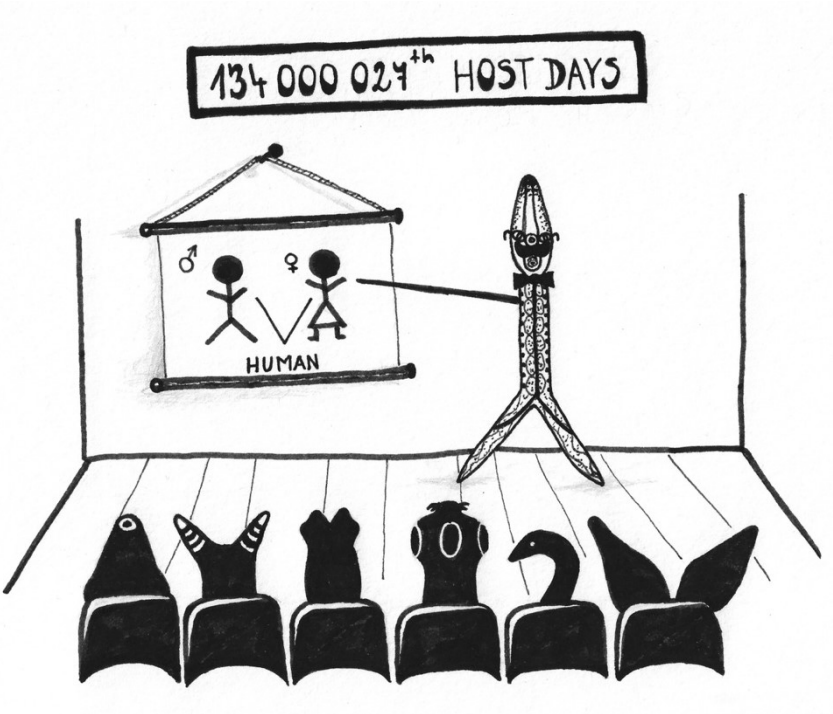
Czech Society for Parasitology

Charles University, Faculty of Pharmacy

27TH HELMINTHOLOGICAL DAYS 2023

Programme & Abstracts

Editors: Pavlína Kellerová & Ivan Vokřál



Hradec Králové 2023

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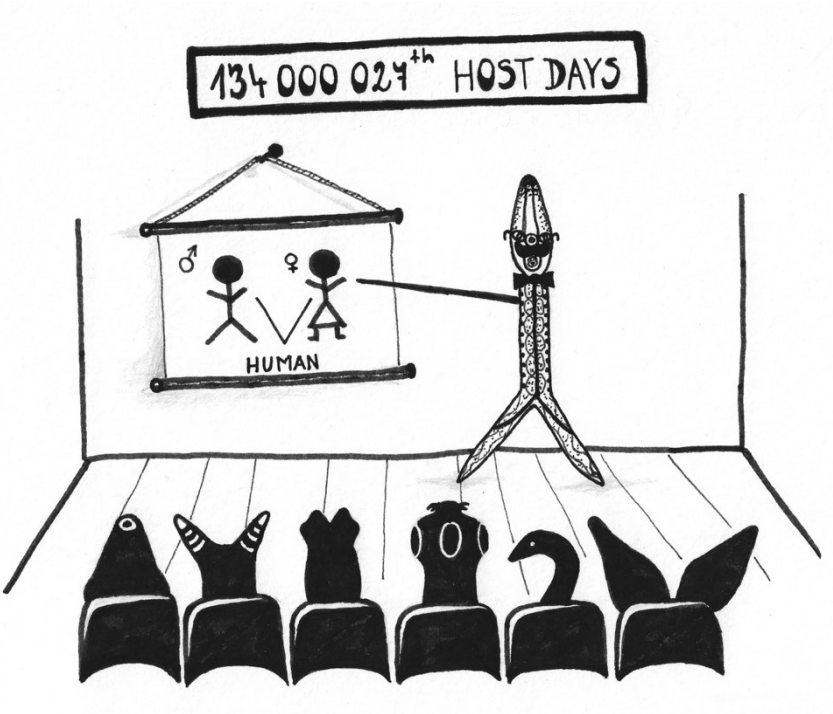

Hradec Králové 2023

27th Helminthological Days

Organised by:	Czech Society for Parasitology, Helminthological Section
Venue & date:	Horská chata Radost, Plasnice 124, Czechia, 8 – 12 May 2023
Organising committee:	Petra Matoušková (coordinator), Pavlína Kellerová, Ivan Vokřál, Lucie Raisová Stuchlíková, Karolína Štěrbová, Martina Navrátilová, Linh Thuy Nguyen, Martin Ambrož, Martin Žofka, Nikola Rychlá, Josef Krátký (Faculty of Pharmacy, Charles University, Prague, Czechia)
Conference website:	https://portal.faf.cuni.cz/HD2023
Conference email:	hd2023@faf.cuni.cz

ISBN 978-80-908308-3-7

PROGRAMME OF 27TH HELMINTHOLOGICAL DAYS

Invited talk: **25 min** + 5 min for discussion
Regular talk: **10 min** + 5 min for discussion
Poster talk: **2 min** and individual discussion at the poster

BSc/MSc or **PhD**

Oral and Poster Presentations included in a student competition will be evaluated by a conference committee. The winners will be announced and awarded during the closing ceremony.

MONDAY, MAY 8

15:00 – 18:00	**Arrival and registration of the participants**
18:00 – 18:45	**Dinner**
19:00 – 00:00	**Get-together-evening**

TUESDAY, MAY 9

08:00 – 08:45	**Breakfast**
09:00 – 09:15	**Opening Ceremony (*P. Matoušková, I. Vokřál*)**
Session I	**Immunology I: Host Defence Mechanisms *(Chairman: T. Macháček)***
09:15 – 09:45	A. Ehrens, C. Nieto Perez, A. Krez, C. Paffenholz, B. Lenz, F. Risch, N. Offermann, M. Koschel, E. Latz, M. Capasso, A. Hoerauf, M. P. Hübner: Catching worms - eosinophil ETosis and protective mechanisms against filariae **(Invited talk)**
09:45 – 10:00	B. Šmídová, C. Nieto Peréz, A. Ehrens, T. Macháček: The hunters called eosinophils: Are *Trichobilharzia regenti* and *Mesocestoides corti* a prey worthy of their extracellular traps? **(PhD)**
10:00 – 10:15	M. Vargová, E. Dvorožnáková, Z. Hurníková, A. Lauková: Role of enterocins and their producing strains in the gut defense of the mice to *Trichinella spiralis* infection
10:15 – 10:30	O. Vosála, B. Šmídová, J. Novák, J. Svoboda, P. Horák, T. Macháček: Live and let live? Amyloid beta does not act as a toxic agent for the larvae of *Toxocara canis* neither in murine brain nor *in vitro* **(BSc/MSc)**

