showing a positivity rate of 12% (10/84). Later, the samples were checked using Real-time PCR and results were compared. Real-time PCR amplification and target detection were performed using the Bio-Rad CFX96 Dx system (USA). RT-PCR proved to be more sensitive and in 33% (28/84) of samples *Rickettsia* spp. DNA was detected. Further analysis is underway to identify the specific pathogen species, including *Babesia*, and to determine the prevalence rates in the tick populations of Vilnius. This research will provide data on the risk of tick-borne diseases such as rickettsiosis and babesiosis and will contribute to a better understanding of the epidemiology of these pathogens in urban areas.

Keywords: *Ixodes ricinus*, *Rickettsia* spp., urban areas, Lithuania

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PREVALENCE OF *BORRELIA* SPP. IN URBAN AND PERI-URBAN TICK POPULATIONS OF KAUNAS, LITHUANIA

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Lyme disease, caused by *Borrelia burgdorferi* sensu lato (s.l.), is one of the most prevalent tick-borne infections in Europe, with Lithuania reporting one of the highest prevalence rates. Urbanisation is increasingly altering the ecology of tick populations and the epidemiology of tick-borne diseases. To assess the risk posed by ticks in urban green spaces in central Lithuania, we collected ticks from urban and peri-urban habitats in Kaunas city during 2023–2024. A total of 597 questing ticks were analysed, including *Ixodes ricinus* (n = 540) and *Dermacentor reticulatus* (n = 57). Ticks were screened for the presence of *Borrelia* spp. using real-time PCR, targeting the *23S rRNA* gene. *Borrelia* species then were identified through amplification and sequence analysis of the partial *ospA* gene, the chromosomal flagellin gene, and the *16S–23S rRNA* intergenic spacer region. *Borrelia* DNA was detected in 22.45% of the collected ticks, exclusively in *I. ricinus*. Four *Borrelia* species were identified: *B. burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*, and *B. miyamotoi*. The presence of these pathogenic species in densely populated green urban areas of Kaunas city indicates a potential exposure risk for Lyme disease. Our findings highlight the epidemiological importance of continuous monitoring and the need for integrated tick management and public awareness strategies.

Keywords: *Borrelia burgdorferi* s.l., ticks, green urban spaces, Lithuania

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REGULATORY CYTOKINES IN DOGS NATURALLY INFECTED WITH *BABESIA CANIS*

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Canine babesiosis, a tick-borne disease caused by *Babesia canis* protozoan, constitutes a significant and widespread veterinary problem in Poland. The pro-inflammatory immune response to the infection induces numerous clinical symptoms, which can often lead to death. Cytokines with regulatory properties play a crucial role in inhibiting inflammation and inducing regenerative processes. The aim of this study was to evaluate serum concentrations of key regulatory cytokines, IL-10 and TGF-β, and to analyse their correlations with other clinical parameters. Cytokine levels were measured in the serum of dogs from Poland naturally infected with *B. canis*. Blood samples were collected from 34 dogs diagnosed with babesiosis and 28 healthy dogs. Cytokine concentrations were determined using the ELISA method. Our results showed increased IL-10 levels and decreased TGF-β levels in the serum of dogs with babesiosis compared to healthy controls. Moreover, IL-10 and TGF-β levels correlated with the age of *B. canis*-infected dogs. IL-10 levels were also associated with immune cell counts (neutrophils, lymphocytes, monocytes, and eosinophils) as well as mean corpuscular haemoglobin concentration (MCHC). These findings indicate that regulatory cytokines may play an important role in the course of canine babesiosis.

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Keywords: *Babesia canis*, dogs, Poland, TGF-β, IL-10

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MOLECULAR DETECTION OF *HEPATOZOON CANIS* IN DOGS IN LITHUANIA

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The rising global average temperatures have created more favourable conditions for the survival of arthropod vectors, such as ticks [1]. This contributes to the increased risk of vector-borne infectious diseases, including the *Hepatozoon canis* infection. This protozoan parasite is primarily transmitted through ingestion of infected brown dog ticks (*Rhipicephalus sanguineus*) [2, 3]. Warmer winters enhance tick survival, reproduction, and geographic spread, consequently elevating the risk of *H. canis* transmission. The aim of this study was to investigate the presence of *H. canis* in ticks and domestic dogs from Lithuania.

A total of 84 blood samples of Lithuanian hounds were obtained from a veterinary clinic and 196 *Ixodes ricinus* ticks were collected from different urban areas in Lithuania. DNA was extracted from both sample types, followed by PCR amplification targeting the *H. canis 18S rRNA* gene. Amplified products were visualised via electrophoresis, and positive samples were submitted for sequencing.

The parasite was not detected in any of the tick samples. However, *H. canis* DNA was identified in 24 out of 84 (28.6%) blood samples of Lithuanian hounds. These findings suggest the presence of *H. canis* infection among domestic dogs in Lithuania, underscoring the need for continued surveillance and vector control.

