



# PARADNI24

PARASITES OF 21. CENTURY

**XV. SLOVAK AND CZECH  
PARASITOLOGICAL DAYS**



**13 - 17 May, 2024  
TESÁRE - NOVÉ MLYNY, SLOVAKIA**



## **“PARADNI24 Parasites of 21 Century” XV. Slovak and Czech Parasitological Days**

Abstract Book

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**Cover Illustration:** Alžbeta Šujanová

**Publisher:** Institute of Zoology, Slovak Academy of Sciences, Bratislava

**<http://www.paradni2024.sav.sk>**

Corresponding authors are responsible for the scientific content and language of their contributions.

**ISBN 978-80-972239-1-5**



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# Plenary lectures

**OP01 *Haemoproteus* parasites and *Culicoides* biting midges, complex interactions still to be unravelled**

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The avian parasites of the genus *Haemoproteus* present a high diversity of species and lineages, being widely studied worldwide. They are transmitted by *Culicoides* biting midges, however, the relationship between these pathogens and their vector is still poorly understood. Their natural vectors were identified only for a few parasite species, and the mechanisms driving the feeding preference of *Culicoides* species in terms of *Haemoproteus* infections are still unknown. A combination of molecular methods, dissection of parous females, and microscopical analyses of thorax preparation is the most recommended approach to address this issue. Despite that, this integrative methodology is being used by only a few research groups in this field.

To investigate the interactions between *Haemoproteus* and *Culicoides* ~4000 *Culicoides* parous females were sampled in Lithuania from 2021 to 2023. Thorax preparations were done for the investigation of the presence of sporozoites in their salivary glands, and the remnants were used for molecular analysis. These preparations were analyzed by microscopy when the samples were PCR-positive to confirm if the insect supports sporogonic development of *Haemoproteus* parasites and if infective stages are present in their salivary glands. Engorged *Culicoides* females were analyzed separately to identify molecular the source of their blood meals.

Concerning *Culicoides* parous females, 7.3% of the analyzed insects were PCR-positive for *Haemoproteus* parasites, with 13 species and 39 different genetic lineages identified. Parasite species commonly found in Turdidae birds were the most common ones in the analyzed samples. *Culicoides reconditus* was confirmed as a competent vector of *Haemoproteus* parasites for the first time and several new interactions between parasite lineages and vector species were identified. Of the PCR-positive samples, 35.9% of them had sporozoites in their thorax preparations. The capacity to support the sporogonic development of *Haemoproteus* parasites was confirmed for four *Culicoides* species. Concerning engorged *Culicoides* females, *C. kibunensis* and *C. segnis* fed only in birds; while *C. festivipennis* and *C. pictipennis* fed mainly on birds and occasionally on mammals. The mechanisms limiting *Haemoproteus* transmission in the wild are far from being completely understood, and studies targeting this issue should be encouraged.

*This research was funded by the Research Council of Lithuania (grant no. S-MIP-21-55).*

**OP02 Unveiling biohazard threats: Molecular surveillance of *D. repens*, *D. immitis*, and *A. reconditum* in stray animals amidst military war in Ukraine**

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The 2022 war in Ukraine not only precipitated a humanitarian catastrophe with widespread displacement, but also amplified existing public health challenges, notably the surge in stray animal populations. These animals, often carriers of parasitic diseases, present a heightened biohazard risk in the context of escalating vector-borne disease spread, further intensified by climate change. Among these, dirofilariasis poses a significant threat to both animal and human health, yet comprehensive epidemiological insights, particularly for *Dirofilaria repens* and *Dirofilaria immitis*, remain scarce within Ukraine, highlighting a critical gap in current research.

This investigation aims to elucidate the biohazard risk presented by the ongoing war through an analysis of *Dirofilaria* species prevalence in Ukraine's stray animal populace. Conducted from March to December 2023 in collaboration with ESCCAP and several Ukrainian NGOs, this study involved parasitological screenings of 465 stray animals (229 dogs and 236 cats) across Kharkiv, Sumy, Zvenyhorodka, and Berdychiv. Employing Real-Time PCR, we extracted genomic DNA from blood samples for initial screening against *Onchocercidae* family's conservative *cox1* gene fragment, followed by species-specific differentiation among *D. repens*, *D. immitis*, and *A. reconditum*.

Our findings revealed a notable incidence of *Dirofilaria* spp., affirming their endemic presence across the studied locales, with DNA traces detected in 28.38% of dogs and 3.39% of cats. The discovery of *A. reconditum*, marking the first reported instances in Ukraine, adds a new dimension to the complexity of local vector-borne diseases. This study set against the backdrop of ongoing war, underscores the indispensable role of a One Health approach in addressing such biohazards. It advocates for robust diagnostic measures and timely interventions for companion animals to curb potential human transmission risk.

*This research was supported by The National Centre for Research and Development, Poland under grant LIDER IX 0106/L-9/2017, National Science Centre, Poland (R-2022/01/3/NZ6/00089), MSCA4Ukraine (AvHID 1233593; <https://cordis.europa.eu/project/id/101101923>) and ESCCAP <https://www.esccap.org/>.*

### OP03 Ticks population genetics: tools and future venues

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The study of the genetic structure of tick populations has evolved enormously in the past 20-25 years with new molecular tools becoming available. Some of these methods are easier to use or cheaper to apply than others, but direct comparisons of different methodologies are rarely available. In this presentation we will give an historical overview of the different approaches used to study instar-specific genetic diversity and structure in ixodid tick species. We will also discuss some of their applications and compare their usefulness based on more recently published data and ongoing research. In particular, we will use two American well-studied tick species, *Ixodes scapularis* and *Amblyomma maculatum*, to illustrate how our understanding of their population structure has evolved over the years.

# Oral presentations



## **O01 Gaps in parasitological research in the molecular & omics era**

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We live in the age of molecular biology and -omics, and molecular methods have opened up unprecedented opportunities for biological research, including all areas of parasitology. However, there are two sides to every coin, and too one-sided a focus on new approaches can lead to major gaps as less “cool”, i.e. “old-fashioned” topics are neglected. In recent decades, much emphasis has been placed on the study of parasites and their interactions with the host at the molecular level. While this is useful, it ignores the fact that parasitology is primarily an ecological discipline. In this talk, I will give a brief overview of selected areas of parasitological research and highlight the achievements and problems of current research, which focuses heavily on molecular and “-omics” methods. These areas include taxonomy and biodiversity studies, classification and evolution of parasites, parasitological surveys and screening, routine diagnostics of parasites, life cycles, epidemiology/epizootiology, and parasite ecology. It is recommended to combine both “classical” and modern methods (molecular and “omics” approaches), without neglecting the complexity of parasite-host interactions and the environment (One Health concept), which is even more urgent now, in the rapidly changing world. The younger generation should be more involved in field studies and multidisciplinary assessment of parasites to understand the complexity of parasitology.

*Financial support of the Czech Science Foundation (project No. 19-28399X) is appreciated.*

## 002 The sample size matters: evaluating minimum and optimum values in prevalence studies

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Estimating the sample size for studying the prevalence of parasite populations is important as it directly influences the precision of the measured parameter and the validity of the research outcomes. While most recommendations advocate using a large number of elements in a sample to reduce uncertainty, the sample sizes used in the field of general parasitology tend to be relatively small. Our objective was to highlight important limitations in the assessment of minimum sample size and to provide guidance on the delineation of minimum and optimum sample sizes in the prevalence studies. Based on the analysis of the allowable margin of error, we suggest that a sample size of 15 hosts could be used for a rough estimate of true prevalence between 20% and 80%. However, caution should be exercised with a such small sample size as the prevalence could be under- or overestimated and will have a high degree of uncertainty. Approximately 30 elements represent the next threshold, which allows researchers to work within a true prevalence range of 10% to 90%. With a sample size of 75-100 individuals, researchers can be confident that they have not missed any species with a prevalence of 4%-5%. At least 400 elements are required to assess prevalences of 1% or 99%. The design of an optimum sample size should be based on a flexible strategy that takes into account the study objectives, available resources and precision. This strategy may be based on finding the plateau phase within the precision or CIs curves. As the uncertainty in prevalence decreases rapidly with increasing sample size up to 70-100 individuals, but not much more with further increasing sample efforts, opting for a sample size exceeding this threshold, could be considered an optional choice within the prevalence range of 10% to 90%. We advise authors, editors and reviewers to track sample size in conjunction with the true populational prevalence of parasite or pathogen. The sample size should meet at least the minimum criteria. In case it is not possible to obtain the minimum sample size because of objective reasons authors should note this fact, as any sampled data is important for understanding parasite and host distribution.

*V.S., S.S. and O.L. are funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under project Nos. 09I03-03-V01-00017, 09I03-03-V01-00029 and 09I03-03-V01-00016, respectively. This study was partially supported by the Slovak Research and Development Agency (APVV SK-CZ-RD-21-0078).*

### 003 Neglected taxa shed light on the diversity and evolution of parasitism strategies in Apicomplexa

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Apicomplexa is a group of very successful parasitic protists, occurring in a wide spectrum of invertebrates and vertebrates. The knowledge of Apicomplexa, however, is mainly based on studies of etiologic agents that cause significant diseases (malaria, toxoplasmosis, etc.) and belong to evolutionary advanced lineages. Here, we place our findings on neglected groups (blastogregarines, gregarines, agamococcidia, protococcidia) into a broader context, offering new insights into parasitism strategies in Apicomplexa.

This study is based on our published data obtained using parasitological and protistological approaches, *in vitro* experiments, light and electron microscopy, immunocytochemistry, and molecular phylogeny.

It is assumed that ancestral apicomplexans parasitised marine annelids and then spread to other marine invertebrates followed by freshwater and terrestrial invertebrates, and finally vertebrates. Apicomplexa have evolved unique adaptations for invading and surviving within hosts. This is especially true for the enormously diversified deep-branching groups, which in various ways realise the extracellular, epicellular and intracellular parasitism in different organs and cavities of invertebrates and vertebrates. Basal lineages differ from other Apicomplexa in that their large trophozoites and gamonts are usually motile and their locomotion differs from substrate-dependent, actin/myosin-based gliding described for highly motile apicomplexan zoites. They use several motility mechanisms that represent specific adaptations to parasitism in different environments.

Apicomplexans demonstrate two main determinative evolutionary trends: i) the origination of epicellular parasitism, observed mostly in gregarines and cryptosporidia, with significant modifications to the attachment apparatus and motility mode at the trophozoite stage; and ii) origination of intracellular parasitism, typical of coccidia and Aconoidasida, accompanied by rejection of trophozoite polarity and motility. We propose a possible scenario for emergence of parasitism in Apicomplexa, where evolution progressed from myzocytotic predation (“cellular vampirism”) to myzocytotic extracellular parasitism, and finally to intracellular parasitism.

Our observations emphasise that only the reconciliation of data obtained from all apicomplexan lineages, including those of no economic or medical significance, will make it possible to reconstruct paths of origin and evolution of parasitism in Apicomplexa.

#### **004 Blood parasites of the order Haemosporida and their possible impact to physiology stress of avian host**

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Haemosporidia constitute a group of unicellular protozoan parasites that infect a wide range of vertebrate species. Our research centered on identifying three genera of these parasites — *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* — in avian hosts. Physiological stress, a significant indicator of vertebrate health, can be assessed through leukocyte profiling. This method evaluates the ratios of various white blood cell types in a blood sample, with the heterophil (or neutrophil) to lymphocyte ratio (H/L or N/L) serving as a key stress indicator. Typically, elevated lymphocyte counts suggest optimal health, whereas increased heterophil counts may indicate potential infections.

The objective of our study was to assess the presence, prevalence, and intensity of blood parasite infections and their correlation with physiological stress levels in birds. We utilized leukocyte profiles to evaluate the birds' immune condition. The study was conducted at the Ornithological Station Drienovec in Eastern Slovakia and included birds captured during the breeding season as part of the CES program. Additional sampling sites included the Bratislava Zoo and the campus of the Faculty of Natural Sciences at Comenius University in Mlynská dolina, Karlova Ves, Bratislava, Western Slovakia. At these locations, birds were captured in nest boxes from October to February. Blood samples were collected from the wing vein, stained using the Giemsa-Romanowski method, and parasites were identified morphologically under a light microscope. We analyzed leukocyte profiles to compute the H/L ratio, indicating physiological stress.

Our analysis of 143 avian blood samples revealed a 51% infection rate with haemosporidia. Notably, 18.31% of these cases showed high infection intensity, signalling an acute infection phase. Statistical analysis highlighted a significant increase in H/L ratios among infected birds, with a notable rise in heterophil counts indicating a negative correlation between infected and uninfected individuals. These findings suggest that blood parasite infections contribute to elevated physiological stress levels in birds.

## 005 *Bartonella* in red deer and deer keds

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Red deer (*Cervus elaphus*) are common woodland animals and popular game in Czechia. They are parasitized by two species of keds, the well-known *Lipoptena cervi* and the recently expanding *L. fortisetosa*. Both deer and keds are known to harbor *Bartonella* spp. from lineage 2 (e.g., *B. bovis*, *B. schoenbuchensis*), and although human cases are rare, some of these bartonellae have zoonotic potential. We screened red deer and their keds for bartonellae to determine their role as a potential reservoir.

Tissue samples and keds were collected by hunters from red deer shot in the Krkonoše National Park in 2016-2022. In total, 470 samples were screened by PCR of the *gltA* gene, grouped into genotypes, and multi-locus sequence typing (*gltA*, ITS, *nuoG*, *rpoB*) was performed for each genotype, followed by phylogenetic analysis. Samples containing multiple infections were analysed by nanopore sequencing (ONT) of PCR products to identify individual sequences.

Altogether 17 genotypes belonging to the ruminant-associated *Bartonella* lineage 2 were detected. Six of the genotypes cluster with sequences of unnamed *B. spp* isolated in Japan from sika deer and *L. fortisetosa*. Multiple infections were found in 245 (46%) of *Bartonella*-positive samples, and up to five genotypes of three *Bartonella* species were found in a single deer. There was no correlation between the infection status of a deer and its keds.

The high prevalence and occurrence of multiple infections show that exposure to *Bartonella* is very common in deer. *Bartonella* species originally detected in the native sika deer and *L. fortisetosa* in Japan has been detected in Czechia in both ked species and red deer, showing evidence of a pathogen spillover.

To test the epidemiological significance of deer-associated bartonellae, we also prepared serologic immunofluorescence tests using *Bartonella* strains isolated from keds. We plan to use these tests to look for antibodies in sera of bartonellosis patients and healthy donors.

*We want to thank Jiří Tureček from the Krkonoše National Park administration and all hunters who provided us with deer samples. The project was supported by the Ministry of Health of the Czech Republic, grant No. NU23-05-00511 and Charles University, grant No. 284423.*

**006    Wanted alive: using citizen science to explore diversity of monoxenous trypanosomatids in invasive true bugs (Heteroptera)**

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Citizen science (i.e. involvement of public in research) is a powerful tool for monitoring invasive or endangered species of both animals and plants. However, protozoan parasites have rarely been subjects of such surveys due to their small size and difficult identification. In our ongoing project, with the help of over one hundred volunteers, we have collected more than 300 specimens of economically important invasive bugs (*Leptoglossus occidentalis*, *Nezara viridula* and *Halyomorpha halys*) from more than fifty localities and examined them for the presence of trypanosomatids (Kinetoplastea: Trypanosomatida). So far, *Phytomonas serpens* and an undescribed species of *Obscuromonas* have been found in *L. occidentalis* and an undescribed species of *Phytomonas* in *N. viridula*. The surprisingly frequent occurrence of *P. serpens*, an enigmatic tomato parasite, is particularly peculiar, as its host feeds almost exclusively on conifers. In the future course of this project, we aim to continue collecting data from citizen scientists in the following years, especially after the invasive bugs become established in natural or seminatural habitats and might become infected with native species of trypanosomatids.

## O07 Gut protists in gut-healthy humans and animals: Challenging existing paradigm

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This project investigates the epidemiology of selected intestinal protists in asymptomatic individuals and their animals in the Czech Republic as an example of an industrialized nation. We focus on their prevalence, genetic diversity, effective diagnostics, and possible zoonotic transmission. Although their existence has been known for more than a century, the definitive role of these protists in the gut microbiome remains elusive. Our innovative study breaks the traditional paradigms by shifting the focus from symptomatic individuals with gastrointestinal complaints to the study of individuals with a healthy gut.

In this large-scale study, we successfully collected almost 300 samples from asymptomatic individuals and their animals in 14 Czech regions, using various methods of popularization and involvement of the Czech media. We focused on the protists *Blastocystis* sp., *Dientamoeba fragilis* and *Giardia intestinalis*. Our results show a relatively high prevalence of *Blastocystis* sp. (30%) and *D. fragilis* (24%) in asymptomatic individuals, which challenges the assumption that they are mainly associated with symptomatic individuals. In addition, we identified several *Blastocystis* subtypes, including those with zoonotic potential. Our analysis of *G. intestinalis* in individuals with a healthy gut provides new insights into its epidemiology and ecology.

Our methodological approach involves advanced molecular diagnostics, juxtaposing conventional PCR, qPCR and NGS to uncover the complex genetic diversity of protists of interest and the low colonization intensity. Our study highlights the need for a multi-faceted diagnostic approach and the need to obtain quantitative data on colonization intensity. In the future, such data would help to understand the differences in the functioning of these protists between gut-healthy individuals and symptomatic patients. Furthermore, our data have shown that the diagnosis of *G. intestinalis* and *D. fragilis* is difficult.

Our research not only contributes to a more accurate understanding of gut protists in health and disease and within the One Health Concept, but also encourages a re-evaluation of their importance in the intestinal ecosystem. By studying asymptomatic subjects, we uncover potential reservoirs of transmission and challenge the conventional wisdom about the strict pathogenicity of these protists.

*Acknowledgement:* We thank for the valuable help of K. J. Petrželková and P. Samaš from the Institute of Vertebrate Biology (Brno, CZ), Dana Květoňová and Martin Kolísko from BC CAS (České Budějovice, CZ), Ondřej Cinek from the Motol University Hospital (Prague, CZ) and C. R. Stensvold from the Statens Serum Institut (DK).

*Funding:* This work was financially supported by a grant from the Czech Science Foundation (grant no 22-04837S) to K. Jirků and by grants from the Student Grant Agency (SGA) at the Faculty of Science of the University of South Bohemia to Z.L. (2018), K.B. (2019), A.K. (2020, & M.Š. (2021)



## **O08    *Isospora* and *Lankesterella* parasites (Eimeriidae, Apicomplexa) of passerine birds in Europe**

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Wild birds serve as common hosts for various intracellular parasites, including single-celled eukaryotes of the family Eimeriidae (order Eucoccidiorida, phylum Apicomplexa). This study explores the prevalence, phylogeny, and pathogenicity of *Isospora* and *Lankesterella* parasites in both wild and captive passerine birds.

A total of 815 wild and 15 deceased captive birds, belonging to 66 species, from Austria, Lithuania, Slovakia, and Hungary were subjected to PCR analysis and partial sequencing of mitochondrial *CytB* and *COI*, as well as nuclear *18S* rRNA genes. The overall prevalence of infection in wild birds was 18%, with *Lankesterella* exhibiting a higher prevalence of 11% compared to *Isospora* that presented a 6% prevalence. *Isospora* and *Lankesterella* infections were identified in 64 species of wild passerine birds by PCR and chromogenic *in situ* hybridization (CISH).

CISH using probes targeting the parasites' *18S* rRNA allowed to detect parasites in multiple organs, while haematoxylin-eosin staining facilitated visualization of parasite stages and assessment of associated lesions. *Isospora* parasites were predominantly present in the intestine, spleen, and liver, with associated lymphohistiocytic inflammation observed in varying degrees as well as necrosis in the spleen and liver in a few cases. Systemic isosporosis, formerly known as atoxoplasmosis, resulted in notable pathological lesions in host organs, indicating a potential health risk primarily for ground-dwelling species such as *Passer domesticus*. On the other hand, the detection of fewer *Lankesterella* parasites in tissue sections by CISH, coupled with the absence of corresponding pathological changes in haematoxylin-eosin stains, suggests more frequent subclinical infections in wild passerines.

*Funding and acknowledgment: This research received no external funding.*



## 009 Diversity, phylogeny, and phylogeography of *Dactylogyrus* (Monogenea) in the Middle East: what to expect from?

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In recent years, numerous studies have focused on exploring the diversity and phylogeny of *Dactylogyrus* (Monogenea) parasites found in cyprinoid fish within the peri-Mediterranean region. Despite the Middle East being recognized as a crucial crossroad in the historical dispersion of cyprinoid fish into the peri-Mediterranean, the diversity and phylogeny of their host-specific *Dactylogyrus* species in this region have been overlooked. The objective of the present study was to investigate the diversity, phylogeny, and host specificity of *Dactylogyrus* in the Middle East, aiming to reveal cyprinoid fish's historical dispersion through their *Dactylogyrus* species' phylogenetic relationships.

Through extensive field data collection conducted in Iran, Iraq, and Turkey between 2018 and 2023, we examined the gills of 89 cyprinoid species for the presence of *Dactylogyrus* species. Seventy-two *Dactylogyrus* species were identified and used for the diversity analysis and phylogenetic reconstruction. *Dactylogyrus vistulae*, with 24 host species, has the widest host range in the Middle East. On the hosts, *Squalius* spp. harbored most *Dactylogyrus* species. For phylogenetic reconstruction, we used partial 18S rDNA, 28S rDNA, and the complete ITS1 region. Phylogenetic analyses, incorporating DNA sequences of *Dactylogyrus* obtained from GenBank and species collected from the Middle East, revealed the presence of seven major clades. Middle Eastern species were categorized into four clades alongside European, North African, and East Asian species, thus confirming the significant role of the Middle East in the diversification of *Dactylogyrus* and providing additional insights into the historical dispersion of cyprinoid fish.

During this investigation, we uncovered 16 potentially new *Dactylogyrus* species for science originating from the Middle East. Surprisingly, several *Dactylogyrus* species (e.g., *D. vistulae*, *D. goktschaicus*, *D. linstwoi*) exhibited high genetic intraspecific variabilities. The genetic intraspecific variability associated with geographical distribution and host phylogeny, highlighted the underexplored diversity of *Dactylogyrus* in the Middle East.