10:30 – 11:00	**Coffee break**
Session II	**Immunology II: Helminthic Therapy *(Chairman: J. Dvořák)***
11:00 – 11:15	M. Majer, B. Šmídová, A. Revalová, P. Horák, T. Macháček: Not all worms are created equal: The story of avian schistosomes vs. lifestyle diseases **(PhD)**
11:15 – 11:30	M. Schreiber, T. Macháček, M. Majer, B. Šmídová, V. Vajs, O. Tolde, J. Brábek, D. Rösel, P. Horák: Immune response changes in tapeworm-infected mice may lead to cancer suppression **(PhD)**
11:30 – 11:45	V. Vajs, M. Schreiber, P. Horák: The effect of tapeworm infection on the growth and metastasis of tumours **(PhD)**
11:45 – 12:00	L. Panská, Š. Nedvědová, V. Vacek, J. Dvořák: Orthologs of human glutamate carboxypeptidase 2 in *Caenorhabditis elegans*: Physiological studies revealing their essential roles
12:15 – 13:15	**Lunch**
Session III	**Epidemiology & Diversity of Helminths & Diagnostics *(Chairman: T. Scholz)***
13:30 – 14:00	D. Modrý: *Angiostrongylus spp.*: A small genus of nematode with huge ambitions **(Invited talk)**
14:00 – 14:15	D. Pandian, D. Modrý, T. Najer: *Angiostrongylus cantonensis* (Nematoda: Angiostrongylidae) an emerging cause of human angiostrongyliasis in India **(PhD)**
14:15 – 14:30	J. Procházka, R. Leontovyč: LAMP – a new method in cercarial dermatitis monitoring **(BSc/MSc)**
14:30 – 14:45	Z. Bartoníček, J. Dvořák, F. Allan, A. M. Emery, P. Isingoma, B. L. Webster: Fish faecal xenomonitoring as a tool for schistosomiasis transmission monitoring **(PhD)**
14:45 – 15:00	Y. Ashong, E. M. Boateng, F. T. Aboagye, F. Kwarteng, S. Armoo, A. Y. Debrah, M. Chanova, I. Ayi, B. L. Webster, M. Y. Osei-Atweneboana: The potential of xenomonitoring as a tool for indirect monitoring of schistosomiasis and evaluating community-wide mass drug administration campaign **(PhD)**
15:00 - 15:15	L. Škorpíková, J. Ilgová, J. Vadlejch, J. Magdálek, R. Plhal, J. Drimaj, O. Mikulka, M. Kašný, N. Reslová: Monitoring of alien parasites *Ashworthius sidemi* and *Fascioloides magna* in wild ruminants in the Czech Republic using real-time PCR
15:15– 15:45	**Coffee break**
Poster session	**Poster talks *(Chairman: P. Matoušková)***
15:45 – 17:15	**Individual discussion at the posters** **P1** M. Komáromyová, M. U. Dolinská, Ľ. Burcáková, A. Königová, M. Várady: Prevalence of gastrointestinal parasites of sheep in Slovakia diagnosed by different coprological methods

P2 C. Paffenholz, A. Hoerauf, M. P. Hübner, A. Ehrens: Identification of calcium-influx and kinase activation during microfilariae-induced NADPH-oxidase-dependent and -independent eosinophil ETosis **(BSc/MSc)**
P3 M. Benovics, P. Mikulíček, P. Papežík, Š. Mašová: A new species of filariid nematode from Epirus water frogs (*Pelophylax epeiroticus*) in Greece
P4 M. Benovics, A. Klimešová, E. Nosková, L. Škorpíková, L. Seidlová, O. Mikulka: Helminth diversity of nutria in Czech Republic
P5 D. Barčák, M. Oros, D. Antolová, M. Kožená, T. Scholz: Marine nematode *Anisakis simplex* s.s. in fish products in Ccntral Europe: Epizootiology, risks for consumers and serological survey
P6 O. I. Lisitsyna, O. B. Greben, V. L. Sarabeev, A. Rashydov, M. Oros: *Gammarus balcanicus* and G*ammarus fossarum* (Crustacea, Amphipoda) as the intermediate hosts of helminth parasites in eastern Slovakia **(PhD)**

17:30 – 18:15 **Dinner**

18:30 – 19:30 D. Modrý: Vets Beyond Borders **(Short seminar)**

20:00 – 00:00 **Campfire evening**

WEDNESDAY, MAY 10

08:00 – 08:45 **Breakfast**

Session IV **Molecular Biology & Transcriptomics of Helminths *(Chairman: R. Leontovyč)***

9:00 – 9.30 O. Puckelwaldt, S. Gramberg, T. Schmitt, S. Haeberlein: A gene expression atlas by single-cell and spatial transcriptomics as valuable resource in liver fluke research **(Invited talk)**

9:30 – 9:45 M. Žofka, L. T. Nguyen, P. Matoušková: *In silico* metabolic model of the parasitic nematode *Haemonchus contortus* **(PhD)**

9:45 – 10:00 K. Peterková, L. Konečný, L. Panská, L. Jedličková, M. Sombetzki, J. Dvořák: Winners vs. losers - comparative transcriptomic analysis of *Schistosoma mansoni* eggs from gut and liver **(PhD)**

10:00 – 10:15 L. Konečný, K. Peterková, L. Jedličková, J. Dvořák: Role of eggs specific venom allergen-like proteins during the infection with *Schistosoma mansoni* **(PhD)**

10:15 – 10:45 **Coffee break**

Session V	**Immunology III: Pathogenesis & Host Immune Response *(Chairman: L. Mikeš)***
10:45 – 11:00	A. Revalová, M. Majer, P. Horák, T. Macháček: *Trichobilharzia* species affect the course and the severity of cercarial dermatitis in a species-specific manner **(PhD)**
11:00 – 11:15	T. Macháček, C. Fuchs, F. Winkelmann, M. Sombetzki, M. Trauner: *BSEP/ABCB11* knockout ameliorates *Schistosoma mansoni* liver pathology by reducing parasite fecundity
11:15 – 11:30	J. Ilgová, S. Šreibr, J. Vorel, P. Dobeš, J. Hurychová, M. Kašný, L. Škorpíková, P. Hyršl: Searching for proteins involved in the infection of the entomopathogenic nematode *Heterorhabditis bacteriophora*
11:30 – 11:45	A. Tymich, L. Mikeš: Kunitz proteins of *Eudiplozoon nipponicum* **(BSc/MSc)**
11:45 – 12:45	**Lunch**
13:00 – 17:15	**Half-day trip to Neratov**
18:00 – 18:45	**Dinner**
19:00 – 22:00	M. Veljačiková: Time management for work and life **(Workshop in Czech)**

