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O1 ★ Keynote Speaker

EFSA risk assessment for animal and plant health: a One Health methodological approach

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The European Food Safety Authority (EFSA) provides independent scientific advice on risks along food and feed chains, including animal health and welfare and plant health. These risks often involve interactions among domestic and wild hosts, vectors, environmental drivers and human activities. From a risk assessment perspective, One Health can be interpreted not only as a framework for cross-sectoral collaboration but also as a methodological approach for integrating heterogeneous evidence and supporting decisions under uncertainty. A broader biosecurity perspective connects these domains through the shared objectives of anticipation, prevention, early detection and mitigation of biological threats. Representing interconnected biological systems within a single assessment is methodologically demanding. Relevant processes span populations, spatial scales and time horizons, while evidence is often incomplete, heterogeneous or unevenly distributed among sectors. Parasites, vector-borne diseases and plant pests are relevant examples; their introduction, establishment and spread may depend on interactions among hosts, vectors, environmental conditions, trade and human activities. Their impacts may extend beyond directly affected hosts and immediate production or health outcomes, affecting population dynamics, ecological communities, biodiversity, ecosystem services, human and animal health, livelihoods and society. Despite this complexity, timely and transparent assessments can be produced by structuring evidence around decision-relevant components. These may include hazard occurrence, introduction pathways, potential establishment and spread, impacts and the expected effects of surveillance or control measures. One Health is operationalised when evidence, assessment and decision-making are connected across sectors. Complementary approaches include horizon scanning and systematic monitoring, pathway analysis, risk-based surveillance and methods for estimating surveillance sensitivity, substantiating freedom from a hazard and allocating sampling effort according to risk. Some of these methods can be transferred or adapted between animal and plant health, providing a common methodological basis for surveillance, risk assessment and biosecurity decision-making. These approaches also rely on harmonised evidence and interoperable data. Standardised information on hazards, sampling, analytical results and metadata supports the comparison and reuse of evidence across surveillance and risk assessment contexts, while greater interoperability among human, animal, plant and environmental data streams can facilitate integrated assessments and cross-sectoral signal detection. Examples from EFSA activities in parasitic and vector-borne diseases, risk-based surveillance and plant pest impact assessment illustrate how these methodological principles can be applied in practice.

Keywords: One Health; risk assessment; biosecurity; parasitic and vector-borne diseases; risk-based surveillance; plant health; ecosystem services; data interoperability.

O2

Alveolar and cystic echinococcosis: two parasitic infections with different epidemiology, clinical manifestation and management

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Echinococcosis is caused by larval stages of several *Echinococcus* species. *E. multilocularis* causing alveolar echinococcosis (AE) is an emerging infection in Central and Eastern Europe. *E. granulosus* causing cystic echinococcosis (CE) is endemic in Southern and Eastern Europe, North Africa, Central Asia

and Latin America. CE is localized in liver and lung predominantly. CE is relatively benign and surgical removal of active cysts with albendazole co-administration is the basic treatment. AE is a serious, potentially life-threatening due to progressive tumor-like growth in liver and risk of metastatic dissemination. We conducted a prospective descriptive study of 38 AE cases and 25 CE cases diagnosed and managed between 2005 and 2025 at three Czech referral centers: University Hospital Bulovka and Motol in Prague (n=56) and Regional Hospital Liberec (n=7). A total of 38 patients with AE were managed: 21 females (median age 48 years) and 16 males (median age 61 years). Patients originated from Czechia (33), Ukraine (3), and Slovakia (2). One patient acquired AE after an infected liver graft. The most common symptoms were abdominal pain (11), fever (4), jaundice (3) and a palpable abdominal mass (3). 17 patients were asymptomatic. Lesions were confined to the liver only in 27 cases. Liver and adjacent organs were affected in 11 patients. Diagnosis was based on characteristic CT/MRI findings, positive serology (36 of 37 cases), histology, and PCR. Immunosuppression was presented in 8 of 37 (22%) Eosinophilia was absent, but total IgE was elevated in 23 of 37 cases. Curative treatment combining surgery and long-term continuous albendazole or mebendazole was performed in 17 patients. 17 persons had inoperable lesions, five patients underwent liver transplantation. Outcomes were favorable in most cases, five elderly patients died from AE unrelated reasons. A total of 25 patients with CE were treated at our departments: 12 females (median age 35 years) and 13 males (median age 36 years). Patients originated from South-East Europe (9), Ukraine (6), and Central Asia (6) predominantly. Lesions were localized in the liver in 22 cases, in lungs in 2 patients, and in both, liver and lung in one patient. Diagnosis was based on characteristic US/CT/MRI findings and positive or borderline serology (24/25). Eosinophilia was in 9 of 25 patients, and total IgE was elevated in 12 of 25 cases. Successful surgery was performed in 18 patients. Diagnostics of CE and AE is difficult. Infections are asymptomatic for many years and there are not useful screening tests available. AE remains a serious, potentially life-threatening disease in the Czech Republic, yet is often underestimated. Increasing clinical awareness, along with early diagnosis and timely treatment, is essential to improve outcomes.

This study was partially supported by the Scientific Board of the Regional Hospital Liberec and by the Czech Ministry of Health Grant NU23-05-00441.

O3

Coexistence of primary hepatic neuroendocrine neoplasm and alveolar echinococcosis: diagnostic challenges and radiological diagnostic dilemmas – a case report

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Introduction: The differential diagnosis of space-occupying lesions in the liver often presents clinicians with a dilemma: is it a neoplastic process or a parasitic infection? The medical literature describes cases in which metastases of cystic neuroendocrine tumors (NETs) almost perfectly mimic echinococcosis, as well as situations where alveolar echinococcosis (AE) is misdiagnosed as a malignant liver tumor. Furthermore, primary neuroendocrine tumors of the liver (PHNEN) are extremely rare pathologies, accounting for only 0.46% of all primary liver tumors. Moreover, in a broader oncological context, these tumours account for only 0.3% of all neuroendocrine tumours. Although cystic echinococcosis (CE) predominates in the European population with an incidence of 0.50–0.64 per 100,000 people, the much rarer AE – with an estimated 1,600 cases annually in the region of Europe, Central Asia, and Russia – represents the most serious diagnostic pitfall for PHNEN. AE caused by *Echinococcus multilocularis* is a rare parasitic disease that, due to its infiltrating "tumor-like" growth pattern, is difficult to diagnose; in nearly one-third of cases, it is initially misdiagnosed as a malignant liver lesion. The statistical rarity of PHNEN and the specific epidemiology of echinococcosis in Europe mean that their co-occurrence is almost entirely neglected in routine diagnostics. Case report: The aim of this study is to present a case of a 50-year-old female patient with a giant liver tumor in which an extremely rare coexistence of PHNEN and AE occurred. This phenomenon created a complex diagnostic challenge in which the tumor and the parasite not only mimicked each other but also coexisted within a single lesion. The study focuses on the phenomenon of

radiological mimicry and serological pitfalls that complicate the final diagnosis, particularly in cases with an atypical clinical profile. The patient was initially hospitalized in 2020 due to abdominal pain and the detection of a large solid-cystic lesion (12 × 13 cm) in the right lobe of the liver, which was managed as a presumed hematoma due to complicated drainage attempt. Over the following three years, significant tumor progression was observed, with the lesion reaching approximately 20 cm in size and the appearance of numerous satellite lesions, radiologically suggestive of an echinococcal cyst or cystadenoma. Due to the enormous size of the lesion and compression of the portal structures, a two-stage treatment was employed: embolization of the branches of the portal and hepatic veins, followed by an extensive right-sided hemihepatectomy extended to segment 4 of the liver. The key finding was the histopathological examination of the resected material, which revealed a highly differentiated neuroendocrine tumor (NET G1) with a low Ki-67 index (<1%). Additional parasitological consultation of previously prepared histopathological sections at the University Centre in Poznań showed the presence of a typical alveolar structure whose morphological appearance corresponded to a coexisting *E.multilocularis* infection. Subsequent Western-blot peripheral blood serology confirmed *Echinococcus* spp. Infection. Conclusions: Space-occupying lesions located in the liver require a thorough clinical, imaging and epidemiological assessment as well as consultations at reference centres of clinical parasitology. The final diagnosis of alveolar echinococcosis does not exclude the coexistence of another serious disease, e.g. liver malignancy.

O4

Therapeutic Drug Monitoring of Albendazole-Sulfoxide in Alveolar Echinococcosis: Experience from a Slovak Cohort

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Alveolar echinococcosis is a rare but potentially life-threatening parasitic disease caused by the larval stage of *Echinococcus multilocularis*. Long-term or lifelong treatment with albendazole represents a cornerstone of patient management in clinical practice, both in patients with inoperable disease requiring lifelong therapy and as adjuvant treatment following major surgical resection of the parasitic pseudotumour. The therapeutic effect of albendazole may be influenced by variable absorption, metabolism, and bioavailability. Albendazole-sulphoxide (ASOX), the main active metabolite of albendazole, may therefore provide valuable information on the patient's systemic exposure to the drug through measurement of its plasma concentrations.
The aim of our study was to assess plasma concentrations of albendazole-sulphoxide (ASOX) in patients treated for alveolar echinococcosis, and thereby evaluate the role of therapeutic drug monitoring in personalizing long-term treatment for individual patients.
Patients with confirmed alveolar echinococcosis were included in the study cohort. Albendazole-sulphoxide (ASOX) concentrations were measured during routine outpatient follow-up visits at our department and were assessed in relation to the dosing regimen, treatment tolerance, treatment adherence, and the patients' clinical status. Plasma ASOX concentrations were detected and analysed using ultra-high-performance liquid chromatography (UHPLC) coupled online with tandem mass spectrometry (MS/MS), performed on a XEVO TQ-S triple quadrupole system (Waters, Czech Republic).
Monitoring revealed high inter-individual variability in ASOX concentrations among the tested individuals, despite comparable dosing regimens. Plasma concentrations ranged from therapeutic to elevated levels. Our results indicate that a considerable proportion of patients were exposed to higher drug concentrations, which may potentially increase the risk of severe adverse effects in some individuals. On the other hand, we did not observe ASOX concentrations below the therapeutic range. These findings highlight the limitations of standard albendazole dosing and support the importance of therapeutic drug monitoring (TDM) in selected patients.
Measurement of albendazole-sulphoxide concentrations is an important tool for personalizing long-term treatment in patients with alveolar echinococcosis. Incorporating therapeutic drug monitoring (TDM) into the routine multidisciplinary management of alveolar echinococcosis may help optimize dosing strategies,

assess treatment adherence, and achieve a better balance between treatment efficacy and safety. Further prospective studies are needed to define optimal target ranges and clarify the relationship between drug exposure, treatment response, and long-term outcomes.

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O5

Fatal case of naegleriosis in Slovakia

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Naegleria fowleri is a free-living, thermophilic amoeba. This pathogenic protozoan naturally occurs in warm freshwater environments such as lakes, rivers, thermal springs, and inadequately maintained swimming pools. Human infection, known as primary amoebic meningoencephalitis (PAM), is extremely rare and usually fatal, occurring when contaminated water enters the nasal cavity and the amoeba migrates to the brain.

Clinical course

An 11-year-old boy attended a swimming course at a thermal pool in Štúrovo. Upon returning home, he reported fatigue, headache, and anorexia but remained in relatively good condition and attended school. Six days later (June 12, 2025), he developed worsening headache, vomiting, and photophobia. A brain CT scan showed no abnormalities, and the patient was discharged fully conscious. On the following day (June 13), recurrent vomiting and decreased responsiveness prompted emergency medical transport to the Emergency Department, followed by transfer to the Department of Pediatric Anesthesiology and Intensive Care. The patient presented with hyperpyrexia (up to 40°C), responsiveness only to strong stimuli, and bilaterally dilated pupils reactive to light. Initial investigations included lumbar puncture, which revealed turbid cerebrospinal fluid (CSF) under increased pressure. Parasitological examination confirmed *N. fowleri* infection, as described in detail below. Despite administration of treatment (amphotericin B deoxycholate, azithromycin, fluconazole, and miltefosine), the patient's condition rapidly deteriorated, with progressive impairment of consciousness. Polyuria developed, accompanied by loss of conscious responsiveness. On June 14, brain MRI demonstrated global ischemia and malignant cerebral edema. On June 15, analgosedation was discontinued, and based on MRI findings and overall clinical status, brain death was declared on June 16. The patient was disconnected from mechanical ventilation on June 17 and subsequently died.

Laboratory identification

On June 13, CSF from the patient was delivered to the parasitology laboratory of Medirex. Microscopic examination of a native CSF preparation revealed structures morphologically consistent with free-living amoebae, highly suggestive of *N. fowleri*, along with numerous leukocytes. Giemsa-stained smears demonstrated characteristic trophozoite stages of the parasite. Definitive diagnosis was established by culture on non-nutrient (NN) agar overlaid with heat-killed *Escherichia coli*, where abundant amoebic growth was observed on June 14. Subsequently, a flagellation test was performed on the amplified culture, in which, after 1.5 hours, typical rapidly motile, biflagellate pear-shaped stages were observed. On June 18, encystment was observed microscopically on culture plates. Following the patient's death, brain tissue samples were subjected to Giemsa staining, confirming the presence of trophozoites. DNA of *N. fowleri* was confirmed in the cultured isolate using PCR.. Scanning electron microscopy (SEM) was used to characterize the ultrastructural morphology of *N. fowleri*. We report the first confirmed fatal *N. fowleri* infection in Slovakia.

O6

Primary Amebic Meningoencephalitis Linked to Public Swimming Pool Activities: An Environmental Investigation

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In June 2025, the first case and death caused by primary amebic meningoencephalitis (PAM) in the modern history of Slovakia was documented. The patient, a previously healthy 11-year-old boy, had participated in swimming lessons at a public swimming pool resort shortly before the onset of the initial symptoms, and this aquatic exposure was considered a probable source of infection by free living amoeba (FLA) *Naegleria fowleri.* Based on cases reported to date, the disease predominantly affects young individuals participating in recreational water activities, such as swimming or diving in warm lakes, rivers, or swimming pools. The Public Health Authority of the Slovak Republic (PHA SR) initiated an epidemiological investigation to confirm the source of infection within the resort. Between June and October 2025, a total of 193 samples were collected and analyzed. Water samples, swabs, and sediment were repeatedly obtained from all areas of the resort, including outdoor and indoor pools, pool gutters, water attractions, recirculation station filters, thermal water source, submerged joints, stairs, and structural defects. In addition, a sample was collected from a natural water reservoir located near the pools and forming part of the resort complex. To increase the probability of detecting the organism, water samples were analyzed in parallel by three laboratories of the PHA SR located in Bratislava, Banská Bystrica, and Prievidza, as well as by three university research institutions in Slovakia and abroad. Both culture-based and microscopic methods, together with advanced molecular techniques, were used to detect the presence of *N. fowleri*. Following successful cultivation of FLA on agar plates, nucleic acids were isolated from the monoxenic cultures using commercially isolation kits. All molecular techniques were used, including conventional PCR targeting the *ITS* region for identification of the genus *Naegleria*; using a specific primer pair targeting the *ITS-1* and *ITS-2* regions for specific detection of *N. fowleri*, real-time PCR targeting the *Mp2Cl5* sequence, which is highly specific for *N. fowleri* and enables reliable differentiation from other *Naegleria* species as well as from other FLA. Finally, real-time multiplex PCR for the simultaneous molecular detection of multiple species of amoebae (*Acanthamoeba* spp., *Naegleria fowleri*, *Balamuthia mandrillaris*, and *Vermamoeba vermiformis*) was performed by international partner teams in Spain and Germany. In 78 culture-positive samples, various saprophytic or bacteriophagous amoebae were identified, including the genus *Naegleria*, and opportunistic pathogenic amoebae of the genera *Acanthamoeba* and *Vermamoeba vermiformis*. However, *N. fowleri* was not confirmed in any of the 109 samples analyzed by molecular methods. The presence of *Naegleria* species and other potentially pathogenic FLA in pool water indicates environmental conditions favorable for the survival of *N. fowleri*; however, it can be assumed that the concentration of the pathogen was likely very low Despite the relatively rare occurrence of PAM worldwide, the documented case highlights the need to consider the potential presence of *Naegleria fowleri* in our recreational waters as well.

O7

Therapeutic challenges in the treatment of imported giardiasis from South Asia – analysis of drug-resistant cases in clinical practice

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Introduction: *Giardia intestinalis* remains one of the most common intestinal protozoa identified in travellers returning from tropical and subtropical areas, with South Asia being the dominant region of acquisition. Standard first-line treatment relies on nitroimidazoles, such as metronidazole, tinidazole or ornidazole. However, data from major European tropical medicine centres indicate a growing problem of treatment failure in this group of travellers. In a retrospective analysis from Hamburg (n = 308), the metronidazole first-line failure rate reached 47.9% in patients returning from South Asia, and the risk of failure was significantly higher compared to other warm climate regions e.g. Central and South America. Similarly, at the Hospital for Tropical Diseases in London, the nitroimidazole failure rate rose from 15.1% in 2008 to 40.2% in 2013, predominantly among travellers from India. Population-based data from Sweden (n = 4,285) show lower overall rates (2.4%) but confirm a striking geographical disparity: 12% failure in cases from India versus approximately 1% from other regions.
Results: In all the patients, monotherapy implemented before hospitalization in an outpatient clinic or by a family doctor with tinidazole (2 g single dose), metronidazole (750-1000 mg daily), or albendazole (400 mg) proved ineffective, leading to months of persistent symptoms. Parasite eradication was only achieved using combination regimens based on nitazoxanide with albendazole, tinidazole, or chloroquine applied for 7-10 days. None of the patients had documented immunoglobulin deficiencies, HIV infection, or confirmed reinfection during the follow-up period of 6 months.
Discussion: Although quinacrine (mepacrine) demonstrates the highest efficacy in second- and third-line treatment – ranging from 81% to 100% cure rates in prospective TropNetEuropstudies and reports from the Karolinska Institute – its limited availability in Central Europe necessitates the use of alternative therapy regimens. Repeating a nitroimidazole course after initial failure rarely results in cure (17-27% efficacy), and switching to a different drug class is strongly recommended. It should be emphasized that the term “drug resistance” in giardiasis is a clinical working definition; routine diagnostics do not include in vitro susceptibility testing or assemblage typing. Treatment failure may result not only from true parasite resistance but also from reinfection, immunodeficiencies (particularly CVID or IgA deficiency), poor compliance, post-infectious IBS, or secondary lactase deficiency.
Conclusions: Giardiasis imported from South Asia is becoming a significant and escalating epidemiological and clinical problem in Central Europe. The high rate of first-line treatment failure among travellers from this region requires an urgent update of diagnostic and therapeutic protocols, an individualized approach to patients, and increased vigilance among primary care physicians and infectious diseases specialists.

O8

Treatment challenges in imported giardiasis: A 22-year retrospective analysis

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Giardiasis is one of the most common enteric infections imported from tropical regions to Europe. Resistance to metronidazole (MTZ) represents an increasing problem, especially in cases imported from Indian Subcontinent. We conducted a retrospective analysis of the epidemiological and clinical characteristics of patients with laboratory-confirmed giardiasis diagnosed at Bulovka University Hospital and University Hospital Motol in Prague, between 2004 and 2025. Diagnosis was performed using stool light microscopy and/or PCR (since 2017). Giardiasis was diagnosed in 406 patients (female:male ratio 0.65). Most of infections were imported from the Indian Subcontinent (234 cases; 57.6%), followed by Sub-Saharan Africa (58 cases; 14.3%), and Southeast Asia (35 cases; 8.6%). Large majority of patients were symptomatic; the most common symptoms were diarrhea, abdominal pain, and bloating. The response to treatment with MTZ was evaluated in two periods. Reduced dose of MTZ (250 mg tid for 7 days) had very low efficacy (18%). The higher dose of MTZ (500 mg tid for 10-14 days) was used in most patients as the first line treatment. Between 2004 and 2015, 89 patients were treated with high dose of MTZ and had documented outcome. 50 of 89 (56.2%) responded and 39 (43.8%) failed. Ornidazole (500 mg bid) plus albendazole (400 mg bid) for 14 days was used as second line treatment successfully. Between 2016 and 2025, 120 patients were treated with high dose of MTZ and underwent parasitological follow-up. 74 (61.7%) of 120 patients were treated successfully and 46 (38.3%) failed. The treatment failure was strongly associated with travel to Indian Subcontinent. Second-line treatment with tinidazole (500 mg bid) plus albendazole (400 mg bid) for 14 days was used in 43 patients with only three documented failures. These cases were treated with paromomycine (500-1000 mg tid for 10-15 days) successfully. Our study revealed low effectiveness of MTZ. MTZ-refractory giardiasis is especially common in travelers returning from South Asia, affecting more than one-third of treated patients. As quinacrine and nitazoxanide is not available in the Czech Republic, paromomycine or tinidazole plus albendazole combination therapy appears highly effective as a second-line regimen.
This study was supported by the Czech Ministry of Health grant NU23-05-00441.

O9

Demodicosis as an interdisciplinary health and aesthetic problem

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Demodex folliculorum and *Demodex brevis* are obligate human parasites that colonise hair follicles and sebaceous glands, including those of the face. While their presence can be asymptomatic, overpopulation leads to demodicosis. This condition can present both a medical problem and a significant aesthetic challenge, negatively affecting patients' well-being and socio-professional functioning. Demodicosis manifests as erythema, papules, pustules, pruritus, skin dryness, and, in the ocular form, blepharitis, Meibomian gland dysfunction, and tear film disorders.

Demodex infestation may manifest in atypical forms with a diverse clinical presentation, which can lead to diagnostic errors and delayed appropriate treatment. Microscopic examination of skin scrapings and eyelashes allows for the detection of the parasites. Effective management of demodicosis requires: (1) increasing awareness among physicians of various specialities regarding the atypical presentations of demodecosis; (2) implementing preventive measures to limit transmission, including through shared cosmetics; (3) applying personalised targeted acaricidal therapy; (4) supplementing antiparasitic treatment with aesthetic medicine procedures, such as medical peelings, mesotherapy, and botulinum toxin.
A holistic, interdisciplinary approach combining parasitology, dermatology, and aesthetic medicine represents the optimal care model for patients with demodicosis, enabling long-term aesthetic benefits and significant improvements in quality of life.

O10

Changes in prevalence and intensity of the head lice infestations in preschool and school children in Poland across 2018-2023 including pandemic COVID-19

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Introduction: Head lice infestation remains an underestimated epidemiological problem in Poland due to the lack of mandatory surveillance and the predominance of indirect assessment methods. Studies based on direct examinations of children attending preschools and primary schools in Poland, covering the pre-pandemic period, the period of COVID-19-related restrictions, and further monitoring in 2021–2023 were synthesized and concluded.
Materials and Methods: Institution-requested examinations were performed by certified nurses in population of children aged 3–14 years. Scalp assessment used individual combs and magnification according to CDC guidance. The first study covered 5008 children examined "for cause" between September 2018 and June 2021 in Warsaw, Krakow and Poznan, with most observations from Warsaw. The second covered 10,943 children examined between September 2021 and December 2023 in Warsaw, Poznan, Gdansk and Wroclaw. Infestation was defined by live lice and/or nits; in the later study, intensity was graded 0–3. Fisher's exact test was used for statistical assessment.
Results: During the pre-pandemic period, the prevalence of head lice infestation in examined preschools was 12.03%, compared with 9.70% during the pandemic. In examined schools, a significant decrease from 10.41% to 5.37% was observed. In 2021–2023, the proportion of children with head lice infestation in schools remained relatively stable at 9.80 - 12.72% and returned to the pre-pandemic years, whereas in preschools it was higher in 2021: 12.66%, and lowered in 2022 and 2023 to 8.77%, and 9.03% respectively. In 2022 and 2023, infestation prevalence was higher in schools than in preschools which is opposite to the pre-pandemic year (2018). At both populations, girls showed higher prevalence and greater infestation intensity than boys. Generally, mild and moderate infestations predominated over severe cases.
Discussion and Conclusions: Reduced peer contact during the pandemic was associated with a decline in head lice prevalence, particularly in schools, whereas in subsequent years transmission persisted at a stable level of approximately 9–13% of the examined populations. The data confirm that direct, periodic screening in educational institutions, together with education of parents and staff, remain essential in the absence of a nationwide surveillance system. Female sex appears to be the most important identified factor associated with both prevalence and intensity of infestation, whereas the influence of age, socioeconomic status, and caregivers' educational level requires further investigation.

O11 ✻ Student Competition

Case report: Zoonotic protozoa in domestic dogs

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Giardia duodenalis and *Cryptosporidium* spp. are gastrointestinal protozoan pathogens with worldwide distribution and zoonotic potential. *G. duodenalis* is common cause of diarrhea in dogs but there is not enough evidence of the prevalece of *Cryptosporidium* spp. in this host group however it is a widespread pathogen with public health concern. This publication describes the case of two dogs (approximately two months old) adopted from the shelter by a family with three children (5 – 11 years old). Dogs suffered from chronic giardiasis with *Cryptosporidium parvum* coinfection. Coprological examination confirmed the presence of *Giardia* cysts with a high intensity of infection. Molecular analysis based on the *gdh* gene identified *Giardia duodenalis* assemblage D in both dogs. Fecal isolates were also used for amplification of the *gp60* gene, specific to *Cryptosporidium* spp.. In one of the dogs, molecular analysis confirmed the presence of *Cryptosporidium parvum subtype* A16G1R1. In this dog, *G. duodenalis* infection persists for 8 months despite repeated treatment with metronidazole and fenbendazole.
Microscopic examination of stool samples from three children living in the same household was negative. However amplification of the *gp60* gene confirmed the presence of *Cryptosporidium parvum subtype* A16G1R1 in one of the children (8 years old). This case demontrates zoonotic transmission of this pathogen from the dog to the child from the same household. *Cryptosporidium* infections in dogs remain underdiagnosed despite its zoonotic potential and close contact of humans and these animals.
This study was supported by the Grant Agency VEGA project No. 1/0709/23.

O12 ✻ Student Competition

The Zoonotic Gap: Why Protozoans Thrive where Helminths Fail in Polish Zoos

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Key words: NHPs, non-human primates, gastrointestinal parasites, protozoans, zoonotic potential, zoo health monitoring
This study aimed to assess the prevalence and diversity of gastrointestinal parasites in non-human primates (NHPs) housed in zoological gardens across Poland, with particular emphasis on protozoa of zoonotic potential. Between 2023 and 2026, a total of 136 faecal samples were collected from individually identified primates representing 37 species and from seven zoological institutions in Poland (Zoo Wrocław, Gdański Ogród Zoologiczny, Śląski Ogród Zoologiczny, Ogród Zoobotaniczny w Toruniu, Nowe Zoo Poznań, Orientarium Zoo Łódź, and Miejski Ogród Zoologiczny w Płocku). Only samples assigned to identified individuals were included; no pooled or unidentified samples were analyzed.
Samples were stored under refrigerated conditions for up to 48 hours or frozen when storage exceeded this period. For each specimen, detailed metadata were recorded, including species, individual identification, age, sex, group size, presence of comorbidities, and information regarding prior antiparasitic treatment. Clinical observations indicated that only a few primates exhibited signs potentially related to infection, such as loose stool, apathy, or weight loss.

Laboratory analyses comprised both microscopic and molecular approaches. Standard parasitological techniques, including stained smears and a modified flotation method, were employed for the detection of helminths and preliminary screening of protozoa. Molecular methods were applied for the detection and characterization of selected protozoan taxa, particularly those with zoonotic potential, including *Blastocystis* spp., *Balantioides* spp., *Giardia* spp., and *Entamoeba* spp.

The results demonstrated a clear predominance of protozoan infections over helminth invasions, the latter not exceeding 8% prevalence. In contrast, protozoan infections reached very high prevalence levels in certain host species and zoological institutions, exceeding 80% in the case of *Blastocystis* sp. or 54% for *Entamoeba dispar*. Notably, within *Balantioides*-like organisms, genetically distinct protozoa consistent with so-called "*Buxtonella*-like" variants were identified in some Old-World primates. Subsequent analyses explored associations between infection status and host-related variables, including age, sex, and group size. The observed prevalence patterns are broadly consistent with findings reported in zoological settings across other European countries.

Overall, the study indicates that protozoan infections are highly prevalent among captive NHPs in Polish zoological gardens, emphasizing the importance of regular monitoring to understand infection dynamics and potential zoonotic risks.

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POSTER PRESENTATIONS

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M1

Hymenolepis nana across wildlife and companion rodents: a remaining public health concern

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Hymenolepis nana (syn. *Rodentolepis nana*) is a hymenolepidid tapeworm of zoonotic importance. Epidemiological data suggest that direct human-to-human transmission is the most common route of infection, particularly in areas with poor hygiene and inadequate sanitation. Nevertheless, *H. nana* is still considered zoonotic because infected free-living or pet rodents serve as a reservoir of infection. Since data on the occurrence of *H. nana* in small mammals in Central Europe are limited, the aim of the study was to evaluate its presence in free-living small mammals and pet rodents in Slovakia and to assess the risk of human infection.

A total of 1,038 small mammals representing 16 species from the orders Rodentia and Soricomorpha were collected in two areas of Slovakia: the municipality of Košice city and protected areas of Tatra National Park (TANAP) between 2020 and 2025. Besides, pooled feacal samples from a total of 568 pet rodents (228 mice, 191 rats, 124 hamsters, and 25 Mongolian gerbils) kept in 119 boxes were collected in pet shops and breeding clubs in Eastern Slovakia. Following the necropsy (free-living small mammals) or coprological examination of faeces (pet rodents), *H. nana* was identified using ITS1 or mitochondrial *nad1* sequence data.

Based on molecular results, *H. nana* was detected in 0.7% free-living small mammals (7/1,038). The tapeworm was recorded exclusively in brown rats (*Rattus norvegicus)* from the urban areas of Košice city. The tapeworm occurrence in urban brown rats from Košice was 10.4% (7/67), while the overall occurrence across all brown rats in both study areas was 7.2% (7/97). No molecularly confirmed records were detected in suburban or rural localities of Košice and TANAP, in either brown rats or any other host species.

In pet rodents, *H. nana* eggs were detected in 25 (21.0%) of boxes with animals. The positivity of animals in pet shops was higher (24.6% positive boxes) than in breeding clubs (17.2%). In pet shops, the parasite was found in 23.5% of boxes with hamsters, 25.0% boxes with mice, and 41.7% boxes with rats. In breeding clubs, the parasite was recorded only in rats (3.7% positive boxes) and mice, where the number of positive boxes was significantly higher (40.9%).

Although the overall number of *H. nana* records in free-living small mammals was low, the positivity rate reached 10.4%. when analysing only brown rats from densely populated urban areas of Košice city. Likewise, the positivity detected in pet rodents was not negligible, highlighting a potential risk of human infection. These findings suggest that synanthropic rat populations, as well as the companion rodents kept in households, may be a relevant source of *H. nana* infection for humans.

The research was financially supported by the project Tubitak-2024-01 and APVV-21-0166.

M2

Prevalence of *Echinococcus multilocularis* in household and shelter dogs from Pomeranian Voivodeship, Poland

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Echinococcus multilocularis is the most dangerous parasite existing in Northern Hemisphere that causes alveococcosis. Humans become infected by ingestion of its eggs excreted by definitive hosts, mainly red foxes (*Vulpes vulpes*). In some areas also dogs can play this role. However, in Poland data on *E.*

multilocilaris infection in dogs is fragmentary. Thus, the aim of the study was to compare the prevalence of *E. multilocularis* infection in dogs living in shelters and households from Pomerania voivodeship.
In total, 517 dog faecal samples were collected with the help of owners and shelter workers along with a questionnaire describing the dog condition and investigated with the use of nested-PCR. We detected *E. multilocularis DNA* in 31 samples, with an overall prevalence of 6%. Presence of the tapeworm was confirmed in 4.63% of samples collected from shelter dogs compared to 8.29% positive of samples collected from household dogs. All samples that tested positive by nested PCR were subjected to molecular sequencing targeting the 12S rRNA gene.
Findings from this study indicate that *E. multilocularis* is present in dogs across both investigated groups. Results contribute to our understanding of the abundance of *E. multilocularis* in dogs living in Poland as well as potential role of these dogs in parasite transmission. In consequence, this study complements knowledge about epidemiology of *E. multilocularis* infection, which is of high both local and world interest. Moreover, biomonitoring of prevalence of *E. multilocularis* infection in dogs helps to understand if, and where dogs may pose a threat for humans, especially owners and shelter workers.
This work was supported by the National Science Centre grant no.2020/37/B/NZ7/03934

M3

Spatial Modelling of European Beaver (*Castor fiber*) Habitat Suitability in the Košice Region

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European beaver (*Castor fiber*) populations have been steadily expanding across Europe, including Slovakia, and are increasingly appearing in urban and peri-urban environments. In Košice, individuals and signs of their activity have been repeatedly observed in close proximity to residential areas, recreational zones, and frequently used riverbank corridors, particularly in the Nad Jazerom district. Although the species is legally protected and generally seen as ecologically beneficial, its increasing presence, along with dam-building activities and extensive tree-gnawing, can damage local habitats and infrastructure. When these activities occur near human dwellings, they also raise important public-health concerns, as the parasitofauna of beavers in Slovakia remains insufficiently characterised, and the potential for infecting pets or humans has not yet been assessed in this area. To address this concern, it is essential to identify where beavers are likely to occur and whether they may expand further into urban environments, thereby helping to pinpoint zones of increasing human–beaver interaction. To enable targeted monitoring and provide a basis for future parasitological surveys, a spatial model of habitat suitability for beaver presence in the Košice town region was created. In this model, three ecologically relevant factors related to beavers' presence were used, such as terrain slope, distance to permanent water bodies, and land-use categories. These factors were reclassified and combined into a weighted suitability map highlighting areas with a higher probability of beaver presence. The resulting maps reveal that the most suitable areas were located on the outskirts of the city, such as the Ťahanovce forest in the northeast and the Čermeľ-Alpinka region in the northwest. Areas with moderately higher suitability were also identified closer to the city, including Myslava in the southwest and the Krásna area in the southeast. Even though the areas where beaver signs were recorded (settlement Nad Jazerom) were classified as unsuitable for beaver occurrence, due to the extent of human development. The proximity of area to highly suitable habitats suggests that beavers may have gradually tolerated human presence and moved closer to dwellings, likely attracted by the availability of food resources. Overall, these areas represent potential hotspots for human-beaver contact and, therefore, priority locations for field surveillance. By identifying where beavers are most likely to occur, the model provides a practical tool for planning parasitological sampling, assessing zoonotic risks, and developing management strategies in urban and suburban environments.
This study was supported by the Interacademic agreement SAS-CNR project No. CNR-SAS-2024-10 and VEGA project No. 2/0069/25.

M4

Acute-on-chronic kidney disease caused by *Babesia gibsoni* infection in dogs

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Babesia gibsoni is an emerging canine pathogen in Europe, characterized by trombocytopenia and anemia in affected dogs. While the clinical course is often chronic and asymptomatic, some cases can involve systemic organ damage, most frequently affecting the kidneys. The extent of organ damage at the time of diagnosis is a critical factor in determining the prognosis during and after therapy.
The aim of this study is to present the clinical course, therapeutic management, and prognosis of complicated cases of *B. gibsoni* infection in dogs in Slovakia.
A total of 30 dogs with *B. gibsoni* infection (confirmed by PCR and 18S rRNA sequencing) were monitored. 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. Follow-up control PCR testing was performed 28 days post-treatment to confirm the elimination of parasitemia. Hematological and biochemical parameters were monitored throughout the study.
Out of 30 PCR-positive dogs, all patients exhibited thrombocytopenia and mild anemia. Elevated renal parameters, consistent with acute kidney injury (AKI) were recorded in three dogs (10%), and only one of them presented with polyuria and polydipsia (PU/PD). Although combined therapy led to complete elimination of parasitemia (PCR-confirmed) and a temporary stabilization of biochemical values in all patients, recurrent uremia developed in all three dogs with initial renal involvement. During a 12-month follow-up period, uremic relapses ocurred 2 to 3 times and were accompanied by anorexia, PU/PD, and vomiting. Despite intensive symptomatic treatment, the final episode resulted in irreversible renal failure, leading to euthanasia in all three dogs.
The combination therapy of Malarone®, azithromycin, and artesunate successfully achieved complete elimination of parasitemia in infected patients. However, these cases suggest that even when molecular resolution is achieved using this 10-day protocol, initial renal involvement may trigger a progressive pathological process. In our study, AKI associated with *B. gibsoni* infection proved to be a poor prognostic factor, potentially leading to acute-on-chronic kidney disease and subsequent fatal renal failure despite successful parasite clearance. This suggests that uremia during *B. gibsoni* infection may serve as a negative prognostic indicator.
This study was supported by the Grant Agency VEGA project No. 2/0016/26.

M5

Parasites of European bison (*Bison bonasus*): comparison of the wild and captive bison populations in Slovakia

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The European bison (*Bison bonasus*) is the largest wild herbivore in Europe. At the beginning of the 20th century, this species was on the brink of extinction; however, restoration efforts have helped preserve its population in many European countries. In Slovakia, the wild bison population is kept in the Poloniny National Park (PNP), and the captive population is kept in the Topoľčianky Bison Park (TBP). Parasitic diseases can pose serious health and welfare problems for bison in both wild and captive populations; therefore, regular examination and monitoring of bison for gastrointestinal (GI) parasites are necessary. Our work aimed to study GI parasites of the wild and captive bison populations in Slovakia using coprological methods.

The study was performed in 2023 and 2025 in the PNP and TBP in Slovakia. In total, 212 faecal samples were collected: 120 samples from wild bison in PNP and 92 samples from the captive bison in TBP.
Flotation (McMaster method) and sedimentation coprological methods were used to detect the eggs and cysts of GI parasites. Pooled faecal samples from both wild and captive populations were used to prepare coprocultures. Third-stage larvae (L3) were harvested by the Baermann technique, and 100 randomly selected L3 larvae were identified according to their morphological features. In the wild bison population from PNP, seven helminth taxa were registered; GI nematodes from the family Trichostrongylidae were the most prevalent (prevalence, P=53.3%), followed by *Dictyocaulus viviparus* (8.3%), *Trichuris* spp. (8.3%), *Toxocara* spp. (5.8%), *Nematodirus* spp. (3.3%), *Moniezia* spp. (2.5%) and *Strongyloides papillosus* (2.5%). Eggs of *Fasciola hepatica* were also found. Cysts of *Eimeria* spp. were detected in 25.8% of samples. Morphological identification of L3 showed that in wild bison, the prevalence of *Ostertagia* spp. was 61.5%, *Haemonchus contortus* 31.5%, *Cooperia* spp. 4.4%, and *Trichostrongylus* spp. 2.5%.
In the captive bison population from TBP, five helminth taxa were registered. The prevalence of all parasites was higher; Trichostrongylidae were the most prevalent (P=68.5%), followed by *Trichuris* spp. (47.8%), *Nematodirus* spp. (39.1%), *D. viviparus* (15.2%) and *Toxocara* spp. (10.9%). No eggs of cestodes (*Moniezia* spp.) or trematodes were detected. Cysts of *Eimeria* spp. were detected in 34.8% of samples. Morphological identification of L3 revealed that the prevalence of *H. contortus* was 54.5%, *Ostertagia* spp. was 33.0%, and *Cooperia* spp. 12.5%.
We can conclude that GI nematodes and *Eimeria* spp. are highly prevalent in both wild and captive bison populations in Slovakia. Considering the high density of the captive bison population in TBP, regular monitoring of the parasite infection levels, along with appropriate parasite control and management, is needed to prevent serious health and welfare problems in the park.
This study was supported by the Grant Agency VEGA project No. 2/0028/26.

M6

Prevalence of *Echinococcus multilocularis* in hunting dogs and red foxes (*Vulpes vulpes*) in hunting areas of Northern Poland

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Echinococcus multilocularis is a parasite causing a dangerous zoonosis, alveolar echinococcosis (AE), which without proper treatment can lead to death. In Poland, the main definitive host of *E. multilocularis*, which releases invasive eggs into the environment, is red fox (*Vulpes vulpes*) and its population is being regulated. Hunting often involves hunting dogs who have direct contact with wildlife. Although it has been shown that in some regions of the world, dogs can also act as definitive hosts of the tapeworm, in Poland the role of these animals in the transmission of the parasite is unclear.
The aim of this study was to compare the prevalence of *E. multilocularis* infection in dogs assisting rangers and hunters and red foxes in chosen hunting areas in the Pomerania Province, northern Poland that is considered moderately endemic region.
In total, 236 faecal samples were collected including 72 samples from dogs of different hunting breeds and 164 samples from hunted red foxes. Fox faeces were collected during dissection directly from the intestine. Canine faecal samples were provided by owners along with a questionnaire characterizing the tested animals. All samples were examined microscopically and molecularly using nested PCR specific to 12SrRNA gene and Sanger sequencing.
Using microscopic investigation, tapeworms eggs of the family Taeniidae were detected in 4 (2.44%) faecal samples collected from foxes and in no dog fecal samples. Molecular study confirmed presence of *E. multilocularis* DNA in 19 (10.98%) and 6 (8.3%) fox and dogs faecal samples, respectively. According to questionnaire, all infected dogs had daily access to the forest, they were often let loose and possibility of close contact with foxes and wildlife carcasses was indicated as high.
Results of this preliminary study show that the prevalence of *E. multilocularis* infection in dogs accompanying hunters and rangers, with direct access to excreta and carcasses of wildlife, is at similar level to living in hunting districts red foxes. In addition, a close co-habitation of humans with dogs,

especially breeds used for hunting, raises the question of whether this could be a significant risk factor for public health.
This work was supported by the National Science Centre grant no.2020/37/B/NZ7/03934.