*This study was supported Czech Science Foundation, project no. GA20-13539S.*

**O10 Unveiling the diversity and evolutionary patterns of *Gyrodactylus* (Gyrodactylidae) communities in Nearctic cypriniform fish hosts: insights from species richness, morphology, and molecular phylogeny**

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Despite the vast diversity of freshwater fishes in the Nearctic region, our understanding of the community composition of viviparous monogeneans belonging to genus *Gyrodactylus*, as well as their phylogenetic relationships within congeners worldwide, remains limited. In this study, we conducted morphological and genetic analyses of *Gyrodactylus* spp. parasitizing a wide range of cypriniform fish species found in North American watersheds across the USA and Canada. Our comprehensive examination, combining taxonomically significant haptor features with sequencing of the ITS regions and the 18S rDNA, identified 25 *Gyrodactylus* spp. parasitizing members of Catostomidae and Leuciscidae. Remarkably, ten of these monogenean species were newly discovered. Phylogenetic analysis based on ITS region sequences revealed the paraphyletic origin of Nearctic *Gyrodactylus* lineages and their close relationship with Palearctic congeners, suggesting dispersal events between continents.

In the Nearctic region, we observed a trend in the evolution of haptor structures in *Gyrodactylus* spp., from relatively simple forms to more complex ones, mirroring patterns seen in other monogenean taxa. Haptor sclerites of *Gyrodactylus* spp. exhibited a spectrum of morphotypes, ranging from structures resembling those found in *Gyrodactylus* communities with global distributions to configurations predominantly seen in Nearctic lineages. Characteristic features of "Nearctic" morphotypes included a median knob in the ventral bar and plate-like membranes or additional filaments attached to the handles of marginal hooks.

Our integrative approach also provided insights into ongoing gene flow, host-switching in generalist *Gyrodactylus* spp., and the regional translocation of monogenean fauna through fish introductions. These findings shed light on the evolutionary dynamics and ecological interactions shaping *Gyrodactylus* communities in North American freshwater ecosystems.

*This study was funded by the Ministry of Education, Youth and Sports of the Czech Republic, project no. LTAUSA18010 (2019-2022) and project no. LUAUS23080 (2023).*

## **O11 Host-specific parasites revealing the biogeographical contacts of freshwater fish**

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Host-specific parasites exhibit close coevolutionary associations with their hosts. They may reflect the historical biogeography of their hosts, especially those exhibiting disjunctive or fragmented distribution, such as freshwater fish. Monogeneans of the genus *Dactylogyrus* are gill ectoparasites almost exclusively restricted to cyprinoid fish, often exhibiting strict or phylogenetic host specificity. We used host-specific parasites to reveal the historical biogeographical contacts of cyprinoid fish between North America and Europe and the more recent contacts of leuciscid fish in North America.

*Dactylogyrus* species were collected from cypriniforms (Leuciscidae and Catostomidae) in North America. Other *Dactylogyrus* species included in the phylogenetic analyses were obtained from our previously published studies. Molecular phylogeny based on three nuclear markers (18S, 28S and ITS1) was reconstructed. Mapping of geographical distribution and fish lineages onto phylogenetic tree was applied to investigate the origin of Nearctic *Dactylogyrus* and host switches of *Dactylogyrus* between clades of Holarctic cyprinoids.

Host-specific *Dactylogyrus* parasitizing Nearctic cypriniforms formed two independent clades with different origins likely associated with different historical routes of cyprinoid dispersion to North America. In the Nearctic region, *Dactylogyrus* switched catostomid fishes. Within two Nearctic clades, *Dactylogyrus* species did not reflect the phylogenetic relationships among leuciscid clades. We showed that the historical contacts between European and North American leuciscids were accompanied by the host switching of *Dactylogyrus*.

We highlight the importance of host-specific monogeneans as a suitable tool to infer the historical biogeography of freshwater fish exhibiting disjunctive distribution.

*This study was supported by the Ministry of Education, Youth and Sports of the Czech Republic, project no. LUAUS23080.*

## O12 Parasites as biological tags of self-spreading populations of neolimnetic fish species

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The neolimnetics is a group of brackishwater species which spread their ranges into freshwaters, mainly along the riverine flows (Kvach & Kutsokon 2017). The monitoring of tag markers of the neolimnetic populations, e.g. parasitological, population and genetic ones, allow to discriminate a certain population within a water body and river basin, assessing its invasive potential. It makes possible to determine with a high probability the individuals outside the native range, which are originated from a studied indigenous population.

Ukraine is located at the intersection of transport corridors and routes of aquatic alien species spread. These so-called aquatic invasion corridors are used by neolimnetics to expand their ranges, i.e. by Ponto-Caspian gobies, Ukrainian stickleback (*Pungitius platygaster*), black-striped pipefish (*Syngnathus abaster*), big-scale sand smelt (*Atherina boyeri*).

For each neolimnetic species, the marker parasites that discriminate populations were delineated based on published data, as well as own study. The monogenean *Gyrodactylus proterorhini*, digenean *Nicola skrjabini*, and cestode *Ligula pavlovskii* were proposed as marker monitoring species for Ponto-Caspian gobies. The Ukrainian stickleback populations could be discriminated by the presence of microsporidia *Glugea* sp., monogenean *Gyrodactylus arcuatus*, digenean *Bunocotyle cingulata*, nematodes *Dichelyne minutus*, *Raphidascaris acus*, and parasitic copepod *Thersitina gasterostei* (Gaevskaya et al. 1975). But only last one, *Th. gasterostei*, could be used as a marker species. The parasite fauna of the black-striped pipefish contains only one marker species – *Trichodina partidisci* (common in sea ecosystems), which can be an informative indicator. The big-scale sand smelt is a host of number of specific species of monogeneans, in particular *Gyrodactylus ginestrae*, which was recorded from the Gulf of Odesa. This species is considered as the marker.

Thus, populations of neolimnetic fish species, as 'youngest' migrants to fresh water bodies, bring their specific parasites to new habitats and become infected with aboriginal species during their spread from their native brackishwater range to freshwater ecosystems and vice versa.

*Funding and acknowledgment. This study was partly supported by the European Union NextGenerationEU under the "Scholarships for excellent researchers threatened by the conflict in Ukraine" program, specifically through project No 09I03-03-V01-00004.*

**O13 New discovery and phylogenetic position of *Salsuginus* parasite (Monogenea: Dactylogyridae) in Western mosquitofish (*Gambusia affinis*)**

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The Western mosquitofish, *Gambusia affinis* (Baird and Girard, 1853), belonging to Poeciliidae, is naturally found across the Mississippi and Gulf Coast drainages in North America. However, it has been widely introduced globally to control mosquito-borne diseases. In our study, we document the presence of a commonly distributed dactylogyrid monogenean, *Salsuginus seculus* (Mizelle and Arcadi, 1945) Murith and Beverley-Burton, 1985, parasitizing *G. affinis* in the North Fork Guadalupe River in Texas, USA. This species exhibits high host specificity and is predominantly associated with *G. affinis*. Our discovery marks the fourth recorded instance of *S. seculus* in Texas.

The meristic characteristics of *S. seculus* largely coincide with those observed in previous studies conducted in Texas. However, we observed intraspecific variability when comparing our findings with type-material collected from the Western USA, as well as with specimens of *S. seculus* found to parasitize non-indigenous *G. affinis* in Japan. Analysis of 28S rDNA sequences places *Salsuginus* as a sister taxon to a monophyletic group of dactylogyrids (*Gussevia*, *Sciadicleithrum*, and *Parasciadicleithrum*) associated with Neotropical Cichlidae. This group likely originated from the Great American Biotic Interchange facilitated by the closure of the Panama Isthmus, which promoted the mixing of diverse freshwater fish lineages.

Our study emphasizes the importance of investigating the potential co-introduction of monogeneans along with *G. affinis* into new freshwater systems and its impact on native fish populations.

*This study was funded by the Ministry of Education, Youth and Sports of the Czech Republic, project no. LUAUS23080. Fish sampling and processing was funded by US Fish and Wildlife Service's Sport Fish Restoration Grant to Texas Parks and Wildlife Department.*

**O14 *Diplostomum pseudospathaceum* (Trematoda) inducing differential immune gene expression in asexual and sexual gibel carp (*Carassius gibelio*)**

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Parasite-mediated selection is considered one of the potential mechanisms contributing to the coexistence of asexual-sexual complexes. Gibel carp (*Carassius gibelio*), an invasive fish species in Europe, currently often forms populations composed of asexual (reproducing by gynogenesis) and sexual specimens.

We performed the experimental infection of eye-fluke *Diplostomum pseudospathaceum* (Trematoda) in gynogenetic and sexual gibel carp. The transcriptome profile of the fish spleen was analyzed to reveal the differentially expressed immunity-associated genes (DEGs) related to trematode infection focusing on differences in DEGs between gynogenetic and sexual gibel carp.

Gynogenetic fish were more parasitized than sexuals. Although metacercariae of *D. pseudospathaceum* are situated in an immune-privileged organ, our results showed that eye trematodes may induce a host immune response. DEGs induced by eye-fluke infection were revealed, with various impacts on gynogenetic and sexual gibel carp. Specifically, the majority of DEGs were upregulated in sexual gibel carp, whilst downregulated in gynogenetic gibel carp. Differences in gene regulation between gynogenetic and sexual fish were evidenced in many immunity-associated genes. The following GO terms associated with immunity were recognized for DEGs: immune function, Notch signaling pathway, MAP kinase tyrosine/threonine/phosphatase activity, and chemokine receptor activity. KEGG analyses revealed the importance of the genes involved in 12 immunity-associated pathways: FoxO signaling, adipocytokine signaling, TGF-beta signaling, apoptosis, Notch signaling, C-type lectin receptor signaling, efferocytosis, intestinal immune network for IgA production, insulin signaling, virion - human immunodeficiency virus, Toll-like receptor signaling, and phosphatidylinositol signaling system.

Our study indicates the limited potential of asexual fish to cope with higher parasite infection (likely a loss of capacity to induce an effective immune response) and highlights the role of molecular mechanisms associated with immunity for the coexistence of asexual and sexual fish.

*The study was funded by the Czech Science Foundation, Project No. 22-27023S.*



**O15 Comparative analysis of paratenic hosts' infectivity by *Angiostrongylus cantonensis*: Exploring varied host responses**

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The rat lungworm *Angiostrongylus cantonensis* is a zoonotic metastrongyloid nematode, currently considered an emerging pathogen approaching Europe. In tropics and subtropics, the parasite is an important food-borne neurotropic pathogen of medical and veterinary importance. Key sources of infection for mammals and birds (aberrant hosts) include gastropod intermediate hosts and paratenic hosts, both terrestrial and aquatic. Many poikilotherms are referred to as paratenic hosts of the parasite, however we hypothesize their effectivity in infection transmission to definitive or aberrant hosts varies greatly.

We performed a series of experiments to evaluate the relevance of two purported paratenic hosts, fish and reptiles, as potential sources of infection. Two fish species (*Oreochromis niloticus*, n = 24 and *Clarias gariepinus*, n = 30) were infected with 10,000 third-stage larvae (L3) each. After one, two and three weeks, no live L3 were found in musculature or viscera. Three days post infection, tens of live L3 were present predominantly in guts and were proven to be infective for Wistar rats.

Twenty leopard geckos (*Eublepharis macularius*) were infected with varying doses of L3: 100 in G1 (n = 5), 1000 in G2 (n = 5) and 10,000 in G3 (n = 10). Live L3 were collected from all infected geckos euthanized one and two months after the infection. Live L3 were present mostly in musculature (in G3, hundreds of L3). Live larvae from geckos were proven to be infective for Wistar rats. Three geckos from G3 were fed to three corn snakes (*Pantheropsis guttatus*) to assess the possibility of intermediation. Snakes were euthanized one-month post-infection and 11, 3 and 10 live L3 were present predominantly in guts and viscera. No reptiles showed significant changes in haematological and biochemical parameters in their blood tests, and they remained clinically healthy throughout the study.

Our findings demonstrate considerable variability in the relevance of potential sources of *A. cantonensis* infection for humans and animals. While both fish and reptiles are considered important paratenic hosts, our experiments demonstrate differences in larval survival, infectivity and tissue migration. These results underscore the complexity of *A. cantonensis* transmission dynamics and emphasize the need for further research to elucidate host-parasite interactions.

*Experimental procedures followed animal welfare standards and regulatory permissions (approval of the Ministry of Education, Youth and Sports 15292 and 22064/2022). Funding: SEAEUROPEJFS19IN-053 grant and Czech Science Foundation grant 22-26136S.*

## O16 Fast and curious: the role of agile snails in *Angiostrongylus cantonensis* life cycle

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*Angiostrongylus cantonensis*, also known as the rat lungworm, is a metastrongyloid nematode with zoonotic potential. Its life cycle involves rats as definitive hosts, various species of gastropods as intermediate hosts and a range of other invertebrates may serve as paratenic hosts. Humans, dogs and other warm-blooded vertebrates can become accidental hosts by ingestion of either the intermediate host or the paratenic host containing the L3 larvae. However, the infective L3 larvae can also be shed by gastropods and contaminate water and food sources. In accidental hosts, the larvae mature in the brain, where they cause potentially life-threatening eosinophilic meningitis. This parasite was originally described in Southeast Asia, but is gradually expanding its distribution globally, including Europe. Many gastropods have been described as possible intermediate hosts, yet the role of individual species in the parasite's life cycle has been rather understudied, although some differences can be expected.

In the present study, we compared the prevalence of *A. cantonensis* in two gastropod species endemic to Canary Islands but differing in ecology: a snail *Hemicycla bidentalis* (Helicidae) and a semi-slug *Insulivitrina lamarckii* (Vitrinidae). Overall, 139 gastropod specimens were collected at a model locality in an endemic area of the parasite in Tenerife (Canary Islands, Spain). We screened for *Angiostrongylus cantonensis* DNA in the snail tissue and rat DNA in the snail feces.

We detected the rat lungworm DNA in 70.4% of the fast-moving semi-slug *I. lamarckii*, whereas in the slower-moving snail *H. bidentalis* the prevalence was only 39.66%. These results suggest that agile gastropod species are able to encounter and ingest rat feces in the wild more frequently and thus increasing the chance of getting infected by *A. cantonensis*. Different prevalence in individual intermediate host species is an important factor in the epidemiology of the parasite and may be useful in screening for the rat lungworm presence and infection burden in the environment.

*The field sample collection was possible thanks to the Internal Mobility Agency at University of Veterinary Sciences Brno which funded JK's internship at the Universidad de la Laguna. Part of the subsequent laboratory analyses was funded by the Czech Science Foundation grant 22-26136S.*



## O17 Newly established parasites and new challenges for veterinary practice in Slovakia

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Parasitic infections remain a global problem to human and animal health. Anthropogenic activities (globalization, deforestation, urbanization, extensive farming, etc.) together with climate change are increasingly affecting the structure and functioning of parasite ecosystems which in response to them are constantly changing concerning their geographical distribution, host range, and the emergence of new or unexpected problems. In Slovakia, as in most of Europe, new parasitic infections, mainly vector-borne, have emerged in recent years as a result of such changes, posing significant challenges for veterinary as well as human diagnostics and treatment.

In Europe, for several decades are of great concern mosquito-transmitted filaroid nematodes, *Dirofilaria repens* and *D. immitis*, causing subcutaneous, or severe cardiopulmonary disease in canines, but also humans. In Slovakia, canine dirofilariosis was reported for the first time in 2005 (Svobodová et al., 2005). For nearly 15 years *D. repens* has been the main causative agent of infection in the dog population in our territory. However, the situation has recently turned significantly and a fundamental change in the *Dirofilaria* species distribution of occurred. Nowadays, *Dirofilaria immitis* has become the dominant causative agent responsible for 60 % of all cases, often with a fatal course.

Another life-threatening vector-borne parasite of dogs, the spread of which has been recorded within Europe in recent years, is *Angiostrongylus vasorum*. Slovakia, the first canine angiostrongylosis cases were reported in 2012 and 2013 from eastern Slovakia (Hurníková et al. 2013; Miterpáková et al. 2014). The following copro-epidemiological research in the territory of Slovakia revealed a relatively high prevalence of *A. vasorum* in dogs and red foxes (4.13 % and 5.43 %, respectively) (Miterpáková et al. 2015; Čabanová et al. 2018a, 2018b). *A. vasorum* causes cardio-respiratory diseases manifested by chronic, coughing, exercise intolerance, dyspnea and tachypnea, coagulopathies, and neurological damage. However, symptoms are often non-specific and many infections have a lethal demise.

A new diagnostic challenge for veterinary practice is posed by infections with parasites that are not yet present in our territory. One such example is canine leishmaniasis, caused by protozoan parasites of the genus *Leishmania*, which is expected to become increasingly common in the foreseeable future, particularly in association with imported infections associated with travel with dogs to endemic areas in southern Europe or with the importation of dogs from these areas. In the context of a rapidly warming climate and considering that Phlebotomine sand flies have already been reported in Slovakia (Dvořák et al., 2016), it can be assumed that epidemiologists and veterinarians can also anticipate the occurrence of autochthonous cases in Slovakia in the near future.

In this contribution three fatal case-reports will be presented, caused by *Dirofilaria immitis*, *Angiostrongylus vasorum*, and *Leishmania* spp., respectively.

*Acknowledgment: The work was funded by the project VEGA 2/0014/21.*

## O18 Human dirofilariasis in Slovakia – or "Hey, Doctor, something is moving under my skin!"

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Human dirofilariasis is considered an emerging parasitosis on the territory of Central Europe, including Slovakia, with globalisation and climate change playing a significant role in its spreading. The life cycle of *Dirofilaria* spp. involves carnivores, especially dogs, as definitive hosts and different species of mosquitoes as intermediate hosts or vectors of infection. The aim of the study is to summarize published and unpublished cases human of *Dirofilaria* infection in Slovakia with an emphasis on their clinical picture and epidemiology.

The first human case of dirofilariasis in Slovakia was diagnosed in 2007, two years after the infection had been observed in the Slovak dog population. Since then, 29 human cases caused by *Dirofilaria repens* have been confirmed at the Institute of Parasitology SAS. Of them, 19 cases presented as subcutaneous form, in 6 patients the worm was located in subconjunctival space/tissues (ocular form), one patient suffered from very rare pulmonary form of infection, in one case the parasite was located in the left testicle and in one patient a live worm was found in the sputum. In 2021, the first autochthonous case of human *D. immitis* infection manifesting by the formation of nodule in right lung parenchyma was confirmed in a woman from Trnava region. Moreover, several other cases of dirofilariasis were reported, but without any details necessary to confirm the diagnosis.

The majority of patients came from the southern regions of Slovakia, which are considered endemic for *D. repens*. On the other hand, recent data from several European countries, including Slovakia, indicate dramatic increase of *D. immitis* in the canine population. Therefore, we can assume the rise of both, *D. repens* and *D. immitis* human cases in Slovakia in the near future, pointing out the need of appropriate awareness of the disease in clinicians as well as in dog owners and general public.

*Funding and acknowledgment. Study was financially supported by the project VEGA 2/0014/21.*

## O19 Initial satellitome analysis of two *Acanthocephalus* species: searching for new potential cytogenetic markers

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Our study represents the first satellitome analysis of two *Acanthocephalus* species, *A. anguillae* and *A. lucii*, focusing on the identification of species-specific satellite DNA sequences. Satellites are known for their considerable variability in both sequence composition and copy number, even within closely related species. These characteristics make them invaluable for the study of genome diversity at both intra- and interspecific levels.

For this purpose, we used RepeatExplorer2 pipeline, a bioinformatic tool for identification and characterization of repetitive elements in low-throughput Illumina sequenced data. The analysis characterized the majority of repeats in each species as satellite DNA, with a considerable number identified as species-specific. Subsequent filtering using a threshold of a minimum of 700 reads within a repeat cluster led to the identification of 11 species-specific satellites in the *A. anguillae* and 16 in the *A. lucii* genome. The physical mapping by fluorescence in situ hybridization (FISH) on the chromosomes of *A. anguillae* was successful for five satellites. Each showed a cluster pattern at a specific chromosomal position, in centro-pericentromeric, telo-subtelomeric or interstitial regions. FISH using *A. lucii*-specific satellites as probes was successful with only two repeats, with hybridization signals observed in centromeric or telomeric positions. No FISH-positive signals were found on the B chromosomes of either *A. anguillae* or *A. lucii*, supporting the hypothesis of a recent origin of the supernumerary chromosome in this group of parasites.

Species-specific repeats and their distribution can be valuable to distinguish morphologically similar species and to identify specific chromosomes and homologous chromosome pairs within karyologically challenging species. Due to the small size and ambiguous morphology of the chromosomes in the species studied, the process of pairing homologous chromosomes is complicated. The newly identified satellites have demonstrated their significance as valuable cytogenetic markers and have proved useful in karyotyping. However, for accurate identification of homologues, especially in the case of *A. lucii*, the inclusion of additional markers is required.

*Acknowledgements:* The study was supported by the Scientific Grant Agency of the Ministry of Education, Research, Development and Youth of the Slovak Republic and the Slovak Academy of Sciences (VEGA No. 2/0093/23)

## O20 Role of hedgehogs in the life cycle of *Angiostrongylus cantonensis*

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The ongoing invasion of the zoonotic metastrongyloid nematode *Angiostrongylus cantonensis* is not only a threat to humans but also to a range of hosts such as mammals and birds that can be affected as aberrant hosts. In Mallorca, increased incidence of neurological disorders caused by *A. cantonensis* has been observed in free-ranging hedgehogs *Atelerix algirus*. Based on published findings, a series of experimental infections of closely related *A. albiventris* was conducted to determine the course of infection and to evaluate the role of the hedgehog in the parasite life cycle. Five and six captive-borne hedgehogs divided into two experimental groups were inoculated orally with two different doses (200 and 2000) of third-stage larvae (L3) of *A. cantonensis* (Tenerife strain). The behavior and health status were monitored for 50 days with repeated blood sampling. From day 39 post-infection (DPI), feces were collected and examined by Baermann's method. Neuropathological symptoms were observed only in the high-dose group, which had to be gradually euthanized on 5 DPI, 15 DPI, 23 DPI, and 44 DPI. The low-dose group showed no clinical findings, half of the group was euthanized on 30 DPI and the remaining on 50 DPI. The tissues isolated during necropsy were preserved for further qPCR analysis and histology. No alterations in blood parameters were observed in hematological and biochemical examination. DNA of *A. cantonensis* was present in organs of both groups, with observable neurotropic migration only in the high-dose group, as confirmed by presence of cross-section of *A. cantonensis* subadult stages in brain tissue. The results of the study confirm the migration of subadult stages to CNS, as typical in aberrant hosts of *A. cantonensis*. Neither necropsy nor larvoscopy confirmed the presence of adult stages in the lungs and first-stage larvae in the feces. Based on these results, we hypothesize that free-ranging Algerian hedgehog in Mallorca enter the parasite life cycle as aberrant hosts. However, the course of infection is likely to depend on the number of ingested larvae.