THURSDAY, MAY 11

08:00 – 08:45	**Breakfast**
Session VI	**Anthelmintic Resistance & Xenobiochemistry *(Chairman: L. Skálová)***
09:00 – 09:30	J. Vadlejch: Will anthelmintic resistance threaten the sustainability of small ruminant production in the Czech Republic? **(Invited talk)**
09:30 – 09:45	K. Štěrbová, N. Rychlá, L. R. Stuchlíková, P. Matoušková, L. Skálová: Flubendazole-induced changes in the expression of selected SDR genes throughout the developmental stages of *Haemonchus contortus* **(PhD)**
09:45 – 10:00	N. Rychlá, P. Matoušková, L. R. Stuchlíková: Heterologous expression of SDR genes from *Haemonchus contortus* **(PhD)**
10:00 – 10:15	E. Kohoutová, N. Rychlá, L. R. Stuchlíková, P. Matoušková: *In vitro* inhibition of enzymes reducing the anthelmintics flubendazole in *Haemonchus contortus* **(BSc/MSc)**
10:15 – 10:30	J. Krátký, D. Bierdemann, K. Štěrbová, P. Matoušková, B. Szotáková: Potential anthelmintic effect of silybins **(PhD)**
10:30 - 11:00	**Coffee break**

Session VII	**Taxonomy, Diversity & Phylogeny of Helminths *(Chairman: M. Oros)***
11:00 – 11:15	J. Brabec, E. D. Salomaki, M. Kolísko, T. Scholz, R. Kuchta: New scenario of the evolution of Neodermata based on transcriptomic data
11:15 – 11:30	C. J. Kibet, R. Kuchta, C. Selbach, P. Kundid, D. Barčák, M. Soldánová: Diversity of bird schistosomes in Europe with three new lineages **(PhD)**
11:30 – 11:45	C. Rahmouni, M. Seifertová, A. Šimková: Species richness, morphological diversity, and molecular phylogeny of *Gyrodactylus* communities (Monogenea: Gyrodactylidae) from Nearctic cypriniform fish hosts
11:45 – 12:00	F. Nejat, M. Benovics, A. Šimková: Morphology and phylogeny of *Dactylogyrus* in the Middle east revealing unexpected diversity **(PhD)**
12:00 – 12:15	M. Benovics, F. Nejat, C. Rahmouni, E. Řehulková, J. Hernandéz-Orts, A. Šimková: Diversity of freshwater fish parasites in Iraq
12:30 – 13:45	**Lunch**
Session VIII	**Host-Parasite Interactions: Ecology & Evolution *(Chairman: R. Kuchta)***
14:00 – 14:15	M. Oros, D. Barčák, D. Uhrovič, D. Miklisová, T. Brázová: Fish-parasite sentinel system as emerging tool for environmental health biomonitoring: Wels catfish and its cestodes
14:15 – 14:30	M. H. Fuad, M. Ondračková, L. Vetešník, A. Šimková: *Diplostomum spathaceum* infection inducing differential immune gene expression in sexual and gynogens of gibel carp (*Carassius gibelio*): Parasites as potential mechanism facilitating the coexistence of two reproductive forms **(PhD)**
14:30 – 14:45	P. Kundid, M. Soldánová: Cercarial emergence patterns and impact on snail host longevity: Transmission strategies of larval trematodes **(PhD)**
14:45 – 15:00	M. Soldánová, P. Kundid, A. M. Ćmiel, E. Żbikowska, A. Stanicka: The role of the invasive zebra mussel in the dilution of trematode cercariae
15:00 – 15:30	**Coffee break**
15:30 – 17:00	**Presentation of conference partners**
17:00 – 00:00	**Closing ceremony and social event**

FRIDAY, MAY 12

08:00 – 08:45	**Breakfast**
09:00 – 10:00	**Departure of participants**

ABSTRACTS

The abstracts, ordered according to the conference programme, are published as received from the authors who are fully responsible for the content. No editing or corrections were made except for minor changes in formatting to keep the layout unified.

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TUESDAY, MAY 9

Session I

Immunology I: Host Defence Mechanisms

(*Chairman: T. Macháček*)

CATCHING WORMS - EOSINOPHIL ETOSIS AND PROTECTIVE MECHANISMS AGAINST FILARIAE

A. Ehrens[1,2], C. Nieto Perez[1], A. Krez[1], C. Paffenholz[1], B. Lenz[1], F. Risch[1], N. Offermann[3], M. Koschel[1], E. Latz[4], M. Capasso[3], A. Hoerauf[1,2], M. P. Hübner[1,2]

[1]Institute for Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Germany
[2]German Center for Infection Research (DZIF), partner-site Bonn-Cologne, Bonn, Germany
[3]Department of Immunregulation, German Center for Neurodegenerative Diseases (DZNE) within the Helmholtz Association, Bonn, Germany
[4]Institute for Experimental Immunology, University Bonn, Bonn, Germany

Eosinophils are important effector cells mediating protective immunity against parasitic filarial nematodes. This is clearly shown in eosinophil-deficient mice infected with the rodent filarial nematode *Litomosoides sigmodontis*, which have a significantly higher adult worm and microfilaria (MF), the filarial progeny, burden compared to immunocompetent wildtype mice. The exact effector mechanism mediating protection has not been identified yet. Eosinophils have been shown to release extracellular DNA traps spiked with toxic granules during a process called ETosis, which mediates entrapping and killing of pathogens. We demonstrated that *L. sigmodontis* MF induce eosinophil ETosis *in vitro*, which is mediated by the dectin-1 receptor and the canonical inflammasome pathway. *In vivo*, the *L. sigmodontis* infection increases local DNA concentration, while blood circulating MF increase plasma DNA levels, which is dependent on eosinophils. Moreover, DNA traps facilitate the clearance of MF from the peripheral blood, indicating that ETosis is an essential protective mechanism by eosinophils. Since murine and human eosinophils release DNA traps in response to MF of the rodent filariae and of *Dirofilariae immitis*, the dog heartworm, filariae-induced ETosis appears to be a conserved mechanism. However, the role of ETosis in pathology development is less clear. Immune responses by granulocytes towards dead MF have been shown to contribute to dermatitis and vision impairment in onchocerciasis patients, while viable MF rarely elicit inflammation. Therefore, we identified the role of the NADPH oxidase and calcium-dependent ETosis during MF stimulation. Interestingly, we observed that viable and dead MF induce different ETosis signaling cascades, which could lead to the altered immune response in onchocerciasis patients towards viable and dead MF. In summary, we demonstrate the role of eosinophil ETosis during a filarial infection and the molecular signalling mechanism occurring during MF-induced eosinophil ETosis.