M7

Imported canine leishmaniosis as an emerging diagnostic and therapeutic challenge in a non-endemic country

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Canine leishmaniosis, caused by *Leishmania infantum*, is an emerging concern in non-endemic European countries, including Slovakia. To date, 26 confirmed cases of the infection have been reported, primarily associated with travel or importation from endemic regions, though epidemiological data have often been incomplete. The absence of confirmed vectors in Slovakia suggests that most cases are imported, although alternative transmission routes cannot be excluded. The present study describes a clinically confirmed case of imported canine leishmaniosis in a 7-year-old dog relocated from Crete. The affected dog presented with dermatological and hematological abnormalities. The diagnosis was established by serological testing and PCR-based molecular identification via kinetoplast DNA sequencing. Treatment with miltefosine and allopurinol resulted in clinical remission, although relapses and treatment-related complications were observed. This report underscores the diagnostic complexity and highlights therapeutic limitations and the underreporting of canine leishmaniosis in non-endemic regions.
This study was supported by the Grant Agency VEGA project No. 2/0016/26 and the organisation ESCCAP SK&CZ.

M8

Nineteen years of dirofilariosis in Poland: a preliminary One Health study from the Warsaw metropolitan area

M. Pękacz [1], A. Pawełczyk [2], R. Welc-Falęciak [1], E. Ganci [3] A. Sędzikowska [4], J. Werszko [4], A. Kawińska [4], R. Sałamatin [4, 5], A. Kaczmarek [5, 6], D. Młocicki [3], Diakou A.[7], A. Zawistowska-Deniziak [1]

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Dirofilariosis is a vector-borne disease transmitted by mosquitoes. Due to climate change and human activity, this parasitosis has rapidly spread throughout Europe. Although carnivores, especially dogs, remain the main reservoir of the disease, dirofilarial worms — particularly *Dirofilaria repens* — exhibit a high zoonotic potential. Several thousand human cases have been reported in Europe; however, the actual number of infections is likely underestimated. The first human case of dirofilariosis in Poland was reported in 2007, whereas the first canine case was described in 2009. Since then, the disease has become established in the region, with an increasing probability of occurrence not only of *D. repens* but also *D. immitis*. Despite

the growing number of reported human clinical cases, no serological screening studies on human dirofilariosis have previously been conducted in Poland. Therefore, the aim of this study was to perform a comprehensive One Health investigation assessing exposure to dirofilarial infections in humans and dogs, together with xenomonitoring of mosquito vectors in the Warsaw metropolitan area between 2023 and 2025.

A total of 325 serum samples collected from clinically healthy blood donors were examined for the presence of specific anti-DSA (*Dirofilaria* Somatic Antigen) antibodies in order to estimate the real risk of dirofilariosis transmission in the human population. In parallel, approximately 5,500 mosquitoes representing different genera, including *Culex*, *Aedes*, *Culiseta*, *Anopheles*, and *Mansonia*, were screened for the presence of *Dirofilaria* DNA using Real-Time PCR. As dogs remain the principal reservoir hosts, more than 500 dogs from Warsaw and surrounding suburban areas were examined using molecular methods, microscopy (Knott's test), and serological assays detecting antibodies against DSA.

Our findings demonstrate the active circulation of *Dirofilaria* spp. in the Warsaw metropolitan region and emphasize the need for integrated surveillance programs combining veterinary, medical, and entomological monitoring to better assess and control the emerging risk of dirofilariosis in Central Europe.

This study was supported by the National Science Centre Poland, grant number 2023/49/N/NZ6/03309 and 2022/06/X/NZ6/00852.

M9

Histopathological evidence of *Sarcocystis* spp. infection in the muscles of European bison (*Bison bonasus* L.) from the Bieszczady Mountains, Poland

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Protozoa of the genus *Sarcocystis* are intracellular parasites belonging to the phylum Apicomplexa. They are characterized by a complex life cycle reflecting a predator–prey relationship. The parasite's intermediate hosts are mainly herbivorous animals, including ruminants, whereas the definitive hosts are carnivorous and omnivorous animals. Intermediate hosts become infected by consuming food contaminated with sporulated oocysts, while sarcocysts containing bradyzoites present in meat constitute the infective stage for definitive hosts. The European bison is the largest terrestrial mammal in Europe. It has a Near Threatened conservation status and is protected in Poland. Although this species may serve as a potential intermediate host for *Sarcocystis* spp., available data are scarce and do not include the population inhabiting the Bieszczady Mountains.

This study aimed to determine the occurrence of *Sarcocystis* spp. infection in free-ranging European bison from the Bieszczady Mountains based on histopathological examination of the tongue, esophagus, diaphragm, and heart muscles.

A total of 16 individuals were examined. Complete sets of tissue samples were collected from 12 European bison. In one case, the esophagus sample was missing, while in two cases, samples of the diaphragm and heart muscle were unavailable. Altogether, 59 muscle samples from European bison were analyzed. The samples were fixed in 10% buffered formalin, dehydrated through graded ethanol and xylene baths, and embedded in paraffin wax. Sections of 4 µm thickness were stained with hematoxylin and eosin.

The overall prevalence of *Sarcocystis* was 93.8%, as sarcocysts were observed in all but one individual. Cysts were detected in 31 of the 59 muscle samples (52.5%). They were most prevalent in the cardiac muscle (prevalence: 85.7%; positive: 12/14; mean: 4.5; range: 1–13), while they were found in 56.3% of tongue samples (positive: 9/16; mean: 2.3; range: 1–7) and in 46.7% of esophageal samples (positive: 7/15; mean: 1.7; range: 1–4). The diaphragm was the least affected (prevalence: 21.4%; positive: 3/14; mean: 1; range: 1–1). The observed cysts were oval, round, or spindle-shaped and were filled with banana-shaped bradyzoites (infective stage). In 19 of the 31 positive muscle samples, mild to moderate focal inflammation

was observed, with mononuclear cells (lymphocytes and plasma cells) present between muscle fibers. Additionally, mild to moderate degeneration, necrosis, and fragmentation of muscle fibers were noted, along with congestion and fibrosis associated with the presence of sarcocysts. In some cases, focal muscle fiber atrophy, focal hemorrhages, and edema were also observed. Molecular analysis aimed at identifying the *Sarcocystis* species involved in infection is currently in progress.
Participation in the conference was funded by: Moduł 4 projektu MODULE4NCU Podniesienie kompetencji kadry dydaktycznej UMK w Toruniu w latach 2024-2026.

M10

What excites infectious diseases doctors: second case of Human African Trypanosomiasis (HAT) detected in Poland: how a blood smear can save a life

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Human African Trypanosomiasis (HAT) – a parasitic disease transmitted by tsetse flies, is rarely diagnosed in non-endemic countries, with only 24 cases found in Europe between 2011–2020. Due to its rarity, treatment with first-line therapy can be challenging.
We present a clinical case of a patient with HAT recently admitted to our hospital.
A 54-years old male with hypertension and metabolic syndrome was admitted with fever to Hospital for Infectious Diseases in Warsaw after a 14-days trip to Zambia and Malawi. Five days after returning the patient noticed a painful erythema on his calf, had headache, transient blurred vision.
On admission the patient was in good general state. Computed tomography and magnetic resonance imaging of brain showed no abnormalities. The patient refused to have a lumbar puncture. Rapid diagnostic tests excluded: COVID-19, influenza, malaria, dengue. *Plasmodium* spp. was not found in blood smear. Laboratory results showed no signs of anemia and platelet count was within typical range, CRP was 67.5 mg/l. During hospitalization fever continued, the erythema was enlarging. On the third day the patient's state deteriorated, with hypovolemia, oedema of lower extremities, somnolence. CRP and D-dimer levels increased up to 265 mg/l, 20227 ng/ml appropriately. Thrombocytopenia decreased to 60 G/l. Blood smear showed *Trypanosoma brucei*. The first-choice treatment for HAT (fexinidazole) is not registered in Europe, so pentamidine was given immediately as interim treatment following WHO guidelines. The next day (Friday) we contacted WHO headquarters in Geneva to obtain fexinidazole. However, due to weekend transport delays and Polish customs procedures, we received the medication four days later. The patient's condition improved on pentamidine, and three days after switching to fexinidazole the patient was discharged home in good general state.
Molecular characterization of the parasite isolate was performed by analyzing the haptoglobin-hemoglobin receptor (HpHbR) gene following Symula et al. (2021; doi:10.1371/journal.pntd.0001728). The protein sequence analysis indicated that the patient was infected with *Trypanosoma brucei rhodesiense*, consistent with the geographical origin of the infection.
HAT is rare in Europe but should be considered in travelers with fever returning from countries where HAT is endemic. Blood smear and close cooperation with a trained parasitologist is essential for diagnosis. A simple operating procedure should be created in Poland to receive life-saving medication from WHO without any delays.

M11

Novel Avian *Trypanosoma* Haplotype Identified in a Mallard Duck (*Anas platyrhynchos*)

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Wild birds play an important role in the maintenance and dissemination of vector-borne pathogens, including numerous agents of veterinary and public health importance. Due to their mobility, ecological plasticity, and interactions with hematophagous arthropods, birds may contribute to the spread of infectious agents across local and intercontinental scales.

The mallard duck (*Anas platyrhynchos*), one of the most abundant and ecologically adaptable waterfowl species in Europe, may serve as a reservoir host for multiple vector-borne pathogens. Due to its high population density, ecological plasticity, and frequent contact with other wildlife, domestic animals, and humans, the mallard plays an important role in the epidemiology of infectious diseases. Studies on blood-parasites in mallard ducks remain limited, and in Poland our knowledge of their occurrence and diversity in this host species is still scarce. Therefore the aim of this study was to investigate the occurence of *Trypanosoma spp*., *Babesia spp*., *Borrelia burgdorferi* s.l., *Anaplasma phagocytophilum*, and *Bartonella spp*. in mallard ducks from Poland by molecular methods.

The research material consisted of spleens collected from 102 wild mallard ducks hunted in natural habitats within the Lubelszczyzna region of eastern Poland. DNA from spleen samples was isolated. The detection of pathogens was based on PCR amplification of: an 18S rRNA gene fragment (*Trypanosoma sp*.), the *flaB* gene (*B. burgdorferi* s.l), the 16S rRNA gene (*A. phagocytophilum*), the rpoB gene (*Bartonella sp*.) and 18S rRNA (*Babesia sp*.). Statistical and phylogenetic analysis were also performed.

Trypanosoma spp. was detected in two samples, whereas no DNA of the remaining investigated pathogens was detected in the current study. Amplification of the 18S rRNA gene yield two distinct haplotypes (534 bp) belonging to *Trypanosoma melophagium*. Haplotype H1 (ID 46W) was identical to 11 haplotype previously reported from the sheep ked *Melophagus ovinus*. Haplotype H2 represents a novel variant of *T. melophagium*. In the phylogenetic reconstruction, haplotypes obtained in the present study clustered together with a haplotype of *T. melophagium* previously detected in *Ursus arctos* from Poland. Both haplotypes identified in this study belonged to the TthII phylogenetic lineage within the *Trypanosoma theileri* complex.

The obtained results indicate a low prevalence of the investigated vector-borne pathogens in wild mallards from Poland; however, the detection of a novel *Trypanosoma melophagium* haplotype highlights previously unrecognized genetic diversity of avian trypanosomes and suggests a potential role of mallards in the ecology of these parasites. Understanding the prevalence of these pathogens in wild birds may provide valuable insights into the epidemiology of vector-borne diseases and their potential transmission risks to humans and domestic animals.

M12

Temporal dynamics of trematode community in a recreational waterbody in western Slovakia

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Trematoda is a class of strictly parasitic flatworms that contains about 18,000 valid species. Among these, some taxa have zoonotic potential, making their monitoring in endemic areas crucial for health risk assessment. As various biotic and abiotic factors affect their spatiotemporal distribution, an establishment of preventive measures requires complex approach. Standard detection methods involve parasitological examination of intermediate hosts, which often act as a source of infection. Novel techniques rely on detecting parasite DNA in environmental samples, i.e., samples containing various genetic materials collected with the environmental matrix (water, soil, air, etc.).

In this work, the composition of the trematode community and its seasonal changes were studied in a water reservoir, with a primary focus on avian schistosomes (AS) as agents of human cercarial dermatitis. The sampling was performed twice a month from April to November using two techniques. (1) The aquatic snails were collected in the litoral zone and examined for both patent and pre-patent infections. The cercariae and sporocysts/rediae were examined under light microscope and representative individuals were genotyped using ITS marker. (2) The environmental (e)DNA samples were collected by filtration of water through a plankton net from five sites along the shore. The species composition of AS and their seasonal abundance were studied by metabarcoding and digital (d)PCR quantification, respectively.

A total of 313 European ear snails *Radix auricularia* were examined, and eight trematode species were detected. Among these, the only avian schistosome was *Trichobilharzia franki*, with an overall prevalence 6 %. The infected snails were recorded from late June, when the water temperature reached 25 °C to late October, with a peak in mid-July. Other potentially zoonotic trematodes, *Echinoparyphium recurvatum* and *Echinostoma revolutum*, were detected during a similar period as *T. franki*, but their temporal patterns differed. Prevalence of echinostomatids was relatively low from late June to late July, but they became the dominant group from mid-August to late October. As their rediae consume intra-snail stages of other trematodes, their increase might contribute to the decrease of *T. franki* after its mid-July peak. Similar negative correlation was observed between echinostomatids and *Plagiorchis* spp. as well as between echinostomatids and *Cotylurus* sp.. Other trematodes, *Australapatemon* sp. and *Notocotylus* sp., were detected in prevalences too low for a seasonal dynamics study.

The eDNA metabarcoding confirmed *T. franki* as the only AS species in the locality. The dPCR quantification detected AS from late May to early October, with a peak in mid-June followed by a steep decline in mid-July. Comparing with AS prevalence in snails, eDNA-based quantification showed similar seasonal pattern but the peak was shifted a month earlier than the peak of prevalence. This decline may be related to the massive mechanical removal of macrophytes from the water reservoir in late June, which temporally reduced the snail population and complicated their sampling. Together with intra-snail competition of trematode larval stages and other environmental factors, trematode communities are dynamic units, and their study requires a multi-method approach in which eDNA is a promising source of data, especially when snail collection is difficult.

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M13

Q fever Outbreak in Ráztočno, Slovakia, 2025

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Coxiella burnetii is an obligate intracellular Gram-negative bacterium with a global distribution, replicating primarily in macrophages and monocytes. It is the causative agent of Q fever in humans and coxiellosis in animals, with domestic ruminants and wild vertebrates serving as the principal reservoirs. Infection in animals is usually subclinical; however, in livestock, it can lead to reproductive disorders, particularly abortions. Transmission to humans occurs via aerosols, while ticks contribute to the maintenance of the pathogen in natural cycles. It was identified in more than 40 infected species. Importantly, *C. burnetii* co-circulates in environments shared with other tick-borne pathogens, including tick-borne encephalitis virus (TBEV), highlighting the potential for overlapping exposure risks in endemic regions.

We investigated a Q fever outbreak in Ráztočno in 2025 using combined serological and molecular approaches, alongside parallel surveillance of TBEV exposure. Antibodies against *C. burnetii* phase II were assessed in suspected patients and volunteers, while pathogen DNA was detected in human sputum, ticks, and environmental samples.

Thirty-eight human cases were laboratory-confirmed by PCR and serology. Molecular screening revealed *C. burnetii* and *Coxiella*-like endosymbionts in 13.8% of *Ixodes ricinus* and 53.2% of *Dermacentor* spp. ticks. Sequencing confirmed the presence of a pathogenic strain of *C. burnetii* in patient samples; and identified strains RMSF (Acc. No. CP115461; 99% identity), DRM95 (Acc. No. MG860513; 99% identity) and a *Coxiella* endosymbiont of *Dermacentor marginatus* (Acc. No. KP985488; 97% identity) in *I. ricinus* and *Dermacentor* ticks.

These findings demonstrate substantial, previously unrecognized circulation of *C. burnetii* within the local population and vector community in Ráztočno. Despite the absence of a clearly identified primary source, the high tick infection prevalence and confirmed human cases indicate active transmission. Further research is ongoing, focusing on monitoring the dynamics of the antibody response in seropositive individuals and pathogen diversity, which will be essential for clarifying transmission pathways and improving surveillance and control strategies.

This study was supported by the Grant Agency VEGA project No. 2/0011/25 and APVV-23-0325, APVV-23-0379.

M14

Reassessing the Role of Dogs in *Neospora caninum* Transmission on Dairy Farms in Poland

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Neospora caninum is an apicomplexan protozoan parasite and one of the primary infectious causes of reproductive disorders in cattle, including abortion and neonatal mortality.

The parasite is transmitted predominantly through vertical transmission from dam to foetus but can also spread horizontally via feed or water contaminated with oocysts shed by the definitive host, the dog.

Although the presence of dogs on dairy farms is common, awareness of their potential role in the epidemiology of *N. caninum* infection is essential for implementing effective disease prevention strategies. This study presents a retrospective analysis of the health status of a dairy herd with a history of abortions associated with *Neospora caninum* infection. The objective was to assess the current epidemiological situation by determining the seroprevalence of antibodies against *N. caninum* in selected cattle. In addition,

dogs living on the farm and in its vicinity were tested to evaluate their potential role in parasite transmission.
Blood samples were collected from cows of reproductive age and from dogs residing on or near the farm. Serum samples were analyzed using a competitive enzyme-linked immunosorbent assay (cELISA) with the *Neospora caninum* Antibody Test Kit (VMRD, USA). The cELISA results were subsequently confirmed by Western blot analysis.
Neosporosis is a complex and persistent disease, and its detection and control in affected herds remain challenging, often requiring several years of monitoring and intervention. The overall prevalence of *N. caninum* infection in Polish dairy cattle has not been comprehensively investigated and is currently documented only for selected regions. Available serological data indicate widespread herd-level exposure, resulting in substantial economic losses for dairy producers.
In the absence of an effective vaccine or treatment, continuous surveillance and integrated control measures are essential. These should include regular serological monitoring of both cattle and farm dogs, together with strict biosecurity practices aimed at reducing both vertical and horizontal transmission of the parasite.

M15

The ongoing spread of *Ashworthius sidemi* (Nematoda: Trichostrongylidae) in northeastern Slovakia

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The blood-sucking nematode *Ashworthius sidemi* is a primary parasite of Asian cervids, especially the sika deer (*Cervus nippon*). This species was originally restricted to Central, East and South-East Asia, but due to the introduction of Asian deer, *A. sidemi* has been found in almost all species of European wild ruminants. Domestic ruminants are also at risk, as *A. sidemi* has been molecularly detected in faecal samples from farmed cattle (*Bos taurus*). Therefore, it represents a potential health risk for some predisposed individuals, such as young or weakened animals, very sensitive endangered species (like European bison), and may reduce livestock productivity. A high intensity of *A. sidemi* can occur especially in farmed fallow deer and free-living roe deer. On the parasite level, due to its rapid development and high adaptability, it poses a threat as it displaces native species and disrupts biodiversity. This is evidenced in studies from Czech Republic showing that *A. sidemi* is becoming the dominant species and is likely to replace the most common species of abomasal nematodes in cervids in Europe.
Monitoring conducted within the years 2022-2026 revealed into the 23 abomasal samples obtained from wild ruminants of the northeastern Slovakia, to be examined for the presence of trichostrongylid nematodes. The analyzed material comprised red deer (*Cervus elaphus*; n=20), fallow deer (*Dama dama*; n= 3). The average age of the red deer was 6 years (1-12) and 4 years for fallow deer (3-5). The female-to-male sex ratio in the sampled cervids was 19:4. To detect the presence of *Ashworthius sidemi*, a necropsy of the digestive tract of all cervids was performed. The gastrointestinal tracts were transported to the laboratory after evisceration and divided into pre-stomach, abomasum, small intestine, and large intestine. Parasitological examinations were performed using the gravity sedimentation method. A total of 1 827 abomasal nematodes were recovered from the examined animals. The overall prevalence of trichostrongylid nematodes in abomasa reached 86% (20/23), with *Ashworthius sidemi* being the dominant species (1489 individuals). The maximum intensity of infection was 1000 individuals of *A. sidemi* per sample (mean-74.5; min.-1). A significant pathological effect of the parasites on the abomasal mucosa was observed in the form of extensive hemorrhagic tissue lesions.
The results confirm the necessity of continuous monitoring of this invasive parasitosis due to the risk of transmission to domestic ruminants and indicate a high level of infestation of the monitored sites with the invasive species *Ashworthius sidemi*, which requires increased attention in terms of game population management.
This study was supported by the Grant Agency VEGA project No. 1/0452/25.

Ecology and Population Biology of Parasites

ORAL PRESENTATIONS

O13 ★ **A.-S. Cârstolovean, A. M. Ionică, B. Pafčo, Ș. O. Rabei, C. D. Cazan, C. M. Gherman, A. Corduneanu, A. M. Aldea, C. C. Șerban, S. Cătănoiu, A. Drașovean, M. Druga, G. B. Chișamera, V. Ilik, D. Hodor, A. D. Hașaș, V.-D. Cotuțiu, A.-G. Irimia, M. Taulescu, D. Modrý, A.-D. Mihalca** — The wildlife-livestock strongyle nemabiome: a hidden dimension of European bison conservation

O14 ✻ **I. B. Mitrea, A. D. Iani, C. M. Gherman, C. D. Cazan, A. M. Ionică, Ș. O. Rabei, G. Deak, M. S. Cernea, V. Alexe, G. B. Chișamera, M. Marinov, A. D. Mihalca** — Ancylostomatidae in carnivores from Romania: new host associations, haplotype diversity and presence of *Ancylostoma caninum*

O15 **A. M. Pyziel, K. Slivinska, R. Sałamatin, J. Werszko, M. Kloch, M. Świątek, O. Zhytova, D. Klich** — Between turbines and parasites: wind farm effects on gastrointestinal parasites of roe deer (*Capreolus capreolus* L.)

O16 ✻ **L. Švantnerová, O. Mikulka, M. Benovics** — Parasites of raccoons in the Czech Republic

O17 **A. Žákovská, M. Budíková, E. Bártová** — Climate-driven predictors of zoonotic pathogen occurrence in wild small mammal populations in the Czech Republic

O18 **D. Vörös, D. Boukal, C. Selbach, M. Soldánová** — Data-driven ecological modeling of trematode species in a subarctic lake

O19 ✻ **K. Lesiak, P. Ciapka, A. Cichy, A. Stanicka, J. Templin, J. Żbikowski, M. Augustyniak, E. Żbikowska** — Does climate warming benefit the parasite or the host? Insights from the *Diplostomum pseudospathaceum*–*Lymnaea stagnalis* system

O20 **I. Fiala, M. Lisnerová, H. Pecková, A. Lövy, E. Lazárková, M. Bürgerová, G.S. van Beest** — Hidden parasites in changing rivers: eDNA metabarcoding reveals the impact of human disturbance

O21 **A. Lövy, E. Lazárková, M. Lisnerová, H. Pecková, I. Fiala** — How human impact shapes amoebae communities in freshwater ecosystem: an eDNA metabarcoding approach

O22 ✻ **M. Bürgerová, M. Lisnerová, I. Fiala** — Tracking parasite diversity in an urban-impacted freshwater stream

O23 ✻ **J. Urbaniak** — Invasive amphipods as a novel link in the transmission of oviduct fluke infection

O24 **A. Vetešníková Šimková, E. Laval, M. Seifertová, L. Vetešník, M. Benovics** — Parasite load and microbiota in crucian carp (*Carassius carassius*) threatened by the invasive activity of gibel carp (*Carasius gibelio*)

O13 ★ Keynote Speaker

The wildlife-livestock strongyle nemabiome: a hidden dimension of European bison conservation

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The recovery of the European bison is usually framed through genetics, habitat, and demography, yet parasitism adds a less visible dimension with direct relevance to reintroduction success. In human-shaped landscapes, the strongyle nemabiome offers a particularly informative view because it reflects not only parasite diversity, but also connectivity between wildlife, livestock, and shared grazing environments. Recent data from Romania show that European bison harbor a diverse gastrointestinal strongyle community, with 32 taxa across 16 genera identified by ITS2 metabarcoding, including dominant species such as *Haemonchus contortus*, *Ostertagia ostertagi*, *Cooperia oncophora*, *Trichostrongylus colubriformis*, and *T. axei*. This pattern is highly suggestive of a strong livestock-related transmission risk, especially as broad overlap is evident with cattle and sheep, while parasite community structure appears to be shaped more by host association than by geography. Rather than reflecting a closed and conserved parasite fauna, the bison nemabiome appears connected to a wider multi-host transmission network at the wildlife-livestock interface. The same interface is also reflected by other recent findings from Romanian bison populations: fatal dictyocaulosis caused by *Dictyocaulus viviparus*, ocular infection with *Thelazia rhodesi* and *T. skrjabini*, and the detection of *Anaplasma phagocytophilum* and *Babesia divergens* in reintroduced animals. These observations point in the same direction, that livestock-associated parasites and pathogens are part of the ecological reality into which European bison are being restored. This has important conservation implications for a post-bottleneck species with low genetic variability and potentially reduced resilience to infection. Monitoring the strongyle nemabiome but also other parasites and pathogens in re-introduced species could provide an early-warning tool for spillover risk and a practical framework for integrating wildlife health into conservation, and highlighting the need for parasite surveillance and control in sympatric livestock.

Keywords: European bison; nemabiome; strongyles; livestock interface; conservation

O14 ⁕ Student Competition

Ancylostomatidae in carnivores from Romania: new host associations, haplotype diversity and presence of *Ancylostoma caninum*

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Hookworms (Ancylostomatidae) significantly impact on the health of both domestic animals and humans worldwide, with some species capable of causing zoonotic infections. While hookworms in pets are frequently reported in Europe primarily by coproscopy, there is limited data regarding their molecular identification. To address this, this study aimed to assess the diversity, prevalence, and distribution of hookworms in both domestic and wild canids and felids from Romania through both morphological and molecular analyses.

From 2011 to 2025, 319 carcasses belonging to 6 species of wild canids and felids from Romania (23 gray wolves (*Canis lupus*), 137 golden jackals (*Canis aureus*), 79 red foxes (*Vulpes vulpes*), 2 raccoon dogs (*Nyctereutes procyonoides)*, 70 European wildcats (*Felis silvestris*), and 8 Eurasian lynxes (*Lynx lynx*)) were collected as road kills or legally hunted. Hookworms were recovered from the intestinal tract during necropsy, preserved in formalin for morphological examination and in absolute ethanol for genetic analysis. Genomic DNA was extracted and analyzed using a PCR targeting a barcode region of the second nuclear ribosomal internal transcribed spacer (ITS-2), followed by sequencing. In domestic carnivores, faecal samples were screened for hookworm eggs using standard coproscopic methods, and positive samples were subsequently confirmed through molecular analysis.

The overall hookworm infection rate in wild carnivores was 14.1%, with hookworms detected in 4 wolves (17.4%), 23 golden jackals (16.8%), 11 European wildcats (15.7%), 4 red foxes (5.1%), 2 raccoon dogs (100%), and 1 lynx (12.5%). Three hookworm species were identified: *Uncinaria stenocephala*, *Ancylostoma caninum*, and *A. tubaeforme*. Molecular analysis revealed 14 unique sequences, comprising nine haplotypes of *U. stenocephala*, three of *A. caninum*, and two of *A. tubaeforme*. Additionally, *A. caninum* and *U. stenocephala* were confirmed in domestic dogs in Romania.

This study provides the first comprehensive molecular assessment of hookworm diversity in European wild carnivores and the first molecular evidence of *A. caninum* in domestic dogs from Romania, showing new host-parasite associations and highlighting the importance of these hosts as reservoirs. The detected haplotypes in wild carnivore showed high similarity to isolates from Europe, Asia, and the Americas, indicating broad global connectivity of hookworm populations.

Keywords
hookworms, wild carnivores, *Ancylostoma*, *Uncinaria*

O15

Between turbines and parasites: wind farm effects on gastrointestinal parasites of roe deer (*Capreolus capreolus* L.)

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In 2022–2024, the digestive tracts of 102 legally hunted roe deer from two voivodeships in central Poland were collected for parasitological analysis. Notably, 39 of the animals included in this study originated from wind farm areas. Parasites were recovered from the contents of the abomasum, small intestine, large intestine, and cecum of each animal. The specimens were preserved in vials containing 70% ethanol before morphological and molecular examination. The anterior and posterior parts of nematode specimens were cleared in lactophenol to visualize the oral cavity and spicules. For molecular identification, nematodes morphologically assigned to the genera *Oesophagostomum* and *Chabertia* were analyzed based on the ITS2 gene region, whereas the cox1 gene was used for cestodes of the genus *Moniezia*.

In total, seven species of abomasal nematodes were identified, with the hematophagous *Haemonchus contortus* being the most prevalent. Notably, the invasive species *Ashworthius sidemi* was detected in roe deer at one location. Other nematodes identified in the abomasum included *Mazamastrongylus dagestanica*, *Ostertagia kolchida*, *O. leptospicularis*, *Spiculopteragia boehmi*, and *Trichostrongylus capricola*. Among nematodes found in both the small intestine (seven species) and the large intestine (four species), *Chabertia ovina* was the dominant species. Additionally, tapeworms of the genus *Moniezia* were found in the small intestine of four animals.

Other nematodes recorded in the small intestine included *Aonchotheca* sp., *Bunostomum phlebotomum*, *B. trigonocephalum*, *H. contortus*, *Nematodirus europaeus*, and *Oesophagostomum venulosum*. In the large intestine, besides *Ch. ovina*, the species *B. trigonocephalum*, *Oe. venulosum*, and *Trichuris* sp. were identified. *Trichuris* sp. was the most common parasite of the cecum. Interestingly, in addition to *Ch. ovina*, *Oe. venulosum*, and *Trichuris* sp., *H. contortus* was also detected in the cecum of one roe deer.

Excluding *Trichuris* sp., both the prevalence and species richness of gastrointestinal nematodes were higher in roe deer inhabiting wind farm areas. Moreover, *Moniezia* spp. were also more prevalent in animals from wind farm locations compared to those from areas outside these sites.

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O16 ✻ Student Competition

Parasites of raccoons in the Czech Republic

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The raccoon (*Procyon lotor* (Linnaeus, 1758)) is an invasive species in Europe originating from North America. Free-living populations became established during the first half of the 20th century, primarily due to escapes from fur farms and intentional releases associated with hunting activities. Nowadays, raccoons are considered a growing ecological and epidemiological concern because they can act as carriers of numerous pathogens and parasites with zoonotic significance. To date, around 90 helminth species have been documented within the species' native distribution area, while only 36 have been reported from introduced populations in Europe and Asia. This study focused on the occurrence and diversity of parasites in raccoons from the Czech Republic. Between 2024 and 2025, 22 raccoon individuals originating from six localities were parasitologically examined for the presence of endoparasitic helminths. Helminth specimens obtained during necropsy were subsequently identified using a combination of morphological characteristics and molecular analyses. Among nematodes, three species were detected, including two representatives of the genus *Porrocaecum* and the zoonotically important *Baylisascaris procyonis*, which is strongly associated with raccoons as its definitive host. Among Platyhelminthes included the trematodes *Plagiorchis koreanus* and *Metorchis bilis*, together with the cestode *Atriotaenia incisa*. Our findings indicate that non-native raccoons have retained specific parasite species from their native distribution range. Especially important are the raccoons infected with *B. procyonis,* which may pose a potential zoonotic risk to humans. The presence of this zoonotic species in Czech raccoons highlights the importance of monitoring invasive species from both ecological and public health perspectives.
The research was funded by the Technology Agency of the Czech Republic (project no. SS07020021), the Ministry of Agriculture of the Czech Republic, (project no. QL24020333).

O17

Climate-driven predictors of zoonotic pathogen occurrence in wild small mammal populations in the Czech Republic

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Wild small mammals are key reservoirs of zoonotic pathogens, yet the role of climate in shaping their infection dynamics remains insufficiently quantified. This study identifies climatic predictors of the occurrence of *Borrelia burgdorferi* sensu lato, *Coxiella burnetii*, and *Francisella tularensis* in wild small mammal populations in the Czech Republic.
A total of 679 individuals from six species were sampled across three localities between 2010 and 2014. Serological analyses (ELISA) detected antibodies to *B. burgdorferi* s.l. in 12.4 % (84/679), *C. burnetii* in 9.7 % (55/568), and *F. tularensis* in 4.6 % (26/568) of individuals. Co-infections were detected at low frequency, with higher prevalence observed for IgM combinations e.g., *B. burgdorferi* s.l. and *F. tularensis* 2.8 % (16/568). Logistic regression revealed significant climate-pathogen relationships. The occurrence of *B. burgdorferi* s.l. increased with the number of frost and ice days combined with low snow cover ($p < 0.05$), while higher water vapor pressure was associated with decreased occurrence ($p < 0.05$). The probability of *C. burnetii* infection increased with the number of summer days and ice days ($p < 0.05$), whereas males showed a lower infection risk ($p < 0.05$). The occurrence of *F. tularensis* was significantly

higher in spring compared to summer and autumn ($p < 0.05$) and was positively associated with temperature and precipitation ($p < 0.05$).
These findings demonstrate that climatic variability significantly shapes zoonotic pathogen occurrence, primarily through indirect effects on host population dynamics and vector activity. Integrating climate-driven predictors into risk models may improve forecasting of zoonotic disease emergence and support targeted prevention strategies.
This study was supported by the Masaryk University internal grant (MUNI/A/1631/2025), and the University of Veterinary Sciences Brno internal grant (2026ITA23).

O18

Data-driven ecological modeling of trematode species in a subarctic lake

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Parasites are important components of ecosystems, linking multiple compartments and trophic levels within ecological networks. However, due to their often complex life cycles, the effects of individual interactions are difficult to disentangle using statistical analyses alone. Parasites can influence the fitness and behaviour of their hosts, while their prevalence is strongly shaped by both host and non-host species of the community through a diverse set of ecological interactions. Trematodes, in particular, are well-known for their complex life cycles involving multiple host species from different functional guilds, making them ideal model organisms for studying parasite ecology.
The aim of this study was to investigate how different types of ecological interactions and feedback loops influence trematode prevalence and diversity within a lake community. The primary focus was on the predation of first intermediate snail hosts by fish predators that may also serve as second intermediate hosts (containing metacercariae), depending on the trematode taxon examined. Two trematode genera, *Diplostomum* and *Plagiorchis*, were chosen as model organisms because both utilise the same snail species as their first intermediate host, yet differ in their life cycles: *Diplostomum* spp. use fish species inhabiting the lake (which also prey on snails) as subsequent hosts, while *Plagiorchis* spp. do not. The study was conducted in the oligotrophic lake Takvatn in subarctic Norway, a system characterised by high trematode diversity and a complex interaction network. Importantly, this lake has been the subject of several previous ecological studies, which have revealed much of its ecological structure, making it a particularly suitable model system for this study.
Using empirical data, we sought to identify the main ecological processes driving the lake community and subsequently used ecological modeling using systems of ordinary differential equations to explain the role of individual ecological interactions. Trematode species were modeled explicitly through compartments representing infected and uninfected host populations. Model parameters were estimated from experimental results, while missing rates were obtained from the literature. Model predictions were then compared with empirical findings to evaluate model performance. The effects of individual interactions were assessed by species removal and by modifying interaction strengths within the model framework.
Our preliminary results suggest that the model qualitatively reproduces observed empirical patterns and helps identify the roles of specific ecological interactions, particularly the influence of fish predators on trematode prevalence. Ongoing work aims to further investigate the mechanisms that may contribute to the unusually high diversity of trematodes observed in this subarctic lake system.
This study was supported by the Czech Science Foundation project no. 24-11738S.

O19 ✻ Student Competition

Does climate warming benefit the parasite or the host? Insights from the *Diplostomum pseudospathaceum–Lymnaea stagnalis* system

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Climate warming is expected to reshape freshwater host-parasite interactions, but the response of parasites with complex life cycles remains difficult to predict. Species of the genus *Diplostomum* use freshwater snails, including *Lymnaea stagnalis*, as first intermediate hosts, fish as second intermediate hosts and water birds as definitive hosts. In fish, metacercariae develop in the eye lens, impairing vision and reducing the host's condition. This makes *Diplostomum* sp. an important model for studying temperature-dependent transmission in freshwater ecosystems.

This study assessed whether long-term water warming disrupts or supports the transmission of *Diplostomum pseudospathaceum* in *L. stagnalis populations*. Thermally polluted lakes in the Konin-Pątnów (Poland) power plant cooling system were used as a natural model of chronic water warming and were compared with lakes exhibiting a natural thermal regime. During the 2015-2017 and 2023-2024 seasons, 3350 *L. stagnalis* individuals were examined, including 1511 from heated lakes and 1839 from natural lakes. Digenean infections were detected by cercarial shedding and dissection, while selected *Diplostomum* sp. isolates were identified using mitochondrial cytochrome c oxidase subunit I (COX1), internal transcribed spacer 2 (ITS2) and 28S rDNA markers. Seasonal prevalence, thermal preferences of infected and uninfected snails, snail survival at constant temperatures, cercarial release and cercarial survival were also analysed.

Molecular analyses confirmed that all examined isolates belonged to *D. pseudospathaceum*. Sequences from heated and natural lakes formed a single phylogenetic lineage, with no stable subdivision corresponding to lake thermal regime. However, field data showed contrasting seasonal patterns of patent infections. In heated lakes, *D. pseudospathaceum* prevalence peaked in summer, whereas in lakes with a natural thermal regime it was lowest during summer and higher in spring and autumn.

Laboratory experiments showed that infection altered snail thermal behaviour. Infected *L. stagnalis* selected lower temperatures than uninfected individuals, suggesting a behavioural anapyrexia-like response. Snails originating from heated lakes survived longer than those from natural lakes, regardless of experimental temperature and infection status. Elevated temperature increased daily cercarial release but reduced the survival of free-living cercariae, with shorter median lethal time (LT50) values at 25°C than at 16°C.

Our results indicate that long-term water warming does not eliminate the *D. pseudospathaceum–L. stagnalis* system from heated lakes. Instead, elevated temperature modifies the seasonality of transmission, while host plasticity or local adaptation may allow the parasite life cycle to persist under thermal stress. Climate warming may therefore not necessarily reduce the transmission potential of *Diplostomum* sp.; rather, it may shift transmission dynamics and maintain, or even increase, infection risk for subsequent hosts such as fish.

O20

Hidden parasites in changing rivers: eDNA metabarcoding reveals the impact of human disturbance

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Understanding biodiversity at species and genetic levels remains a major challenge in ecology, particularly for microscopic parasites such as myxozoans, aquatic microsporidians, and amphizoic amoebae. As

freshwater ecosystems face increasing pressure from habitat degradation, river regulation, intensive aquaculture, and climate change, assessing parasite diversity and community change has become increasingly urgent.
Because parasites are difficult to detect, they are often overlooked in conventional ecological surveys. Environmental DNA (eDNA) metabarcoding offers a powerful alternative by enabling sensitive detection directly from environmental samples. Here, we used eDNA metabarcoding to test how anthropogenic disturbance shapes the occurrence and diversity of aquatic microscopic parasites.
We study parasite diversity in two river systems undergoing contrasting types of environmental change: the Lužnice River in the Czech Republic and the Klamath River at the Oregon/California border, USA. The Lužnice River provides a natural contrast between two hydrologically distinct sections: an undisturbed, meandering stretch and a regulated stretch connected to a system of intensively managed fishponds. Both sections are exposed to comparable climatic conditions, allowing us to assess differences in parasite communities between a near-natural river section and a section strongly influenced by fishpond management. In contrast, the Klamath River is undergoing large-scale restoration following the removal of major hydroelectric dams, with the aim of restoring natural river flow and enabling salmonids to return to their historical spawning grounds. In this system, we focus on comparing parasite communities before and after dam removal, particularly to determine whether fish parasites reappear in river sections where their hosts had previously been absent or severely limited due to dam-related barriers. To assess parasite diversity in both systems, we analyzed environmental DNA from water and sediment samples. Amplicon sequencing was performed using group-specific primers targeting Myxozoa, microsporidians, and amphizoic amoebae, with sequencing carried out using both Illumina and Oxford Nanopore Technologies platforms.
We revealed unexpectedly high parasite diversity, including numerous previously uncharacterized taxa. Parasite communities differed markedly between disturbed and undisturbed sites. Alongside native parasites, we detected taxa likely introduced through fish stocking in the Lužnice river system. Several species were reduced in heavily impacted areas, whereas overall parasite richness was higher in sites linked to intensive aquaculture. Our results show that anthropogenic disturbance strongly shapes parasite communities and may disrupt host–parasite balance.
This project was supported by MEYS (LUAUS24281).

O21

How human impact shapes amoebae communities in freshwater ecosystem: an eDNA metabarcoding approach

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Free-living amoeboid protists represent a morphologically defined group of microbial eukaryotes characterized by amoeboid movement and the formation of pseudopodia, spanning multiple evolutionary lineages, with most species belonging to Amoebozoa but with additional diversity in other groups such as Heterolobosea. They play key roles in freshwater ecosystems as predators of bacteria and contributors to nutrient cycling, yet their diversity and community structure remain poorly understood, particularly in relation to anthropogenic disturbance.
In this study, we investigated amoeboid protist community composition in two freshwater systems in the South Bohemian region of the Czech Republic: the Lužnice River and the Zlatá stoka channel, representing environments with contrasting levels of human impact. Environmental DNA (eDNA) metabarcoding was used to assess diversity from both water and sediment samples collected across multiple sites.
A total of ten primer pairs targeting SSU rRNA and mitochondrial cox1 markers were tested to evaluate their efficiency in detecting amoeboid protists. Sequencing was performed using Oxford Nanopore Technologies (ONT), followed by bioinformatic processing and phylogenetic analysis. Primer performance differed substantially, with cox1-targeting primers showing the highest specificity and detection efficiency. The detected community was dominated by Amoebozoa, particularly members of the genera *Parvamoeba* and *Cochliopodium*, while additional diversity was represented by Heterolobosea, including a high diversity of *Naegleria*. Overall, diversity was higher and more consistent in the Lužnice

River, whereas the Zlatá stoka exhibited reduced diversity, likely reflecting the impact of intensive fish farming and associated environmental conditions. Phylogenetic analyses further suggest that many detected taxa represent previously undescribed species.
These results demonstrate that anthropogenic influence can significantly shape amoeboid protist communities and highlight the importance of marker choice in eDNA-based studies. The study also demonstrates the potential of eDNA metabarcoding to reveal hidden diversity and ecological patterns in freshwater microbial eukaryotes.
This study was supported by the Ministry of Education, Youth and Sports of the Czech Republic (project no. LUAUS24281 to I.F.)