Keywords: dog, Lithuanian hound, *Hepatozoon canis*, *Ixodes ricinus*

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PREVALENCE OF TICK-BORNE PATHOGENS IN PET DOGS IN LITHUANIA

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Tick-borne pathogens (TBPs) represent a growing veterinary and public health challenge in the Baltic region, where climatic conditions support abundant populations of *Ixodes ricinus* and *Dermacentor reticulatus* ticks [1]. As the most common companion animals in Lithuania, dogs are frequently exposed to tick habitats, increasing the risk of infections with *Anaplasma phagocytophilum* and *Babesia canis* [2, 3]. Both pathogens are clinically relevant due to non-specific clinical signs, which complicate diagnosis in veterinary practice. This study aimed to investigate the prevalence of *A. phagocytophilum* and *B. canis* in Lithuanian dogs and to characterise their genetic diversity through cytological, serological, and molecular analysis. A total of 203 blood samples from pet and hunting dogs were analysed. *A. phagocytophilum* prevalence reached 25% in pet dogs and 4.4% in hunting dogs. Serology and PCR results did not always coincide, underscoring the importance of molecular testing, while *B. canis* DNA was detected in 66.3% (62/107) of pet dogs. Importantly, discrepancies were observed between microscopy and PCR: out of 75 dogs diagnosed microscopically, only 54 were PCR-positive, while eight PCR-positive dogs were smear-negative. Four sequence variants of *B. canis* were identified, with Lithuanian isolates clustering with strains from Latvia, Germany, and France. Sequencing revealed multiple *A. phagocytophilum* genotypes, with Lithuanian isolates clustering alongside strains from Hungary, Spain, Germany, Sweden, Slovenia, and local *Ixodes ricinus* ticks. *B. canis* isolates grouped closely with strains from Latvia, Germany, and France. No significant associations were found between pathogen prevalence and host factors such as sex, age, or breed. This study demonstrates that Lithuanian dogs serve as important hosts for both *A. phagocytophilum* and *B. canis*. The molecular and phylogenetic findings confirm genetic links between local and European isolates, emphasising the need for regular screening, tick prevention strategies, and molecular surveillance to mitigate the veterinary and zoonotic risks of TBPs.

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MOLECULAR SCREENING FOR TICK-BORNE ENCEPHALITIS VIRUS IN URBAN TICK POPULATIONS

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Climate change is reshaping tick ecology by prolonging their seasonal activity and enabling expansion into previously unsuitable habitats [1]. Consequently, the incidence of tick-borne diseases is increasing in regions once considered low risk, creating growing public health challenges. Tick-borne encephalitis (TBE) remains one of the most important vector-borne diseases in Europe, with the Baltic countries accounting for nearly one-third of reported cases [2, 3]. This study investigated the prevalence of tick-borne encephalitis virus (TBEV) in questing ticks in Lithuania and evaluated key transmission risk factors. During 2023–2024, 544 ticks (*Ixodes ricinus*, n = 525; *Dermacentor reticulatus*, n = 19) were collected by flagging method in urban habitats and pooled into 47 groups by species and developmental stage. TBEV detection was carried out using real-time RT-PCR and virus isolation in two cell lines (Neuro-2a and MARC-145). TBEV RNA was detected in 15.26% (83/544) of individual tick samples. Virus isolation proved more sensitive than direct RT-PCR, yielding a total minimum infection rate (MIR) of 2.8% across both cell lines, compared with 1.47% by direct RT-PCR. MIR was 1.3% (7/525) for *I. ricinus* and 5.3% (1/19) for *D. reticulatus*. Positive samples included two nymphs, three females, and three males, indicating active TBEV circulation across developmental stages and sexes. These findings highlight a potentially greater role of *D. reticulatus* in TBEV transmission than previously recognised and emphasise the need for continued tick surveillance, robust public health interventions, and the inclusion of virus isolation techniques to enhance detection accuracy.

Acknowledgements: this study was supported by the Research Council of Lithuania (Grant No. S-MIP-23/19).

Keywords: tick-borne encephalitis, *Ixodes ricinus*, *Dermacentor reticulatus*, questing ticks

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OCCURRENCE OF *RICKETTSIA* SPP. IN TICKS AND MITES INFESTING SMALL RODENTS IN LITHUANIA