*Experimental procedures adhered to animal welfare standards and regulatory permissions. The research was supported by the project of Czech Science Foundation No. 22-26136S. Permit number for working with animals: 22063.*

## 021 Modulation of M1/M2 macrophages by excretory-secretory products of *T. spiralis* larvae

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Macrophages are effector cells of the innate immune system that are divided into two groups according to their activation: M1 or M2 macrophages. M1 type are classically activated macrophages by IFN- $\gamma$  or lipopolysaccharide (LPS), produce proinflammatory cytokines and initiate an immune response. M2 type are alternatively activated macrophages, typically by IL-4 or IL-10 production and are associated with tissue repair. Th2 immune response is dominant in *T. spiralis* infection when new born larvae begin to migrate in muscle phase. M2 macrophages are related to the Th2 immune response in anti-helminthic defence. In our study we focused on influence of the excretory-secretory products of *Trichinella spiralis* (ES T.sp.) on the macrophage M1/M2 polarization.

Mouse RAW 264.7 macrophage cells were grown in Dulbecco's modified eagle medium (DMEM) with 10% fetal bovine serum (FBS) and antibiotics (Penicilin-Streptomycin) at 37°C and 5% CO<sub>2</sub>. After cells were achieved in 90% confluency, they were stimulated with excretory-secretory products of *T. spiralis* (7 $\mu$ g/ml) and IL-4 (20 ng/ml) for 18 hours. Then, cells were incubated with LPS (200 ng/ml) for 12 hours. At the end, mRNA was isolated for cDNA transcription. The changes in mRNA expression of M1 (TNF- $\alpha$ , IL-1 $\beta$ , IL-23, CCL2) and M2 (TGF- $\beta$ , IL-10, Arg-1, CD206, Fizz-1, CXCL10) markers were detected with qPCR method. Macrophages incubated with ES T. sp. significantly decreased proinflammatory IL-1 $\beta$ , IL-6, IL-23, TNF- $\alpha$  and CCL2 production. These Th1 cytokines and CCL2 chemokine are effective in *T. spiralis* larvae destruction. However, ES T. sp. induced high level of two major M2 markers Arg-1 and Fizz-1 in macrophages, that has a role in tissue repair and wound healing. We found increased level of CD206 (Man receptor of macrophages) in cells treated with ES T.sp. Production of CXCL10 was also upregulated by ES T. sp., but the level of anti-inflammatory cytokines TGF- $\beta$  and IL-10 was not significant. As the result, *T. spiralis* infection increased M2 population of macrophages.

We confirmed that excretory-secretory products of *T. spiralis* larvae induce M2 macrophage polarization by increasing the expression of Arg-1, CD206, Fizz-1, CXCL10 and inhibit M1 markers (IL-1 $\beta$ , IL-6, CCL2). *T. spiralis* larvae enhance M2 macrophage polarization as part of their escape mechanisms from immune response.

*The study was supported by VEGA 2/077/23.*

## O22 Molecular characterization of *Dictyocaulus* spp. lungworms in wild ungulates in Lithuania

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*Dictyocaulus* spp. are lungworms of significant economic importance. Infected animals can transmit parasitic disease between wild and domestic ruminants. These parasites are an agent causing distyocaulosis characterized by symptoms of bronchitis, with infection leading to fatal outcomes in heavily-infected individuals.

The aim of this study was to perform the identification of lungworms collected in red deer *Cervus elaphus*, roe deer *Capreolus capreolus*, moose *Alces alces*, and European bison *Bison bonasus* using morphological and molecular methods. 28 red deer, 37 roe deer, 11 moose, and 31 European bison lungs were analysed for the presence of lungworms in 2021-2024. Lungworms were collected from the trachea, bronchi, and bronchioles. Identification of lungworms until genus level was done by morphological criteria. DNA was extracted from the adult lungworms by using a QIAGEN QIAamp DNA Mini Kit. The *cox1*, *SSU*, and *cytB*, genes were amplified by PCR and sequenced. A total of 1109 lungworms were collected (1028 in red deer, 28 in roe deer, 43 in moose, and 10 in European bison).

The morphological characteristics of the lungworms showed that ungulates were infested with *Dictyocaulus* spp. Additionally phylogenetic analysis was conducted. Our study underlines the importance of molecular analyses for lungworm identification, specifically for *Dictyocaulus* species in wild ungulates and domestic ruminants.



## O23 Helminth diversity in *Pelophylax* spp. and *Rana temporaria* in Lithuania

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Amphibians are essential subjects for parasitological studies due to their wide range of trophic connections and adaptability to various ecosystems. To the date, no comprehensive studies on helminths of frogs have been performed in Lithuania, except one survey of helminth species from anurans in Kaunas performed over 60 years ago.

Our study aims to assess the current diversity, occurrence and abundance of helminths in common frog (*Rana temporaria*) and green frogs (*Pelophylax* spp.) in Lithuania. In total, 60 amphibians (22 *R. temporaria*, 38 *Pelophylax* spp.) were sampled from 13 localities. Helminths were cleared in lactophenol, examined and identified under the light microscope. Further molecular analysis is required to confirm morphological identifications of some species.

In the entire sample, we found 23 helminth species: trematodes (16), nematodes (5), monogeneans (1), and acanthocephalans (1). Out of 9 species infecting *R. temporaria*, nematodes (4) were the most abundant and prevalent group: each species infected more than half of hosts. *Cosmocerca ornata* and *Oswaldocruzia bialata* reached the infection prevalence of over 70%. The monogenean *Polystoma integerrimum* infected half of the frogs. Dominance of monoxenous nematodes and monogeneans in *R. temporaria* is attributed to its terrestrial lifestyle.

Among 18 species parasitizing *Pelophylax* sp., trematodes (14) were the most abundant and widespread group. Three trematode species were represented by metacercariae; besides, *Codonocephalus urniger* and *Tylodelphys excavata* reached the highest abundance values among all helminths in the entire sample (>1000 specimens). Adult *Opisthioglyphe ranae* infected more than 60% of hosts. Among the nematodes infecting green frogs, *Icosiella neglecta* was the only heteroxenous species; it reached the prevalence of 20% and was found in 3 localities, for the first time in Lithuania. Higher helminth diversity in green frogs and dominance of species with complex life cycles is attributed to semi-aquatic lifestyle of these hosts. Results of this study match the statement of Koprivnikar that helminth diversity in frogs is related to the host order and ecology (Koprivnikar et al., 2012).

*The study is supported by the Nature Research Centre and the European Molecular Biology Organization (EMBO) Solidarity Grant. The author is grateful to Dr. Vytautas Rakauskas for the help with sampling frogs and to Dr. Oleksii Marushchak for the identification of frogs.*

## O24 Diversity of trematode parasites in European water frogs of genus *Pelophylax*

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Although the species distribution of water frogs in the Western Palaearctic is relatively well-investigated, little is known about the species richness of the parasite fauna associated with individual species. Our research expands knowledge about the species diversity of trematodes parasitizing water frogs *P. esculentus* and *P. ridibundus* in southern Slovakia, as well as *P. epeiroticus*, *P. kurtmuelleri*, and *P. shqipericus* in the Balkans, and provides the first molecular data for numerous local trematode taxa. A total of 264 water frogs were collected at 23 localities in Slovakia and the Balkans and examined for the presence of trematode parasites. Altogether, 17 trematode genera were found in the host frogs. A combination of morphological and molecular data was used to identify individual species, revealing five potentially new species. The highest species diversity was observed among *Haematoloechus* lung flukes, with three species identified. To quantify parasitic infection, basic epidemiological parameters were determined for individual species of trematodes, and diversity indices were calculated to assess alpha diversity. The highest mean prevalence was recorded for *Opisthioglyphe ranae* found in the intestine of frogs, the only representative of this monotypic genus. A phylogenetic analysis using partial 28S rRNA divided the found species of trematodes, belonging to eight families, into five well-supported groups. The partial sequences of the *cox1* mitochondrial gene were used to investigate genetic variability among collected taxa, and substantial intraspecific genetic variability was recorded in species with a wide distribution range (e.g., *Diplodiscus subclavatus*, *Haematoloechus variegatus*, or *Opisthodiscus diplodiscoides*). Our novel molecular data provide a new perspective on the taxonomy of frog trematodes and further highlight the underexplored diversity in Europe.

*The research was carried out under the following permits: Romania: no. 362, 27 June 2023, Administratia Rezervati ei Bios Ferei Delta Dunarii; Greece: No. 123199/3356/01-02-2021, Directorate of Forest Management, Ministry for Environment and Energy of the Hellenic Republic; Albania: no. 480/2019, National Agency of Protected Areas, Ministry of Tourism and Environment of the Albanian Republic; Slovakia: No. 519/2022, The Ministry of Environment of the Slovak Republic.*

*Acknowledgement. This study was financially supported by projects VEGA 1/0583/22 and APVV DS-FR-22-0006.*



## O25 The little-known *Metagonimus romanicus* with zoonotic potential

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The trematode *Metagonimus romanicus* was described by Ciurea (1915) as *Loossia romanica* from a domestic dog from the Danube Delta in Romania. In the course of its long and confused taxonomic history, this species, endemic to Europe and neighbouring regions (northwestern Turkey), was usually misidentified as *M. yokogawai* (Katsurada, 1912), a human parasite in East Asia.

In the present work, we have redescribed *M. romanicus* on the basis of the type material from domestic dogs (*Canis familiaris*) in Romania, and vouchers from experimentally infected cats (*Felis catus*). Additionally, adult trematodes were obtained from golden hamsters (*Mesocricetus auratus*) infected with metacercariae from the scales of the chub (*Squalius cephalus*) and common nase (*Chondrostoma nasus*). The two species, *M. romanicus* and *M. yokogawai*, differ considerably from each other, both genetically and morphologically, e.g. in the position of the ventral sucker, the presence of the prepharynx, the anterior extent of the vitelline follicles and the posterior extent of the uterus. A molecular phylogenetic analysis of *M. romanicus*, based on three genetic markers (18S rRNA, 28S rRNA and COI gene sequences), revealed that it is the earliest diverged taxon of *Metagonimus*.

A literature search revealed that *M. romanicus* is a widespread intestinal parasite of a variety of piscivorous mammals and birds in central and southeastern Europe. It is particularly widespread in the lower reaches of the rivers in the Black Sea-drainage area (mainly the Danube, Dniester and Dnieper), while it is apparently absent from the upper reaches of the Dnieper and Danube. This distribution corresponds to the distribution of the first intermediate hosts, *Esperiana esperi* and *Microcolpia daudebartii acicularis*. The most suitable definitive hosts are dogs and cats. The zoonotic significance of *M. romanicus* is unclear, but appears to be low in Europe, mainly because raw or undercooked, whole fish with scales are generally not consumed.

*This study was supported by the Slovak Research and Development Agency (APVV SK-CZ-RD-21-0078) and the Ministry of Education, Youth and Sports of the Czech Republic (project LUASK 22045)*

## O26 *Collyriclum faba* infestation in the Great reed warbler

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The cutaneous monostome trematode *Collyriclum faba* (Bremser in Schmalz, 1831) is an avian parasite with a specific life cycle. Most cases of clinical collyriclosis are reported by field ornithologists, bird ringers and by the staff of wildlife rehabilitation stations.

In our work, we report a case of collyriclosis in an adult male of the Great reed warbler *Acrocephalus arundinaceus* (Linnaeus, 1758) from Drienovská wetland, Slovakia (GPS: 48.61515, 20.91516).

Infested bird was recorded and trapped by ornithological mist-nets twice (24<sup>th</sup> May 2023 and 20<sup>th</sup> June 2023) during regular monitoring of breeding avifauna by Constant effort site ringing method. Infected Great reed warbler was in a good condition but without morphological signs of breeding (absent cloacal protuberance).

Dermal cysts of *C. faba* (n=22) were located on ventral parts of infested host. All cysts were excised and trematodes were extracted after the second trapping of infested specimen. After the extraction, trematodes were stored in a sampling tube with 70% ethanol. Wounds by cysts were disinfected and the bird was released on the place of trapping.

Six specimens of extracted *C. faba* were determined morphologically and two specimens were identified by molecular method.

The DNA from ethanol-fixed trematode specimens was isolated using the DNeasy® Blood & Tissue kit (Qiagen). Trematode species was determined by amplifying of two nuclear DNA loci: a fragment of 18S rDNA and a full-length internal transcribed spacer 2 (ITS2) with the flanking conserved 5.8S and 28S rDNA region sequences.

Sequencing of ITS2 suggested that both specimens share a similarity ranging from 100% to 92.5% with *C. faba* isolates deposited in the GenBank database. Sequence data of partial 18S rDNA and ITS2 of both *C. faba* isolates from this study were uploaded to the GenBank database under accession numbers PP316965, PP316966 and PP317288, PP317289 respectively.

*Acknowledgment: This work was supported by the project VEGA 1/0316/23 granted by Ministry of Education, Research, Development and Youth of the Slovak Republic.*

**O27 Proven performance in diagnostics of Lyme Borreliosis and Neuroborreliosis**

K. Ryyänänen, M. Šišák

ABSTRACT NOT PROVIDED

**O28 Active water vapour uptake in unfed and engorged *Dermacentor marginatus* (Acari, Ixodidae)**

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Active water vapour uptake from unsaturated air is the principal source of water for unfed ticks in a mostly dehydrating atmosphere, i.e., at  $\leq 99\%$  RH. Engorged larvae and nymphs of at least some species in the genera *Ixodes*, *Haemaphysalis* and *Argas* are capable of gaining atmospheric water in this way. The critical lower threshold for this oral process is usually 80–85% RH. It is unknown, however, whether engorged *Dermacentor* ticks are able to do so.

This was investigated by exposing unfed and engorged immatures and engorged males of the Eurasian 3-host tick *D. marginatus* sequentially to low and high RHs and weighing them intermittently on a microbalance or continuously on a recording ultramicrobalance. Usually, active water vapour uptake results in a measurable body mass gain in ticks and some other terrestrial arthropods.

When partially dehydrated, unfed *D. marginatus* larvae and nymphs compensated for water losses by active water vapour uptake at 95% RH. However, engorged larvae did not. There was also no evidence of active water vapour uptake in engorged nymphs when weighed intermittently. However, when their body mass was recorded continuously there were relatively short phases with distinct gains, which suggests that active water vapour uptake occurs in the engorged nymph but is masked for most of the time, particularly by a high rate of spiracular and also some integumental water loss. Engorged *D. marginatus* males were also found to be capable of active water vapour uptake.

These results will be briefly discussed in an ecological context.

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## O29 Visualization of the distribution of Central European tick species on the virtual globe

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Digital maps, particularly displayed on virtual globes such as Google Earth, will represent one the most important sources of geographical knowledge in the future. The use of Google Earth to show the distribution of ticks is demonstrated here. This includes the compilation of overlays for tick reports, the calculation of habitat suitability maps, the overlay of tick locations on climate maps, or the display of additional information such as photos of sampling locations, pathogens detected, references or climate diagrams. Technical details are explained using 12,000 georeferenced locations of *Ixodes ricinus*. In addition, a first published application for the distribution of *Dermacentor reticulatus* is presented. For this purpose, 2,200 locations were georeferenced, providing insight into the historical and current global distribution, but also the urban occurrence of *D. reticulatus* in public parks, fallow land, and recreational areas. Special emphasis is placed on the ticks (Acari: Argasidae, Ixodidae) found in the Czech Republic and Slovakia. Maps of the following 15 species recorded in the Czech Republic are shown: *Argas polonicus*, *A. reflexus*, *Carios vespertilionis*, *D. reticulatus*, *Haemaphysalis concinna*, *I. apronophorus*, *I. arboricola*, *I. canisuga*, *I. frontalis*, *I. hexagonus*, *I. lividus*, *I. ricinus*, *I. simplex*, *I. trianguliceps*, and *I. vespertilionis*. The soft tick *A. polonicus* does not occur in Slovakia, but 6 additional tick species have been mapped. These are: *A. persicus*, *D. marginatus*, *Ha. inermis*, *Ha. punctata*, *I. acuminatus*, and *I. laguri*. Thus, 20 tick species have been reported in Slovakia. In addition, three species of Ixodidae in the genera *Hyalomma* (each spring imported by migratory birds) and *Rhipicephalus* (occasionally imported by dogs returning from abroad with their owners) are reported from in the Czech Republic and Slovakia. Regarding the national distribution of ticks, the global distribution is also discussed, for which a new map for *I. laguri* is presented. All maps and associated content have been integrated into Keyhole Markup Language (KML) files and can be used with the free Google Earth application by scientists from various disciplines, students, and lay people interested in the geographical distribution of ticks.

### **O30 The distribution of Ixodid ticks and their infection with *Borrelia* spp. in urban and peri-urban parks in Lithuania**

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It has been known for over thirty years that *Ixodes ricinus* ticks can be found in green areas within European cities and carry various diseases affecting humans and animals. Urban green areas have recently been identified as high-risk environments for exposure to infected ticks. The genus *Borrelia* is a large assemblage of bacterial species that now is the most commonly reported cause of arthropod-borne illness in the US and Europe. Lithuania has one of the highest prevalence rates of Lyme disease per country in Europe. The distribution of ticks and their infection with *Borrelia* have been studied in Lithuania in natural habitats, while the situation in the urban areas remains largely unknown. However, the National Public Health Center of Lithuania states in its annual report that the incidence of Lyme disease in urban residents is higher than in rural. The present study aimed to investigate the distribution of Ixodid ticks and their infection with *Borrelia* in urban and peri-urban parks in Lithuania.

From 2021 to 2023, questing ixodid ticks were collected by flagging method at 11 urban and 10 peri-urban parks in Lithuania. A total of 3180 ticks were collected, and all of them identified as *I. ricinus*. Each tick was examined individually for the presence of *Borrelia* spp. by a 23S rRNA gene-based real-time PCR assay. Positive samples were further analyzed using three different targets of the bacteria genome: the linear plasmid *ospA* gene, the chromosomal flagellin gene, and the 16S (*rrs*)—23S (*rrlA*) rRNA intergenic spacer (IGS) region.

*Borrelia* DNA was detected in 26.6% (848/3180) of collected ticks. The prevalence of infection differed among years (32.0%, 21.5%, and 29.8%), urban (22.5%) and peri-urban (31.9%) parks, and it was higher in adults (32.7%) than in nymphs (19.5%). Four *Borrelia* species were identified: *B. garinii*, *B. burgdorferi* sensu stricto and *B. afzelii* from *B. burgdorferi* s.l. complex, and *Borrelia miyamotoi* from the relapsing fever group.

This study reports new data on the distribution of ixodid ticks in city parks in Lithuania and the prevalence of the causative agents of Lyme disease and relapsing fever in urban areas. Further evaluation of tick distribution and the prevalence of *Borrelia* spp. is necessary to monitor how climate change and urbanization affect pathogen circulation and infection risk.

*This study was supported by the Research Council of Lithuania (Grant No. S-MIP-23/19) and Vytautas Magnus University (Grant. No. P-G-22-10).*

### 031 The role of big game in the epidemiology of tick-borne diseases in Slovakia

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Free-ranging ungulates are important feeding hosts for all active developmental stages of the tick *Ixodes ricinus* and contribute to the maintenance of its abundant populations. They also serve as reservoirs for tick-borne pathogens of medical and veterinary importance. Because they carry infected ticks and/or are serologically positive, they can be used as sentinels to monitor the presence of ticks and tick-borne pathogens in the environment, allowing seasonal monitoring of tick activity. With their increasing occurrence in urban and suburban habitats, free-ranging ungulates are becoming an important component in the developmental cycle of urban *I. ricinus* populations and of tick-borne microorganisms such as *Anaplasma phagocytophilum* and *Babesia venatorum*. On the other hand, cervids are known for their dilution role in the circulation of *Borrelia burgdorferi* s.l. and tick-borne encephalitis virus. In Slovakia, knowledge on free-ranging ungulates as reservoirs of tick-borne pathogens is limited. In south-western Slovakia, almost 100% of cervids, including roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), and fallow deer (*Dama dama*), were found to be infected with *Theileria* spp. The infection with these hemoparasites is considered asymptomatic. Candidate vectors for transmission are *Haemaphysalis* spp., but confirmation is necessary. Comparative molecular phylogeny of the 18S rRNA gene of *Theileria* spp. isolated from infected cervid species suggests that parasite-host co-speciation has occurred. The provisional designation *Theileria* sp. (Elaphus) has been suggested for the novel taxon isolated from red deer and fallow deer, while the species isolated from roe deer does not segregate into a single well-defined clade and further genetic analysis is required. The presence of several genetic variants of *A. phagocytophilum* has been reported in free-ranging ungulates with an overall prevalence of over 50%. Variants associated with roe deer are unlikely to be pathogenic to humans and domestic animals, whereas red deer are thought to be reservoirs for variants pathogenic to domestic ruminants and horses. Importantly, the 16S rRNA and *groEL* gene sequences of the variant isolated from wild boar (*Sus scrofa*) showed identity to those of the causative agent of human granulocytic anaplasmosis. Presence of roe deer was also found to affect the diversity of *A. phagocytophilum* variants in questing *I. ricinus*. *Rickettsia helvetica*, *R. monacensis*, *R. raoultii*, *Coxiella burnetii*, *Neoehrlichia mikurensis*, *B. burgdorferi* (s.l.), *Babesia venatorum*, *B. crassa*, and *Babesia* sp. hc-hlj212 were identified in ticks feeding on free-ranging ungulates. The presented findings show that the knowledge of the role of wildlife as pathogen reservoirs and carriers of pathogen-infected ticks contributes important insights into the epidemiology of tick-borne zoonotic diseases.