O22 ⁕ Student Competition

Tracking parasite diversity in an urban-impacted freshwater stream

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Freshwater ecosystems are increasingly threatened by anthropogenic pressures, including wastewater discharge, urbanization, and habitat degradation, which can strongly alter aquatic community composition. Despite their high diversity and widespread occurrence in aquatic hosts, myxozoan and microsporidian parasites remain poorly studied lineages of freshwater biodiversity, particularly in relation to environmental disturbance. The Živný Stream in South Bohemia, Czechia, influenced by urban activities and effluent from the Prachatice sewage treatment plant, represents a suitable model system for studying anthropogenic effects on parasite communities. Using environmental DNA metabarcoding, we investigated spatial and temporal patterns in myxozoan and microsporidian diversity along the stream across four sampling campaigns spanning 2024–2025. Particular attention was given to changes in parasite diversity and community composition associated with seasonal turnover and urban influence.
Samples were collected from 10 sites along the Živný stream during four sampling campaigns (March, June, and September 2024; March 2025). Water and sediment samples were collected at all sites during each sampling campaign for detection of myxozoans and microsporidians. Additionally, benthic invertebrates (*Gammarus* spp., Trichoptera larvae) were collected in June and September 2024 for targeted detection of microsporidians. Environmental DNA was extracted and used for PCR amplification of a 350–400 bp long region of SSU rDNA targeting Myxozoa and Microsporidia with barcoded primers, followed by Illumina NovaSeq sequencing (250 bp paired-end). Sequence data were processed using a bioinformatic pipeline including the merging of forward and reverse reads, quality filtering, demultiplexing, chimera removal, OTU clustering, and taxonomic assignment. Community analyses in R focused on spatial and temporal variation in parasite assemblages associated with urban influence and seasonal turnover.
A total of 22 myxozoan and 230 microsporidian OTUs were detected, including numerous unassigned OTUs within both parasite groups. Although the stream is influenced by urban activities and wastewater discharge, parasite community composition appeared to be shaped more strongly by temporal turnover than by anthropogenic influence. Microsporidians showed substantially higher diversity than myxozoans across all sampling periods and sites. Temporal variation in OTU richness was observed in both groups. Differences between sample types were also apparent: myxozoans were detected predominantly in water samples, whereas microsporidians occurred more evenly across water, sediment, and invertebrate samples. Preliminary alpha and beta diversity analyses suggested temporal differences in parasite assemblages between sampling periods, while spatial patterns among localities were generally weaker. Our results highlight the usefulness of eDNA metabarcoding for assessing spatiotemporal dynamics of freshwater parasite communities under anthropogenic influence.
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O23 ✻ Student Competition

Invasive amphipods as a novel link in the transmission of oviduct fluke infection

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Biological invasions constitute one of the major threats to contemporary biodiversity and ecosystem functioning. Invasive species may affect the environment through competition, predation, habitat modification, and the transmission of parasites and pathogens. Their spread is closely associated with human activity, including global trade and transport.
Pontogammarus robustoides is an example of an organism whose dispersal is primarily attributed to aquatic transport, including transfer via ballast water and interconnected canal systems. This species is an amphipod crustacean which is native to the Ponto-Caspian region and has successfully colonised European inland waters. It has been demonstrated that *P. robustoides* exerts a significant impact on native invertebrate communities, for instance, through competition and predatory pressure. However, its ecological role is not limited to direct interactions. This species may also act as the second intermediate host for digenean parasites of the genus Prosthogonimus, thereby constituting an important component of their life cycle.
Oviduct flukes of the genus *Prosthogonimus* infect definitive hosts - poultry - mainly through metacercariae inside dragonflies. However, the use of amphipods as a rich source of protein, omega-3 fatty acids and chitosan in poultry farming (including chickens) carries the possibility of a new route of parasite transmission from aquatic environments to terrestrial livestock systems.
This example demonstrates that the impacts of biological invasions extend beyond direct interspecific interactions and may involve complex, multi-level relationships resulting in unexpected economic and veterinary consequences.

O24

Parasite load and microbiota in crucian carp (*Carassius carassius*) threatened by the invasive activity of gibel carp (*Carasius gibelio*)

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The crucian carp (*Carassius carassius*) is a native freshwater fish species widespread in European rivers. However, its population density, genetic integrity, biological condition and ecological valence are currently being significantly negatively affected by the invasion of the non-native gibel carp (*Carassius carassius*). The gibel carp currently represents a serious threat to global freshwater biodiversity and the functioning of aquatic ecosystems. Current knowledge about the effect of the invasive gibel carp on native crucian carp in Europe remains limited, and practically no information exists in the Czech Republic.
This study aimed to investigate the impacts of the invasive species on the native species, i.e., to reveal the potential transfer of parasites and microbiota associated with the invasive gibel carp to the native crucian carp. We applied invasive screeing of parasite and microbial communities, as well as we developed non-invasive methods for parasite and microbiota sampling. Populations of crucian carp and gibel carp from both allopatric and sympatric populations were investigated.
The populations of allopatric crucian carp harboured less abundant monogenean communities than allopatric populations of invasive gibel carp. In allopatric populations of crucian carp, the *Gyrodactylus* parasites were relatively rare, whereas these parasites reached high infection intensities in allopatric gibel carp populations. Sympatric populations of native crucian carp and invasive gibel carp differed in parasite diversity, with the highest monogenean diversity observed at the site with higher hybridization rate. At this

site, monogenean load tended to be higher in hybrids when compared to crucian carp. In contrast, at the site with lower hybridization rates, parasite load tended to be lower in invasive gibel carp than in crucian carp and hybrids.
Microbial DNA metabarcoding revealed that allopatric populations of crucian carp and gibel carp exhibited a higher number of unique bacterial sequences than sympatric populations. In sympatric populations, the number of unique bacterial sequences detected in crucian carp was lower than that detected in gibel carp. Partial sharing of bacterial communities at this site may reflect the influence of the shared environment or is potentially a consequence of the effect of the invasive species on the native species or their mutual direct or indirect interactions.
We expect that the outputs of this study will be directly applicable to crucian carp reintroduction programmes, as they will allow for prediction of the biological vigour of crucian carp populations based on monitoring of parasite and microbial loads, as well as estimation of the risk associated with coexistence with invasive gibel carp.
This study was supported by the Technological Agency of the Czech Republic, project SQ01010194.

Taxonomy, Systematics and Biodiversity of Parasites

ORAL PRESENTATIONS

O25 ★ **Piotr Tryjanowski** — Winged minds and hidden manipulators: parasites and cognition processes

O26 ◆ **Louis du Preez** — Evolutionary Ingenuity: Reproductive Strategies of Polystomatid Flatworms

O27 M. Benovics, L. du Preez — Dwarves and giants of the African frog world - what do they hide within?

O28 R. Bodzíková, C. Pantoja, P. Papežík, M. Benovics — Diversity and distribution of pleurogenid trematodes in European water frogs

O29 F. Nejat, M. Benovics, J. Vukić, R. Šanda, A. Vetešníková Šimková — Host Switching Drives Diversification of *Dactylogyrus* in Middle Eastern Cyprinoid Fishes

O30 J. Omondi Outa and O. Kudlai — Elusive and taxonomically problematic: unravelling the hidden diversity of blood flukes in freshwater fish from Lithuania

O31 ✻ **S. Salonová, D. Barčák, M. Orosová, A. Kiššová, Miroslav Urošević, D. Gombitová, M. Oros** — Survey for fish helminths in the Danube River basin with focus on zoonotic species: first results

O32 I. Přikrylová, P.S. Molokomme, L.P. Lukhele, A. Tripathi, J.R. Sara, W.J. Luus-Powell — Parasites associated with the ornamental fish industry: current status and emerging risks

O33 J. Omondi Outa, O. Kudlai, F. Jirsa — Trematodes of snails from Lake Victoria: diversity, new records, broad geographic connections and human health risks

O34 V. Sarabeev, O. Lisitsyna, O. Greben, A. Rashydov, M. Oros — Hidden co-invasions and enemy release: contrasting helminth communities in native and invasive amphipod

O35 G. Zaleśny, J. Gabrysiak, G. Kanarek, J. Hildebrand — More than meets the eye: cryptic diversity of Strigeidae trematodes revealed in swan populations

O36 ✻ **J. Gabrysiak, G. Kanarek, G. Zaleśny, J. Hildebrand** — New insights into the diversity of *Australapatemon* and *Apatemon* species in Central Europe (Digenea, Strigeidae) - an integrative approach

O37 A. Šujanová, J. Harl, T. Himmel, M. Duc, M. Pacheco, G. Valkiūnas — Exo-erythrocytic development and tissue tropism of *Haemoproteus* parasites in Eurasian blackcaps

O38 D. Damnjanovic, M. Lisnerová, M. Perlík,K. Svobodová, S. Nayak, A. Lövy, M. Burgerová, H. Pecková, Š. Zeman, J. Votýpka, I. Fiala, A. Krejčí, P. Bartošová-Sojková — Hidden in the honeycomb: Microsporidian and trypanosomatid parasites in bees identified by metabarcoding approach

O25 ★ Keynote Speaker

Winged minds and hidden manipulators: parasites and cognition processes

Piotr Tryjanowski

Faculty of Veterinary Medicine, Poznan University of Life Sciences, Poland

Infection can empty not only the lungs, and it can also weaken the mind. In medicine this is obvious; in the cognitive ecology of birds it remains an open puzzle. This presentation argues that the link between parasites and avian cognition deserves a programmatic research agenda, even though field data are still scarce and contradictory. The evolutionary intuition is strong: spatial memory, song and egg recognition are tightly tied to fitness in birds. The candidate mechanisms - neuroinflammation, the hypothalamic–pituitary–adrenal axis, energetic costs, and developmental windows in song nuclei (HVC) - are well described in mammals and in one classic experiment on canaries. In wild adult birds, however, parasite removal does not improve problem solving, and a poorer test score may reflect lost motivation rather than lost competence. The thesis is simple: conflicting evidence is not a reason to set the topic aside, but a reason to design an experiment that separates the direction of causality, motivation from competence, and the hippocampus from HVC.
Keywords: avian cognition; parasites; Plasmodium; hippocampus; neuroinflammation; sickness behaviour; developmental stress hypothesis; One Health

O26 ◆ Invited Speaker

Evolutionary Ingenuity: Reproductive Strategies of Polystomatid Flatworms

Louis du Preez

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Polystomatid flatworms are known from the Australian lungfish; all three orders of amphibians namely salamanders, frogs and caecilians; freshwater turtles; the American alligator and from the eye of the common hippopotamus. Of the 31 currently known polystome genera, 19 are known from amphibians, nine from freshwater turtles, one each from the lungfish, the alligator and the hippopotamus. Parasite transmission can only occur in an aquatic environment, involving the oncomiracidium. As a result, the diversity of host species is limited to aquatic or semi-aquatic hosts. Polystomes co-evolved with their amphibious hosts and unique life cycles evolved in close synchronisation with that of the host. Exposure to the aquatic environment may be permanent or semi-permanent like the fully aquatic clawed frog *Xenopus* and freshwater turtles, or may be a brief period of a few hours per year for frogs adapted to arid conditions, like *Scaphiopus*. When hosts are permanently or semi-permanently in water, polystome egg production is usually slow and takes place over an extended period of time. However, in hosts that spend a brief period in water, polystomes adopted various reproductive strategies. Two morphological features that play a key role are the attachment organs to secure a firm grip to the various micro-environments and the ability to store eggs to maximise reproductive capacity. Polystomes attach by means of haptoral suckers and in most of the known genera also by hamuli. On soft substrates like in the urinary bladder, the hamuli appear to provide a more secure grip while on firm substrates such as the oral region of freshwater turtles or on the eye of turtles and the hippopotamus, haptoral suckers have skeletal elements build into the suckers to secure a firm grip. The development of an extensive uterus and with that intra-uterine development of eggs enabled polystomes to infect even explosive breeders that would enter the water once a year for a few hours. Polystomes serve in many respects as a model parasite group to demonstrate various principles of parasitism.

O27

Dwarves and giants of the African frog world - what do they hide within?

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South Africa harbours a remarkable amphibian diversity, with 138 frog species occurring across a wide range of habitats and climatic regions. However, despite this diversity, the parasitic fauna of South African frogs remains insufficiently investigated, and available data on helminth diversity and host associations are still limited for many taxa. In our research we focus on shedding new light on the diversity of amphibian helminths in southern Africa using an integrative approach. The present study focused on helminth parasites of two distinct frog groups: rain frogs (*Breviceps*), a group of small terrestrial breeding frogs, endemic to southern Africa, and African bullfrogs (*Pyxicephalus*), which in contrast, are among the largest frog species on the continent. Parasitological examinations revealed apparent differences in parasite diversity and infection patterns between the investigated hosts. In rain frogs, only nematodes were detected. These occurred in the lungs, gastrointestinal tract, and on the stomach wall, where larval stages were observed. In contrast, African bullfrogs exhibited a considerably richer helminth community. Besides nematode species, infections included cestodes of the genus *Batrachotaenia* and potentially a new trematode taxon for science. The observed differences in parasite assemblages likely reflect ecological and trophic differences between the examined hosts, including body size, habitat use, diet, and life-history strategies. Our findings contribute to the still limited knowledge of amphibian parasites in southern Africa and highlight the importance of further surveys targeting both common and understudied amphibian hosts in this amphibian diversity rich region.
Molecular part of this study was supported by the Grant Agency VEGA project No. 1/0399/26.

O28

Diversity and distribution of pleurogenid trematodes in European water frogs

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Trematodes of the family Pleurogenidae are common intestinal parasites of amphibians. They are widely distributed across the Palearctic, with occasional records from adjacent regions. However, their diversity, host specificity, and phylogenetic relationship are still not well understood. In this study, we investigated parasites of this family collected from water frogs (genus *Pelophylax*) using an integrative approach, combining morphological and molecular methods. We examined frogs belonging to five *Pelophylax* species (*Pelophylax ridibundus, P. esculentus, P. kurtmuelleri*, *P. caralitanus*, and *P. epeiroticus*) from 12 localities in southwestern Slovakia, three Balkan countries: Greece (four localities), Romania (three localities), and Bulgaria (two localities), and two localities in Malta. Selected specimens were analysed using three genetic markers (28S rDNA, ITS regions, and the mitochondrial partial COI gene). The molecular and morphological data revealed eight phylogenetic lineages among the collected pleurogenids and confirmed the presence of *Prosotocus confusus* in Slovak, Balkan, and Maltese water frogs. This species exhibited a relatively low genetic variability in the 28S rDNA region among the specimens from Slovakia. The Slovak specimens formed a phylogenetically sister group to the specimens from the Balkans collected from *P. kurtmuelleri* (Greece). Moreover, species *Pleurogenoides medians* was recorded exclusively from Balkan frogs. Molecular data obtained from collected pleurogenid specimens also revealed the presence of at least two morphologically almost indistinguishable taxa, which may represent a cryptic species complex. Overall, our findings highlight the limits of morphology-based identification

among pleurogenid taxa. This study opens a new chapter in understanding parasite diversity and host-parasite interactions in European amphibians.
This study was supported by the Grant Agency VEGA project No. 1/0399/26.

O29

Host Switching Drives Diversification of *Dactylogyrus* in Middle Eastern Cyprinoid Fishes

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Host–parasite coevolution is driven by several processes, particularly cospeciation, host switching, duplication and lineage sorting. Monogeneans of the genus *Dactylogyrus*, which are highly specific parasites of cyprinoid fishes, offer an excellent study system for investigating patterns of diversification and host-parasite evolutionary congruence. Although the Middle East is an important biogeographic region for the diversification and historical dispersal of cyprinoids, the cophylogenetic relationships between these fishes and their monogenean parasites remain under investigated. In this study, we reconstructed the phylogenyof 63 *Dactylogyrus* species parasitizing 58 cyprinoid host species from Iran, Iraq, and Turkey. To reconstruct the parasite phylogeny, partial 28S and 18S rDNA were used and the phylogeny of cyprinoid hosts were reconstructed using the complete cytochrome *b* of mtDNA. Host–parasite evolutionary congruence was assessed using both global-fit approaches (PACo and ParaFit) and event-based methods (Jane and eMPRess). The results indicate that host switching was the dominant evolutionary mechanism, while cospeciation represented the second most common historical process. Frequent parasite transfers among sympatric cyprinoid lineages, especially within *Capoeta* and *Paracapoeta*, likely contributed substantially to parasite diversification.
This study was supported by the Grantová Agentura České Republiky, GA20-13539S.

O30

Elusive and taxonomically problematic: unravelling the hidden diversity of blood flukes in freshwater fish from Lithuania

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Fish blood flukes inhabit the cardiovascular system of their hosts and are known to cause mortalities of infected fish. Unfortunately, blood flukes of fish, especially from freshwater ecosystems, are underreported worldwide. Until now, only seven species, belonging to the genus *Sanguinicola* (Digenea: Sanguinicolidae) were known in Europe. In Lithuania, only three species (*Sanguinicola armatus*, *S. inermis* and *S. intermedius*) had been reported. The current study is a report of blood flukes from 10 fish species belonging to families Cyprinidae, Leuciscidae and Tincidae, that were obtained from rivers, reservoirs, lakes and a lagoon in Lithuania. The flukes were characterised using morphological data and partial sequences of nuclear 28S rDNA and mitochondrial *cox*1 DNA. Contrary to previous reports of only three sanguinicolid species in Lithuania, morphological and phylogenetic analyses of the specimens from this study revealed 10 sanguinicolid species. Two species occurred in more than one host species while the others appear to be species specific. The current study provides novel genetic data and supplemental morphological descriptions of *S. armatus* (type species), *S. inermis* and *S. intermedius*. Also, we provide new locality records for *Sanguinicola volgensis*. Finally, we describe new sanguinicolid species and assess the phylogenetic relationships within the family Sanguinicolidae.
This research received funding from the Research Council of Lithuania (LMTLT) (S-PD-24-132).
Keywords: Sanguinicolidae, Species descriptions, Morphology, Phylogenetics, Host affiliations

O31 ✻ Student Competition

Survey for fish helminths in the Danube River basin with focus on zoonotic species: first results

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The Danube is the second-longest river in Europe and one of the continent's most important river systems. It flows through ten countries and serves as a major migration corridor for numerous species of fish, molluscs, and other aquatic organisms. Due to its biodiversity and strong ecological connectivity, the Danube River basin provides a suitable environment for the circulation of various helminths.

The aim of this study was to determine the occurrence of helminth larval stages, focusing on species of zoonotic importance. Fish sampling was conducted in the Danube River in Austria, Romania, Serbia, and Slovakia, as well as in its major tributary, the Sava River in Serbia. Fish were caught by electrofishing, nets, and angling rods in cooperation with local authorities. In total, 220 fish representing 31 species from 12 families were examined under a stereomicroscope for helminth infections in the eyes, scales, skin and fins. Subsequently, the muscles, scales and fins were digested in artificial gastric fluid to liberate metacercariae from the tissues. Some of encysted metacercariae were excysted using a pancreatin-based medium, heat-killed and preserved in 70% ethanol for morphological examination, whereas cysts were fixed in 96% ethanol. Metacercariae were identified by genotyping using the complete ITS as genetic marker. Additionally, surface water was filtered through a plankton net to collect environmental (e)DNA samples for eDNA metabarcoding.

Seven genera of digenean trematodes and one nematode species were recorded. The most prevalent was *Metagonimus romanicus* (average overall prevalence P_o: 26.8%), found in scales of 15 fish species, predominantly members of the family Leuciscidae. The highest infection was found in *Squalius cephalus*, *Chondrostoma nasus*, *Alburnus alburnus* and *Leuciscus idus*; the invasive goby *Neogobius melanostomus* was also infected. Another common parasite was *Diplostomum* sp. (P_o: 14.1%) detected in eye lenses of 12 species from all sampled localities except Slovakia. The highest prevalence was recorded in Romania (42.9%) and the most heavily infected fish belonged to the family Leuciscidae. Metacercariae of *Apophallus* spp. (P_o: 10.6%) were found in the fins and muscles of nine fish species from the families Leuciscidae and Percidae across all investigated localities. *Tylodelphys* sp. occurred in the vitreous humour of the eye in eight fish species, mainly in Austria, and *Holostephanus* sp. was detected in muscles of nine species with highest occurrence in Romania. These two species had equal prevalence (P_o: 8.2%). The only nematode detected, *Eustrongylides* sp. (P_o: 5.5%), was found in the muscle of five species from Serbia and Romania, especially in *Perca fluviatilis*. The least common (P_o: 1.8%) were *Clinostomum* sp. found under the skin of *P. fluviatilis* in Slovakia and Romania, and *Posthodiplostomum* sp. recorded mainly in the skin of the same fish in Slovakia and Austria.

The results indicate that the Danube River is an important area for the circulation of helminths with zoonotic potential, namely *Apophallus* spp., *Clinostomum* sp., *Eustrongylides* sp., and *Metagonimus romanicus*, and highlights the need for further monitoring fish-borne parasites in European rivers.

This study was supported by Slovak Research and Development Agency (APVV 24-0140) and Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and Slovak Academy of Sciences (VEGA No. 2/0130/24).

O32

Parasites associated with the ornamental fish industry: current status and emerging risks

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The aquarium fish sector is a significant global component of international trade, fisheries, and aquaculture, with more than 1.5 billion fish and 4,000 freshwater species traded annually, the majority originating from Asia. Despite this scale, the role of the ornamental fish trade in the dissemination of parasites remains underexplored.

Between October 2020 and November 2025, a total of 785 ornamental fish, representing 39 species from 14 families, were imported from Indonesia, Sri Lanka, and Thailand through an established trading company and examined for ichthyoparasites. Standardised protocols were followed when transporting, handling, and dissecting fish, and an integrated approach combining morphometric and molecular characterisation was used to identify parasites. Overall, 44% (17) of the fish species examined were infected with 31 parasite species representing five parasitic groups, *i.e.* Ciliophora (1 sp.), Monopisthocotyla (19 spp.), Cestoda (6 spp.), Nematoda (3 spp.), and Crustacea (2 spp.). Monopisthocotylans were the most diverse and prevalent, occurring on 15 host species, with high infection intensities observed in several cases. Notably, atypical host associations were recorded for species such as *Cichlidogyrus tilapiae* and *C. furu*, likely reflecting intensive aquaculture and stocking practices. A new genus of monopisthocotylan parasites from *Glossolepis incisa* (imported from Indonesia) and *Melanotaenia boesemani* (from India) was found, demonstrating host specificity within the Melanotaenidae. Taxonomic challenges within the *Thaparocleidus* spp. from *Pangasianodon hypophthalmus*, suggest the need to synonymise *T. sianensis* and *T. campylopterocirrus*. Endoparasite infections were generally less frequent, although *Camallanus cotti* exhibited 100% prevalence in *G. incisa*. The nematode *Capillaria pterophyll*i was found in the intestines of three cichlid species, *Andinoacara pulcher*, *Pterophyllum scalare*, and *Uaru amphiacanthoides*, representing another example of how rearing facilities and stocking practices facilitate the spread of parasitic infections to atypical hosts. Larval stages of Bothryocephalidae, Diphyllobotriidae, and Gryporhynchidae were occasionally observed, gryporhynchid metacestodes (i.e. *Dendrouterina herodiae* and *Neogryporhynchus cheilancristrotus*) reaching a prevalence of up to 40% in *Trichopodus leerii.* Parasitic crustaceans were rare, with low prevalence recorded for *Lernaea cyprinacea* (P = 4.8%) on *G. incisa* and *Argulus japonicus* (P = 6.7%) on *Carassius auratus*.

These findings highlight the role of the ornamental fish trade as a pathway for parasite introduction, host-switching, and taxonomic complexity. The study underscores the importance of routine parasitological screening, strict quarantine measures, and integrative taxonomic approaches to mitigate biosecurity risks and improve our understanding of parasite biodiversity and life cycles.

This study was supported by DSTI-NRF SARChI Chair (No.101054).

O33

Trematodes of snails from Lake Victoria: diversity, new records, broad geographic connections and human health risks

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Molluscs are typically the first intermediate hosts for most digenean trematodes. Unfortunately, comprehensive studies on trematode larvae, especially using molecular data, are few in many parts of the world. The current study provides taxonomic, phylogenetic and human health risk information on snail-

borne trematodes from Lake Victoria. The data is based on examination of snail specimens that were collected at Rusinga Island (Kenya) in 2023 and 2026. The snails and their trematodes were characterised using morphological data, and fragments of the mitochondrial *cox*1 and ribosomal 28S DNA. The snail community consisted of native species *Bellamya unicolor*, *Biomphalaria choanomphala*, *Bulinus* aff. *ugandae*, *Melanoides tuberculata*, *Pila ovata* and *Radix natalensis*, and the invasive *Pseudosuccinea columella*. This appears to be the first report of *P. columella* in Lake Victoria. Genetic analyses revealed that *P. columella* sequences from the current study are closely related to a highly invasive haplotype from USA that has invaded water bodies in South America (Argentina and Colombia), Europe (Portugal), Africa (Egypt, South Africa and Zimbabwe), and Australia. The introduction of *P. columella* at Rusinga Island appears to be recent, since it was observed only during the 2026 survey. Overall, 10 digenean trematode species, representing families Echinostomatidae, Heterophyidae, Lecithodendriidae, Paramphistomidae, Schistosomatidae and Thapariellidae were recorded. Two species from this study (*Schistosoma mansoni* and *Haplorchis pumilio*) are known to infect humans. *Haplorchis pumilio* from the current study showed close genetic relationships with isolates from North America and southern Asia. Genetic data also show intercontinental distributions of two echinostomatid genera, that can be linked to the cosmopolitan distribution of their final hosts (birds). Sequences of two species (one echinostomatid and a lecithodendriid) did not correspond to any known genera. Future work will focus on assessing the distribution of *P. columella* and studying its role in trematode transmission in the Lake Victoria basin.
Keywords: Cercariae, Metacercaria, Phylogenetics, Intercontinental distributions, Invasive snail

O34

Hidden co-invasions and enemy release: contrasting helminth communities in native and invasive amphipod

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The helminth fauna of the Ponto-Caspian invasive amphipod *Dikerogammarus villosus* in Central Europe remains insufficiently studied, with the existing literature predominantly showing a taxonomic bias toward Acanthocephalan infections. A detailed inventory of this pervasive invader's parasite assemblage is crucial for quantifying its ecological impact across European waters.
A comprehensive parasitological survey was conducted at 18 localities across distinct macro-regions, primarily within the Middle Danube basin and the Carpathian Mountains. A total of 7,004 amphipod specimens were examined, including the invasive host *D. villosus* (n = 1,374) and the native species *Gammarus balcanicus* (n = 5,507) and *G. fossarum* (n = 123). Parasite identification used an integrative taxonomic approach, combining detailed morphological evaluation with molecular methods.
The analysis revealed fundamental disparities in helminth community composition and species richness between the invasive and native hosts occupying ecologically distinct biotopes within the same geographical macro-region (the Middle Danube versus lentic systems and mountain streams). We identified 19 helminth taxa in total. The native *G. balcanicus* harbored 14 taxa (*Maritrema neomi*, *Microphallidae* gen. sp., *Cephalotrema elasticum*, *Triodontolepis torrentis*, *T. skrjabini*, *Coronacanthus integrus*, *C. longicirrosus*, *C. vassilevi*, *Vaucherilepis trichophorus*, *Polymorphus* cf. *minutus*, *P. cincly*, *Pomphorhynchus tereticollis*, Nematoda gen. sp., and Nematomorpha gen. sp. larvae). *G. fossarum* hosted 8 taxa (*M. neomi*, *Microphallidae* gen. sp., *T. torrentis*, *Triodontolepis hamanni*, *C. integrus*, *P.* cf. *minutus*, *P. tereticollis*, and Nematoda gen. sp.). Conversely, the invasive *D. villosus* exhibited a significantly reduced parasite species richness, hosting only 5 taxa (*Maritrema dikerogammari*, *Plagioporus skrjabini*, *Microsomacanthus* sp., *Pomphorhynchus bosniacus*, and Nematomorpha gen. sp. larvae). Notably, while Cestoda dominated overall taxonomic diversity with 8 identified species, they were rare faunal elements with low prevalence. In contrast, Trematoda overwhelmingly dominated in terms of abundance and intensity of infection across both native and invasive host populations.
The significantly lower helminth diversity in *D. villosus* compared to native amphipods in the studied region supports the enemy release hypothesis. The invader appears to have left behind most of its native

parasite fauna, and has not yet acquired local parasite species in the recipient area. At the same time, the invasive host retained a distinct subset of co-introduced taxa (*M. dikerogammari*, *P. skrjabini*, and *P. bosniacus*). Their introduction and presence are intrinsically linked to the simultaneous range expansion of the broader Ponto-Caspian complex, particularly the intermediate and definitive hosts (hydrobiid snails and gobiid fishes) required to sustain their life cycles. Furthermore, this co-invasion pattern reflects the host's active self-dispersal through the Danube's fluvial corridor.
This study was supported by grants APVV-24-0140 and SAS SoE/2025/61/MSCA4Uk, and by the International Visegrád Scholarship No. 52610360.

O35

More than meets the eye: cryptic diversity of Strigeidae trematodes revealed in swan populations

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Trematodes of the genus *Cotylurus* (family Strigeidae) form a relatively small yet highly specialized group of parasites that inhabit the gastrointestinal tract and the bursa of Fabricius in aquatic and wading birds. For many decades, the prevailing belief in the parasitological literature and among researchers was that, in swans, we are dealing almost exclusively with a single, widespread trematode species specific to these avian hosts. This assumption, although historically deeply rooted, has generated numerous taxonomic problems over the years.
Classical identification of *Cotylurus* representatives based solely on morphological features is immensely difficult. This stems primarily from the lack of clear, unambiguous diagnostic traits in adult specimens, coupled with their exceptionally high phenotypic plasticity. This morphological variability, a direct response to diverse environmental factors, such as parasite crowding or the host's specific immune response, significantly affects the absolute body dimensions of the trematodes. Over the years, this plasticity has led to frequent misinterpretations and the establishment of multiple taxa of questionable validity.
To resolve this long-standing issue and clarify the true structure of the parasitic fauna, we undertook comprehensive research using an integrative taxonomy approach. In our analyses, we used an extensive collection of material obtained directly from naturally infected swans. Classical methods, including morphometric studies, were supplemented with analyses of allometric indices, which exhibit significantly greater evolutionary stability. However, the application of advanced molecular tools, combining analysis of the conserved nuclear 28S rDNA marker and the highly variable mitochondrial COI gene fragment, proved to be the key factor.
The results completely overturned the existing dogma. Our research unequivocally demonstrated that the trematodes traditionally lumped into a single species category constitute a complex of cryptic taxa. Phylogenetic analysis, strongly supported by morphometric data, enabled the confident delimitation of up to four distinct evolutionary lineages within European swan populations. Each lineage is characterized by a unique genetic signature and represents a fully biologically independent species.
Our studies conclusively demonstrate that the true species diversity of Strigeidae trematodes parasitizing seemingly well-studied definitive hosts, such as swans, has been drastically underestimated and remains far from fully understood. The case of the genus *Cotylurus* serves as an excellent example that, in modern parasitology, the application of integrative taxonomy is essential for the correct delimitation of taxa and the discovery of cryptic diversity in the natural environment.

O36 ✻ Student Competition

New insights into the diversity of *Australapatemon* and *Apatemon* species in Central Europe (Digenea, Strigeidae) - an integrative approach

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Australapatemon and *Apatemon* are closely related genera of digenean trematodes within the family Strigeidae parasitizing waterfowl. Originally described as a single genus, they were later separated by Sudarikov (1959) based on differences in life-cycle patterns and the structure of cercarial protonephridial systems. The genus *Apatemon* was defined by cercariae possessing ten flame cells and metacercariae encysted in fish, meanwhile *Australapatemon* was established for species with cercariae with fourteen flame cells and metacercariae encysted in leeches. However, due to the high morphological similarity of adults, the taxonomic status of these genera has remained controversial, frequently leading to species misidentifications. In Europe, the most representative species include *Apatemon gracilis* parasitizing fish-eating waterfowl, as well as *Australapatemon minor* in ducks and *Au. fuhrmanni* in swans. Because morphological differences between species of both genera are subtle and often overlap, identification based solely on morphology is difficult and misleading. Recent molecular analyses have revealed distinct lineages and significant hidden species diversity, highlighting the genetic variability and richness of the genus across different regions.
The aim of this study was to investigate the diversity of *Australapatemon* and *Apatemon* species in Central Europe using an integrative taxonomic approach. Phylogenetic analyses were performed on extensive material comprising adult and larval stages collected from birds and invertebrate hosts in Poland. Additional material from Switzerland was included through collaboration with the Natural History Museum of Geneva. Analyses were based on mitochondrial COI and nuclear 28S rDNA markers and included both phylogenetic reconstruction and haplotype network analyses.
Our results, based on more than 90 COI sequences (~820 bp), provide new insights into the phylogeny and biology of these taxa, including the description of the life cycle of *Australapatemon fuhrmanni* and clarification of the taxonomic status of *Apatemon fuligulae* (= *Australapatemon fuligulae*). Furthermore, species delimitation analyses together with estimates of intra- and interspecific genetic diversity revealed at least three previously unrecognized lineages within *Apatemon* and at least seven lineages within the *Australapatemon* clade. The results emphasize that the actual species diversity of Strigeidae is still far from known, and further comprehensive studies using an integrative approach are required.

O37

Exo-erythrocytic development and tissue tropism of *Haemoproteus* parasites in Eurasian blackcaps

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Avian haemosporidians are widespread apicomplexan parasites of birds and close relatives of the malaria-causing genus *Plasmodium*. Their life cycle includes an obligate exo-erythrocytic phase developing within

host tissues, yet these tissue stages remain poorly characterized, particularly under natural conditions involving mixed infections.
We examined tissue development of *Haemoproteus* parasites in 62 Eurasian blackcaps (*Sylvia atricapilla*) using an integrative methodological framework combining histopathology, chromogenic in situ hybridization (CISH) with genus- and lineage-specific probes, lineage-specific PCR assays, and laser capture microdissection followed by sequencing.
Exo-erythrocytic stages were identified in 20 birds and showed clear organ-specific distribution patterns. The kidneys and heart represented the most frequently infected organs, while skeletal muscle and gizzard were affected less commonly. Meronts detected in kidneys and cardiac tissue were linked to the *Haemoproteus parabelopolskyi* complex and were frequently associated with inflammatory responses. In contrast, *H. pallidulus* exhibited marked tropism for skeletal muscle, where numerous meronts developed within individual muscle fibres. Megalomeronts assigned to *H. majoris* were detected in the gizzard wall. Mixed infections predominated in infected birds, with 17 of 20 individuals carrying multiple cytb lineages. Notably, co-infecting lineages frequently occupied different organs within the same host, suggesting lineage-specific tissue specialization and within-host niche segregation.
This study provides the first lineage-specific characterization of exo-erythrocytic development in *S. atricapilla*. Furthermore, it documents tissue stages of *H. pallidulus* and *H. parabelopolskyi* for the first time and highlights tissue tropism as an important lineage-dependent trait in mixed haemosporidian infections.
The research presented at the conference is supported by the COST Action CA22108 – Wildlife Malaria Network (WIMANET), as well as by Grant No. P-PD-23-029 from the Research Council of Lithuania (LMTLT), the Scientific Grant Agency of the Ministry of Education and the Slovak Academy of Sciences (VEGA Grant No. 2/0148/24), and the Austrian Science Fund (FWF; Grant-DOI 10.55776/P37076).

O38

Hidden in the honeycomb: Microsporidian and trypanosomatid parasites in bees identified by metabarcoding approach

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Bees (*Apidae*) are essential pollinator taxa that support both natural ecosystems and agricultural production, yet they are exposed to a wide range of parasites that can influence their health and population dynamics. Abundance of floral resources affects the density and diversity of pollinating communities and engages a network of interspecific interactions that can facilitate transmission from one host taxa to another. While parasites of honeybees and bumble bees have been relatively well studied, those infecting wild bee species remain poorly explored. This project examines the diversity, prevalence and evolutionary relationships of microsporidian and trypanosomatid parasites across honeybees, bumble bees and wild bees using a metabarcoding approach. Moreover, inference of shared and host-specific parasite taxa between hosts are explored. More than 1500 bee tissue samples from 90 hymenopteran species (including 944 samples of *Apis mellifera*) and 155 honeybee product samples were analyzed. DNA was extracted, followed by parasite-group-specific PCR amplification and high-throughput sequencing using the Oxford Nanopore MinION platform. The metabarcoded data were used to assess parasite diversity across hosts and environmental sources. Microsporidian parasites showed high diversity across bee hosts. In *Apis mellifera*, the most prevalent species was *Nosema ceranae*, detected in approximately 40% of samples. Other taxa, including *Nosema bombi* and *Nosema invadens*, were rare, whereas *Nosema apis* was absent. *N. ceranae* DNA was also found in bee products (honey). Across all bees, 47 microsporidian species or phylogenetic lineages were identified, greatly expanding known diversity of this parasite group. Wild bees represent a major reservoir, with many lineages found exclusively in solitary species. Trypanosomatid parasites were moderately prevalent, with ten species detected, including *Lotmaria passim*, *Crithidia mellificae* and *Crithidia bombi*. Overall sample positivity in bee tissues was about 26%, with *L. passim*

reaching 67% sample prevalence in honeybees. These parasites were not detected in bee products. Overall, our results reveal a much broader diversity of bee-associated microsporidian parasites than previously recognized and highlight wild bees as significant hosts of parasite diversity. Future research will clarify host-parasite co-evolution and impacts of emerging parasites on pollinator health.
This study was supported by the Czech Sience Foundation (25-16047S, PI: P. Sojková. 2025-2027).

POSTER PRESENTATIONS

TU1 A. Čerevková, M. Jakubcsiková, M. Čižmár, M. Renčo — Tree Identity and Seasonal Dynamics as Drivers of Soil Nematode Functional Structure in Temperate Forests

TU2 G. Kanarek, J. Gabrysiak, J. Hildebrand, G. Zaleśny — Freshwater leeches (Annelida: Hirudinea) as intermediate hosts for avian digenean parasites: a neglected link in the transmission of helminths in the water environment

TU3 J. Kozica, M. Drážovská, K. Hudáková, L. Gogoľová, K. Franková, P. Petroušková, J. Lipinský, M. Prokeš, Ľ. Korytár, A. Pelegrinová, M. Kostičák, P. Tryjanowski, A. Ondrejková — Dynamics of *Borrelia* spp. occurrence in ticks during spring and autumn in different environment

TU4 R. Onufriienko, M. Oros, O. Lisitsyna, M. Špakulová, V. Sarabeev, — Long-term epizootic dynamics of *Pomphorhynchus tereticollis*: climatic lags, host buffering, and parasite-induced mortality in a warming lake

TU5 M. Renčo, E. Gömöryová, M. Čižmár, A. Čerevková — Nematode response to non-native black locust

TU6 A. Žákovská, H. Nejezchlebová, E. Bártová — Selective blood group-associated behavioural responses in *Ixodes ricinus* nymphs under controlled experimental conditions

TU7 I. Dmytriieva, R. Svitin, R. Horlach, O. Marushchak, T. A. Kuzmina — Helminth diversity in the common frog *Rana temporaria* in Ukrainian and Lithuanian localities

TU8 O. Greben, O. Lisitsyna, Ya. Syrota, T. Kuzmina — Morphological and molecular examination of *Gruitaenia latissima* (Cestoda; Dilepididae) from the Common Crane (*Grus grus*)

TU9 O. Marushchak, Y. Kuzmin, T. Brázová, E. Čisovská Bazsalovicsová, Ľ. Juhásová, R. Svitin, I. Dmytriieva, T. A. Kuzmina — Helminth infection of the European common toad (*Bufo bufo*) in Slovakia and Ukraine

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TU11 E. Řehulková, F. Nejat, A. Vetešníková Šimková — Hidden diversity of Ligictaluridus (Monopisthocotyla: Dactylogyridae) parasitizing North American ictalurid catfishes

TU12 L. Ihnacik, I. Papajová, J. Šmigová, I. Imrich, M. Oros — Endoparasite diversity in free-living animals in Slovakia

TU13 N. Pipová, B. Pavľáková, M. Balogová, I.Majláth, P. Mikulíček, V. Majláthová — Blood parasites of water frogs (*Pelophylax esculentus* complex)

TU14 L. de Klerk, I. Přikrylová, N.J. Smit — Three's company: Introducing a new species of *Udonella* as a new epibiont on *Caligus tetrodontis* off the coast of South Africa.

TU15 J. Radzijevskaja, E. Kaminskienė, M. Stanko, A. Paulauskas — Species diversity of mesostigmatid mites (Acari: Mesostigmata) collected from small rodents in suburban habitats in Lithuania

TU16 Y. Syrota, R. Svitin, V. Dupak, P. Komorová, Z. Hurníková, O. Greben, O. Lisitsyna, M. Miterpákova — Preliminary insights into the molecular phylogeny of *Microtetrameres* and *Tetrameres* (Nematoda: Tetrameridae), parasites of the avian proventriculus

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TU18 M. Frątczak*, P. Tryjanowski — Beyond endocrine disrupting-compounds: parasites as overlooked modulators of hormonal balance and development in amphibians

TU1

Tree Identity and Seasonal Dynamics as Drivers of Soil Nematode Functional Structure in Temperate Forests

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Tree species identity is a key driver of belowground ecological processes, yet its effects on soil nematode communities remain insufficiently understood, particularly under biological invasion. This study examines how two native broadleaf species, *Fagus sylvatica* and *Quercus robur*, and the invasive *Negundo aceroides* influence nematode community composition, trophic structure, and functional indices in temperate forests of Central Europe.

Soil samples were collected from rhizospheres in spring and autumn 2023. Nematodes were identified to genus level, and analyses included diversity metrics, trophic composition, coloniser–persister (c–p) structure, and nematode-based indices. A total of 51 genera were recorded. Bacterivores dominated overall, whereas plant-parasitic nematodes were more abundant in spring under *N. aceroides*. Fungivores were mainly associated with *F. sylvatica*, suggesting a stronger fungal decomposition pathway. Higher trophic groups, such as omnivores and predators, remained relatively stable across tree species. Functional indices indicated generally mature soil food webs, only moderately disturbed, with dominance of c–p 3–5 groups. Seasonal dynamics strongly modulated these patterns, with invasion effects most pronounced in spring and reduced differentiation among tree species in autumn. Soil pH emerged as a key environmental driver shaping nematode distribution and trophic structure.