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Rickettsia spp. are best known as human pathogens transmitted mainly by arthropods and causing public health problems worldwide [1]. However, the distribution, natural cycle and associations of *Rickettsia* spp. with various species of ectoparasites are not completely understood [2]. This study aimed to investigate the presence and prevalence of *Rickettsia* spp. in ticks and mites collected from small rodents in Lithuania and to molecularly characterise the *Rickettsia* strains obtained from different ectoparasites. A total of 596 *Ixodes ricinus* ticks and 550 mites of five species (*Laelaps agilis*, *Hyperlaelaps microti*, *Haemogamassus nidi*, *Eulaelaps stabularis*, *Myonyssus gigas*) were collected from 238 rodents in Lithuania during 2013–2014. Analysis of the samples revealed that the infection rate varied among ectoparasites and was found to be higher in *I. ricinus* ticks (MLE = 26.5%; 95% CI: 22.2–31.3%) compared to mites (MLE = 9.3%; 95% CI: 7.0–12.2%). Sequence analysis of partial *gltA* and *17kDa* genes revealed the presence of *R. helvetica*, *R. felis* and *Rickettsia* sp. in Laelapidae mites, while only *R. helvetica* was detected in *I. ricinus* ticks. Phylogenetic analysis of the *Rickettsia* strains isolated from *I. ricinus* ticks and different mite species demonstrated genetic heterogeneity and provided evidence for host-specific strain variation. To our knowledge, this is the first evidence of the presence of *R. felis* in *L. agilis* and *H. microti* mites and the first report of the occurrence and molecular characterisation of *Rickettsia* spp. in ticks and Mesostigmata mites from rodents in the Baltic region.

Keywords: *Rickettsia*, *Ixodes ricinus*, Laelapidae, rodents, Lithuania

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MOLECULAR INSIGHTS INTO ITP-LIKE (ITPL) NEUROPEPTIDE SIGNALLING IN THE *IXODES RICINUS* TICK

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Ticks are obligate hematophagous ectoparasites that must maintain water balance during extended off-host periods and excessive blood ingestion. Osmoregulation in ticks is primarily mediated by organs under the control of neuropeptides produced by the central nervous system, among others. Among these, ion transport peptide (ITP) and its alternatively spliced isoforms, ITP-like peptides (ITPLs), are regulators of development and water homeostasis in insects [1, 2], yet in ticks they remain poorly understood. In this study, we investigated the ITP gene of the tick *Ixodes ricinus*. We identified two alternatively spliced transcripts, ITPL1 and ITPL2, with no evidence of the canonical ITP isoform. Transcript quantification showed both ITPLs are confined to synganglion with differential localisation among neurons. Furthermore, immunohistochemical staining with ITPL1-specific antiserum revealed axonal projections from ITPL-expressing neurons extending beyond the synganglion to innervate peripheral tissues. We characterised three putative ITP/L receptors: two G protein-coupled receptors (GPCR) homologous to *Bombyx mori* BNGR-A2 and BNGR-A24, and a guanylyl cyclase receptor related to *Drosophila melanogaster* GYC76c. Expression profiling revealed the GPCRs are expressed in the synganglion, female gonads, and gut, whereas GYC76c is restricted to female gonads. These patterns suggest functional roles for ITPL signalling both within the CNS and in peripheral tissues.

Our results confirm the presence of ITPL signalling, and absence of the insects' major regulator of water homeostasis, ITP, in *I. ricinus* ticks.

Acknowledgements: this study was supported by SAS PostdokGrant No. APD0086.

Keywords: *Ixodes ricinus*, Neuropeptide, Neurophysiology

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BORRELIA INFECTIONS IN RODENT-ASSOCIATED IXODID TICKS FROM LITHUANIA

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Borrelia bacteria are tick-borne pathogens that cause zoonotic diseases such as Lyme borreliosis and tick-borne relapsing fever [1, 2]. Rodents serve as important hosts for the larval and nymph stages of ixodid ticks, contributing to their life cycle and maintenance in the ecosystem [3]. In this study, a total of 800 immature ticks, identified as *Ixodes ricinus* ($n = 673$) and *Dermacentor reticulatus* ($n = 127$), were collected from 141 rodent hosts captured in the Curonian Spit, Lithuania. Ticks were analysed for the presence of *Borrelia* DNA using real-time PCR amplifying the partial *23S rRNA* gene. For the identification of *Borrelia* species and strains, conventional and nested PCRs were applied, targeting the *ospA* gene and the 16S–23S intergenic spacer (IGS) region, followed by sequence analysis. *Borrelia* DNA was detected in 11.8% of *D. reticulatus* and 7.7% of *I. ricinus* ticks. Sequence analysis revealed the presence of *Borrelia afzelii* and *Borrelia miyamotoi* in both tick species. This study is the first report of *Borrelia* bacteria in *D. reticulatus* ticks from rodents in Lithuania. Our findings highlight the significant role of rodents as hosts for immature ticks, contributing to the maintenance and transmission of the agents causing Lyme borreliosis and tick-borne relapsing fever in Lithuania.