*Acknowledgement.* The study was financially supported by project APVV 22-0372 and by the National Institute of Agricultural Technology, Argentina (INTA; 2023-PD-L06-I114, 2023-PE-L01-I044).



### **O32 Citizen science in tick research in Slovakia (INFOTICK)**

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Data and research material (ticks) obtained from the public and non-scientific community can be successfully used in research (Földvári et al. 2022). 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. CS is used in a wide range of areas of study including biological research and conservation (Kullenberg and Kasperowski 2016; Vohland et al. 2021). This methodology is particularly useful to generate information on human–tick encounters and may also contribute to geographical tick records to help define species distributions and/or abundance of ticks across large areas (Eisen and Eisen 2021).

In an ideal case, not only citizens serve science, but also science serves to citizens. This is also a main mission in our project: 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 (MA) 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 MA will inform the public on the seasonal activity of ticks and the risk of acquiring tick-borne diseases in Slovakia.

At the same time, we will increase the probability of finding non-native tick species in Slovakia.

The research team in our project is multidisciplinary and includes zoologists, parasitologists, ecologists as well as rickettsiologists. The multidisciplinary research team is completed by participants from the Institute of Informatics SAS, who will focus on the development of technological devices that we will use for monitoring ticks. 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.

*Acknowledgement: Research was supported by project APVV 22-0372.*



### **O33    Impact of microhabitats on species richness and tick densities in karst habitats of Slovakia**

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The authors present data on seasonal dynamics, changes in tick population structure during the year, and fluctuation changes in tick densities within several years. The results are based on the authors' long-term monitoring survey (2013 - 2023). Stationary research was conducted in eastern Slovakia, in the surrounding of the Slovak Karst National Park, near the village of Hrhov (48°34.899 N, 20°46.743 E, altitude 200 – 220 m a.s.l.). Ticks were collected by flagging vegetation, each sampling represented 100-meter transects (the relative density of ticks is expressed as the number of ticks per 100 m<sup>2</sup>). Flagging of vegetation was usually performed in 1-2 month intervals on two parallel lines: 1. Lines on the edge of oak-hornbeam forest and pastures; 2. Lines inside the forest parallel to the edge. The third microhabitat compared was the edges of the shrubs on pasture. In this type of biotope, tick flagging was carried out per unit of time (30 or 60 minutes). In total, approximately 16.500 adult ticks and nymphs (and in additional more than 5.700 larvae of ticks were not included in the population density evaluation) of six species were collected: *Ixodes ricinus* (74.2 %), *Haemaphysalis inermis* (10.3 %), *H. concinna* (7.4 %), *Dermacentor marginatus* (5.0 %), *D. reticulatus* (3.0 %), and very rarely *Ixodes frontalis* (3 individuals). Research has confirmed the uniqueness of the studied area in terms of species diversity and joint occurrence of tick species with different habitat requirements. The highest tick species diversity was registered in late March, April, and in May, when on the individual lines the co-occurrence of 3-5 tick species was recorded. The average relative density of ticks in winter amounted to 3- 42 ticks per 100 m<sup>2</sup> (the highest values in February 2014 and again in February 2020). In the spring season densities were significantly higher: 32-238 ticks (the highest value registered in April 2017; on the ecotone lines). We compared the average number of tick species and the average densities of ticks registered on the 100 m<sup>2</sup> in the forest and the edge of the forests. The significantly higher densities of ticks on the lines on the forest border were in spring, while in the forest were registered in summer. Research confirmed a significant impact of microhabitats on the number of ticks and tick species richness during the season.

*This study was financially supported by projects VEGA 2/0014/21 and VEGA 2/0051/24.*

**O34 Antibodies against EGF-like domains in *Ixodes scapularis* BM86 orthologs impact tick feeding and survival of *Borrelia burgdorferi***

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*Ixodes scapularis* ticks transmit multiple pathogens, including *Borrelia burgdorferi* sensu stricto, and encode many proteins harboring epidermal growth factor (EGF)-like domains.

We show that *I. scapularis* produces multiple orthologs for Bm86, a widely studied tick gut protein considered as a target of an anti-tick vaccine, herein termed as Is86. The data suggest that Is86 antigens feature at least three identifiable regions harboring EGF-like domains (termed as EGF-1, EGF-2, and EGF-3) and are differentially upregulated during *B. burgdorferi* infection. Although the RNA interference-mediated knockdown of *Is86* genes did not show any influences on tick engorgement or *B. burgdorferi* sensu stricto persistence, the immunization of murine hosts with specific recombinant EGF antigens marginally reduced spirochete loads in the skin, in addition to affecting tick blood meal engorgement and molting. However, given the borderline impact of EGF immunization on tick engorgement and pathogen survival in the vector, it is unlikely that these antigens, at least in their current forms, could be developed as potential vaccines.

Further investigations of the biological significance of Is86 (and other EGF-like tick antigens) would enrich our knowledge of the intricate biology of ticks, including their interactions with resident pathogens, and contribute to the development of anti-tick measures to combat tick-borne illnesses.

*This study was supported by the Slovak Research and Development Agency, grant number APVV-18-0201, and the National Institute of Allergy and Infectious Diseases, Award Numbers R01AI080615, R01AI116620, and P01AI138949.*

### O35 Midgut immunity of *Ixodes ricinus* – What is behind the unstable midgut microbiome of the tick?

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Ticks are obligate hematophagous arthropods that transmit a wide range of pathogens to humans and animals. They also harbor a non-pathogenic microbiota, mainly in the ovaries and midgut. In our previous study on the midgut microbiome of *Ixodes ricinus*, we found a diverse but quantitatively poor bacterial microbiome and the absence of a core microbe. Experiments with artificial infection by capillary feeding with model bacteria from the environment (Gram-positive *Micrococcus luteus* and Gram-negative *Pantoea* sp.) showed a surprisingly rapid clearance of these microbes from the midgut of unfed females. In search of the molecules that might be responsible for this rapid defense effect, we performed RNAseq analysis of unfed *I. ricinus* midgut (Bioproject PRJNA685402), complemented by proteomic analysis. These high-throughput approaches led to the identification of potential antimicrobial molecules that hypothetically act as “sentinels” against infections with environmental microbes. In addition to the recently characterized domesticated amidase effector2 (dae2), we also identified two different types of midgut-specific defensins: (i) early defensin, which is mainly present in unfed midgut, and (ii) late defensin, whose expression increases during blood feeding. These cationic peptides, approximately 4 kDa in size, have been commercially synthesized and exhibit a conserved pattern of three disulfide bridges. Antibacterial assays performed in microtiter plates in the concentration range of defensins from 250 µM to 8 nM showed their antibacterial potency against Gram-positive bacteria (*Staphylococcus aureus* or *Staphylococcus epidermidis*) with a minimum inhibitory concentration (MIC) of about 2 µM, while for *M. luteus* it was even at least two orders of magnitude lower in the nanomolar range. In contrast, no antimicrobial activity was exerted against Gram-negative bacteria. The role of the conserved disulfide bridges for defensins was also tested by reducing them with dithiothreitol (DTT). This experiment revealed surprising differences between the two defensins. While the antimicrobial activity of the reduced late defensin decreased significantly (~250-fold), DTT treatment had no effect on the activity of the early defensins, the MIC of which remained the same as the untreated peptide. Whether the activity of the defensins and other antimicrobial molecules such as Dae-2 is mainly due to the almost sterile environment of the tick midgut or whether other factors (such as epithelial immunity) play a role remains to be investigated.

*This project was supported by the Czech Science Foundation No. 22-12648J to P.K. and L.Ž.*

### O36 Insight into the dynamics of the *Ixodes ricinus* nymphal and adult midgut proteome

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The tick midgut plays a crucial role as the main tissue for the storage and digestion of host blood, which serves as the exclusive source of energy and nutrients essential for the tick overall development and reproduction. During feeding at each stage of development, the midgut epithelium undergoes dynamic changes reflecting the changes in physiological processes in this tissue. Furthermore, the midgut serves as the primary interface between the tick and tick-borne pathogens, which determines the tick vector competence. This work will provide the first insight into the proteomics of the midgut during tick development.

Label-free quantitative (LFQ) proteomics was used to elucidate changes during the blood meal and development of *Ixodes ricinus*. Midguts from different feeding stages of nymphs (unfed, fed for 2 days, fully-fed, 14 days after detachment, and before molting) and adults (unfed, fed for 1, 3, 5 days, fully-fed, 4 and 6 days after detachment) were dissected, thoroughly washed to remove the excess of the host blood, and homogenized. In-solution digestion using trypsin was followed by the peptide analysis carried out on the timsTOF Pro (Bruker) mass spectrometer coupled to an Ultimate 3000 RSLnano System (Thermo Fisher Scientific). Obtained raw data were submitted to the actual database available on UniProt for the *I. ricinus* and searched in MaxQuant software. Data were further analyzed using Perseus and Blast2GPro programs or in-house written scripts.

A comprehensive proteomic mapping of the midgut of *I. ricinus* was performed. Protein levels at different feeding and developmental timepoints were compared using LFQ proteomics. An additional functional analysis reveals major groups of proteins involved in tick ontogeny. Proteins related to the poorly understood lipid metabolism of the tick were identified. Furthermore, a manual scrutiny has been performed to deeply investigate the proteins associated with the key metabolic processes.

In summary, several transcriptomics data of *I. ricinus* have been published, however only few studies have investigated the tick proteomes. In contrast the transcriptomics, proteomics provides a deeper understanding of the key cellular processes occurring in the investigated tissues. Additionally, potential targets for drug or vaccine treatment might be developed.

*Acknowledgement:* Supported by GACR 21-08826S and ERDFunds (No.CZ.02.1.01/0.0/0.0/16\_019/0000759).

### **O37    Function of neuropeptides and their receptors in ticks**

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*Ixodes* ticks are important vectors of dangerous pathogens (e.g. arboviruses, *Anaplasma*, *Borrelia*, *Babesia*) but mechanisms controlling their transmission into the host are poorly understood. The Lyme disease spirochetes *Borrelia* spp. are multiplied and stored in the midgut, and transmitted into the suitable vertebrate hosts during tick feeding. Therefore, the digestive system serves as the most important reservoir of these pathogens. However, physiological processes controlling function of the gut and transmission of spirochetes during tick feeding in different developmental stages have not been elucidated. We found that peptidergic neurons in the tick brain innervate muscles of the digestive system, while numerous enteroendocrine cells are distributed in the midgut epithelium. Using molecular, immunological and physiological techniques we detected increased expression of multiple neuropeptides and their receptors in the gut at different physiological stages of feeding and repleted ticks. Our observations and RNAi experiments suggest that neuropeptides released from specific neurons and enteroendocrine cells regulate activity of the gut and associated transmission of pathogens. These results may provide tools to control tick development and suppression of pathogen transmission.

*Acknowledgements:* Supported by the Slovak grant agencies: APVV-14-0556, APVV-18-0201, VEGA-2/0080/18.

**O38 Mapping expression of neuropeptides revealed unique peptidergic cells in the sheep tick *Ixodes ricinus***

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The sheep tick *Ixodes ricinus* is a widespread haematophagous arthropod in Europe, notorious for transmitting a variety of dangerous pathogens such as encephalitis viruses, *Borrelia* sp., *Rickettsia* sp. and *Babesia* sp. Many critical aspects of tick biology, including blood uptake, digestion and reproduction, are regulated by neuropeptides. These signalling molecules are both evolutionarily conserved and extremely diverse.

Although 43 genes coding for neuropeptides in the tick have been identified to date, their functions are still poorly understood. As a prerequisite for functional analysis, we have used in situ hybridisation and immunohistochemistry to determine the sources and targets of neuropeptides in the tick. To date, we have identified 30 different peptide-producing cells by combining hybridisation probes targeting specific gene transcripts with antibodies against tick and insect peptides. These peptides are produced in various neurons and endocrine cells. In the central nervous system of the tick, the synganglion, we observed an abundance of different neurones. Some of them have been characterised as neurosecretory cells that release their peptide content into the haemolymph, while few others project their axons to release peptides specifically in peripheral organs such as the salivary glands, hindgut, reproductive organs and accessory glands.

Until now, little was known about the gut endocrine cells and the peripheral neurones of ticks. Through our research, we have identified several types of endocrine cells in specific regions of the midgut as well as peripheral neurons associated with the foregut, hindgut and legs. Remarkably, some of these cells and their peptide content represent a unique signalling system not found in model arthropods or blood-feeding insects. Our study sheds new light on the complex regulatory mechanisms that control the physiology and behaviour of ticks.

*Funding and acknowledgment. We thank the Slovak grant agencies for financial support of projects: APVV-21-0431, VEGA-2/0070/23, VEGA-2/0037/23, VEGA-2/0040/23 and Doktograd APP0227.*

### O39 Fat body of tick *Ixodes ricinus* and immune-related genes

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The tick *Ixodes ricinus* is the most important vector of tick-borne pathogens in Europe. The transmission of pathogens depends on their ability to evade or overcome the immune response of the vector. Immune molecules, which are mainly produced in the fat body or hemocytes, play a crucial role in innate immunity. The role of the fat body in tick immunity is not yet well characterized, which is why we focus on it.

The fat body of the tick is a diffuse organ that is associated to the tracheal trunks or surrounds other internal organs. We performed an RNAseq analysis of the fat body/trachea (FB/Tr) complex (NCBI Bioproject PRJNA748353) of partially fed adult *I. ricinus* females and processed the data obtained. In total, we identified about 45,000 transcripts, most of which were annotated as "unknown", "secreted" or "storage" class. The most important class of interest to us was the "immunity" class, which accounts for only 1% of the contigs. Transcriptomic data on secretory products of FB/Tr specifically involved in humoral immunity were complemented by proteomic analysis of tick hemolymph. Comparison of immune peptides and proteins from the fat body with those produced by hemocytes revealed that the fat body serves as a unique producer of certain immune components.

Twenty abundantly expressed transcripts representing tick immune proteins of different classes were analyzed in response to experimental challenges with model microbes at different time points. The highest immune response was detected after bacterial infection with Gram (-) *Escherichia coli* for 13 out of 20 selected genes. Only 4 genes were significantly upregulated after infection with Gram (+) bacteria *Micrococcus luteus* and 4 genes after infection with the yeast *Candida albicans*. The expression of 7 immune genes was not affected by any microbe.

In conclusion, the tick FB/Tr complex is involved in humoral immunity through the synthesis of effector molecules such as antimicrobial peptides, thioester-containing proteins and putative immune genes. Taken together, this may contribute to a better understanding of immune processes and their regulation in ticks.

*Acknowledgment: This work was supported by the Grant Agency of the Czech Republic (grants No. 20-05736S to P.K.)*



## O40 Hard tick Cathepsin L3 - the evolutionary adaptation to the extreme bloodfeeding lifestyle of females

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Hard tick females consume massive quantities of blood, rapidly converting it into thousands of eggs during off-host digestion. To safeguard the bloodmeal from clotting and infections, hard ticks have developed anti-clotting and intestinal microbial defense mechanisms associated with cathepsin L (CL) activities. In this study, we identify and characterize a newly separated clade of hard tick-specific CL3 enzymes, distinct from the previously described digestive L1 (CL1).

We compare the biochemical properties of recombinantly expressed IrCL1 and IrCL3, prototype members of *Ixodes ricinus*, focusing on their effective degradation and cleavage profile of hemoglobin and serum albumin. While both enzymes share basic biochemical properties, we uncover significant disparities in their expression and distribution: IrCL1 is intracellularly produced and expressed in multiple tissues, including partially engorged female guts, whereas IrCL3 is solely expressed in the midgut in off-host digesting tick females. IrCL3 is secreted into the gut lumen where we demonstrate its exclusive ability to degrade plasma thrombin and its substrate fibrinogen and to exclusively produce antimicrobial peptides from host hemoglobin as shown both in in vitro assays and in vivo using E64 inhibitor/PBS control injected ticks and subsequent mass-spectrometry and analysis.

We establish the crucial function of CL3 in preventing clotting and inhibiting microbial growth within the bloodmeals consumed by mated hard tick females, facilitating the massive egg production and the rapid expansion of tick populations in nature. Our study enhances the understanding of tick biology and their reproductive success, offering insights into potential targets for tick control strategies.

**Acknowledgements:** This work was supported by the grants 21-08826S and 24-10659S from the Czech Science Foundation (GAČR)

# Posters

**P01 Taxonomic evaluation of the digeneans (Trematoda) of *Gammarus balcanicus* and *G. fossarum* in eastern Slovakia**

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This study focuses on the taxonomic and molecular diversity of digeneans found in gammarid hosts (Crustacea: Amphipoda) in eastern Slovakia. Sampling and examination of helminth parasites were conducted at 9 different localities in 2022 and 2023: 1) Hornad River, 2) Hýľov Stream, 3) Veľké Morske Oko (inflow stream), 4) Veľké Morske Oko (outflow stream), 5) Veľké Morske Oko (lake), 6) Male Morske Oko, 7) Strážske stream, 8) Dunajec I (Červený Kláštor) and 9) Dunajec II (Spišská Stará Ves). A total of 4012 specimens of *G. balcanicus* and 120 specimens of *G. fossarum* were examined. Based on morphological data, three species of trematodes were identified: *Maritrema neomi* Tkach, 1989, *M. pyrenaica* Deblock et Combes, 1965 and *Cephalotrema minutum* Baer, 1943. The prevalence of *Maritrema* spp. was 1.49%, 1.95%, 9.92%, 0.55%, 0.37%, and 12.14% for localities #1-4, 6 and 7, respectively. The *C. minutum* was recorded from localities #3 and #4 with a prevalence of 1% and 0.14%. We isolated and extracted a whole amount of DNA from 2, 7 and 9 individuals of *C. minutum*, *M. neomi* and *M. pyrenaica*, respectively. DigI2F (5'-AAG CAT ATC ACT AAG CGG-3') and L0R (5'-GCT ATC CTG AG(AG) GAA ACT TCG-3') primers were used for the PCR reaction and sequencing. Raw forward and reverse sequences were analysed and assembled with UGENE ver. 48.1. The sequences obtained with a length of 1190-1260 bp were subjected to a BLAST search in GenBank. The closest 40 species were selected to create phylogenetic trees. Our results confirm the allocation of the studied species to the Plagiorchiida and of *Maritrema* spp. to Microphallidae. The *C. minutum* showed sister relationships with the frog parasite *Opisthioparorchis* sp. and showed a remarkable similarity of 95.1%. Despite their high morphological and molecular similarity, *Cephalotrema* and *Opisthioparorchis* are currently classified in different families (Phaneropsolidae and Batrachotrematidae) and even orders. their taxonomic position requires further refinement. Although morphological differences in the ratio of ventral and dorsal suckers, two species of *Maritrema* form a monophyletic clade with the polyphyletic position of *M. neomi* and *M. pyrenaica* individuals and with more than 99.5% similarity between specimens. Based on the 28S gene results, it appears to be a genetically homogeneous species. Further work will concern the sequences of the COI and SSU genes to refine the taxonomy of digeneans from gammarids, as well as the molecular characterization of *M. pyrenaica* from its type locality in southern France to clarify the taxonomic validity of *M. neomi*.

*The study was supported by the Next Generation EU through the Recovery and Resilience Plan for Slovakia (No. 09I03-03-V01-00144, 09I03-03-V01-00016 and 09I03-03-V01-00017) and VEGA (No. 2/0093/23).*

## P02 Is uncinariosis zoonotic diseases?

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Infections caused by hookworms are globally widespread in the population of dogs, cats, wild animals, and pose a risk to public health. The genera *Ancylostoma* and *Uncinaria* belong to the family Ancylostomatidae and have zoonotic potential. *Uncinaria stenocephala* is the causative agent of the most common ancylostomiasis in temperate climates and is known as the fox hookworm. From the perspective of climatic conditions, species of *Ancylostoma* spp. prefer warmer environments compared to *U. stenocephala*. Hookworms are usually transmitted orally by ingesting filariform larvae, which live freely in the soil, but percutaneous transmission is also possible. Species of the Ancylostomatidae family are responsible for the syndrome known as *cutaneous larva migrans* (CLM) in humans.

In the period from 2021 to 2023, 103 faecal samples were examined to diagnose hookworm infections in dogs from shelters, household pets, hunting dogs, as well as foxes. The flotation method was used to confirm the presence of eggs of the Ancylostomatidae family, which was the case in 17 individuals, corresponding to 17% prevalence. The prevalence in pet dogs was 20%; in dogs kept in shelters it was 20%; and in foxes it was 17%. As for hunting dogs, occurrence of hookworms was not confirmed. To confirm the species *U. stenocephala*, coprocultures, intestinal dissections, and PCR diagnostics were utilized. Larvae at various stages were morphologically identified according to the available Gibbs 1961 key. For precise species identification, a one-step nested PCR analysis targeting the cytochrome c oxidase subunit I (cox1) gene was employed. In foxes, following parasitological dissection of the gastrointestinal tract, five adult individuals of *U. stenocephala* were detected in one of them, whose presence was also confirmed through coproculture and PCR diagnostics. Currently, global warming primarily contributes to the spread of parasites, which may impact the occurrence of newly emerging species of hookworms in Slovakia, especially *Ancylostoma caninum*, posing a risk to the health of both animals and humans.