The study was supported by DZIF Translational Thematic Unit: Novel Antibiotics grants #09.701, the Deutsche Forschungsgesellschaft (DFG, German Reaearch Foundation grant HU2144/3-1) and the DFG under Germany's Excellence Strategy – EXC2151 – 390873048.

THE HUNTERS CALLED EOSINOPHILS: ARE *TRICHOBILHARZIA REGENTI* AND *MESOCESTOIDES CORTI* A PREY WORTHY OF THEIR EXTRACELLULAR TRAPS?

B. Šmídová[1], C. Nieto Peréz[2], A. Ehrens[2,3], T. Macháček[1]

[1]Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic
[2]Institute for Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Bonn, Germany
[3]German Centre for Infectious Research (DZIF), partner side Cologne-Bonn, Germany

In the recent years, eosinophils have been more and more studied for their ability to protect the organism from parasites by releasing DNA traps containing cytotoxic granules. These traps significantly decrease movement and viability of various life stages of filaria. Here we tested trap release induction by two different parasites: the avian schistosome *Trichobilharzia regenti* and the tapeworm *Mesocestoides corti*. They both attract large numbers of eosinophils to the site of infection, suggesting their possible role in parasite elimination. *T. regenti* activated bone marrow-derived eosinophils, induced mitochondrial ROS production, DNA trap release and later caused eosinophil apoptosis. These results support our former *in vivo* findings suggesting a host-protective role of eosinophils in the *T. regenti*-infected spinal cord. As for the *M. corti*, we discovered a DNAse activity of larval excretory-secretory products destroying formed DNA traps suggesting that the tapeworm could actively fight against the defence mechanisms of the host. However, *M. corti* still stimulated ROS release and, when the DNAse activity was inhibited, traps were formed. Taken together, we have shown that both parasites stimulate eosinophils to release DNA traps but *M. corti* can protect itself from the deadly effect of the traps by excreting/secreting DNAse.

The study was supported by ERASMUS+, DZIF Translational Thematic Unit: Novel Antibiotics grants #09.701 and Jürgen-Manchot PhD scholarship.

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ROLE OF ENTEROCINS AND THEIR PRODUCING STRAINS IN THE GUT DEFENSE OF THE MICE TO *TRICHINELLA SPIRALIS* INFECTION

M. Vargová[1], E. Dvorožnáková[1], Z. Hurníková[1], A. Lauková[2]

[1]Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovak Republic
[2]Institute of Animal Physiology, Centre of Biosciences, SAS, Košice, Slovak Republic

Enterocins are extracellular proteins produced by *Enterococcus* strains, which represent the microbiota in the gut of humans, mammals and other animals. In our study we focused on the role of beneficial enterococci and their prime metabolites – enterocins in the gut defence to *Trichinella spiralis* infection. Gut microbiota strongly interfere with the pathophysiology of parasitic infections, modulate the host immune response and have an anti-parasitic effect. They also regulate intestinal motility and mucus secretion, two major components of intestinal physiology that are essential in defending the host against helminths. We detected changes in mRNA expression of transmembrane MUC-1 and MUC-17, MUC-2 and secretory Immunoglobuline A in the gut after Enterococci/Enterocins therapy.

The strains *Enterococcus faecium* CCM8558 and *E. durans* ED26E/7 (10^9 CFU/ml) and their enterocins Enterocin M and Durancin-like (50 µl) were administered daily and mice were infected with *T. spiralis* (400 larvae) on 7^{th} day of treatment. We detected antiparasitic effect of both *Enterococcus* strains and their enterocins on *T. spiralis* infection. The application of the *E. faecium* CCM8558 and its Enterocin M resulted in a significant reduction in the number of adults in the gut on day 11 p.i. (reduction 55 and 47%). *E. durans* ED26E/7 showed also effective reduction 36%, but its Durancin-like did not maintain antiparasitic efficacy, reached only 17%. A significant reduction of larval burden was detected in muscles of treated mice (reduction by *E. faecium* CCM8558 = 56%, Enterocin M = 40%, *E. durans* ED26E/7 = 36%, Durancin-like = 15%).

The gene expression of MUC1 and MUC17 protecting epithelial cells was affected by treatment. The mRNA levels for MUC1 and MUC17 were significantly increased on day 5 p.i. in all treated groups, but the stimulatory effect was kept up to day 18 p.i. only by Enterococci and Enterocin M. The expression of MUC2 from goblet cells was stimulated by *E. faecium* CCM8558 and Enterocin M from 11 to 25 day p.i. The expression of intestinal secretory IgA was stimulated during the intestinal phase of trichinelosis only by strains *E. faecium* CCM8558 and *E. durans* ED26E/7. Durancin-like increased the sIgA expression during the migratory phase of trichinellosis (18 – 25 day p.i.). The results indicate that Enterococci/Enterocins positively activated the mucosal immune response of the host - the expression of mucin and sIgA, thus protected intestinal mucosa from the parasite invasion, inhibited worm development and reduced female fecundity. The Enterococci and their enterocins offer a prospective strategy for the prevention and control of *T. spiralis* infection.

The study was supported by VEGA 2/077/23 and APVV-17-0028.