Keywords: Lyme borreliosis, tick-borne relapsing fever, *Ixodes ricinus*, *Dermacentor reticulatus*, Lithuania

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IMPAIRED REGULATORY IMMUNE RESPONSE IN DOGS WITH BABESIOSIS

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Canine babesiosis, caused by the protozoan parasite *Babesia canis*, is one of the most dangerous parasitic diseases in veterinary medicine [1]. Similar to malaria, canine babesiosis is associated with the development of a T helper type 1 (Th1)-dependent immune response that triggers a strong inflammatory reaction [2]. Regulatory T cells (Tregs) are essential for limiting excessive immune responses. Their immunosuppressive effect is mediated by the production of regulatory cytokines and direct cell-to-cell interactions. Impairment of this suppressive response may contribute to disease severity and even lead to death [3]. The aim of this study was to evaluate the regulatory immune response in dogs naturally infected with *B. canis*. Blood samples were collected from 22 dogs diagnosed with babesiosis and from 28 healthy controls. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood, and the expression of CD5, CD4, CD8, and CD25 surface markers, as well as intracellular transcription factor Foxp3, was assessed by flow cytometry. The percentage of CD4$^+$CD25$^+$Foxp3$^+$ and CD8$^+$CD25$^+$Foxp3$^+$ Tregs, as well as CD4+CD25$^+$Foxp3$^-$ and CD4$^+$CD25$^-$Foxp3$^+$ subpopulations, were analysed. The analyses revealed a reduced percentage of CD4$^+$CD25$^+$Foxp3$^+$ Tregs, CD4$^+$CD25$^+$Foxp3$^-$, and CD4$^+$CD25$^-$Foxp3$^-$ subpopulations in the blood of infected dogs compared to healthy controls. These findings indicate significant deficiencies not only in suppressive CD4$^+$ Tregs but also in other CD4-positive T-cell populations in dogs with babesiosis, which may contribute to the severe course of the disease and increased mortality.

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Keywords: *Babesia canis*, dogs, Poland, Tregs, T cells

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RED FOXES (*VULPES VULPES*) AS HOSTS OF TICK-BORNE PATHOGENS IN LITHUANIA

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Ticks are among the most important vectors of infectious diseases in Europe, transmitting a wide range of pathogens to humans and animals [1]. Their distribution and abundance are strongly influenced by climate change, land use alterations, and the increasing presence of wildlife in suburban and urban habitats [2]. Wild carnivores, particularly canids such as the red fox (*Vulpes vulpes*), are key hosts for ticks and tick-borne pathogens. Red foxes are highly adaptable: they thrive in both rural and urban environments and serve as reservoirs of multiple pathogens due to their role as hosts to numerous ectoparasites, including ticks [3]. This study aimed to investigate the presence of tick-borne pathogens in red foxes (*Vulpes vulpes*) from Lithuania and to evaluate their potential role as reservoirs in the epidemiology of tick-borne diseases. Spleen tissues from red foxes were analysed for the presence of *Anaplasma* spp., *Borrelia* spp., and *Babesia* spp. DNA using PCR assays. The results showed that 5.3% of foxes were infected with *Anaplasma* spp., 1.8% with *Borrelia* spp., and 3.5% with *Babesia* spp. Sequencing further revealed the presence of *Anaplasma phagocytophilum*, *Babesia vulpes*, and *Borrelia burgdorferi* sensu lato. These findings demonstrate that red foxes in Lithuania harbour several tick-borne pathogens, indicating their potential role as natural reservoirs. Further studies are needed to better understand their contribution to pathogen circulation and transmission dynamics.

Keywords: *Vulpes vulpes*, *Borrelia*, *Anaplasma*, *Babesia*

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EXPERIMENTAL INFECTIONS WITH *RICKETTSIA HELVETICA*: WHY *IXODES RICINUS* POSES A GREATER RISK THAN *DERMACENTOR MARGINATUS*?

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Ticks are among the most important arthropod vectors of human and animal pathogens, second only to mosquitoes. Beyond their role as vectors, they also serve as reservoirs of various pathogens [1, 2]. This study investigated the vector competence of *Ixodes ricinus* and *Dermacentor marginatus* for the emerging pathogen *Rickettsia helvetica* through experimental infection.

Pathogen-free ticks from a lab colony were used for experimental infection. One hundred females of *I. ricinus* and *D. marginatus* were injected with 50 nL of *R. helvetica* suspension (~500 bacteria). Ticks were sampled every four days over 32 days to detect pathogens, and quantitative PCR was used to track bacterial growth in whole ticks. Sterile PBS-injected ticks served as controls. Pathogen presence in tissues was visualised by light and scanning electron microscopy.

Over 24 days, *R. helvetica* showed strong replication in *I. ricinus* (1324-fold increase), with microscopy confirming its presence in tracheae, synganglion, and salivary glands. In contrast, *D. marginatus* showed limited bacterial replication, with a 24-fold increase over the same period. These findings show the role of *D. marginatus* only as a mechanical vector for *R. helvetica*, and the minimal proliferation of the pathogen aligns with our long-term field observations, where questing *D. marginatus* repeatedly show zero prevalence of *R. helvetica*.

We demonstrated that *I. ricinus* is a competent vector for *R. helvetica*, while *D. marginatus* is not. Although both tick species may acquire the pathogen while feeding on an infected host, only *I. ricinus* makes *R. helvetica* successfully replicate and colonise the salivary glands – a critical step for subsequent transmission to a new host. This study enhances the understanding of the transmission dynamics of this pathogen and aids in informing public health risk assessments related to tick-borne diseases.