*Aknowledgements. This research was funded by the projects of the Scientific Grant Agency of the Ministry of Education of the SR and the Slovak Academy of Sciences, VEGA 1/0709/23.*

**P03 Preliminary study on the helminths of rodents of the Finisterre Range, New Guinea**

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In New Guinea, two spatially overlapping but temporally separate radiations have occurred after over-sea colonization of the island by two closely related rodent tribes, the Hydromyini ('Old Endemics', arrived ~ 8 mya ago) and the Rattini ('New Endemics', arrived ~ 2 mya ago). The helminth communities of these rodent species have been only partially explored and several aspects are still unknown. In this study, rodents were sampled at three altitudes (700 m, 1700 m and 2700 m) in the Finisterre Range in 2023. Our sampling includes at least 12 genera of Hydromyini and 2 different species of *Rattus*. We first investigated the helminths of the caecum and colon. We provide a preliminary taxonomic characterization of these helminths and explore the distribution of the helminth community in Hydromyini and Rattini. Of 107 rodents examined, 89 were Hydromyini and 18 Rattini. Forty-seven (43.9%) rodents were positive for at least one helminth species. Infection rates of *Syphacia* spp in Rattini and Hydromyini were 66.6% and 28.8%, respectively. Prevalence of Heterakids in Rattini and *Trichuris* spp in Hydromyini were 27.7% and 5.6% respectively. The genus *Syphacia*, with a direct life cycle, was the most common helminth of the caecum and colon for both Rattini and Hydromyini. It has been found at altitudes 1700 m and 2700 m in the Finisterre Range. Further research is needed to determine the role of different helminth species in regulating rodent population dynamics at different altitudes of the Finisterre Range.

## **P04 Intestinal parasites of European ground squirrel (*Spermophilus citellus*) from selected parts of Slovakia**

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In the conservation of endangered species, understanding the health status of their populations is imperative. Despite increasing efforts to comprehend the parasites of European ground squirrels (*Spermophilus citellus*) in Slovakia and globally, our knowledge of their intestinal parasite fauna remains relatively limited. Given the protected status of the European ground squirrel, it is essential to employ minimally invasive research methods. Therefore, fecal samples were collected in seasons 2022 and 2023 to confirm the presence of intestinal parasites. Using the flotation method with Sheather's solution, 603 samples from 18 locations in various regions of Slovakia were examined. The presence of several parasitic groups was confirmed, including *Eimeria* sp., *Capillaria* sp., *Hymenolepis* sp., and the representatives of Trichostrongylidae family, as well as pseudoparasites such as *Monocystis* sp. and *Adelina* sp. Partial statistical results also demonstrated a certain influence on parasite prevalence in the samples, such as environmental factors associated with seasonal fluctuations. Additionally, during field work, the remains of 22 ground squirrels were found. The necropsy revealed the presence of nematodes and cestodes. Molecular methods combined with morphometrics identified the presence of the genus *Capillaria* (Nematoda), according to 18S rDNA sequences, the most likely the species *Capillaria plica*, which commonly infects foxes. Another identified parasitic nematode was *Trichostrongylus colubriformis* (Nematoda). The cestode genus *Hymenolepis* was also represented. Although the literature suggested the possible presence of *Hymenolepis diminuta*, this was not confirmed. According to the molecular data, we hypothesize the species is *Hymenolepis citelli*; however, its taxonomy remains unresolved due to morphological similarities with the former species. The next step will involve applying molecular methods directly to fecal samples and trying to capture a broader spectrum of parasitic species. This study may contribute to forming a comprehensive understanding of European ground squirrel populations' fitness in Slovakia and potentially reveal parasites with zoonotic potential.

*The research is supported by the project – LIFE Sysel, Conservation of European Ground Squirrel (LIFE19 NAT/SK/001069).*

**P05 Molecular identification of bloodmeals and species composition in *Culicoides* biting midges in the Bird ringing station Drienovec**

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*Culicoides* (Diptera: Ceratopogonidae) play an important role as vectors in the transmission of avian blood parasite species of the order Haemosporida. Identification of species composition and host preference is essential for understanding the interaction between the vector and the host. The aim of this study was to determine the species composition of *Culicoides* and host preference at the Bird Ringing Station Drienovec. The ornithological station is located in the Drienovec marsh in the southeast of Slovakia and belongs to the protected area of Slovak Karst, which monitors and studies bird migration primarily on the southeastern Euro-African migration route. We captured midges using CDC UV light traps between June 4th and July 30th every 10 days in 2022 at two locations (pond, riparian forest). Out of a total of 2,344 captured individuals, we identified 17 species of *Culicoides*. The most commonly captured species were *C. clastrieri* (n=727/31.05%), *C. kibunensis* (n=457/19.52%), *C. festivipennis* (n=282/12.04%), *C. alazanicus* (n=239/10.20%), *C. obsoletus*/ *C. scoticus* (n=234/9.99%), and *C. segnis* (n=211/9.01%). In smaller numbers of captured species were *C. punctatus* (n=60/2.56%), *C. pulicaris* (n=55/2.35%), *C. pictipennis* (n=40/1.70%), *C. impunctatus* (n=16/0.70%), *C. simulator* (n=6/0.26%), *C. lupicaris* (n=3/0.13%), *C. newsteadi* (n=2/0.08%), *C. circumscriptus* (n=2/0.08%), *C. picturatus* (n=1/0.04%), *C. deltus* (n=1/0.04%), *C. shaklawensis* (n=1/0.04%), and *Culicoides* spp. (n=4/0.17%). Molecular identification of the host blood meals from blood-engorged female was performed by sequencing part of the cytochrome b gene (cyt b) mitochondrial DNA. The dominant hosts for the species *C. festivipennis* and *C. alazanicus* were *Turdus philomelos* (Song Thrush) and *Turdus merula* (Eurasian Blackbird). The results of this study can contribute to a better understanding of the ecological interactions between *Culicoides* and their hosts, as well as to the improvement of monitoring and management of the potential risk of disease transmission in this area.



**P06 Preliminary results of molecular screening of the presence of *Ixodiphagus* spp. in ticks (Ixodida) across urban and mountain areas**

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*Ixodiphagus hookeri* is a parasitoid wasp that parasitizes various tick species, including members of genera *Haemaphysalis*, *Dermacentor*, and *Ixodes* which are abundant in the territory of Slovakia. *Ixodiphagus hookeri* lays eggs in the bodies of unfed tick nymphs. The embryonic development of the wasps' eggs is induced by blood circulation in the body of the tick's nymph after a blood meal. Wasp larvae feed on the internal tissue of the tick. Adult wasps bite a hole in the nymph's body, and they fly away through, causing the death of their host. Thus, parasitized ticks die before the start of their reproduction. It has been shown that parasitoid wasps have the potential to contribute to the biological control of ticks and regulation of the transmission of pathogens they carry.

To study the prevalence of *I. hookeri*, ticks were collected using the flagging method from urban and mountain areas. The ticks were sorted by species and developmental stage, and the genomic DNA was extracted using the alkaline hydrolysis method. *Ixodes ricinus* ticks were analyzed for the presence of *I. hookeri* by PCR amplification of *cox1* and 28S rRNA gene fragments. The PCR products were separated using gel electrophoresis, and positive amplicons were purified and sent for sequencing. The nucleotide sequences were then compared with similar sequences in the GenBank database.

This study presents the results of molecular screening of *I. ricinus* tick DNA samples collected in urban and mountain areas. A representative sample of ticks collected in 2019 from four sites in Košice was chosen, including Anička Park, Bankov, Čičky Majer, and Kavečany Observation Tower. The overall prevalence of *I. hookeri* was 13.3%, ranging from 6.7% to 20% in individual locations. For the initial screening, a representative sample of ticks was collected in 2016 and 2017 at various locations of Poľana, Smrekovica, Slánske Mts., Volovské Mts., and several model sites in the High Tatras. However, no wasps were found in higher altitudes, suggesting that the occurrence of wasps is influenced by environmental conditions.

*The work was created with the financial support of the VEGA 2/0051/24 project.*

**P07 Close-to-Nature forest management alters soil nematode communities and microbial activity in pine plantations on aeolian sands**

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Close-to-nature forest management combines the economic use of forests and nature conservation in forest, promoting stability, productivity, diversity and continuity. While close-to-nature management is expected to have a positive effect on tree growth, its impact on the belowground micro-biota is poorly investigated. Nematodes and microbes are species-rich and abundant soil microorganisms, that have long been used as ecological indicators of soil health. In this study, was assessed the impact of close-to-nature forest management on soil nematode communities and microbial activity in the pine plantations on aeolian sands in Southern Slovakia, was assessed 10 years after the management introduction. Fifteen stands in close-to-nature forest managed and fifteen stands with applying standard forest management were chosen. Close-to-nature forest management significantly increased the abundance of soil nematodes and nematode biomass in each of the plots. Moreover, close-to-nature forest management significantly increased the mean abundance of omnivores and predators as stress sensitive nematodes (c-p4, c-p5). Furthermore, close-to-nature forest management reduces the proportion of stress tolerant nematodes (c-p1) and enrichment opportunists (c-p2), increased all maturity and structure indices as well as composite, structure, predator and omnivore footprints. Additionally, close-to-nature forest management significantly increased soil moisture, soil carbon content, microbial biomass carbon, basal respiration and N-mineralization, and understory vegetation diversity. The data suggest, that close-to-nature management resulting in forest of different ages positively influenced nematode communities and microbial activity by changing the quantity and quality of resources associated with increased understory cover and diversity.

*Acknowledgements: We thank project APVV-19-0142 (0.8) and VEGA 2/0007/24 (0.2) for financial support of this study.*

**P08 *Bartonella* in bat flies from the Egyptian fruit bat in the Middle East**

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*Bartonella* belonging to the alpha-2 subgroup of the class *Proteobacteria* (order Rhizobiales, family Bartonellaceae) are fastidious, Gram-negative, facultative intracellular pathogens with a unique intraerythrocytic lifestyle. Bartonellae are zoonotic bacteria transmitted by a diverse range of hematogenous arthropods, including fleas, lice, deer keds, and sandflies. In the last decade, several new strains/genotypes of *Bartonella* were detected in bats and their flies throughout the world. The search for *Bartonella* in the populations of *Rousettus aegyptiacus* brought detection of the genotype *Candidatus* *Bartonella rousetti* from the bat fly *Eucampsipoda africana* Theodor, 1955, a frequent ectoparasite of this fruit bat. This strain was found in this bat fly collected in Nigeria and Zambia and was also confirmed in the blood of *R. aegyptiacus* from Kenya. The absence of any evidence of *Bartonella* in the Palaearctic populations of the Egyptian fruit bat and its monoxenous arthropod parasite was an impulse for a more detailed study of *Eucampsipoda aegyptia* (Macquart, 1850), an obligatory ectoparasite of *R. aegyptiacus*. DNA from bat flies was extracted using the QIAamp DNA Mini kit (Qiagen) or by Chelex. The presence of DNA of *Bartonella* was analysed by the real-time PCR amplification of the 16S rRNA gene fragment. For the study of genetic diversity 16S-26S rRNA gene region and *gltA* gene sequences were compared. Altogether, 176 *E. aegyptia* individuals were analyzed for the presence of *Bartonella*; these flies were collected from 68 individuals of *R. aegyptiacus* (Egypt: 44; Iran: 37; Jordan: 13; Lebanon: 5; Oman: 68; UAE: 5; Yemen: 4). Based on the real-time PCR analysis, a total of 65 of the bat flies DNA samples (36.9%; 95% CI: 29.80–44.06) were found positive for *Bartonella*, and further characterized. The *Bartonella* DNA was found both in females (40.91%; 95% CI 29.05–52.77) and males (34.55%; 95% CI 25.66–43.43). The difference between the presence depending on sex was not significant ( $p=0.423$ ). The *Bartonella*-positive bat flies were collected from 37 individuals of *R. aegyptiacus*, with a frequency between one and five positive bat flies per fruit bat individual. An analysis of the ITS and *gltA* genes indicated the presence of an uncultured *Bartonella* sp., belonging to the *Ca. B. rousetti* genogroup, identified from populations of the Egyptian fruit bat in Africa. These results support the hypothesis that *Bartonella*'s diversity corresponds to its host's diversity.

*This study was financially supported by the project Vega 2/0021/21, APVV-19-0066, APVV-19-0519.*

## P09 Strongylids of domestic horses in Slovakia: species diversity and strongylid community structure

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Strongylid nematodes are the most prevalent and pathogenic group of horse parasites worldwide, including Slovakia. However, till now, any reliable data on the strongylid species parasitized equines have not been published in the country. The aim of our study was to examine the species diversity and structure of the strongylid community in domestic horses in Slovakia. Also, possible resistance in strongylids to two anthelmintic drugs – fenbendazole (FBZ) and ivermectin (IVM) was examined.

Twenty-seven horses, 1.5–21 years old, from two farms in eastern Slovakia were included in the study. Strongylids were collected using *in vivo* method of diagnostic deworming. The horses were dewormed with IVM drug Noromectin (0.2 mg ivermectin/kg). Faecal samples (500 g each) were collected from every horse 24, 36 and 48 hours after treatment; all strongylid nematodes (66,170 specimens) were collected, fixed in 70% ethanol and identified. Faecal egg count reduction test (FECRT) was performed after horse treatments with FBZ and IVM.

Twenty-four strongylid species, including 3 species of Strongylinae and 21 species of Cyathostominae, were found. Horses were infected with 6 to 16 species (average=11.7). Six cyathostomin species (*Cylicocyclus nassatus*, *Cyathostomum catinatum*, *C. pateratum*, *Cylicostephanus longibursatus*, *C. goldi*, *C. calicatus*) were the most prevalent (prevalence,  $P > 80\%$ ); they accounted for 85% of the total strongylid number. General structure of the strongylid community was multimodal with dominant ( $P > 80\text{--}100\%$ ), subdominant ( $P > 50\text{--}80\%$ ), background ( $P > 20\text{--}50\%$ ), and rare ( $P \leq 20\%$ ) species. FECRT revealed presence of strong resistance in strongylids to FBZ (FECRT= was 22.7–36.4%). Resistance to IVM was not observed (FECRT=100%).

Our study is the first report on the strongylid nematodes parasitizing domestic horses in Slovakia that extended our knowledge of the current state of horse strongylid community structure in central Europe. We believe that our results can be used as the basis for planning further studies of horse parasites and provide new information on potentially resistant strongylid species in Slovakia and Eastern Europe.

*The study was supported by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia (projects No. 09I03-03-V01-00015 and 09I03-03-V01-00046).*

## P10 Anoplocephalosis in horses: immunological versus coprological methods and risk factor analysis

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Insufficient attention to diagnostics and control of equine tapeworm *Anoplocephala* spp. causes increases in horse anoplocephaloses worldwide. Our study aimed to examine the occurrence and distribution of *Anoplocephala* spp. infection in horses kept under different conditions using coprological and serum or saliva-based antibody detection methods and to analyse various risk factors associated with the presence of infection in domestic horses in Slovakia.

Coprological (n=401), serological (n=423), and saliva samples (n=299) were collected from 427 horses of different age groups from 31 farms in six regions of Slovakia. Additionally, a questionnaire study was conducted to investigate the prevalence and risk factors for tapeworm infection. Faecal samples were examined using the mini-FLOTAC and the double centrifugation / combined sedimentation-flotation techniques; serum and saliva samples were analyzed by ELISA to determine antibody levels against *Anoplocephala* spp. The effects of variables at individual horse level were assessed for a positive outcome of the saliva ELISA test for *Anoplocephala* spp. *Anoplocephala* spp. eggs were detected in 1.99% of faecal samples (farm prevalence 12.5%), with no differences between the two methods. Serum-based tapeworm ELISA results revealed that 39.11% of horses tested positive (farm prevalence 81.25%); while saliva-based tapeworm ELISA results revealed 56.95% positive horses (farm prevalence 93.75%). Both ELISA-based methods reported a greater number of infected animals than were identified by coproscopy. A highly significant positive Spearman's rank correlation between serum and saliva scores ( $p = 0.75$ ) was observed. In the classification of cases (i.e. comparing horses that fell below or above the treatment threshold in the test) between the serum and saliva-based ELISAs, there was a significant difference (McNemar's test;  $p$ -value  $< 0.001$ ). This could be due to the saliva test detecting more horses with low intensity infections (i.e.,  $<20$  tapeworms) compared to the serum-based assay. Binary logistic regression analysis revealed four meaningful predictors that significantly impacted the likelihood of detecting tapeworm infection in horses using the saliva test: horse age, pasture size, anthelmintic treatment scheme, and access to pasture.

The study was supported by EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under projects No. 09I03-03-V01-00015 and No. 09I03-03-V01-00046 and VEGA 2/0099/22.

## P11 Small mammals as a reservoir of zoonotic parasites *Trichinella* spp. and *Capillaria hepatica*

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Within the research of zoonotic pathogens in small mammals 715 liver samples were examined for the presence of *Capillaria hepatica* eggs and the same number of muscle samples were used for detection of *Trichinella* spp. larvae.

Both tissue parasites were confirmed by artificial digestion method and the obtained *Trichinella* larvae were molecularly identified by PCR, performed according to Zarlenga et al. (1999).

Overall 18 species of small mammals were included in our research – *Apodemus agrarius* (64), *A. flavicollis* (290), *Arvicola terrestris* (1), *Circetus circetus* (1), *Clethrionomys glareolus* (124), *Crocidura suaveolens* (11), *Micromys minutus* (1), *Microtus agrestis* (5), *M. arvalis* (7), *Microtus* spp. (1), *Mus musculus* (70), *Neomys anomalus* (1), *N. fodiens* (3), *Rattus norvegicus* (43), *R. rattus* (5), *Sorex alpinus* (1), *S. araneus* (62), *S. minutus* (19) and *Talpa europea* (6). Small mammals were collected in two different areas – Košice region, that included samples from Košice city, botanical garden and ZOO, and High Tatras region.

Capillarid eggs were confirmed in the livers of 23 individuals of small mammals belonging to the 7 species (*A. flavicollis*, *A. terrestris*, *C. glareolus*, *Microtus* sp., *N. fodiens*, *R. norvegicus*, *S. araneus*). The prevalence in both monitored areas together was 3.22% (23/715), however respectively it was higher in High Tatras (4.09%; 22/538) than in Košice (0.56%; 1/177), where only one *R. norvegicus* was infected with *C. hepatica*.

Larvae of *Trichinella* spp. were found in two individuals of *A. flavicollis* and one *A. agrarius* and *C. glareolus* (0.56%; 4/715). The same prevalence (0.56%) was confirmed in High Tatras (3/538) and also in Košice region (1/177). Regarding species identification two individuals of *A. flavicollis* from High Tatras were infected with *Trichinella pseudospiralis*. In one *C. glareolus* from the same region *Trichinella britovi* was detected. This species was found also in Košice region in one *A. agrarius*.

The last data on the occurrence of *C. hepatica* in Slovakia come from the sixties and seventies of the 20th century, when several cases of human hepatic capillariosis were also recorded. Our research confirmed that *C. hepatica* is still present in its hosts, both in natural ecosystems and in urban areas. Also epidemiological studies of *Trichinella* spp. provide very valuable data needed to monitor this zoonotic parasite posing a risk to human health. Small mammals, especially rodents, represent an important reservoir of *Trichinella* species in diverse types of ecosystems, which is confirmed also in our study.

*The work was supported by projects APVV-21-0166 and APVV-SK-PL-21-0026.*



**P12 B chromosomes occurrence in *Acanthocephalus lucii* and *A. anguillae*: Effect of environmental pollution?**

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Supernumerary B chromosomes are present in addition to the standard karyotype and have been described in about 15% of cytogenetically examined eukaryotes. B chromosomes mostly consist of repetitive DNA sequences, especially rDNA, satellite DNA and mobile elements. Most likely source of B chromosomes is the host genome itself.

To extend the limited data on the chromosomal organization of multigene families and the structure and origin of B chromosomes, standard and fluorochrome staining (CMA<sub>3</sub>/DAPI) and fluorescence in situ hybridization (FISH) with 18S rDNA and histone H3 probes were performed on the standard karyotype and B chromosomes in three populations of two acanthocephalan species from sites with different environmental exposures. The diploid number found in all populations of both species was  $2n = 7/8$  (male/female). Individual chromosome pairs in *A. lucii* and *A. anguillae* showed slight differences, namely in the morphology of the third chromosome pair. At the intraspecific level, the populations differed by the presence of B chromosomes, which were found in all specimens of *A. lucii* from Zemplínska Šírava and in 89.4% of specimens from the Laborec River and in 34% of specimens of *A. anguillae* from Zemplínska Šírava, but not at the reference site. FISH revealed one 18S rDNA locus on the X chromosome and one locus of H3 histone genes on the first pair of chromosomes in *A. lucii*. In *A. anguillae*, FISH revealed the presence of three rDNA loci on the first two autosomes and the dispersion of H3 histone genes on all chromosomes, including the X chromosome. An interesting finding is the presence of the H3 gene on the B chromosome.

The investigated sites, Zemplínska Šírava and the Laborec River, are known for their high toxin contamination. It is known that an environment permanently polluted with chemicals leads to an increased incidence of chromosomal rearrangements. Based on this fact, as a possible scenario for the B chromosome origin, we propose chromosomal breaks due to the mutagenic effect of pollutants in the aquatic environment. The present data contribute to the very limited data on the distribution of repetitive DNA sequences in the genome of Acanthocephala.

*Acknowledgements: The study was supported by the Scientific Grant Agency of the Ministry of Education, Research, Development and Youth of the Slovak Republic and the Slovak Academy of Sciences (VEGA No. 2/0093/23) and by the Slovak Research and Development Agency (No. APVV 18-0467)*



### **P13 Molecular and phylogenetic analysis of *Crenosoma vulpis* in dog**

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*Crenosoma vulpis* (Dujardin, 1845) is a nematode lungworm that is highly prevalent in the red fox population of Europe and the Northern America. Dogs and canines are susceptible to infection with clinical signs consisting primarily of a chronic cough. The Small Animal Clinic of the University Veterinary Hospital in Košice have admitted and treated a patient young dog presented with persistent cough associated with difficulty in breathing and even asphyxia. Adult lungworms were discovered and collected during bronchoscopic examination. On the basis of morphological diagnosis, lungworms were classified in the genus *Crenosoma*. Molecular analyse of partial mitochondrial DNA cytochrome c oxidase subunit I gene (cox1) confirmed the species *Crenosoma vulpis*. This study also examines the phylogenetic relationships among species of the *Crenosoma* genus from 8 different hosts (*Canis lupus familiaris*, *Vulpes vulpes*, *Martes foina*, *Martes martes*, *Meles meles*, *Atelerix algirus*, *Erinaceus europaeus*, *Procyon lotor*). The phylogenetic tree was constructed based on gene *cox1* by the Neighbor-joining method with all the sequences of *Crenosoma* spp. (*C. vulpis*, *C. petrowi*, *C. melesi*, *C. striatum* and *C. goblei*) available in the GenBank. The sequences of *C. vulpis* and *C. petrowi* formed a common clade in a phylogenetic tree in which they split into different branches. Phylogenetic analysis is necessary for precise taxonomic classification and identification of *C. vulpis* and *C. petrowi* species, which showed a high percentage of identity when compared in the GenBank database.

