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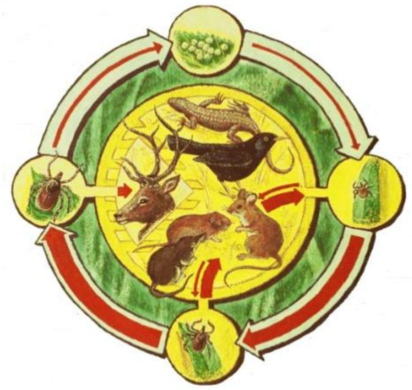

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PLENARY PRESENTATIONS

EVOLUTION OF TICKS AS REFLECTED BY AMBER ARTEFACTS

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Ticks are the oldest known parasites and exhibit the broadest spectrum of transmitted pathogens. The evolution of ticks is only partly known. Most of our knowledge about the oldest ticks is based on some specimens discovered in the Burmese amber of Myanmar. New fossil ticks (Arachnida: Parasitiformes: Ixodida) have recently been described from mid-Cretaceous (ca. 100 Ma) deposits. *Ixodes antiquorum* is the oldest representative of the most species-rich extant tick genus *Ixodes*. This extinct species still appears to be related to modern extant Australian *Ixodes* ticks. More remarkable is *Khimaira fossus* which combines a body resembling that of a soft tick (Argasidae) with a basis capitulum more like that of a hard tick (Ixodidae). We referred it to a new family, Khimairidae, as a possible transitional form between the two main families of ticks alive today. The extinct species *Cornupalpatum burmanicum* is associated with a dinosaur feather barb. Two nymphs of *Bothriocroton muelleri* represent the oldest fossil records of this genus, living members of which are still restricted to Australia. A female *Archaeocroton kaufmani* shares its basis capitulum shape with the tuatara tick *Archaeocroton sphenodonti*, the only extant member of this genus and an endemic species for New Zealand. The Deinocrotonidae has been found in Burmese amber from Myanmar and Iberian amber from Spain, suggesting a wider distribution of the lineage composed of Deinocrotonidae and Nuttalliellidae. Recent studies describe eight fossils from mid-Cretaceous Burmese amber: two *Deinocroton* species, five *Nuttalliella* species, and a new genus and species. These data show that *Deinocroton* of the former family Deinocrotonidae was considered an extinct family, but forms part of the extict family Nuttalliellidae comprising now three genera, *Deinocroton*, *Nuttalliella*, and a new genus, *Legionaris*.

The occurrence of these ticks with Australasian affinities strongly supports the current geological hypothesis that the Burma terrane rifted from Australia and moved across the ocean before it accreted to Asia. This would imply that extension all lineages postulated to have diversified prior to the origin of these genera (include the prostriates, *Ixodes*, basal metastriates, the soft ticks and the Nuttalliellidae) would be older than 150 million years. Finding of *Nuttalliella* in Burmese amber suggest that *Nuttalliella* had a much wider distribution than its current limited distribution. The distribution of *Nuttalliella* likely stretched from Africa over Antarctica and much of Australia, suggesting that extant members of this family may still be found in Australia. Our data strongly support previous suggestions that the origin of ticks is probably located in Gondwanaland and is older than 150 Ma. Considerations for the geographic origin of tick in the Afrotropic origin are becoming less probable, but are still in discussion and may be excluded when analyzing new artifacts of ticks in amber.

PAVLOVSKY’S CONCEPT OF NATURAL FOCALITY AND TRANSMISSION OF TBE VIRUS REVISITED

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Tick-borne encephalitis (TBE) is the most important tick-borne viral disease in Europe and Asia. The TBE virus is circulating in a natural transmission cycle between ticks (vectors) and certain small mammals (reservoir hosts). Historically, the transmission of an arbovirus is via the bite of the vector and the uptake of the pathogen by feeding vector ticks via viremic blood. Although this fundamental knowledge is well accepted for TBE virus, many details of the transmission cycle in tick-borne viruses are still unclear. The fact that only a small portion of ticks in a natural focus is infected and only a low percentage of small mammals is found to be infected with the TBE virus caused doubts about the long-existing ideas on the importance of viremic transmission in analogy to mosquito-borne arboviruses.

These doubts were strengthened when in the late 1980s and 1990s non-viremic transmission of tick-borne viruses, among them also TBE virus, via co-feeding of ticks was detected. Accepting this new mode of transmission, there was common agreement among many tick virologists that this may explain how low tick infection rates may maintain the virus transmission cycle. However, so far, no field evidence on the importance of non-viremic transmission by co-feeding ticks has been detected. All available direct evidence resulted from laboratory experiments or from indirect evidence in the field.

In a recent study over 5 years a TBE virus natural focus was studied and the virus prevalence rates in ticks and infection rates in small mammals were monitored. The data do not show any evidence for the importance of co-feeding and/or non-viremic transmission. The results show a constant infection rate of 0.5 to 5% in nymphs and adult *Ixodes ricinus* and a constant seroprevalence in bank voles of about 20% over the whole study period. However, we detected some unusual features in infected bank voles which, together with some historical data about the distribution of infected ticks in the field, caused us to postulate a new mode of transmission cycle for TBE virus in nature. Applying this new mode of transmission, many of the so far unexplained features of transmission of TBE virus in the field may now become clear. It has to be seen whether this new concept may also apply to other tick-borne viruses.

INTRINSIC POPULATIONS OF MICROBIOME AND THEIR ADAPTATION THROUGH MOSQUITO LIFE CYCLES

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Paratransgenic control of mosquitoes aims at introducing effector genes carried by mosquito-associated microbes into mosquito populations that affect the pathogen transmission. Although in several genera, such as *Asaia* and *Serratia*, effector microbes and their genetic pathways have been identified, little is known about what microbes can colonize mosquitoes stably and their mechanisms. Therefore, we compared the microbiome of different mosquito life stages reared under different conditions (caught mosquito's, reared in field water, reared in sterilized water) by metagenomics. We analyzed both based on reads to get the diversity data and binning for functions for different species' genomes. We identified 42 core bacterial species that are more abundant in all mosquito stages than in the water in all three rearing conditions. The 14 qualified core bacteria species genomes showed their Horizontal Gene Transfer (HGT) genes were most functional in environmental sensing and bacteria recognition pathways. There were only 2 fungal core species. All Fungi species could be detected both in the water and mosquito samples while only a few common bacterial species could be detected. The results suggest only a few bacteria may colonize mosquitoes, especially for those that interact well with other bacteria. This work is the first step in understanding mosquito symbionts, allowing us to choose mosquito paratransgenetic control tools. The identified core bacterial species and their genomes lay the foundation for further research in this field.

ORAL PRESENTATIONS

FIELD INVESTIGATIONS ON THE DEVELOPMENTAL DIAPAUSE OF ENGORGED LARVAL AND NYMPHAL *IXODES RICINUS*: COMPARING NEW AND OLD FINDINGS

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Immature *Ixodes ricinus* feed and detach over the whole growing season in Central Europe. Engorged individuals then develop to the next life stage. They go through a complex physiological process called moult during which they are immobile and not able to overwinter. Engorged immatures detached in spring or early summer moult in the same year before the following winter, yet, those detached in late summer or autumn first enter a developmental diapause, overwinter, and moult only in the next summer. Aim of the present study was (i) to determine the seasonal time of the switch from direct development to diapause and (ii) to find out whether this seasonal switch changed from 1986 to 2018, i.e. over a span of 32 years with gradually increasing temperatures. These results might help assess whether the duration of the life cycle of *I. ricinus* will distinctly change in Central Europe in the coming decades under the influence of probably further increasing temperatures. Field-collected larval and nymphal *I. ricinus* were allowed to feed to repletion on Mongolian gerbils at different times of the year (1986, 1987, 2013, and 2018). The freshly engorged and detached ticks were then placed into field plots in the forest leaf litter, where they were checked regularly for their development to the following life stage. The switch from direct development to diapause in engorged nymphs occurred in July/early August, always 2–3 weeks earlier than in the engorged larvae. In 2013 and 2018, the switch in both stages occurred 2 weeks later than in 1986 and 1987, seemingly a clear and simple result, but which needs careful biological interpretation.

EXPERIENCES FROM SEVEN YEARS OF TICK-BORNE ENCEPHALITIS INCIDENCE FORECASTS IN CENTRAL EUROPE

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In the Greater Alpine Region (GAR, which here includes Austria, Germany and Switzerland), the number of human tick-borne encephalitis (TBE) cases increased by 1,100 within the period 1991-2024. The highest number was recorded in the COVID-19 year 2020 with 1,367 notified TBE cases, although the numbers can fluctuate by up to 100% from year to year. The analysis of the 40-year TBE time series in Austria showed that these fluctuations of TBE cases are by no means random (Rubel et al., 2020). Accordingly, the 12-year cycle (maxima in 1994, 2006 and 2018) can be explained by the large-scale atmospheric circulation, which is represented by the teleconnection index SCAND (Scandinavian Index). For the TBE endemic areas in GAR high SCAND values indicate cooler and wetter periods. Low SCAND values, on the other hand, describe above-average warm and drier periods. Annual TBE fluctuations can be explained by the sequence of mast years, i.e. the extent of fructification of forest trees, which drive the natural TBE transmission cycle between small mammals and ticks. In particular, the TBE incidence correlates strongly with beech mast two years ago. This allows a TBE forecast to be made for the next two years by applying a series of generalized linear models (Rubel & Brugger, 2020). For the first time, the performance of these forecasts for Austria, Germany, and Switzerland, which has been operational for seven years (Rubel & Brugger, 2021), is determined by verifying the forecasts with official TBE case numbers. At the conference, initial results from the forecast extension to TBE endemic areas in Northern Italy and Eastern France will be presented. Finally, the increasing number of TBE cases due to climate change (more frequent mast years), climate adaptation (forest transformation), and changing recreational behaviour (during COVID-19) will be discussed.

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SEASONAL ACTIVITY OF TWO EPIDEMIOLOGICALLY IMPORTANT TICKS IN SLOVAKIA, *IXODES RICINUS* AND *DERMACENTOR RETICULATUS* AND THEIR INFECTION WITH TICK-BORNE AGENTS

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Ixodes ricinus and *Dermacentor reticulatus* ticks were monitored during 2023-2025 in Bratislava region. The seasonal activity and abundance of ticks and their infection with selected pathogens were investigated. Questing ticks (n = 1594) were collected by flagging the vegetation at three study sites. All samples were screened for *Borrelia burgdorferi* sensu lato (19.2%), *Anaplasma phagocytophilum* (2.1%), *Babesia* spp. (3.5%) by molecular methods. Tick gardens (TGs) were placed in different habitats. Questing activity of *I. ricinus* ticks was monitored in four TGs, *D. reticulatus* in two TGs. The different seasonal activity among monitored tick groups in TGs was recorded. Winter activity of ticks was confirmed. Host-feeding ticks (n = 1865) were obtained from 44 fallow deers, 12 roe deers, 2 wild boars and 1 mouflon. Ticks were recorded in 88% of the wild animals. Animals were infested with three tick species (*I. ricinus*, *D. reticulatus*, *Haemaphysalis concinna*) with the predominance of *I. ricinus* (96%). In the context of global changes we are monitoring the spread of non-native tick species to Slovakia. In July 2022, one questing *Hyalomma marginatum* female, climbing cloths, was collected in Púchov. One semi-engorged nymph of *Ixodes frontalis* was collected from vegetation in the Botanical Garden in February 2024.

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TICK-BORNE PATHOGENS IN DIVERSE URBAN HABITATS OF CENTRAL LITHUANIA: ASSESSING RISK OF TICK-BORNE DISEASES IN POPULAR RECREATIONAL AREAS

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Urban green spaces offer well-known benefits, but there is growing evidence pointing to potential health risks, particularly due to increased exposure to vector-borne diseases. Lithuania is a highly endemic area for Lyme borreliosis and tick-borne encephalitis. The *Ixodes ricinus* tick, which is the primary vector for medically and veterinary significant pathogens, is widespread throughout the country. Traditionally, the risk of infected tick bites has been associated with natural habitats, such as deciduous or mixed forests and meadows. Urbanization, population growth, changes in human behaviour such as encroachment into natural habitats, climate change, environmental shifts, as well as increasing wildlife populations in urban and peri-urban areas have increased the risk for various vector-borne diseases affecting public health. However, there is a lack of data on the prevalence of tick-borne pathogens (TBPs) in urban areas of Lithuania. This study aimed to investigate the prevalence of TBPs in ticks collected from recreational, leisure, and frequently visited memorial sites in urban and peri-urban areas of Kaunas County, central Lithuania. A total of 1328 *I. ricinus* ticks were collected by flagging in eight urban green areas, which included a botanical garden, city parks, urban forest parks, a national regional park, and a cemetery, during the spring of 2022-2024. Each tick was examined individually for the presence of *Borrelia* spp., *Anaplasma phagocytophilum*, *Ca*. Neorlichia mikurensis, *Rickettsia* spp. and *Babesia* spp. using real-time PCR assays targeting the *23S rRNA*, *msp2*, *groEL*, *gltA*, and *18S rRNA* genes. Positive samples were further analyzed using conventional and nested PCRs and sequencing of different targets of the bacteria genomes. Of all the ticks analyzed, 41.6% tested positive for at least one species of tick-borne pathogen. Between sites, the prevalence of *Borrelia* spp. varied from 13.4% to 38.9%, *A. phagocytophilum* from 0.8% to 7.1%, *Ca*. Neorlichia mikurensis from 1.7% to 16.2%, *Rickettsia* spp. from 1.2% to 4.0% and *Babesia* spp. from 1.0% to 5.6%. All tested pathogens were found across the tick collection sites except at one location. The highest prevalences of pathogens were detected in the green area of the cemetery and Nemunas Loops Regional Park. Double and triple co-infections with different pathogens were detected. The present study highlights that urban and peri-urban green areas can serve as important habitats for ticks carrying multiple medically important TBPs and demonstrate the potential public health risks associated with human activities in popular recreational and often-visited memorial sites in Kaunas County.

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MONITORING OF *RICKETTSIA* SPP. IN TICKS IN TWO ZOOS IN THE CZECH REPUBLIC

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Zoos are ideal environments for ticks and for the spread of vector-borne diseases, as many animal species are found in a relatively small space with many natural habitats. The aim of the study was to detect *Rickettsia* spp. in ticks from the zoological gardens in the Czech Republic.

A total number of 1612 *Ixodes ricinus* ticks (1062 nymphs and 550 adults) were collected by flagging in areas of 2 zoos (1239 in Zoo Ostrava and 373 in Zoo Brno) during years 2023 and 2024. From these ticks, 904 samples were created (691 from Zoo Ostrava, and 213 from Zoo Brno), with adults analyzed individually and nymphs in groups of three.

The overall positivity rate (MIR) of *Rickettsia* spp. was 11% (173/1612), without difference in zoos, because the same positivity was obtained in Zoo Ostrava (11%, 133/1239) and Zoo Brno (11%, 40/373). The positivity of ticks was elevated in year 2024 comparing the previous year: MIR in Zoo Ostrava was 10.5% (67/641) in 2023 and 11% (66/598) in 2024; in Zoo Brno it was 6.1% (8/131) in 2023 and 13.2% (32/242) in 2024. Tick positivity was influenced by season with the highest positivity (MIR) in spring (13% in Ostrava) or summer (19% in Brno). Other factors (localities, and sex of stage of ticks) did not influence tick´s positivity to *Rickettsia* spp.

This is the first study focusing on detection of *Rickettsia* spp. in ticks collected in area of zoos in the Czech Republic.

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VECTORS AND RESERVOIRS OF *RICKETTSIA* SPP. IN BALTIC REGION

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Rickettsiae are intracellular bacteria with complex life cycles and are transmitted to vertebrate hosts through arthropod vectors such as ticks, fleas, and lice. Some species can pose public health threats in many countries worldwide. Spotted fever group (SFG) rickettsiae have previously been reported in questing Ixodidae ticks from Lithuania and Latvia: *R. helvetica* has been identified in *Ixodes ricinus* and *R. raoultii* in *Dermacentor reticulatus*. In Latvia, both *R. helvetica* and *R. raoultii* were detected in host-seeking *Ixodes persulcatus, I. ricinus* and *D. reticulatus*, while *R. monacensis* was detected only in *I. ricinus* (Capligina et al. 2020). Three *Rickettsia* species – *R. helvetica*, *R. monacensis* and *Candidatus* R. tarasevichiae – were detected in *Ixodes* tick populations from Estonia (Katargina et al. 2015).