Keywords: tick-borne pathogens, vector competence, *Rickettsia helvetica*, *Ixodes ricinus*, *Dermacentor marginatus*

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NYMPHAL TICKS FROM WILD BIRDS: MICROBIOME DIFFERENCES LINKED TO *RICKETTSIA* INFECTION

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Nymphal ticks play a vital role in transmitting pathogens to humans and large mammals. They are small and often overlooked, but they are highly effective vectors. Compared to adult ticks, whose microbiomes often change after blood-feeding and are frequently dominated by a few endosymbionts, nymphs typically harbour more diverse bacterial communities. Wild birds serve as common hosts for immature ticks, providing the blood meals necessary for their development, and can carry ticks with their pathogens over long distances through migration routes. As a result, bird-associated ticks form an important ecological link between wildlife, humans, and domestic animals.

For microbiome analysis, 51 nymphs were chosen. The ticks were obtained in Latvia from *Turdus merula* and *T. philomelos* and initially screened for *Rickettsia* by PCR targeting the *gltA* gene. The sample set included both *Rickettsia*-positive and negative samples. Bacterial communities were characterised by *16S rRNA* gene (V3–V4) amplicon sequencing using an Illumina MiSeq platform. Sequencing data were processed using standard bioinformatic workflows, involving quality filtering, denoising, and taxonomic assignment to generate microbial profiles and diversity metrics. Comparative analyses were conducted to evaluate the differences associated with the presence of *Rickettsia* and to explore potential host-related effects.

The results indicated variation in microbial composition based on the presence of *Rickettsia*, including changes in the abundance of symbiotic bacteria. This suggests that *Rickettsia* may influence the structure and functional potential of tick microbial communities. Additionally, host-related effects were observed, indicating that avian hosts may further influence shaping the tick microbiome.

This study underscores the importance of considering both the pathogen infection status and host factors when studying the microbiome. This work also highlights the role of bird-associated nymphs in pathogen circulation, providing new insights into the complex relationships between ticks, their endosymbionts, pathogens, and avian hosts.

Keywords: ticks, *Rickettsia*, microbiome, birds, nymphs

MOLECULAR EVIDENCE OF ZOONOTIC *RICKETTSIA* SPP. IN CATS AND THEIR ECTOPARASITES IN LITHUANIA

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Domestic cats and their ectoparasites are increasingly recognised as potential reservoirs and vectors of zoonotic *Rickettsia* species, yet their role in pathogen maintenance and transmission remains insufficiently defined [1]. Among these, *R. felis* is of particular concern as a cosmopolitan pathogen primarily maintained by the cat flea (*Ctenocephalides felis*), although over 20 other arthropod species, including fleas, ticks, and mites, have been shown to harbour it [2]. To characterise the presence and genetic diversity of *Rickettsia* spp. in Lithuania, we performed molecular screening of domestic cats and their ectoparasites between 2015 and 2023. Blood samples were collected from 543 owned cats, together with 153 fleas (137 *C. felis*, 15 *C. canis*, 1 *Nosopsyllus fasciatus*) from 28 cats and 321 ticks (*Ixodes ricinus* and *Dermacentor reticulatus*) from 59 cats across multiple regions. DNA was extracted and analysed using nested PCR targeting the *gltA* and 17-kDa antigen genes, followed by sequencing and phylogenetic analysis. *Rickettsia* DNA was detected in 4.6% (25/543) of cat blood samples. Three human-pathogenic species – *R. helvetica*, *R. conorii* subsp. *raoultii* and *R. felis* – were identified. Based on *gltA* and 17-kDa gene sequences, we distinguished four and three genotypes of *R. helvetica*, four and one genotypes of *R. conorii* subsp. *raoultii*, and two and three genotypes of *R. felis*, respectively. Prevalence was higher in ticks (25.2%; 81/321) than in fleas (15%; 23/153). *R. helvetica*, *R. conorii* subsp. *raoultii* and *R. felis* were detected in fleas, while only *R. helvetica* and *R. conorii* subsp. *raoultii* occurred in ticks. These findings provide the first evidence of zoonotic *Rickettsia* species circulating in domestic cats and their ectoparasites in Lithuania and highlight the potential of cat-associated ticks and fleas to act as reservoirs of rickettsial pathogens, underscoring the need for continued surveillance and public health monitoring.

Keywords: Zoonoses, domestic cat, ectoparasite, genetic diversity

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DETECTION AND CHARACTERISATION OF *BABESIA* SPP. IN RED DEER (*CERVUS ELAPHUS*) FROM LITHUANIA

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Tick-borne diseases are increasingly reported across Europe and parasites of the genus *Babesia* are of particular concern due to their impact on animal health and zoonotic potential [1]. Wild cervids, especially red deer (*Cervus elaphus*), are recognised as important hosts that may contribute to maintaining transmission cycles in natural ecosystems [2, 3].