Invasive tree species reorganise nematode trophic structure and diversity without substantially affecting functional stability, highlighting the resilience of soil food webs and the importance of temporal dynamics.

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TU2

Freshwater leeches (Annelida: Hirudinea) as intermediate hosts for avian digenean parasites: a neglected link in the transmission of helminths in the water environment

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Key words: leeches, intermediate host, metacercariae, Cyathocotylidae, Diplostomidae

Leeches (Hirudinea) constitute a widely distributed group of aquatic invertebrates. In freshwater ecosystems, they play an important ecological role as predators and prey; moreover, they serve as second intermediate hosts for several trematode taxa belonging to the families Diplostomidae Poirier, 1886 (genera *Australapatemon* Sudarikov, 1959 and *Cotylurus* Szidat, 1928) and Cyathocotylidae Mühling, 1898 (genus *Cyathocotyle* Mühling, 1898). Studies on the ecological role of leeches as intermediate hosts of avian digenean trematodes remain absent in the literature.

In the presented study, we describe the identity and diversity of digenean metacercariae detected in two species of freshwater leeches (*Erpobdella octoculata*, *Haemopis sanguisuga*), collected from small rivers and drainage canals in the Gdańsk Pomerania region (northern Poland). In 2023-2024, 103 leeches, including 94 specimens of *H. sanguisuga* and 9 of *E. octoculata,* were examined for the presence of digenean metacercariae. The sampling was divided into two periods: autumn and spring. While *H. sanguisuga* was present in both seasons, *E. octoculata* was recorded only in spring, suggesting differences in seasonal activity or microhabitat preferences.

In necropsied leeches, metacercariae of three genera were detected: *Australapatemon, Cotylurus* and *Cyathocotyle*. Data clearly revealed, that all three recorded trematode genera showed significantly higher

prevalence rates during the spring season, e.g., *Australapatemon* reached 100% prevalence in spring, suggesting that every individual in the population acts as a transmission node for this parasite. This seasonal surge likely corresponds with the spring migration and nesting of waterfowl (definitive hosts) and the increased shedding of cercariae by snails (first intermediate hosts) as water temperatures rise. Moreover, when comparing the two species in spring, distinct ecological patterns emerge (generalist vs. specialist): *H. sanguisuga* serves as a "collector" species, harboring a diverse and highly prevalent community of all studied trematode taxa. A significant finding is the complete absence of *Cotylurus* sp. metacercariae in *E. octoculata*, in sharp contrast to their high prevalence in *H. sanguisuga*. This suggests a strong host-selection mechanism or the existence of physical and/or physiological barriers, where *Cotylurus* metacercariae preferentially or exclusively infect the larger, semi-aquatic *H. sanguisuga*. while *Australapatemon* is the dominant parasite in *Haemopis*. *Erpobdella* shows a higher relative affinity for *Cyathocotyle*.
To sum up, the obtained results demonstrate that spring is the key period for transmission of leech-transmitted avian digeneans in the analyzed aquatic ecosystems. *H. sanguisuga* plays a central role as a major reservoir for multiple trematode lineages, particularly those cycling through waterfowl. In contrast, *E. octoculata* exhibits a more restricted parasite profile, highlighting that even within the same habitat, different leech species occupy distinct functional niches in the parasite's life cycle. Acquired data underscore the importance of leeches as bioindicators of ecosystem connectivity, linking the health and presence of snail populations to bird migratory patterns.

TU3

Dynamics of *Borrelia* spp. occurrence in ticks during spring and autumn in different environment

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Ticks are ectoparasites, which can transfer pathogens such as those of the genus *Borrelia*, posing a threat to the health and lives of humans and animals. Although ticks can act as vectors for Borrelia spp., the prevalence of the pathogen within tick populations may vary depending on the ticks' location and the season. The aim of our study was to compare tick populations collected at two different times of year in Košice, with those collected in the vicinity of Košice, in terms of the number of individual infected with *Borrelia* spp. And the location from which they were collected.
Ticks from vegetation were collected in spring and autumn 2025 at two localities in Košice region (Košice and Slanec) and three different areas – urban parks, tourist places and around the farms on each locality. Ticks were collected by flagging method and identified under a trinocular stereozoom microscope according to morphological key. Subsequently genomic DNA was isolated using InnuPREP DNA/RNA Tick Kit (IST Innuscreen GmbH, Berlin, Germany). Conventional PCR method was used to amplify 250bp fragment of *IgS* gene. PCR products were visualized on 1.0% agarose gel with a Gel Red Nucleic Acid Stain (Biotinum, Fremont, CA, USA).
In total, 288 ticks were collected, 195 in spring season and 93 in autumn season. The most prevalent tick species was *Ixodes ricinus* (n = 260). Other species were *Dermacentor reticulatus* (n = 25), *Dermacentor marginatus* (n = 2) and *Haemaphysalis concina* (n = 1).
Out of the total 288 collected ticks, only *I. ricinus* ticks were *Borrelia* spp. positive, with an overall prevalence 32.29%. The results of our study indicate that the population of ticks infected with *Borrelia* spp. is larger during the spring season. In spring, 31.53% of ticks collected in Košice and 42.85% of ticks collected in Slanec were tested positive, whereas in autumn the prevalence decreased to 22.22% and 25.64%, respectively. In Košice, the majority of *Borrelia* spp. positive ticks collected during spring originated from urban parks (14 infected ticks) and farms (13 infected ticks). In contrast, during the autumn season, most positive ticks were collected from farms, where 12 infected individuals were identified. In the vicinity of Košice, most *Borrelia* spp. positive ticks collected in spring were also found in urban parks,

with 19 infected ticks detected. During autumn, positive ticks were most frequently recorded in parks and tourist areas, with five infected ticks identified at each location.
Our study suggests that the seasonal variation influences the prevalence of ticks infected with *Borrelia* spp. in the studied population. In addition, a higher proportion of infected ticks was observed in suburban and peri-urban areas compared to urban environments.
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TU4

Long-term epizootic dynamics of *Pomphorhynchus tereticollis*: climatic lags, host buffering, and parasite-induced mortality in a warming lake

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Small, closed lake ecosystems provide an ideal model system for observing the population dynamics of parasites, enabling a profound understanding of fundamental ecological processes and host-parasite interaction theory necessary for predicting ecosystem responses to global climatic change. This study investigates the microecological, interannual, and climatic drivers of acanthocephalan abundance (*Pomphorhynchus tereticollis*) in its two consecutive hosts, intermediate (*Gammarus balcanicus*) and definitive (*Phoxinus phoxinus*), in Lake Male Morské Oko (Slovakia).
We used modern parasitological surveys (2022–2026) with a unique historical dataset collected by M. Špakulová (1999–2001). The comprehensive combined dataset includes infracommunity abundance data sampled from 2,502 *G. balcanicus* and 159 *P. phoxinus*. To disentangle the complex transmission dynamics, we applied Generalized Additive Mixed Models (GAMMs) and Generalized Linear Mixed Models (GLMMs via *glmmTMB*) fitted with negative binomial error distributions. An information-theoretic approach (AIC) was utilized to test multiple hypotheses regarding host traits, temporal phenology, and climatic triggers.
Microecological modeling revealed fundamentally contrasting accumulation strategies. In *G. balcanicus*, parasite abundance plateaued in larger individuals (>10 mm), a classic signature of parasite-related mortality that removes heavily infected hosts from the population. Conversely, *P. phoxinus* exhibited exponential parasite accumulation with body size, along with a significant sex bias: females were more heavily infected. Temporally, the analysis revealed highly stable seasonal phenology in *G. balcanicus*, with no intra-annual peaks, and weak seasonal variability in *P. phoxinus* during the warm months. However, inter-annual dynamics revealed a massive shift: historically high infection levels in 2001 were followed by a severe collapse in population abundance in the modern period (2022–2026). Climatic modeling, which compared current weather with past extremes, provided unambiguous evidence that current conditions do not drive population dynamics but are driven by the thermal extremes of the preceding year. Past summer thermal stress acts as the principal catalyst for transmission. Furthermore, we detected a significant non-linear synergy: current infection risk is maximized under a specific combination of high temperatures and low precipitation.
A unique and pivotal finding of our long-term analysis is the identification of a "Predator Accumulation Lag" (buffering effect). Following the near-zero collapse of parasite prevalence in the intermediate host in 2022, the definitive fish host unexpectedly maintained high infection burdens throughout 2023. *P. phoxinus* functioned as an ecological sink, buffering the parasite population against environmental shocks. However, a population surge of the noble crayfish (*Astacus astacus*) observed in 2024 introduced intense competition for the ecological niche, displacing the fish from their typical benthic habitats. This spatial segregation disrupted the food chain required for parasite transmission and, by negatively affecting the reproductive success of the fish, led to a decline in their population, ultimately causing the system to enter a deep depression by 2026. These results demonstrate that parasite outbreaks and collapses are complex

ecosystem responses driven by historical thermal stress and critically modulated by trophic disruptions and host bioaccumulation capacity

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TU5

Nematode response to non-native black locust (*Robinia pseudoacacia* L.) at the regional scale

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Invasive tree species are known to modify aboveground vegetation and soil properties; however, their influence on soil biota remains less well documented. This study examined the effects of the invasive *Robinia pseudoacacia* L. on soil nematode communities in temperate forests of Europe (southern Slovakia), using native pedunculate oak (*Quercus robur* L.) stands as a reference. A paired-site design was applied across nine localities to compare nematode abundance, richness, diversity, trophic and functional structure, ecological indices, and metabolic footprints. A total of 107 nematode genera were identified across all sites. Overall, no significant differences were found between black locust and oak forests in total nematode abundance, diversity, biomass, or most ecological indices, indicating similar soil food web structure and stability. Bacterivorous nematodes dominated in both forest types, followed by plant parasites, omnivores, fungivores, and predators. A significantly higher plant parasitic index was observed in black locust stands, driven by increased abundance of obligate plant-parasitic nematodes. These findings suggest that, despite its invasive status, *R. pseudoacacia* does not cause major shifts in soil nematode community structure under the studied conditions.

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TU6

Selective blood group-associated behavioural responses in *Ixodes ricinus* nymphs under controlled experimental conditions

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Ixodes ricinus is a primary vector for numerous zoonotic pathogens across Europe. While sensory mechanisms for host detection are documented, the impact of host blood group variability on tick behaviour is not yet fully understood. This study aimed to evaluate potential color preferences during field sampling and to investigate selective behavioral responses of *I. ricinus* nymphs to stimuli associated with human ABO blood groups under controlled conditions.

Field experiments included flagging with white, red, and green flags to assess color preference. Laboratory assays involved 640 active nymphs exposed to human ABO blood group-associated stimuli in a standardized petri dish quadrant test. Field results showed no significant differences in nymph distribution among colored flags ($p=0.73$), suggesting visual signals do not influence sampling efficiency. Conversely, laboratory tests revealed a highly significant association between behaviour and blood groups ($\chi2=219.49$, $df=3$, $p<0.0001$). Groups O (39.8 %) and A (38.0 %) were most preferred, while group B was the least attractive (4.7 %). These findings suggest that blood group–associated chemical stimuli may influence host selection behaviour in *I. ricinus*.

Our results provide new insights into tick sensory ecology and highlight a previously underexplored factor that may influence vector–host interactions and differential exposure risk. Further research is required to elucidate the chemical mechanisms underlying these behavioural responses and their ecological relevance under natural conditions.
This study was supported by the Masaryk University internal grant (MUNI/A/1631/2025), and the University of Veterinary Sciences Brno internal grant (2025ITA23).

TU7

Helminth diversity in the common frog *Rana temporaria* in Ukrainian and Lithuanian localities

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The composition and structure of amphibian helminth fauna depend on the host biology and behavior, including habitat preferences and the connection with intermediate hosts. Our study aimed to characterise and compare the helminth fauna in the common frogs (*Rana temporaria*) collected in Ukraine (UA) and Lithuania (LT). Helminth occurrence and abundance were analysed by calculating the prevalence, intensity, and mean abundance of infection.
In total, 124 frogs (102 in UA, 22 in LT) were collected. In the entire host sample, at least 15 helminth species from 4 taxonomic groups were found: monogeneans (1 species), trematodes (8), acanthocephalans (1), and nematodes (5). 13 species were recorded in *R. temporaria* in UA and 9 in LT, with almost half of the total helminth fauna (7 species) shared between the two countries (the Sorensen similarity index = 0.64, the Jaccard similarity index = 0.47).
Despite the greater number of trematode species, nematodes represented the most widespread and abundant group of parasites of *R. temporaria*. The intestinal nematode *Oswaldocruzia bialata* was found in most localities, and reached the highest infection prevalence (P, %) and mean abundance (MA) in frogs in both UA (P=72.55%; MA=7.34) and LT (P=77.27%; MA=5.09). The occurrence of the intestinal nematode *Cosmocerca ornata* was similarly high in the samples: it infected more than half of the examined hosts in UA (P=60.78%) and in LT (P=77.27%). Despite its slightly lower prevalence (P=43.14% in UA; P=59.09% in LT), the lung nematode *Rhabdias bufonis* reached a maximum infection intensity of 30 specimens in a single frog in the LT sample, whereas the intestinal nematode *Oxysomatium brevicaudatum* (P=41.18% in UA; P=54.55% in LT) showed the highest abundance, with 802 specimens in total and 168 worms in a single host in UA. The dominance of monoxenous nematodes in *R. temporaria* is attributed to its terrestrial lifestyle.
Among the other parasites, the urinary-bladder-dwelling monogenean *Polystoma integerrimum* (P=28.43% in UA; P=54.55% in LT) and the intestinal trematode *Pleurogenes claviger* (P=11.76% in UA; P=13.64% in LT) were shared between the two samples. Other trematode species infected only a few hosts and were recorded exclusively in frogs from UA (*Diplodiscus subclavatus*, *Opisthioglyphe ranae*, *Pleurogenoides medians*, *Gorgodera cygnoides*) or from LT (*Gorgoderina vitelliloba*, *Haplometra cylindracea*).
Most helminth species (13) found in this study reach maturity in amphibian hosts, whereas the nematodes of the family Acuariidae (P=0.98% in UA) and the trematodes of the family Strigeidae (P=8.82% in UA; P=9.09% in LT) use *R. temporaria* as an intermediate host. Thus, the common frog may contribute to the transmission of parasites that infect birds in their adult stages. Infection with the acanthocephalan *Acanthocephalus ranae* is associated with hibernation and reproduction of common frogs in aquatic habitats, where frogs may become infected by ingesting intermediate hosts. In our material, the presence of *A. ranae* exclusively in hosts from the UA sample (P=19.61%) is most likely due to the smaller frog sample size in LT.
The study was supported by the National Research Foundation of Ukraine (project 2023.03/0068).

TU8

Morphological and molecular examination of *Gruitaenia latissima* (Cestoda; Dilepididae) from the Common Crane (*Grus grus*)

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The helminth fauna of the Common Crane (*Grus grus*) includes 22 species, of which only one cestode, *Gruitaenia latissima* Spasskii, Borgarenko et Spasskaya, 1971, is known. This cestode species was described from cranes in Tajikistan (Central Asia) based on specimens without scolices (Spassky and al., 1971). The unique morphology of the strobilae allowed the erection of a new cestode species and a new monotypic genus, *Gruitaenia*, within the family Dilepididae. Later, *G. latissima* was recorded only in Germany in the same host; the morphology of the scolex was not examined. In this study, we examined 50 common crane individuals that died in winter and during spring migration in January–March 2021 in the Kherson region, South of Ukraine. Seven birds were found to be infected with *G. latissima*. The prevalence was 14.0% (95% CI: 6.7–26.8%); infection intensity: 2.3 (1–5), median: 1; mean abundance 0.32 (95% CI: 0.12–0.72). This is the first record of *G. latissima* in Ukraine; also, the morphology of the scolex was studied for the first time. The very small and delicate scolex is with four unarmed suckers, well developed rostellum armed with a double-row crown of 38–42 hooks, each measuring 18–25 µm. The study of strobila morphology has established that it corresponds to the morphology of the cestode from Tajikistan. Examination of the strobila also showed that its length, the position of the testes, and the lateral margins of the ovary lobes vary. The morphology of *G. latissima* was compared with the description of *Gruitaenia gruis*, the second species of the genus, described from *Grus canadensis* in the USA (Rausch and Rausch, 1985). It has been established that the only difference between these two species is the shape and position of the cirrus-sac: in *G. latissima,* the cirrus-sac is rounded and never reaches the poral osmoregulatory canals, while in *G. gruis,* the cirrus-sac is elongated, often reaching the poral osmoregulatory canals. However, this criterion is not sufficient for species differentiation. The remaining characteristics, including the armament of the scolex, are similar for both *G. latissima* and *G. gruis*. However, we refrain from synonymizing these two geographically distant species until we have had the opportunity to carefully examine the original material from cranes from North America. The Bayesian analysis of the mitochondrial NAD1 gene fragment from our *G. latissima* specimen confirmed the place of the species within the order Cyclophyllidea. Specifically, the Bayesian inference nested our material within the family Dilepididae, a result perfectly consistent with morphological predictions. In the future, obtaining the DNA sequences for *G. gruis* from North American cranes will be instrumental in resolving the taxonomic status and potential synonymy of these two species.

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TU9

Helminth infection of the European common toad (*Bufo bufo*) in Slovakia and Ukraine

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The study is aimed to update the information about helminth species infecting the European common toad (*Bufo bufo* L.) in Eastern Europe. Toads were collected in Košická Belá, Slovakia (n=35), and in five localities across Ukraine: in Lviv (n=10), Khmel'nytskyi (n=17), Kyiv (n=14), and Kharkiv (n=20) regions, and in the city of Kyiv (n=18). In a combined sample of 114 toads, 13 helminth species were

recorded, including 9 species of nematodes, 3 species of trematodes and 1 species of acanthocephalans. From 5 to 8 helminth species were recorded in separate host samples, and from 1 to 5 (mean 3.3, median 3) species were found in each examined host.
The nematode *Oswaldocruzia filiformis* (Goeze, 1782), which parasitises the small intestine, was found in toads from all localities; it infected 93.0% of the examined hosts, with infection prevalence (P) ranging from 50.0% to 100% across localities. Two species of lung-dwelling nematodes of the genus *Rhabdias* Stiles et Hassall, 1905 were identified; *R. sphaerocephala* Goodey, 1924 was found in all host samples and infected 93.0% of examined hosts (P=83.3–100% in separate localities); *R. bufonis* (Schrank, 1788) was found in 5 of 6 samples, in 44.7% of hosts, in total (P=5.0–88.8% in separate localities). Co-infection with two *Rhabdias* species was observed in 40.3% of toads. Three species of cosmocercid nematodes were found in the large intestines of toads. Males of *Cosmocerca ornata* (Dujardin, 1845) and *Neyraplectana schneideri* (Travassos, 1931) were found in 5 of 6 localities, infecting 14.9% and 23.7% of toads, respectively. Females of the two species were morphologically indistinguishable and were observed in all samples, infecting 65.8% of hosts. Overall, 68.4% of toads were found infected with either or both of the two species (P=7.1–90.0% in separate localities). *Oxysomatium brevicaudatum* (Zeder, 1800) was found in 4 localities, in 17.5% of all examined toads (P=11.8–64.3% in separate localities). Unidentified larvae of three nematode species of the order Spirurida were found each in the stomach wall of one toad in 3 separate localities. Adult trematodes *Opisthioglyphe ranae* (Froelich, 1791) and *Pleurogenes claviger* (Rudolphi, 1819) were found in the small intestine of toads in 1 and 2 localities, respectively, infecting one host individual at each locality. An unidentified trematode metacercaria was found in the forelimb muscles of one toad. One larva of acanthocephalan from the family Centrorhynchidae was found encysted on the mesentery of one toad.
We conclude that monoxenous nematodes predominated among the helminths of *B. bufo* in the studied regions of Eastern Europe and in helminth communities from all studied localities; of them, *O. filiformis* and *R. sphaerocephala* had the highest infection prevalence. This predominance is characteristic of amphibian hosts with a mostly terrestrial mode of life.
The study was supported by the National Research Foundation of Ukraine project No. 2023.03/0068 and by the Scientific Grant Agency of the Ministry of Education, Research, Development and Youth of the Slovak Republic, no. VEGA 2/0052/24.

TU10

Blood Parasites in *Emys orbicularis* from Lublin Voivodeship, Eastern Poland

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The European pond turtle (*Emys orbicularis*) is classified as an endangered animal species, facing the threat of extinction in some areas. However, in certain parts of Poland, there are numerous populations of *E. orbicularis*, for example in the Leczna-Wlodawa region and in the Polesie National Park in the eastern part of Poland. The aim of this study was to detect and characterize intracellular blood parasites of the phylum Apicomplexa in European pond turtles (*E. orbicularis)* captured from three natural areas in the Lublin Voivodeship in eastern Poland. Turtle capture took place in June 2024, during the egg-laying period. Examination of 20 adult females using microscopy revealed a prevalence of 70%, and using molecular methods, 85%. Microscopic analysis identified two developmental stages: gamonts and premerонts belonging to the genus *Haemogregarina*. Sequencing of isolates from the samples confirmed the presence of *Haemogregarina stepanowi*, *Haemogregarina* sp., and *Hepatozoon* sp. The average parasitemia was 29.7 parasites per 10,000 erythrocytes, with a maximum parasitemia of 94.89 and a minimum of 13.05. Phylogenetic analyses supported a close genetic relationship of the isolates from this study with haemogregarines found in other species of freshwater turtles.
This study was supported by the Grant Agency VEGA project No. 1/0762/25 and APVV project no. APVV-23-0320.

TU11

Hidden diversity of *Ligictaluridus* (Monopisthocotyla: Dactylogyridae) parasitizing North American ictalurid catfishes

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The catfishes of Ictaluridae represent a diverse lineage of freshwater fishes endemic to North America. Several ictalurid species have been introduced outside their native range together with their associated parasite fauna. Monopisthocotylans associated with ictalurid catfishes belong predominantly to *Ligictaluridus* Beverley-Burton, 1984 and *Gyrodactylus* von Nordmann, 1832. Species of *Ligictaluridus* represent the most species-rich group parasitizing ictalurids; however, their diversity, host associations and phylogenetic relationships remain insufficiently resolved.

A survey focused on the diversity of monopisthocotylans parasitizing North American ictalurid fishes was initiated in 2018. Between 2018 and 2023, seven species and one unidentified species of Ictaluridae were collected from multiple localities in the United States (Arkansas, Illinois, Michigan, Mississippi, Texas and Wisconsin), Canada (Québec) and northern Mexico (Chihuahua and Durango). Recovered monopisthocotylans were examined using an integrative approach combining morphological and molecular analyses, with species identification based primarily on the morphology of sclerotised structures. To date, seven nominal species of *Ligictaluridus* have been reported from 14 species of North American ictalurid catfishes. However, previous descriptions and illustrations of the frequently reported species *L. pricei* and *L. mirabilis* reveal substantial variability in the morphology of sclerotised structures, including both haptoral and reproductive hard parts, suggesting that their current taxonomic delimitations may encompass overlooked diversity. Preliminary analyses of the newly collected material support this hypothesis and indicate a considerably higher diversity of *Ligictaluridus* than previously recognised. At least 11 putative species or morphologically distinct taxa were detected, including *L. bychowskyi*, *L. michaelalicea*, *L. mirabilis* and *L. pricei*, together with several potentially undescribed species. Distinct morphological lineages were identified within forms corresponding to *L. pricei* and *L. mirabilis*, differing primarily in the morphology of haptoral attachment structures and the male copulatory organ, respectively. Ongoing phylogenetic analyses aim to clarify species boundaries and assess the evolutionary position of *Ligictaluridus* among siluriform-associated dactylogyrids.

Overall, the present results suggest that the diversity of *Ligictaluridus* associated with North American ictalurid catfishes has been considerably underestimated, particularly within forms currently assigned to *L. pricei* and *L. mirabilis*.

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TU12

Endoparasite diversity in free-living animals in Slovakia

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Wild animal faeces often contain developmental stages of various endoparasites, many of which can persist in the environment for extended periods, and some have zoonotic potential. In areas where wildlife, livestock, and human activities overlap, faecal contamination may facilitate cross-transmission of parasites and increase exposure risk for animals and humans. However, limited knowledge of wildlife's parasitofauna in such environments may lead to underrecognition of epidemiologically important and zoonotic species. This can pose a risk not only to other animals but also to humans who may come into contact with contaminated environments or infected wildlife. In this study, we analysed the occurrence of endoparasites in wildlife across three localities in Slovakia. Gápel in western Slovakia, along with Abrahámovce and Ladmovce in eastern Slovakia. A total of 219 faecal samples were collected and examined using standard concentration coprological methods for the presence of helminth eggs and

protozoan cysts. Overall, 47% of samples were positive for at least one parasite species. Prevalence varied markedly among localities, ranging from 8% in Ladmovce to 64% in Abrahámovce, while Gápel showed positivity rates of 36%. Statistical analysis confirmed this significant difference in parasite prevalence. Abrahámovce showed a substantially higher likelihood of positivity and a higher proportion of positive samples (OR = 4.31; 95% CI: 2.44–7.60), whereas Ladmovce and Gápel exhibited a significantly lower probability compared to the other localities (OR = 0.08; 95% CI: 0.01–0.36 and OR = 0.47; 95% CI: 0.27–0.83, respectively). Most samples originated from ruminants (n = 105; *Cervus elaphus* = 57, *Dama dama* = 40, *Capreolus capreolus* = 8) and wild boar (*Sus scrofa;* n = 96). The parasite spectrum varied across the study sites. In Gápel, *Trichostrongylus axei* was the most frequent finding (35.7%), while in Abrahámovce, the dominant parasites were *Metastrongylus elongatus* (29.8%) and *Oesophagostomum* spp. (18.3%). Several species occurred exclusively in a single locality, suggesting the influence of local environmental or host-specific factors (e.g., *Trichostrongylus axei* or *Monienzia* spp., found only in Gápel in ruminant faeces samples). In total, more than 25 parasite taxa were identified, indicating considerable parasite diversity despite generally low prevalence of individual taxa. These findings provide an overview of parasites present in free-living animals in selected localities in western and eastern Slovakia. This underscores the importance of long-term monitoring to better understand parasite transmission dynamics and to evaluate potential risks for wildlife, livestock, and human health. The study also establishes a foundation for future ecological modelling and spatial analyses of parasite distribution.
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TU13

Blood parasites of water frogs (*Pelophylax esculentus* complex)

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Water frogs of the genus *Pelophylax* serve as hosts for a wide range of parasites, ranging from unicellular organisms to helminths. The most commonly reported blood parasites in frogs include hemogregarines belonging to the phylum Apicomplexa, nematodes, and several species of the genus *Trypanosoma*. In this study, we focused on blood parasites of water frogs inhabiting the Danube Delta in Romania.
A total of 74 individuals of *P. ridibundus* and 8 individuals of *P. esculentus* from six localities were examined. Blood parasites were detected microscopically and by molecular analysis using the 18S rDNA marker. Overall, 89.77% of frogs from all studied localities were infected with at least one group of parasites, specifically hemogregarines (84.09%), nematodes (1.14%), and trypanosomes (63.64%).
Parasitemia of hemogregarines and trypanosomes differed significantly among the studied localities. No statistically significant differences in parasitemia were found between male and female hosts. However, adult individuals infected with hemogregarines exhibited significantly higher parasitemia compared to subadults, and a significant positive correlation between parasitemia and frog body length was detected (r = 0.226).
Molecular analyses confirmed the presence of the genus *Hepatozoon*. Comparison of partial 18S rDNA sequences with corresponding sequences available in GenBank revealed a similarity of 98.1–99.8% with *Hepatozoon magna*. Trypanosome sequences showed the highest similarity to *Trypanosoma* sp. isolate R10 clone L2–3 identified in *P. ridibundus* frogs from Ukraine, as well as to *Trypanosoma ranarum* and *Trypanosoma cobitis*.
This research was supported by the VEGA grant no. 1/0762/25.

TU14

Three's company: Introducing a new species of *Udonella* as a new epibiont on *Caligus tetrodontis* off the coast of South Africa.

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Udonellids are monopisthocotylan flatworms that occupy a unique epibiotic niche, attaching to parasitic copepods while feeding on the fish host's epithelial surface. Despite their broad distribution, species diversity within the genus *Udonella* Johnston, 1835 remains underexplored, particularly in African waters, where new species discoveries are essential for refining its phylogeny. This study presents the integrative description of a new species of *Udonella* collected from *Caligus tetrodontis* Barnard, 1948, parasitising the evileye blaasop, *Amblyrhynchote honckenii* (Bloch), off the coast of South Africa. Twelve specimens were recovered from three copepods infecting a single fish. Morphological analyses revealed distinct diagnostic features, including body proportions, haptor size, gonad arrangement, and caudal gland configuration, separating the new species from all known congeners. Molecular characterisation based on partial 18S and 28S rDNA sequences supported species delimitation, with the new species from South Africa most closely related to *U. australis* Carvajal & Sepulveda, 2002. Although 18S rDNA divergence was low, this is consistent with the marker's highly conserved nature. This study also provides the first molecular characterisation of a *Udonella* species from the southwestern Indian Ocean and highlights the importance of integrative taxonomy for resolving species boundaries in morphologically conservative parasites.
This study was supported by the National Research Foundation (NRF) of South Africa (grant 132805; L. van der Spuy; grant CPRR240430216732; N.J. Smit).

TU15

Species diversity of mesostigmatid mites (Acari: Mesostigmata) collected from small rodents in suburban habitats in Lithuania

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Mesostigmatid mites (Acari: Mesostigmata) represent a diverse group widely distributed across different environments and are of ecological interest due to their potential role as vectors or reservoirs of microorganisms causing diseases in humans and animals. Small rodents serve as important hosts for mesostigmatid mites and contribute to the maintenance and distribution of these parasite communities. However, the species diversity and distribution of mesostigmatid mites associated with small rodents in suburban habitats remain poorly documented. The aim of this study was to investigate the species diversity and infestation patterns of mesostigmatid mites in small rodents inhabiting suburban areas of Lithuania. A total of 916 Mesostigmata mites were collected from small rodents captured at twenty suburban sites in Lithuania during 2017-2020. Mites were recorded from 934 small rodents representing eight different species. Based on morphological characteristics, fourteen species of mesostigmatid mites, belonging to eight genera (*Androlaelaps*, *Cyrtolaelaps*, *Eulaelaps*, *Haemogamassus*, *Hirstionyssus*, *Hyperlaelaps*, *Laelaps*, and *Myonyssus*), were identified. Abundance and mean intensity of mite infestation varied among rodent species and study sites. Mesostigmatid mites were detected on 24.7% of the examined rodents, with *A. flavicollis* harboring the highest mite abundance. *L. agilis* was the most abundant mite species. *Laelaps hilaris* and *Haemogamassus nidi* were also common, whereas all remaining mesostigmatid species together accounted for less than 12% of collected mites. This study reports the first record of *Cyrtolaelaps mucronatus* in Lithuania. The findings contribute to a better understanding of Mesostigmata mite diversity

in suburban habitats and provide a foundation for future research on host–parasite interactions and pathogen-related processes.

TU16

Preliminary insights into the molecular phylogeny of *Microtetrameres* and *Tetrameres* (Nematoda: Tetrameridae), parasites of the avian proventriculus

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Tetrameridae is a taxonomically diverse family of spirurid nematodes primarily associated with avian hosts. Representatives of two of its most species-rich genera, *Microtetrameres* and *Tetrameres*, parasitise the proventriculus, where adult females are embedded in the proventricular glands. Current taxonomy of these genera relies heavily on male morphology; although females of the two genera are strikingly different, they lack sufficient diagnostic features for species-level identification. Although these nematodes are cosmopolitan and widely recorded worldwide, their molecular diversity and phylogenetic relationships remain poorly studied. This is largely due to the scarcity of suitable material for molecular studies, as many known hosts are protected species and parasite samples are often available only opportunistically. In the present study, we obtained partial sequences of the mitochondrial cytochrome c oxidase subunit (*cox1*) gene and the nuclear 18S rRNA gene from morphologically identified tetramerid nematodes. Newly generated sequences were obtained for *Microtetrameres cloacitectus* from birds of prey, *Microtetrameres helix* from the rook, *Microtetrameres* sp. from the Eurasian sparrowhawk, *Tetrameres fissispina* from the mallard, and *Tetrameres paradisea* from the common crane. For most studied species, specimens from multiple spatio-temporal points were included to provide a preliminary estimate of intraspecific genetic variability. The sequences were compared with the few available GenBank records, and combined datasets of public and newly generated sequences were analysed using Bayesian phylogenetic inference. The analysis of the *cox1* dataset separated *Microtetrameres* and *Tetrameres* into distinct, well-supported, and close-related lineages and supported a clear distinction among species. The analysis of the 18S rRNA dataset also confirmed the generic assignment of specimens and distinct differences among species, but failed to cluster all included tetramerids into a single clade. Thus, while used fragments are useful for molecular identification of tetramerid nematodes, they are insufficient on their own for resolving deeper phylogenetic relationships within the family. To our knowledge, the study is the first to analyse both *Microtetrameres* and *Tetrameres* together, providing an initial test of the hypothesis of their close relationship, as expected from their morphology and ecological peculiarities. Further advances in understanding the phylogeny of Tetrameridae will require DNA extraction from morphologically verified vouchers, especially diagnostically informative males, across most species of these genera, together with the development of primer sets suitable for amplifying longer mitochondrial and nuclear fragments. Given the diversity of Tetrameridae and the challenge of obtaining fresh material, a complete phylogeny of this group is unlikely to emerge from a single study. Instead, progress will rely on the steady accumulation of morphologically and molecularly verified specimens collected as opportunities arise, along with their inclusion in growing molecular datasets.

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TU17

Multiple origins, secondary radiation, and morphological innovation of Nearctic *Gyrodactylus* (Platyhelminthes: Monogenea) revealed by global phylogenetic reconstruction

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The evolutionary history and biogeographic origin of Nearctic *Gyrodactylus* remain poorly understood despite the remarkable diversity of viviparous monogeneans infecting North American freshwater fishes. Here, we investigated the phylogenetic position, historical origins, host associations, and evolution of selected haptoral characters in Nearctic *Gyrodactylus* within a global framework. Between 2015 and 2024, gyrodactylid specimens were collected from freshwater fishes in North America and Europe. Partial ITS1–ITS2 sequences were generated and combined with GenBank data, resulting in a dataset of 193 sequences representing 189 *Gyrodactylus* species and four *Swingleus* species, including 69 Nearctic taxa and 36 newly generated sequences from undescribed species. Phylogenetic relationships were inferred using Bayesian Inference (BI) and Maximum Likelihood (ML), and ancestral state reconstructions of geographic distribution, host associations, and haptoral morphology were performed in Mesquite. Both BI and ML analyses recovered highly congruent topologies and showed that Nearctic *Gyrodactylus* represents a paraphyletic assemblage. Reconstruction of geographic origins revealed multiple independent evolutionary origins of Nearctic lineages rather than a single endemic radiation. Nearctic taxa occurred in basal, intermediate, and derived positions throughout the phylogeny. Basal and intermediate Nearctic lineages were interspersed with Western and Eastern Palearctic taxa, suggesting historical biogeographic connections between North American and Eurasian parasite faunas, whereas the terminal portion of the tree contained a well-supported monophyletic Nearctic radiation interpreted as a secondary diversification event within North America.

Ancestral host reconstruction identified Cypriniformes as the principal ancestral host group for *Gyrodactylus*, whereas parasites infecting Cyprinodontiformes formed a more derived and phylogenetically structured assemblage. Other host groups appeared sporadically throughout the phylogeny, indicating repeated host-switching events and possible fish translocations rather than extensive host-associated radiations. Mapping of haptoral characters revealed multiple independent gains and losses across the phylogeny. The plate-like ventral bar membrane, ventral bar median knob, and terminal hook filament were restricted mainly to derived Nearctic and Neotropical lineages, suggesting that these diagnostic haptoral features evolved secondarily within derived American taxa rather than representing ancestral states. Overall, the study highlights a complex evolutionary history shaped by repeated dispersal, historical interchange with Palearctic faunas, host switching, regional diversification, and convergent morphological evolution.

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TU18

Beyond endocrine disrupting-compounds: parasites as overlooked modulators of hormonal balance and development in amphibians

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Amphibians are increasingly exposed to multiple environmental stressors capable of interfering with endocrine regulation, development and reproduction. Endocrine-disrupting compounds (EDCs) such as widespread in the environment plastic residues, pharmaceuticals and pesticides are recognized as important

drivers of altered hormonal pathways, somatic development and sex differentiation in amphibians. However, less attention has been paid to the potential interaction between EDCs exposure and parasitic infestations. This review highlights the possibility that parasites may represent an overlooked but biologically relevant co-factor influencing endocrine homeostasis and development in amphibians. Recent studies indicate that environmental contamination may indirectly increase susceptibility of amphibians to parasites and infectious diseases through endocrine and immunological stress pathways. Simultaneously, some parasites themselves appear capable of directly interfering with endocrine-related processes. Trematode infections, particularly by *Ribeiroia ondatrae*, may cause somatic abnormalities, resembling endocrine disruption phenomena through disturbances of retinoic signaling pathways. Moreover, gonad-invading parasites *Myxobolus* spp. have been reported in the amphibian testes and ovaries, in causing extensive destruction of gonadal tissue. Other parasites may affect sex steroid levels indirectly through systemic physiological stress, immune activation or altered reproductive investment. Evidence from fish studies further suggests that parasitic infections can reduce gonadotropin expression, suppress sex steroid production and impair gonadal development, potentially leading to reproductive dysfunction at the population level. At the same time, controlled laboratory studies on amphibians frequently focus on single stressors, whereas natural populations are simultaneously exposed to chemical pollutants, parasites, pathogens and changing environmental conditions. Such multi-stressor interactions may complicate interpretation of biomarkers commonly attributed solely to EDCs exposure. We propose that future studies on amphibian endocrine disruption should incorporate parasitological assessment as a component of experimental and field-based investigations. Integrating parasitology, ecotoxicology and reproductive biology may improve interpretation of endocrine biomarkers and provide a more realistic understanding of the mechanisms underlying amphibian developmental abnormalities and population declines in the Anthropocene.

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Vectors and Vector-Borne Diseases (Labuda Days)

ORAL PRESENTATIONS

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O40 **A. Šujanová, E. Gatial, Z. Balogh, M. Derdáková, D. Selyemová, S. Purgatová, V. Rusňáková Tarageľová** — InfoTick: a citizen-science web platform for monitoring tick occurrence in Slovakia

O41 ✻ **S. Purgatová, B. Mangová, D. Selyemová, Z. Krumpálová, M. Chvostáč, Y. M. Didyk, P. Rajská, M. Kazimírová, V. Rusňáková Tarageľová** — Sympatric occurrence of five exophilic tick species in the Levice region (southwestern Slovakia) and their infection with tick-borne pathogens

O42 **M. Kazimírová, M. Ličková, Yu. M. Didyk, B. Mangová, M. Chvostáč, V. Rusňáková Tarageľová, D. Selyemová, S. Fumačová Havlíková, V. Vaňová, P. Rajská, S. Purgatová, O. Zhovnerchuk** — Monitoring tick-borne encephalitis foci in Slovakia based on tick collections and rodent trapping

O43 ✻ **B. Annerová, B. Mangová, M. Chvostáč, O. Zhovnerchuk, Y. M. Didyk** — Hedgehogs as reservoirs of tick-borne pathogens in urban areas in western Slovakia

O44 ✻ **M. Poľanská , M. Chvostáč, M. Kazimírová, B. Mangová , S. Purgatová, Y. Didyk , P. Rajská , V. Rusňáková Tarageľová** — Diversity and Host-Associated Patterns of Ectoparasites in Wild Ungulates from the Malé Karpaty Mountains (Slovakia)

O45 ✻ **M. Ruivo , Y. Didyk , S. Purgatová , B. Mangová , M. Kazimírová , Ľ. Korytár , A. Ondrejková , M. Prokeš , J. Lipinský ,M.Wijnveld** — Improving the surveillance of zoonotic pathogens associated with bats in Central Europe

O46 ✻ **H. Comic, M. Ruivo, M. Wijnveld** — Serotonin as a Tool for Artificial Feeding of *Dermacentor marginatus* Ticks based on Silicon Membranes

O47 **M. Baňas, L. Šofranková, L. Šimo, J. Kurimský, R. Cimbala, V. Majláthová, N. Pipová, I. Majláth** — Tick Behavior: Electromagnetic Radiation and Pathogens

O48 **A. Paulauskas, D. Šakienė, A. Aleksandravičienė, L. Griciuvienė, V. Mažeika, R. Vaitkevičiūtė, J. Radzijevskaja** — Helminths and vector-borne pathogens in wild mustelids from the Baltic countries

O39 ◆ Invited Speaker

Borrelia and ticks, adaptive strategies

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Among the most prevalent tick-borne pathogens in the northern hemisphere are spirochaetes of the *Borrelia burgdorferi* sensu lato (s.l.) complex, often reaching a prevalence of 25-30% in questing *Ixodes ricinus* s.l. ticks. While several aspects of survival and adaptability are known for *B. burgdorferi* sensu stricto (s.s.) B31 strain, much remains unknown for other (European) species.
The genome of *B. burgdorferi* s.l. consists of a linear chromosome and several circular and linear plasmids, with around 1400 predicted genes. Many of these genes still have unknown functions and show no homology with other organisms.
To explore gene functions, especially tick-pathogen interactions, manipulating the genetics of these spirochaetes is essential. Although the *B. burgdorferi* s.s. B31 strain has frequently been successfully modified, many other borrelial species have not been efficiently transformed. This led us to study the transformation efficiency in *B. afzelii* and *B. garinii*, particularly their restriction-modification system (RMS). This RMS provides protection against invading (foreign) DNA elements, such as those originating from bacteriophages. In *B. burgdorferi* s.l., the RMS comprises a methyltransferase and an endonuclease. The bacteria methylate their own DNA, and foreign DNA, which lacks this methylation pattern, is degraded by the endonuclease.
The study aimed to improve the transformation efficiency in early-passage patient isolates. To this end, we premethylated the plasmids using recombinant borrelial RMS genes and assessed the effect on the transformation efficiency. Following premethylation, we observed increased transformation efficiency for several *B. burgdorferi* s.l. species. The most significant increase was observed in *B. garinii*, where no transformation was observed with unmethylated plasmids, whereas 21% of spirochetes were transformed with premethylated plasmids.
The next step in the study of borrelial adaptability is to infect ticks with fluorescent spirochetes by artificial feeding through silicone membranes.
This research is funded by the Austrian Science Fund (FWF) Grant DOI: 10.55776/P33867 and Grant DOI 10.55776/PAT8439224.