Human-pathogenic *R.helvetica*, *R. felis*, *R. monacensis* were identified in four species of fleas (*Ctenophthalmus agyrtes*, *Hystrichopsylla talpae*, *Megabothris turbidus*, *M. walkeri*), while *R. helvetica*, *R. felis* were identified in four species of Laelapidae mites (*Laelaps agilis*; *Hyperlaelaps microti*; *Myonyssus gigas*; *Eulaelaps stabularis*) collected from rodents in Lithuania. Only *R. helvetica* was identified in *I. ricinus* (Radzijevskaja et al. 2018). *R. helvetica*, with high prevalence, was detected in *Micromys minutus*, *Apodemus flavicollis*, *Clethrionomys glareolus,* suggesting that these rodents may play an important role as potential reservoir hosts and contribute to the maintenance of this pathogen in nature (Mardosaitė-Busaitienė D et al. 2018). In rodent-attached ticks from Estonia *R. helvetica* and *Candidatus* R. uralica were detected (Vikentjeva et al. 2021).

In Lithuania, human pathogenic *R. helvetica*, *R. conorii* subsp. *raoultii* and *R. felis*, detected in domestic cats, and their ectoparasites: *Ctenocephalides felis* and *Ct. canis* fleas, as well as *D. reticulatus* and *I. ricinus* ticks. Three species – *R. helvetica*, *R. monacensis, R. raoultii* – were also detected in *I. ricinus*, *I. persulcatus* and *D. reticulatus* ticks collected from dogs in Latvia (Namina et al. 2011).

The SFG rickettsial pathogen *R. helvetica* and *R. monacensis* were detected in *I. ricinus* ticks removed from migrating birds. Furthermore, a newly identified species, *R. vini*, was confirmed in Lithuania in *I. lividus* ticks from *Riparia riparia* nests (Matulaitytė et al. 2020). *R. helvetica* was also detected in *I. ricinus* ticks collected from migrating birds in Latvia (Capligina et al. 2014).

Further studies are necessary to understand the epidemiology of Rickettsiae and their potential links to human and animal infections.

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TICK-BORNE ENCEPHALITIS VIRUS IN RODENTS AND TICKS FROM ENDEMIC REGIONS OF LITHUANIA

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Tick-borne encephalitis virus (TBEV) is the most prevalent in Lithuania among European countries, with incidence rates continuing to rise and remaining the highest in Europe. Rodents play a crucial role in TBEV transmission as reservoir hosts and are the main hosts of larval and nymphal ticks. Ticks act as both reservoirs and vectors of TBEV. In this study, a total of 8,846 questing ticks were collected, including 7,170 *Ixodes ricinus* (2,754 adults, 4,036 nymphs, and 380 larvae) and 1,676 adults *Dermacentor reticulatus* (Sidorenko et al. 2021). In addition,139 rodents representing six species (*Apodemus flavicollis*, *Clethrionomys glareolus*, *Microtus arvalis*, *Mus musculus*, *Apodemus sylvaticus*, and *Apodemus agrarius*) were trapped at various locations across Lithuania (Simkute et al. 2024). Ticks and rodents were analysed for TBEV using real-time PCR. Genotypic characterisation of the detected viruses was performed based on the sequence of partial E protein and NS3 genes. TBEV was detected in 0.4% of *I. ricinus* (across all three developmental stages) and 0.4% of *D. reticulatus*. The highest infection rate was observed in adult *I. ricinus* (0.6%) compared to nymphs (0.3%) and larvae (0.3%) (Sidorenko et al. 2021). TBEV RNA was identified in 74.8% (104) of rodents, and 100% of *M. arvalis* specimens were infected (Simkute et al. 2024). TBEV isolates revealed that they were specific to Lithuania, and all belonged to the European subtype. The analysis showed that certain TBEV strains were region-specific and exhibited significant regional genetic diversity. The detected genotypes were not associated with specific tick species and were unevenly distributed within the tick populations. At least six distinct virus lineages were found circulating in ticks, and two in rodents (Sidorenko et al. 2024). Further whole-genome sequencing studies are needed to better understand the regional genetic diversity of TBEV strains.

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CURRENT STATE OF KNOWLEDGE ON THE EPIDEMIOLOGICAL SIGNIFICANCE OF THE MOUND- BUILDING MOUSE (*MUS SPICILEGUS*, RODENTIA) IN SLOVAKIA – OVERVIEW OF RESULTS

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This rodent species, with a significant occurrence in the Mediterranean region, reaches its northern limit of distribution in southern Slovakia. The results of many years of research (2002 - 2015) are summarized, while were obtained in cooperation with several scientific institutions in Slovakia and abroad. Large samples of parasites, sera and host tissues were gradually processed and continuously published at several conferences and in several papers. Theriological and parasitological research of *M. spicilegus* was carried out at 16 localities in four orographic units of southern Slovakia - from Košická kotlina basin, the Východoslovenská rovina plain, Ipeľská and Hronská pahorkatina upland. A total of 391 individuals of *M. spicilegus* were captured and parasitologically examined. In addition, a total of 137 *M. spicilegus* nests were excavated and examined for several groups of arthropods. Low intensity of mound-building mice parasitation was confirmed. Examination of the mice yielded 417 mites (Mesostigmata) of nine species (the most abundant *Laelaps algericus*), 40 fleas (Siphonaptera) belonging to four species (the most abundant *Ctenophthalmus assimilis*), as well as 24 specimen of lice from three species (the most abundant *Hoplopleura captiosa*). No ticks were obtained from hosts. In the nests we found 3,760 fleas of nine species, more than 67 thousand individuals of mesostigmatic mites belonging to 79 species, 36 specimens of two tick species (Ixodida), from which *Ixodes acuminatus* represent new species for fauna in Slovakia. Information is summarized on what species of beetles, isopods, millipedes, and centipedes were recorded in the nests. A significant part of the hosts was examined serologically. Antibodies against *Leptospira* sp. (P = 12.3 %), against *Toxocara* sp (P = 11.0 %) were confirmed. Antibodies against *Trichinella* sp were confirmed in four samples. A total of 134 individuals of *M. spicilegus* were examined for the presence of gastrointestinal helminths. There were two nematode species and one cestode species were found in approximately one-third of the examined mice. Coprological examination of 48 mice confirmed positivity on three helmints (eggs and tetrathyridium). Comprehensive, long-term research has confirmed the significant epidemiological role of *Mus spicilegus* in the agricultural landscape.

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TICKBORNE OR NOT? EXPLORING THE ROLE OF TICKS IN NEGLECTED ZOONOTIC BACTERIAL TRANSMISSION

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Francisella tularensis, *Coxiella burnetii*, and *Brucella suis* are important but often neglected zoonotic bacteria (Michelet et al. 2016). Although transmission typically occurs via aerosols, ticks are also considered to be vectors of these pathogens. Detection is further complicated by the presence of non-pathogenic endosymbionts, such as *Francisella*-like endosymbionts (FLE), which can interfere with molecular diagnostics (Sun et al. 2000). To complement the surveillance of less frequently studied zoonotic bacteria, *Borrelia burgdorferi* s.l. was included as a well-established tick-borne pathogen, enabling a comparison between infection prevalence in ticks and seroprevalence in wildlife.

In this study, samples were collected from 16 sites across the Czech Republic. A total of 437 hare sera, 106 wild boar sera, 554 wild boar spleens, and 1,284 questing ticks were analyzed. Wildlife samples originated from 12 hare sites and 4 wild boar sites. Ticks were collected by flagging at four of these locations (two hare and two boar sites). All sera were tested for antibodies against *F. tularensis*, *B. suis*, and *C. burnetii* using ELISA. Spleens were screened by PCR: *B. suis* was detected by Bruce-ladder multiplex PCR; *F. tularensis*, FLE and *C. burnetii* were targeted by multiplex qPCR (targeted genes TUL4, fopA, ICD); and *B. burgdorferi* s.l. was detected in ticks using singleplex qPCR (targeted gene OspA). To minimize the risk of false-positive results caused by surface bacteria, all ticks were washed in 10% (NaOCl) solution prior to homogenization. Ticks were pooled by 8 for multiplex qPCR.

Serological screening revealed substantial exposure of wildlife to zoonotic bacteria: 23% of hares were seropositive for *F. tularensis*, 8% for *B. suis*, and none for *C. burnetii*. Among wild boars, 7% were seropositive for *F. tularensis*, 17% for *B. suis*, and 0% for *C. burnetii*. PCR analysis of 554 wild boar spleens revealed a single positive individual for *B. suis* and one for *F. tularensis*. *C. burnetii* DNA was not detected in any spleen samples. In addition, *B. burgdorferi* s.l. DNA was identified in 3.36% of wild boar spleens (11/554), suggesting occasional systemic infection in this host.

None of the 1,284 tested questing ticks were positive for *B. suis*, *F. tularensis*, FLE, or *C. burnetii*. In contrast, *B. burgdorferi* s.l. was frequently detected, with a mean prevalence of 23%, ranging from 5% to 40% across sites. Collected tick genera included *Ixodes*, *Dermacentor*, and *Haemaphysalis*.

The results suggest low active infection rates but a notable level of prior exposure to *F. tularensis* and *B. suis* in wild mammals. While ticks were not carriers of the more neglected pathogens, they remain important vectors of *Borrelia*. The study highlights the need for combined serological and molecular approaches to fully understand pathogen circulation in natural ecosystems and assess potential zoonotic risks.

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VECTOR-BORNE BLOOD PATHOGENS IN REPTILES FROM SOUTH CAROLINA, USA

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Reptiles represent a diverse group of animals that have successfully adapted to different types of environments throughout their evolutionary history. In parallel, various groups of ectoparasites have also evolved, some of which can act as potential vectors of a wide range of pathogens. These pathogens include intracellular and extracellular blood parasites, mainly from the class Kinetoplastida, phylum Apicomplexa, microfilariae, as well as viruses and bacteria. The aim of this study was to identify the spectrum of vector-borne pathogens in various reptile species from different sites in South Carolina (USA). The research focused mainly on determining the prevalence and parasitemia rates of parasites from the phylum Apicomplexa depending on host species and locality. A total of 305 individuals of various reptile species were captured and subsequently examined both microscopically and molecularly for the detection of intracellular and extracellular blood parasites. 279 individuals were subjected to microscopic examination, while 256 individuals were analyzed molecularly. Microscopically, the presence of parasites from the Apicomplexa phylum was confirmed in 21.5 % of the individuals examined. Molecular examinations confirmed the presence of parasites in 29.3 % of all individuals examined. Within the Serpentes group, 37.8 % were positive, compared to 2.3 % in Sauria and 40% in Testudines. Statistically significant differences in parasitemia were recorded between individual localities and between host species, with the highest parasitemia in *Nerodia fasciata* (3504.85) and the lowest in *Coluber constrictor* (3.21). Using NGS method, several tick-borne bacteria, including *Rickettsia, Neoehrlichia, Borrelia*, and *Candidatus* Midichloria, were detected in *Anolis carolinensis* and *Eumeces laticeps*. By comparing the 18S rDNA sequences with the corresponding GenBank sequences, *Hepatozoon* species identified in reptiles showed a close similarity to *Hepatozoon ophisauri*, *Hepatozoon cyclemydis*, *Hepatozoon cuestensis*, *Hepatozoon* cf. *sipedon*. *Haemogregarina* species identified in our samples showed similarity with *Haemogregarina daviesensis.* These results contribute to a better understanding of the occurrence of pathogens in reptile populations in the South Carolina region and highlight the potential importance of reptiles as reservoirs of zoonotic pathogens. Understanding the interactions between vectors, pathogens, and hosts is crucial for understanding the epidemiology and prevention of reptile-associated diseases.

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„KLÍŠŤAPKA“: A MOBILE APPLICATION FOR PREDICTING TICK ACTIVITY, INFECTION RISK AND TICK REPORTING BY THE PUBLIC

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Tick-borne diseases are the most prevalent vector-borne illnesses in Central Europe, with the Czech Republic reporting some of the highest rates of Lyme neuroborreliosis (LNB) across the continent. The primary vector, *Ixodes ricinus*, is traditionally associated with forested habitats rich in shrub and herbaceous vegetation, yet it is increasingly encountered in urban areas—where public awareness and preventive behavior are typically limited.

Our long-term objective is to develop *Klistapka*, a mobile application designed to predict tick activity and infection risk using geo-referenced data. This data originates from an extensive national surveillance program that monitors tick abundance and pathogen prevalence in both urban and forest ecosystems across the Czech Republic. Since 2014, we have collected and analyzed 4,845 ticks from 12 forest parks in Prague. In 2023, our monitoring efforts expanded to 12 regional cities (6,620 ticks) and 30 smaller towns (4,032 ticks). Additionally, 60,416 ticks have been sampled from 150 forested sites nationwide since 2021.

Preliminary analyses reveal a higher tick density in urban green spaces of regional cities (19.0 ticks/100 m^2) compared to forested areas (16.2 ticks/100 m^2). More strikingly, urban ticks exhibit significantly higher infection rates. *Borrelia burgdorferi* sensu lato was detected in 25.8% of urban ticks, along with *Borrelia miyamotoi* (2.4%), *Anaplasma phagocytophilum* (8.5%), *Neoehrlichia mikurensis* (2.0%), and *Rickettsia* spp. (7.0%). In contrast, forest ticks showed lower infection rates for *B. burgdorferi* s.l. (10.4%) and *A. phagocytophilum* (1.8%).

Klistapka integrates these surveillance data with real-time weather forecasts and seasonal patterns to model and predict tick activity and infection risk—especially for *B. burgdorferi* s.l., which demonstrates increased prevalence in urban areas. Set for release in April 2025 on Android and iOS platforms, the app also facilitates citizen science. Users can report tick encounters, upload tick photographs, and contribute to a growing, crowdsourced tick database. Submitted data are continually updated and visualized within the app.

In addition to real-time risk mapping, *Klistapka* offers educational resources on tick prevention, safe removal practices, and symptoms of tick-borne diseases. By combining predictive analytics with public engagement and health education, *Klistapka* serves as both an innovative public health tool and a nationwide tick surveillance platform.

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INFOTICK APP - MOBILE APPLICATION FOR TICKS MONITORING

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The increasing prevalence of tick-borne diseases mainly occurring in temperate regions has highlighted the need for effective, real-time systems for tick population monitoring, including the invasive species. Traditional methods of tick surveillance, often based on periodic field sampling and manual data reporting, face significant limitations in terms of spatial coverage, timeliness, and scalability. In response, the widespread use of smartphones and advancements in mobile technologies offer a promising mean to overcome these challenges through citizen science and automated data acquisition. Mobile applications have already proven effective in this domain. For instance, the TickPhone App (Zhiheng Xu et al., 2021) utilizes automated tick image categorization, while eTick (Khov et al., 2024) has been deployed in Canada for monitoring invasive tick species. Similar approaches have also been successful in related fields, such as mosquito monitoring, through applications like Mosquito Alert (Palmer et al., 2017) and the GLOBE Observer: Mosquito Habitat Mapper (Low et al., 2021). These tools have significantly enhanced the spatial and temporal resolution of vector data, while also promoting public engagement and awareness. Inspired by these successful implementations, our project introduces a mobile application designed for real-time collection, geotagging, and visualization of tick encounters. By leveraging smartphone sensors (GPS, camera, timestamp), intuitive user interfaces, and scalable data storage, the proposed application facilitates seamless data acquisition both by the public and researchers. Mobile application relies on the backend services for spatiotemporal data lookup and store, information flow management, real-time messaging and user account handling. These services are designed with the regard to possible scalability ensuring the system can be expanded to cover wider regions and handle real-time data processing. The content of the mobile application contains a screen for interactive vector map geolocating the reported information, a message screen containing timely restricted user reports in the nearby location, a channel screen allowing to search and manage information channels, and a user screen configuring user's preferences. Proposed mobile application allows collecting the tick information from public (also assuming the users have no knowledge about the ticks) providing tick photos, approximate location of tick occurrence, place and source of extraction; whereas it also allows to be used be the experts allowing them to information include additional information about the tick species, development stage and gender. Moreover, educational modules are planned to provide useful tips and frequently asked questions included into the information channels. This digital approach enables high-resolution mapping of tick distributions and seasonal activity patterns, empowering users with immediate feedback, such as tick species information and relevant health guidance. Additionally, aggregated data can support epidemiological research and guide public health interventions.