The objective of this study was to detect and characterise *Babesia* spp. in red deer from Lithuania using molecular methods. During the hunting seasons (2023–2025), spleen samples from 62 animals were collected and analysed by PCR targeting the *18S rRNA* gene. PCR products were checked by agarose gel electrophoresis and two samples (2/62; 3.2%) showed bands of the expected size (~380 bp), indicating the presence of *Babesia* DNA. The amplicons were sequenced to determine the *Babesia* species present in the samples. Two distinct *Babesia* species were identified: *B. divergens* and *B. odocoilei.* These findings confirm that red deer in Lithuania can be infected with *Babesia* spp. and emphasise the importance of wildlife monitoring in understanding the circulation of tick-borne pathogens in Europe.

Keywords: red deer, *Cervus elaphus*, *Babesia divergens*, *Babesia odocoilei*.

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LINKING MOOSE CALF MORTALITY TO *ANAPLASMA PHAGOCYTOPHILUM* INFECTIONS

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Over recent decades, records from South Norway have shown a decline in moose calf carcass weights [1]. In addition, hunters have been increasingly reporting sightings of moose cows without calves, a trend confirmed through entries in the Norwegian Deer Register system (https://hjorteviltregisteret.no) [2], where all observations and harvests during the hunting season are logged. One possible contributing factor to this trend is the geographic spread of ticks and the diseases they transmit [3]. This study focused on assessing the prevalence of *Anaplasma phagocytophilum* in juvenile moose population in Norway. Spleen samples were collected from dead calves and hunted animals and analysed for *A. phagocytophilum* DNA using PCR targeting the *msp2* and *16S rRNA* genes. Results revealed that 100% of the dead animals and 90% of the hunted individuals tested positive for *A. phagocytophilum* using RT-PCR. Analysis based on the *16S rRNA* gene indicated the bacterium was present in 78% samples from dead calves and 69% from the hunted moose. Sequence analysis of the *16S rRNA* gene identified nine genetic variants of the bacterium. The high prevalence of *A. phagocytophilum* may contribute to weakened immunity in moose calves, potentially impacting their body condition, disease susceptibility, and overall survival.

Keywords: moose, *Alces alces*, *Anaplasma phagocytophilum*, Norway

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SEASONAL ACTIVITY OF *IXODES RICINUS* TICKS UNDER THE IMPACT OF GLOBAL CHANGES IN BRATISLAVA, SLOVAKIA

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The distribution areas of some tick species have changed, and many tick-borne diseases have emerged (or re-emerged) within the past two or three decades due to global changes.

This study deals with seasonal activity of *Ixodes ricinus* in Bratislava (south-western Slovakia). From June 2023 to August 2025, questing activity of ticks has been monitored. First, questing ticks were monthly collected by flagging at 12 urban/suburban sites. Second, activity of ticks was monitored in 'tick gardens' (TG) placed at three study sites located in the forest [1]. Third, ticks feeding on humans were collected.

In our collections, three tick species were detected with the dominance of *I. ricinus*, followed by *Haemaphysalis concinna* and *Dermacentor marginatus*.

In total, 4883 questing *I. ricinus* ticks were collected. Nymphs were the dominant (47%), followed by larvae (38%) and adult ticks (15%). The highest activity was recorded in June 2023, April and October 2024, and in May 2025. August was the month with the lowest tick activity during all three study years. Low winter activity of ticks were confirmed in all study year.

Questing activity of ticks in TG was different compared to the flagging method because the different groups of ticks were used in TG. Winter activity of ticks was confirmed.

From humans, 485 ticks were collected with the dominance of *I. ricinus* (98%). Nymphs (73%), adult ticks (21%), and larvae (6%) were collected. *D. reticulatus* was determined in 12 samples.

A great deal of work has been done on tick molecular systematics, population genetics, and TBD. Researchers tend to put aside studies of fundamental ecological aspects of ticks and their phenology, which are currently changing alongside with changing climatic and non-climatic conditions including globalisation and urbanisation [2, 3]. Our study presents a picture of the current risk of infection with tick-borne diseases.

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Keywords: seasonal activity, tick, *Ixodes ricinus*, global changes

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BORRELIA SPP. AND *RICKETTSIA* SPP. IN URBAN PARKS OF BRATISLAVA (WESTERN SLOVAKIA): ESTIMATION OF RISK AND TREATMENT OF INFECTIONS

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Due to climate changes and human activity, the occurrence of ticks in higher altitudes and urban areas has increased. The aim of this study was to analyse the prevalence and heterogeneity of tick-borne pathogens (*Borrelia* spp., *Rickettsia* spp.) in questing *Ixodes ricinus* ticks from urban parks in Bratislava. *I. ricinus* is the most important vector of tick-borne pathogens in Europe. This species is the main vector for *Borrelia burgdorferi* sensu lato (s.l.), *Rickettsia* spp. and other pathogens with major epidemiological significance. Doxycycline is the first-line antibiotic therapy used to treat infections caused by Rickettsiae and Borreliae. However, certain tick-borne pathogens have demonstrated resistance to specific antibiotics. Consequently, there is an increasing imperative to investigate and develop alternative therapeutic strategies.