*Acknowledgments:* This research was funded by the project VEGA 1/0709/23.

## P14 Engaging of environmental DNA approach in monitoring of avian schistosomes in Slovakia and the Czech Republic

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Avian schistosomes are flukes of the family Schistosomatidae (Trematoda), which use mainly waterfowl as definitive hosts and snails as their intermediate hosts. Free-swimming cercariae of some species may cause an allergic reaction known as cercarial dermatitis, when they penetrate the skin of people engaged in water activities. While biodiversity of avian schistosomes has been relatively well-studied in the Czech Republic, only a single record of an agent of cercarial dermatitis is known in Slovakia. The objective of this study is to broaden the knowledge regarding species diversity within this trematode group in both countries by a new cutting-edge metabarcoding method applied on environmental DNA (eDNA) samples. The samples were collected by filtering water at selected localities through plankton net, which was followed by extraction of total eDNA. The PCR amplification of  $\approx 350$  bp long part of ITS region was performed using barcoded primers specific for avian schistosomes. The amplicon of each sample contained a unique combination of barcodes and a library of pooled amplicons was sequenced on a NGS platform.

Of the 35 localities sampled, avian schistosomes were detected in the Velký Hatínský, Hornobranský and Loužek ponds, and the Medard and Most lakes in the Czech Republic, as well as in the Jazero Lake in Košice, and in the ponds near Iňačovce in Slovakia. A total of 16 OTUs (Operational taxonomic units) were recognised and *Trichobilharzia franki* Müller & Kimmig, 1994 was the most common species (6 localities including the Jazero lake in Košice), followed by unidentified avian schistosome (5 localities). *T. szidati* Neuhaus, 1952 was confirmed only in the Velký Hatínský pond. The other OTUs represented unidentified, and in some cases new genetic lineages.

These findings confirmed the suitability of this method for a rapid, non-invasive and cost-effective survey of the biodiversity of avian schistosomes. The presence of numerous unidentified lineages indicates a lack of genetic data or the potential existence of previously unknown taxa. Novel data will help to identify health risks associated with these parasites and thus reduce the risk of cercarial dermatitis in recreational areas.

*This study was supported by Slovak Research and Development Agency (APVV SK-CZ-RD-21-0078), the Ministry of Education, Youth and Sports of the Czech Republic, project LUASK22045, and Scientific Grant Agency of the Ministry of Education, Science and Research of the Slovak Republic and the Slovak Academy of Sciences (VEGA No. 2/0130/24).*

## P15 Impact of pasture enriched with chicory on experimental haemonchosis

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The health and production of small ruminants is constantly menaced by parasitic infections, especially those caused by the blood-sucking gastrointestinal nematode *Haemonchus contortus* whose presence causes severe anemia and the loss of proteins. For maintaining parasitic population at levels that will not affect the health of the host population is necessary to use synthetic anthelmintics or alternative ways, such as the use of plants and their compounds or adjustment of feed rations with trace elements.

In our study, we monitored the effect of pasture enriched with chicory (*Cichorium intybus*) on lambs experimentally infected with *Haemonchus contortus*. Twenty infected lambs were divided into two groups: one group grazed on pasture enriched with chicory (supplemented group) and a control group grazed on meadow grassland. Supplementation started on day (D) 34 post infection and lasted for 110 days with dosage *ad libitum*. Antiparasitic effect of pasture was determined by monitoring intensity of infection (faecal egg count) and total number of parasites in abomasum. Intensity of infection was expressed as number of eggs per gram (EPG) of faeces examined by McMaster method.

Weight gains were monitored as a production indicator and significantly differed ( $p < 0,05$ ) between supplemented and control group from day D89 for the rest of experimental period. Significant difference in faecal egg counts between groups was not recorded during experiment, however we observed significant reduction ( $p < 0,05$ ) in EPGs within both experimental groups from D48. The highest peak of EPGs was reached on D34 with EPGs up to 31 000. At the end of experiment on D144, faecal egg counts in experimental group were reduced by 58% in comparison with control group, however the level of EPGs in both groups was very low (approx. 100 EPG). These results were confirmed by necropsy where most animals were negative for the presence of parasites.

The supplementation of infected lambs with chicory for 110 days affected the dynamics of infection and improved parameters of production. Furthermore, our results are also showing that time and meadow grassland are also beneficial factors positively influencing the course of parasitic infection.

*The study was supported by Slovak Research and Development Agency Project APVV-18-0131.*

## **P16 Down-regulation of neuronal form of Nitric oxide synthase in the Nurse cell of *Trichinella spiralis***

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The free radical nitric oxide (NO) and Ca<sup>2+</sup> are critical regulators of skeletal muscle exercise performance and fatigue. The major source of NO in skeletal muscle cells is the neuronal form of the enzyme Nitric oxide synthase (nNOS). One of the most peculiar characteristics of the Nurse cell of *Trichinella spiralis* is the complete loss of the contractile capabilities of its derivative striated muscle fiber.

The aim of the present study was to clarify the expression of nNOS protein and mRNA in striated muscles during the muscle phase of *T. spiralis* infection in mice. Muscle tissue samples were collected from mice at days 0, 14, 24, and 35 post infection (d.p.i.). The expression of nNOS was investigated by immunohistochemistry, and the expression levels of mRNA of mouse Nitric oxide synthase 1 (*Nos1*) by real-time PCR.

In healthy mouse muscle fibers, nNOS showed regular sarcoplasmic distribution. At day 14 p.i. the expression of the protein was still well presented in the disintegrated sarcoplasm, however, the cytoplasm of the developing (24 d.p.i.) and mature (35 d.p.i.) Nurse cells showed the absence of nNOS. The relative expression analysis of the qPCR data detected slight down-regulation of *Nos1* in skeletal muscles at days 14, 24 and 35 p.i. in comparison with the non-infected muscle samples.

At least at the beginning of the Nurse cell development, *Trichinella* uses the same repairing process of skeletal muscle cell, induced after any trauma. At a later stage, however, we could suggest that the down-regulation of nNOS in the Nurse cell of *T. spiralis* either serves a protective function or is an outcome of the genetic identity of the Nurse cell.

Increasing scientific evidence suggests the ability of *T. spiralis* to influence the expression of the host cell genes that are relevant to striated muscle development, myogenesis and differentiation. This phenomenal ability of *Trichinella* could be applied in the fields of biotechnology, tissue engineering and regeneration.

*This work was financially supported by the Bulgarian Academy of Sciences and the Slovak Academy of Sciences under grant IC-SK/03/2023-2024.*

## P17 Successful treatment of *Babesia gibsoni* infection in dogs

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Canine babesiosis caused by *Babesia gibsoni* is increasingly occurring in non-endemic areas within Europe. The increased prevalence of infection in recent years is mainly related to the import of American Pit Bull Terriers (APBT) from endemic countries. We previously conducted research on *B. gibsoni* treatment and developed a new treatment protocol that was found to be highly effective in eliminating parasitaemia. Thanks to a published study, the therapy has been extended to other European countries. To verify the efficacy of the therapy in a wider range of patients, we decided to subject some patients to further testing.

During the years 2019–2023, we monitored 156 APBT dogs from Slovakia (SK; n=66), Czech Republic (CZ; n=52), Serbia (RS; n=24) and Bulgaria (BG; n=14). Molecular detection of *B. gibsoni* in dogs from SK and CZ was performed using conventional PCR amplification of 18S rRNA gene fragments using genus- and species-specific primers. In dogs from RS and BG, the samples were examined in commercial IDEXX Laboratories. The PCR analysis was conducted before treatment, 30 and 365 days post-treatment. PCR-positive 18S rRNA samples were further analyzed using cytochrome b PCR assay to check the presence of mutations responsible for drug resistance. Positive PCR amplicons (18S rRNA and cyt b) were sequenced and further analyzed. All dogs were treated with Malarone® (13.5 mg/kg of atovaquone PO q24 h), azithromycin (10 mg/kg PO q24 h) and artesunate (12.5 mg/kg PO q24 h) for 10 days.

Molecular diagnostics demonstrated a negative PCR result in 98.7% (154/156) of patients enrolled in the study at 30 and 365 days post-treatment. Three dogs from this group experienced vomiting on the first day of therapy and these patients received maropitant without treatment discontinuation. Two dogs (1.3%; 2/156) from CZ tested positive for *B. gibsoni* 30 days post-treatment. Analyzing nucleotide sequences of *B. gibsoni* cytochrome b gene revealed the presence of M121 mutation in these patients. Treatment with Malarone®, azithromycin and artesunate is highly effective in the elimination of *B. gibsoni* parasitaemia and prevention of infection recurrence for at least one year. It can be considered safe in terms of the incidence of adverse effects. However, the therapy is not able to eliminate parasitaemia caused by *B. gibsoni* in the presence of cytochrome b mutations, as this represents resistance to the atovaquone contained in the Malarone®.

*Funding:* APVV 19-0440, VEGA 1/0569/24, VEGA 2/0051/24

**P18 Protist colonization alters the gut microbiome and accelerates recovery from gut inflammation in the animal model**

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*Blastocystis* sp. is a widespread intestinal protist in humans and animals, but its effects on health and its interactions with the microbiome are not well understood. Although it is occasionally associated with inflammation and gastrointestinal symptoms, there is growing evidence that *Blastocystis* is commonly found in asymptomatic individuals and is more prevalent in healthy conditions than in diseases such as Inflammatory Bowel Disease (IBD) or Irritable Bowel Syndrome (IBS). This dichotomy underscores the existing knowledge gaps regarding the factors that influence host colonization and the protist's interactions with the gut microbiome.

In our research, we have investigated the effects of *Blastocystis* ST3 colonization on the immune system and gut bacterial microbiota, both in isolation and in association with chemically induced colitis (via DNBS). We inoculated outbred rats with *Blastocystis* ST3 and induced colitis after three weeks (short-term exposure) and three months (long-term exposure) of colonization. We evaluated the intensity of inflammation in the colonized rats compared to control groups and analyzed cytokine gene expression, macroscopic and microscopic observations, clinical data, and changes in the bacterial microbiome.

The study found that short-term colonization with *Blastocystis* ST3 had no effect on gut inflammation after colitis induction. However, long-term colonization appeared to enable faster recovery from colitis in rats. This was evidenced by a significant decrease in inflammatory markers (TNF $\alpha$ , IL-1 $\beta$ ) and pathology two days after colitis induction in the colonized group, as well as improved clinical scores. We also investigated for the first time the effects of colonization duration on the gut microbiota and characterized the bacterial community in fecal, colon and caecum samples by next-generation sequencing. Before colitis, colonization with *Blastocystis* ST3 did not lead to an increase in bacterial diversity. However, two days after colitis, bacterial diversity increased in the long-term colonized group. We observed shifts in the bacterial community due to *Blastocystis* colonization before and after colitis induction, with the effects increasing over time and after colitis.

Our results suggest that *Blastocystis* ST3 functions primarily as a benign colonizer and bacteriome stabilizer and may support recovery from chemically induced colitis.

*Funding:* This work was financially supported by a grant from the Czech Science Foundation (grant no 22-04837S) to K. Jirků and by grants from the Student Grant Agency (SGA) at the Faculty of Science of the University of South Bohemia to Z.L. (2018).



## P19 Effect of selected Indonesian plants on *Giardia intestinalis* in experimental *in vitro* model

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Numerous gastrointestinal pathogens pose a problem in human health, including the ubiquitous waterborne species of *Giardia*. Parasite infections can have a serious impact on the health of an individual, just as the general health of an individual can affect their susceptibility to parasite infection. The investigation of interactions between parasites and hosts is necessary to understand how organisms fight diseases in their natural environment. Based on research of the feeding behaviour of orang-utans with the emphasis on specific plant food items consumed to overcome parasitoses, plants with potential anti-parasitic activity were selected for further testing.

*In vitro* anti-giardial activity of four Indonesian plants with promising anti-parasitic activity, selected on the basis of collected behavioral data and ability to decrease parasite load in Sumatran orang-utans was evaluated. Aqueous, methanol and methanol-tetrahydrofuran derived extracts of *Archidendron fagifolium*, *Diospyros sumatrana*, *Piper betle* and *Shorea multiflora* together with *Arabidopsis thaliana* as negative control were tested at different concentrations and compared with a standard giardicidal drug, metronidazole. *Giardia* cultures were counted in a hemocytometer using light microscopy and the anti-giardial effect was statistically evaluated.

We recorded a statistically significant decrease in giardia trophozoites in both methanolic extract of *A. fagifolium* and the aqueous extract of *P. betle*, with the effective concentration 400 µg/ml and 100 µg/ml, respectively. No effect was observed in *D. sumatrana*, *Shorea multiflora* and *A. thaliana*. No evidence of cytotoxicity was shown.

The results of our study showed that extracts from *A. fagifolium* and *P. betle* could have a potential for an alternative treatment against *G. intestinalis*. These findings can serve as a basis for further research concerning their potential use in systemic therapy and/or prophylaxis of *G. intestinalis* infections, as well as the structure and activity of the active compounds.

*This work was supported by the UMI – Saving of Pongidae Foundation project “Parasites and Natural Antiparasitics in the Orang-utan” and by the Czech Science Foundation (23-06571S) and Scholarship program to support creative activity - research collaboration (PřF\_04\_27.10.2006).*



## **P20 *Cryptosporidium mortiferum*: first case in human and synanthropic rodents as source of infection in the Czech Republic**

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*Cryptosporidium*, a globally distributed protist parasite, poses a health risk to both humans and various vertebrate hosts. While *C. parvum* and *C. hominis* are still the predominant pathogens in humans, there has been an increase in the number of rodent-borne *Cryptosporidium* infections, including those caused by *C. mortiferum*. Its first occurrence in Europe was documented in Eurasian red squirrels in Italy in 2008, and its presence was linked with the introduction of the eastern gray squirrel to Italy in the mid-20th century. Experimental studies reveal diverse clinical manifestations among different squirrel species, ranging from asymptomatic or mild signs in eastern gray squirrels to severe diarrhea, apathy, and mortality in Eurasian red squirrels.

Fecal samples were screened for the presence of oocyst/DNA *Cryptosporidium* spp. by specific Ziehl-Nielsen staining and nested PCR amplifying a small subunit of rRNA. The intensity of infection was quantified by qPCR. Human samples were investigated by PCR and bacteriological examination for the presence of bacteria (*Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, and *Clostridium*) and parasitic protists (*Encephalitozoon* spp., *Enterocytozoon bieneusi*, *Giardia intestinalis*, *Blastocystis hominis* and *Cyclospora cayetanensis*).

A 42-year-old urban resident suffered from gastroenteritis, with molecular analysis confirming *C. mortiferum* and genotyping revealing subtype XIVaA20G1T1. Simultaneously, young Eurasian red squirrels exhibited fatal infections, while European ground squirrels remained asymptomatic, indicative of host-specific susceptibility. Genetic analyses demonstrated identical *C. mortiferum* subtypes in both humans and squirrels. Notably, subtype XIVaA20G2T1 was exclusive to Eurasian red squirrels and humans in Scandinavia and the Czech Republic, challenging previous assumptions about the role of eastern gray squirrels in infections. This study highlights the alarming increase of *C. mortiferum* infections across Europe.

The detection of asymptomatic European ground squirrels suggests broader susceptibility among rodent species, requiring comprehensive surveillance and management strategies to mitigate this new threat to public health. The findings prompt further exploration into the dynamics of *Cryptosporidium* infections in wildlife, urging collaborative efforts for effective control and prevention.

*This work was supported by grant from the Grant Agency of the Czech Republic (21-23773S).*

**P21 Rodents as reservoirs for *Toxoplasma gondii* in different ecosystems in Slovakia – seroprevalence study**

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*Toxoplasma gondii* is a ubiquitous intracellular parasite with felids as definitive hosts and a broad range warm blooded animals of intermediate hosts. Rodents are considered suitable sentinels for prevalence studies of many infections, including toxoplasmosis. Study aimed at estimating the seroprevalence of *T. gondii* in rodents from different ecosystems of Slovakia and investigating the correlation between the seropositivity and the species, age, and sex of animals.

Altogether 1009 wild rodents belonging to nine species were trapped in 2015 and 2019 and antibodies to *T. gondii* were detected in 6.7% animals. Seropositivity was detected in seven species, ranging from 0.0% in *Micromys minutus* and *Apodemus sylvaticus* to 7.7% in *A. flavicollis*. The seropositivity differed significantly among localities, higher positivity was detected in suburban and touristic areas (12.2%) than in localities with lower level of human activities, i.e. in agrocenoses, forests, hunting grounds and alluvia (5.5%). Females reached significantly higher seropositivity (9.7%) than males (3.8%) and adults were positive significantly more often (9.2%) than subadults (4.9%).

Results showed that occurrence of *T. gondii* varies significantly in rodents from habitats with various environmental conditions and different level of anthropic use. Several biological and ecological factors, such as soil contamination, soil conditions, susceptibility of rodent species etc., may influence this variability.

*Acknowledgment.* Study was financially supported by the projects VEGA 2/0024/24 and APVV-21-0166.

## P22 Unravelling host selection and pathogen diversity through bloodmeal analysis

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Ticks, as vectors of various pathogens, pose significant health risks to humans and animals. With urbanization, the prevalence of ticks in urban areas has increased, elevating the risk of tick encounters for city residents and their pets. Urban green spaces can also serve as tick habitats, especially neglected areas frequently visited by humans and their pets. This study aims to monitor tick abundance and species composition in neglected recreational areas of Košice, Eastern Slovakia.

Questing ticks were collected from 4 urban localities 1. Zberne suroviny, 2. Tahanovce sídlisko, 3. Tahanovce kostol, and 4. Hornbach in Košice during March and April 2022. We collected 216 ticks in total. We identified 2 species: *Ixodes ricinus*, *Dermacentor reticulatus*. From the 216 questing ticks we found 72 males, 68 females and 34 nymphs, 2 larvae *I. ricinus* and 11 males and 29 females of *D. reticulatus*. Notably, *D. reticulatus* was discovered directly in the city center, indicating a stable population in urban environments. The presence of ticks, particularly *I. ricinus* and *D. reticulatus*, highlights the high risk of tick encounters and the potential transmission of tick-borne diseases in urban green spaces.

Ticks were collected by the flagging method with white cotton flag (1 m<sup>2</sup>). They were examined by molecular biology methods - reverse line blot hybridization assay (RLB) for detection of hosts and polymerase chain reaction (PCR) for pathogen detection.

Prevalence of *Babesia* spp. in *D. reticulatus* was 7.5%, however in *I. ricinus* ticks we did not find any positive sample. Prevalence of *Rickettsia* spp. was 7.5% in *D. reticulatus* and 10% in *I. ricinus*.

The most represented host in *D. reticulatus* were birds and for *I. ricinus* ticks were small rodents, artiodactyls (*Sus scrofa*, *Capreolus capreolus*), birds (*Turdus* sp., *Parus* sp.) and *Erinaceus europaeus* on which they fed in the previous developmental stages.

These findings underscore the complex ecology of ticks in urban environments and highlight the importance of understanding the interactions between ticks, pathogens, and hosts in these settings. Urban green spaces, such as parks and recreational areas, serve as hotspots for tick activity, presenting a potential risk to public health. Further investigation into tick-borne pathogens and their hosts in these urban areas is warranted to comprehensively assess the risk posed by tick bites and formulate appropriate preventive measures.

*Acknowledgments: This research was supported by VEGA 1/0287/22.*

## **P23 Seasonal activity of *Ixodes ricinus* ticks and role of game animals in the circulation of tick-borne pathogens in southwestern Slovakia**

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The castor bean tick, *Ixodes ricinus* is the most widespread tick species in Europe. It is a competent vector for various pathogenic microorganisms such as viruses, bacteria and protozoan parasites.

Ticks were collected in Žemberovce village (southwestern Slovakia). The seasonal activity and abundance of ticks and their infection with selected pathogens were investigated. Questing ticks (n = 458) were collected by flagging the vegetation on seven plots during 2019-2022. Feeding ticks were collected from hunted animals. *I. ricinus* prevailed among questing ticks (55%) and in feeding ticks on wild animals (63%, n = 334). In addition, *Dermacentor marginatus*, *Dermacentor reticulatus*, *Haemaphysalis concinna* and *Haemaphysalis inermis* were found on vegetation as well as feeding on deer and wild boars. *Ixodes canisuga* and *Ixodes kaiseri* were obtained from a fox and a European badger, while *I. kaiseri* was recorded in Slovakia for the first time.

Spleen samples from 12 red deer, 2 roe deer, 19 wild boars, one red fox, and one European badger were also obtained from local hunters. The samples were screened for piroplasmids, *Anaplasma phagocytophilum* and *Rickettsia* spp. by molecular methods. Cervids were positive for *Theileria capreoli* (85%), *A. phagocytophilum* (100%) and wild boars for *A. phagocytophilum* (56%). A *Babesia* species related to *Babesia* sp. badger from China was detected in the badger spleen.

20% of feeding ticks were positive for *Babesia/Theileria* spp. *Theileria* spp. (65%) were detected in *I. ricinus* and *H. concinna* from infected cervids. *Babesia microti* and *Babesia crassa* were confirmed in one engorged *I. ricinus* and one *H. concinna*, respectively. *A. phagocytophilum* was found in 42% of feeding ticks.

Wildlife animals, mainly ungulates are key reservoir hosts mainly for *A. phagocytophilum* and *Theileria* spp. and contribute significantly to the maintenance of these pathogens in natural habitats of southwestern Slovakia.

In further research, we will focus on monitoring of host-parasite-pathogen relationships in two epidemiologically important tick species *I. ricinus* and *D. reticulatus* and monitoring changes in the bionomics of ticks under the influence of global changes in natural and urban areas.