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LIVE AND LET LIVE? AMYLOID BETA DOES NOT ACT AS A TOXIC AGENT FOR THE LARVAE OF *TOXOCARA CANIS* NEITHER IN MURINE BRAIN NOR *IN VITRO*

O. Vosála[1], B. Šmídová[1], J. Novák[2], J. Svoboda[3], P. Horák[1], T. Macháček[1]

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[2]Institute of Immunology and Microbiology, First Faculty of Medicine, Charles University, Prague, Czech Republic
[3]Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic

Amyloid (Aβ) is a small peptide native to the central nervous system (CNS) of vertebrates. Although being infamous for forming "senile plaques", a typical hallmark of Alzheimer's disease (AD), it also has several physiological functions including antimicrobial activity as a part of CNS innate immunity. Its cytotoxic effect was observed on bacteria and yeasts, but there is still a lack of complex data describing the similar phenomenon in multicellular neuropathogens. To test the protective function of Aβ against neurotropic helminths, we selected the dog roundworm, *Toxocara canis* (Tc), as it has also been suspected to be associated with AD etiology. Firstly, we studied the effect of Aβ on Tc larvae in the murine brain using the transgenic mice (B6C3-Tg(APPswe, PSEN1dE9) 85Dbo/Mmjax) which naturally overproduce human Aβ. Preliminary data suggest generally lower susceptibility of this murine strain to Tc infection. It shows that Aβ overproduction did not affect Tc viability. The subsequent part of our project aims to study possible direct toxic effects of Aβ on Tc larvae *in vitro*. We incubated them with the physiological concentration of the peptide 48 hours and 7 days. Viability changes were then evaluated by observing morphological alterations, uptake of vital and non-vital dyes, and by quantifying lactate secretion, which mirrors the larvae metabolic activity. Our results show no significant changes in Tc viability, suggesting there is no toxic effect of the peptide on the larvae. Altogether, our data generated from both *in vivo* and *in vitro* experiments do not support an eminent effect of Aβ on the viability of Tc.

The study was supported by Charles University Grant Agency, project GAUK 405022.

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Session II

Immunology II: Helminthic Therapy

(Chairman: J. Dvořák)

NOT ALL WORMS ARE CREATED EQUAL: THE STORY OF AVIAN SCHISTOSOMES VS. LIFESTYLE DISEASES

M. Majer, B. Šmídová, A. Revalová, P. Horák, T. Macháček

Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic

Helminthic therapy has been a hot topic for almost two decades. The beneficial effects were proven even in several clinical studies. On the other hand, the detrimental effects caused by either parasite-induced tissue damage or host immune reaction are noteworthy. Most of the experimental studies are focused on systemic immunomodulation by parasites and their products, but the effect of the parasite presence in the specific tissues is usually neglected. Schistosomula of *Trichobilharzia regenti* migrate through the central nervous system (CNS) and *T. szidati* through the lungs. Both species indicated tissue-specific anti-inflammatory potential in previous studies. We implemented the mouse models for multiple sclerosis (experimental autoimmune encephalomyelitis, EAE) and ovalbumin-induced asthma and tested the effect of avian schistosomes on these diseases, respecting the natural migratory routes of the parasite (i.e., neurotropic *T. regenti* vs. EAE, and viscerotropic *T. szidati* and asthma). *T. regenti* migration through the spinal cord of mice with EAE increased the recruitment of the eosinophils to the CNS, polarized systemic immune response towards Th1, and worsened the disease symptoms. On the other hand, the presence of *T. szidati* in the asthmatic lungs led to the downregulation of the local Th2 response, induction of the regulatory milieu, and the reduction of leukocyte infiltration into the lungs. Taken together, the beneficial effects of the avian schistosomes were proven only for *T. szidati* against allergic asthma. On the contrary, *T. regenti* worsened the manifestations of the multiple sclerosis model. Undoubtedly, our comparative study clearly shows that not all helminths (even within the same genus) positively impact the course of lifestyle diseases. This diversity should be kept in mind when data are extrapolated or generalized in the field of helminthic therapies.

The study was supported by Charles University, project GAUK (580120), Institutional Grants (Charles University COOPERATIO BIOLOGY (2022-2026), UNCE/SCI/012 - 204072/2018 (2018-2023), SVV 260563/2020).

IMMUNE RESPONSE CHANGES IN TAPEWORM-INFECTED MICE MAY LEAD TO CANCER SUPPRESSION

M. Schreiber[1], T. Macháček[1], M. Majer[1], B. Šmídová[1], V. Vajs[1], O. Tolde[2], J. Brábek[2], D. Rösel[2], P. Horák[1]

[1]Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic
[2]Department of Cell Biology, Faculty of Science, Charles University, Prague, Czech Republic

Some helminth species, such as *Trichinella spiralis* or *Echninoccocus granulosus*, have been shown to protect against cancer. Although the mechanisms are still widely speculative, most of the existing research suggests the involvement of a parasite-altered murine immune response. For our work, we use different strains of mice infected with the tapeworms *Mesocestoides corti* and *Taenia crassiceps*, which are then intraperitoneally injected with B16F10 melanoma cells. Various levels of melanoma growth suppression were observed in infected mouse strains with different Th1/Th2 backgrounds, indicating an involvement of the murine immune system. Flow cytometry was used to determine selected immune cell populations associated with cancer, with a focus on the peritoneal cavity and organs (lungs and liver), which are primary sites of B16F10 metastases. *Ex vivo* restimulation of splenocytes with tapeworm and B16F10 antigens was used to assess the impact on systemic Th1/Th2 response. Infected mice show higher numbers of granulocytes and NK cells, especially in the peritoneal cavity; changes in the myeloid populations have also been observed. The results suggest that infection with *M. corti* and *T. crassiceps* could induce an unfavorable immune response for cancer development. Especially NK and CD8+ cells, eosinophils, and peritoneal macrophages could contribute to the observed effect.

The study was supported by the Czech Science Foundation (21-28946S), the Charles University Grant Agency (B-BIO 283823), and the Charles University institutional support (Cooperatio Biology, UNCE/SCI/012 - 204072/2018, and SVV 260563/2020).

THE EFFECT OF TAPEWORM INFECTION ON THE GROWTH AND METASTASIS OF TUMOURS

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The two tapeworm species - *Taenia crassiceps* and *Mesocestoides corti* - are parasites of carnivores with the ability to extensively asexually multiply in their larval stage. They have already proven themselves capable of reducing the growth and metastasis of the B16F10 melanoma cell line in a mouse model. While the underlying mechanisms are under intense investigation, which includes, for example, the exploration of the role of cell-mediated immune response, they remain yet unclear. Experiments regarding the role of antigen cross-reactivity in this effect have yielded negative results. Therefore, we moved onto the study of various excretory-secretory products released by these two species on the previously mentioned melanoma cell line, while expanding the current studies by two more cancer cell lines. The newly established cell lines – the Hepa 1-6 hepatocarcinoma and the ID8 ovarian carcinoma – will also be studied using new methods, especially *in vivo* imaging, as they have both been genetically modified to produce the luciferase enzyme. Upon the injection of the mouse with luciferin, this enzyme emits detectable light, which clues us in on the size and extent of cancer growth in the still-living model animal throughout the entire course of infection without the need of dissection. This method will then be used to determine the effect of the infection by these two tapeworm species on the growth and metastasis of the two new cell lines. If the infections also affect these two cell lines, the effect of various fractions of tapeworm products, such as whole worm homogenate, cystic fluid, different size-fractions, etc., will be studied. The experiments will include the study of the cells' ability to grow, divide and migrate, as well as the differences in activation levels of various cancer-relevant cell signalling pathways.