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THE PCR TARGETING THE LARGE SUBUNIT OF PHAGE TERMINASE (TERL) IS NOT SUITABLE FOR *BORRELIA* DIAGNOSTICS IN EUROPE

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In March 2021 a paper was published suggesting that a real-time PCR targeting the multiple copies of the large subunit of phage terminase (*terL*) located on cp32 plasmids of *Borrelia* could improve detection of spirochetes in blood (Shan et al. 2021). Though an interesting idea, a critical appraisal revealed serious methodological flaws (van de Schoor et al. 2021).

Subsequently, we have established this real-time PCR in-house and used it to re-analyse serum and tissue samples, all from patients diagnosed with Lyme borreliosis according to established criteria and with an active *Borrelia burgdorferi* s.l. infection (i.e. before start of antibiotic therapy). Furthermore, we analysed DNA from *in vitro* cultures of *Borrelia* species (n=33) and a DNA panel consisting of serial dilutions of *Borrelia* DNA from 15 species and specificity controls (n=95).

Serum samples (n=15) from patients with active disease remained negative in *terL* PCR. Out of 22 tissue samples that were positive in control PCRs (*flaB*, 5S-23S IGS), 12 (54%) remained negative (no Ct value) using the phage terminase PCR. Species determination revealed that the negative tissue samples belonged to *B. afzelii* (n=10, 45%), and/or to *B. garinii/B. bavariensis* (n=2, 9%). Notably, *B. afzelii* and *B. garinii/B. bavariensis* are the main causative agents of Lyme borreliosis in Europe. DNA analyses using cultured isolates of various species of *Borrelia* including *B. afzelii*, *B. burgdorferi* sensu stricto, *B. bavariensis*, *B. garinii*, *B. spielmanii* and *B. valaisiana* showed that some isolates of *B. garinii* and *B. afzelii* remained negative in *terL* PCR, even when as many as 10^4 DNA equivalents/PCR reaction were used and control PCRs were positive. We have shown using whole genome sequences that these samples contained cp32 plasmids. These data suggest that the PCR targeting the *terL* locus in its current form is unsuitable as diagnostic tool for *Borrelia* in Europe, especially from serum samples.

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GAPS AND CHALLENGES IN RESEARCH ON THE PHYSIOLOGY OF THE TICK MIDGUT

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Ticks are obligate hematophagous ectoparasites that have developed a highly specialized feeding strategy that allows them to maintain long attachment times while ingesting blood and transmitting a variety of pathogens. The tick midgut not only plays a central role in the extraordinary parasitic lifestyle of ticks, but also determines the vector competence of ticks.

This talk will focus on recent discoveries in the physiology of the tick midgut, both from a structural (morphological) and functional perspective. A particular focus will be on the processing of blood meal, heme/iron homeostasis and immune responses to transmitted pathogens and the midgut microbiota. Despite the great progress we have made over the past two decades in our understanding of tick midgut physiology, there are still many unknown gaps that define future perspectives and challenges for further research: (i) a better understanding of the specific role of different cell types and compartments in nutrient processing by light and electron microscopy, which has been limited by a lack of specific markers and antibodies; (ii) nutritional sensing and signaling pathways that control blood meal processing; (iii) the utilization of lipids from host blood or from long-term lipid stores; (iv) the export of heme and iron from digestive cells and their transport between tissues; (v) immune mechanisms that prevent bacterial degradation of ingested blood.

A detailed understanding of these physiological processes is essential to find new targets for disrupting tick feeding and pathogen transmission, thus contributing to the development of effective tick control strategies.

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IDENTIFICATION AND CHARACTERIZATION OF NEUROPEPTIDES AND THEIR RECEPTORS IN THE TICK *IXODES RICINUS*

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Ticks are among the most important vectors of various pathogens, including viruses, bacteria, and protozoans, which pose serious threats to human and animal health worldwide. With the increased prevalence of ticks and their spread to new areas, the incidence of tick-borne diseases is also expected to rise. Understanding the mechanisms that govern tick physiology and pathogen transmission is therefore crucial for developing new strategies to combat tick-borne diseases. Neuropeptides are bioactive substances widespread across the animal kingdom and represent one of the most diverse groups of signaling molecules in terms of structure and function. They regulate almost all aspects of animal life, including numerous physiological and behavioral processes in insects. They act as neurotransmitters or hormones and are an essential part of regulating many processes including growth, development, reproduction, metabolism, or digestion. Despite extensive research, we still have insufficient information on the function of these neuropeptides in vectors carrying pathogens such as ticks. In this work, we focus on the identification and functional analysis of selected neuropeptides and their receptors in the tick *Ixodes ricinus* using various methods such as molecular cloning, aequorin-based receptor bioassay, immunohistochemical staining, *in situ* hybridization, RT-qPCR, and RNA interference.

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ARE STRUCTURAL CELLS OF THE HUMAN SKIN KEY PLAYERS IN THE FIRST PHASE OF TICK-BORNE ENCEPHALITIS VIRUS INFECTION?

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Tick-borne encephalitis virus (TBEV), a neurotropic flavivirus transmitted by ticks, is the most prevalent arbovirus in Europe and north-eastern Asia. Although brain represents a targeting organ for TBEV, the skin is the first host tissue exposed to the virus during tick feeding. The human skin hosts a number of different cell types that play a crucial role in fending off invading pathogens, however in the context of early phase of TBEV infection their role is poorly defined. In this study, we examined the susceptibility and response of human skin structural cells, such as keratinocytes and fibroblasts, to TBEV (strain Hypr) infection *in vitro*.

Using HaCaT (keratinocytes) and BJ (fibroblasts) cell lines, we demonstrated that both cell types permit viral replication without evident cytopathic effect. Real-time PCR showed increasing amount of viral RNA copies; immunocytochemistry confirmed the presence of viral particles in infected cells, and plaque assays verified productive infection. Transmission electron microscopy (TEM) provided ultrastructural evidence of active infection, including the presence of virus-induced vesicles, membrane rearrangements, autophagy in both cell types.

To characterize the interferon-mediated response, we analysed the expression of type I and III interferons and their receptor subunits at various time points and multiplicities of infection. In HaCaT cells, TBEV infection led to the induction of IFN-β1 and IFN-λ1, - λ2/3 gene expression, with peak levels observed at 48 hours post-infection,7 followed by a decline. Interestingly, this IFN induction was accompanied by downregulation of the first subunits of their corresponding receptors, suggesting potential viral interference with interferon signalling.

In contrast, BJ fibroblasts responded to TBEV infection primarily through the induction of type I IFN-β1 and upregulation of its receptor subunit, with no detectable induction of type III IFN. This suggests a cell-type-specific divergence in IFN pathway activation, with fibroblasts relying predominantly on type I IFN responses, while keratinocytes mount a broader IFN-mediated defence involving both type I and III IFNs. These responses were influenced by the multiplicity of infection and time post-infection, with the strongest responses generally occurring at 48 h post-infection.

Our results indicate that both structural skin cells, keratinocytes and fibroblasts, play an active role in the early stages of TBEV infection by supporting viral replication and mounting a distinct IFN-mediated antiviral response, that may shape local immunity at the skin–virus interface. These findings provide new insight into the role of the skin as an immunologically active interface, highlight the importance of non-immune skin cells and underscore the need to identify key molecular players. A deeper understanding of these early cellular responses could provide insights for the development of preventive strategies.

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SEROSURVEYS OF SMALL RODENTS FOR ZOONOTIC VIRUSES IN SLOVAKIA WITH EMPHASIS ON TICK-BORNE ENCEPHALITIS VIRUS

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Wild rodents are reservoirs for a number of zoonotic pathogens, including viruses. We examined sera of live-trapped rodents for antibodies against orthohantaviruses, lymphocytic choriomeningitis virus (LCMV), cowpox virus (CPXV), Ljungan virus (LV) (only in 2012-2014) and tick-borne encephalitis virus (TBEV) with the aim to identify their presence in different habitat types (peri-urban, natural, agricultural) of Slovakia. Study sites were located in western Slovakia: the Small Carpathian Mts - Bratislava forest park (BFP) (peri-urban habitat), Fúgelka (natural habitat), the Tríbeč Mts – Topolčianky (natural habitat)) and in eastern Slovakia – Rozhanovce (game reserve, agricultural habitat). Rodents were live trapped in 2006-2007 (Topolčianky, Rozhanovce) and in 2012-2014 (BFP, Fúgelka, Rozhanovce). Sera of 1069 rodents (102 and 967 in 2006-2007 and 2012-2014, respectively) including 723 mice (553 *Apodemus flavicollis,* 2 *A. sylvaticus*, 167 *A. agrarius,* 1 *Micromys minutus*), and 346 voles (315 *Clethrionomys glareolus,* 27 *Microtus arvalis*, and 4 *Microtus subterraneus*) were examined using immunofluorescent antibody test (IFAT). Antibodies against hantaviruses were detected in 11.8% of mice and 1.8% of voles. Seropositivity to LCMV was found in 5% mice and 10.3% voles, and to CPXV in 15.4% mice and 65.1% voles. Antibodies against these viruses were present in rodents from all habitat types, whereas antibodies against LV were detected only in rodents from the Small Carpathians and against TBEV in animals from Topolčianky (*C. glareolus* – 10%) and from the Small Carpathians (*A. flavicollis* – 0.3%, *C. glareolus* – 0.5%, *M. arvalis* – 21%).

In frame of an ongoing project aimed to monitor TBEV foci in natural forests and in the vicinity of farms where alimentary infections with TBEV were registered, forest habitats at Topolčianky and Fúgelka, and sites in the districts Detva and Banská Bystrica (central Slovakia) were chosen for screening wild rodent blood for antibodies against TBEV. In autumn 2024 and spring 2025, in total 22 *A. flavicollis,* 8 *A. agrarius,* 1 *M. minutus*, and 1 *C. glareolus* were trapped at

Topolčianky, 8 *A. flavicollis*, 1 *M. arvalis* and 1 *Apodemus* sp. were trapped at Fúgelka, 5 *A. flavicollis*, 35 *A. agrarius*, 1 *M. minutus*, 1 *C. glareolus* and 5 *M. arvalis* were trapped at Detva and 8 *A. flavicollis* and 2 *C. glareolus* were trapped at Banská Bystrica. Preliminary results suggest that the long-term TBEV focus at Topolčianky is still active (weak seropositivity was detected by microneutralization assay in two *A. agrarius* specimens), although the structure of the focus may have changed due to the westward expansion of *A. agrarius*. In the other study sites, seropositivity of rodents against TBEV has not been confirmed yet.

Our results show that multiple zoonotic rodent-borne viruses are present in different habitat types of Slovakia, including peri-urban sites. Occurrence of infected rodents in areas used by people for outdoor activities poses an increased risk of spread of the diseases to humans and domestic animals.

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OPHIDASCARIASIS IN FROGS - A VETERINARY PERSPECTIVE

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Ophidascaris species are parasitic nematodes with a complex, indirect life cycle involving a reptilian final host, typically a snake, and at least one amphibian, rodent, or marsupial intermediate host (De Oliveira Simones et al. 2022). At least one species, *O. robertsi*, is additionally zoonotic (Hossain et al. 2023). These helminths primarily circulate among wild animal populations in tropical and subtropical Asia, Australia, and America. Still, globalization and human activities such as the exotic pet trade and animal collections at zoological gardens or wildlife conservation significantly increase these nematodes' veterinary relevance. All involved hosts can develop severe and sometimes fatal pathological changes because of the parasitoses. Adult *Ophidascaris* sp. typically burrow into the gastric mucosa, consequently inducing trauma, granulomatous gastritis, anorexia, and progressive debilitation in their final snake hosts (Gonzalez-Astudillo et al. 2019). In the other host species, migrating *Ophidascaris* sp. larvae primarily cause pathological changes (Hossain et al. 2023). The consequences of *Ophidascaris* sp. infections on the health status of frog species serving as intermediate or accidental intermediate hosts are still poorly understood. In the present study, over four years, 115 post metamorphic, wild-caught, and imported *Occidozyga lima* frogs were examined post-mortem for the presence of parasites. All frogs originated from the European pet trade and had naturally succumbed during quarantine before dissection, gross pathological examination, light microscopically examination of native tissue samples, and histopathology. Out of the 115 frogs, 16 frogs were infected with *Ophidascaris* sp. larvae. The infection intensity per individual frog ranged from 1 to 15 up to 2.2 cm long nematode larvae. The larvae were found in various anatomical predilection sites, including the coelomic cavity, liver, skeletal musculature, serosa, connective tissue, and the central nervous system. All detected *Ophidascaris* sp. larvae caused associated pathological changes ranging from acute trauma, necrosis, and bleeding to severe chronic granulomatous inflammations. The results of the present study reveal that larva migrans visceralis and neuralis by parasitic *Ophidascaris sp.* can induce profound pathological changes in *Occidozyga lima* frogs. Veterinarians dealing with exotic pets and wild animals should be especially aware of this parasitic infection as the problem can also be present in European frog collections.

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HUNT FOR PARASITES: A MULTI-CRITERIA APPROACH TO IDENTIFYING RISK FACTORS AND HIGH RISK AREAS FOR ENDOPARASITES TRANSMISSION

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Intestinal endoparasitoses (including parasitozoonoses) remain an important health problem in Central European countries, including Slovakia. The prevalence of these diseases is especially high in areas where the population lives in poor environmental and hygienic conditions. In Slovakia, such conditions are typical for marginalised population groups represented by the Roma population. Targeted prevention is the most effective protection against endoparasitic diseases. This is only possible if the most risky factors and areas of endoparasite occurrence are identified. Therefore, the work is focused on defining the risk factors and risk areas for the spread of endoparasites in the human population in localities with a predominantly minority population in the area of the so-called hungry valleys in Slovakia (districts of Rožňava, Lučenec, Revúca and Rimavská Sobota). In addition, the prevalence of serious endoparasitic diseases in the population was also monitored in this area. In humans, endoparasitic diseases occurred mainly in the minority population, in rural people and in the child population. The most common diseases were caused by *Ascaris lumbricoides* and *Trichuris trichiura*. For the first time in Slovakia, risk factors that influence the occurrence and spread of endoparasites in humans were identified using analytic hierarchy process (AHP), multi-influence factor (MIF) and correlation coefficient methods. It was confirmed that these factors mainly include ethnic group affiliation, poor hygiene conditions, access to drinking water, sanitation and the use of cesspools. These factors were assigned importance, and the choice of method depended on the availability of epidemiological data. Based on the results obtained, it can be concluded that if data on the prevalence of parasitic infections are not available, the AHP method is recommended to be used in determining the importance of the factors, but it is more difficult to implement. However, if epidemiological data are available, a more appropriate method is to determine the importance of factors based on correlation analysis. At the same time, a risk assessment model based on spatial analyses of the prevalence of endoparasitic diseases was proposed and tested for the first time in Slovakia. Using the proposed model and multicriteria analysis tools in GIS, the risk areas of the hungry valleys were determined, which will enable better identification and clarification of endoparasite transmission pathways among people in marginalized and majority communities. In addition, by using these multidisciplinary approaches, it will be possible to predict the prevalence of endoparasites in animals as well as prevalence of the developmental stages of endoparasites in the environment, opening up new possibilities for the use of risk mapping also in the field of veterinary medicine and environmental health.