*This study was financially supported from the Scientific Grant Agency by the project VEGA 2/0137/21 and by the Slovak Research and Development Agency APVV-22-0372.*

## P24 Diversity of blood parasites in *Pelophylax* sp. from Lithuania

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Blood parasites can be found in all vertebrates, but the ones infecting amphibians are less studied. Lithuanian fauna records 13 species of amphibians and five of them are listed in the Lithuanian Red Book. For more than two decades, no study on blood parasites in amphibians in Lithuania has been done. In it, only *Trypanosoma* was found in one amphibian species and the situation in the country is currently unknown. Amphibians are considered important bio-indicators of a healthy ecosystem due to their high sensitivity to environmental changes (e.g. pollution). This makes studies with amphibians relevant, especially in a climate change scenario, given that ecosystems have been transformed over time by the expansion of human activities, impacting the distribution and ecology of several species and their pathogens. This study aimed to investigate the presence of blood parasites in *Pelophylax* sp. in Lithuania. Thin blood smears were collected from 20 individuals in 5 different localities in Lithuania. These thin blood smears were dried at room temperature, fixed in absolute methanol and stained with a 10% Giemsa solution. Further, they were analyzed by microscopy.

In total, 11 individuals (55%) were positive for at least one blood parasite. Nine of them presented co-infections between *Lankesterella* sp. (45%), and other parasites such as *Trypanosoma* sp. (n=6), *Dactylosoma* sp. (n=1), or microfilaria (n=2). Single infections were detected in only two individuals, one with *Lankesterella* sp. and one with *Trypanosoma* sp. Further molecular analysis and detailed microscopical investigation are required to confirm parasite species. *Lankesterella* was the parasite with the higher prevalence infecting *Pelophylax* sp. in Lithuania. This represents a change from the previous studies, and might indicate an expansion in vectors range that needs to be better investigated. This study also presents the first report of *Lankesterella*, *Trypanosoma*, *Dactylosoma* and microfilaria in *Pelophylax* sp. in the country. More studies with amphibian blood parasites should be conducted in the future to better understand their prevalence, pathogenicity and distribution in Lithuania.

*The study is supported by the Nature Research Centre and the European Molecular Biology Organization (EMBO) Solidarity Grant. The authors are grateful to Dr. Vytautas Rakauskas for the help with sampling amphibians and to Dr. Oleksii Marushchak for the identification of frogs.*

## **P25 Molecular diversity of *Cryptosporidium* species in the early stage of development in calves**

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Cryptosporidiosis is a zoonotic disease that causes significant losses in the cattle population, especially in calves and young individuals. In cattle, the infection is most commonly caused by species such as *C. parvum*, predominantly found in pre-weaned calves, while *C. bovis* and *C. ryanae* are mostly diagnosed in calves post-weaning, and *C. andersoni* is the most common species infecting adult cattle.

The study aimed to molecularly identify and genotype the protozoan parasite with zoonotic potential *Cryptosporidium* spp. in calves in the early stage of development on a dairy farm in Zemplínska Teplica, Eastern Slovakia. Twenty-five Holstein-Friesian calves were included in the study, monitored from birth to their fourth week of life (0 – 4 weeks). Fresh fecal samples were collected from the same group of calves each week, except during the third week when sampling was not feasible. All fecal samples were examined for *Cryptosporidium* spp. coproantigen using a commercially available kit CRYPTOSPORIDIUM (FAECAL), Diagnostic Automation, INC, Calabasas, USA as a screening method. The highest incidence of cryptosporidiosis occurrence was recorded in the first week of life, where the antigen was confirmed in 21 (84%) calves. Overall, 29 calves tested positive for coproantigen over the four weeks. Positive isolates were molecularly characterized. Species and subgenotypes of *Cryptosporidium* in positive isolates were identified using PCR and sequence analysis of the small subunit ribosomal RNA (ssu rRNA) and 60 kDa glycoprotein (gp60) genes of the parasite. Sequence analysis of 29 isolates at the ssu rRNA loci confirmed the presence of two *Cryptosporidium* species, including *C. parvum* and in two cases, *C. ryanae*. Of the 29 isolates, 27 were assigned to the species *C. parvum*, with the gp60 locus identified as subgenotype IIaA17G1R1.

Among individual animal groups, calves are the most common reservoir of the zoonotic species *C. parvum*. The disease has significant public health implications, as contact with livestock, feces, or working with barn manure are major sources of infection not only for other animals but also for humans. Farm workers, zootechnicians, veterinarians, and veterinary medicine students are among the most at-risk groups.

*Acknowledgements.* This research was funded by the projects of the Scientific Grant Agency of the Ministry of Education of the SR and the Slovak Academy of Sciences, VEGA 1/0709/23.



## P26 *Cryptosporidium* and cryptosporidiosis in reptiles

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*Cryptosporidium*, single-celled parasites infecting the gastrointestinal and respiratory tracts of vertebrates, are long recognized as significant pathogens. This study aimed to elucidate the genetic diversity and biology of *Cryptosporidium* in reptiles from the Squamata order.

Fecal samples were collected immediately after defecation, microscopically screened for *Cryptosporidium* oocysts using aniline-carbol-methyl violet staining, molecularly tested using nested PCR targeting SSU and GP60 genes and immunochromatographically based on coproantigen testing. Selected animals with cryptosporidial infection were monitored weekly for 120 days. Reptile carcasses, along with clinical information of screened animals, were provided by the veterinary clinics. Tissue samples from deceased animals were molecularly, histologically, and electron-microscopically examined. Out of 901 samples from 74 lizard species and 12 snake species, 26 were positive for *Cryptosporidium* by microscopy and 97 by nested PCR. Sequence analysis identified *C. varanii* (n=68), *C. serpentis* (n=21), *C. tyzzeri* (n=3), *C. parvum* (n=2), *C. muris* (n=1), *Cryptosporidium* sp. lizard genotype (n=1), and *Cryptosporidium* sp. (n=1). Infected reptiles shed oocysts with intensities ranging from 10,000 to 2,000,000 OPG. Coproantigen tests verified *C. varanii*, *C. serpentis*, and *C. muris* even in samples with low infection intensity (<2,000–3,000 OPG). Out of twenty naturally infected cadavers from seven reptile species, developmental stages of *C. varanii* were found in thirteen animals in various organs including the small intestine, liver, stomach, and cloaca. *Cryptosporidium serpentis* was detected in 6 individuals in the stomach, while DNA of the lizard genotype was found in the heart, lungs, stomach, and small intestine of a single lizard. Long-time monitoring of *C. varanii*-positive animals revealed infection only by PCR. Despite low infection levels, retarded gain weight, appetite loss, and regurgitation were described. Snakes with *C. serpentis* had occasional appetite loss but thrived better than those with *C. varanii*. The study found high rates of *C. varanii*, in captive snakes and lizards in the Czech Republic. Besides the lizard genotype, we identified a new *Cryptosporidium* genotype in reptiles needing further investigation. Rapid coproantigen testing could help breeders identify and manage infections effectively.

*The study was supported by the grant of GAJU (049/2023/Z).*



**P27 *Cryptosporidium muris* in domestic cat differs from other murine isolates**

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*Cryptosporidium muris* is a gastric species of *Cryptosporidium* naturally infecting rodents such as the mouse, rat, gerbil, or vole, but natural infections in sheep, goats and camels have also been described. In this study an isolate of *Cryptosporidium muris* was obtained from naturally infected domestic cat and a moderate intensity of *Cryptosporidium* infection was detected ( $3\text{--}4 \times 10^4$ /gram of feces).

The infected cat was followed up for 110 days from the first examination. Biological and molecular characteristic including oocyst size, host specificity, course and location of infection, and pathogenicity was studied. To determine the susceptibility of various model hosts to this isolate of *Cryptosporidium muris*, SCID, BALB/c, IFN- $\gamma$  KO mice and southern multimammate mice (*Mastomys coucha*) were used for experimental infections. Mucosal smears and tissue samples from *Cryptosporidium*-infected animals were stained with Wright methods, prepared for histology, and scanning and transmission electron microscopy, respectively, to visualize the characteristic morphological structures of developmental stages.

All mice were susceptible to infection, and they shed microscopically detectable oocysts, which was not statistically different from those obtained from domestic cat. The oocyst measured  $8.43 \times 6.22 \mu\text{m}$  with a shape index of 1.37 ( $n = 90$ ). The intensity of infection was different in different strains of mice. Southern multimammate mice and IFN- $\gamma$  KO mice had high intensity of infection that ranged from 3,000 to 3 million OPG (oocysts per gram) with a prepatent period of 11-18 days. BALB/c mice had very low intensity of infection 700 to 3,000 OPG with a prepatent period of 19-24 days, but a patent period lasted up to 30 days. The infection was exclusively localised in glandular part of the stomach. Using Wright staining and transmission electron microscopy we were able to identify trophozoites, Type I and Type II meronts, merozoites, macrogamonts, microgamonts, zygotes and oocysts.

The study revealed that while *C. muris* CAT21 shows no molecular distinction from other isolates, it possesses distinct biological characteristics. It triggers a robust immune response, causes a milder infection in immunodeficient mice, induces mucus production like *C. proliferans*, and displays limited oocyst viability, leading to rapid degradation.

*This work has been supported from GACR project (21-23773S).*

## P28 Non-antibiotic elimination of rickettsial infections

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Tick-borne rickettsioses, which belong to zoonoses, are among the oldest known tick-borne diseases. *Ixodes ricinus*, *Dermacentor reticulatus* and *Dermacentor marginatus* are epidemiologically important vectors transmitting various species of *Rickettsia* including *R. helvetica*, *R. slovaca* and other pathogenic alphaproteobacteria throughout Europe. The growing prevalence of microbial infections and the rapid emergence of drug resistance to antibiotics represent a health threat worldwide. One of the possibilities for treating bacterial infection shortly after the tick bite is the use of methylene blue (MB) or carbon quantum dots from citric acid (CA-CQD) for photodynamic therapy (PDT). They have great potential to serve as an effective alternative to antibiotics for the treatment of bacterial infections. Our work aimed to confirm and optimize the bactericidal efficacy of PDT of *R. conorii* infection in the presence of the MB and CA-CQD after irradiation.

*Rickettsia conorii* was propagated on VERO cell line in 3% Dulbecco's modified medium. CA-CQD and MB were used as photosensitizers. AllPrep DNA/RNA Mini Kit (Qiagen) was used for DNA/RNA isolation, and First Strand cDNA Synthesis Kit (Thermo Scientific) was for reverse transcription of the isolated genomic RNA to cDNA. To determine the number of viable *R. conorii*, quantitative real-time PCR was performed using CS-r and CS-f primers and the CS-p probe targeting the synthesis of the *gltA* gene fragment encoding citrate synthase.

Photodynamic inactivation can reduce up to 100% rickettsial infection *in vitro* and changes in proteomic level were observed.

These findings show that CQDs have great potential to serve as antibacterial agents for bacterial infection treatment, and as an effective and complementary alternative to conventional antibiotics for early tick-borne infection.

*This study was financially supported by the project Vega 2/0021/21, APVV-19-0066, APVV-19-0519.*

## P29 Urban hedgehogs as important hosts for hard ticks in Slovakia

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Growing urbanization and related environmental changes significantly affect the structure, density, and dynamics of animal communities serving as hosts for ectoparasites. The population of ticks, which are agents of a large number of tick-borne pathogens, is also affected secondarily. The maintenance of the ticks and the circulation of tick-borne pathogens are ensured by the presence of native, synanthropic and introduced species of animal as well as companion and stray animals. Green urban zones pose a risk of human contact with infected ticks and the transmission of dangerous diseases. Ticks as vectors of diseases as well as identification of reservoirs, hosts, and the circulation of pathogens in the natural environment are in the center of scientific attention. However, there are only a few studies from the urban environment of some European cities. The occurrence of ticks on wild animals, close to human settlements, increases the risk of transmission of pathogens from the reservoir host by infected ticks.

The aim of our study was to investigate the presence of hard ticks and the circulation of medically and veterinary important tick-borne pathogens in hedgehogs in urban areas in Slovakia.

During 2021-2023 we captured 40 Northern white-breasted hedgehogs in the city parks of Bratislava and Stupava. All animals were checked, carefully cleaned from hard ticks and returned to their habitat.

In total, we collected 495 hard ticks: 138 females, 20 males, 219 nymphs and 118 larvae. We found two species: *Ixodes ricinus* (55%) and *Ixodes hexagonus* (36%). For some ticks (9%), it was possible to make an identification only to the genus (*Ixodes* spp.). Totally 49.5% of hard ticks from hedgehogs were infected with tick-borne pathogens: four species from *Borrelia burgdorferi* sensu lato (s.l.) complex (*B. afzelii*, *B. bavariensis*, *B. spielmanni*, *B. lusitaniae*), *B. myiamotoi*, *Rickettsia helvetica*, *Anaplasma phagocytophilum* and two species of *Babesia* spp. (*B. capreoli* and *B. venatorum*). Among the infected *I. ricinus* ticks, 88% were co-infected, and carried up to three different pathogens. The most common association was between *B. burgdorferi* s.l. – *A. phagocytophilum* (45%), followed by *Rickettsia* spp. – *A. phagocytophilum* (5%). Three pathogens co-infections were between *B. burgdorferi* s.l. – *A. phagocytophilum* – *Babesia* spp. (2%).

This is the first study of hard ticks and tick-borne pathogens from hedgehogs from urban environment in Slovakia.

*Acknowledgement: This study was financially supported by VEGA 2/0004/22, 2/0137/21 and APVV – 22-0372.*

### P30 The first molecular detection of *Borrelia miyamotoi* in *Ixodidae* ticks in the Chornobyl Exclusion Zone, Ukraine

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*Borrelia miyamotoi* is a bacterium of the spirochete phylum in the genus *Borrelia*, causing tick-borne relapsing fever. It is considered a pathogen of high significance to public health. Increasing number of *B. miyamotoi* infections in Europe, Japan and USA were reported. On the territory of Ukraine, *B. miyamotoi* has not been recorded to date. The objective of this study was to investigate the presence of *B. miyamotoi* infection in *Ixodidae* ticks collected in the Chornobyl area, Ukraine.

A total of 113 *Ixodes ricinus* ticks and 1176 *Dermacentor reticulatus* ticks were gathered in the vicinity of Chornobyl city (51°16'47"N; 30°12'47"E) and the abandoned regions: "Ostrov" (51°17'03"N; 30°12'57"E), "Puso" (51°17'52"N; 30°11'17"E), "Altanka" (51°16'41"N; 30°10'47"E), and Cherevach former village (51°12'44"N; 30°07'45"E) in 2019. The isolated DNA underwent screening for *B. miyamotoi* sensu lato presence via a two-step nested PCR approach.

The resulting phylogenetic tree of *B. miyamotoi*, based on the glpQ gene, depicted the percentage of replicate trees in which related taxa clustered together in the bootstrap test (1000 replicates) next to the branches. Values below 70% were excluded to ensure accuracy and reliability of the analysis.

The presence of *B. miyamotoi* was identified in two (0.17%) out of the 1176 *D. reticulatus* tested ticks. These specimens were collected in "Altanka" (1 male) and Cherevach former village (1 female). The incidence of *B. miyamotoi* infection was 2.04% and 0.52%, respectively. No *I. ricinus* ticks exhibited infection.

The PCR products, 425 bp in size, were purified, sequenced, and analyzed using Blast version 2.2.13. The sequences obtained were submitted to GenBank with accession numbers: MZ365312-MZ365314. Two obtained sequences (MZ365312, MZ365314) are identical. The sequence MZ365313 differs from the other received sequences by one nucleotide.

This marks the first instance of *B. miyamotoi* being detected among *D. reticulatus* ticks in the Chornobyl area, Ukraine.

*This research was supported by National Institute of Public Health – National Institute of Hygiene (BP-1/2020); National Science Centre, Poland (R-2022/01/3/NZ6/00089) and MSCA4Ukraine (AvHID 1233593; <https://cordis.europa.eu/project/id/101101923>).*

### **P31 Analysis of patient sera for the presence of specific antibodies against tick-borne antigens**

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Vector-borne pathogens (VBPs) are a global health concern that affect both humans and animals. These diseases are caused by a variety of pathogens that are transmitted by arthropod vectors, such as ticks. Lyme disease, bartonellosis, and Q fever are among those that are spreading into previously non-endemic areas and overlapping the vectors' territory. This study aimed to assess the seroprevalence of selected VBPs in sera of Slovak patients.

Using the IFA method, sera from Slovak patients were examined at the National Reference Centre in Marseille, France and the National Reference Centre for Surveillance and Laboratory diagnosis of Rickettsioses, Slovakia for the presence of specific antibodies against chosen VBPs (*Borrelia* sp., *Coxiella burnetii*, *Bartonella quintana* and *Ba. henselae*).

An overall seroprevalence of 1.63% (8/492) for at least one VBP was recorded in patients enrolled. We detected specific antibodies against *C. burnetii* in three samples, and against *Borrelia* sp. in 5 samples. Presence of antibodies against *Ba. quintana* and *Ba. henselae* were not found.

There is less information on the seroprevalence of such a wide spectrum of VBPs in Slovakia. Overall, these data may stimulate interest in confirming and expanding patient seroprevalence studies in Slovakia to better assess the clinical implications of these VBPs.

There exists a paucity of information regarding the seroprevalence of a diverse range of VBPs in Slovakia. These findings underscore the importance of conducting further seroprevalence studies among patients in Slovakia to enhance our understanding of the clinical ramifications associated with these VBPs, thereby stimulating interest in their confirmation and subsequent expansion.

*Funding and acknowledgement: APVV-19-0519, APVV-19-0066, VEGA 2/0021/21*

**P32 Monitoring of the seasonal activity of *Ixodes ricinus* and *Dermacentor reticulatus* ticks in Bratislava using tick gardens**

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Ticks transmit a wide range of pathogenic microorganisms. In humans, the diseases caused by these agents include Lyme borreliosis (LB), spotted fever group rickettsioses, human granulocytic anaplasmosis, tick-borne encephalitis, babesiosis, and many others. Many of these diseases have emerged (or re-emerged) within the past two or three decades. New foci of tick-borne diseases can be formed due to climatic changes and spread of ticks to new areas. Research on tick ecology and the diseases transmitted by ticks should be the subject of integrative multidisciplinary approach (biological, ecological, veterinary, medical and public health).

We investigated changes in the distribution and seasonal activity of two epidemiologically important species, *Ixodes ricinus* and *Dermacentor reticulatus*.

Seasonal activity has been monitored at several sites in Bratislava since July 2023. Ticks are collected at monthly intervals by flagging vegetation in each site. In addition to collections, we monitor the activity of ticks directly using tick gardens (TG) where exact numbers of ticks of each developmental stage are released.

The first results indicate a significant change in the seasonal activity of ticks compared to the situation in the past. The highest number of *I. ricinus* ticks was recorded in June (n = 550) and in November 2023 (n = 2286), but in this sample larvae predominated (97.6%). In August there was a decrease (n = 8), and in the fall (September and October) a few nymphs and adult ticks were collected (n = 64) and larvae predominated (86.3%). When daytime temperatures were above 10 °C in December, ticks were still active in some study sites (Železná studnička city forest park, n = 77) out of which the most abundant were larvae (n = 73). A similar situation was recorded in January 2024: 98 ticks were flagged including 96.9% of larvae. In February and March, the number of nymphs and adults increased to 91 and 96, respectively. The number of larvae decreased to 6 and 0, respectively.

Monitoring of *D. reticulatus* in TG confirmed tick activity even at sub-zero temperatures and also during the snow cover (December and January).

The study of the changes in tick seasonal activity and prevalence, as well as the genetic variability of tick-borne pathogenic agents will show the current risk of infection with tick-borne diseases.

*This study was financially supported from the Scientific Grant Agency by the project VEGA 2/0137/21 and by the Slovak Research and Development Agency APVV-22-0372.*



**P33 Genetic variability of multi-hosts pathogen: *Anaplasma phagocytophilum* in southern Slovakia**

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*Anaplasma phagocytophilum* is an intracellular bacterium and the causative agent of granulocytic anaplasmosis of both medical and veterinary importance. In Europe, the main vector of this pathogen is *Ixodes ricinus* tick with a wide range of hosts. The objective of this study was to determine the spectrum of animal species involved in the circulation of *A. phagocytophilum* (AP) in southern Slovakia and to analyze the genetic variability of the obtained AP genotypes.

During 2011-2023, 3626 feeding *I. ricinus* ticks from various hosts (humans, dogs, cats, sheep, wild ruminants, hedgehogs, birds) were collected. 1177 questing *I. ricinus* ticks were collected by standard blanket dragging. AP was detected in *I. ricinus* ticks by using a real-time PCR assay targeting the *msp2* gene (77 bp). Positive samples were genotyped by sequencing of *msp4* and *groEL* genes. Infection prevalence in host feeding ticks was: 5.3% in ticks from humans, 17.2% from dogs, 14.4% from cats, 33.3% from sheep, 52.4% from goats, 59.7% from wild ruminants, 60% from hedgehogs and 4.6% in ticks feeding on birds. Preliminary results suggest that prevalence of AP in questing *I. ricinus* ticks was 5.8% (Bratislava region).

From an epidemiological point of view, Cervidae and hedgehogs probably play the most important role in the circulation of this pathogen. Pets and farm animals can serve as sentinel animals in expressing the epidemiological risk for humans of being bitten by infected ticks. Overall, a wide range of animal species are involved in the maintenance and the circulation of AP in natural foci, because transovarial transmission in ixodid ticks has not been confirmed. Further genetic analyses are needed to identify the genetic variants of *A. phagocytophilum* circulating in Slovakia and their associations with reservoir hosts.

*This study was financially supported from the Scientific Grant Agency by the project VEGA 2/0137/21, VEGA 2/0004/22 and by the Slovak Research and Development Agency APVV-22-0372.*



### P34 The first report of *Ixodes ventalloi* on migratory birds in Slovakia

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*Ixodes ventalloi* is considered a three-host, endophilic, and monotropic tick. Historically, it has been associated with host specificity, primarily targeting lagomorphs, hence its designation as the 'rabbit tick'. However, its host range is more extensive and includes carnivores, rodents, birds, and even humans. Despite being relatively understudied, *I. ventalloi* is associated with at least 13 pathogenic agents, including viruses such as Eyach and Erve, bacteria like *Anaplasma phagocytophilum*, *Rickettsia Helvetica*, and *Coxiella burnetii*, as well as protozoans including *Leishmania infantum* and *Theileria annulata*.