ORTHOLOGS OF HUMAN GLUTAMATE CARBOXYPEPTIDASE 2 IN *CAENORHABDITIS ELEGANS*: PHYSIOLOGICAL STUDIES REVEALING THEIR ESSENTIAL ROLES.

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Glutamate carboxypeptidase 2 (GCP2) is a member of the M28B metalloprotease family and is a promising target for therapy in neurological disorders and an important tumor marker. However, the physiological functions of GCP2 are still not fully understood. In this study, we used the simple model organism *Caenorhabditis elegans* to investigate the physiological roles of GCP2. By genetic manipulation of the three existing GCP2 orthologs, we found that the knockouts had significant effects on gene expression and metabolite levels, as well as on the pharyngeal physiology, reproduction, and structural integrity of the worms. The three GCP2 orthologs showed distinct localization patterns of their gene expression, with *gcp-2.1* being most abundant in muscles, intestine, and pharyngeal interneurons class SAA, *gcp-2.2* restricted to the phasmid neurons (PHAL/R, PHBL/R), and *gcp-2.3* located in the excretory cell and its bilateral channels. Our findings provide novel insights into the unique phenotypic manifestations of GCP2 gene knockouts in *C. elegans*, which have not been previously described in the animal kingdom in association with the M28B metalloprotease group. This study highlights the potential for elucidating the roles of GCP2 in non-mammalian organisms like *C. elegans* to shed light on important issues related to human GCP2 physiology and its involvement in pathophysiological processes.

The study was supported by the Czech Science Foundation (Grant No. 18-14167S) and the Czech Ministry of Education, Youth, and Sports (Grant No. LTAUSA19023).

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Session III

Epidemiology & Diversity of Helminths & Diagnostics

(Chairman: T. Scholz)

ANGIOSTRONGYLUS CANTONENSIS (NEMATODA: ANGIOSTRONGYLIDAE) AN EMERGING CAUSE OF HUMAN ANGIOSTRONGYLIASIS IN INDIA

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Angiostrongyliasis is an emerging zoonotic disease caused by larvae of metastrongyloid nematode *Angiostrongylus cantonensis*. Its obligatory heteroxenous life cycle involves rats as definitive, mollusks as intermediate, amphibians and reptiles as paratenic hosts. In humans, infection manifests as Angiostrongylus eosinophilic meningitis (AEM) or as an ocular form. As there is no comprehensive account on the disease in India, our study aims to evaluate distribution of human angiostrongyliasis, its clinical course and possible sources. Systematic literature search identified 17 reports describing 36 human cases from 1966 to 2022. AEM accounted for 29 cases (82 %), 5 cases were reported as ocular, 1 case was combined, 1 case unspecified. The presumed source of infection was reported in only 5 cases. Importantly, 19 AEM patients reported a history of eating raw monitor lizard tissues. As top predators, varanids (Varanus spp.) cumulate high numbers of L3, responsible for acute disease in humans. The source was not identified in ocular cases. Absolute majority of cases were diagnosed based on findings of nematodes and clinical pathology (dominated by CSF eosinophilia). Only 2 of the cases were confirmed as *A. cantonensis,* one is by immunoblot and the other by q-PCR. Cases of angiostrongyliasis were reported in Delhi, Karnataka, Kerala, Maharashtra, Madhya Pradesh, Puducherry, Telangana, and West Bengal. With more than 1.4 billion inhabitants, India represents one of the least studied areas regarding *A. cantonensis*. Many cases probably remain undiagnosed/unreported. As most cases have been reported in the state of Kerala, further research can address this region. Besides examination of rodent and mollusk hosts, monitor lizards can be exploited as effective sentinels. DNA-based diagnostic methods, such as q-PCR and LAMP should be introduced into clinical diagnostics of suspect cases, as well as into studies addressing the genetic diversity and species identity of nematodes tentatively identified as *A. cantonensis*.

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LAMP – A NEW METHOD IN CERCARIAL DERMATITIS MONITORING

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Since 2021 collecting and shedding of water snails is part of the mandatory monitoring of water quality in the Czech Republic due to the increased number of case reports of cercarial dermatitis (CD). Even though this method is officially recommended it has some limitations for routine implementation, such as time requirements and low sensitivity. To address these issues, we developed a new cost-effective, rapid, and sensitive assay, that combines Loop-mediated isothermal amplification method (LAMP) with filtration of free-floating environmental DNA (eDNA). We designed set of primers for detection of genus *Trichobilharzia*, the predominant causative agents of CD. In order to reduce the costs, the reaction volume has been reduced to 15µl. Specificity testing of this method has been carried out on the most common species within the genus (*T. szidati, T. franki, T. regenti, T. filifromis*) and it showed no cross-reactivity with other commonly found trematodes. Furthermore, the sensitivity testing has demonstrated that our LAMP assay is suitable for eDNA detection. The capability and detection limit of eDNA approach was tested on dilution series of cercariae and it confirms the revolutionary potential of this method in routine monitoring.

FISH FAECAL XENOMONITORING AS A TOOL FOR SCHISTOSOMIASIS TRANSMISSION MONITORING

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Schistosoma mansoni causes intestinal schistosomiasis in humans. Its complex lifecycle depends on zooplanktonic larvae (cercariae and miracidia) and intermediate freshwater snail hosts, all of which are a part of freshwater food webs. Traditionally, environmental transmission monitoring is achieved via malacological surveys where snails are collected and screened for emerging cercariae or parasite DNA. Although informative, these can be laborious and insensitive, and new, more sensitive monitoring methods are needed. This study developed and field-tested a new DNA-based approach – Fish Faecal Xenomonitoring (FFX), detecting *S. mansoni* DNA in the faeces of *Oreochromis niloticus*.

We conducted laboratory fish-feeding experiments under variable conditions, offering *S. mansoni* cercariae to juvenile *O. niloticus* fish (SL 2-5 cm). We analysed the effects of fish size, the number of cercariae, time after consumption and availability of alternative food on the parasite consumption and subsequent detection of *S. mansoni* DNA in the fish faeces by qPCR. Additionally, at Lake Albert (Uganda), endemic for *S. mansoni*, juvenile *O. niloticus* were caught, and their faeces were collected and analysed for the presence of *S. mansoni* DNA. Results were compared to the detection of transmission by traditional snail xenomonitoring.