O40

InfoTick: a citizen-science web platform for monitoring tick occurrence in Slovakia

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Tick-borne diseases are among the most important vector-borne infections affecting humans and animals in Europe. Their incidence has increased in recent decades, highlighting the need for effective surveillance systems capable of capturing fine-scale spatial and temporal patterns of tick occurrence. Traditional monitoring approaches are often geographically limited and resource demanding, creating opportunities for citizen-science-based initiatives.
We present InfoTick, a Slovak citizen-science web platform designed for public reporting and visualization of tick occurrence data. The platform enables users to submit georeferenced tick observations together with photographs and contextual information, including habitat type, host association, and date of observation. Submitted records are displayed on an interactive online map, allowing real-time visualization of tick activity across the country.

Beyond data collection, the platform serves as an educational and awareness-raising tool by providing accessible information about tick biology, tick-borne pathogens, prevention measures, and species identification. The project actively engages the public in participatory surveillance while simultaneously increasing awareness of tick-related health risks.
Since its launch, the platform has accumulated hundreds of reports from different regions of Slovakia, demonstrating the strong potential of citizen-generated data for complementing conventional surveillance approaches. User-submitted photographs additionally provide valuable material for monitoring tick species distribution, including records of less commonly reported species.
Future development will focus on expert validation of submitted records, integration of AI-assisted species identification, development of dynamic risk visualization tools, and expansion of public engagement activities.
The research presented at the conference is financially supported by the Slovak Research and Development Agency under contract No. APVV-22-0372.

O41 ✻ Student Competition

Sympatric occurrence of five exophilic tick species in the Levice region (southwestern Slovakia) and their infection with tick-borne pathogens

S. Purgatová[1], B. Mangová[1], D. Selyemová[1], Z. Krumpálová[2], M. Chvostáč[1], Y. M. Didyk[3], P. Rajská[1], M. Kazimírová[1], V. Rusňáková Tarageľová[1]

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Of the 24 tick species identified in Slovakia, *Ixodes ricinus* is the most widespread and abundant. In certain areas, multiple epidemiologically significant tick species (2–5) occur together, creating important hotspots for investigations of tick-borne pathogen circulation. The co-existence of five exophilic tick species – *I. ricinus*, *Dermacentor reticulatus*, *D. marginatus*, *Haemaphysalis concinna*, and *H. inermis* – was confirmed in the vicinity of Žemberovce village (Levice region of south-western Slovakia). The study in this locality focused on the seasonal activity and abundance of questing ticks, together with the extent of tick infestation in sheep and wild game animals.
Questing ticks, spleens of game, and ticks removed from game and sheep were examined for the presence of tick-borne pathogens (*Babesia* spp., *Theileria* spp., *Anaplasma phagocytophilum*, *Rickettsia* spp., *Borrelia burgdorferi* s.l., and *Borrelia miyamotoi*) by molecular methods. Among the questing tick species, *I. ricinus* was identified as the predominant. The analyses confirmed the occurrence of several pathogens (*Babesia crassa*, *B. microti*, *Rickettsia helvetica*, *R. raoultii*, *A. phagocytophilum*, *Borrelia afzelii*, *B. garinii*, *B. valaisiana*, *B. burgdorferi* sensu stricto and *B. miyamotoi*).
Dermacentor marginatus, *I. ricinus* and *H. concinna* were collected from sheep. *A. phagocytophilum*, *A. ovis*, *R. slovaca*, and *R. raoultii* were determined in these ticks. *Anaplasma phagocytophilum* was identified in all examined red deer and roe deer and in 55.6% of wild boar. All cervids were positive for *Theileria* spp. The presence of all five monitored tick species on wild game animals was confirmed, with *I. ricinus* representing the dominant species. In these ticks, *A. phagocytophilum*, *R. helvetica*, *R. raoultii*, *Babesia* sp. hc-hlj212, *B. crassa*, *B. microti*, *Babesia* spp. and *B. miyamotoi* were detected. This research confirmed the presence of *B. crassa* in Slovakia for the first time. Due to the simultaneous presence of five exophilic tick species and the broad diversity of detected tick-borne pathogens, the studied locality can be considered an epidemiologically significant hotspot posing a potential risk of infection for both humans and domestic animals.
This study was supported by the Slovak Research and Development Agency (project APVV 22-0372) and the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic, as well as the Slovak Academy of Science (projects 2/0083/25 and 2/0092/26).

O42

Monitoring tick-borne encephalitis foci in Slovakia based on tick collections and rodent trapping

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In Slovakia, a significant proportion of tick-borne encephalitis (TBE) cases is caused by alimentary transmission. Knowledge of the structure of TBE foci where domestic ruminants acquire the infection is still limited. In a current project, TBE foci in natural forests known from the past are revisited (Topolčianky and Fúgelka, western Slovakia), and areas close to farms where alimentary infections with TBE virus were registered (districts Detva and Banská Bystrica, central Slovakia, and Považská Bystrica, western Slovakia) are monitored. Sera of 150 sheep from farms in Detva and P. Bystrica districts were screened for TBEV antibodies using ELISA, with over 50% prevalence in Detva. Sera of live-trapped rodents are screened for antibodies against TBEV by microneutralization assay and questing and rodent-attached ticks are analysed for the presence of the virus by PCR-based molecular methods. From October 2024 to May 2026, 327 rodents were live-trapped. *Apodemus flavicollis* prevailed in all sites except for Víglaš (district Detva), where *Apodemus agrarius* dominated. *Microtus arvalis, Clethrionomys glareolus* and *Micromys minutus* were also present. The structure of the focus in Topolčianky has changed from the 2010s – the proportion of *C. glareolus* decreased, while *A. agrarius* expanded there. Rodents were infested mainly by tick larvae, co-feeding of larvae and nymphs was rare. Among the over 5000 questing ticks, *Ixodes ricinus* prevailed in all sites except for Víglaš, where *Dermacentor reticulatus* was dominant. *Dermacentor reticulatus* was found also in Topolčianky, Visolaje and Sverepec (district P. Bystrica), Fuggerov Dvor (district B. Bystrica), while *Dermacentor marginatus* was found in Visolaje, Sverepec, Prečín (district P. Bystrica), and in Fuggerov Dvor and Víglaš. *Haemaphysalis concinna* occurred in low numbers in all sites except for Kostolec. Presence of *Haemaphysalis inermis* was confirmed in Topolčianky and Víglaš. Molecular screening of part of questing ticks from Topolčianky, Fuggerov Dvor, Fúgelka and Víglaš did not confirm TBEV presence. Seropositivíty of rodents has been confirmed in Topolčianky (one *A. flavicollis*) and Víglaš (one *C. glareolus*). Preliminary results suggest that the long-term TBEV focus at Topolčianky may still be active.

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O43 ✻ Student Competition

Hedgehogs as reservoirs of tick-borne pathogens in urban areas in western Slovakia

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Hedgehogs are common synanthropic mammals frequently occuring in urban and peri-urban environments, where they often come into contact with humans and domestic animals. Due to their active foraging behaviour, they serve as suitable hosts for ticks and may contribute to the circulation of tick-borne pathogens in urban ecosystems.

The aim of this study was to evaluate the role of hedgehogs as reservoirs of tick-borne pathogens in urban environments in western Slovakia.

In 2022–2026, a total of 1828 ticks were collected from 169 hedgehogs in Bratislava (Dúbravka and Devínska Nová Ves), Stupava, Malacky and Nitra. Hedgehogs were captured, examined for the presence

of ticks, and released back into their habitat. Collected ticks were morphologically identified to species, developmental stage and sex. Genomic DNA was isolated from tick samples and analyzed for the presence of tick-borne pathogens using molecular methods.
The most frequently found tick species were *Ixodes hexagonus* (55 %) and *Ixodes ricinus* (45 %). Molecular analyses confirmed the presence of the following pathogens: *Borrelia burgdorferi* s.l. complex, *Borrelia miyamotoi*, *Anaplasma phagocytophilum*, *Rickettsia* spp., and *Babesia* spp. The most prevalent pathogens were *Anaplasma phagocytophilum* (45,6 %) and *Borrelia burgdorferi* s.l. complex (26,4 %), with *Borrelia afzelii* and *Borrelia spielmanii* among the most frequently detected species.
Our results show that hedgehogs may contribute to the circulation and maintenance of medically and veterinary important tick-borne pathogens in urban areas in western Slovakia.
This study was supported by VEGA 2/0092/26, 2/0083/25 and APVV-22-0372.

O44 ✻ Student Competition

Diversity and Host-Associated Patterns of Ectoparasites in Wild Ungulates from the Malé Karpaty Mountains (Slovakia)

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From October 2023 to February 2025, a total of 157 skins of ungulate game animals (84 fallow deer, 42 wild boar, 26 roe deer, 5 mouflon), hunted in the Malé Karpaty Mountains, were obtained from a hunting association. A total of 7,496 ticks belonging to three species were identified: *Ixodes ricinus* (73.15%), *Haemaphysalis concinna* (26.60%) and *Dermacentor reticulatus* (0.25%). Tick infestation was recorded in 91.67% of fallow deer, with a mean intensity of 60.55 ± 98.56 ind/host and a maximum of 609 ind/host. Wild boars were infested in 60.98% of cases, with a mean intensity of 5.78 ± 7.49 ind/host (max. 25 ind/host). In roe deer, 77.78% of individuals were infested (30.19 ± 60.90 ind/host; max. 263), while all mouflons were infested (148.40 ± 135.01 ind/host; max. 335). Tick activity peaked in spring and autumn but persisted during winter. A total of 3,011 deer keds were identified: *Lipoptena fortisetosa* (83.53%), *Lipoptena cervi* (16.14%), and *Hippobosca equina* (0.33%). Infestation occurred in 88.10% of fallow deer (20.67 ± 36.16 ind/host; max. 243) and 96.15% of roe deer (49.00 ± 77.42 ind/host; max. 350). A single specimen of *L. fortisetosa* was found on mouflon. A total of 1,127 lice (*Haematopinus apri*) were recorded, parasitizing 69.05% of wild boar (26.76 ± 58.27 ind/host; max. 320). Three individuals were also found on fallow deer. Additionally, 214 chewing lice (*Bovicola bovicola longicornis*) were identified, infesting 5.95% of fallow deer (0.39 ± 2.87 ind/host; max. 26), 19.23% of roe deer (6.38 ± 20.42 ind/host; max. 84), and 20% of mouflon (3.00 ± 6.71 ind/host; max. 15). Seventy larvae of botflies (*Hypoderma* sp.) were detected in subcutaneous tissues. Parasitism was observed in 3.57% of fallow deer (0.17 ± 1.32 ind/host; max. 12) and 19.23% of roe deer (2.15 ± 6.40 ind/host; max. 29). A single specimen of the flea *Pulex irritans* was recorded on wild boar. A statistically significant difference was detected among parasite communities of host species ($F = 20.72$, $p < 0.05$). These findings indicate that wild ruminants play a key role in maintaining populations of ticks and deer keds in the environment. Although wild boar exhibited lower tick infestation levels, they contribute to tick persistence and are essential hosts for the survival and transmission of *Haematopinus apri*.
Key words: wild ungulates, ectoparasites, ticks, deer keds, Slovakia
Acknowledgments: This research was funded by APVV 22-0372, VEGA 2/0083/25 and 2/0092/26.

O45 ✻ Student Competition

Improving the surveillance of zoonotic pathogens associated with bats in Central Europe

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Bats (Chiroptera) are an important part of the Central European wildlife, with over 25 species recorded across Slovakia. They are commonly found in caves in regions such as the Slovak Karst, as well as in forests, old buildings, and attics. Species such as the greater mouse-eared bat (*Myotis myotis*) are among the most widespread. These nocturnal mammals play a key role in controlling insect populations, making them beneficial for agriculture and ecosystems. In contrast, bats are important reservoirs of various zoonotic pathogens and parasites. A wide variety of ectoparasites associated with bats includes bat flies, bat bugs, fleas, ticks, and mites.

This study was a collaboration between Slovak and Austrian institutes and aimed to investigate zoonotic diseases by studying bats and their ectoparasites in Central Europe. In total, blood samples of 69 bats, samples of five different organs from 25 bats, 45 bat flies, 155 bat mites, and 1 soft tick (*Carios verpertilionis*) were analysed during this project. The bats were trapped using nylon monofilament mist-nets at several localities within the municipal cadastre of Drienovec village in the Slovak karst. Subsequently, DNA was isolated from all samples, followed by PCR- reverse line blot analysis to identify the presence of pathogens.

Of the 69 bats, 18 were collected in 2021, of which 2 species were identified: *Myotis myotis* and *Pipistrellus pipistrellus*. Of these, 83 % (15/18) were positive for at least one pathogen. The most prevalent pathogens were *Rickettsia* spp. and *Babesia* spp. This last was identified in 7 bats, and subsequent bidirectional sequencing revealed and confirmed the presence of *Babesia vesperuginis* in Slovakia for the first time.

The remaining 51 blood samples were collected between 2023 and 2024. A wider variety of bat species was collected, with 7 different species being identified. Of these, 59% (30/51) were positive for at least one pathogen. Several samples were found to be coinfected with different pathogens. The most prevalent pathogens detected in these blood samples were *Rickettsia* spp., *Candidatus* Neoehrlichia mikurensis, *Borrelia burgdorferi* sensu stricto, and *Babesia* spp., with *B. vesperuginis* being again detected. Regarding the organ samples, it was observed that 84% (21/25) of the bats had at least one organ infected with at least one pathogen. DNA of several pathogens was detected, such as *Rickettsia* spp, *Babesia venatorum, B. burgdorferi* sensu stricto, and *Candidatus* N. mikurensis. From the organs tested, the most affected were the kidneys.

A wide variety of pathogens were detected in all ectoparasites tested at high prevalences. It was observed that 64% (25/39) of the bat flies and 94% (68/72) of the bat mite pools were positive for at least one pathogen. In all three cases, the most prevalent one was *Rickettsia* sp.

Overall, pathogens were found in all types of samples, including the bat blood, organs, and ectoparasites. This indicates that bats can potentially be reservoir hosts for the pathogens tested in this project. This way, this project demonstrates the importance of surveillance of not only the vectors but also the hosts, since bats are still very understudied.

This study was supported by the project of Action Austria-Slovakia SAIA 2023-10-15-001.

O46 ✻ Student Competition

Serotonin as a Tool for Artificial Feeding of *Dermacentor marginatus* Ticks based on Silicon Membranes

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Artificial tick feeding systems are a valuable tool for studying tick physiology and pathogen transmission while reducing the need for vertebrate hosts, supporting the 3Rs in animal research. However, this technique requires optimisation to meet the specific needs of different tick species and life stages. *Dermacentor marginatus*, an expanding vector species in Europe, has proven challenging to maintain under *in vitro* conditions. Previous studies have shown symbiont-regulated serotonin biosynthesis can modulate tick feeding activity, suggesting that serotonin may be a promising tool for the optimisation of artificial feeding of *D. marginatus* ticks.
In this study, we used field-collected *D. marginatus* adults to initiate the optimisation of artificial feeding conditions and identify whether the addition of serotonin to the feeding substrate significantly enhances the feeding success.
We tested silicone membranes with increased thickness (approximately 120 µm) compared to previous trials. To provide mechanical stimuli, horsehair was added to the feeding units. Serotonin was added to the feeding substrate in half of the units, while the remaining units served as controls. As field-collected ticks were used, both the ticks and the blood they fed upon were screened for the presence of pathogens, enabling assessments of vector capacity.
Using thicker membranes enabled continuous feeding without leakage. We determined that, unlike *Ixodes ricinus*, *D. marginatus* requires mechanical stimuli to initiate attachment. The addition of horsehair to the feeding unit supplied this mechanical cue and revealed this species-specific requirement. In three artificial feeding trials, only one showed a significant difference between units with and without serotonin supplementation. The trials were conducted in late fall, winter, and spring, respectively.
Overall, this work demonstrates that for successful *in vitro* feeding of *D. marginatus,* thicker membranes are required as well as mechanical stimuli which led us to the first successful artificial feeding of *D. marginatus* in our lab. The observed variability between trials suggests potential season-specific feeding behaviour, highlighting the need for further research.
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O47

Tick Behavior: Electromagnetic Radiation and Pathogens

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Tick behavior is a critical factor in the development of effective strategies for tick control and the prevention of tick-borne diseases. Artificial electromagnetic radiation is a new environmental factor that affects tick development and behavior, with significant differences observed in various parameters. Aim of our study was to monitor the effect of electromagnetic radiation on the behavior of four tick species: *Ixodes ricinus, Dermacentor reticulatus, Dermacentor marginatus* and *Haemaphysalis inermis*. In total 1,200 ticks, 300 of each species, were tested in modules allowing the choice of an exposed or shielded area. During the test, the ticks were exposed to electro-magnetic radiation of 900 MHz for 24 h. The position of the individuals was recorded and we evaluated the obtained data statistically. We observed a significant preference to the exposed area in both sexes of *I. ricinus*. Males of *D. reticulatus* and *D. marginatus* also showed an affinity to radiation, but not females of both species, nor females and males of *H. inermis*. The results of the study support the assumption that ticks perceive the electromagnetic field and the observed differences in their response have the potential to help understand the mechanism of

perception. Molecular analyses (PCR and DNA sequencing) identified prevalent tick-borne pathogens. In *D. reticulatus*, we detected four species of *Rickettsia*. The occurrence of species not typically detected in *D. reticulatus*, namely *R. aeschlimanii* and R. *monacensis*, is noteworthy. The outcomes of statistical analyses examining the correlation between the presence of a particular genus of pathogens and the tick's preference under radiation in the RST test demonstrated significance solely in the context of *Rickettsia* infection among female *D. reticulatus* ticks. Infected ticks were more frequently present in the shaded section of the apparatus. In conclusion, while our study indicates a potential link between *Rickettsia* infection and tick behavior under electromagnetic radiation, further investigations are required to confirm and understand this phenomenon.

This study was supported by the Grant Agency VEGA project No. 1/0762/25 and APVV project no. APVV-23-0320 and APVV-17-0372.

O48

Helminths and vector-borne pathogens in wild mustelids from the Baltic countries

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Wild mustelids are widespread mesocarnivores in the Baltic region and may contribute to the maintenance of helminths and vector-borne pathogens in natural ecosystems. However, data on helminths and pathogen occurrence in these hosts remain limited, particularly in Latvia and Lithuania. The aim of this study was to assess the prevalence and diversity of helminths and vector-borne pathogens in wild mustelids from the Baltic countries. Tissue samples from pine martens (*Martes martes*), stone martens (*Martes foina*), American mink (*Neovison vison*), European polecats (*Mustela putorius*), Eurasian otter (*Lutra lutra*) and European badgers (*Meles meles*) were collected in Lithuania and Latvia between 2013 and 2025. Samples were screened by real-time PCR for *Anaplasma/Ehrlichia* spp., *Babesia* spp., *Bartonella* spp., *Borrelia* spp., and *Rickettsia* spp. Selected positive samples were further analysed using conventional, nested, and semi-nested PCR targeting the 17 kDa, *gltA*, ITS, *groEL*, *rpoB*, 16S rRNA, and *msp4* gene fragments, followed by sequencing. The screening revealed the presence of diverse vector-borne microorganisms in wild mustelids, including *Babesia*, *Anaplasma/Ehrlichia*, *Rickettsia*, *Bartonella*, *Borrelia*, and *Mycoplasma* species. The detected pathogens varied depending on host species and tissue type. Sequence analysis confirmed the presence of *Anaplasma phagocytophilum* in European polecats, *Ehrlichia muris* in American mink, and *Rickettsia helvetica* and *Bartonella* sp. in pine martens. European badgers tested positive for *Babesia* sp. type A, *A. phagocytophilum*, and *Mycoplasma* spp.

Helminthological necropsies were performed and found helminths, were identified using identification keys. Molecular identification of *Uncinaria* sp., was also performed using the mitochondrial cytochrome c oxidase subunit I (COX1) gene method. A total of 11 helminth taxons were detected: in otter – 1, in pine marten – 4, in badger – 4 and in polecat – 7. Based on infection parameters the most frequently found helminth species was *Aoncotheca putorii*. Molecular results showed that the studied *Uncinaria* sp., is *Uncinaria criniformis.*

The obtained results demonstrate that wild carnivores inhabiting the Baltic region may contribute to the maintenance and circulation of helminths and several vector-borne pathogens of veterinary and zoonotic importance.

Keywords

Mustelidae, helminths, vector-borne pathogens, Baltic region

POSTER PRESENTATIONS

W1 P. Bartíková, I. Štibrániová, D. Palenčár, J. Džubara, K. Donátová, M. Mladá, L. Kudlová, — Optimisation of human skin explant culture conditions for the study of tick-borne encephalitis virus–skin interactions.

W2 Paweł Błaszkiewicz, Aleksandra Błaszkiewicz, Weronika Maślanko, Marek Asman, Katarzyna Bartosik — Increase in *Dermacentor reticulatus* (Acari: Ixodidae) abundance over a five-year period in an area unaffected by anthropogenic pressure in Eastern Poland

W3 E. Bártová, A. Žákovská, A. Pechová — Detection of selected pathogens in *Ixodes ricinus* ticks removed from children attending summer camps

W4 E. Bártová, H. Pavelková, L. Plecháčková, J. Kejnovská, K. Račka — Occurence of *Rickettsia* spp. in *Ixodes ricinus* ticks in zoological gardens in the Czech Republic and Slovakia

W5 E. Fencáková, B. Peťko, I. Slepáková, I. Majláth, V. Majláthová — Ecology of vector-borne pathogens in Eastern Slovakia

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W1

Optimisation of human skin explant culture conditions for the study of tick-borne encephalitis virus–skin interactions.

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Tick-borne encephalitis virus (TBEV) is a medically important flavivirus transmitted to humans through the bite of infected ticks. Following transmission, the skin represents the primary site of viral establishment and an essential environment for the early stages of viral replication and host immune responses. These initial interactions, which have been studied very little to date, are likely critical for the virus's subsequent spread within the body and thus for the further progression of the viral infection. Human skin explants constitute a physiologically relevant *ex vivo* model that preserves the native architecture and cellular heterogeneity of the skin, enabling the investigation of virus–host interactions under controlled laboratory conditions. The aim of this study was to optimise culture conditions for full-thickness human skin explants intended for subsequent TBEV infection experiments. Skin explants were obtained from a fully informed (written informed consent) patient undergoing abdominal plastic surgery at the Department of Plastic Surgery. After removing the hypodermal (adipose) layer, skin samples were cut into small pieces and cultured for up to 10 days under multiple conditions that varied in (i) culture medium, (ii) incubation temperature, and (iii) submerged versus air–liquid interface culture. Throughout the ten-day culture period, explants were regularly evaluated for preservation of tissue morphology and overall physiological integrity. Histological and macroscopic examinations demonstrated that the skin explants maintained comparable structural integrity across all tested conditions. No significant differences were observed in the architecture of the epidermis or dermis, suggesting that all evaluated culture protocols maintained tissue viability for at least 10 days. These results establish reliable and flexible culture conditions for human skin explants and support their use as a relevant *ex vivo* platform for investigating the early stages of TBEV infection, including viral replication, cellular tropism, and innate immune responses in human skin.
This study was supported by the Grant Agency VEGA project No. 2/0136/25.

W2

Increase in *Dermacentor reticulatus* (Acari: Ixodidae) abundance over a five-year period in an area unaffected by anthropogenic pressure in Eastern Poland

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In recent years, the distribution range of *Dermacentor reticulatus* has expanded throughout Europe, now encompassing meadow and forest ecosystems, recreational suburban areas, and the outskirts of large cities. While this tick species primarily feeds on wild and domestic animals, reports of human infestations have also emerged. The increasing geographical range of this arthropod vector is epidemiologically significant, as *D. reticulatus* is a confirmed vector of tick-borne pathogens (TBPs), including *Babesia* spp., *Rickettsia* spp*., Anaplasma marginale, Coxiella burnetii*, and Tick-borne encephalitis virus.
The study aimed to compare the abundance of *D. reticulatus* in two biologically diverse habitats within an area with low anthropogenic impact, over a 5-year period. Field research was carried out in 2013 and 2018 in habitats located within Parczew Forest District. Ticks were collected from the vegetation during their

spring (April-May) and autumn (September-November) activity peaks using the flagging method. During tick sampling, temperature and relative humidity were measured in each habitat. Ticks collected from vegetation were identified in the laboratory to the species level, developmental form and sex.
As part of the study, 32-hour tick collections were performed in the studied habitats, 4 in each season at each sampling site. During the course of the study, a total of 1,186 adult *D. reticulatus* were collected from the vegetation at both study sites, including 381 individuals in 2013 and 805 in 2018. In both study years, the largest number of ticks were collected during the autumn activity period (273 and 541, respectively), while during spring collections were smaller (108 and 264, respectively). The highest values of saturation deficit recorded during tick collections were recorded during spring in both studied habitats (133.88 and 106.08, respectively). The study showed an over two-fold increase in the *D. reticulatus* abundance in both studied habitats within the 5-year period, and considered the environmental aspects of this phenomenon. High prevalence of TBPs detected in *Dermacentor reticulatus* in Lubelskie Province, according to literature data, correlated with notifications about its increased abundance, needs urgent interventions aimed at protecting human and animal health.

W3

Detection of selected pathogens in *Ixodes ricinus* ticks removed from children attending summer camps

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The aim of this study was to detect selected bacterial pathogens (*Anaplasma phagocytophilum, Borrelia burgdorferi* sensu lato, *Coxiella burnetii, Ehrlichia chaffeensis,* and *Francisella tularensis*) in ticks removed from children during summer camp activities in the Czech Republic.
A total of 101 attached ticks, predominantly *Ixodes ricinus*, were collected from children aged 7–13 years at two summer camps (Brno-Soběšice, South Moravian Region; n = 20, and Horní Lhotice, Vysočina Region; n = 81) during the summer of 2022. DNA was extracted using a commercial insect DNA isolation kit. Detection of *B. burgdorferi* s.l. was performed by conventional PCR using specific primers targeting the *hbb* gene. The remaining four pathogens were analyzed using multiplex PCR with species-specific primers. Amplified products were visualized by gel electrophoresis and identified based on expected fragment sizes. The overall pathogen positivity reached 44.6 % (45/101). *B. burgdorferi* s.l. was detected in 41.6 % (42/101) of ticks, representing the dominant pathogen. *Ehrlichia chaffeensis* was identified in 2.9 % (3/101) of samples. No mixed infections were observed. The remaining pathogens (*A. phagocytophilum, C. burnetii,* and *F. tularensis*) were not detected. A marked difference between localities was observed with only 5 % (1/20) of ticks to be positive (all *B. burgdorferi* s.l.) in Brno-Soběšice, whereas in Horní Lhotice 54 % (44/81) of ticks were positive, including *B. burgdorferi* s.l. (51 %) and *E. chaffeensis* (5 %).
The study revealed a relatively high prevalence of *B. burgdorferi* s.l. in ticks parasitizing children in recreational settings and the sporadic occurrence of *E. chaffeensis*. The results highlight significant spatial variability in pathogen occurrence and underline the importance of preventive measures against tick bites, particularly in children attending outdoor activities.
This study was supported by the Masaryk University internal grant (MUNI/A/1631/2025), and the University of Veterinary Sciences Brno internal grant (2025ITA23).

W4

Occurence of *Rickettsia* spp. in *Ixodes ricinus* ticks in zoological gardens in the Czech Republic and Slovakia

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Ticks are haematophagous ectoparasites and important vectors of pathogens, including *Rickettsia* spp., causative agents of rickettsial diseases. This study aimed to detect *Rickettsia* spp. in ticks from zoological gardens in the Czech Republic and Slovakia and to evaluate associated risk factors.
Ticks were collected by flagging in three Czech zoos (Ostrava, Jihlava, Brno) and one Slovak zoo (Košice) in 2023–2025. Sampling was performed monthly (March–November) at five sites per zoo. In total, 4,960 ticks were collected (Ostrava 2,095; Jihlava 1,589; Košice 720; Brno 556). All collected ticks were determined as *Ixodes ricinus*. Adults were analysed individually; larvae and nymphs were pooled (3 per sample), yielding 2,782 samples. DNA was extracted and analysed by PCR targeting the *gltA* gene. The overall minimum infection rate (MIR) was 7.2 % (358/4,960). Positivity was the highest in Košice (12.6 %, 91/720), followed by Brno (8.3 %, 46/556), Ostrava (8.2 %, 172/2095), and Jihlava (3.1 %, 49/1589), with significant differences among zoos ($p<0.001$). Interannual differences were observed in all zoos; in Jihlava and Košice, positivity also varied by season, developmental stage, and location. Tick activity peaked in spring in Brno and Jihlava (57 % and 50 %) and in summer in Ostrava and Košice (42 % and 55 %). Of 358 positive samples, 223 (62.3 %) were successfully sequenced. Most were *Rickettsia helvetica* (92.4 %), followed by *Rickettsia monacensis* (7.2 %); one sample matched to *Rickettsia conorii*/ *Rickettsia slovaca*.
This study provides a long-term overview of *Rickettsia* spp. in zoo environments and indicates that zoological gardens may act as important foci for their circulation, highlighting the need for continuous monitoring due to potential risks to animals and humans.
This study was supported by the Internal Grant Agency (IGA) of the University of Veterinary Sciences Brno (No. 212/2025/FVHE). We would like to thank the zoological gardens for their cooperation and for allowing us to collect ticks.

W5

Ecology of vector-borne pathogens in Eastern Slovakia

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Vector-borne pathogens are not the exclusive preserve of tropical regions, where the lay public most often associates them with arthropods such as mosquitoes and tsetse flies. In Europe, their occurrence is often associated with ticks, which pose a threat at any time of the year. Slovakia is not an exception in the rule. Thanks to increasingly intensive use of molecular techniques for nucleic acid isolation from pathogens located in blood or tissue, a number of previously unknown or sparsely studied genera and species have been identified. This study draws focus on vector-borne pathogens on the region of eastern Slovakia.
A total of 117 ticks (*Dermacentor marginatus, D. reticulatus, Ixodes ricinus*) were collected by tick dragging method at the location Košická Belá. The prevalence of *Babesia* sp. in *D. reticulatus* was 11.4%, *Anaplasma* sp. occurred in 20% of individuals. The presence of *Babesia* sp. in *I. ricinus* was confirmed in 30% of samples, the presence of *Anaplasma* sp. was present in 20% of samples. No pathogens were confirmed in the *D. marginatus* species.
A total of 27 ticks (species *D. reticulatus, I. ricinus*) were collected by tick dragging method at the location Košice – South. The prevalence of *Babesia* sp. in *D. reticulatus* was 50%, the occurrence of *Anaplasma*

sp. was confirmed in 20% of samples. The prevalence of *Babesia* sp. in *I. ricinus* samples was 23.5%, *Anaplasma* sp. was not present in the samples. Female ticks showed a higher prevalence of *Babesia* sp. in samples than males of the same species, the prevalence of *Anaplasma* sp. in females was approximately the same as in males.
Small animals (lizards, songbirds, small rodents) were captured at the model sites Drienovec and Zádiel. Obtained blood was subjected to molecular-genetic methods. The positive samples showed the presence of *Hepatozoon* sp., *Trypanosoma* sp. and the little-known genus *Lankesterella*.
This study was supported by the Grant Agency VEGA project No. 1/0762/25 and APVV project no. APVV-23-0320.

W6

Between Ticks and Wildlife: The Ecology of Infection

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Wildlife plays an important role in the ecology of tick-borne diseases because it serves as a reservoir for various pathogens. We investigated the role of wildlife in natural foci of tick-borne diseases. Using the PCR method, 97 blood samples of wild ungulates and wild carnivores from three different locations in Slovakia (Gápel, Považský Inovec, Ladmovce) were analyzed. Simultaneously, the collection and morphological identification of attached ticks were performed. The presence of at least one pathogen was confirmed in 61.86% of the examined animals. The most frequently detected pathogen was the parasite *Theileria capreoli* (42.30%), restricted exclusively to ungulates, primarily red deer from the Gápel locality. The bacteria of *Anaplasma* genus (37.11%) demonstrated a broader host spectrum, with their presence also confirmed in expansive carnivore species: the golden jackal and the raccoon dog (Ladmovce). Co-infection with both pathogens occurred in 19.59% of the individuals. The presence of *Rickettsia* spp. in the blood samples was not confirmed. Among the 235 collected ticks from all localities, *Ixodes ricinus* was the significantly dominant species. However, in the lowland locality of Ladmovce, *Dermacentor reticulatus* was also recorded, parasitizing mainly on golden jackals. The study confirms the key role of wild ungulates as stable reservoirs of infections and highlights the rapid integration of carnivore species into the transmission cycles of tick-borne pathogens. The monitoring of pathogens in wildlife is important not only for biodiversity conservation but also in the prevention of zoonoses that may threaten domestic animals and consequently humans.
This study was supported by the Grant Agency VEGA project no. 1/0762/25 and APVV project no. APVV-19-0440.

W7

Rodent Reservoirs of Vector-Borne Pathogens in the High Tatras, Slovakia

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Small mammals play an important role as reservoirs of zoonotic vector-borne pathogens and contribute significantly to the circulation of infectious agents affecting both human and animal health. This study investigated the prevalence and diversity of selected vector-borne pathogens in rodents and insectivores

collected in the High Tatra Mountains region and Tatra National Park, Slovakia. A total of 156 small mammals representing 10 species were screened for the presence of *Bartonella* spp., *Borrelia* spp., *Anaplasma phagocytophilum*, and *Babesia* spp. using conventional PCR and sequence analysis.
Overall pathogen prevalence reached 74.35%. *Bartonella* spp. were the most prevalent pathogens, detected in 57.7% of the animals examined, particularly in *Apodemus flavicollis* and *Clethrionomys glareolus*. Sequence analysis confirmed the presence of *Bartonella grahamii* and *Bartonella* sp. *Borrelia burgdorferi* sensu lato was detected in 11.5% of rodents, with *Borrelia afzelii* identified as the dominant genospecies. *Babesia microti* and related *Babesia* spp. were detected in 3.2% of samples, mainly in *A. flavicollis, C. glareolus,* and *Mus musculus*. The lowest prevalence was observed for *A. phagocytophilum* (1.9%).
The findings confirm that wild and synanthropic small mammals inhabiting protected mountain ecosystems act as important reservoirs of multiple vector-borne pathogens. The occurrence of zoonotic agents in recreational and protected areas of the High Tatras highlights the epidemiological importance of wildlife monitoring and the need for continued surveillance of pathogen circulation in Central European mountain habitats.
This study was financially supported by the projects Tubitak-2024-01, VEGA 2/0051/24, and APVV-21-0166.

W8

Ochlerotatus excrucians as a potential vector of *Dirofilaria immitis* in Slovakia

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Hairworms (*Dirofilaria*) of the family Onchocercidae are transmitted by mosquitoes of various genera, such as *Culex* (*Cx. pipiens*, *Cx. modestus*, *Cx. nigripalpis*), *Anopheles* (*An. maculipennis*), *Aedes* (*Ae. vexans*, *Ae. albopictus*), and *Ochlerotatus* (*Oc. caspius*) (Capelli et al. 2018). As early as 2013, the first entomological and molecular study was published with the aim of identifying potential vectors of *Dirofilaria* in eastern Slovakia by Bocková et al. (2013). The authors confirmed that *Aedes* (*Aedimorphus*) *vexans* can transmit *Dirofilaria repens*. Two years later, *D. immitis* was also confirmed in *Ae. vexans* (Bocková et al., 2015), and DNA from both *Dirofilaria* species was repeatedly confirmed in western Slovakia as well (Čabanová et al., 2018).
We performed an entomological survey in South-western Slovakia (Palárikovo, Komjatice, and Nové Zámky) in 2024–2025. We collected, identified, and stored mosquitoes using standard methods. We isolated genomic DNA from the abdomens of randomly selected mosquitoes. For species identification, we selected individuals that were damaged. To confirm the presence of dirofilariae, we processed the head and a portion of the thorax containing the salivary glands of engorged and gravid females. DNA extraction was performed using the commercial DNeasy Blood and Tissue Kit (QIAGEN, GmbH Hilden, Germany). For molecular confirmation of filariae, we used the 5.8S rRNA and mtDNA cox1 gene sets. We also used the 700-bp cox1 mtDNA gene. Sequencing was performed using the Sanger method, and the resulting sequences were analysed and edited using MEGA X software version 10.1.5 build 10191107 (Kumar et al. 2018).
We confirmed the presence of 19 species of mosquitoes in Palárikovo, Komjatice and Nové Zámky. The most abundant and most frequently occurring species were the *Culex pipiens* complex (41%) and *Ochlerotatus excrucians* (35.7%); among other minor species, we identified of *Oc. caspius*, *Oc. rusticus*, *Oc. flavescens*, *Oc. cantans*, *Oc. geniculatus*, *Oc. sticticus*, *Oc. annulipes*, *Oc. punctor*, *Ae. vexans*, *Ae. cinereus*, *Anopheles plumbeus*, *An. claviger*, the *An. maculipennis* complex, *An. messeae*, *Culex modestus*, and *Culiseta annulata*, with a prevalence of 0.1–9.6%.
Dirofilaria immitis DNA we confirmed in the salivary gland of *Oc. excrucians* from Komjatice, 99.71% match with NC_005305.1 Australia host: *Canis lupus familiaris*. The larval stages of this mosquito appear mainly in late spring and early summer; their development is linked to both permanent and temporary open water bodies shaded by vegetation, as well as to wet meadows and the edges of ponds. The territory of southwestern Slovakia is characterized by a warm lowland climate, a long growing season, and the presence of floodplain forests and extensive river systems. It is the region with the highest average

temperatures in the country, and ambient temperature is an important factor influencing the development of microfilariae in the mosquito. To our knowledge, this is the first record of *D. immitis* detection in this mosquito species in Slovakia. This finding suggests the potential involvement of *Oc. excrucians* in the local transmission of *Dirofilaria immitis*. Although this is an isolated finding, the dominant presence of the species during the study period and in the study area increases its epidemiological significance. However, further targeted research will be necessary to definitively confirm the vector competence of *Oc. excrucians* under Slovak conditions.
This study was supported by the Grant Agency VEGA project No 1/0793/25.

W9

Behavior of Pathogen-Infected *Dermacentor* Ticks

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Successful transmission of pathogens to their hosts depends on vector behavior, including locomotor activity and the ability to search for and attack a host. Pathogen-induced alterations in vector behavior may increase pathogen survival, transmission efficiency, or completion of the pathogen life cycle. The aim of this study was to investigate the influence of pathogens on the behavior of ticks as their vectors using two experimental setups designed and optimized for tick testing: a modified open field (MOF) and a light–dark test (LDT).
Using Smart software (Panlab, Spain), we analyzed and compared locomotor activity and light/dark preference in females of *Dermacentor reticulatus* (n = 88) infected and uninfected with *Rickettsia* spp. pathogens (prevalence 36.36%). In the LDT, both groups of ticks showed a significant preference ($p < 0.0001$) for the illuminated part of the apparatus. Although ticks explored the center of the MOF significantly more during the first 15 minutes of the experiment (out of a total duration of 24 h; $p < 0.0001$), most individuals subsequently adopted a questing or resting position at the periphery of the apparatus ($p < 0.01$). Moreover, elevated positions were significantly preferred for this behavior ($p < 0.001$).
Ticks infected with *Rickettsia* spp. exhibited significantly increased exploratory activity ($p = 0.0221$), reflected by longer movement trajectories. Our results indicate a possible influence of pathogens on specific behavioral traits of ticks as vectors, potentially enhancing the probability of pathogen transmission.
This study was supported by the Grant Agency VEGA project No. 1/0762/25 and APVV project no. APVV-23-0320.

W10

The Expanding Threat of Dermacentor reticulatus: Pathogen and Microbiome Analysis

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Ticks represent significant vectors of pathogens of both medical and veterinary importance, as they transmit a wide range of viruses, bacteria, and parasites. The ornate cow tick, *Dermacentor reticulatus*, is a tick species that is spreading intensively into new areas as a result of climate and socioeconomic changes. This species transmits the causative agents of serious diseases such as canine babesiosis, TIBOLA/DEBONEL, tularemia, and Q fever. The aim of our study was to identify pathogens transmitted by *Dermacentor reticulatus* ticks collected from various localities in eastern Slovakia using molecular-genetic methods. In the samples, we detected the presence of pathogenic bacteria of the genus *Rickettsia* in 39% of the ticks. The most frequently detected species was *Rickettsia conorii* subsp. *raoultii* (26.24%). We also identified *R. monacensis*, *R. slovaca*, and *R. aeschlimannii*. In one sample, we detected *Babesia*

venatorum and *Babesia odocoilei*. Further analyses using next-generation sequencing (NGS) revealed a total of 851 different bacterial genera in the ticks. The most frequently represented were bacteria from the phylum Proteobacteria, class Gammaproteobacteria, order Pseudomonales, and genus *Pseudomonas*. This was followed by the genera *Coxiella*, *Candidatus* Midichloria, *Neoehrlichia*, *Anaplasma* and *Rickettsia*. All studied groups also contained symbiotic or environmentally common genera such as *Rickettsiella*, *Lactobacillus*, *Variovorax*, *Rhodococcus*, *Stenotrophomonas*, *Acinetobacter*, *Brevibacterium*, *Arsenophonus*, *Prevotella*, *Sphingomonas*, *Morganella*, and *Wolbachia*. Differences between sexes were also observed — for example, bacteria of the genus *Coxiella* were more frequently found in male *D. reticulatus*.