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VARIABILITY OF GROWTH CHARACTERISTICS DURING CULTIVATION OF THE SEXUALLY TRANSMITTED PARASITE *TRICHOMONAS VAGINALIS*; OCCURRENCE OF TRICHOMONOSIS IN SLOVAKIA

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The flagellated parasitic protozoon *Trichomonas vaginalis* affects the urogenital system and is the cause of the sexually transmitted disease – trichomonosis. The aim was to characterize the growth abilities of trichomonads and also to evaluate occurrence of trichomonosis in selected areas of Slovakia during a 7-year period. The culture examination (28191 patients) and DNA proof of *T. vaginalis* by PCR (1677 patients) were used. If the initial inoculum of *T. vaginalis* was increased, the generation time (GT) was also increased. This was confirmed by evaluating the growth curves of 42 strains: 12.9 hours GT for inoculum of 1 – 5000 cells, 17.3 hours GT for inoculum of 5001 – 10000 cells, 25.8 hours GT for inoculum of 10001 – 20000 cells and 55.5 hours GT for inoculum from 20001 cells. Also, the maximum multiplication time was increased with the increase of the inoculum: 2.1 days for inoculum of 1 – 5000 cells, 3.6 days for inoculum of 5001 – 10000 cells, 5.2 days for inoculum of 10001 – 20000 cells. The prevalence of trichomonosis using culture was 1.8 %. Significantly more women suffer from this disease. The highest rate of infection was determined in the age category of 41 – 60 years. The most patients infected with trichomonosis came from eastern Slovakia. These observed differences were statistically significant ($p \leq 0.05$). The real-time PCR does not achieve such a detection capability compared to the cultivation method (1.3 % and 1.8 % prevalence, respectively). Cultivation evidence is a diagnostic method that is fully sufficient for the diagnosis of trichomonad infection and can still be considered the gold standard. Further studies will be focused on the quantification of trichomonads using real-time PCR and the comparison of quantitative data in growth curves using PCR and the Neubauer counting chamber.

NEMATODE *CAENORHABDITIS ELEGANS* AS A MODEL ORGANISM IN THE COMPARATIVE STUDY OF BISPHENOL A AND BISPHENOL S EFFECTS

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Endocrine disruptors represent a heterogeneous group of exogenous chemical substances that interfere with natural hormonal processes in organisms, thereby potentially affecting a wide range of physiological functions. One of the most studied and widespread substances in this category is bisphenol A (BPA). It is an organic chemical compound widely used in the production of polymers, particularly polycarbonates and epoxy resins, which are present in everyday plastic consumer products. BPA exhibits a broad spectrum of biological effects, including reproductive, metabolic, and neurological dysfunctions across various animal species, including humans. In an effort to mitigate the potential risks associated with BPA exposure, its use has been progressively restricted and replaced with structurally related compounds, such as bisphenol S (BPS). However, an increasing number of studies suggest that BPS may not represent a safer alternative, as it demonstrates comparable adverse effects on biological systems. The present study focuses on the in vivo characterization of the impact of BPA and BPS on selected biological parameters of the nematode species *Caenorhabditis elegans*. Two strains were used in the experiment: the standard wild-type strain N2 and the mutant strain bli-1, which is characterized by altered expression of the *bli-1* gene encoding one of the collagen proteins essential for proper cuticle formation, the protective outer layer of the organism. Nematodes of both strains were exposed to various concentrations of BPA and BPS (0.1; 0.5; 1; and 5 µMol), after which we examined several biological parameters including larval hatching rate, sensitivity to mechanical stimuli (habituation to anterior touch), and body length. The analysis revealed significant changes in all monitored parameters in both *C. elegans* strains after exposure to selected concentrations of BPA and BPS, compared to the unexposed control groups. These effects were observed not only in the wild-type N2 strain but were even more pronounced in the bli-1 mutant strain, indicating increased sensitivity in organisms with compromised cuticle integrity. The data suggests that mutant *C. elegans* strain may exhibit changes more readily when exposed to low levels of potentially harmful substances. In conclusion, although BPS has been proposed as a safer alternative to BPA, it exhibits comparable harmful effects on the behavior and development of *C. elegans*. Our results contribute to the growing body of evidence emphasizing the necessity of rigorous safety assessments of chemical alternatives prior to their widespread use.

TOXOPLASMA GONDII IN DOMESTIC RUMINATS IN SLOVAKIA – SEROEPIDEMIOLOGICAL STUDY

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Toxoplasmosis is a foodborne infection of global concern, posing a great risk especially for pregnant women and immunosuppressed people. People can become infected after the consumption of oocysts from the environment or water, or after the consumption of tissue cysts in undercooked meat from infected intermediate hosts, e.g. domestic animals or game. *Toxoplasma gondii* tachyzoites were detected also in the milk of sheep, goats and cows and direct connection between human toxoplasmosis and consumption of unpasteurised goat milk and cheese has been confirmed. The study aimed to monitor the seroprevalence of *T. gondii* in domestic ruminants, namely cattle, sheep and goats in Slovakia. Serum samples were collected in cooperation with the Veterinary and Food Institute in Bratislava between January and December 2024. The results showed that IgG antibodies to *T. gondii* were detected in 88 (36.8 %) out of 239 tested sheep and in 56 (41.8 %) out of 134 goats. A total of 384 cow samples were tested, out of which 78 yielded positive results, accounting for 20.3%. Our results confirmed that the main risk to humans arises from the handling and consumption of meat, milk and animal products from goats and sheep.

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THE OCCURRENCE OF AVIAN SCHISTOSOMES IN SLOVAKIA AND THE CZECH REPUBLIC DETECTED BY ENVIRONMENTAL DNA METABARCODING

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Avian schistosomes are parasitic flatworms of the family Schistosomatidae (Trematoda) that uses water-fowls as definitive hosts and aquatic snails as intermediate hosts. Humans may become an accidental host, when free-swimming cercariae penetrate the skin and cause local allergic reaction called cercarial dermatitis (or swimmer´s itch). In Europe, there is a growing number of clinical cases in people engaged in water sports, therefore surveillance of the etiological agents of this parasitic zoonosis became necessary. For that purpose, we have modified the environmental DNA metabarcoding approach to detect cercariae in surface water by its filtration through a plankton net with 20 µm pores. These environmental water samples containing cercariae and miracidia were used for the extraction of DNA, which served as a template for an PCR amplification of partial ITS1 region of avian schistosomes. The PCR products of expected length were pooled for high-throughput sequencing, raw reads from Illumina platform were bioinformatically analysed, and OTUs (Operational Taxonomic Units) were identified by BLAST supplemented with phylogenetic analysis. Water samples were collected at 17 localities in the Czech Republic and 35 localities in Slovakia in summer and early autumn 2021 and 2024. Avian schistosomes were detected at 12 localities where 17 OTUs were identified. In the Czech Republic, three OTUs were assigned to the species *Bilharziella polonica*, *Trichobilharzia franki* and *T. szidati*, while 6 OTUs clustered with an unidentified isolate (Avian schistosomatid sp. I4) from snail *Anisus vortex* from the Czech Republic. The most widespread species were *T. franki* and *T. szidati*, which occurred at 6 localities. The localities with the highest number of OTUs were Loužek pond (8) followed by Hornobranský pond (7) and Lake Most (7). In Slovakia, 10 OTUs were detected, which were assigned to two known species. *T. franki* was present in all positive samples, while *T. szidati* was only detected in the Iňačovce ponds. The remaining OTUs detected in the samples from both countries could not be unambiguously assigned to any known species and might represent undescribed taxa. In summary, this eDNA-based method has the potential to be a rapid and cost-effective tool for large-scale detection of species diversity of avian schistosomes with eventual modification for other trematode taxa.

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POSTERS

DERMACENTOR RETICULATUS IN SLOVAKIA IN SPACE AND TIME AT THE BEGINNING OF THE MILLENNIUM

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The *Dermacentor reticulatus* (DR) tick, one of the two representatives of the *Dermacentor* genus found in Europe, has become one of the fastest-spreading parasites since the beginning of the new millennium. The list of confirmed pathogens associated with DR includes viruses, bacteria, rickettsiae, and unicellular parasites—agents that are primarily dangerous to livestock and domestic animals, particularly dogs, due to the transmission of *Babesia canis*.

The geographical distribution of DR is highly focal and fragmented, encompassing two main macro-regions. The Western European macro-region stretches from northern Spain to western Poland, with isolated outbreaks reported in the Czech Republic, Hungary, and Slovakia. The Eastern European macro-region extends from eastern Poland through the Baltic States to Russia (Rubel et al. 2015). A notable feature of DR is its mosaic distribution across lowland to upland areas, with a particular affinity for riverine environments, as also observed in Slovakia.

Despite extensive knowledge of its ecology, the limits of DR’s spatial distribution remain unclear. A distinctive feature of this tick is its mixed ecological developmental cycle. Adult ticks are exophilic, inhabiting the soil surface and ground vegetation, while immature stages—larvae and nymphs—are endophilic, developing in the burrows of small terrestrial mammals. During the summer months, these developmental stages progress from host-carried larvae to nymphs, ultimately maturing into adult ticks.

Adult DR ticks exhibit high resilience to climatic extremes, capable of surviving up to three winters, extended periods of flooding, hot summers, and summer diapause. In contrast, larvae and nymphs are highly sensitive to both drought and excessive soil moisture.

Based on records of DR occurrence in Slovakia up to 2015 (Rubel et al. 2015), and our new findings from 2021–2025 in eastern Slovakia, when mapped onto the ecological soil moisture types from 1999 (Granec, Šurina 1999), DR appears to exhibit a strong preference for semi-moist soil types. Locally, their presence also extends into adjacent moist and/or semi-arid soils. We did not observe DR in areas with long-term average annual precipitation exceeding 800 mm, or at altitudes above 600 meters above sea level, except sporadically. We suggest that this may reflect changes in climate and soil hydrology over the past 25 years across various regions of Slovakia.

Given the mixed ecological development of DR, we propose that soil moisture—crucial for larval and nymphal development—is one of the key limiting factors for the tick’s expansion into new areas in Slovakia, with important epidemiological implications.

Molecular screening for the presence of *Rickettsia* of 141 adult DR ticks from eastern Slovakia revealed the presence of *Rickettsia* spp., with a predominance of *R. conori* subsp. *raoultii* (26.2%), *R. monacensis* (2.1%), and *R. slovaca* (0.7%). Additionally, *R. aeschlimannii* (0.7%) was recorded for the first time in DR in Slovakia. Moreover, in localities from southwestern Slovakia the presence of unicellular parasites *Babesia capreoli* was found.

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BENEATH THE TICK'S CUTICLE: A MICROSCOPIC EXPEDITION USING SEM

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Ticks are vectors of numerous pathogens, and their internal anatomy remains a key focus in parasitological and morphological research. High-resolution imaging techniques, such as scanning electron microscopy (SEM), offer impressive opportunities for detailed visualisation of their internal structures. Our aim was to gently remove the dorsal part of the cuticle of *Dermacentor marginatus* in order to visualise the internal tissue composition in the most natural state possible using SEM. Additionally, we aimed to dissect individual tissues and visualise them separately. The resulting SEM images successfully captured the internal anatomy of *Dermacentor marginatus* in situ, with preserved spatial relationships between tissues. By isolating and imaging individual organs, we created a visual map linking dissected structures to their original positions within the tick's body. This approach provides a clear and informative overview of tick internal anatomy, demonstrating the potential of SEM for detailed morphological studies in acarology.

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DETECTION OF SIX TICK-BORNE PATHOGENS IN RODENTS CAUGHT NEAR TO MUNICIPAL WASTE LANDFILL

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Free-living rodents are significant reservoirs of infectious pathogens, posing health risk to human and animals. The aim of study was detection of 6 tick-borne bacterial pathogens in rodents caught near to municipal waste landfill Žabčice during years 2020–2022. Tissue samples (spleen or kidney) of 167 small free-living mammals (95 *Microtus arvalis*, 31 *A. sylvaticus*, 23 *Sorex araneus*, 20 *A. uralensis*, and 1 *Crocidura suaveolens*) were used. Multiplex PCR targeting specific genes was used to detect *Anaplasma phagocytophilum* (16S rRNA gene), *Coxiella burnetii* (*IS1111* gene), *Ehrlichia* spp. (*Omp* gene), and *Francisella tularensis* (fopA gene) and single PCR was used to detect *Rickettsia* spp. (*gltA* gene), and *Borrelia* spp. (*hbb* gene). Out of 167 individuals tested, 21 (12.6%) were negative, single infection was proved in 38 (22.8%) animals, and coinfections (2 or more pathogens) in 108 (64.7%) animals. The highest positivity was reached for *F. tularensis* (54%, 91/167), followed with *Rickettsia* spp. (43%, 71/167), *A. phagocytophilum* (31%, 52/167), *Ehrlichia* spp. (20%, 33/167), *Borrelia* spp. (15%, 25/167), and *C. burnetii* (0%, 0/167). Coinfection of 2 pathogens was in 39.5% (66/167) of the animals with the most frequent combinations of *Ehrlichia* spp. and *F. tularensis* (15%, 25/167), and *A. phagocytophilum* and *F. tularensis* (11.4%, 19/167). Coinfection of 3 pathogens was in 19.8% (33/167) of the animals with the most frequent combination of *Rickettsia* spp., *Ehrlichia* spp, and *F. tularensis* (7.8%, 13/167). Coinfection of 4 bacteria was in 3.6% (6/167) of the animals, all included *Borrelia* spp. and *Rickettsia* spp. with other bacteria. Despite our expectations, the results showed that area near municipal waste landfill does not present high risk of *Borrelia* spp., *Ehrlichia* spp. and *C. burnetii*, however *Rickettsia* spp., *F. tularensis* and *A. phagocytophilum* exhibited higher positivity than it is in natural environment.

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RICKETTSIA SPP. PREVALENCE IN *IXODES RICINUS* AND *DERMACENTOR RETICULATUS* TICKS FROM SLOVAKIA AND NEIGHBOURING COUNTRIES SINCE 2000: A META-ANALYSIS

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Rickettsioses are zoonoses caused by bacteria of the genus *Rickettsia* transmitted by arthropods such as lice, fleas, ticks and mites. They are commonly divided into two groups: the spotted fever group (SFG) and the typhus group (TG). Mediterranean Spotted Fever (MSF) and MSF-like syndromes transmitted by *Ixodes ricinus*, *Rhipicephalus* and *Hyaloma* ticks, along with Tick-Borne Lymphadenopathy (TIBOLA) / *Dermacentor*-Borne Necrosis Erythema Lymphadenopathy (DEBONEL) syndromes associated with *Dermacentor* ticks, represent the most prevalent forms of rickettsial infections in Europe (Portillo et al 2015). In Central and Eastern Europe, *Ixodes ricinus* and *Dermacentor reticulatus* are the most abundant tick species.

The objectives of this meta-analysis were to determine whether the prevalence of *Rickettsia* spp. in questing *I. ricinus* and *D. reticulatus* from Slovakia and its neighbouring countries (Austria, Czech Republic, Hungary, Poland, Ukraine) has changed since 2000, and/or whether it varies between countries.

A total of 498 studies published between 2003 and 2025 were identified in PubMed, of which 41 met the inclusion criteria. Statistical analysis showed that *Rickettsia* prevalence in *I. ricinus* ticks is significantly higher in Austria than in Slovakia, Poland, and the Czech Republic. Prevalence was also higher in the 2010s than in the 2000s and early 2020s. Conversely, no significant differences in *Rickettsia* prevalence were observed in *D. reticulatus* ticks between countries and decades.

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EVALUATING THE ROLE OF *DERMACENTOR* TICKS IN THE CIRCULATION OF *RICKETTSIA HELVETICA*

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Rickettsia helvetica, a tick-borne intracellular pathogen belonging to the spotted fever group of Rickettsiae, causes flu-like rickettsiosis with symptoms such as fever, rash, headache, and myalgia. Although, it has been associated with more severe conditions such as sarcoidosis, perimyocarditis, and meningitis. In Europe, *Ixodes ricinus* is recognized as the primary vector and potential reservoir for this pathogen, with prevalence rates in questing *I. ricinus* ticks ranging from 0.5% to 66%. However, the role of *Dermacentor* species in the transmission of *R. helvetica* remains unclear, with no conclusive evidence supporting their epidemiological relevance. Although *R. helvetica* DNA has been detected in *Dermacentor* ticks in Croatia, Austria, and the Czech Republic, sporadic findings in *D. reticulatus* do not confirm its role as a competent vector. The study aimed to evaluate the presence of R. helvetica in questing ticks and compare its reproducibility in I. ricinus and Dermacentor ticks.