In laboratory trials, detection of *S. mansoni* DNA in the fish faeces was achieved when as little as one cercaria was consumed (positivity 16.7 %), and positivity increased with the number of cercariae offered. In microcosm experiments, where fish were offered alternative food sources as well as cercariae, the positivity was 83.3%. *S. mansoni* DNA was detectable in the fish faeces at 12-21 hours post-feeding. At Lake Albert, *S. mansoni* DNA was detected in the faeces of fish collected at nine out of ten sites, whereas snails shedding *S. mansoni* cercariae were only found at four sites.

Our findings show that juvenile *O. niloticus* readily consume *Schistosom*a cercariae with DNA detectable in their faeces post-consumption. We also demonstrate that the FFX method can detect schistosomiasis transmission in water bodies such as Lake Albert, perhaps with higher sensitivity than traditional methods, enhancing schistosomiasis transmission monitoring.

The study was supported by NERC London DTP, NE/L002485/1; the Fisheries Society of British Isles Small Grants programme, FSBI-RG21-2040; fieldwork was supported by the LCNTDR travel award.

THE POTENTIAL OF XENOMONITORING AS A TOOL FOR INDIRECT MONITORING OF SCHISTOSOMIASIS AND EVALUATING COMMUNITY-WIDE MASS DRUG ADMINISTRATION CAMPAIGN

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Schistosomiasis continues to affect hundreds of millions of people in sub-Saharan Africa. One of the ways to ensure effective control and elimination is through monitoring and evaluation of control programs. This study explored the potential of snail xenomonitoring as an indirect tool for monitoring and evaluating schistosomiasis mass drug administration. Freshwater snails were collected from human contact sites of schistosomiasis endemic areas along the Weija Lake in Ghana, before and after (1, 2, 6, 12, and 18 months) communitywide praziquantel treatment during 2017-2019. Snails were investigated for the production of cercariae of various morphotypes and further molecular analysis for schistosomes by amplification of ITS and COX-1 genes using genus and species-specific primers respectively. Amplicons were verified by gel electrophoresis and Sanger sequencing. The prevalence of schistosomes and non-schistosome infected snails pre- and post-PZQ MDA were statistically analyzed using simple ratios and frequencies in Microsoft Excel 2016 version. In all, 4223 snails were collected, which were made up of four different genera, and 289 (6.84%) shed any of 7 morphologically distinct types of cercariae. Out of these, 71 snails were identified as shedding cercariae of schistosome morphotype. Subsequent DNA analysis revealed that 77 snails were infected with schistosomes. Based on prevalence by morphotype of schistosome infection in susceptible snails, there was a significant decline from 4.11% at baseline to 0% at 12 months post MDA, while that of non-schistosomes appreciated during the study period. However, molecular studies showed a significant decline in schistosome prevalence of 0.6% at 2 months post-MDA and then rises sharply at 6- and 12 months post-MDA. This preliminary result reveals snail xenomonitoring as a potential tool for evaluating and monitoring schistosomiasis MDA, however, this must be validated by parallel studies in humans.

MONITORING OF ALIEN PARASITES *ASHWORTHIUS SIDEMI* AND *FASCIOLOIDES MAGNA* IN WILD RUMINANTS IN THE CZECH REPUBLIC USING REAL-TIME PCR

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Infections by non-native parasites can endanger entire native host populations. An example of such a parasite introduced into the Czech Republic is a pathogenic blood-sucking nematode *Ashworthius sidemi*, which has been imported via the East Asian sika deer (*Cervus nippon*). Similarly, the liver fluke *Fascioloides magna* spread to the Czech territory during the introduction of the North American white-tailed deer (*Odocoileus virginianus*). Ignorance of the parasite burden and prevalence, coupled with a lack of reliable diagnostic methods, has led to a significant underestimation of the impact of these parasitoses in the past. In fact, invasive species can cause severe to fatal infections especially in new, atypical hosts (e.g., endangered species or farm ruminants) and predisposed individuals (young, sick animals). It has also been confirmed that non-native parasites may displace native parasite species and significantly affect the parasitofauna of native wild ruminants. The aim of this study was: i) to provide a comprehensive insight into the current spread of the invasive parasites *Ashworthius sidemi* and *Fascioloides magna* and ii) to reveal impact of these parasites on populations of the endangered European bison (*Bison bonasus*) and other wild ruminant species (e.g., red deer *Cervus elaphus*, roe deer *Capreolus capreolus*, fallow deer *Dama dama*, and others) in game reserves and hunting grounds in the Czech Republic. To achieve this objective, an intravital diagnostic tool based on molecular detection of parasite DNA directly from animal faeces by real-time PCR was designed and optimised.

The study was supported by the Technology Agency of the Czech Republic, project no. SS05010070.

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Poster Session

(Chairman: P. Matoušková)

P1: PREVALENCE OF GASTROINTESTINAL PARASITES OF SHEEP IN SLOVAKIA DIAGNOSED BY DIFFERENT COPROLOGICAL METHODS

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As grazing animals, sheep are highly susceptible to parasitic diseases caused mostly by gastrointestinal helminths. Presence of parasitic worms has negative impact on production parameters, fertility and overall animals´ health. Monitoring and resulting effective control could help to eliminate negative outcomes associated with parasites. During 2020 – 2021 we examined 500 samples from sheep collected on 25 sheep farms across Slovakia for presence of internal parasites. Diagnosis was performed by using different coprological methods – flotation with miniFLOTAC, quantitative sedimentation method and larval differentiation technique. A representative number of samples was taken from each herd, which provided a picture of parasitological status of the entire farm. Out of 25 farms, 100 % farms were positive for eggs of Trichostrongylidae species, 88 % for coccidia, 72 % for *Moniezia* spp., 60 % for *Dicrocoelium dendriticum*, 44 % for *Nematodirus* spp., 40 % for *Fasciola hepatica*, 40 % for *Trichuris ovis* and 12 % for *Strongyloides* spp. As eggs from Trichostrongylidae family were diagnosed in 349 animals and 100 % of examined farms with EPGs range up to 7830, larval differentiation for species identification was performed. Dominant species was *Teladorsagia/Ostertagia* spp. (present in 100 % of farms) followed by *Haemonchus contortus* (84 %), *Trichostrongylus* spp. (80 %), *Chabertia ovina/Oesophagostomum* spp. (64 %), *Cooperia* sp. (16 %) and *Bunostomum* sp. (8 %). Larvoscopic examination of pooled samples for lungworms presence showed positivity in 80 % of farms with species *Muellerius capillaris* and *Protostrongylus rufescens*. Our results showed that gastrointestinal helminths are prevalent across whole country however a prominent part of the farms did not have a high parasite burden. The key to eliminate negative effect of parasites is not only regular deworming but also compliance with adequate pasture management.