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W11

Unexpected *Babesia canis* detection in *Ixodes ricinus* ticks collected from dogs in Silesia, Poland

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Babesia canis belongs to large species of *Babesia* protozoans, pathogenic for dogs. It is an etiological agent of canine babesiosis, the disease which may cause many symptoms including lethargy, apathy, dehydration, anorexia, jaundice, splenomegaly, hemoglobinuria, renal failure, diarrhea, and tachycardia. The main vector of this parasite is *Dermacentor reticulatus*, a tick species, which is gradually expanding its range across Europe. Nevertheless, the most frequently detected tick species in Poland is the castor bean tick (*Ixodes ricinus*), whose role in the *B. canis* transmission remains unclear. This study is one of the few, which shows the occurrence of this pathogen in *I. ricinus* and the first, in which positive ticks were collected from hosts.

In spring 2023, ticks have been collected from companion animals during the routine examination in veterinary clinic from Zawiercie (Silesia, Poland). The material was captured from domestic dogs and cats directly from their skin. The material was conserved in plastic tubes with 70% ethyl alcohol. Ticks were identified under the stereomicroscope (Zeiss Stemi 305, Warsaw, Poland) using the identification keys. Then, DNA was isolated from ticks using the ammonium hydroxide method, and the concentration of DNA was measured spectrophotometrically by the Nanospectrophometer PEARL (Implen, Munich, Germany). To detect *Babesia* spp. in ticks, a pair of primers specific to the 18S rRNA gene was used. The amplification products were separated electrophoretically in 2% ethidium bromide-stained gels and visualized under ultraviolet light. The PCR product was isolated from the gel with the use of an Agarose-Out Kit (EURx, Gdańsk, Poland) according to the manufacturer protocol. Then, the samples were sequenced (Genomed, Warsaw, Poland), and the obtained sequences were compared with the sequences found in the GenBank database.

Overall, 41 *I. ricinus* ticks were collected from 33 companion animals, including 30 dogs and 3 cats. 80.5% of collected ticks were females (N=33), 17.1% were males (N=7) and 2.4% (N=1) was nymph. In total, 4.9% (2/41) of examined ticks (females) were *Babesia canis*-positive. The sequencing analysis showed that obtained sequences were 100% identical to the sequence of *Babesia canis canis* (Accession number: AY072926.1). *Babesia*-positive ticks were collected from purebred dogs - Polish Greyhound and Akita Inu, which due to the lack of disease symptoms, were not tested for babesiosis.

The obtained results showed the possible *I. ricinus* participation in the *B. canis* circulation in the natural habitat and among hosts. Moreover, infection with *Babesia* in dogs from Silesia (Poland) should be taken into consideration during the differential diagnosis of tick-borne diseases at veterinary clinics. Future studies are required to investigate the role of *I. ricinus* in the transmission of *B. canis*.

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W12

Soil moisture as an ecological factor in the spread of the tick *Dermacentor reticulatus*

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The *Dermacentor reticulatus* tick is one of the fastest-spreading tick species in Europe, and its distribution varies across regions. It typically inhabits shrubby meadows near rivers and wetlands in lowland areas. It is not entirely clear what factors cause the uneven distribution of this tick.

A field experiment was conducted in the Eastern Slovak Lowland over an area of 25 × 35 km to assess the effect of soil moisture on the occurrence of *D. reticulatus*. The study included 5 sites near rivers where the presence of adult ticks on vegetation was confirmed in 2024 and 2025, and 3 sites distant from watercourses where this tick species was not detected under the same sampling conditions. At each of the 8 sites (1-8), five soil samples were collected in July and August 2025 at two-week intervals using a special drilling device to a depth of 1 m in 10-cm increments.

The water content of each sample was determined in the laboratory, and based on this, a soil moisture graph was compiled according to four standard hydrological limits: (a) soil at field water capacity (FWC), at which the soil pores remain filled with water; (b) soil between FWC and the point of reduced availability (PRA), also referred to as “semi-moist”; (c) soil between the PRA and the wilting point (WP), also referred to as “semi-dry”; and (d) soil with a water content below the WP, also referred to as “dry” soil.

In March 2026, a follow-up tick collection was conducted in the vicinity of the eight borehole sites mentioned above. Adult *D. reticulatus* were recorded at all monitored sites.

At sites near rivers, soil moisture in July and August remained between FWC and PRA (semi-moist) to a depth of 40 cm (1), 80 cm (2), and 90 cm (3), while deeper soil layers were with FWC. At site (4), soil moisture was with FWC throughout the entire profile until mid-July; thereafter, it remained “semi-moist” to a depth of 30 cm until the end of August. At site (5), the soil was “semi-moist” throughout the entire soil profile in July, and in August the FWC extended only to a depth of 50 cm.

At three sites located away from rivers, the soil was drier in July and August, ranging between FWC and WP. At the first site (6), the soil was “semi-moist” to a depth of 30 cm in both months, and “semi-dry” to “dry” at greater depths. At the last two sites (7) and (8), the soil was “semi-moist” throughout the entire soil profile in July and “semi-dry” in August.

These results, together with current knowledge of the life cycle of *D. reticulatus*, suggest that a key ecological factor for its successful development is soil moisture conditions, particularly in July, ranging from FWC to RAP (“semi-moist”) to a depth of at least 30 cm, when the larvae and nymphs develop in rodent burrows. Since *D. reticulatus* was recorded at all monitored sites in March 2026 as well, it was not possible to assess soil moisture conditions at sites with a long-term absence of this species; however, soil moisture at these sites significantly supplements our understanding of the extent to which larvae, and especially nymphs, require soil moisture for their development.

Soil moisture in July is significantly influenced by precipitation, particularly the summer monsoon, which typically lasts from June 8 to mid-July (“Medard’s total”) and soil type (proportion of sand, loam, and clay), which supports further research into the ecological factors influencing the occurrence and spread of *D. reticulatus.*

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W13

Urbanization Shapes the Prevalence of Tick-Borne Pathogens

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Representatives of the family Ixodidae serve as vectors of numerous pathogens causing a wide range of animal and human diseases. This study aimed to compare the prevalence and co-infection patterns of selected tick-borne pathogens in *Ixodes ricinus* and *Dermacentor reticulatus* ticks collected from environments differing in the level of anthropogenic disturbance. Since urban habitats are often associated with modified host–parasite interactions and enhanced pathogen circulation, ticks were sampled from both natural and urban localities in Slovakia.

A total of 579 *I. ricinus* ticks (305 collected from a natural locality and 274 from an urban locality) were screened for the presence of *Borrelia* spp., *Anaplasma* spp., and *Rickettsia* spp. using PCR-based methods. In the natural locality Biele vody (Muránska planina National Park), the overall pathogen prevalence reached 34.5%, including *Borrelia* spp. (13.1%), *Anaplasma* spp. (12.5%), and *Rickettsia* spp. (8.9%). In contrast, ticks collected in the urban area of Košice showed a higher overall prevalence of 43.5%, with *Borrelia* spp. detected in 15.7% of samples, while both *Anaplasma* spp. and *Rickettsia* spp. were identified in 13.9% of ticks. Both single infections and co-infections were more frequent in ticks from the urban environment compared to the natural locality.

In addition, 120 *D. reticulatus* ticks collected from Streda nad Bodrogom and Jablonov nad Turňou were analyzed for the presence of *Rickettsia* spp., *Ehrlichia* spp., and *Babesia* spp. The total pathogen prevalence reached 36.7%, with *Rickettsia* spp. detected in 32.5% of ticks and *Ehrlichia* spp. in 4.2%; *Babesia* spp. were not detected.

The obtained results demonstrate differences in the prevalence of tick-borne pathogens between urban and natural environments and emphasize the epidemiological significance of urban habitats in the circulation of vector-borne pathogens.

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W14

Tick-Borne Pathogens in Urban Companion Animals and Their Ticks in Lithuania

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Tick-borne pathogens (TBPs) are an increasing threat to companion animals and public health in urban environments. This study investigated the prevalence, clinical significance, and diversity of TBPs in dogs and ticks collected from dogs in Lithuania. A total of 132 client-owned dogs presented to veterinary clinics with suspected tick-borne diseases, 91 hunting dogs (assessed as healthy after examination and all protected against ticks), and 147 shelter dogs were examined using molecular and serological methods. *Babesia canis* was the predominant pathogen, detected in 57.9% of client-owned dogs, while *Anaplasma phagocytophilum* was identified in 8.4%. Serological evidence of *Borrelia* spp. exposure was found in 5.3% of dogs. Coinfections with *B. canis* and *A. phagocytophilum* were confirmed in five dogs. The most common clinical signs of canine babesiosis were lethargy, fever, and anorexia. In dogs with suspected or confirmed anaplasmosis, the most common symptoms were vomiting, diarrhea, and lack of appetite. Shelter dogs showed lower molecular prevalence of *B. canis* (11.7%) and *A. phagocytophilum* (6.8%), although serology indicated broader exposure. The dogs did not have clinical signs of TBPs; molecular testing identified subclinical infection. Hunting dogs had a low prevalence of infection: 4.3% were infected with *A. phagocytophilum*. A total of 257 ticks (*Ixodes ricinus* and *Dermacentor reticulatus*) collected from 50 dogs were analyzed. *A. phagocytophilum* was detected in 11% of *D. reticulatus* and 6.7% of *I. ricinus*. *Rickettsia* spp. were detected at a higher prevalence in *I. ricinus* (14.9%) than in *D. reticulatus* (7.7%). *R.*

helvetica identified in *I. ricinus* and *R. conorii* subsp. *raoultii* in *D. reticulatus*. *Babesia* spp. were detected in 8.9% of ticks, with *B. canis* identified in *D. reticulatus* and *B. microti* in *I. ricinus*. *Borrelia burgdorferi* s.l. was detected only in *I. ricinus* (2.9%). Coinfections of *B. canis* and *A. phagocytophilum* were identified in *D. reticulatus* ticks collected from eight dogs. Our findings demonstrate substantial circulation of TBPs in dogs and their ticks in Lithuania. Companion animals may serve as important sentinels and epidemiological bridges between wildlife reservoirs and humans, underscoring the need for continuous surveillance and a "One Health" approach to tick-borne disease prevention.
This study was supported by the Research Council of Lithuania (Grant No. S-MIP-23/19).

W15

Current prevalence of *Borrelia burgdorferi* s.l. and seasonal activity of *Ixodes ricinus* ticks in Bratislava, Slovakia

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Across Europe, Lyme borreliosis is caused by the *Borrelia burgdorferi* sensu lato (s.l.) complex and is mainly spread by the tick *Ixodes ricinus*. In recent years, environmental and climate-related changes have contributed to shifts in tick occurrence as well as their bionomics. The aim of this study was to investigate the prevalence of *B. burgdorferi* s.l. (Bbsl) and to monitor changes in the distribution patterns and seasonal dynamics of *I. ricinus* populations in Bratislava.
Questing ticks were sampled monthly from 2023 to the present at eleven locations in Bratislava using the flagging method along 100-m transects. Collected ticks were subsequently identified according to species and developmental stage. Genomic DNA was extracted from the ticks by alkaline hydrolysis. Detection of Bbsl was performed by amplification of the 23S rRNA gene and the 5S–23S (rrfA–rrlB) rRNA intergenic spacer region. PCR products from positive samples were purified with the QIAquick Spin PCR Purification Kit and subsequently analysed by sequencing.
A total of 8 759 *I. ricinus* ticks were collected. In our collections, larvae predominated (58.4%), followed by nymphs (31.2%) and adults (10.4%). The highest tick activity was observed in July and November for larvae and from April-June for nymphs and adults. Winter tick activity was confirmed in all study years. So far, 2 170 tick samples have been tested for the presence of Bbsl. In 2023, 13.8% of tested samples were positive by conventional PCR. Five genospecies were identified, with *B. afzelii* and *B. garinii* being the most prevalent, followed by *B. valaisiana*, *B. spielmanii*, and *B. lusitaniae*. In 2024, 13.4% of tested samples were positive. We detected the same species as in 2023, with the addition of *Borrelia burgdorferi* sensu stricto. The prevalence of infected ticks varied among the sampling sites, ranging from 3% to 29%. Analysis of the remaining samples is ongoing.
Our study demonstrates that both, the prevalence and the composition of *Borrelia* species, differed among the eleven sampling sites within the city. Our findings indicate that global changes affect both tick abundance and their seasonal activity. Milder winters lead to prolonged tick activity, which also increases the risk of human infection.
This study was supported by the Slovak Research and Development Agency under contract No. APVV-22-0372 and the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic, 2/0083/25.

W16

Avian Haemosporidian Parasites in *Culicoides* Biting Midges from the Bird Ringing Station Drienovec, Slovakia

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Biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae) are a diverse group of insect vectors transmitting a wide range of pathogens affecting humans, domestic animals, and wildlife. They are recognized vectors of avian blood parasites of the order Haemosporida, which may cause disease, pathology, and mortality in birds. To date, more than 200 haemosporidian species have been described within the genera *Plasmodium*, *Haemoproteus*, and *Leucocytozoon*. In Europe, 15 *Culicoides* species have been identified as potential vectors of avian haemosporidians.

The aim of this study was to identify *Culicoides* species involved in the transmission of avian haemosporidian parasites and to determine the parasite species circulating in wildlife populations in Slovakia. The research was conducted in a wetland ecosystem at the Bird Ringing Station in Drienovec, an important stopover site for migratory birds during spring and autumn migration. Biting midges were collected between 2022 and 2024 using UV light traps suspended from trees. A total of 919 parous female *Culicoides* were collected, of which 225 females were morphologically identified and screened for avian haemosporidian parasites using PCR-based methods.

Nested PCR amplification of the mitochondrial cytochrome b (*cyt b*) gene revealed infection with avian haemosporidian parasites in 21 parous females (9.33%). Since parous females have completed at least one gonotrophic cycle, they are considered the only individuals capable of transmitting pathogens, indicating active circulation and ongoing transmission within the studied ecosystem. Haemosporidian DNA was detected in seven *Culicoides* species: *Culicoides kibunensis, Culicoides alazanicus, Culicoides segnis, Culicoides pictipennis, Culicoides simulator, Culicoides festivipennis,* and *Culicoides obsoletus*. Three haemosporidian species were identified: *Haemoproteus minutus* (genetic lineage TURDUS2) in *C. kibunensis* (n = 2), *C. pictipennis* (n = 1), *C. festivipennis* (n = 1); *Haemoproteus asymmetricus* (genetic lineage TUPHI01) in *C. kibunensis* (n = 4), *C. alazanicus* (n = 2), *C. pictipennis* (n = 1), *C. simulator* (n = 2), *C. segnis* (n = 1) and *Haemoproteus sp.* (genetic lineage CCF6) in *C. obsoletus* (n = 1); and 3 unidentified distinct sequences which, when compared in database GenBank (NCBI), could only be assigned to the order Haemosporida with 98.06% – 98.38% similarity, in *C. segnis* (n = 2) and *C. kibunensis* (n = 1). In addition, *Plasmodium ashfordi* (genetic lineage GRW02) was detected in *C. alazanicus* (n = 2) and *C. kibunensis* (n = 1).

The obtained results contribute to the currently limited knowledge on the occurrence and circulation of avian haemosporidian parasites in *Culicoides* biting midges, while also providing the first evidence of *Plasmodium ashfordi* circulation in Slovakia and the presence of three potentially novel genetic lineages of Haemosporida.

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W17

First Record of Thelaziosis in the European Bison (*Bison bonasus*) from Poloniny National Park, Slovakia

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Nematodes of the genus *Thelazia* (Spirurida: Thelaziidae) cause a serious parasitic ophthalmic disease, thelaziosis, in various mammalian hosts, including humans. The causative agents are spirurid nematodes

(eyeworms) infecting the eyes and associated tissues, particularly the conjunctival sac, lacrimal ducts, and corneal surface; they may also occur beneath the third eyelid or in other ocular structures. Infection may result in excessive tearing, severe inflammation, corneal opacity, and, in advanced stages, complete blindness.
Thelaziosis represents a growing problem in both wild and domestic ruminants. Thelaziosis cases have been reported in populations of European bison (*Bison bonasus*) in Poland and Romania. So far, three species have been confirmed in European bison: *Thelazia gulosa*, *Thelazia skrjabini*, and *Thelazia rhodesi*. Transmission of these parasites is likely mediated by several species of secretophagous non-biting flies acting as vectors, including *Musca autumnalis*, *Musca larvipara*, *Musca osiris*, *Musca domestica*. These flies feed on ocular secretions, tears, and conjunctival tissues of various animal species, including humans.
In December 2025, a deceased male European bison was found in Poloniny National Park, and four nematodes, including adult males and females, were collected from its eye. The eyeworms were identified based on morphometric analysis using a Nikon SMZ 1500 stereomicroscope, with an average body length of 1.5 cm. Subsequently, molecular analysis of the mitochondrial cytochrome c oxidase subunit I (*cox1*) gene was performed using specific primers targeting filaroid nematodes. Comparison of the obtained sequences with reference sequences available in the GenBank (NCBI) database revealed only 89.20–89.70% similarity to the eyeworm of carnivores, *Thelazia callipaeda*, which was insufficient for precise species identification. Phylogenetic analysis of the *cox1* gene demonstrated that the newly obtained sequences formed a distinct clade separated from other reference sequences of *Thelazia* spp. available in GenBank.
Studies conducted in Europe indicate that *Thelazia* spp. are common parasites of the European bison and are widely distributed across different populations regardless of habitat, geographic region, season, or host-related factors. This suggests that thelaziosis may occur endemically in European bison populations, particularly in Poland. Our findings from Slovakia indicate the possible presence of a previously unidentified *Thelazia* species whose sequences have not yet been deposited in the GenBank database. However, it remains unclear whether these findings represent a truly novel species or a species that has not yet been genetically characterized and documented in publicly available databases.
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W18

Temporal dynamics of *Borrelia burgdorferi* sensu lato prevalence in two urban parks in Bratislava

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Due to climate changes and human activity has increased the occurrence of ticks in higher altidutes and urban areas, too. *Ixodes ricinus* is the most important vector of tick-borne pathogens in Europe. This tick species is the main vector for *Borrelia burgdorferi* sensu lato (s.l) and other pathogens, with major epidemiological significance. Monitoring tick abundance and pathogen prevalence over time is essential for assessing the risk of tick-borne diseases.
Study areas Železná Studnička (ŽS) and SAS campus (SAS) are situated in urban parks in Bratislava frequently used for recreational activities. Questing ticks were collected using the flagging method. Collected ticks were identified to species and developmental stage and tested for the presence of *B. burgdorferi* s.l. using PCR methods. Pathogen prevalence were compared among study years.
Borrelia prevalence showed temporal changes at both localities between 2011–2013 and 2023–2024.
At the ŽS locality, the prevalence increased from 13.5% in 2011–2013, 2017 and 2019 to 19.6% in 2023, indicating a substantial rise over time. Although the prevalence decreased slightly to 18% in 2024, it still remained higher than the values recorded during 2011–2013, suggesting an overall long-term increase in *Borrelia* occurrence at this locality. In contrast, the SAS locality showed a different trend. The prevalence decreased from 6.7% in 2011–2013 to 4.1% in 2023, indicating a temporary decline. However, in 2024, the prevalence increased markedly to 10.7%, exceeding the values observed during 2011–2013. This suggests that *Borrelia* prevalence at SAS has also changed significantly over time, particularly due to the marked increase recorded in 2024. Overall, the comparison indicates that *Borrelia* prevalence has varied considerably over the years at both localities, with ŽS consistently exhibiting higher prevalence than SAS.

Temporal increases in *Borrelia* prevalence may reflect changing environmental conditions, host dynamics, and climate-related effects on tick populations. Interannual dynamics in the tick population was driven primarily by microclimatic changes, although methodological differences may also have contributed. Long-term monitoring is important for evaluating the epidemiological risk of Lyme borreliosis.
This study was financially supported by Project of Slovak Research and Development Agency APVV 22-0372 and VEGA project No. 2/0083/25.

W19

Rickettsia and rickettsia-like bacteria presence in bat obligate ectoparasites of the western part of the Old World

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Bat ectoparasites represent a highly diverse assemblage of insect and acarine taxa exhibiting considerable ecological and trophic diversity. Among the microorganisms associated with these hematophagous parasites are medically important bacteria such as *Rickettsia*, obligate intracellular α-proteobacteria capable of infecting both humans and animals. Current knowledge indicates that the occurrence of *Rickettsia* spp. in bat-associated parasites has mainly been documented in blood-feeding ectoparasites of cave-dwelling bats; however, data remain fragmented and geographically limited.
To expand current knowledge on the distribution of *Rickettsia* and rickettsia-like bacteria in bat ectoparasites, more than 270 obligate hematophagous parasites collected between 2001 and 2026 by Czech and Slovak chiropterologists were analyzed. Samples originated from the Palearctic and Afrotropical regions, including continental Europe, the Middle East, northern Africa, and several Mediterranean islands. Two major parasite groups were investigated: hard and soft ticks (Ixodida), including genus *Ixodes*, *Reticulinasus*, and *Secretargas*, and dipteran bat flies (Nycteribiidae), including genus *Penicillidia*, *Phthiridium*, and *Nycteribia*.
DNA was extracted using nucleotides extraction kits. The presence of *Rickettsia* spp. and rickettsia-like bacteria was assessed by PCR amplification of the *gltA* gene using primers RpCS.877p and RpCS.1256n. Positive samples were subsequently analyzed by amplification of the *ompA* gene using primers Rr190.70p and Rr190.701.
Based on *gltA* detection, *Rickettsia* DNA was identified in 62.1% of analyzed ectoparasites, with the highest prevalence observed in *Secretargas transgariepinus*, and the lowest in *Eucampsipoda aegyptia*. Overall prevalence was higher in ticks than in bat flies, and northern African and Middle East localities showed a higher positivity rate than European regions and Mediterranea islands. In contrast, *ompA* amplification confirmed the presence of *Rickettsia* in 24.3% of samples, not all positive samples having been tested at the time. Sequencing of *gltA* and *ompA* amplicons is currently in progress and will provide further insight into the detected taxa, although precise species identification may require multilocus sequence analysis.
These findings expand current knowledge by providing new geographical records and novel host–parasite associations involving *Rickettsia*. Nevertheless, the available evidence remains insufficient for a comprehensive reconstruction of transmission pathways, evolutionary relationships, or for confirming the investigated ectoparasites as competent vectors. The poster will summarize both previously published and newly obtained records of *Rickettsia* occurrence in bat ectoparasites.
This study was supported by the VEGA project No. 2/0011/25.

W20

Occurrence of selected vector-borne pathogens in deer keds of the genus *Lipoptena* (Diptera: Hippoboscidae) in Slovakia

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Deer keds (*Lipoptena* spp.) are obligate hematophagous ectoparasites of mammals. Although they receive relatively little attention, they are increasingly recognised as potential vectors of a wide range of vector-borne pathogens. The main objective of this study was to analyse the presence of selected vector-borne pathogens in collected ectoparasites and compare the prevalence of these pathogens between two groups of deer keds collected across several localities in Slovakia. The first group consisted of winged individuals collected from the environment, whereas the second group consisted of wingless individuals collected from legally hunted wildlife (13 red deer and 2 fallow deer).

A total of 782 individuals of the genus *Lipoptena* were collected during the years 2023 and 2024. Specimens were identified using a morphological key as two species: *Lipoptena cervi* (179 females, 166 males) and *Lipoptena fortisetosa* (214 females, 223 males). Samples were analysed for the presence of DNA of selected vector-borne pathogens using molecular methods.

Molecular analyses confirmed the presence of several bacterial agents in deer keds collected from hosts, including *Bartonella* spp. (88,33%), *Anaplasma phagocytophilum* (24,58%), and haemotropic *Mycoplasma* spp. (4,56%). In addition, protozoan pathogens such as piroplasmids *Babesia/Theileria* spp. (60,83%) and members of Kinetoplastida (20,42%) were detected. Furthermore, the presence of filarial nematode *Setaria cervi* DNA was confirmed in wingless *L. cervi*, with a prevalence of 2.5% (2/80 tested specimens). In contrast, winged deer keds collected from the environment showed a considerably lower prevalence of pathogens, with the detection of *Bartonella* spp. (7,38%), *Rickettsia* spp. (0,92%) and haemotropic *Mycoplasma* spp. (0,37%). The genus *Borrelia* spp. was not detected in any of the study groups.

Despite their widespread distribution and frequent contact with wildlife, deer keds have historically received limited scientific attention, and their role as potential mechanical or biological vectors in pathogen transmission within wildlife populations remains poorly understood. The broad spectrum of pathogens detected in *Lipoptena* spp. underscores their potential epidemiological importance in wildlife ecosystems and suggests possible implications for both animals and public health.

This work was supported by the projects VEGA 2/0033/25, VEGA 2/0051/24, APVV SK-PL-25-0018, and by the bilateral project of the Slovak Academy of Sciences PAS-SAS 2024-16.

Epidemiology, Diagnostics and Control of Parasitic Diseases

ORAL PRESENTATIONS

O49 ★ **L. Goterris, P.C. Köster, A. de Lucio, B. Bailo, L. Castro-Companioni, G.J. Muñoz-de-Mier, S. Sánchez, D. Carmena** — Impact of the COVID-19 restrictions on the epidemiology of *Cryptosporidium hominis* in Spain

O50 **L. Ihnacik, I. Papajová, F. D. Javan, C. Anthonj, J. Šmigová, J. Foellmer, P. Janeka, P. Blišťan** — Insights into Marginalised Roma Settlements from an Integrated Geo-Socio-Parasitological Assessment in Eastern Slovakia

O51 **A. Lass, I. Kontogeorgos, K. Baranowicz, B. Szostakowska, P. Karani, X. Zhang** — Detection of *Echinococcus multilocularis* in wastewater and water sources and possible implications for human health – 10 years of research experience in Poland and China

O52 **R. Kuchta** — *Dibothriocephalus latus* (diphyllobothriosis) in Europe

O53 **T. Macháček, J. Procházka, Z. Bartoníček, R. Leontovyč, P. Pumann, J. Bulantová, P. Horák** — Cercarial dermatitis in Czechia: Tracking avian schistosomes in the laboratory and in the field

O54 **D. Dwużnik-Szarek, R. Mysłajek, S. Nowak, I. Kwiatkowska, M. Figura, M. Szewczyk, K. Kurek, M. Jackowiak, E. J. Mierzejewska, M. Krokowska-Paluszak, P. Opalińska, Ł. Stańczak, G. Górecki, A. Bajer** — Cestodes occurrence in wild carnivores (wolves, foxes and martens) in Poland

O55 **K. Slivinska[1], M. Pękacz[3], E. M. Ganci[4], A. Vyniarska[5], U.-L. Hunda[5], A. Wesołowska, V. Kharchenko, A. Zawistowska-Deniziak[3]** — Molecular screening of dirofilariosis in dogs and cats along the Ukrainian border with European Union countries

O56 ✻ **K. Kováčová, S. Blahút, Y. Syrota,, M. Miterpáková , Z. Hurníková** — Investigating the occurrence and species composition of *Eimeria* spp. in broiler chickens: The role of farm-related factors

O57 **J. Vadlejch, A. Khlebnikova, L. Škorpíková, J. Ilgová** — Multidrug resistance in strongylids of Bighorn Sheep: A case study from the Pilsen ZOO

O49 ★ Keynote Speaker

Impact of the COVID-19 restrictions on the epidemiology of *Cryptosporidium hominis* in Spain

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Cryptosporidium hominis is a major cause of human cryptosporidiosis, primarily transmitted via anthroponotic routes. In Europe, subtype IbA10G2 historically dominated, reflecting stable transmission networks. The COVID-19 pandemic and associated non-pharmaceutical interventions (NPIs) disrupted gastrointestinal pathogen circulation, particularly human-to-human transmission cycles. In Spain, a marked increase in cryptosporidiosis cases was reported in 2023, including a predominance of the rare *gp60* subtype IfA12G1R5. This study investigates temporal changes in *Cryptosporidium gp60* subtype distribution in Spain before and after the pandemic.

We conducted a retrospective and multicentre molecular epidemiological analysis of human *Cryptosporidium* infections in Spain. Pre-pandemic data (2000–2019) were compiled from published literature, a multicentre hospital study (2013–2019), and the national microbiology reference laboratory. Post-pandemic data (2023) were obtained from a multicentre non-outbreak study. All samples were confirmed and genotyped by nested PCR amplification and Sanger sequencing of the *gp60* gene, followed by subtype assignment through sequence analysis. A total of 1,321 isolates were analysed.

Pre-pandemic data (*n*=1,195) showed consistent dominance of *C. hominis* (76–87%), mainly subtype family Ib (90–97%), with IbA10G2 as the predominant subtype (96–99%). *Cryptosporidium parvum* represented 13–24% of cases, mainly IIa subtypes. In contrast, post-pandemic samples (*n*=126) showed a marked shift in species and subtype distribution, with *C. hominis* accounting for 96.0% of cases. Subtype IfA12G1R5 represented 67.5% of all isolates and 70.2% of *C. hominis* diversity, whereas IbA10G2 declined to 1.6%. *Cryptosporidium parvum* remained rare (4.0%) and showed reduced subtype diversity.

In conclusion, the COVID-19 pandemic disrupted established transmission patterns of *Cryptosporidium* in Spain, followed by a post-pandemic reconfiguration of circulating subtypes. The rapid expansion of the hyper-transmissible IfA12G1R5 lineage indicates sustained community transmission and possible introduction or selection advantage. These findings highlight the dynamic nature of *Cryptosporidium* populations and underscore the importance of continuous molecular surveillance to detect emerging lineages with epidemic potential..

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O50

Insights into Marginalised Roma Settlements from an Integrated Geo-Socio-Parasitological Assessment in Eastern Slovakia

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Even today, marginalised Roma communities in Slovakia face persistent environmental and health challenges shaped by social or political discrimination, which are connected with inadequate water, sanitation, and waste management. These conditions ultimately increase health risks by compromising the overall hygiene of inhabitants and increasing their exposure to environmental contamination and potentially to endoparasites. To characterise health risks in these settings, we implemented an integrated approach by combining unmanned aerial vehicle (UAV) remote sensing, parasitology, microbiology and social science-based community-based research methods across three selected settlements with different living conditions. First, UAV imagery allows the identification of environmental hotspots (such as waste accumulation and standing water), enabling targeted sampling of dog faeces and well water. Across the three settlements, a total of 123 dog faecal samples were collected. Standard concentration-based coprological analytical methods confirmed the presence of helminth eggs in 34% of them. In Settlement 1, the positive sample rate was 67%; in Settlement 2, it was 20%; and in Settlement 3, it was around 31%, with *Toxocara canis*, *Trichuris vulpis*, and *Ascaris* spp. as the dominant species. The detection of *Ascaris* spp. in dogs, a parasite atypical of dogs, indicates potential faecal contamination of the environment by human or wild-animal faeces. Also, water testing of 11 wells from two settlements further indicated severe faecal contamination. In settlement 1, elevated *E. coli* concentrations were detected in one well, while in settlement 3, several wells exhibited high nitrate levels, and they were classified as undrinkable. Across all sampled wells, elevated concentrations of coliform bacteria were detected. Lastly, survey questionnaire using the Household Water Insecurity Experiences (HWISE) and Sanitation-related Quality of Life (SanQoL) scales, photovoice data and group discussions supported these findings, documenting widespread faecal contamination of living spaces, the presence of stray dogs, and frequent exposure of children to these conditions. Our results demonstrate that combining parasitology, microbiology with UAV imagery and participatory methods provides a feasible and ethically acceptable strategy for assessing environmental contamination and potential health risks in hard-to-reach communities where conventional sampling is socially or logistically difficult. This integrated approach represents the first study of its kind conducted in Slovakia and one of the earliest applications of such a combined methodology for assessing environmental and health risks in marginalised communities in Europe.

This study was supported by the HealthyWatersIntegrated project, funded by the Dutch Science Foundation (#406.X5.24.01.042), Grant Agency VEGA project No. 2/0069/25, and by the Slovak Research and Development Agency under contract No. APVV-18-0351.

O51

Detection of *Echinococcus multilocularis* in wastewater and water sources and possible implications for human health – 10 years of research experience in Poland and China

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Echinococcus multilocularis is a parasite causing a dangerous zoonosis, alveolar echinococcosis (AE), which can be acquired by swallowing parasite eggs excreted into the environment with the feces of infected definitive hosts. It has been estimated that 90% of AE cases recorded in China, especially in the Qinghai Tibet Plateau (QTP) area. In Poland, Warmia-Masuria is considered the most endemic province. Although water has been indicated as possible source of infection with *E. multilocularis*, data on the distribution of the tapeworm in water reservoirs is very limited.
The aim of the study was to determine the occurrence of *E. multilocularis* in different types of wastewater and water supplies in the area of QTP, China as well as Pomerania and Warmia-Masuria provinces, Poland. In China, 510 samples were collected, including 186 wastewater samples and 326 samples collected from streams and shallow waterholes that serve as sources of drinking water consumed unprocessed by humans and animals. In Poland, 105 samples were collected from rivers, lakes and surface wells. 40 L surface water samples were either filtered on site with capsule filters (Poland) or first collected in sterile vessels and transported to the laboratory for further filtration with membrane filters (China). Wastewater samples, in contrary, were treated using flocculation method. Final pellets were subsequently analysed with molecular detection methods including real-time PCR, nested PCR and DNA sequencing.
Of the samples examined, *E. multilocularis* DNA was confirmed in 11 (5.9%) wastewater samples and 3 (0.9%) stream water samples from Qinghai province, China and in 2 (1.9%) lake water samples from Warmia-Masuria province, Poland.
Results indicate the potential role of surface water in dissemination of *E. multilocularis* in endemic areas of the QTP, China and Warmia-Masuria, Poland and contamination of drinking water sources. This raise a question about the risk for humans and animals echinococcosis living in at-risk regions.
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O52

Dibothriocephalus latus (diphyllobothriosis) in Europe

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Diphyllobothriosis is a human parasitic infection caused by the broad fish tapeworm *Dibothriocephalus latus*. The disease is widespread across the Northern Hemisphere, including Europe, and is typically associated with the consumption of raw or undercooked freshwater fish. Common sport fish such as pike (*Esox lucius*) and perch (*Perca fluviatilis*) are recognized sources of human infection.
We report the first autochthonous case of diphyllobothriosis in the Czech Republic from a popular tourist destination in Central Europe. The infection was linked to consumption of raw pike roe (caviar). This occurrence likely reflects a recent colonization of the parasite, with the Lipno Reservoir potentially representing a new local focus of this fish-borne zoonotic disease.

O53

Cercarial dermatitis in Czechia: Tracking avian schistosomes in the laboratory and in the field

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Cercarial dermatitis (CD) is a non-communicable water-borne parasitic disease that develops shortly after exposure to cercariae, the free-swimming larval stages of schistosomes. Avian schistosomes are the principal etiological agents of CD worldwide, with species of the genus *Trichobilharzia* being the most frequent causative agents of outbreaks reported from both natural and artificial water bodies. To address existing gaps in the epidemiology, pathobiology, and detection of CD, we combined retrospective field surveillance, experimental infections, and molecular diagnostic approaches. First, we conducted a retrospective analysis of CD outbreaks in Czechia to identify major epidemiological patterns. Overall, we recorded nearly 200 outbreaks over the past 60 years, with incidence markedly increasing during the last two decades. Second, we investigated in mice the migration and pathogenicity of *Trichobilharzia franki*, the species most frequently associated with recent outbreaks. Infection resulted in hyperkeratotic skin crust formation and transient pulmonary hemorrhages, providing the first experimental evidence of *T. franki* behavior in mammals. Moreover, we demonstrated remarkable immune cross-reactivity between *T. franki* and other avian schistosome species, suggesting broader epidemiological and diagnostic implications. Third, we developed a molecular toolkit combining loop-mediated isothermal amplification (LAMP), quantitative PCR (qPCR), and multiplex PCR for rapid, sensitive, and accurate detection and identification of *Trichobilharzia* spp. from biological and environmental samples. Altogether, our findings provide an integrated overview of the current epidemiological situation of CD in Czechia, improve understanding of host-parasite interactions during avian schistosome infections, and introduce new molecular tools applicable to surveillance, outbreak monitoring, and future diagnostic strategies.
This study was supported by the Czech Science Foundation (24-11031S), and by Charles University institutional funding (Cooperatio Biology, UNCE24/SCI/011, SVV 260678 and 260796).

O54

Cestodes occurrence in wild carnivores (wolves, foxes and martens) in Poland

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Wild carnivores, such red fox (*Vulpes vulpes*), and wolf (*Canis lupus*), constitute an important reservoir of parasites, including tapeworms of both medical and veterinary importance. One of the most dangerous tapeworms transmitted by these carnivores is *Echinococcus multilocularis*, the causative agent of alveolar echinococcosis in humans — one of the most severe parasitic zoonoses occurring in Europe.
The aim of this study was to determine the prevalence of tapeworm infections, with particular focus on *E. multilocularis*, in foxes and wolves originating from natural habitats, as well as in foxes and martens from urban areas of Warsaw, and to assess their role in maintaining the parasite's life cycle.
The study material consisted of fecal samples collected from foxes (108 samples collected between 2016 and 2018) and wolves (93 samples; 2016 - 2023), obtained during animal necropsies, as well as fecal samples from the Warmian-Masurian Voivodeship (69 samples; 2021 - 2023) collected from the field. Additionally, fecal samples from foxes (44 samples) and martens (*Martes* sp.; 24 samples) collected in urban areas of Warsaw were examined. Two genes: fragment of *nad1* gene (529 bp), and fragment of *rrnS* gene (200 bp) were amplified to molecular identification of cestodes. Selected products were seguenced and phylogenetic analysis were conducted.
A significantly higher prevalence of tapeworm presence was detected in foxes from natural habitats (32.4%; 35/108) compared to wolves (11.11%; 18/162). A higher prevalence of *E. multilocularis* in wolves was detected in fecal samples from the Warmian-Masurian Voivodeship (17.4%; 12/69) compared to the remaining samples (4.3%; 4/93). *Echinococcus* sp. and *Taenia* spp. were also detected in wolf samples. DNA of *E. multilocularis* was detected in foxes from natural habitats but was not found in foxes or martens from urban areas. DNA of *Mesocestoides litteratus* tapeworm was detected in one marten sample (4.2%; 1/24), two fox samples from urban area (4.5%; 2/44) and 3.7% (4/108) from foxes from natural area.
The obtained results highlight the importance of monitoring wild animal populations in the particular context of the of *E. multilocularis* transmission.

O55

Molecular screening of dirofilariosis in dogs and cats along the Ukrainian border with European Union countries

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Keywords: *Dirofilaria*, *Acanthocheilonema,* molecular epidemiology, dog, cat, Ukraine

The rapidly increasing population of stray dogs and cats in Ukraine represents a growing challenge from a One Health perspective, as these animals may serve as reservoirs for vector borne zoonotic parasites. Of particular concern are *Dirofilaria repens*, *Dirofilaria immitis*, and *Acanthocheilonema reconditum*, which affect both animal and human health. The ongoing war in Ukraine has severely disrupted veterinary services, animal population management, and disease surveillance systems, leading to the uncontrolled movement and accumulation of stray animals particularly in border regions with European Union countries. Despite numerous reports of human dirofilariasis, epidemiological data on filarial infections in companion animals in Ukraine remain limited. In this context, this study aimed to conduct a molecular epidemiological survey of filarial parasites in dogs and cats from western Ukraine, focusing on areas bordering European Union countries. Between February and July 2025, a total of 383 animals, including 339 dogs and 44 cats, were collected in five administrative regions which are bordered with European Union countries - Zakarpatska, Chernivetska, Ivano-Frankivska, Lvivska, and Volynska oblasts. Molecular screening was performed using real time PCR with species specific primers targeting *Dirofilaria* spp. and *Acanthocheilonema reconditum*. No filarial DNA was detected in the examined cats; therefore, feline samples were excluded from prevalence calculations.

In dogs, *D. repens*, *D. immitis*, and/or *A. reconditum* were detected in 67 of 339 samples, yielding an overall prevalence of 19.76%, with both mono and co infections observed. Only mono infections were identified in the majority of positive cases, including *D. repens* in 46 dogs (13.57%), *D. immitis* in 6 dogs (1.77%), and *A. reconditum* in 1 dog (0.29%). Co infections were detected less frequently and involved *D. repens* + *D. immitis* in 12 dogs (3.54%) and *D. repens* + *A. reconditum* in 2 dogs (0.59%). Co infection with *D. immitis* + *A. reconditum* was not recorded.

Geographically, the highest prevalence was recorded in Zakarpatska oblast at 40.0% (40 infected dogs out of 100 examined). Lower prevalence rates were observed in Volynska oblast (15.87%; 10/63), Lvivska oblast (14.04%; 8/57), and Ivano-Frankivska oblast (10.29%; 7/68). The lowest prevalence was noted in Chernivetska oblast at 3.92% (2 infected dogs out of 51 examined).

These findings demonstrate substantial circulation of comlex zoonotic filarial parasites in dogs in western Ukraine. Under wartime conditions, which exacerbate public and veterinary health challenges, the results highlight the urgent need to strengthen an integrated One Health approach. Furthermore, the implementation of effective vector control strategies is essential to reduce the risk of zoonotic transmission, particularly in border regions adjacent to European Union countries with increased cross border movement of people and animals.

This study was supported partially by NGO ESCCAP.