The presence of *R. helvetica* in *Dermacentor marginatus* and *D. reticulatus* collected from vegetation in various localities in Slovakia was not confirmed. A laboratory colony of *I. ricinus* and *D. marginatus*, confirmed negative for *Rickettsia* spp., were artificially infected via injection with 500 live *R. helvetica* bacteria. In laboratory-infected *I. ricinus*, the bacterial load significantly increased, reaching a 1,324-fold rise to 1.88×10^6 copies per tick by day 24 post-infection. In contrast, *R. helvetica* persisted in *D. marginatus*, but the bacterial quantity increased only 24-fold by the same time points. Additionally, a comparison of defensin gene expression in *I. ricinus* and *D. marginatus* revealed distinct differences with overexpression of *def1* and *def2* observed exclusively in *I. ricinus* ticks. Our results do not support the hypothesis that *Dermacentor* species could serve as vectors or reservoirs for *R. helvetica*. The sporadic detection of this pathogen in *Dermacentor* ticks may be attributed to co-feeding on *R. helvetica*-infected hosts rather than direct transmission.

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SYMPATRIC OCCURRENCE OF *IXODES RICINUS* AND *DERMACENTOR* SPP. TICKS IN SOUTHERN SLOVAKIA AND THE POTENTIAL RISK TO HUMAN AND ANIMAL HEALTH

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Ixodes ricinus and *Dermacentor reticulatus* ticks belong among the most widespread tick species in Central Europe. In our area, they represent a serious medical and veterinary problem, as vectors of a wide range of pathogenic agents (protozoa, bacteria, viruses). Tick-borne diseases include babesiosis, borreliosis and tick-borne encephalitis. We monitored the simultaneous occurrence of our two most common ticks at the Váh River (locality 1, Vinohrady nad Váhom), at the Malý Dunaj River (locality 2, Jelka) and in Žemberovce (locality 3). Questing ticks were collected by the flagging method. In total, we flagged 184 ticks, which we subsequently examined for the presence of *Borrelia burgdorferi* s.l., *Anaplasma phagocytophilum* and *Babesia* spp. using the PCR method. *D. reticulatus* was detected at the Malý Dunaj and Žemberovce sites. *I. ricinus* was the dominant species at site 2 (80.5%), the abundance of *D. reticulatus* was 19.5%. *Borrelia* infection was detected in 19% at site 1 and in 22% at site 2. Other pathogens were not detected at site 1. *A. phagocytophilum* was found in 5% of tested ticks at the Malý Dunaj site and 12.5% of *D. reticulatus* was positive for *Babesia* spp. At the Žemberovce site, the dominant species was *I. ricinus* (69%), followed by *D. marginatus* (25.5%) and *D. reticulatus* (5.5%). Preliminary results point only one positive *I. ricinus* tick for *A. phagocytophilum*. At site 2, *D. reticulatus* we recorded for the first time. This tick species is a vector of *Babesia canis*, the causative agent of babesiosis, a serious and fatal disease in dogs. Sequencing of positive samples is necessary to identify genetic variants of individual pathogens circulating in this area. Eco-epidemiology of tick-borne diseases is a key part of accurate diagnosis, treatment and estimation of the risk of infection.

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SEASONAL ACTIVITY OF *IXODES RICINUS* AND CITIZEN SCIENCE IN TICK RESEARCH IN SLOVAKIA (INFOTICK)

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Ticks transmit a wide range of pathogenic microorganisms. Many of tick-borne diseases have emerged (or re-emerged) within the past 2 or 3 decades.

We focused on the study of changes in the distribution and seasonal activity of *Ixodes ricinus* (IR). Seasonal activity was monitored in Bratislava since June 2023 until now. Ticks have been collected monthly by flagging the vegetation. Moreover, we are monitoring the activity of ticks directly using tick gardens (Purgatová et al. 2025).

Our results indicate changes in the seasonal activity of ticks compared to the situation in the past. A total, 6,256 IR ticks were collected. The highest number of nymphs/adult ticks was flagged in 2023 in June (n = 408), in April 2024 (n = 309) and in April 2025 (n = 311). In August, the questing activity of nymphs and adults significantly decreased in both years. In autumn (September 2023 and October 2024) the number of collected nymphs/adults increased slightly. Larvae predominated in our collections in July 2023 (n = 279), November 2023 (n = 2232) and in 2024 in January (n = 95), July (n = 263) and August (n = 86). Larvae were recorded in all collections in 2023 and 2024, except October 2023. So far in 2025, larvae have been recorded in March (n = 15). Ticks, mainly larvae (n = 140) were still active during the warm December 2023 and January and December 2024. From February and March, the number of nymphs/adult ticks increased in both years (2023 and 2024).

We also involve the public in the research. Citizen science (CS) projects have the potential to address hypotheses requiring large datasets that cannot be collected with the financial and labour constraints of most scientific projects. In an ideal case, not only citizens serve science, but also science serves to citizens. This is also a main mission in our project „INFOTICK“: to provide evidence based scientific data on ticks and tick-borne diseases for the public. People come into contact with ticks quite often, but they cannot identify the tick, or/if they are at risk of tick bites and tick-borne infections. Therefore, the aim of our project, is to develop a mobile application (MApp) for the identification of ticks, and the interactive website with the map, where the public will provide us with data on the occurrence of ticks. Moreover, the website as well as MApp will inform the public on the seasonal activity of ticks and the risk of acquiring tick-borne diseases in Slovakia (Gatial et al. 2025).

Based on retrospective and new data we will study how the changes in the environment over the last 20 years have affected the distribution and ecology of ticks.

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SEROPREVALENCE AND RISK FACTORS FOR ZOONOTIC PATHOGENS *TOXOPLASMA GONDII*, *BORRELIA BURGDORFERI* SENSU LATO, *COXIELLA BURNETII*, AND *FRANCISELLA TULARENSIS* IN OWNED DOMESTIC CATS IN THE CZECH REPUBLIC

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Cats (*Felis catus* L.) are undoubtedly one of the most popular pets however, close coexistence of humans and pet cats is linked with the risk of transmission pathogens that are causative agents of medically important diseases. The aim of this study was detection of antibodies to 4 selected pathogens in cats from the Czech Republic. Sera of 276 owned domestic cats were examined by modified indirect sandwich ELISA for the presence of IgG and IgM antibodies against parasite *Toxoplasma gondii*, and against 3 bacteria *Borrelia burgdorferi* sensu lato, *Coxiella burnetii*, and *Francisella tularensis*. In total, 67 % (184/276) cats were seropositive, with single infection in 33 % (92/276) cats and coinfection of 2 or more pathogens in 67 % (184/276) cats. Combination of antibodies to *T. gondii* and some of the bacteria was proved in 30 % (84/276) cats. Total antibodies to *T. gondii* were found in 44 % (121/276) cats with IgM in 37 % (101/276) and IgG in 29 % (80/276) cats. Antibodies to *B. burgdorferi* s.l. were found in 42 % (116/276) cats with IgM in 34 % (95/276) and IgG in 35 % (96/276). Antibodies to *C. burnetii* were found in 21 % (59/276) cats with IgM in 12 % (34/276) and IgG in 18 % (50/276) cats. Antibodies to *F. tularensis* were found in 13 % (37/276) cats with IgM in 9 % (26/276) and IgG in 8 % (23/276) cats. The way of keeping had strong effect on seropositivity to all of the above-mentioned pathogens ($p \leq 0.05$), however age category and sex had influence on seropositivity of some of the infections.

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PRELIMINARY DETECTION OF CANINE PARVOVIRUS IN RED FOXES (*VULPES VULPES*) IN SLOVAKIA

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Canine parvovirus type 2 (CPV-2) is a highly contagious viral pathogen primarily affecting domestic dogs as the main hosts. Due to its remarkable environmental stability and broad host spectrum, CPV-2 represents a significant epidemiological threat not only to domestic dog populations but also to various wild animals, especially members of the order Carnivora (Leopardi et al. 2022). Among them, the red foxes (*Vulpes vulpes*) had emerged as a potential reservoir species capable of sustaining and circulating the virus in the environment (Van Arkel et al. 2019, Ndiana et al. 2021, Kimpston et al. 2022). In Slovakia, a steady annual increase in red fox populations may facilitate the long-term maintenance and spread of the virus in both natural and anthropogenic habitats with limited vaccination coverage. Identifying the role of wildlife reservoirs is crucial for managing CPV-2 in domestic and wild animal populations. This preliminary study aimed to investigate the presence of CPV-2 in red foxes (*V. vulpes*) in Slovakia using real-time PCR and conventional PCR. Rectal swab samples were collected from red foxes (n = 30) legally harvested by hunters during the regular hunting season as part of authorized wildlife population control measures. The animals were obtained from multiple hunting districts across various regions of Slovakia. Viral DNA was extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen). The presence of parvoviral DNA was detected by the amplification of a 93 bp fragment of the VP2 gene using real-time PCR. Positive samples were subsequently retested by conventional PCR targeting a 573 bp fragment of the VP2 gene and subjected to sequencing. The detection of CPV-2 in 6 out of 30 samples supports the hypothesis that red foxes may harbor the virus asymptomatically or subclinically, thereby playing a silent epizootiological role. These findings highlight the need for continued molecular surveillance of wildlife, especially species like red foxes, that may bridge the gap between wild and domestic environments.

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BARTONELLA AND *RICKETTSIA* PATHOGENS IN LAELAPID MITES (ACARI: MESOSTIGMATA) OF SMALL RODENTS IN LITHUANIA

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Laelapidae mites include many species that are general parasites of small rodents, which are considered carriers and reservoir hosts of vector-borne bacteria such as *Bartonella* and *Rickettsia* spp. Although Laelapid mites found on small rodents are supposed as potential vectors of infectious diseases, the role of these ectoparasites in transmission of *Bartonella* and *Rickettsia* bacteria among rodent hosts have been poorly studied. The aim of this study was to investigate the prevalence and and diversity of these pathogens in mites parasitizing various species of small rodents. Sequence analysis of the partial *gltA* and *17kDa* genes exposed the presence of three *Rickettsia* species in Laelapidae mites feeding on rodents: *R. helvetica* in *Laelaps agilis*, *Eulaelaps stabularis* and *Myonyssus gigas* mites, *R. felis* in *Laelaps agilis* and *Hyperlaelaps microti* mites, and unidentified *Rickettsia* sp. in *Eulaelaps stabularis* mite. Analysis of the 16S–23S rRNA ITS region sequences revealed the presence of two *Bartonella* species in Laelapidae mites: *Bartonella taylorii* in *Laelaps agilis*, *Haemogamasus nidi* and *Myonyssus gigas* mites, and *B. grahamii* in *L. agilis.* The overall rate of *Rickettsia* infection in mites was 9.3%, while infection rate of *Bartonella* in mites was 14.4%. This study provides the first detection of *R. felis* in *L. agilis* and *H. microti* mites and *Bartonella* spp. in *L. agilis*, *H. nidi* and *M. gigas* mites. To our knowledge, this is the first report of the occurrence of *Bartonella* spp. and *Rickettsia* spp. in mites from small rodents in Baltic countries.

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DIVERSITY OF *BORRELIA* SPECIES IN RODENTS AND THEIR IXODID TICKS IN LITHUANIA

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Borrelia spp. are tick-borne pathogens responsible for zoonotic diseases such as Lyme borreliosis and tick-borne relapsing fever. Rodents play an important role in the ecosystem as reservoir hosts and serve as key hosts for ectoparasites, particularly the immature stages of ixodid ticks. In this study, a total of 141 rodents representing five species (*Apodemus flavicollis*, *Microtus arvalis*, *Microtus oeconomus*, *Clethrionomys glareolus* and *Micromys minutus*) were captured in the Curonian Spit of Lithuania. Ticks were removed from the rodents, identified morphologically and using molecular methods. In total, 673 *Ixodes ricinus* and 127 *Dermacentor reticulatus* were collected. Urine bladders samples from 85 rodents and 800 ticks were analysed for *Borrelia* DNA using real-time PCR, as well as conventional and nested PCR targeting 16S rRNA, *ospA* genes and 16S–23S intergenic spacer (ITS) region. *Borrelia* species were identified through sequence analysis of the partial *ospA* gene and 16S-23S ITS region. *Borrelia* DNA was detected in 4.7% of rodent urine bladder samples, 11.8% of *D. reticulatus* and 7.7% of *I. ricinus* ticks. Sequence analysis revealed the presence of *Borrelia afzelii* and *Borrelia miyamotoi* in *M. arvalis* and *M. oeconomus* rodents, as well as in both tick species. This study is the first report of *B. afzelii* and *B. miyamotoi* in *D. reticulatus* ticks from rodents in Lithuania.

Our findings demonstrate the important role of rodents and their ectoparasites in the maintenance and transmission of the pathogens responsible for Lyme borreliosis and tick-borne relapsing fever in Lithuania.

TICK ABUNDANCE AND TICK-BORNE PATHOGENES PREVALENCE IN DIFFERENT ECOSYSTEMS IN THE CZECH REPUBLIC

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Tick-borne bacterial diseases are among the most common vector-borne diseases in Central Europe, including the Czech Republic. The most common habitats for *I. ricinus* are deciduous and mixed forests as well as scrubland areas characterised by a substantial herb layer. Ticks are more abundant at the edges of forests and in areas proximate to water sources within a fragmented landscape. However, they also colonise suitable urban habitats, including parks, gardens and cemeteries.

The aim of our study was to determine tick abundance and prevalence of tick-borne pathogens (*B. burgdorferi* s.l., *B. miyamotoi*, *A. phagocytophilum*, *N. mikurensis* and *Rickettsia* spp.) in selected urban and forest areas in the Czech Republic.

A total of 150 forest areas across the Czech Republic have been selected for the study. In June 2024, a total of 15,958 ticks were collected by flagging. In addition, 4,767 ticks were collected from 31 small towns, distributed across all regions of the Czech Republic, in June 2024; and 3,297 from all 13 large regional cities in May 2024.

The highest observed abundance of ticks was recorded in large regional cities, with an average of 19 ticks per 100 m^2. The average density in forest areas was found to be 16.2 ticks per 100 m^2, while in small towns, the average was 11.7 ticks per 100 m^2.

The mean proportion of ticks from small towns that were positive for *B. burgdorferi* s. l. was 19.8%. The presence of other tick-borne pathogens was detected in 16.8% of ticks; 1.7% of ticks were positive for *B. miyamotoi*, 2.4% for *A. phagocytophilum*, 2.6% for *N. mikurensis*, and 10.1% for *Rickettsia* spp.

Ticks collected from forest areas were found to be infected with *B. burgdorferi* s.l. in 10.4% of cases and with *A. phagocytophilum* in 1.8%. The highest prevalence of *B. burgdorferi* s. l., exceeding 27.6%, was observed in regional cities.

These results show high infection rates with *B. burgdorferi* s.l. and other tick-borne pathogens in urban areas in the Czech Republic. The risks associated with exposure to ticks and tick-borne pathogens appear to be even higher in urban areas than in natural ecosystems.

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EVIDENCE OF ZOONOTIC *BABESIA* SPP. IN TICKS COLLECTED FROM COMPANION ANIMALS IN EASTERN SLOVAKIA

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Companion animals can act as both reservoirs and sentinels for zoonotic tick-borne pathogens due to their frequent contact with humans and exposure to tick habitats (Day, 2011). In urban environments, the close proximity of humans, pets, and ticks facilitates the transmission of zoonotic agents, including *Babesia* species. *Babesia* are intraerythrocytic protozoan parasites of the family Babesiidae that cause babesiosis, an infectious disease posing significant health risks to both humans and animals (Krause, 2019).

This study aimed to detect the presence of *Babesia* spp. in ticks collected from companion animals in urban environments. Ticks were obtained from pet dogs and cats at three veterinary clinics and one dog grooming salon located in the Prešov and Košice regions over the course of 2024. In total, 185 ticks were collected from 123 companion animals, comprising 96 dogs of various breeds and 27 European Shorthair cats. Upon arrival at the clinic, information regarding each animal's ectoparasite protection status was also recorded. All ticks were morphologically identified to species and sex using a standard morphological key. Three tick species were identified: *Ixodes ricinus*, *Ixodes hexagonus*, and *Dermacentor reticulatus*.

Following morphological identification, each tick was individually tested for *Babesia* spp. using PCR targeting the 18S ribosomal DNA gene. *Babesia* DNA was detected in 8 out of 185 ticks (4.37%). Sequence analysis confirmed the presence of two zoonotic species: *Babesia microti* and *Babesia venatorum*. No additional *Babesia* species were identified in the analysed samples. The positive ticks were collected from six cats and two dogs. *Babesia* -positive specimens included engorged adult females and one non-engorged male of *Ixodes ricinus*, the dominant tick species identified in this study.