Study areas (9) are situated in urban parks and botanical garden in Bratislava with the interest for recreation. In 2023 and 2024, questing ticks were collected and tested for the presence of *B. burgdorferi* s.l. and *Rickettsia* spp.

Prevalence of *B. burgdorferi* s.l. varied from 5.4% to 34.4%. A total of five genospecies was identified. *Borrelia afzelii*, *B. garinii*, *B. valaisiana*, *B. lusitaniae*, and *B. spielmanii* all are known to be associated with Lyme disease (1). Prevalence of *Rickettsia* spp. was 12.5% in 2023, *R. helvetica* (6.3%) was identified in these *Rickettsia*-positive ticks by species-specific PCR (2, 3). The documented levels of tick infestation and pathogen prevalence demonstrate that humans are at risk of acquiring tick-borne diseases even under urban conditions. A comprehensive understanding of the eco-epidemiology of these infections is essential for accurate diagnosis, appropriate treatment, and reliable risk assessment. In parallel, we are exploring nanobiotechnology-based approaches as potential alternative strategies for therapy. These include the application of nanoparticles and novel polymeric materials such as carbon quantum dots employed as photosensitizers in photodynamic inactivation (4).

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Keywords: *Borrelia* spp., *Rickettsia* spp., treatment, nanobiotechnology

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CLINICAL MANIFESTATIONS OF CANINE BABESIOSIS AND THEIR ASSOCIATION WITH HEMATOLOGICAL FINDINGS AND OUTCOME OF DISEASE

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Canine babesiosis, a protozoan tick-borne disease caused by *Babesia canis*, has become an important health concern worldwide. The parasite invades and lyses erythrocytes, leading to haemolytic anaemia. Because of its nonspecific clinical presentation, diagnosis remains challenging [1]. The aim of this study to characterise the most common clinical and haematological findings in dogs naturally infected with *B. canis*.

Forty-seven dogs with babesiosis confirmed by blood smear microscopy were retrospectively analysed. Clinical signs, haematological, and biochemical parameters were evaluated.

The predominant clinical signs included lethargy (100%), anorexia (87.2%), fever (51.1%), pale mucous membranes (42.6%), vomiting (34%), and diarrhoea (17%). Thrombocytopenia was the most frequent haematological abnormality, observed in 97.9% of dogs (mean platelet count 45.3 ± 50.5 K/μL). Lymphopenia (68.1%) and anaemia (66%) were also common. Decreases in erythrocyte count, haematocrit, and haemoglobin, together with elevations in creatinine and blood urea nitrogen (BUN), were significantly associated with poor outcomes ($p < 0.05$). More severe clinical signs were positively correlated with an increased likelihood of unfavourable prognosis ($p < 0.05$). Mortality was 2.1%, while 12.8% of dogs were euthanised.

B. canis infection in dogs is most often characterised by lethargy, anorexia, and fever, accompanied by thrombocytopenia, anaemia, and lymphopenia. Elevated renal parameters are linked to more severe disease and poorer prognosis.

Keywords: babesiosis, clinical signs, thrombocytopenia, anaemia, dog

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DETECTION OF TICK-BORNE PATHOGENS IN MUSTELIDAE FROM LITHUANIA AND LATVIA

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Wild mustelids may play an important role in the maintenance and transmission of tick-borne pathogens; however, data on their role as reservoir hosts in the Baltic region remain scarce. The aim of this study was to investigate the presence of *Babesia*, *Rickettsia*, and *Bartonella* spp. in different mustelid species from Lithuania and Latvia. During 2023–2025, tissue samples were collected from free-ranging mustelids, including European pine martens (*Martes martes*), stone martens (*Martes foina*), American mink (*Neovison vison*), and European polecats (*Mustela putorius*). A total of ~110 organ samples (spleen, lungs, liver, kidneys, and muscle) were tested using real-time PCR assays targeting specific gene loci. The results revealed that *Babesia* DNA was detected in one pine marten (Latvia, lungs), *Rickettsia* spp. DNA in three pine martens (two from Latvia, lungs; one from Lithuania, spleen), and *Bartonella* spp. DNA in two pine martens (one from each country, spleen). All other tested samples from martens, mink, and polecats were negative. This study demonstrates that pine martens may serve as occasional hosts for *Babesia*, *Rickettsia*, and *Bartonella* spp., while no evidence of infection was found in other mustelid species examined. These findings provide new insights into the epidemiology of tick-borne pathogens in mustelids from the Baltic region and highlight the need for further research.