O56 ✻ Student Competition

Investigating the occurrence and species composition of *Eimeria* spp. in broiler chickens: The role of farm-related factors

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The poultry industry is among the fastest-growing sectors of animal production and plays a significant role in ensuring a supply of high-quality protein for the human population. Within the European Union, the breeding of domestic chickens (*Gallus gallus domestica*) predominates, with broilers representing the main source of poultry meat. Intensive broiler chicken production is associated with several serious challenges at the farm management level. One of the major problems in the modern poultry industry is intestinal coccidiosis, caused by intracellular parasitic protozoa of the genus *Eimeria*, which induce intestinal diseases with varying degrees of morbidity, ranging from subclinical infections to acute hemorrhagic diarrhea. Our study represents a pilot investigation focused on coccidia occurrence, farm hygiene conditions, animal health management, and the analysis of critical points in infection transmission.
Sample collection was carried out from January to May 2026 on two commercial intensive broiler chicken farms rearing Ross 308 breed chickens, each with a maximum stocking capacity of approximately 280,000 birds. Fresh fecal samples were collected from individual poultry houses on each farm during two production cycles at weekly intervals. In the laboratory, samples were examined for the presence of parasitic eggs and oocysts using concentration flotation methods, and the intensity of infection was determined using the quantitative McMaster method. Contamination of litter with oocysts was investigated using the standardized method for diagnosing parasite eggs and oocysts in the environment. For DNA extraction of *Eimeria* spp., oocysts from positive samples were collected using a combined sedimentation and flotation method, transferred onto Petri dishes containing a 1 % potassium sorbate solution to allow sporulation at a temperature of 28°C and 70–80% humidity for 72 hours, and subsequently stored at −20 °C for molecular analyses. Oocysts were mechanically disrupted using vortexing with glass beads, followed by DNA extraction using a commercial silica-membrane kit. Species identification was performed by amplification of the internal transcribed spacer 1 (ITS1) region of the ribosomal RNA gene cluster using PCR with species-specific primers. Statistical analyses of the results obtained from the epidemiological investigation in the studied farms were performed in the R computing environment.
The study discusses findings on the occurrence and species composition of the genus *Eimeria* on the investigated farms in the context of zoohygienic conditions and health management practices in the studied flocks (external farm temperature and humidity, temperature and humidity within poultry houses, litter type, litter temperature, ventilation, CO_2 concentration, stage of chicken production, nutrition, disinfection, and others), enabling the identification of risk factors for infection transmission.
The study was supported by the Grant Agency VEGA project No. 2/0058/26.

O57

Multidrug resistance in strongylids of Bighorn Sheep: A case study from the Pilsen ZOO

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Strongylid nematodes are globally distributed parasites in grazing ruminants. In the specific environment of zoological gardens, high animal density often leads to severe parasitic burdens, which can jeopardize conservation and reintroduction programs. This study describes a critical case of anthelmintic failure in a herd of five bighorn sheep (four females and one male) at the Pilsen Zoo, which had faced a prolonged

history of thriftlessness, adult mortalities, and reproductive failures, including abortions during late pregnancy.

Initial spring coprological examinations confirmed a massive parasitic load (mean: 5,212 EPG; range: 2,820–8,000). In response to the herd's poor clinical condition, a whole-group intervention was performed using oral moxidectin (Cydectin, Zoetis) at the standard dosage recommended for domestic sheep; this agent had never previously been administered to these animals. Precise dosing was ensured by weighing all animals during routine hoof trimming. Anthelmintic efficacy was evaluated via the Faecal Egg Count Reduction Test (FECRT) according to WAAVP recommendations, where resistance is indicated when the FECR is below 95% and the upper limit of the 90% confidence interval is below 95%. This *in vivo* test revealed a profound treatment failure (FECR 60.2%; 90% CI: 36.7–83.7%). A subsequent Larval Development Test was used to determine resistance levels against three main anthelmintic classes compared to established discriminating doses (0.0.08 µg/ml for thiabendazole, 21.6 ng/ml for ivermectin aglycone, and 2 µg/ml for levamisole). The results confirmed resistance to benzimidazoles (LD_{50} = 0.32 µg/ml) and an alarmingly high level of resistance to ivermectin (LD_{50} = 178 ng/ml), which exceeded the discriminating dose more than eightfold. Levamisole remained the only effective drug (LD_{50} = 0.25 µg/ml). Molecular identification (qPCR) of infective larvae cultured during the larval development test confirmed the predominant presence of *Haemonchus contortus*, which was resistant to both ivermectin and benzimidazoles. The resistant population also included *Trichostrongylus* spp. to a lesser extent. Retrospective analysis indicated that this selection was driven by long-term, high-frequency prophylactic treatments administered as powder in communal feed troughs.

This study is among the first to demonstrate multiple anthelmintic drug resistance in zoo ruminants. Furthermore, the data suggest the possibility of emerging cross-resistance of strongylid nematodes to macrocyclic lactones. These findings highlight the inadequacy of current deworming strategies in zoological settings and underscore the urgent need for a transition toward evidence-based parasite management to prevent the selection of highly resistant populations.

This study was supported by the National Agency for Agricultural Research project No. QL24010306.

Host–Parasite Interactions and Immunology

ORAL PRESENTATIONS

O58 **M. Soldánová, O. Tolstenkov, M. Benovics, P. Kundid, C. Pantoja, M. Oros, L. Konečný, C. Selbach** — Do trematode infections alter anti-predator behaviour of intermediate hosts in a subarctic lake?

O59 **R. Stryiński, E. Fiedorowicz, M. Maździarz, A. Niestępska, M. Stawicka, E. Łopieńska-Biernat** — Transcriptomic reprogramming of intestinal epithelial cells by helminth extracellular vesicles: implications for early host–parasite interaction

O60 ✻ **M. Stawicka , A. Niestępska , R. Stryiński** — A dual-action modulator: extracellular vesicles from *Anisakis simplex* suppress pro-inflammatory signaling and induce antioxidant defenses

O61 ✻ **M. Stawicka, A. Dudek, P. Rutkowski, A. Niestępska, G. Jawilak, R. Stryiński** — *Anisakis simplex*-derived EVs reshape early inflammasome signalling in an in vitro model of intestinal inflammation

O62 ✻ **Alicja Laskowska, Mateusz Pękacz, Katarzyna Basałaj, Alicja Kalinowska, Kinga Sekuła, Anna Zawistowska-Deniziak** — Manipulating Immune Responses: Chemokine Modulation During *Fasciola hepatica* Infection

O63 **M. Pękacz, A. Laskowska, K. Basałaj, E. Carretón, R. Morchón, E. Ganci, M. Klockiewicz, A. Zawistowska-Deniziak** — From zero to hero: uncovering immunobiology of *Dirofilaria repens*

O64 **M. Maruszewska-Cheruiyot, M. Machcińska, M. Kretshmer, K. Donskow-Łysoniewska, A. Bajer, R. Homanowski, P. Wrzodak-Zajdel, M. Komorowski, D. Dwużnik-Szarek** — Impaired regulatory immune response in dogs naturally infected with *Babesia canis*

O58

Do trematode infections alter anti-predator behaviour of intermediate hosts in a subarctic lake?

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Digenean trematodes are widespread parasites with complex life cycles that can alter host behaviour, potentially affecting predator-prey interactions, parasite transmission and food-web structure in aquatic environments. Parasite-mediated behavioural changes may create an ecological trade-off: increased host exposure can enhance parasite transmission to suitable hosts but also increase the risk of concomitant predation, in which infected hosts and their parasites are consumed together. While parasite-mediated behavioural changes are well documented, comparative experimental studies under predator cues remain limited, especially for trematodes with contrasting transmission strategies and particularly in subarctic freshwater ecosystems.

We investigated whether trematode infection alters the anti-predator behaviour of two common intermediate hosts in subarctic Lake Takvatn (northern Norway): the freshwater lymnaeid snail *Ampullaceana balthica*, naturally infected with *Plagiorchis* spp. (Plagiorchiidae), *Apatemon* spp. (Diplostomidae), *Diplostomum* spp. (Diplostomidae) and *Trichobilharzia* sp. “peregra” (Schistosomatidae), and the amphipod *Gammarus lacustris*, naturally infected with *Plagiorchis* spp. metacercariae. Behavioural experiments were conducted outdoors in August and October 2025 using chemical predator cues from Arctic charr (*Salvelinus alpinus*), one of the two dominant salmonid fish species in the lake and an important predator of both intermediate hosts.

Snail hosts were repeatedly exposed to treatments with and without fish cues. Snail anti-predator responses were assessed as changes in activity and refuge use under standardized conditions with food and shelter available, using more than 70 infected individuals and corresponding uninfected controls. Amphipod behaviour (vertical position and refuge use) was assessed in 80 individuals, each tested under both treatments. Since infection status could only be confirmed after experiments by parasitological dissection, trematode presence and identity in snails and metacercarial infection intensity in amphipods were determined post hoc, and trials involving misclassified controls were repeated when necessary.

We predict that behavioural responses will reflect different parasite transmission strategies. Snails infected with trematodes for which fish are unsuitable hosts (*Plagiorchis*, *Trichobilharzia*) are expected to show stronger avoidance, whereas infections involving parasites that use fish as transmission intermediate hosts (*Diplostomum*, *Apatemon*) may reduce snail-avoidance behaviour, despite the increased risk of infected host mortality. By comparing multiple host-parasite systems under common predator cues, this study will improve understanding of how parasite-associated behavioural changes influence concomitant predation and parasite transmission dynamics in subarctic freshwater food webs.

This study was supported by the the Czech Science Foundation project no. 24-11738S

O59

Transcriptomic reprogramming of intestinal epithelial cells by helminth extracellular vesicles: implications for early host–parasite interaction

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Anisakis simplex is a parasitic nematode and an etiological agent of human anisakiasis. Recent studies have shown that parasitic nematodes release extracellular vesicles (EVs) capable of entering host cells and participating in host–parasite communication. However, the molecular mechanisms underlying EV-mediated modulation of host intestinal responses remain poorly understood. Therefore, this study aimed to investigate the impact of *A. simplex* EVs on the transcriptomic profile of human intestinal epithelial cells and to compare EV-induced responses with those triggered by direct parasite exposure.
Extracellular vesicles released by L3 larvae of *A. simplex* (Anis-EVs) were isolated by ultracentrifugation. Human Caco-2 intestinal epithelial cells were exposed either to purified EVs at two concentrations (LOW and HIGH) or directly to live L3 larvae. High-throughput RNA sequencing (Illumina) combined with bioinformatic analyses was used to identify differentially expressed genes (DEGs) and parasite-derived miRNAs potentially involved in host gene regulation.
Transcriptomic profiling revealed substantial modulation of host cellular pathways following exposure to both Anis-EVs and live larvae. Comparison of control cells with EV-treated cells identified 57 DEGs under Anis-EVs LOW exposure and 554 DEGs under Anis-EVs HIGH exposure, indicating a clear dose-dependent transcriptomic response. Direct exposure to live larvae resulted in the identification of 300 DEGs.
In addition, analysis of the Anis-EVs cargo identified 39 miRNAs, including several highly abundant regulatory candidates potentially involved in host immune modulation. Bioinformatic prediction of host–parasite interactions indicated that these miRNAs may target genes associated with inflammatory signalling, epithelial barrier integrity, and immune regulation pathways.
Overall, the obtained results demonstrate that Anis-EVs are biologically active mediators capable of reshaping host intestinal transcriptomic responses. These findings provide new insight into the role of helminth-derived EVs and miRNAs in host–parasite communication and contribute to a better understanding of the molecular mechanisms underlying anisakiasis.
Keywords: *Anisakis simplex*, extracellular vesicles, parasite-host interactions, miRNA.
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O60 ✻ Student Competition

A dual-action modulator: extracellular vesicles from *Anisakis simplex* suppress pro-inflammatory signaling and induce antioxidant defenses

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Extracellular vesicles (EVs) secreted by parasitic helminths have emerged as key mediators of inter-kingdom communication. Although these cargo-carrying vesicles are increasingly recognized for their capacity to modulate host immunity, the functional impact of EVs from the zoonotic nematode *Anisakis simplex* on the human intestinal barrier remains poorly defined. Here, we investigated the immunomodulatory and biochemical effects of L3-stage *A. simplex* EVs (Anis-EVs) using a human Caco-2 epithelial cell model.

Anis-EVs were isolated from larval culture supernatants and subjected to quality control by nanoparticle tracking analysis, transmission electron microscopy, and determination of the particle-to-protein ratio to confirm highly enriched vesicular fractions. Caco-2 monolayers were then exposed to increasing Anis-EV concentrations, followed by assessment of cytokine profiles and oxidative stress–related markers.

Anis-EVs acted as potent immunomodulators, inducing a dose-dependent reprogramming of the epithelial cytokine signature. We observed a significant suppression of the pro-inflammatory chemokine IL-8, accompanied by a marked upregulation of the anti-inflammatory mediators IL-10 and IL-13. In contrast, IL-4, IL-6, and IFN-γ levels remained unchanged, indicating a selective rather than broad, systemic effect.

In parallel, Anis-EVs fine-tuned cellular redox homeostasis. Rather than triggering oxidative injury, Anis-EV treatment was associated with increased total antioxidant capacity (TAC) and glutathione (GSH) levels, enhanced superoxide dismutase (SOD) activity, and a pronounced reduction in lipid peroxidation.

Together, these results indicate that Anis-EVs are not passive metabolic byproducts but multifunctional effectors that concurrently dampen epithelial inflammatory signaling and strengthen antioxidant defenses. This dual activity may support parasite persistence by limiting excessive inflammatory and oxidative stress at the host–parasite interface.

Keywords: *Anisakis simplex*, extracellular vesicles, parasite-host interactions, oxidative stress, ELISA.

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O61 ✻ Student Competition

Anisakis simplex-derived EVs reshape early inflammasome signalling in an in vitro model of intestinal inflammation

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Parasitic helminths release extracellular vesicles (EVs) that mediate host–parasite communication and can modulate host immune responses. *Anisakis simplex*, a marine nematode responsible for human anisakiasis, induces inflammation in the gastrointestinal tract; however, the contribution of parasite-derived EVs to intestinal inflammatory signaling remains poorly defined. Recent attention has focused on the NLRP3 inflammasome, a cytosolic multiprotein complex that promotes caspase-1 activation and the maturation and secretion of the pro-inflammatory cytokines IL-1β and IL-18.

This study aimed to determine whether *A. simplex*–derived EVs (Anis-EVs) influence NLRP3 inflammasome–associated responses in an in vitro model of intestinal inflammation. Human Caco-2 cells were exposed to EVs, a pro-inflammatory cytokine cocktail, or a combined treatment. The expression and secretion of IL-1β and IL-18, along with the chemokines IL-8 and monocyte chemoattractant protein-1 (MCP-1), were assessed using real-time PCR and enzyme-linked immunosorbent assay (ELISA). Caspase-1 activity was measured as an additional readout of inflammasome-related signaling.

Anis-EVs altered both cytokine and chemokine responses in Caco-2 cells. The most pronounced modulatory effects were observed during the early phase of inflammation and included changes in IL-1β and IL-18 expression and secretion, accompanied by altered caspase-1 activity. Anis-EV exposure also affected IL-8 and MCP-1 levels, suggesting a potential impact on epithelial inflammatory signaling and

immune cell recruitment cues in the intestinal environment. Notably, discrepancies between transcript and protein profiles indicate that EVs-mediated regulation may occur at multiple levels, including post-transcriptional mechanisms.
Overall, these results suggest that Anis-EVs can modulate NLRP3 inflammasome–associated inflammatory responses in intestinal epithelial cells and may represent a mechanism of host immune regulation during anisakiasis.
This research was funded by the Rector's Student Grant (6th Edition) of the University of Warmia and Mazury in Olsztyn (5/SGRVI/2024) and as part of a project funded by the National Science Centre, Poland (2023/49/B/NZ6/02078). The conference registration fee for M. Stawicka was covered by the Polish Parasitological Society.

O62 ✻ Student Competition

Manipulating Immune Responses: Chemokine Modulation During *Fasciola hepatica* Infection

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Parasitic helminths are masters of immune manipulation, capable of reshaping host responses. Among them, *Fasciola hepatica* a foodborne trematode and causative agent of fasciolosis, causes major economic losses in animal production and poses an emerging threat to human health. The parasite is highly effective at establishing chronic infections due to its remarkable ability to modulate host immune responses from the earliest stages of infection. This immunomodulatory capacity is considered one of the major reasons underlying the limited efficacy of currently tested vaccine strategies. Although cytokine-mediated immunoregulation during fasciolosis has been extensively studied, considerably less is known about the role of chemokines in parasite-driven immune evasion and the disruption of immune cell communication. Therefore, understanding how parasite-derived molecules influence chemokine signaling may provide important insight into the mechanisms underlying immune modulation during infection.
The aim of this study was to investigate how *F. hepatica* excretory–secretory products (*Fh*ESPs) influence chemokine and cytokine secretion by immune cells such as macrophages and dendritic cells. As an initial experimental system, human monocyte-derived dendritic cells (moDCs) and macrophages (moMACs) were generated from peripheral blood monoclear cells (PBMCs) and stimulated with whole *Fh*ESPs, parasite-derived extracellular vesicles (EVs) and extracellular vesicle-depleted fractions (*Fh*ESP w/o EVs). Cytokine and chemokine secretion profiles were analyzed using ELISA test. To further validate these observations in a model more closely reflecting parasite infection in natural mammalian hosts, comparative studies using murine bone marrow-derived dendritic cells (BMDCs) and macrophages (BMMACs) were additionally performed.
Our findings demonstrate that *F. hepatica* antigens significantly modulate the secretion of multiple inflammatory mediators, including CXCL11, CXCL1, CCL20, IL-6 and TNF-α in human immune cells, indicating active interference with early inflammatory signaling and immune cell communication. The strongest immunomodulatory effects were primarily associated with the soluble fraction of *Fh*ESPs rather than extracellular vesicles. Ongoing experiments using mouse bone marrow-derived immune cells are expected to determine whether similar mechanisms of chemokine modulation are conserved in the model that reflects host immune responses observed during fasciolosis in ruminants.
Collectively, these findings support the hypothesis that *F. hepatica* manipulates chemokine-mediated immune responses to promote parasite survival and long-term infection. A better understanding of these mechanisms may help identify immunological pathways limiting vaccine efficacy and contribute to the development of more effective anti-helminth strategies.
Financial support for this study was provided by NCN, project nr: 2021/43/D/NZ6/01555

O63

From zero to hero: uncovering immunobiology of *Dirofilaria repens*

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Helminths are highly adapted parasites capable of establishing chronic infections through modulation of host immune responses. Their evolutionary success is associated with the ability to fine-tune both innate and adaptive immunity, limiting excessive inflammation while promoting parasite survival and long-term host tolerance. This immunomodulatory capacity is largely mediated by excretory-secretory products (ES), which interact with professional antigen-presenting cells, including dendritic cells (DCs) - key regulators of adaptive immunity. Beyond classical type 2 responses, helminth-derived molecules are increasingly recognized as modulators of immunometabolic pathways and chronic inflammation. *Dirofilaria repens* is a mosquito-borne filarial nematode primarily infecting dogs, but increasingly recognized as the major causative agent of human dirofilariosis in Europe. Despite its growing epidemiological significance, the immunobiology of *D. repens* remains largely unexplored.

Therefore, the aim of this study was to evaluate the immunomodulatory potential of *D. repens*-derived antigens, with particular emphasis on excretory-secretory products (*Dr*ES), and to perform the first proteomic characterization of *Dr*ES composition using a mass spectrometry-based approach.

In this study, we explored *Dr*ES, influence the functionality and immunological properties of human monocyte-derived dendritic cells (moDCs). Exposure to *D. repens* antigens altered the maturation profile of moDCs, as reflected by reduced expression of MHC II and co-stimulatory molecules, suggesting impaired capacity for classical pro-inflammatory T-cell activation. Cytokine profiling performed using ELISA and proteome profiler analyses revealed suppression of inflammatory mediators, including TNF-α and IL-6, accompanied by modulation of several non-canonical cytokines. Interestingly, increased expression of Dkk-1 and ApoA1 was observed, indicating that *D. repens*-derived molecules may influence pathways involved in tissue remodeling, cholesterol efflux, and metabolic homeostasis. In addition, the cellular functionality of moDCs was assessed based on phagocytic activity, with no significant differences observed following stimulation. To identify candidate molecules responsible for the observed immunomodulatory effects, we additionally performed the first proteomic characterization of DrES using LC-MS/MS-based analysis combined with bioinformatic annotation against closely related filarial species.

Our preliminary findings provide novel insights into the largely unexplored immunobiology of *D. repens* and highlight parasite-derived molecules as promising targets not only for the development of anti-parasitic vaccines, but also as potential immunomodulatory agents for helminth-inspired therapies in chronic inflammatory and metabolic disorders.

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O64

Impaired regulatory immune response in dogs naturally infected with *Babesia canis*

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Canine babesiosis, caused by the protozoan parasite *Babesia canis*, is one of the most dangerous parasitic diseases in veterinary medicine. Similar to malaria, canine babesiosis is associated with the development of a T helper type 1 (Th1)-dependent immune response that triggers a strong inflammatory reaction. Regulatory T cells (Tregs) are essential for limiting excessive immune response. One of the key mechanisms of Treg activity is the production of regulatory cytokines, such as IL-10 and TGF-β1. Impairment of suppressive function may contribute to increased disease severity and can lead to death.
The aim of this study was to evaluate the regulatory immune response in dogs naturally infected with *B. canis*.
Blood samples were collected from dogs diagnosed with babesiosis and from healthy controls. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood, and the expression of CD5, CD4, CD8, and CD25 surface markers, as well as intracellular transcription factor Foxp3, was assessed by flow cytometry. Concentarion of regulatory cytokines: IL-10 and TGF-β1 was determined using the ELISA method.
The percentage of $CD4^+CD25^+Foxp3^+$ and $CD8^+CD25^+Foxp3^+$ Tregs, as well as $CD4^+CD25^+Foxp3^-$ and $CD4^+CD25^-Foxp3^+$ subpopulations, were analysed. The analyses revealed a reduced percentage of $CD4^+CD25^+Foxp3^+$ Tregs and $CD4^+CD25^+Foxp3^-$ subpopulation in the blood of naturally infected dogs compared to healthy controls. In addition decreased TGF-β1 levels in plasma of *Babesia*-infected dogs were obsered. These findings indicate significant deficience in suppressive immune response in dogs with babesiosis, which may contribute to the severe course of the disease and increased mortality.
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Molecular Parasitology, Physiology and Biochemistry

ORAL PRESENTATIONS

O65 **Z. Fečová, R. Gašparovičová, A. Marková, M. Orosová** — Initial Repeatome Analysis of *Echinorhynchus* borealis (Acanthocephala): Searching for Novel Cytogenetic Markers

O66 ✻ **R. Gašparovičová, Z. Fečová, M. Orosová** — Research on *Khawia japonensis*: decoding the repeatome

O67 **P. Bartošová-Sojková, T. Dobai, S. Nayak, F. Dyčka, M. Horn[3], L. Bieliková, B. Kaščáková,G. G. Ferenczy, A. S. Holzer, Z. Franta** — Blood on the menu: Proteases-mediated hematophagy in the common carp pathogen Sphaerospora molnari

O68 **L. Jedličková, L. Konečný, J. Dvořák, P. Horák, T. Macháček** — Tissue laser microdissection of infected mouse liver and intestine reveals true *Schistosoma mansoni* eggs-cretome

O69 ✻ **T. Dejmková, J. Votýpka, K. Poloprutská, P. Mikulíček** — Unravelling the diversity of trypanosomes in European water frogs (*Pelophylax*) and their development in mosquitoes

O65

Initial Repeatome Analysis of *Echinorhynchus* borealis (Acanthocephala): Searching for Novel Cytogenetic Markers

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Echinorhynchus borealis is a widespread Palaearctic acanthocephalan and one of the most common intestinal parasites of cold-water freshwater fishes. This obligate endoparasite has a complex life cycle involving arthropod intermediate hosts and vertebrate definitive hosts. Acanthocephalans are generally underexplored in terms of genome architecture, cytogenetics, and the composition of their repetitive DNA fractions. In this study, we integrated cytogenetic, molecular, and bioinformatic approaches to characterize the repetitive genome fraction of *E. borealis* and to identify novel cytogenetic markers. Cytogenetic analyses revealed a karyotype comprising three pairs of metacentric autosomes and one pair of sex chromosomes, with males exhibiting 2n = 7 = 6m + 1m(X) and females 2n = 8 = 6m + 2m(XX). Differential staining with chromomycin A3 demonstrated that GC-rich regions are predominantly located in the terminal regions of the chromosomes. Fluorescence *in situ* hybridization (FISH) analysis revealed single cluster of 18S rDNA and 5S rDNA per haploid genome. Specifically, the 18S rDNA cluster was situated on the largest chromosome No 1 in the pericentromeric region, whereas the 5S rDNA cluster was located on chromosome No 3 in the subtelomeric region. Repetitive elements were identified and quantified using the RepeatExplorer2/TAREAN pipeline within the Galaxy platform. This graph-based clustering approach analyzes low-coverage next-generation sequencing reads to characterize repetitive DNA without requiring a reference genome assembly, making it especially valuable for non-model organisms such as parasitic helminths. Analysis of concatenated low-coverage datasets grouped 351,268 reads into 9,487 clusters, of which 335 clusters represented more than 0.01% of the genome. Annotation showed that transposable elements form the dominant repetitive component. LINE retrotransposons were the most abundant (10.68% of the genome), followed by Ty3/Gypsy LTR retrotransposons (4.46%) and satellite DNA (7.14%). Other detected elements included Penelope-like retroelements, Tc1/Mariner DNA transposons, and Helitrons, although a substantial proportion of repeats remained unclassified. Subsequent FISH localized selected repetitive sequences on chromosomes. While most repeats displayed dispersed hybridization patterns, several elements exhibited distinct localized accumulations, representing promising new cytogenetic markers for chromosome identification and comparative cytogenomics. Overall, these results significantly expand the limited cytogenetic and genomic resources available for Acanthocephala. They demonstrate the effectiveness of combining bioinformatics with classical cytogenetics for investigating genome organization and chromosome evolution in non-model parasites, and provide valuable markers for future comparative studies of chromosomal structure and evolution within the group.
This study was supported by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences (VEGA No. 2/0093/23) and the Postdoctoral Fellowship Programme of the Slovak Academy of Sciences, grant No. APD0178.

O66 ✻ Student Competition

Research on *Khawia japonensis*: decoding the repeatome

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Khawia japonensis is a monozoic cestode of the order Caryophyllidea parasitizing common carp (*Cyprinus carpio*) and other cyprinid fishes. It is native to East Asia and is now widely distributed in Europe. For the

first time, the karyotype of *K. japonensis* was characterized using classical and molecular cytogenetic approaches, revealing eight pairs of metacentric and acrocentric chromosomes (2n = 16, n = 5m + 3t).
Clustering analysis identified 2,046,935 reads grouped into 119,479 clusters, of which 293 represented genomic proportions greater than 0.01%. RepeatExplorer annotation showed that the *K. japonensis* genome is dominated by transposable elements, particularly LINEs (32.44%) and Penelope retrotransposons (9.39%), along with a substantial fraction of satellite DNA (10.1%). The satellitome comprised monomers ranging from 149 bp to 3,093 bp, representing classical short, long, and very long satellite repeats. LTR retrotransposons accounted for 4.26% of the genome, whereas SINEs were rare (0.17%). Approximately 4.92% of the genome consisted of unclassified repeats. Although RepeatExplorer did not identify DNA transposons, additional GIRI/RepBase annotation detected similarities to Tc1/Mariner, Helitron, and hAT families.
Of the 20 repetitive elements selected for mapping, six have been successfully localized to date by fluorescence in situ hybridization (FISH): KjapSat199-281, KjapSat214-1711, KjapSat29-603, KjapSat209-1133 (snDNA U1), KjapSat161-2113 (snDNA U2), and KjapSat205-797. Each showed specific chromosomal localization at single loci on different chromosomes. FISH with an 18S rDNA probe revealed four clusters of major ribosomal genes. Hybridization signals were stronger on one chromosome pair, indicating a higher number of rDNA repeats at this nucleolar organizer region (NOR). This finding is surprising, as all *Khawia* species studied to date have exhibited only one cluster per haploid genome. These results are part of ongoing research, and additional repetitive sequences will be mapped in future studies.
This study was supported by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences (VEGA No. 2/0093/23) and by the Postdoctoral Fellowship Program of the Slovak Academy of Sciences, grant No. APD0178.

O67

Blood on the menu: *Proteases-mediated hematophagy in the common carp pathogen Sphaerospora molnari*

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Myxozoans are highly reduced cnidarian endoparasites with complex life cycles and specialized strategies for exploiting invertebrate and fish hosts. Despite expanding genomic resources, their metabolism and nutrient acquisition remain poorly understood. The heme biosynthesis pathway (HBP) is essential for aerobic metabolism, yet many blood-feeding parasites acquire heme from host hemoglobin (Hb). Hb degradation is mediated by cysteine, aspartic, and metallopeptidases. *Sphaerospora molnari*, a myxozoan parasite of common carp *Cyprinus carpio*, proliferates as motile blood stages (SMBS) and forms spores in gills and skin, causing anemia and osmoregulatory dysfunction. SMBS attach to red blood cells (RBCs) and internalize Hb, suggesting that host RBCs provide amino acids and heme. Here, we characterize the hemoglobinolytic cascade of *S. molnari*, assess its HBP, and identify druggable proteolytic targets.
We combined genome, transcriptome, and proteome mining with qPCR profiling to identify genes involved in HBP, heme/iron-related handling, and Hb degradation. SMBS lysate activities were assessed using fluorogenic substrates, specific inhibitors, activity-based probes, and Hb digestion assays. The dominant cathepsin L-like cysteine peptidase, SmolCathL2, was recombinantly produced and characterized for substrate specificity, pH optimum, and Hb-cleaving activity. Antibodies against recSmolCathL2 were used for confocal and TEM immunogold localization. Its structure was solved by X-ray crystallography and used with docking/simulations to identify parasite-selective compounds.
S. molnari encodes all core HBP enzymes but shows reduced HBP gene expression in SMBS relative to sporogonic stages, suggesting lower reliance on *de novo* heme production during blood parasitism. No canonical heme oxygenase was identified, whereas genes linked to heme/iron transport and heme-binding

redox functions were detected. SMBS proteomics identified 4,855 proteins, including 72 peptidases from 7 enzyme classes. Activity assays and inhibitor profiling showed that C1 cysteine and A1 aspartic peptidases dominate SMBS proteolytic activity. Hb digestion required both cathepsin L-like cysteine and cathepsin D-like aspartic peptidases: single-class inhibition partially blocked degradation, whereas combined inhibition abolished Hb cleavage. SmolCathL2 was the most abundant and active cathepsin L-like enzyme in SMBS. RecSmolCathL2 showed an acidic pH optimum, cleaved Hb *in vitro*, and localized to lysosome-like compartments. Its 2 Å structure revealed high similarity to host cathepsin Ls but also uncovered parasite-specific Ser-206 near the active site, used for selective inhibitor design.
S. molnari combines endogenous HBP with host Hb exploitation during SMBS development. Its hemoglobinolytic cascade depends on coordinated cysteine and aspartic peptidase activity, resembling strategies of other hematophagous parasites. SmolCathL2 is the first recombinant and structurally characterized protease from a myxozoan parasite and a promising target against this pathogen.
This study was supported by the Grant Agency of the Czech Republic (GAČR) project No. 24-13238L.

O68

Tissue laser microdissection of infected mouse liver and intestine reveals true *Schistosoma mansoni* eggs-cretome

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Schistosoma mansoni eggs are the central drivers of schistosomiasis pathology and transmission, yet the proteins they release within host tissues remain poorly defined due to limitations of conventional in vitro approaches. Here, we provide the first in situ characterization of egg-derived proteins present in host tissues. Using laser microdissection, we selectively isolated periovular granuloma microenvironments from the livers and small intestines of infected mice during the acute and chronic phases of infection, followed by high-resolution mass spectrometry. Across all samples, we identified a conserved core set of 149 parasite proteins, representing a true in vivo egg excretory/secretory repertoire. Egg-associated protein profiles varied markedly by organ and infection phase. In the liver, acute infection was characterized by a richer and more abundant repertoire of immunomodulators (including omega-1 and IPSE), proteases, protease inhibitors, antioxidant proteins, and micro-exon gene and venom allergen-like proteins, whereas chronic liver granulomas showed a reduced and altered profile. In contrast, intestinal granulomas exhibited fewer qualitative changes between phases and a more uniform protein composition over time, despite an overall decline in protein detectability. These patterns likely reflect fundamental differences in egg fate and tissue context, as hepatic eggs remain permanently trapped whereas intestinal eggs transit out of the host. This study provides the first in situ snapshot of schistosome egg-derived proteins within host tissues, revises assumptions about intestinal granuloma chronicity, and defines core and context-dependent egg proteins that are likely to shape *S. mansoni* granuloma biology, host modulation, and parasite transmission.
This study was supported by the Czech Science Foundation (23-06638S), by the Schistosomiasis Research Grant (FR7060453) by ARES Trading S.A., an affiliate of Merck KGaA, Darmstadt, Germany, and by Charles University institutional funding (Cooperatio Biology, UNCE24/SCI/011, SVV 260678 and 260796).

O69 ✻ Student Competition

Unravelling the diversity of trypanosomes in European water frogs (*Pelophylax*) and their development in mosquitoes

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Trypanosomes are widespread protozoan parasites infecting all vertebrate groups. While research has been primarily focused on species affecting humans and livestock due to their medical and economic impact, amphibian trypanosomes remain largely neglected. In our study, we examined 1,283 individuals of the genus *Pelophylax*, representing eight host species, for the presence of trypanosomes. The overall prevalence was 73 %, and molecular analyses revealed 13 distinct *Trypanosoma* species, including eight previously undescribed species. The highest diversity was observed in frogs from the Balkan Peninsula, whereas no evidence of host specificity was observed. To investigate the development of anuran trypanosomes in potential insect vectors, we experimentally infected three mosquito species (*Culex molestus*, *Cx. quinquefasciatus* and *Aedes aegypti*). Based on our results, four out of the nine tested *Trypanosoma* species successfully survived defecation, attached to the hindgut wall via their flagella forming hemidesmosomes, and proliferated. No migration to either the salivary glands or the stomodeal valve was observed, indicating that transmission to amphibian hosts likely occurs through contaminative rather than inoculative routes. By integrating large-scale screening of *Pelophylax* for trypanosome diversity with experimental infections of mosquitoes, our study provides a comprehensive view of anuran trypanosome biology. The remarkable diversity of trypanosomes uncovered in the genus *Pelophylax*, together with evidence of development in the mosquito hindgut and contaminative transmission, offers new insights into the ecological and evolutionary processes shaping host–parasite–vector interactions.

POSTER PRESENTATIONS

TH1 **B. Fedorková, A. Schreiberová, G. Štrkolcová** — Host specificity and zoonotic potential of *Giardia duodenalis*

TH2 **J. Górska, A. Lass** — Prevalence of Giardia intestinalis in privately owned and shelter dogs from Pomeranian Voivodeship, Poland

TH3 **K. Lesiak, J. Urbaniak, R. Maciaszek, A. Cichy, E. Żbikowska, A. Stanicka** — Imported ornamental molluscs as a source of invasive Digenea larvae: preliminary research results

TH4 **A. Paulauskas, M. Laukutė, I. Ražanskė, U. Medikaitė, L. Griciuvienė, I. Lipatova, R. Špinkytė, J. Radzijevskaja** — Molecular Detection and Serological Evidence of Protozoan Parasites in Free-Ranging Grey Wolves (*Canis lupus*)

TH5 **K. Sołowińska, L. Holec-Gąsior** — Development of bacterial expression systems for the production of a novel *Borrelia burgdorferi* glycosaminoglycan-binding protein with potential diagnostic utility

TH6 **K. Sołowińska, L. Holec-Gąsior** — Novel Recombinant Dense Granule Proteins in the Detection of specific anti-*Toxoplasma gondii* IgG antibodies in Sera of Small Ruminants

TH7 **E. Dvorožňáková, M. Petrová, A. Kandričáková, A. Lauková** — Cytokine production *ex vivo* of mice treated with postbiotic substances (Enterocins-like) and infected

TH8 **E. Dvorožňáková, M. Petrová, A. Kandričáková, A. Lauková** — Lymphocyte subpopulations in mice treated with postbiotic substances (Enterocins-like) and infected

TH9 **A. Kandričáková, E. Dvorožňáková, M. Petrová, A. Lauková** — Protective effect of postbiotic substances (Enterocins-like) from beneficial/probiotic strains on *Echinococcus multilocularis* infection

TH10 **A. Kandričáková, M. Petrová, G. Hrčková, E. Dvorožňáková, A. Lauková** — Modulatory effects of enterocin-producing strains on cytokine's expression and production in mice with alveolar echinococcosis

TH11 **S. Braun, A. Kočišová** — Big flukes infecting small frogs: Histopathological evidence of parasite host interactions in lung fluke infested *Occidozyga lima* frogs.

TH12 **A. Wesołowska, K. Slivinska, K. Basałaj, A. Laskowska, A. Kalinowska, M. Pękacz, A. Zawistowska-Deniziak, T. Pniewski, M. Przewoźnik, H. Pudelska, K. Karczmarzyk, M. Kęsik-Brodacka** — Sheep oral vaccination against fasciolosis using plant-produced cysteine protease of *Fasciola hepatica* delivered via virus-like particles

TH13 **L. Koščová, M. Oros, D. Barčák, K. Holovská, J. Fedorčák, J. Koščo, J. Čurlík** — Infection of Black bullhead with metacercariae of *Hysteromorpha triloba*

TH14 **O. Kousal, T. Kozelková, V. Urbanová, P. Kopáček** — Unraveling Tick Lipid Metabolism

TH15 **A. Marková, V. Kulinich, A. Mášová,P. Abaffy, L. Valihrach** — Unlocking parasite biology: Comprehensive transcriptomic services at GeneCore facility

TH16 **P. Matoušková, N. Slaninová, L. Skálová** — Can NHR-3 be part of the xenobioitic response mechanisms *in Haemonchus contortus?*

TH17 **N. Slaninová, P. Matoušková, L. Skálová** — Nuclear Hormone Receptors in *Haemonchus contortus*: Transcriptomic Profiling and Optimization of Ligand Interaction Analysis

TH18 **V. Šnábel, T.A. Kuzmina, A. Königová, Ľ. Burcáková, S. Cavallero, Y. Syrota, Z. Vasilková** — Genetic diversity assessed in the equine ascarids from central-eastern Europe, with *Parascaris univalens* being

TH19 **A. Stachyra, M. Pękacz, A. Laskowska, K. Basałaj, A. Zawistowska-Deniziak** — Exploring effector-like proteins in *Trichinella*: recombinant production of genus-specific immunoreactive candidates

TH20 **A. Laskowska, M. Pękacz, K. Basałaj, A. Kalinowska, A. Zawistowska-Deniziak** — *Fasciola hepatica* FABP1 Reprograms Human Dendritic Cells Through PPARγ/RXR/AhR Signaling to Promote Tolerogenic Immune Responses

TH1

Host specificity and zoonotic potential of *Giardia duodenalis*

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Giardia duodenalis is a protozoan pathogen with worldwide distribution. It is one of the most common cause of parasitic diarrhea in animals and humans. The species complex of *G. duodenalis* consists of eight assemblages, designated A - H, which differ in host specificity. Only assemblages A and B are considered to be zoonotic. In this study we focused on the molecular identification of *G. duodenalis* isolates in various animal groups and their role in zoonotic transmission of this protozoan.
The first group was pet animals where we examined dogs, cats and pet rodents. After previous coprological examination we performed PCR method on the *Giardia* positive samples using *tpi, gdh* and *bg* gene primers. We identified zoonotic assemblage B in pet chinchillas which were the source of infection for their owner. In dogs the most common were assemblages C and D which are specific to canids. In cats the most prevalent assemblage was assemblage F but we also identified assemblage C which is considered to be host specific.
We also focused on the giardiasis in livestock. These animals represent an important reservoir of *G. duodenalis* but this pathogen has not been sufficiently studied in this host group. We microscopically examined 72 fecal samples from calves from a farm in the east Slovakia out of which 17 were positive for *Giardia* cysts (23,61 %) and mixed samples of sheep and lambs from 5 farms. On the two farms lambs were positive for *Giardia.* By multilocus sequence analysis we identified assemblage E which is typical for ungulates but has also been descibed in humans and assemblage C in lambs. Assemblage C is considered to be specific to candis but there have also been reports of human giardiasis caused by this genotype. *Giardia* assemblages seem not to be strictly host specific and thus are able to spread among various host groups and represent risk to humans and public health.
This study was supported by the Grant Agency VEGA project No. 1/0709/23 and Internal Grant Agency Project No. 03/2026.

TH2

Prevalence of *Giardia intestinalis* in privately owned and shelter dogs from Pomeranian Voivodeship, Poland

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Giardia intestinalis is a cosmopolitan intestinal protozoan of significant zoonotic importance. The nonspecific clinical presentation in humans complicates the implementation of targeted treatment. Dogs are considered a potential reservoir of the parasite; however, data regarding the prevalence of *G. intestinalis* among dogs in Poland remain limited.
The aim of this study was to assess the prevalence of *G. intestinalis* in privately owned and shelter dogs in Pomeranian Voivodeship and to determine the influence of the dogs' living environment on the occurrence of this parasitic protozoan.
The study included 300 canine faecal samples collected in Pomeranian Voivodeship (150 samples from privately owned dogs and 150 from shelter dogs). DNA was isolated from faecal material and subsequently analyzed using the nested-PCR method. Amplification products were evaluated by electrophoresis in 1,5% agarose gel.
G. intestinalis was detected in both shelter and privately owned dogs. In shelter dogs, 11/150 samples tested positive (7.3%), whereas among privately owned dogs, 3/150 samples were positive (2.0%). The overall prevalence of *G. intestinalis* in dogs from Pomeranian Voivodeship was 4.7% (14/300).
The obtained results, which are preliminary*, indicate a markedly higher prevalence of *G. intestinalis* in the shelter dog population compared with dogs kept under household conditions. The observed differences

are likely associated with the influence of environmental factors and animal density on the transmission dynamics of the parasite. Determination of the zoonotic potential requires molecular sequencing of the analyzed samples to identify *G. intestinalis* subtypes, which will be carried out as part of the project.
**The results are part of the project "Prevalence analysis of parasitic protozoa (Giardia intestinalis and Cryptosporidium spp.) in privately owned and shelter dogs in Pomeranian Voivodeship and assessment of the potential risk of transmission to humans and other animals"; Young Scientist Program, IDUB Programme.*

TH3

Imported ornamental molluscs as a source of invasive Digenea larvae: preliminary research results

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Exotic snails are among the most valued ornamental molluscs in aquaristics. Many of these animals are collected from natural habitats in regions where they occur endemically and may be exposed to diverse pathogens and parasites. Among snail-associated parasites, digenean trematodes are of particular interest because molluscs act as intermediate hosts in their complex life cycles, which are completed in various vertebrate groups, including species of veterinary or medical importance.
This preliminary study aimed to detect developmental stages of Digenea in ornamental molluscs imported from Thailand and commercially available in Poland. A total of 742 snails representing nine taxa were subjected to parasitological examination: *Anentome helena* (100 individuals), *Clithon corona* (78), *Faunus ater* (80), *Filopaludina martensi* (77), *Neritina natalensis* (87), *Neritina pulligera* (120), *Plotia scabra* (80), *Tylomelania* sp. "Golden Rabbit" (60), and *Tylomelania* sp. "Yellow Spot" (60). Each individual was examined for cercarial emergence and then dissected to detect larval or adult parasite stages. The isolated parasites were preliminarily identified morphologically, and selected digenean material was preserved for further molecular verification.
Parasites were detected in 29 molluscs, corresponding to 3.91% of the examined material. Digenean trematodes were the dominant group, occurring in 25 individuals and accounting for 86.21% of all detected parasitic infections. Digenean stages were found in three snail taxa. *Tylomelania* sp. "Yellow Spot" acted as a first intermediate host, releasing xiphidiocercariae, whereas *C. corona* harboured metacercariae and therefore served as a second intermediate host. *Filopaludina martensi* was the most important source of digenean larvae, as both cercariae and metacercariae were detected in this species.
The results show that imported ornamental snails can retain viable parasite stages despite transport and commercial handling. Consequently, they may represent a potential source of non-native parasites and pose a risk to aquarists, sellers and other aquarium organisms. If released or accidentally introduced into natural habitats, infected exotic molluscs may contribute not only to biological invasions, but also to the spread of parasites with possible veterinary, medical and epidemiological significance.
This study was supported by grant no. 4101.000000261 under the Excellence Initiative – Research University programme, Nicolaus Copernicus University in Toruń.