This study provides the first molecular confirmation of the presence of *Babesia microti* and *Babesia venatorum* in ticks collected from companion animals in the city of Prešov. Notably, 85.8% of the animals examined were not protected against ectoparasites, underscoring the importance of preventive measures. These findings highlight the critical need for increased awareness, routine tick control in pets, and ongoing surveillance of tick-borne pathogens in urban environments.

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ZOONOTIC *BABESIA* SPECIES AS A NEGLECTED TICK-BORNE PATHOGEN IN *IXODES RICINUS* FROM AN URBAN AREA OF NORTHERN SLOVAKIA: FIRST MOLECULAR DETECTION IN THE CITY OF ŽILINA

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Ixodes ricinus is the most common tick species occurring throughout Europe and is known for its capacity to transmit a broad spectrum of pathogens, including bacteria, viruses, and protozoa (Medlock et al. 2013). Among the protozoan agents, babesiosis, particularly human babesiosis, is considered a rare but emerging disease, with nonspecific symptoms that frequently lead to misdiagnosis or diagnostic delay. As a result, it remains one of the most overlooked protozoan infections of public health relevance (Hildebrandt et al., 2013).

This study was conducted in Žilina, a city located in northern Slovakia at an altitude of 342 m a.s.l., characterized by a colder climate with an average annual temperature of approximately 8 °C. Despite these conditions, urban green areas may still support tick populations.

Questing ticks were collected from urban vegetation in the city centre between March and November 2024 using the flagging method. Of the total 264 specimens, 67 were females, 85 were males, and 112 were nymphs. Larvae were not collected. All ticks were morphologically identified as *I. ricinus*.

All samples were processed individually. Molecular detection of tick-borne pathogens, with a focus on *Babesia* spp. was performed by PCR. Sequencing of PCR-positive samples confirmed the presence of *Babesia microti* in three ticks and *Babesia venatorum* in two, both of which are recognized zoonotic species.

This study presents the first molecular detection of zoonotic *Babesia* species in ticks collected from the urban environment of the city of Žilina. Despite the relatively low prevalence (1,9%), the detection of these two zoonotic species, *B. microti* and *B. venatorum*, in urban *I. ricinus* ticks highlights the potential risk of human babesiosis even in colder urban environments.

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BIOFARM AS A POTENTIAL MONITORING AREA FOR THE OCCURRENCE OF TICK-BORNE PATHOGENS IN THE ENVIRONMENT

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Free-ranging wild and domestic animals may serve as effective sentinels for monitoring the presence and circulation of ticks and tick-borne pathogens within a given habitat. Between 2020 and 2022, ticks were collected from domestic animals (goats, sheep, horses, dogs, cats, rabbit) and hedgehogs, as well as from vegetation on a family-operated farm situated at the interface between the White Carpathians and Myjava Hills (western Slovakia). The study area comprises a heterogeneous mosaic of pastures, meadows, and forest fragments. A total of 391 ticks were collected, including 163 host-feeding ticks and 228 questing ticks. *Ixodes ricinus* predominated (94.9%), followed by *Dermacentor reticulatus* (4.9%) and *Haemaphysalis concinna* (one specimen). All specimens were screened for *Borrelia burgdorferi* sensu lato (*B.b.*s.l.), *Borrelia miyamotoi*, *Anaplasma phagocytophilum*, *Babesia* spp., and *Rickettsia* spp. using PCR-based methods and subsequent sequencing. *B.b.*s.l. was detected in 17.1% of ticks, predominantly in *I. ricinus* nymphs and females, with no significant difference in prevalence between host-associated and questing ticks. Six genospecies were identified, with the dominance of *B. afzelii*. *Borrelia miyamotoi* was detected in 3.3.% of *I. ricinus* ticks, primarily in questing specimens. *Anaplasma phagocytophilum* was identified in 18.2% of ticks, mainly in *I. ricinus* females and nymphs collected from animals. Four genotypes were distinguished, clustering into two phylogenetic clades. *Babesia* spp. were detected in 5.6% of ticks from various hosts. Identity of *B. microti*, *B. venatorum*, and *B. capreoli* was confirmed. *Rickettsia* spp. were present in 10.2% of ticks, more frequently in *D. reticulatus* and in host-feeding specimens. Identified species comprised *R. helvetica*, *R. monacensis*, and *R. raoultii*. Co-infections involving two to three pathogens were observed in several ticks. Multivariate analyses revealed associations between *Borrelia* spp. and *B. miyamotoi* with nymphs, *Anaplasma* spp. and *Babesia* spp. with adult females of *I. ricinus*, and *Rickettsia* spp. with *D. reticulatus*. These results underscore the considerable diversity and active circulation of tick-borne pathogens in agroecosystems characterized by the presence of free-ranging animals.

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GEOGRAPHIC VARIATION IN THE MICROBIOME OF *VARROA DESTRUCTOR* IN THE NEIGHBOURING COUNTRIES SLOVAKIA AND CZECHIA

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Varroa destructor mite is a major threat to honeybee (*Apis mellifera*) populations, contributing to colony losses through parasitism and pathogen transmission. While extensive research has focused on *Varroa* biology and its role as a virus vector, its microbiome remains poorly understood, particularly regarding geographic variation. Here, we investigated the microbial diversity, composition, and functional potential of *Varroa* mite microbiota collected from two neighboring countries, Czechia and Slovakia. Using high-throughput sequencing and network analysis, we assessed alpha and beta diversity metrics, microbial co-occurrence patterns, and predicted metabolic functions. Our results revealed significant differences in microbial diversity between the two regions, with distinct bacterial taxa defining each population. Network analysis indicated that microbial communities in *Varroa* mites exhibit varying structural stability, suggesting that geography influences microbial interactions. Functional profiling further highlighted differences in metabolic potential, with key bacterial taxa contributing to region-specific pathways. These findings provide new insights into the *Varroa* microbiome and its potential ecological role, emphasizing the importance of geographic factors in shaping microbial assemblages. Understanding these microbial dynamics could help develop novel strategies for *Varroa* mite management and honeybee health conservation.

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TABANIDAE: A NEGLECTED RESEARCH SUBJECT IN SLOVAKIA

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Horseflies (Diptera: Tabanidae) are hematophagous insects of significant veterinary and medical importance (Baldacchino et al. 2014). Despite their relevance and widespread occurrence, they remain a largely understudied in Slovakia. Recent faunistic data are scarce, and the species diversity of Tabanidae remains unknown in many regions of the country. This study aims to enhance the current knowledge of the distribution and diversity of Tabanidae in selected localities across Slovakia. The field research was carried out in June and July 2024 at six sites located in eastern Slovakia (Betlanovce, Letanovce, Hrhov, Rozhanovce, Šiba, and Fijaš). Tabanids were collected by sweeping with an entomological net and into a stationary car, which acted as a visual and thermal attractant. Identification of tabanids was based on morphological characteristics using taxonomic keys (Chvála 1980; Krčmar et al. 2011; Zeegers, Schulten 2022) and by partial amplification and sequencing of the mitochondrial cytochrome c oxidase gene I (COI) (Folmer et al. 1994). A total of 527 specimens were collected and classified into 15 species of tabanids belonging to the five genera. Species representation was highest in the genus *Tabanus* with 7 species, followed by *Haematopota* with 3 species, *Chrysops* and *Atylotus* both with 2 species, and *Silvius* with one species. The most abundant species at all study sites was *Tabanus bromius* (70,6 %), followed by *Haematopota pluvialis* (10,4%), *Atylotus loewianus* (2,8 %), *Atylotus rusticus* (2,8 %), *Haematopota italica* (2,6 %), *Tabanus sudeticus* (2,3 %) and *Chrysops caecutiens* (2,1 %). The greatest diversity of species of tabanid was recorded at the Hrhov site, where we have confirmed the occurrence of 11 species. This study provides valuable baseline data for future biodiversity assessments and underlines the need for more systematic research on horseflies, especially considering their ecological roles and potential impact on both wildlife and livestock.

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SMALL MAMMALS AS RESERVOIR HOSTS OF *TOXOCARA* SPP. – PRELIMINARY RESULTS

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Toxocara spp., an aetiological agent of toxocariasis, a serious human zoonosis, is a common roundworm of domestic and wild carnivores worldwide. Small mammals serve as its important intermediate and paratenic hosts and play a significant role in the circulation of the parasite. The aim of the study was to find out the occurrence of *Toxocara* spp. in different species of small mammals from the Tatra National Park and Košice city and its surroundings. From 2021–2024, muscle samples from 950 small mammals were individually examined for the presence of *Trichinella* muscle larvae using the artificial digestion method. In five animals, larvae different from *Trichinella* spp. were found and subsequently identified using molecular methods. Amplification of ITS1 and ITS2 gene fragments of *Toxocara* spp. was successful in one sample derived from the European mole (*Talpa europea*) from surroundings of Košice city. After the sequence analyses of isolates, the larvae were identified as *Toxocara cati* and nucleotide sequences were deposited in GenBank under the accession numbers PV329859 and PV329881.

Our results confirmed the presence of *Toxocara* spp. in small mammals in monitored localities, but further investigations on its occurrence in wild and domestic environments should be carried out.

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DIPLODISCUS MEHRAI, AN INTESTINAL TREMATOD IN PET FROGS

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Trematodes of the genus *Diplodiscus* circulate in freshwater ecosystems all over the world. Their complex, dixenous life cycles involve one vertebrate final host, typically amphibians, and one intermediate invertebrate host, typically an aquatic snail. Frog hosts in India and Russia (Shinad and Prasadan 2018, Besprozvannykh et al. 2018) have described *Diplodiscus mehrai*. To date, no information has been published on the pathogenicity of *Diplodiscus* species in the final frog hosts, and therefore, the veterinary relevance of these flukes remains unknown. The presented poster provides novel insights into the veterinary relevance of *Diplodiscus mehrai* in the pet frog species *Occidozyga lima*. A total of 115 post metamorphic, wild-caught, and newly imported *Occidozyga lima* frogs that naturally succumbed during routine quarantine were examined post mortem for the presence of parasites. During dissection, the dead frogs were examined macroscopically, followed by a light microscopical examination of native gastrointestinal tracts and a histopathological examination of stained tissue sections of the latter. Collected trematodes were individually measured and photographed, then preserved in 70% ethanol and later used for DNA extraction, PCR, and sequencing to confirm the parasite species. Of the 115 examined frogs, 29 were infected with *Diplodiscus mehrai*. The mean infection intensity per frog was 1.3 Trematodes. Adult diplodiscid flukes were found within the lumen of the small and the large intestines. Characteristic morphological features, such as the large acetabulum and the large transparent operculated eggs, were found to aid veterinary diagnosis. Egg morphology is especially of great value for veterinary clinicians as these are the only developmental stages shed by living frog patients in the outer environment. In vivo observations of living flukes within the anuran gastrointestinal tract, together with histopathology, revealed parasite- host interactions involving the large acetabulum of the fluke and its impact on the intestinal mucosa of the frog host and the large body size of some *Diplodiscus* specimens in relation to the diameter of the hollow organ they are targeting. In conclusion, *Diplodiscus mehrai* is a common intestinal fluke in *Occidozyga lima*, and an infection is well diagnosable in post mortem examinations. Additionally, a temptative diagnosis based on egg morphology is possible in veterinary clinics where live frogs are examined.

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MOLECULAR CHARACTERISATION OF *CEPHALOTREMA ELASTICUM* (PLATYHELMINTHES: DIGENEA) WITH NOTES ON ITS DEFINITIVE HOST

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The ecological importance of digeneans is emphasized by their complex life cycles, host range, and broad distribution. We study the intestinal digenean trematode *Cephalotrema elasticum* of insectivorous mammals, sampled from *Gammarus balcanicus* (Crustacea: Amphipoda) as second intermediate hosts. It is especially important as part of the helminth species assemblage found in protected mammals. Our study is based on an intensive survey of helminths in 8,920 gammarids collected from 38 localities in Slovakia, Poland, and France. The DNA sequences for three markers (28S rDNA, 18S rDNA, and CO1 mtDNA) were analysed using Bayesian analysis. The two partial 28S sequences obtained were identical, differing only in their lengths, whereas the mitochondrial partial CO1 sequences varied by 3 base pairs in TC content. The final alignment lengths were 1230 bp, 1823 bp, and 375 bp for 28S, 18S, and CO1, respectively. The phylogenetic analysis indicates that *C. elasticum* is deeply embedded within Microphalloidea, as supported by all three gene markers. Molecular data suggest that *C. elasticum* represents a distinct genetic lineage closely related to Cryptotropidae and Pleurogenidae. Anatomical peculiarities such as the placement of the marginal or submarginal genital pore may affect both mating behavior and species isolation in Microphalloidea. Genetic and morphological evidence supports the establishment of a new family, within the Microphalloidea, that includes *Cephalotrema*, *Pseudocephalotrema*, and *Opisthioparorchis*. Therefore, a new family is proposed based on our results. The integration of molecular characterization with classical taxonomy provides a deeper insight into species delineation and phylogenetic relationships. The geographical distribution of *C. elasticum* highlights its usefulness as a biological indicator, reflecting the decline of water shrew populations due to climate-induced ecological changes, particularly in the French Pyrenees.

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OCCURRENCE OF GASTROINTESTINAL PARASITES IN RUMINANTS: A PRELIMINARY STUDY FROM KAJIADO COUNTY, KENYA

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Gastrointestinal parasites represent a significant health and economic challenge, particularly in animal husbandry. In countries such as Kenya, where agriculture constitutes an important livelihood for a large portion of the population, parasitic infections are one of the primary causes of reduced productivity in livestock. The objective of this study was to determine the prevalence and diversity of gastrointestinal protozoa and helminths in populations of sheep, goats, and cattle. The study was conducted in the Kajiado region, located in southern Kenya, which is characterized by a typical pastoral way of life. Fecal samples were collected in February 2025 from domestic ruminants on two farms. A total of 88 fecal samples were collected (38 goats, 34 sheep, and 16 calves). On Farm 1, fecal samples were collected from 12 sheep and 14 goats only. On Farm 2, samples were collected from 22 sheep, 24 goats, and 16 calves samples. Feces were collected either directly from the rectum or based on the animals that were defecating at the time. All 88 samples were analyzed for the detection of *Cryptosporidium* spp. coproantigen, using the CRYPTOSPORIDIUM (FAECAL) ELISA test, Diagnostic Automation, Inc., Calabasas, USA. Specific antigens of *Cryptosporidium* spp. in animal feces were detected according to the manufacturer's instructions. For the ELISA test, positivity was indicated above an OD value of 0.15, while all samples below OD 0.149 were considered negative. The McMaster method was used to examine 47 fecal samples: 27 sheep (10 from Farm 1 and 17 from Farm 2), 8 goats (from Farm 1), and 12 calves (from Farm 2).

Cryptosporidium spp. coproantigen was not detected in any of the fecal samples from the studied farms. The presence of gastrointestinal parasites using the McMaster method was found in 96 % (45/47) of the samples. Nematodes from family of Trichostrongylidae were the most frequently detected parasites at 85.10 % prevalence (40/47), followed by *Eimeria* spp. at 57.44 % (27/47), *Strongyloides papillosus* at 6.38 % (3/47), and *Moniezia* spp. at 6.38 % (3/47). On Farm 1, the positivity rate for Trichostrongylidae was 100 % (10/10) in sheep and 62.5% (5/8) in goats. On Farm 2, the positivity rate was 94.11 % (16/17) in sheep and 66.6 % (8/12) in calves. The positivity rate of *Eimeria* spp. was 100% (10/10) in sheep and 75 % (6/8) in goats on Farm 1. On Farm 2, the prevalence was 52.94 % (9/17) in sheep and 41.66 % (5/12) in calves. *Strongyloides papillosus* was detected only in calves from Farm 2 at 6.38 % (3/47) positivity rate. *Moniezia* spp. was confirmed on Farm 1 in sheep at 20% (2/10) positivity rate and on Farm 2 in 1/17 sheep.

This study highlights that gastrointestinal parasites represent a significant issue in domestic ruminants in the studied area, given the high prevalence of these infections. In addition to causing weight loss, reduced milk production and decreased fertility, severe parasitic infections can lead to animal mortality, which directly impacts the livelihoods of local farmers. Research and monitoring of parasites are crucial for the sustainability of livestock farming and the improvement of animal health in the region. As part of this preliminary study, molecular detection of selected gastrointestinal parasite species and helminths will be performed in the subsequent phases of the research.