Keywords: mustelids, pine marten, *Babesia*, *Rickettsia*, *Bartonella*, Baltic region

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OCCURRENCE OF TICK-BORNE PATHOGENS IN EUROPEAN BADGERS FROM LITHUANIA

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European badgers (*Meles meles*) are opportunistic carnivores widely distributed across Europe and are often exposed to a broad range of pathogens due to their omnivorous diet and ecological adaptability. As members of the Mustelidae family, they can harbour and potentially transmit several vector-borne pathogens of both veterinary and public health importance. However, their role as reservoir hosts in the Baltic region has been poorly studied. The aim of this study was to investigate the occurrence of different tick-borne pathogens in European badgers from Lithuania. Specimens were collected from three regions of Lithuania (Radviliškis, Plungė, and Šilutė districts). Tissue samples were examined for the presence of selected pathogens using molecular methods. The results showed that 28.5% of individuals were infected with *Anaplasma* sp., while 71.4% were positive for *Babesia* spp. In contrast, *Bartonella*, *Rickettsia*, and *Borrelia* spp. were not detected in any of the tested samples. Phylogenetic analysis revealed that the *Anaplasma* sequences clustered with *Anaplasma phagocytophilum*, whereas *Babesia* sequences grouped with known species previously described in badgers. This is the first report providing molecular evidence of a high prevalence of *Anaplasma* and *Babesia* spp. in Lithuanian badgers. These findings highlight the potential role of badgers in the epidemiology of tick-borne pathogens and emphasise the need for further studies to explore their contribution to the circulation of vector-borne agents in the Baltic region.

Keywords: European badger, *Anaplasma phagocytophilum*, *Babesia* spp., Lithuania, vector-borne pathogens

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FROM TICKS TO NANODOTS: CARBON QUANTUM DOTS AS A NOVEL STRATEGY AGAINST *RICKETTSIA*

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Ticks and tick-borne pathogens represent a significant global challenge in both human and veterinary medicine. *Rickettsia helvetica*, *R. monacensis*, *R. slovaca*, and *R. conorii* subsp. *raoultii* are among the most common tick-borne pathogens transmitted by *Ixodes ricinus*, *Dermacentor reticulatus*, and *D. marginatus* ticks, not only in Slovakia but across Europe (Sekeyová et al., 2013). These organisms are recognised as etiological agents of spotted fever group rickettsioses. Humans serve as incidental hosts for ticks. Although rickettsioses are generally responsive to antibiotic treatment, antimicrobial resistance in rickettsiae has been reported.

Nanoparticles represent a novel approach to combating bacterial infections, offering a promising strategy to address antibiotic resistance. Their antibacterial activity is mediated through multiple mechanisms, including the induction of oxidative stress, release of metal ions, disruption of membrane integrity, interference with nucleic acids and protein structures, and modulation of host immune responses (Gatoo et al., 2014; Morris et al., 2019).

Molecular analysis of ticks collected from humans during 2023–2024 revealed the presence of rickettsiae in 24.55% of specimens. Serological screening detected IgG antibodies against rickettsiae in 3.70% and IgM in 3.95% of human samples. Investigation of the anti-rickettsial activity of hydrophilic and hydrophobic carbon quantum dots demonstrated efficacy ranging from 40.36% to 99.33% against *R. slovaca* and *R. conorii* infections. Cell viability assays confirmed no cytotoxic effects of the photosensitisers on the Vero cell line. No cytopathic effect attributable to rickettsial infection was observed in any treatment group, in contrast to the infected controls.

Owing to their small size, tunable fluorescence, high photostability, biocompatibility, and low toxicity, carbon quantum dots are widely recognised as a distinct class of nanoparticles with applications in bio-imaging, biosensing, drug delivery, and photodynamic therapy (Kováčová et al., 2020). These findings indicate that nanoparticles hold promise as prophylactic agents against rickettsial infections, particularly in the early stages of tick-borne disease.

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Keywords: ticks, *Rickettsia*, rickettsioses, photodynamic therapy, carbon quantum dots

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DIVERSITY AND DISTRIBUTION OF TICKS COLLECTED FROM VEGETATION AND DOMESTIC ANIMALS IN ZAMBIA

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Ticks are major ectoparasites of livestock and wildlife in sub-Saharan Africa, where they play an important role in the transmission of pathogens affecting animal and human health. With its diverse ecosystems and large cattle population, Zambia is a hotspot for tick diversity, yet systematic surveys are scarce. Previous studies in Southern Africa have reported high prevalence of *Rhipicephalus (Boophilus) microplus*, *Amblyomma variegatum*, and *Rhipicephalus appendiculatus*, species responsible for transmitting pathogens such as *Babesia*, *Theileria* and *Anaplasma* spp. The aim of this study was to assess the tick fauna of selected regions of Zambia, focusing on species composition in different habitats and host associations.

During a field expedition in February 2025, ticks were collected from vegetation (dragging method) and from domestic animals (mainly cattle and dogs) across different habitats in Southern and Eastern Zambia, including farms, pastures, natural reserves and national parks. A total of 2200 ticks were collected and morphologically identified to the species level using established taxonomic keys and molecular methods for confirmation.

Ticks from three genera were identified: *Rhipicephalus*, *Amblyomma*, and *Haemaphysalis*. In total, 11 species were recorded, with *Rhipicephalus microplus*, *R. appendiculatus*, *R. sculptus*, and *Amblyomma variegatum* being the most prevalent. There were marked differences in tick diversity in different localisations and between ticks from hosts and vegetation.

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