TH4

Molecular Detection and Serological Evidence of Protozoan Parasites in Free-Ranging Grey Wolves (*Canis lupus*)

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The European grey wolf (*Canis lupus*) plays a key ecological role as an apex predator, contributing to the stability and balance of natural ecosystems. In addition to their ecological importance, wolves may serve as reservoirs or incidental hosts for several protozoan pathogens of veterinary and zoonotic significance, including *Babesia* spp., *Hepatozoon* spp., and *Toxoplasma gondii*. This study investigated the occurrence, genetic diversity, and exposure to these protozoan parasites in free-ranging wolves from Lithuania using molecular and serological methods.

Spleen samples from 70 wolves were screened for *Babesia* spp. and *Hepatozoon* spp. using PCR and subsequent phylogenetic analyses. Overall, 84.3% (59/70) of wolves were positive for at least one pathogen. *Hepatozoon canis* was highly prevalent, detected in 82.9% (58/70) of individuals, while *Babesia canis* was identified in 30.0% (21/70). Co-infection with both pathogens occurred in 28.6% (20/70) of wolves. Molecular characterization revealed two genotypes of *B. canis* and four genotypes of *H. canis*. To the best of our knowledge, this study provides the first molecular evidence of *B. canis* and *H. canis* infections in wolves from Lithuania and represents the northernmost record of these pathogens in European wolf populations.

In addition, brain samples from 71 wolves were analysed for antibodies against *Toxoplasma gondii* using ELISA. Seropositive results were detected in 25.4% (18/71) of wolves, 7.0% (5/71) were equivocal, and 67.6% (48/71) were seronegative. Sample-to-positive (S/P) values ranged from −0.62% to 77.99%, indicating variable levels of exposure among individuals. Notably, one sampled wolf had been hunted in a village following reports of aggressive behavior, suggesting a possible association between infection and behavioral alterations, although further studies are required to clarify this relationship.

These findings provide important insights into the circulation of protozoan pathogens in wild wolf populations and highlight the importance of continuous wildlife surveillance for zoonotic and vector-borne agents that may affect wildlife, domestic animals, and human health.

TH5

Development of bacterial expression systems for the production of a novel *Borrelia burgdorferi* glycosaminoglycan-binding protein with potential diagnostic utility

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Lyme disease is a multisystemic illness induced by the spirochete bacteria *Borrelia burgdorferi* sensu lato (s.l.), primarily transmitted through the bite of infected *Ixodes* ticks. The only pathognomonic clinical marker is a bull's-eye rash - erythema migrans (EM), occurring in 70–80% of patients within the first two weeks of infection. In the absence of this hallmark rash, clinical diagnosis becomes challenging as other symptoms mimic common viral illnesses. Because *B. burgdorferi* s.l. is present in extremely low concentrations in clinical samples, direct microbiological detection is rarely feasible, making laboratory diagnosis dependent on the detection of the host's humoral immune response.

The current gold standard for diagnosis is two-tiered serological testing designed to optimize accuracy while minimizing cross-reactivity. This approach begins with a sensitive enzyme immunoassay (EIA) utilizing whole-cell sonicate (WCS) or crude fractions of *B. burgdorferi* s.l. as the antigen substrate. However, because these crude preparations often lack specificity and are prone to cross-reactivity, a second-stage confirmatory Western immunoblot is necessitated to ensure diagnostic reliability. Conducting

separate tests for screening and confirmation is both resource-intensive and time-consuming. These challenges are compounded by the diversity and low degree of protein conservation between genospecies of *B. burgdorferi* s.l.
Current research aims to streamline the two-tier diagnostic paradigm to reduce costs and turnaround times. Recombinant antigens, developed by genetic engineering, offer significant promise due to their high diagnostic utility. However, a validated one-stage test utilizing these proteins has yet to be established that can successfully replace the existing two-stage gold standard.
In this research, an *Escherichia coli* expression system was developed for the production of Bgp, a novel *Borrelia* protein. Bgp is a glycosaminoglycan-binding protein secreted into the extracellular environment; it acts as an important virulence factor that facilitates adherence, tissue colonization, and disease severity. Target genes were amplified from *B. burgdorferi* s.l. genomic DNA and cloned into a pUET1 vector to introduce an N-terminal polyhistidine tag, enabling one-step metal affinity chromatography purification. We produced two Bgp fragments: an 8.3 kDa fragment A (amino acids 33–72) and a 22.4 kDa fragment B (amino acids 93–265). After optimizing expression conditions, the systems successfully yielded milligram quantities of purified proteins with a potential diagnostic value.
This study was funded by The National Science Centre, Poland, under the research project no. 2023/49/B/NZ6/02881.

TH6

Novel Recombinant Dense Granule Proteins in the Detection of specific anti-*Toxoplasma gondii* IgG antibodies in Sera of Small Ruminants

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Toxoplasma gondii, an obligate intracellular apicomplexan protozoan, represents both a veterinary and public health challenge. The parasite remains one of the primary agents of reproductive failure in the small ruminant industry. In sheep and goats, toxoplasmosis frequently results in abortion, fetal mummification, or the birth of congenitally infected, non-viable offspring. These reproductive disturbances are a source of considerable economic loss for producers, stemming from reduced flock productivity and the increased costs associated with veterinary intervention and disease management. Beyond the veterinary impact, small ruminants serve as intermediate hosts and reservoirs for human infection. The presence of bradyzoite-containing tissue cysts in the muscles of these animals poses a significant public health risk, as the consumption of undercooked meat products is a major route for zoonotic transmission.
The effective management of *T. gondii* in livestock populations is hampered by the lack of standardized, cost-efficient diagnostic platforms. Currently, serodiagnosis relies heavily on the use of *Toxoplasma* Lysate Antigens (TLA), typically derived from tachyzoites propagated in the peritoneal fluid of infected mice or via *in vitro* cell cultures. While TLA provides a broad spectrum of epitopes, its production is fraught with technical and ethical drawbacks, including high operational costs, the risk of contamination with host-cell proteins (leading to false-positive reactions), and significant batch-to-batch variability. Consequently, considerable effort has been directed toward the development of more reliable, sensitive, and cost-effective diagnostic tools. A widely recognized alternative is the application of recombinant antigens expressed in heterologous systems, which allow for the large-scale production of purified, specific proteins.
The diagnostic performance of novel recombinant GRA29, GRA45, and GRA54 antigens, expressed in a prokaryotic system, was assessed by indirect IgG ELISA using a characterized panel of ovine and caprine sera. Results were subjected to a comparative analysis with a traditional TLA-ELISA to determine sensitivity, specificity, and overall diagnostic accuracy. Among the tested proteins, GRA29 demonstrated the highest diagnostic utility. These findings indicate that single recombinant antigens represent a viable alternative to TLA for detecting specific anti-*T. gondii* antibodies in small ruminants.

TH7

Cytokine production *ex vivo* of mice treated with postbiotic substances (Enterocins-like) and infected with *Echinococcus multilocularis*

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Postbiotics have emerged as a promising alternative to live probiotics and were defined as "a preparation of inanimate microorganisms and/or their components that confers a health benefit on the host". Postbiotics influence physiological, immunological, regulatory, and metabolic processes in the host organism through antimicrobial, antioxidant, and immunomodulatory activities. Enterocins are postbiotic substances from the *Enterococcus* strains, relatively small (30 to 60 aminoacids) cationic molecules. Data about the immunomodulatory activity of postbiotics in parasitic infections are missing. The present study aimed to evaluate cytokine's production in mice after immunomodulation by enterocins and *Echinococcus multilocularis* infection.
Mice were infected intraperitoneally with 3000 *E. multilocularis* protoscoleces. The enterocins Enterocin M (51 200 AU/ml) and Durancin-like (25 600 AU/m) were administered daily in dose 50 µl *per os* and their producing strains *Enterococcus faecium* AL41 (CCM8558) and *Enterococcus durans* ED26E/7 in dose of 10^9 CFU/ml in 100 µl *per os* during the first month of the infection. Mitogen-stimulated production of cytokines was used to assess T-cell function, cytokine concentration in supernatants of splenocyte cultures was detected by capture ELISA (commercial kits).
Th1/Th2 balance is critical during *E. multilocularis* infection and the immune escape mechanisms involves the transition from the protective Th1 response to the non-protective Th2 response. Enterocins/enterococci treatment significantly increased the levels of IFN-γ from 30 dpi. The greatest stimulatory effect on IFN-γ and TNF-α was shown by Enterocin M and *E. faecium* AL41 from 15 to 60 dpi. Enterocins/enterococci inhibited IL-10 production up to 60 dpi compared to untreated infected mice, with the most pronounced effect after application of *E. faecium* AL41, Durancin-like and *E. durans* ED26E/7. Similarly, IL-4 and IL-5 cytokine production were suppressed in all treated groups up to 90 dpi, i.e. two months after the end of treatment. On the contrary, IL-4 production was increased in mice after application of Durancin-like and *E. durans* ED26E/7 from 60 dpi, i.e. up to one month after the end of treatment, which could be caused by growing parasite larvocysts rather than an effect of treatment.
The obtained results reveal promising immunomodulatory activity of the examined enterocins with long-term effect. After the application of enterocins/enterococci, a shift of the Th1/Th2 response towards the desired Th1 profile was observed, indicating the development of active defense and immunological control of the growth of parasite larval cysts. Enterocins, comparable to their producing strains, induced suppression of the Th2 response (IL-4, IL-5, IL-10) and, conversely, intensified the Th1 response (IFN-γ, TNF-α) with a restrictive effect on parasite growth for 2 – 3 months after the end of treatment.
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TH8

Lymphocyte subpopulations in mice treated with postbiotic substances (Enterocins-like) and infected with *Echinococcus multilocularis*

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Probiotic bacteria significantly reduce the pathogenicity of parasites and the development of parasitic infection. Extensive research into the immunomodulatory properties of probiotic bacteria has shown that even non-living bacteria, their cellular components, metabolites, proteins or peptides, referred to as

postbiotic substances, can bring health benefits. Postbiotics have become a promising alternative to live bacteria. Bacteriocins = enterocins produced by *Enterococcus* strains increase their probiotic potential, contribute to the modulation of microbiota and regulate the immune system. The immunomodulatory effect of enterocins and their producing strains in experimental alveolar echinococcosis were tested as a new therapeutic strategy.
Mice were infected intraperitoneally with 3000 *E. multilocularis* protoscoleces. The enterocins Enterocin M (51 200 AU/ml) and Durancin-like (25 600 AU/m) were administered daily in dose 50 µl *per os* and their producing strains *Enterococcus faecium* AL41 (CCM8558) and *Enterococcus durans* ED26E/7 in dose of 10^9 CFU/ml in 100 µl *per os* during the first month of the infection. The changes in lymphocyte subpopulations in the *epithelium* (IEL) and *lamina propria* (LPL) of the small intestine, and in the spleen were determined by flow cytometry.
E. multilocularis infection significantly suppressed IEL and LPL CD4+, CD8+ and CD19+ from 60 dpi, during the advanced phase of larval cyst proliferation. The presence of CD4+T subpopulation was stimulated not only during the application of enterocins/enterococci (first month after infection), but up to 90 dpi with the greatest effect after the application of Enterocin M and *E. faecium* AL41.The subpopulation of LPL CD8+ was not inhibited after the infection, but were restored until 60 dpi in all groups with enterococci/enterocins. *E. durans* ED26E/7 and Durancin-like increased IEL CD8+ subpopulation until 45 dpi. Dynamics of B cells (CD19+) was not changed by enterocins/enterococci treatment. Splenic lymphocytes were affected by enterocins/enterococci. Enterocin M and *E. faecium* AL41 increased CD4+ T cells from 30 to 90 dpi and reduced CD8+ T cells from 60 to 90 dpi. *E. durans* ED26E/7 and Durancin-like stimulated both CD4+ and CD8+ T cell subpopulations in the spleen from 30 to 60 dpi.
Activation of CD4+ T helper cells is associated with the control of *E. multilocularis* infection and the increased proportion of CD8+ T lymphocytes occures with progressive cyst growth. The examined enterocins and enterococci stimulated the CD4+ and CD8+ T cells to improve immune function and positively modulated the lymphocytes into the antiparasitic defense and could contribute to the parasitostatic effect on *E. multilocularis* larval cysts.
This study was supported by the Grant Agency VEGA project No. 2/0077/23.

TH9

Protective effect of postbiotic substances (Enterocins-like) from beneficial/probiotic strains on *Echinococcus multilocularis* infection

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Alveolar echinococcosis (AE) caused by *Echinococcus multilocularis* is a serious zoonoses with high mortality in patients with late treatment. The treatment with available anthelmintics is long-term and relatively little effective, it has only a parasitostatic effect. A surgical extirpation of cysts as well as mini-invasive methods are not always successful owing to ability to form the metastatic foci. In recent years, new approaches in the therapy of AE have been sought. The protective anti-parasitic effect of postbiotic substances Enterocin M and Durancin-like produced by *Enterococcus* strains was examined as an alternative biological therapy for alveolar echinococcosis in experimental murine model.
Mice were infected intraperitoneally with 3000 *E. multilocularis* protoscoleces. The enterocins Enterocin M (51 200 AU/ml) and Durancin-like (25 600 AU/m) were administered daily in dose 50 µl *per os* and their producing strains *Enterococcus faecium* AL41 (CCM8558) and *Enterococcus durans* ED26E/7 in dose of 10^9 CFU/ml in 100 µl *per os* during the first month of the infection. The development of parasitic infection was assessed by the weight of larval cyst mass isolated from mice and the *E. multilocularis* specific antibody response by iELISA.
Our study confirmed the antiparasitic effect of enterocins/enterococci, which limited the proliferation of larvocysts up to 3 months after the end of therapy. Their reducing effect compared to untreated infected mice varied during the development of the infection in individual treatments as follows: Enterocin M 52.4–78.2%, *E. faecium* AL41 48.4–68.2%, Durancin-like 33.2–67.4%, *E. durans* ED26E/7 31.1–88.8%. Specific antibodies are part of the immunopathological mechanisms responsible for the chronic granulomatous course of the disease. Our results document high serum IgM levels at the beginning of

infection with maximum values at 30 dpi, while in all treated mice the production of IgM antibodies was lower compared to untreated mice. IgG antibodies reached high values at 30 dpi and remained elevated until the end of the experiment (4 months after infection) equally in all infected mice without the influence of therapy. A significant decrease in IgG_1 levels compared to the untreated group was observed in mice infected with Enterocin M and *E. faecium* AL41 from 30 to 90 dpi. On the contrary, the formation of IgG2a antibodies was significantly stimulated after the application of Enterocin M, Durancin-like and *E. faecium* AL41 up to 60 dpi. The dynamics of the IgG2b subclass showed a shift in increase in mice infected with Enterocin M, *E. faecium* AL41 and *E. durans* ED26E/7 from 60 to 90 dpi. The specific antibody response reflects the development of E. multilocularis infection in the host and documents the protective role of IgG_{2a} and IgG_{2b} isotypes.
The obtained results confirm the antiparasitic potential of enterocins as promising candidates for improving the effectiveness of AE treatment. Enterocins as a complementary therapeutic approach may contribute to reducing the risk of infection or to reducing the therapeutic dose of anthelmintics.
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TH10

Modulatory effects of enterocin-producing strains on cytokine's expression and production in mice with alveolar echinococcosis

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Alveolar echinococcosis (AE) is a serious parasitic zoonosis without effective larvicidal chemotherapy, and therefore it is necessary to develop new treatment methods. A promising direction is the use of bioactive natural substances with immunomodulatory effects. Antiparasitic potential of probiotic bacteria has been increasingly used in recent years. Probiotic bacteria counteract the development of parasitic infection by strengthening the intestinal barrier and modulating the intestinal microbiota, producing antimicrobials - bacteriocins, modulating the local and systemic immune response, and are involved in maintaining Th1/Th2 balance. The study was focused on the changes in mRNA expression of cytokines IFN-γ (Th1 response) and IL-4 (Th2 response) in the small intestine and the spleen and cytokine's production *in vitro* by splenocytes of mice with *Echinococcus multilocularis* infection and treatment with beneficial enterococci.
Mice were infected intraperitoneally with 3000 *E. multilocularis* protoscoleces and the strains *Enterococcus faecium* AL41 (CCM8558), *Enterococcus faecium* 2019 (CCM7420), *Enterococcus durans* ED26E/7, and *Enterococcus mundtii* EM41/3 were administered daily in dose of 10^9 CFU/ml in 100 µl *per os* during the first month of the infection. Capture ELISA (commercial kits) was used to determine cytokine's production and qPCR (End-point real-time PCR) was used to study gene expression for cytokines in the gut and spleen. The development of parasitic infection was assessed by the weight of larval cyst mass isolated from mice.
Compared to untreated infected mice, IFN-γ gene expression in the intestine was stimulated only at 30 dpi after application of all strains, but in the spleen it was significantly increased from 45 to 90 dpi, the time of intensive larval cyst growth. IL-4 gene expression in the intestine was affected after the end of treatment from 30 to 60 dpi with up-regulation of IL-4 by strains *E. durans* ED26E/7 and *E. mundtii* EM41/3 and marked suppression after treatment with *E. faecium* AL41. Higher IL-4 expression was found in the spleen from 45 to 90 dpi in all treated mice, except for *E. durans* ED26E/7. *In vitro* production of the cytokine IFN-γ was stimulated by all strains from 30 dpi, i.e. at the time of active growth of *E. multilocularis* cysts, and remained high in *E. faecium* AL41 and *E. faecium* 2019 treatment even 3 months after the end of their application. In contrary, IL-4 production was suppressed by all strains compared to infected mice without treatment, with significant inhibition at 90 and 120 dpi.
In addition to the immunomodulatory activity of the beneficial strains, their antiparasitic efficacy was also found. The parasitostatic effect was still evident 3 months after the end of therapy, when slower growth of *E. multilocularis* larval cysts was observed compared to the untreated group of mice with AE. The highest reduction effect was shown by strains *E. faecium* 2019 (85.9%) and *E. faecium* AL41 (68.2%). Lower efficiency was found in strains *E. mundtii* EM41/3 (58.1%) and *E. durans* ED26E/7 (32.1%). The examined

enterococci can influence the host's immune microenvironment and represent a promising tool in the complementary therapy or prevention of AE. The enterococci, particularly *E. faecium* AL41 and *E. faecium* 2019 suggests a potential to modulate the balance toward a Th1 response. The dominant Th1 response (2-3 months after infection) could have activated effector mechanisms of host immunity and slowed the development of parasitic infection.
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TH11

Big flukes infecting small frogs: Histopathological evidence of parasite host interactions in lung fluke infested *Occidozyga lima* frogs.

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Occidozyga lima (Gravenhorst, 1829) are a small, day-active, dicroglossid frog species native to Southeastern Asia. The frogs often live in biodiversity hot spots where the frogs potentially participate in various parasitic life cycles. As there is no commercial breeding of this popular, small frog species in europe, almost all traded specimens are wild-caught and imported frogs, which means parasitic infections in *O. lima* pet frogs are common problems. This study aims to show that *Occidozyga lima* are natural final hosts to haematophagous *Haematoloechus sp.*, frog lung flukes with complex trixenous life cycles, which leads to unique histopathological changes in response to the parasite.
From 2021 to 2025, 140 adult, wild-caught *Occidozyga lima* frogs that had naturally succumbed within the first 3 months post import, were sent by hobbyists and importers for routine postmortem examinations. Following necropsy the left or the right respiratory tract including the lung was removed from each frog and fixed in 10% neutral buffered formalin, dehydrated with a graded ethanol series and embedded in paraffin. 2 to 4 µm thick tissue sections were routinely stained with haematoxylin and eosin. The tissue sections were examined using a Leica DM4B light microscope connected to a Flexacam C3 c-mount camera and a computer equipped with a Leica Application Suite LAS X software.
From 142 examined frogs, 94 frogs had parasites (prevalence 66.2%) and 23 out of 142 frogs were infected with *Haematoloechus* lung flukes (prevalence 16.2 %). The mean infection intensity was 1.4 lung flukes per frog (1 – 3 flukes per frog). 29 out of 32 flukes were located within the lung lumen, 2 were found directly caudally to the larynx and one completely obstructed one main bronchus. The average body size of dissected *O. lima* was 2.39 cm, while the trematods reached a maximum body length of 7.5 mm. In all parasitized frogs, the trematode body length exceeded 50% of the inflated lung length. While fulminant inflammatory reactions were not seen in *O. lima*, the large contact surfaces between parasite and host tissue during respiratory movements lead to compression atrophy of the respiratory epithelium and collapse of alveoles together with acute necroses and epithelial detachment at the luminal lung surface. Later, hypertrophy of the luminar epithelium and sometimes also the connective tissue and smooth muscular components of the frog lung wall were also common. Large amounts of blood cells were detected within the digestive tract lumen of *Haematoloechus* specimens and hyperemia and bleedings were common findings at or close to parasite attachment sites.
Both, parasite and host naturally occur at the same habitats, potentially leading to common lung fluke infections in *O. lima*. The lack of marked inflammatory cell infiltrations in response to the large blood feeding parasite could be due to immune suppression in the stressed, newly imported frogs or potentially also be due to long-term co-evolution of a natural final host and the parasite. However, the histopathologically detected lesions caused by recurrent mechanical contact between parasite and host together with the blood loss are severely impacting frog health and often lead to death of the frog over time.

TH12

Sheep oral vaccination against fasciolosis using plant-produced cysteine protease of *Fasciola hepatica* delivered via virus-like particles

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Fasciolosis, caused by the liver fluke *Fasciola hepatica*, is a globally important zoonotic disease that significantly reduces livestock productivity and poses also a public health concern. Strikingly, despite decades of research, no effective commercial vaccine against *F. hepatica* is currently available. Current control strategies rely mainly on antihelmintic treatment; however, increasing drug resistance and frequent reinfections highlight the urgent need for alternative approaches.

In this context, immunization via intestinal mucosa represents a particularly attractive strategy, as *Fasciola hepatica* infection is initiated via the gastrointestinal tract. Here, we explore an oral vaccination strategy in sheep using *F. hepatica* cysteine protease as the vaccine antigen. To enhance its immunogenicity, the protein was fused to a carrier protein—hepatitis B core antigen, which self-assembles into highly immunogenic virus-like particles with a strong capacity to display attached heterologous proteins.

The vaccine construct was expressed in transgenic lettuce, and the resulting lyophilized plant material was administered to sheep of both sexes. Control and two experimental groups were included: one group was vaccinated exclusively orally three times, while the second received one intramuscular dose followed by two oral booster administrations. All sheep were subsequently infected with *F. hepatica* to evaluate the effect of vaccination on infection progression. The experiment ended with necropsy 16 weeks post-infection.

Statistically significant reductions in worm burdens between groups were not observed. However, female sheep vaccinated with the mixed injection-oral regimen appeared to benefit from immunization. Liver damage observed was considerably reduced compared with other groups, as indicated by lower liver damage scores and reduced levels of liver enzymes. Reduced liver damage supports better preservation of normal physiological liver function. Furthermore, fecal egg counts and the number of eggs recovered from the gallbladders at necropsy were significantly reduced. This suggests that vaccination in this group affected parasite fertility. Notably, egg hatching experiments demonstrated that a substantial proportion of eggs failed to hatch. This finding may have important implications under field conditions, as it could reduce pasture contamination, lower reinfection rates, and protect other animals within the flock.

The vaccine study demonstrated an indirect protective effect in a group of female sheep vaccinated using a mixed regimen. It also revealed an important influence of host sex on vaccine efficacy. The development of such next-generation oral vaccines has the potential to transform not only fasciolosis control, but also other helminthiases, offering a sustainable, scalable, and urgently needed alternative to drug therapy within a One Health framework. However, continued research and refinement are essential to fully realize the potential of this promising vaccination strategy.

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TH13

Infection of Black bullhead with metacercariae of *Hysteromorpha triloba*

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The cercariae and metacercariae stages of the families Diplostomidae, Bolbophoridae, Neodiplostomidae, and Strigeidae are responsible for fish pathology and follow a complex life cycle that includes birds as definitive hosts, snails as first intermediate hosts and fish as second intermediate hosts. The genus *Hysteromorpha* Lutz, 1931 (Diplostomidae) comprises intestinal trematodes that parasitize fish-eating birds, with species of *Phalacrocorax* serving as the main definitive hosts. *Hysteromorpha triloba*, originally described from Europe, utilizes the freshwater snail *Gyraulus albus* as the first intermediate host and a variety of freshwater fish as second intermediate hosts. This genus has a broad geographic distribution and comprises three previously recognized species, namely *H. triloba*, *H. plataleae*, and *H. corti.* During a routine ichthyoparasitological survey in eastern Slovakia, digenean metacercariae were detected in the muscle tissue of the black bullhead (*Ameiurus melas*). This work briefly describes the observed metacercariae and provides data on their distribution, infection rates and histopathology in *A. melas* collected from the Uh River dead arm (Tisa River Basin). At the first detection of *Hysteromorpha* metacercariae in June 2023, prevalence (P) was 33.3% (1/3) with mean intensity of infection 20.7 larvae per 1 cm^2 of muscle tissue (8-29); SL of caught fish was 78-101 mm (mean 89 mm). The only infected female fish was 101 mm long and weighed 5.2 g. One year later (September 2024), a second sampling of *A. melas* from the same locality revealed a P 16.7% (1/6) with mean intensity of infection 2 metacercariae per 1 cm^2 of tissue (1-5); SL of examined fish was 45 -175 mm (mean 109 mm), infected was the smallest female fish from the catch, with SL 45 mm and weight 1.2g. Sampling conducted in spring 2025 revealed 15 positive bullheads out of 15 examined (P-100%) with intensity of infection 1-16 cysts or excysted metacercariae per 1 cm^2 of muscles (mean 5.8). SL of examined fish ranged from 53-130 mm (mean 99.3 mm) and weight 3.5-40.6 g (mean 19.26 g). Condition of the fish was poor. The parasites were localized predominantly in cranial parts of the body and dorsal muscles. Histological examination of the infected host tissue revealed multiple parasitic cysts within skeletal muscle. Encysted metacercariae were seen within round or oval thin fibrous capsules that composed of collagen fibres. Skeletal muscle exhibited signs of hyaline degeneration and variability in muscle fibre size was prominent. In the Americas, metacercariae have been reported primarily from cyprinid fishes as well as from members of four additional fish families: Ictaluridae, Catostomidae, Ariidae and Pimelodidae. The planorbid snail *Gyraulus hirsutus* Gould has been reported as the first intermediate host of *H. triloba.* The most frequent fish species hosting larval stages of *H. triloba* in Europe, were cyprinid fish (12 species) including families Cyprinidae, Leuciscidae and Tincidae. Records of cercariae in first intermediate host from European countries are scarce. Two small freshwater planorbids *Gyraulus albus* and *Segmentina nitida* were identified harboring cercariae of *H. triloba* in European waters.

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TH14

Unraveling Tick Lipid Metabolism

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Host blood represents the only source of nutrient for ticks, and is crucial for their physiology, development, and reproduction. In contrast to the relatively well-studied digestion of proteins, almost nothing is known about the uptake, digestion and utilization of host lipids, which play crucial role in tick physiological processes and even in pathogen transmission.
In ticks, protein digestion is mediated via multienzymatic networks composed of endo- and exopeptidases. By contrast, lipid digestion has never been studied in our model organism hard tick *Ixodes ricinus*.
Several genes encoding lipases have been identified in the midgut transcriptome and proteome of the hard tick *I. ricinus*. For a more detailed characterization of the lipolytic system, the activity of these lipases was investigated.
Total lipase activity (TLA) in tick midgut homogenates from different feeding timepoints was determined using a universal fluorescent substrate (4-Methylumbelliferyl oleate). TLA was found to increase until 5th day of feeding and then decreased rapidly. For a more detailed characterization of the lipolytic system, multiple commercially available lipase inhibitors with diverse substrate specificities were used.
Ticks spend most of their life cycle off-host. During this period, ticks must survive from energy resources accumulated during the ingestion of the host's blood meal. Obtained and digested lipids are utilized to form lipid droplets which serve as the primary energy storage during prolonged periods of starvation. Lipid droplets-dynamics in the midgut was observed in the nymphal midgut using the fluorescent dye LD540, revealing their rapid formation in the early phase of blood intake and the utilization of lipids during the long fasting periods.
This work offers the first in-depth study of poorly understood lipid metabolism in ticks. Fluorescence-based assays using various commercial lipase inhibitors were used to characterize lipolytic enzymes and their activity during blood feeding. Additionally, lipid droplets dynamics was observed using microscopy techniques.
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TH15

Unlocking parasite biology: Comprehensive transcriptomic services at GeneCore facility

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In recent years, advances in transcriptomic technologies have expanded the possibilities across many research areas, including parasitology. Bulk RNA sequencing, single-cell transcriptomics, and spatial transcriptomics provide complementary approaches to investigate gene expression in diverse biological systems, including parasitic helminths, protozoa, or arthropod vectors such as ticks.
Transcriptome analysis enables genome-wide identification of novel genes, protein families, and biological pathways involved in parasite development, life-cycle transitions, host adaptation, pathogenicity, immune evasion, tissue tropism, and responses to treatment. Such analyses can address important biological questions, including which genes are essential for parasite survival, which mechanisms mediate parasite–host interactions, and which processes control parasite reproduction and ensure persistence of the parasite life cycle.
While bulk sequencing offers a broad overview, single-cell RNA sequencing further increases resolution by identifying distinct cell populations, rare cell states, and dynamic developmental processes. Furthermore, spatial transcriptomics adds crucial tissue context by mapping gene expression patterns

directly within the sample. Together, these insights deepen our understanding of complex parasite biology and support the discovery of novel therapies for these pathogens.
At GeneCore, an academic facility specializing in advanced transcriptomics, we support researchers through comprehensive bulk, single-cell, and spatial RNA-seq projects. Our services span the entire workflow, covering experimental design consultation, sample preparation, next-generation sequencing, and basic bioinformatic analysis. In this poster, we present an overview of analytical workflows applicable to parasitological research, demonstrating how our accessible services can transform biological samples into interpretable molecular insights.

TH16

Can NHR-3 be part of the xenobioitic response mechanisms *in Haemonchus contortus?*

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Anthelmintic resistance in *Haemonchus contortus* is a growing global problem, strongly associated with enhanced xenobiotic detoxification capacity, including upregulation of drug-metabolizing enzymes and efflux transporters. However, the regulatory mechanisms underlying this response remain poorly understood. Nuclear hormone receptors (NHRs) are key transcriptional regulators of xenobiotic responses in model organisms, yet their roles in parasitic nematodes are largely unexplored. Here, we focus on NHR-3 as a candidate regulator of detoxification pathways in *H. contortus*. Comparative genomics identified the *H. contortus* orthologue of NHR-3, and expression analyses revealed both constitutive differences between drug-susceptible and resistant strains and dynamic expression throughout the life cycle. Functional and biochemical characterization, including RNA interference, heterologous expression, and ligand-binding assays, is being employed to assess the role of NHR-3 in xenobiotic sensing and response.
Overall, our findings suggest that NHR-3 may act as a regulator of detoxification pathways in *H. contortus*, contributing to adaptive responses and potentially to anthelmintic resistance.
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TH17

Nuclear Hormone Receptors in *Haemonchus contortus*: Transcriptomic Profiling and Optimization of Ligand Interaction Analysis

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Anthelmintic resistance in parasitic nematodes has become an increasingly significant global concern, emphasizing the need to better understand the molecular mechanisms underlying this phenomenon. Previous studies demonstrated that exposure to anthelmintics stimulates the expression and activity of multiple detoxification systems, including drug-metabolizing enzymes (DMEs) such as UDP-glycosyltransferases and carbonyl-reducing enzymes, as well as efflux transporters belonging to the P- glycoprotein family. Moreover, resistant nematode strains frequently exhibit constitutively elevated expression of DME-related genes, however, the molecular pathways responsible for their regulation have not yet been fully characterized. This project aims to investigate the regulation of DME gene expression, focusing on the potential role of transcription factors (xenosensors), including nuclear hormone receptors (NHRs). Upon activation, these receptors bind xenobiotic response elements in DNA and induce transcriptional programs associated with stress responses and xenobiotic metabolism.
In *H. contortus*, 62 NHR genes have been identified, likely representing a key component of adaptive mechanisms that facilitate survival in the chemically complex host environment. These receptors may integrate host-derived signals, including immune-associated metabolites and microbiome-derived

molecules, thereby triggering metabolic adaptation, modulation of stress responses, and immune evasion, which may ultimately contribute to the development of anthelmintic resistance.
In the first phase of the project, candidate NHRs potentially involved in the regulation of xenobiotic responses in *H. contortus*, as well as their homologs in the model organism *Caenorhabditis elegans*, were identified. Gene expression of selected receptors and their ligand-binding domains (LBDs) was subsequently analyzed using RT-qPCR in a susceptible (ISE) and a resistant (IRE) strain of *H. contortus*, with preliminary data already indicating differences in expression profiles between the two strains. In parallel, the induced expression of selected NHR genes in *H. contortus* were evaluated following exposure to anthelmintics (albendazole, ivermectin). Promising candidate genes will be selected based on the results of comparative gene expression analysis and will be cloned and heterologously expressed in a bacterial expression system. Interactions between xenobiotic ligands, i.e., anthelmintics (e.g., albendazole), and receptor ligand-binding domains will be assessed using microscale thermophoresis (MST), and the binding affinity will be determined. The MST method has been optimized using the protein cel_NHR-176 with a known interaction partner, thiabendazole.
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TH18

Genetic diversity assessed in the equine ascarids from central-eastern Europe, with *Parascaris univalens* being the only found species.

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Parascaris spp. are large, whitish roundworms that inhabit the small intestine of horses and other equids and are of major veterinary and economic importance. The currently accepted names for the two most known species of the genus *Parascaris* are *Parascaris univalens*, which has one pair of chromosomes, and *Parascaris equorum* with two pairs of chromosomes. Traditionally, equine roundworms were referred to as *P. equorum* due to the prevailing concept of its different variants during the last century (Lin, 1954). *P. univalens* as a separate species has received only limited attention, although since the 1986 it is the only species in Europe and North America confirmed by karyotyping. The aim of the study was to provide DNA sequence information on ascarids collected from horses from stud farms, riding parks, abattoirs and clinics in central-eastern Europe to better recognize the distribution of *Parascaris* spp. in the region and to indicate possible factors that have influenced the subdivision of the population structure.
Sixty-nine *Parascaris* samples (23 isolates from Poland, 21 from Slovakia, 20 from Ukraine, 3 from Hungary, 2 from the USA) were collected from 16 geographical locations. The isolates from domestic horses originating from Slovakia, Ukraine, Hungary and Poland were obtained *in vivo* from faecal samples of macrocyclic lactones-treated equids. Nineteen isolates were collected from slaughtered domestic horses in the abattoir of Nowy Sącz (Poland). Two specimens from the USA were isolated from dead horses necropsied at Colorado State University. Fragments of the cytochrome *c* oxidase I (*cox1*, 384 bp), the small subunit of mitochondrial ribosomal RNA (12S rRNA, 495 bp) of mitochondrial genes and the internal transcribed spacer 1 of the nuclear ribosomal RNA (ITS1, 504 bp) were amplified.
All examined isolates showed genetic characteristics that could be assigned to *P. univalens*. The haplotype network derived from the sequences of three genes revealed two clusters separated by two mutational steps, with polymorphism detected predominantly in *P. univalens* from Ukrainian horses. The highest average nucleotide diversity was found in Ukraine ($\pi = 0.0108$) and the highest haplotype diversity was observed in Hungary ($\pi = 0.6667$), however, this value may be biased by the small sample size from Hungary and by the fact that horses from the riding school in Miskolc were purchased from different locations. In the *cox1* gene, the examined isolates revealed two major profiles differing by three nucleotide substitutions. In the 12S rRNA gene, all evaluated sequences grouped into a single cluster, with a sequence identity of 99.1-100%. A portion of the ITS1 gene of ribosomal RNA revealed one polymorphic site that divided the analyzed samples into two clusters.

The population structure of *P. univalens* in Poland and Slovakia was more homogeneous than that in Ukraine, probably due to closer geographical distance and higher gene flow between populations implied by horse trade. Several locally adapted isolates appear to have emerged in Ukraine, some of which have affinity to Chinese samples. The relatively low levels of nucleotide and haplotype diversity indices suggest that the global population of *P. univalens* in Europe is rather genetically uniform, likely due to the movement of infected horses within Europe through the trade exchange.
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TH19

Exploring effector-like proteins in *Trichinella*: recombinant production of genus-specific immunoreactive candidates

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The *Trichinella* nematode lives in the muscles of mammals, including humans. It enters the body of the host through the consumption of infected meat containing the invasive larvae. Infections caused by *Trichinella* can be severe, depending the dose of the ingested larvae. To avoid immune reactions, both the larvae and the adult worms produce a range of proteins that facilitate invasion, modulate immune responses, and enable survival in the hostile host environment. These proteins interact with the host's immune system, altering its response and promoting parasite persistence.
In our previous immunoproteomic studies, some immunoreactive *Trichinella* proteins were identified and selected. The group of proteins that appear to be particularly interesting comprises putative effector proteins, proteins which do not have conserved domains and are highly *Trichinella* genus-specific. They resemble effector proteins first discovered and best described in the pathogen-plant interactions, and defined as molecules that manipulate host cell structure and function, thereby facilitating pathogen infection and/or triggering defense responses of the host. Effector candidate-proteins are usually determined based on specific features, including sequence length ≤ 300 amino acids; cysteine richness (>2% cysteine content or >4 cys residues), presence of a secretory signal peptide; absence of transmembrane domains; higher expression in interaction with the host; no or limited sequence similarity to other organisms (species specificity) and encoded by genes with long intergenic regions or in gene-sparse, repeat-rich chromosomes. Despite being investigated mostly in the context of plant pathogens (fungi, bacteria, plant-parasitic nematodes), the conception of effector proteins match well to *Trichinella*. *Trichinella* produce many proteins to communicate with the host at the molecular level, assuring immunological homeostasis that provides survival of both parasite and the host, as it occupies the host's muscle cells without killing them. The aim of the present study was to produce selected putative effector proteins: ES21, P16967 and P13379 in recombinant form in order to characterize them and use *in vitro* studies.
The current and future *in vitro* experiments will provide data on the functions of selected proteins and will contribute to a better understanding of the molecular aspects of *Trichinella* infection, including parasite–host interactions, immunomodulation, thereby improving our understanding of molecular communication between the parasite and its host. Further analysis may help identify potential proteins that can be used in immunodiagnostics, immunotherapy or the selection of new target drugs.
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TH20

Fasciola hepatica FABP1 Reprograms Human Dendritic Cells Through PPARγ/RXR/AhR Signaling to Promote Tolerogenic Immune Responses

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Fasciola hepatica, a helminth parasite with a complex migratory lifecycle, crosses key tissue barriers such as the intestinal wall and liver, relying on immune evasion to establish chronic infection. One of its key immunomodulators, Fatty Acid Binding Protein 1 (FABP1), can induce tolerogenic human monocyte-derived dendritic cells (moDCs), which suppress Th1 responses and promote IL-4 and IL-10 via Thrombospondin-1 (TSP-1). However, the underlying mechanisms remain poorly defined.
In this study, we examined how recombinant FABP1 influences moDC function and T cell responses. Human moDCs were stimulated with FABP1 and LPS, with or without pharmacological inhibitors of PPARγ, RXR, and AhR. Additional experiments tested the effects of retinoic acid (RA) and selected long-chain fatty acids (LCFAs). We assessed cytokine production (IL-6, CXCL11, TNF, TSP-1) and surface markers (CD40, CD80, CD86, MHC II) via spectral flow cytometry.
FABP1 suppressed IL-6 and CXCL11 and induced TSP-1 expression. RA enhanced TSP-1 induction, suggesting synergy. Inhibition experiments showed that TSP-1 induction depends on PPARγ, RXR, and AhR, while CXCL11 suppression is mediated mainly by AhR. Thus, FABP1 engages nuclear receptor pathways to exert distinct immunomodulatory effects.
To assess adaptive responses, FABP1-conditioned moDCs were co-cultured with allogenic naïve $CD4^+$ T cells. After two weeks, increased IL-4/IFNγ ratios and elevated IL-10 production upon restimulation indicated Th2 skewing and regulatory T cell polarization.
Our findings reveal that FABP1 reprograms dendritic cells via nuclear receptors to promote tolerogenic, anti-inflammatory T cell responses, facilitating parasite persistence. This mechanism offers insight into immune regulation at barrier sites and suggests potential for therapeutic modulation of inflammation using helminth-derived molecules.
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