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FIRST RECORD OF THE CRYPTIC TAPEWORM SPECIES *HYDATIGERA KAMIYAI* (CESTODA: TAENIIDAE) IN SLOVAKIA

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A strobilocercus cyst was isolated from the liver of a yellow-necked mouse, *Apodemus flavicollis* (Melchior, 1834), trapped in May 2023 on the territory of the Košice Zoological Garden. Total genomic DNA was extracted from the excysted metacestode, and the near-complete cytochrome c oxidase subunit 1 (*cox1*, ≈ 1,600 bp) was amplified and sequenced. Bayesian inference based on publicly available *cox1* haplotypes of the *Hydatigera taeniaeformis* complex placed the Slovak sequence within the *Hydatigera kamiyai* clade. Thus, this record provides the first confirmation of *Hydatigera kamiyai* Iwaki in Lavikainen et al., 2016 in Slovakia. Definitive hosts of the parasite reported to date include the domestic cat, the European wildcat, and the Eurasian lynx, whereas intermediate hosts are arvicoline voles and *Apodemus* mice (Lavikainen et al., 2016; Bisterfeld et al., 2024). Because these rodents readily occupy peri-urban and even urban habitats, the circulation of *H. kamiyai* in city environments is plausible. Although felid infections are typically subclinical and the zoonotic risk to humans is minimal, continued monitoring is essential for the well-being of domestic cats, tracking the overall urban parasitological situation, and clarifying the parasite's ecology in human-dominated landscapes.

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GENETIC STRUCTURE OF *TOXOPLASMA GONDII FROM* PALLAS´S CATS (*OTOCOLOBUS MANUL*) IN 5 ZOO´S IN THE CZECH REPUBLIC

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Pallas´s cats (*Otocolobus manul*) are wild felids that are highly sensitive to *Toxoplasma gondii* infection. The aim of this study was molecular analysis and genetic characteristics of *T. gondii* isolates, involved in 21 cases of fatal toxoplasmosis in captive Pallas´s cats from 5 zoos in the Czech Republic during the years 2004 – 2015. After full gross necropsy, the tissues (brain, lungs, liver and kidneys) were collected for DNA isolation and conventional PCR with using specific primers for SAG2 gene of *T. gondii*. Samples positive for *T. gondii* were genotyped by multiplex PCR for 15 microsatellites including typing markers (TUB2, W35, TgM-A, B18, B17, M33, MIV, MXI), and polymorphic markers (M48, M102, N60, N82, AA, N61, N83) and then a Neighbor-Joining tree was constructed with genotypes from this study and from literature. *T. gondii* DNA was detected in tissues of 16 animals (76 %). Genotyping was successful in 12 isolates: 11 of them were characterized as the avirulent genotype II, that is the most prevalent in Europe, and 1 isolate was characterized as a recombinant II/I or II TgM-A variant. According to the phylogenetical tree, several strains were almost identical in various markers, that can be the evidence for the same source of infection (strains Manul1-CZ and Manul 2-CZ from zoo Děčín, Manul 5-CZ and Manul 6-CZ from zoo Prague, Manul 12bJ-CZ and Manul 14J-Cz from zoo Jihlava). Identical genotype was isolated from one subadult (died in 2012) and kitten (died in 2014) from zoo Jihlava, but in contrast one kitten (Manul 12aJ-CZ) from the same litter as subadult (Manul 12bJ-CZ), showed genotype different in several markers that could be evidence for different source of infection.

Toxoplasmosis may present serious health problems in captive Pallas´s cats and therefore complex preventive measures should be implemented, including testing of animals, biological control of rodents and using the feed without *T. gondii* contamination.

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SEROLOGICAL SURVEY ON *TOXOPLASMA GONDII* IN RUMINANTS FROM KAJIADO COUNTY, KENYA

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Toxoplasma gondii is a protozoan parasite that is distributed worldwide and significantly affects the health of companion and farm animals, wildlife, and humans. In sheep and goats, toxoplasmosis is considered one of the main causes of abortions and stillbirths.

Recently, very few serological studies on *Toxoplasma gondii* have been conducted in sheep and goats within Kenya. That is why the aim of this study was to detect antibodies to *T. gondii* in serum samples collected in February 2025 from domestic ruminants, located in Kajiado County, Kenya: farm 1 with sheep and goats and farm 2 with sheep, goats and cattle. The animal numbers in this production system are dynamic.

In total, blood samples were collected from 92 animals: 37 sheep (12 in farm 1 and 27 in farm 2), 35 goats (16 in farm 1 and 19 in farm 2) and 20 calves (farm 2 only). Blood was taken from the jugular vein and serum was obtained by centrifugation, which was kept frozen at -20 °C until testing. Antibodies to *T. gondii* were detected by commercial Enzyme-linked immunosorbent assay (ELISA, ID Screen Toxoplasmosis Indirect Multispecies, ID Vet, Montpellier, France), according to the manufacturer's instructions. Optical density values were obtained by automatic plate reader at 450 nm. Value S/P (%), that represents the ratio of antibodies in the sample to antibodies in positive control, was calculated for each sample. Samples with S/P ≥ 50 % were considered as positive.

Antibodies to *T. gondii* were detected in 19 % (17/92) of animals, which means 26 % (9/35) in goats, 22 % (8/37) in sheep and 0 % in calves. In farm 1, total seroprevalence was 39 % (42 % for sheep and 38 % for goats). The S/P values in positive samples ranged from 161 % to 243 %. In six animals, three sheep and three goats, the S/P value was ≥ 200 %, which indicates acute *T. gondii* infection. In farm 2, total seroprevalence was 12 % (12 % for sheep and 11 % for goats). The S/P values in positive samples ranged from 64 % to 166 %.

T. gondii may present serious risk for both animals and humans in tested farms, hence one health concerns. During the acute *T. gondii* infection, tachyzoites could be present in raw milk and blood, which may increase risk of infection due to the traditional diet of local people, consisting almost entirely from milk, meat and blood. Therefore this research area needs further investigations, focusing on molecular analysis of seropositive animals and possible wildlife interactions.

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PRELIMINARY STUDY ON *GIARDIA DUODENALIS* AND *CRYPTOSPORIDIUM* SPP. AS ZOONOTIC PATHOGENS IN DOMESTIC DOGS

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Giardia duodenalis and *Cryptosporidium* spp. are intestinal, opportunistic parasites of humans, companion animals, livestock, and wildlife. Both parasites are excreted in the feces of infected hosts and can infect new hosts through fecally contaminated soil, water, feed, and food. *G. duodenalis* is a relatively common pathogen in comparison to *Cryptosporidium* spp. in dog populations, causing gastrointestinal disorders. *Cryptosporidium* spp. also leads to gastrointestinal issues, particularly in young animals or those with compromised immune systems. The aim of this study was to investigate the prevalence of *G. duodenalis* in domestic dogs and to determine whether *Cryptosporidium* spp. co-occurs in these animals or whether there is an association between infections with both pathogens.

Between September 2024 and the present, a total of 95 fecal samples from domestic dogs were examined using flotation methods. To detect *G. duodenalis* cysts, Faust's solution (33% zinc sulfate solution) was used. Positive fecal samples were subjected to DNA isolation using the ZR Fecal DNA MiniPrep Kit (Zymo Research, Tustin, CA, USA), following the manufacturer's instructions. For confirmation of *G. duodenalis* assemblages, molecular identification was performed by amplification of the beta-giardin (bg) and triosephosphate isomerase (tpi) genes. Positive *G. duodenalis* isolates were subsequently molecularly tested for the presence of *Cryptosporidium* spp. Species and subtypes were identified using PCR and sequence analysis of the 60 kDa glycoprotein (gp60) gene. To date, 13 samples have been molecularly analysed for *Cryptosporidium* spp. The sequencing was performed by the Sanger sequencing method.

Out of the total number of samples examined, the prevalence of *G. duodenalis* cysts in domestic dogs was confirmed to be 35.5% (37/100). Molecular identification based on amplification of the bg and tpi genes confirmed the presence of assemblage C. From the 13 samples that underwent molecular analysis for *Cryptosporidium*, species *Cryptosporidium parvum* subtype IIaA17G1R1 was detected in 3 samples.

Infections caused by *Giardia* and *Cryptosporidium* in dogs are often underestimated; however, their presence can have a significant impact on animal health and public health. Co-infection with these parasites may contribute to the persistence of clinical symptoms, and therapeutic management can be more complex and less effective. It remains a question for future studies whether the presence of *Giardia duodenalis* in the host predisposes to co-infection with *Cryptosporidium* spp., highlighting the need for further research into the relationship and interaction mechanisms between these two pathogens.

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CHICORY PASTURE AND ZINC NANOPARTICLES AND THEIR EFFECT ON ABOMASAL PARASITE *HAEMONCHUS CONTORTUS* IN LAMBS

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Haemonchus contortus, a highly pathogenic gastrointestinal nematode causing the most important of infections of small ruminants, is responsible for economic losses due to losses in productivity, high mortality, anthelmintic resistance, and cost of treatment. Conventional therapy of haemonchosis is based on the use of synthetic anthelmintics, which, however, pose a risk of anthelmintic resistance and burden the environment with drug residues. The development of new preventive, therapeutic and more sustainable strategies for parasite control has increased the interest in plant medicines, trace elements and other alternative methods. Thirty lambs 3–4 months old were evenly divided into 3 groups by 10 animals: control group, chicory group (grazing on pasture with 25% of chicory), and zinc group (supplemented with zinc oxide nanoparticles ZnO-NPs). Lambs were experimentally infected with 5000 *H. contortus* larvae and kept supplemented for whole experimental period. On day 107 post infection, lambs were humanely slaughtered, necropsied and adult nematodes from abomasum of each animal were recovered. Total number of *H. contortus* in abomasum was counted in each group – average 1919±3738 adults in control group, 567±1228 in chicory group and 330±993 in zinc group. Adult *H. contortus* from each group were fixed for scanning and transmission electron microscopy, and ultrastructural differences on the cuticle and internal organs between groups were compared. Length of adult males and females was measured with significant differences ($p<0,001$) between control and experimental groups. Average female length was higher in control group (19,78±3,13 mm) than in chicory (16,24±1,99 mm) or zinc (16,43±2,14 mm) groups. Similarly, male adults were longer in control (14,69±1,45 mm) than in chicory (10,96±2,10 mm) or zinc (11,87±1,20 mm) groups. According to our results we can assume that supplementation with chicory and ZnO-NPs positively affected total number of parasites in abomasum, significantly decreased the size/length of *H. contortus* and worsened fitness of adult parasites. Chicory and ZnO-NPs could be promising alternative for maintaining parasitic infections in lambs on levels that won't negatively affect animal's health however further studies are needed.

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OCCURRENCE OF THE TREMATODE *METAGONIMUS ROMANICUS* IN THE DANUBE RIVER

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Trematodes of the family Heterophyidae use snails as first intermediate hosts, fish as second intermediate hosts, and some species can infect humans by eating raw or undercooked fish. *Metagonimus romanicus* (Ciurea, 1915) was described as *Loossia romanica* in a domestic dog (*Canis familiaris*) from the Danube Delta in Romania. It is a widespread intestinal parasite in a variety of piscivorous birds and mammals in central and southeastern Europe, particularly in the lower reaches of rivers flowing into the Black Sea. In the absence of comprehensive epizootiological and epidemiological studies, snails and fish were investigated to identify intermediate hosts of *M. romanicus* from the Danube River. The non-pulmonate snail *Microcolpia daudebartii acicularis* was the only gastropod found to be infected with *Metagonimus romanicus*. A total of 593 individuals of *M. daudebartii acicularis* were collected from three localities in southern Slovakia, of which 7.6% (n = 45) were infected with *M. romanicus*. In the Danube in Slovakia, Austria and Hungary, a total of 120 fish individuals belonging to 25 fish species from seven families (mainly Leuciscidae) were collected and examined. Of these, only 12 species (48 % of all species examined) in Slovakia and Hungary harbored metacercariae of *M. romanicus*. Leuciscids such as *Blicca bjoerkna*, *Chondrostoma nasus*, *Leuciscus idus* and *Squalius cephalus* seem to be the most suitable hosts, as they show the highest infection intensity and prevalence. The cysts with metacercariae of *M. romanicus* were located exclusively within the scales (on the underside of the scales) of the entire body of the infected fish. The highest number of metacercariae detected in a single fish individual was 387 in *L. idus*. In Europe, where raw fish is traditionally not consumed, and probably due to the exclusive localization of the metacercariae on the fish scales and not in the musculature, no infections with *M. romanicus* have been observed in humans.

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NEW SPOT OF CERCARIAL DERMATITIS IN SLOVAKIA CONFIRMED BY ENVIRONMENTAL DNA METABARCODING

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Cercarial dermatitis (swimmer´s itch) is a parasitic zoonosis caused by avian schistosomes (family Schistosomatidae) using water-fowls as definitive hosts and aquatic snails as intermediate hosts. Humans may serve as accidental host, when free-swimming furcocercariae penetrate the skin and cause allergic reaction. In Slovakia, the disease has only been recorded in Košice, where *Trichobilharzia franki* (Müller and Kimmig 1994) was confirmed as the causative agent. Recently, we have sampled additional localities in Slovakia including Stará Myjava water reservoir, which was selected following a call from a local resident exhibiting clinical signs of cercarial dermatitis. In August 2024, 53 individuals of European ear snail *Radix auricularia* L. were collected in the water reservoir and 43 of them released cercariae after exposure to lamp light for 12 hours. However, no furcocercariae were detected among them. Simultaneously with snail sampling, environmental water samples were collected by filtration of approximately 30 litres of surface water from the reservoir through a plankton net with 20 µm pores. These samples were taken in triplicate from 5 sites to assess the effect of transport and storage conditions on DNA extractability. In the laboratory, total environmental DNA from all samples was extracted by a commercial kit and used as templates for amplification of partial ITS1 (Internal Transcribed Spacer 1). The PCR products were pooled, commercially sequenced on Illumina platform and bioinformatically analysed to determine species composition. The dominant species in the water reservoir was *T. franki*, detected in all samples in high number of Illumina reads. Additionally, five other OTUs (Operational Taxonomic Units) were detected in significantly lower numbers, however, their phylogenetic position within avian schistosomes remains unclear. Regarding the transport and storage measures, the highest DNA concentrations in PCR products were gained when water samples were cooled during the transport and stored at -18°C in laboratory until extraction. Slightly lower average DNA concentration was measured when the cooled water samples were stored at 8°C after transport and eDNA was extracted the following day. Fixation in molecular-grade ethanol to its final concentration 70 % immediately after filtration showed to be the worst option. The zoonotic potential of *T. franki* was supported by information from local residents who repeatedly suffered by cercarial dermatitis, as well as by a skin rush of one of the authors, that occurred after collecting the snails with bare hands.

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DIFFERENTIAL NEUROTROPISM AND INNATE IMMUNE ACTIVATION BY KEMEROVO AND TRIBEČ TICK-BORNE ORBIVIRUSES IN HUMAN ASTROCYTES

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Kemerovo virus (KEMV) and Tribeč virus (TRBV) are neglected zoonotic tick-borne orbiviruses (TBOs) with potential neuroinvasive properties. Their relevance is underscored by epidemiological data from Slovakia, where between 2016 and 2018, up to 40% of viral central nervous system (CNS) infections were classified as unspecified (Kerlik et al. 2018). This study aimed to investigate the neurotropism of KEMV and TRBV, as well as the innate immune responses they elicit in human astrocytes (HA).

HA cells were infected with KEMV or TRBV at a low multiplicity of infection (MOI = 0.1). After a 1-hour incubation at 37 °C in a 5% CO_2 atmosphere, the viral inoculum was removed, and cells were cultured in astrocyte medium for 12, 24, 48, and 72 hours post-infection (hpi). Both viruses replicated productively in HA cells, with increasing viral RNA levels observed over time. Peak viral RNA copy numbers were detected at 48 hpi (TRBV: 2.2×10^6; KEMV: 7.1×10^6 copies/reaction). The highest infectious titers were recorded at 48 hpi for TRBV (1.0×10^5 PFU/mL) and at 72 hpi for KEMV (1.3×10^5 PFU/mL).