Presented study describes the first detection of *I. ventalloi* in Slovakia. Two engorged females of *I. ventalloi* were collected from dunnocks (*Prunella modularis*) captured in eastern Slovakia. The identification of females was based on morphological and molecular 16S rRNA gene features.

Phylogenetic analysis revealed a classification of the females into distinct genogroups. The comparative analysis between our isolates (GenBank accession numbers: PP301986, PP301987) revealed a nucleotide identity of 90.2% within the examined nucleotide fragment, highlighting their distinct genetic backgrounds. Moreover, comparative morphological analysis shows variations between the two females, particularly in the curvature of the auriculae, the shape of coxa I, and the internal spur.

In this research we report the first identification of *Ixodes ventalloi* in Slovakia and its first collection on dunnocks (*Prunella modularis*), expanding the known host range and geographical distribution of this tick species. Furthermore, results indicate morphological diversity among *I. ventalloi* females, challenging previous assumptions of uniform phenotypes and suggesting the presence of distinct morphotypes. These findings support the integration of molecular techniques with traditional morphological identification methods to ensure accurate species identification. Further research is needed regarding the establishment of *I. ventalloi* populations in Slovakia.

*Funding and acknowledgement:* This research was funded by the Slovak Research and Development Agency, grant number APVV-19-0440 and Scientific Grant Agency of The Ministry of Education, Research, Development and Youth of the Slovak Republic and the Slovak Academy of Sciences VEGA 1/0316/23.

### **P35 Partial phylogenetic analysis of Uukuniemi virus strains isolated from ticks collected from vegetation in eastern Slovakia**

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Uukuniemi virus (UUKV, family: *Phenuiviridae*, genus: *Uukuvirus*) is a tick-borne arbovirus, whose genome encompasses three single-stranded segments of negative-sense or ambisense RNA. UUKV shares high sequence homology with Severe Fever with Thrombocytopenia Syndrome virus (SFTSV) and Heartland virus (HRTV), tick-borne viruses implicating in fatal human diseases. However, unlike SFTSV and HRTV, UUKV does not cause disease in humans, making it an ideal model for studying similar, more dangerous arboviruses.

This study aimed to detect orthoflaviviruses, orbiviruses and uukuviruses in ticks collected from vegetation in eastern Slovakia from 2019 to 2021. A total of 502 pools (2966 ticks), were analyzed using molecular and classical virological methods. Positive pools were sequenced and obtained sequences were compared with the GenBank database.

None of the pools tested positive for orthoflaviviruses or orbiviruses. Uukuvirus RNA was confirmed in five tested pools, with subsequent sequence analysis revealing the presence of UUKV. The positive ticks were identified as *Ixodes ricinus*. Two of the positive pools contained females, one contained males and the other two contained nymphs. To date, comparative partial nucleotide sequence analyses for the small (S), medium (M), and large (L) RNA segments of four of our isolates with other strains of UUKV and the Chize virus available in GenBank were conducted to construct phylograms.

A high degree of similarity between UUKV strains from Slovakia, Czech Republic, and the prototype UUKV strain from Finland was demonstrated. These strains formed a separate cluster with a common ancestor. Segment S, coding the nucleoprotein N and the nonstructural protein NSs, showed sequence identity of 96.1% to 99.3%. Segment M, coding the glycoproteins GN and GC, showed a similarity of 92.5% to 98.9% and segment L, coding the viral RNA-dependent RNA polymerase L, 98.2% to 99.2%. Conversely, UUKV strains from the United Kingdom and Norway and Chize virus from France formed separate cluster and exhibited lower sequence similarity in the case of each segment (segment S: 68.1% to 72.4%; segment M: 69.2% to 72.0%; segment L: 77.1% to 77.2%).

This study significantly contributes to expanding the UUKV sequence repository. Presently, GenBank contains merely three sequences of the S segment, six sequences of the M segment, and four sequences of the L segment, underscoring the limited availability of UUKV genomic data.

*Funding and acknowledgement. This research was funded by the Slovak Research and Development Agency, grant number APVV-19-0440.*

### P36 Ticks as potential vectors of microsporidia

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Ticks, ectoparasites of humans, domestic and wild animals, are widespread ectoparasites that can transmit a wide range of pathogens causing a variety of diseases, mainly due to the way it parasitizes when interacting with the host's blood. Since the tick does not exclusively parasitize mammals and its life cycle requires sucking on up to 3 different hosts, it is a potential candidate for the transmission of microsporidia from various hosts.

Microsporidia, unicellular obligate parasites have long been associated with serious infections in immunocompromised individuals, but also occur in immunocompetent ones, and often cause life-threatening complications of common diseases.

This study was conducted to elucidate the prevalence and molecular characterization of microsporidia in ticks in the Czech Republic. The ticks were collected either from various hosts or using flagging low vegetation. A total of 217 samples were obtained from *Ixodes ricinus* (n=203) and *Dermacentor reticulatus* (n=14). Of these, 25 microsporidia positive ticks were molecularly detected. The sequence analyses of the internal transcribed spacer (ITS) of the SSU rDNA gene identified *Enterocytozoon bieneusi* genotype D (n=8), *Encephalitozoon cuniculi* genotype I (n=7) and genotype II (n=10).

According to the results of the study, microsporidia are ranked among the most common pathogens detected in ticks (*Ixodes ricinus*) in the Czech Republic and their possible involvement in the transmission of microsporidia has been suggested. Moreover, it appears that tick feeding may facilitate transmission of infection through the concentration of microsporidia in sites of inflammation induced by the tick.

*The study was supported by the grant of Grant agency of the Czech Republic (No.24-10274S).*

**P37 Prevalence of *Anaplasma phagocytophilum* and *Neorhlichia mikurensis* in *Ixodes ricinus* ticks collected in urban areas of Lithuania**

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*Anaplasma phagocytophilum* and *Neorhlichia mikurensis* are gram-negative intracellular bacteria belonging to the family Anaplasmataceae. The main vector of these bacteria in Europe is *Ixodes ricinus* tick. Both *A. phagocytophilum* and *N. mikurensis* are implicated in human health. The presence of these bacteria in *I. ricinus* ticks was reported from various European countries. However, to date, *N. mikurensis* has never been investigated in Lithuania. Furthermore, the presence of *A. phagocytophilum* in urban areas of Lithuania remains unknown and needs to be discovered. The present study aimed to determine the prevalence and coinfection of these bacteria in questing *I. ricinus* ticks in Lithuanian urban areas.

A total of 2310 questing *I. ricinus* were collected by flagging and dragging vegetation from 15 urban locations (represented by public parks, city forests, and green areas used for recreational activities) in five Lithuanian counties during spring 2022-2023. All the specimens were subsequently processed for DNA extraction and the molecular detection of pathogens. Ticks were screened for the presence of pathogens using duplex real-time PCR assay amplifying *A. phagocytophilum* *msp2* and *N. mikurensis* *groEL* genes. Positive samples were further analyzed by nested PCR and sequencing.

*A. phagocytophilum* was detected in 4.7% (108/2310) of the samples, while *N. mikurensis* DNA was present in 7% (161/2310). Coinfection with *A. phagocytophilum* and *N. mikurensis* was detected in 23 (1%) ticks.

This study reports new data on tick-borne pathogens in urban zones in Lithuania and highlights the presence of *N. mikurensis*, a newly emerging pathogen of significant public health importance that should be monitored. The detection of *A. phagocytophilum* and *N. mikurensis* in *I. ricinus* ticks in Lithuanian urban green areas characterized by human outdoor activities, indicates a potential transmission risk for tick-bitten humans.

*This study was supported by Research Council of Lithuania (Grant No. S-MIP -23/19).*

### **P38 Occurrence of Rickettsiales from questing ticks in urban districts in Košice (Eastern Slovakia)**

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*Rickettsia* is a genus of intracellular bacteria that are transmitted to humans through vectors such as ticks, fleas, lice, and mites. Ticks, major vectors of many pathogens (viruses, bacteria, protozoa and helminths) in urban areas are becoming more prevalent, largely due to factors like increased urban wildlife populations, habitat fragmentation, and changes in land use. In urban areas, ticks are often found in parks, gardens, lawns, and near wooded or grassy areas. In this study, ticks were collected by white cotton flag (1m<sup>2</sup>) method in tree green areas of Košice city. The areas have been selected on the basis of a large green urban area isolated from the surrounding forests, suitable for the survival of ticks and their hosts. Total of 158 ticks of two species (*Dermacentor reticulatus* and *Ixodes ricinus*) were collected. From 158 questing ticks during autumn 2022 we found 58 *D. reticulatus* (46 females and 12 males) and 100 *I. ricinus* (47 females, 45 males and 8 nymphs). Ticks were isolated for DNA with commercial kit (Qiagen, Hilden, Germany) and tested for presence of *Rickettsia* spp. by PCR. PCR was performed using oligonucleotide primer D767f and D1390r which amplify a 623-bp fragment of the surface cell antigen 4 (sca4) gene of *Rickettsia* spp. The prevalence of *Rickettsia*-positive ticks was 10.8% (17 ticks). There was no significant difference between the prevalences in females (3.2%) and males (5.7%). We found 3 positive samples in *D. reticulatus* and 14 in *I. ricinus*. DNA of three different *Rickettsia* species was identified: *R. conorii* subsp. *raoultii*, *R. helvetica* and *R. monacensis*. *Rickettsia helvetica* was dominant species found only in *I. ricinus* ticks. *Rickettsia monacensis* was found in 1 sample of *I. ricinus*. *Rickettsia c. subsp. raoultii* was found in *D. reticulatus* ticks. It is evident that *I. ricinus* and *D. reticulatus* ticks in Košice city are commonly infected with *R. helvetica*, *R. monacensis* and *R. conorii* subsp. *raoultii* that are considered human pathogens. The implication of this finding and its possible association with human disease will require further investigations.

*Acknowledgments: This research was supported by VEGA 1/0287/22.*

### **P39 Functional characterization of receptors in tick *Ixodes ricinus***

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In Europe, the tick *Ixodes ricinus* is the most important vector and reservoir of numerous pathogens that are transmitted during blood feeding on their vertebrate hosts. Understanding the complex mechanisms underlying tick physiology and transmission of pathogens is essential for the development of effective strategies to combat tick-borne diseases. Many physiological functions in ticks are under control of neuropeptides and peptide hormones, which act on target tissues through interaction with specific membrane receptors. Due to their lower expression levels, *receptors represent* promising targets for double-stranded RNA-mediated interference (*RNAi*) enabling the functional analysis of respective *neuropeptide*-receptor signalling in tick physiology. We therefore successfully adopted aequorin-based bioluminescence assays for deorphanization of identified *I. ricinus* G-protein-coupled receptors (GPCRs) and membrane-bound guanylyl cyclase receptors. These characterized receptors can then be effectively targeted in follow-up research to unravel the mysteries of tick biology.

*This work was supported by Slovak Grant agencies, Agentúra na podporu výskumu a vývoja (APVV-18-0201, APVV-21-0431) and Vedecká grantová agentúra (VEGA-2/0070/23, VEGA-2/0037/23, VEGA-2/0040/23).*

**P40 Characterization of insect parathyroid hormone-like peptide in the hard tick  
*Ixodes ricinus***

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Ticks are hematophagous parasites and important vectors of pathogens. In tick's life cycle, the salivary glands produce various bioactive compounds necessary for tick's feeding, function in water homeostasis, or sex, and participate in pathogen transfer. These organs are regulated by neurons of the central nervous system producing neuropeptides, which function as neuromodulators, neurotransmitters, or hormones (Nässel 1993). Only few neuropeptides were verified so far in the innervation of salivary glands (Vancová et al. 2019; Roller et al. 2015; Šimo et al. 2009).

In this study we focused on neuropeptide PTH (parathyroid hormone-like peptide), previously characterized in the beetle *Tribolium castaneum* and later identified in the tick *Rhipicephalus microplus* (Xie et al. 2020; Waldman et al. 2022). Using bioinformatic analysis, we identified PTH and PTH receptors in genome of the hard tick *Ixodes ricinus*. We verified the PTH transcript by cloning and consecutively prepared probe for *in situ* hybridization and peptide-specific antiserum. The *in situ* hybridization and immunohistochemistry revealed expression of PTH in CNS and densely branched innervation of salivary glands, rectal sack, and muscles of the tick. Double-immunohistochemical staining of the organs revealed co-expression of PTH with other neuropeptides. Furthermore, using RT-qPCR we verified expression of PTH receptor in the salivary glands. We thus provide evidence of a novel neuropeptide in the innervation of salivary glands.

*This work was supported with Slovak grant agencies with projects: APVV-18-0201, APVV-21-0431, VEGA-2/0070/23 and VEGA-0037-23.*



#### P41 Winter activity of ticks on game animals in the Small Carpathians (southwestern Slovakia)

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Big game animals represent important hosts for ticks. During late autumn - winter (2023/2024), 59 game animals hunted in Bratislava forest park: 44 fallow deer (*Dama dama*), 12 roe deer (*Capreolus capreolus*), two wild boars (*Sus scrofa*), one mouflon (*Ovis musimon*) were examined for infestation by ticks. The aim of the study was to determine the occurrence and activity of ticks during the cold season. The study material included 1,865 ticks of three species: *Ixodes ricinus* (95.76 %), *Haemaphysalis concinna* (3.38 %) and *Dermacentor reticulatus* (0.86 %). In total, 88.14 % of game individuals (93.18 % fallow deer, 75.00 % roe deer, one wild boar and one mouflon) were infested by ticks. The mean infestation intensity by ticks was  $31.61 \pm 45.52$  ex/ind (MIN-MAX: 0 ex/ind - 203 ex/ind), with significant difference between roe deer and fallow deer ( $\chi^2 = 17.40$ ;  $p = 3.03E-05$ ;  $CV = 3.84$ ). Adults of both sexes (sex ratio F = 61.3 %; M = 38.7 %) and juvenile stages (ratio N = 97.17 %, L = 2.83 %) of *I. ricinus* occurred on deer. Only five nymphs were collected from wild boar and 32 ♀, 24 ♂ and 54 nymphs were collected from mouflon. A fact confirming the ability of *I. ricinus* to quest even on days when the temperature does not reach 10°C is the recording of unfed individuals crawling freely in the animal fur (November - 20 ♀, 42 ♂, 25 nymphs and one larva collected from six fallow deer; December - 1 ♀, 29 ♂, eight nymphs and one larva collected from four fallow deer; January - 2 ♀, 13 ♂, five nymphs and one larva collected from 14 fallow deer and two roe deer). Surprisingly, feeding *H. concinna* males and juveniles (ratio N = 96.67 %, L = 3.33 %) were also recorded on game in winter. On the contrary, only unfed freely crawling adults (sex ratio F = 6.25 %, M = 93.75 %) of *D. reticulatus* were recorded during cold days (December - 1 ♀, 1 ♂ collected from fallow deer; January 2 ♂ collected from fallow deer). The continuous activity of *I. ricinus* during cold days, when the temperature does not reach 10°C, represents to Bratislava citizens visiting the forest park a risk of tick infestation and transmission of tick-borne pathogens during the whole year.

*Acknowledgements. Special thanks go to the members of the hunting association PS Rača. Study was financially supported by VEGA 2/0004/22, 2/0137/21 and APVV 22-0372.*

**P42 *Ixodes laguri*, a grassland and steppe tick that parasitizes critically endangered rodents**

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The range of *Ixodes laguri* Olenov, 1929 is only partially known. It is a nest-dwelling parasite of small mammals that mainly infest rodents of the families Cricetidae, Gliridae, Muridae and Sciuridae. There is no proven vectorial role for *I. laguri*, although it is suggested that it is a vector of *Francisella tularensis*. A first map depicting the entire range of *I. laguri* based on georeferenced locations and a list of main hosts is presented. For this purpose, a digital data set of 142 georeferenced locations from 16 countries was compiled. Particular attention is paid to the description of the westernmost record of *I. laguri* in the city of Vienna, Austria. There, *I. laguri* is specifically associated with its main hosts, the critically endangered European hamster (*Cricetus cricetus*) and the European ground squirrel (*Spermophilus citellus*). These two host species have also been mapped in the present paper to estimate the potential distribution of *I. laguri* in the Vienna metropolitan region. The range of *I. laguri* extends between 16–108°E and 38–54°N, i.e. from Vienna in the east of Austria to Ulaanbaatar, the capital of Mongolia. In contrast to tick species that are expanding their range due to global warming, *I. laguri* has become increasingly rare throughout its range. However, *I. laguri* may be threatened not by climate change, but by anthropogenic influences on its hosts and their habitats, which are typically open grasslands and steppes. Rural habitats are threatened by intensification of agriculture and semi-urban habitats are increasingly being destroyed by urban development. Main hosts are hamsters (*C. cricetus*, *Nothocricetulus migratorius*, *Mesocricetus auratus*, *Mesocricetus newtoni*, *Mesocricetus raddei*, *M. brandti*) and ground squirrels (*S. citellus*, *S. pygmaeus*), but also gerbils (*M. unguiculatus*, *M. tristrami*) and marmots (*Marmota sibirica*). Less frequent *I. laguri* was reported from lemmings (*L. lagurus*), rodents such as mice, shrews and moles, and other small mammals.

### P43 Winter activity of ectoparasites – (deer keds, chewing lice, sucking lice) on game animals in the Small Carpathians (southwestern Slovakia)

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Big game animals represent important hosts for various ectoparasitic arthropods. During late autumn - winter (2023/2024), 59 hunted game animals: 44 fallow deer (*Dama dama*), 12 roe deer (*Capreolus capreolus*), two wild boars (*Sus scrofa*), one mouflon (*Ovis musimon*) were examined for the infestation by ectoparasites. The aim of the study was to determine the occurrence of ectoparasites during the cold season. The study material included 1 286 deer keds (Diptera: Hippoboscidae), 87 sucking lice (Phthiraptera: Anoplura) and 209 chewing lice (Phthiraptera: Ischnocera). Additionally, botfly *Hypoderma* sp. (Diptera: Oestridae) larvae were detected under the skin of two roe deer (12 and 1 ex, resp.) and two fallow deer (1 ex each). Each game individual (96.6 %) except wild boar was infested by deer keds, which were present constantly during the whole winter. The mean infestation by deer keds was  $22.25 \pm 38.68$  ex/ind (MIN-MAX: 1 ex/ind - 243 ex/ind both on fallow deer) without significant difference between roe deer and fallow deer ( $\chi^2 = 0.097$ ;  $p = 0.755$ ;  $CV = 3.84$ ). Two species of deer keds were identified: *Lipoptena forfisetosa* (69.56 %) and *Lipoptena cervi* (29.02 %). A total of 1.42 % deer keds were identified by morphological characteristics only to genera. *L. forfisetosa* was dominant on both fallow deer (70.64 %) and roe deer (65.08 %), however, its infestation rate significantly fluctuated during autumn-winter ( $t = 16.72$ ,  $p = 1.97E-23$ ) similarly as that of *L. cervi* ( $t = 8.18$ ,  $p = 3.93E-11$ ). The sex ratio was similar in both deer keds species (*L. cervi*: ♀ 64.67%; ♂ 35.33%; *L. forfisetosa*: ♀ 66.78%; ♂ 33.22%) and was changing significantly during the observation period (*L. cervi*:  $t = 9.57$ ,  $p = 2.22E-13$ ; *L. forfisetosa*:  $t = 16.48$ ,  $p = 3.81E-23$ ). Regarding lice infestation, the sucking lice *Haematopinus apri* were collected from one wild boar (18 ♂, 33 ♀, 34 nymphs) and one fallow deer (1 ♀, 1 nymph), while the chewing lice *Bovicola longicornis* were present on three fallow deer (2 ♂, 29 ♀), three roe deer (144 ♀, 19 nymphs) and one mouflon (8 ♂, 7 ♀). In this work we confirmed the infestation of big game animals by five ectoparasitic insect species during late autumn and winter. However, active spread of deer keds to new hosts cannot be confirmed due to the absence of winged individuals.

*Acknowledgements. Special thanks go to the members of the hunting association PS Rača. Study was financially supported by VEGA 2/0004/22, 2/0137/21 and APVV 22-0372.*

#### P44 Mosquitoes of selected recreational areas of Slovakia and their host preference

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Recently, we have detected significant changes in the species composition of mosquito populations in Slovakia. In particular, the rapid spread of invasive mosquitoes of the genus *Aedes*, as well as climatic and environmental changes, mean a high risk of introducing exotic pathogens into our territory. Therefore, the aim of our survey was to determine the species composition of mosquitoes in selected recreational areas of Slovakia and their host preference.

In 2023 we trapped mosquitoes at 16 sites, the trapping sites were divided into urban (Košice forest park Popradská, Zoo, UVLF riding school) and suburban (Čaňa, Drienovec, Košické Olšany, Nižný Klátov, Ružín, Krompachy, Rozhanovce, Sered', Kolovo pleso, Martovce, Komárno, Jahodná). We used standard CO<sub>2</sub> baited traps for mosquito imago trapping, which were exposed for about 12 hours (from 19:00 to 07:00 of the following day). Species determination was made on the basis of morphological characters. We subjected morphologically difficult to distinguish species to molecular analysis. In selected female mosquitoes, we detected host preference from the blood-filled gut.

A total of 4036 mosquitoes were collected, 18 species. The highest prevalence was recorded for *Culex pipiens* komplex (70.7%), *Ochlerotatus excrucians* (10.7%), *Culiseta annulata* (4.9%) and *Aedes vexans* (4.1%). For the first time, *Culex quinquefasciatus* was confirmed molecularly in eastern Slovakia. According to the gene bank, this is a 100% match with species from Brazil (MK575480.1) and Tunisia (KY744202.1). We detected host specificity confirming predominant suckling on humans. According to gen Bank, there is 100% concordance with Spain (LC088149.1). At the UVLF equestrian campus mosquitoes sucked on horses with 100% concordance with isolates from Hungary (KT792957.1). We have not yet confirmed pathogens of parasitic origin in mosquitoes at the sites surveyed.

Mosquitoes in the monitored areas are highly anthropophilic and represent a potential risk of transmission of flaviviruses as well as parasitic filarial worms circulating in Slovakia.

*Acknowledgements: We have carried out this research thanks to APVV project no. 19-0440.*



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