To assess the innate immune response, the expression of selected cytokines (IFN-β, IL-1β, IL-6, IL-8) and pattern recognition receptors (RIG-I, MDA5) was analyzed. KEMV induced a markedly stronger IFN-β response compared to TRBV, with fold changes of 2700× vs. 0.7× at 24 hpi, 2000× vs. 220× at 48 hpi, and 2100× vs. 170× at 72 hpi. Proinflammatory cytokines IL-6 and IL-8 were also significantly upregulated in KEMV-infected astrocytes. Furthermore, genes encoding cytosolic RNA sensors RIG-I and MDA5 were upregulated following KEMV infection but were downregulated in TRBV-infected cells.

KEMV and TRBV exhibit distinct replication dynamics and immune activation profiles in human astrocytes. KEMV demonstrated stronger neurotropism and elicited a more robust innate immune response, including high levels of IFN-β and proinflammatory cytokines, as well as activation of cytosolic RNA sensors. In contrast, TRBV showed limited immune activation and even downregulation of key antiviral sensors. These differences may contribute to their varying pathogenic potential and should be considered in future studies on TBO-related CNS infections.

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IMMUNE RESPONSE OF HUMAN KERATINOCYTES TREATED WITH *IXODES RICINUS* TICK SALIVARY GLAND EXTRACT INFECTED WITH TICK-BORNE ENCEPHALITIS VIRUS

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One of the most dangerous and widespread arboviruses is tick-borne encephalitis virus (TBEV), the causative agent of tick-borne encephalitis, which manifests as a common febrile illness but can also lead to serious neurological complications. Transmission of TBEV during a bite by an infected tick is facilitated by components of the tick's saliva. The site of initial contact between the tick, host and tick-borne viruses (TBVs) during inoculation is the skin, which serves as the primary line of defence against tick-borne pathogens, including viruses. Keratinocytes, as the dominant non-immune cell type of the epidermis, play a critical role in viral replication and activation of the early immune response, thereby contributing to viral load and facilitating viral spread to target organs.

The aim of this work was to follow the course of TBEV infection and the effect of substances from I. ricinus tick saliva on human dermal keratinocytes after treatment with salivary gland extract (SGE) from TBEV-infected ticks. Using specific primers and semiquantitative q-PCR, we investigated the effect of TBEV- and TBEV-infected SGE on the expression of selected genes associated with the early antiviral immune response of keratinocytes. Transcriptome analysis was used to further characterise the effect of TBEV- and TBEV-infected SGE on the innate antiviral response of skin keratinocytes in vitro.

We found differences in the expression profiles of the genes studied not only as a function of the concentration of TBEV in the sample, but also as a function of the duration of infection. In the case of TBEV alone, increasing the initial infectious titer in the sample had a suppressive effect on the expression of the genes studied during the first 48 hpi. We observed a more pronounced increase in expression up to 72 hpi. for TBEV with a higher initial concentration compared to less concentrated TBEV. Agents in infectious SGE repressed the expression of almost all TBEV-activated genes, with the exception of OAS1 for TBEV with lower concentration during 48 hpi. The results suggest that 72 hpi. agents in infectious SGE with lower TBEV titer repressed the expression of TBEV-activated genes, whereas those with higher TBEV concentration in SGE had a more activating effect on the genes studied. RNA-Seq analysis revealed downregulation of IFN-a response by agents in TBEV-SGE 48 hpi.

Thus, our results suggest that substances present in the saliva of infected ticks repress the expression of genes activated by TBEV infection.

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EVALUATION OF TWO EXPERIMENTAL METHODS OF INFECTION OF ADULT TICKS WITH TICK-BORNE ENCEPHALITIS VIRUS

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Tick-borne encephalitis virus (TBEV), the most widespread arbovirus in Europe and Asia, causes tick-borne encephalitis (TBE), a serious disease of the central nervous system (CNS). During the last 30 years the number of TBE human cases in endemic regions of Europe has increased by almost 400%, thus TBE has become a growing public health challenge. The risk areas for the disease have expanded and new outbreaks have emerged due to lack of efficient control measures and changes in global socio-economic and climatic conditions leading to a wide spread of tick vectors.

Ticks play an important role not only as vectors of TBEV but also as reservoirs of this virus in nature. For almost 100 years, this duo, tick-TBEV, has been on the epidemiology scene, nevertheless, knowledge about their mutual interactions is insufficient and very limited. Elucidation of these interactions is essential for understanding the biology of tick-borne diseases and may help in the development of new control strategies of both ticks as well as virus. To overcome this gap of knowledge, it is essential to reproduce transmission cycles under controlled laboratory conditions by setting up effective experimental models. Therefore, various laboratory methodologies for experimental tick infection have been developed. In fact, establishing and maintaining laboratory tick colonies and performing experimental infections of ticks remain a challenge.

The aim of this study was to evaluate and compare two methods of tick infection with TBEV: 1. percoxal (injection of the virus under the tick’s 2nd coxa), 2. feeding on infected host (laboratory mice; natural route of infection). Females of two tick species, *Ixodes ricinus* and *Dermacentor reticulatus*, competent vectors of TBEV, were infected, followed by detection of TBEV in the ticks' salivary glands, in the feeding site of infectious ticks in the host skin using RT-PCR, RT-qPCR, immunohistochemistry, replication and titration on permissive cells.

Both methods allowed ticks to acquire TBEV, therefore they are suitable for tick infection in our laboratory conditions. We detected the presence of the virus at the gene level and at the same time we confirmed the presence of entire infectious virus particles in the collected tissues – ticks´ salivary glands and mouse skin. The presence of complete virus capable of infecting was detected by the permissive PS cell line, however the titer/amount of virus in the samples was very low compared to the initial dose of percoxal infection. Further analyzes and experiments are necessary to decide which of the methods we will use for the study of tick-virus interactions. Artificial infection methods of ticks are important tools to study tick-virus interactions. When optimally used under laboratory settings, they provide important insights into tick-borne virus transmission cycles.

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CORRELATION BETWEEN ELEVATED PRO-INFLAMATORY TNF-α LEVELS AND SPECIFIC ANTIBODIES IN PATIENTS WITH LYME DISEASE

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Lyme disease, an infection caused by bacteria of the *Borrelia burgdorferi* sensu lato complex, is one of the most common vector-borne illnesses in Europe. The infection can affect multiple organs and tissues, leading to a wide range of clinical symptoms. Failure to adequately treat Lyme disease can result in long-term consequences that may lead to severe health complications, significantly impacting the patient's quality of life.

The aim of this study was to compare the presence of specific IgM and IgG antibodies in patients with Lyme disease, based on serum testing using the ELISA method with whole-cell and recombinant antigens. Additionally, the study investigated whether both the presence and serum levels of the pro-inflammatory cytokine TNF-alpha correlate with the positivity of these antibodies.

Sera from patients tested for Lyme disease were examined by indirect sandwich ELISA for the detection of specific IgM and IgG antibodies against bacteria *Borrelia burgdorferi* sensu lato. Quantification of TNF-α was performed using the Double Antibody-Sandwich ELISA method. The results were statistically evaluated.

The preliminary results of this study suggest that the use of whole-cell antigen compared to recombinant antigen may lead to different results in detecting specific IgM and IgG antibodies, which could impact the diagnosis of Lyme disease. The results also indicate that serum TNF-α concentrations tend to be higher in samples with positive and border-line results. In these samples, the mean TNF-α concentration was higher than in those with negative results for IgM and/or IgG antibodies.

Elevated serum TNF-alpha concentrations in patients indicate an inflammatory response and the correlation between TNF-α levels and the presence of specific antibodies may provide valuable insight into the dynamics of the immune response. Furthermore, TNF-α levels could potentially imply the stage of Lyme disease, offering additional diagnostic accuracy.

DEVELOPMENT OF A NOVEL SANDWICH ELISA TEST FOR THE DETECTION OF ANTIBODIES AGAINST *RICKETTSIA*

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Rickettsiae are obligate intracellular, Gram-negative, aerobic bacteria responsible for a spectrum of zoonotic diseases affecting humans and animals globally. Rickettsial infections (rickettsioses) often present with non-specific clinical manifestations, which can complicate timely and accurate diagnosis, even among experienced healthcare professionals. Common symptoms include fever, headache, and malaise, and are frequently accompanied by a maculopapular, vesicular, or petechial rash localized at the site of an arthropod bite. Due to the non-specific nature of early clinical signs, laboratory confirmation is essential for diagnosis. Serological testing, particularly the indirect immunofluorescence assay (IFA), remains the gold standard for detecting rickettsial antibodies. However, a definitive diagnosis often requires a fourfold rise in antibody titers between acute and convalescent sera. In addition to IFA, enzyme-linked immunosorbent assays (ELISAs) utilizing recombinant rickettsial proteins have emerged as valuable tools for improving diagnostic sensitivity and specificity. These assays enable more standardized and reproducible detection of antibodies by targeting well-characterized antigens. In this study, we focused on the development and standardization of a novel sandwich ELISA using recombinant proteins from *R. akari* (A8GP63, GroEL, and DnaK) to detect rickettsial antibodies. Critical assay parameters, including antigen concentration, microtiter plate type, buffer pH, and secondary antibody dilution, were systematically optimized to enhance performance. A total of 94 human serum samples were tested. Diagnostic accuracy was assessed through receiver operating characteristic (ROC) curve analysis, demonstrating high sensitivity (ranging from 62% to 94%) and specificity (ranging from 63% to 95%) for the detection of IgG antibodies. The diagnostic odds ratio (DOR), used to quantify the test's discriminative power, ranged from 8.95 to 31.77, indicating strong diagnostic capability. Our results demonstrate that the developed ELISA represents a robust and reliable platform for the detection of antibodies against *Rickettsia*, with significant potential for application in clinical diagnostics and epidemiological surveillance.

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COMPARATIVE ANALYSIS OF LIPID A STRUCTURES IN PATHOGENIC RICKETTSIA

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Representatives of the genus *Rickettsia* are aerobic, strictly intracellular, Gram-negative coccobacilli transmitted by blood-sucking arthropods. Their surface is covered by a lipopolysaccharide (LPS) layer, which is anchored in the outer membrane by a lipid domain. Lipid A is the bioactive part of LPS, induces an innate immune response, and has endotoxic character. The molecular structure of LPS consists of three main subunits. The innermost, hydrophobic part is lipid A, which is responsible for the main toxic properties of bacterial endotoxins, and also serves as a so-called membrane anchor for LPS, connecting via 2-keto-3-deoxyoctonate to the oligosaccharide core. Lipid A is relatively highly conserved among a wide range of Gram-negative bacteria and consists of a diglucosamine backbone substituted with phosphate and acyl groups. Modifications of the lipid A structure to less acylated forms have been observed in some bacterial species. It is hypothesized that such modifications facilitate the escape of bacteria from the host's innate immune response, thereby increasing their pathogenicity.

Despite the apparent importance of lipid A in maintaining the integrity of the bacterial outer membrane, as well as its inflammatory potential during infection, this molecule is still poorly understood in rickettsiae. To address this knowledge gap, we conducted a lipidomic and mass spectrometry-based structural analysis of lipid A from *R. akari*, *R. conorii*, *R. slovaca*, and *R. prowazekii*. Our results demonstrated variations primarily in the length of fatty acyl chains of hexaacylated lipid A molecules. These structural insights are critical for comprehending the immunomodulatory potential of rickettsial lipid A.

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NANOPARTICLE-BASED STRATEGIES FOR COMBATING RICKETTSIAL INFECTIONS: AN ALTERNATIVE TO ANTIBIOTICS

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Bacterial resistance has become a serious problem due to the widespread use of antibiotics, which are often applied prophylactically or therapeutically without proper medical indications. Rickettsiae (*Alphaproteobacteria; Rickettsiales, Rickettsiaceae*) are small (0.3–0.5 × 0.8–2.0 µm), Gram-negative, obligate intracellular bacteria that target vascular endothelial cells and grow within the cytoplasm of eukaryotic host cells. Arthropods, such as the ticks *Ixodes ricinus, Dermacentor reticulatus,* and *D. marginatus,* serve as vectors transmitting various rickettsial species, including *R. helvetica, R. monacensis, R. slovaca,* and *R. conorii* subsp. *raoultii* across Europe.

Rickettsioses are successfully treated with antibiotics; however, resistance of rickettsiae to antimicrobial therapy has been reported. Nanoparticles (NPs) are increasingly being explored as an alternative to antibiotics for targeting bacterial infections, as they can effectively evade microbial drug resistance. The antibacterial mechanisms of NPs are relatively well studied, although some aspects remain under investigation. NPs exhibit antibacterial activity through multiple mechanisms, including oxidative stress induction, metal ion release, direct membrane damage, disruption of DNA and proteins, and indirect immune system enhancement.

We investigated the effectiveness of silver NPs (AgNPs), selenium NPs (SeNPs), and chitosan NPs (ChiNPs) in inactivating rickettsiae. HaCaT and Vero cell lines were infected with *R. conorii* strain Caspia and treated with SeNPs, AgNPs, and ChiNPs at non-cytotoxic concentrations for host cells. The effectiveness of NPs was analyzed every 24 hours using quantitative RT-PCR. The antibacterial activity of AgNPs and SeNPs against rickettsiae in Vero cells reached up to 55% and 76%, respectively. However, ChiNPs demonstrated the highest antibacterial activity, with 94% inhibition; however, some morphological changes in host cells were observed 72 h hours post-infection.

NPs show promising prophylactic efficacy in treating early rickettsial infections, and their effects could be synergistically enhanced. Nanotechnology may offer significant advantages in combating bacterial infections.

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PHOTODYNAMIC INACTIVATION OF *COXIELLA BURNETII*

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Coxiella burnetii, the causative agent of Q fever, is a highly infectious obligate intracellular pathogen that poses significant public health risks to humans and animals. . Its ability to replicate within specialized vacuoles resembling phagolysosomes and its environmental resilience complicate control measures.

Although ticks contribute to the transmission and environmental maintenance of *Coxiella burnetii*, they are not the primary source of human infection. Humans are mainly exposed through inhalation of contaminated aerosols from birth products, excreta, or milk of infected domestic animals, or via dust containing environmentally resistant spores. Ticks acquire *C. burnetii* from infected wild or domestic hosts and can transmit it transstadially and transovarially, acting as both reservoirs and vectors. Some hard ticks (e.g., *Dermacentor*, *Rhipicephalus*, *Ixodes*) can harbor the bacterium for extended periods, supporting its persistence in nature.

This study aims to investigate the effect of photodynamic inactivation (PDI) using carbon quantum dots synthesized from citric acid (CA-CQD) on the growth of *C. burnetii* in axenic conditions. *C. burnetii* was inoculated into ACCM-D axenic medium at a concentration of 10^6 genome equivalents per milliliter. The bacteria were exposed to PDI for five consecutive days. Analysis of optical density, qPCR, and qRT-PCR revealed an inhibitory effect of CA-CQD on *C. burnetii* growth and replication under the specified conditions compared to controls. While a single irradiation showed minimal inhibitory effects, repeated treatment led to markedly stronger inhibition.

Future research will focus on elucidating the mechanisms underlying these changes in bacterial physiology and determining whether *C. burnetii* is killed outright or transitions into metabolically inactive forms, such as small cell variants.

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ANTIBACTERIAL HYDROGELS FOR PLASTERS

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Recently, a new specific class of quantum dots was discovered, referred to as carbon quantum dots (CQDs), which are an effective source of active oxygen. CQDs also work on the principle of photodynamic therapy, which is currently used mainly for the diagnosis and treatment of oncological and skin diseases. After irradiation with light of a given wavelength, carbon quantum dots can produce reactive oxygen species that are capable of disrupting the biological membranes of living organisms, for example, bacteria, and the overall viability of cells. The “power” strength of the effect of CQDs depends mostly on the concentration of CQDs in the selected substrate and the irradiation time. In comparison with known antibacterial materials, e.g., silver, the antibacterial or antimicrobial activities of CQDs are generated only after irradiation with light; thus, CQDs have a controllable biological activity (Kováčová et al. 2020).

Our work presents our preliminary results of hydrophilic CQDs prepared from citric acid. They were mixed with various hydrogels, and their antibacterial activity was evaluated.

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