The study was supported by funds from the Scientific Grant Agency VEGA 2/0099/19 and VEGA 2/0090/22.

P2: IDENTIFICATION OF CALCIUM-INFLUX AND KINASE ACTIVATION DURING MICROFILARIAE-INDUCED NADPH-OXIDASE-DEPENDENT AND -INDEPENDENT EOSINOPHIL ETOSIS

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Eosinophils play a major role during protective immune responses against parasitic filarial nematodes. One mechanism is the explosive release of DNA-containing cytotoxic granules, which entraps and kills pathogens. This effector mechanism is called extracellular DNA trap cell death (ETosis). Previous research on neutrophils has shown that ETosis can be induced through two different pathways: the NADPH-oxidase (NOX)-dependent and the calcium-dependent pathway. The NOX-dependent pathway is indicated by the activation of ERK1/2 and reactive oxygen species (ROS) produced by NOX. The calcium-dependent pathway is indicated by the activation of p38 and Pyk2, the production of mitochondrial ROS and histone citrullination. Previous results from our group have shown that microfilariae (MF) of the rodent filarial nematode *Litomosoides sigmodontis* induce eosinophil ETosis. Interestingly, viable MF induce the NOX-dependent ETosis pathway as determined by NOX-knockout eosinophils, while dead MF trigger the calcium-dependent ETosis pathway, which was determined by mitochondrial ROS production and histone citrullination in eosinophils. To further prove the activation of the two different pathways by viable and dead MF, calcium influx in eosinophils using two different fluorescence dyes was measured by fluorescence microscope as well as flow cytometry in response to the parasite. In addition, western blots were used to determine ERK1/2, p38, and Pyk2 phosphorylation as well as histone citrullination in eosinophils after stimulation.

The study was supported by DZIF Translational Thematic Unit: Novel Antibiotics grants #09.701, the Deutsche Forschungsgesellschaft (DFG, German Reaearch Foundation grant HU2144/3-1) and the DFG under Germany's Excellence Strategy – EXC2151 – 390873048.

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P3: A NEW SPECIES OF FILARIID NEMATODE FROM EPIRUS WATER FROGS (*PELOPHYLAX EPEIROTICUS*) IN GREECE

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Filariae are predominantly tissue-dwelling parasitic nematodes of terrestrial vertebrates belonging to the Onchocercidae family. Only a few species are associated with European amphibians, however some regions are completely unexplored in context of these parasites. Such a region is the Balkan peninsula, where the amphibian parasites diversity is almost unknown. The Balkans is considered a European hotspot of biodiversity and currently we recognize here seven species of water frogs (genus *Pelophylax*) out of which two are considered as endemic.

In years 2021 and 2022, we conducted two field collections in Greece and collected various helminths from endemic *P. epeiroticus* at Lake Ioannina. Besides endoparasitic trematodes we recorded also a new species of nematodes belonging to Onchocerchidae family. The relatively large-bodied adults were present in the mesentery of frogs and larval stages (filaria) were observed in the stained blood samples. Out of the ten examined *P. epeiroticus,* eight were parasitized by this new nematode species. The phylogenetic analyses based on five genetic markers (18S and 28S, rbp1, COI, and hsp70) revealed this new taxon as a close relative to waltonelline onchocerchids of the genus *Ochoterenella*.

The Waltonellinae and Icosiellinae are currently the only two subfamilies encompassing filarial nematodes of anurans. Although the *Icosiella* spp. are common parasites of European frogs, the representatives of Waltonellinae, and especially *Ochoterenella*, exhibit neotropical distribution. The finding of this entirely new taxon in the Balkan peninsula may either be an outcome of the previous biological invasion, or a remark on how underexplored is the diversity and distribution of anuran onchocerchids.

The study was financially supported by VEGA no. 1/0583/22 and APVV-19-0076.

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P4: HELMINTH DIVERSITY OF NUTRIA IN CZECH REPUBLIC

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Nutria was originally introduced to Europe from South America and kept in the fur industry. The first documented introductions date back to the second half of the 19th century, and since then, the animals escaped or were intentionally released into the wild. This semiaquatic rodent is currently a relatively well-established species in the Czech Republic; however, it still poses a threat to the native fauna, not only as the natural competitor but also by the transmission of non-indigenous parasites.
Only little is known about the helminth fauna of nutria in the Czech Republic. Therefore, our research aimed to investigate the diversity of their endoparasitic helminths with a particular focus on assessing the risk posed by helminths with zoonotic potential. During 2022 we collected 41 nutria cadavers from various sources at nine localities in the Morava River basin. In order to collect the endoparasitic helminths, we examined the internal organs (i.e., intestines, liver, spleen, kidneys, hearth, and lungs) under the stereomicroscope and washed them out with the system of sieves. A total of three nematode species were collected by this method, with the highest prevalence recorded in *Strongyloides myopotami* (prevalence = 71%) and *Trichuris myocastoris* (p = 37%). Both species are host-specific parasites of nutria. The diversity of trematodes was, in comparison, lower, as only two taxa were recorded – *Echinostoma* sp. (p = 2%) and unidentified species of the family Psilostomatidae (p = 12%). We also recorded the presence of alveolar hydatid cysts of *Echinococcus multilocularis* in the liver of four nutria specimens collected close to the city of Šumperk.
In the present study, we provide novel molecular data for each collected parasite species, which can be used in the future phylogenetic analyses. Our preliminary results also suggest that nutria is in the Czech Republic a carrier of helminths with zoonotic potential (i.e., *E. granulosus*, *S. myopotami*). Although it is locally a relatively new species, with its often synanthropic distribution the nutria may pose a threat to humans, so handling the animals should be taken with care.

The study was financially supported by AF-IGA2022-IP-030.

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P5: MARINE NEMATODE *ANISAKIS SIMPLEX* S.S. IN FISH PRODUCTS IN CENTRAL EUROPE: EPIZOOTIOLOGY, RISKS FOR CONSUMERS AND SEROLOGICAL SURVEY

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Food safety is an undisputed imperative in developed countries. Strict control of foods before their release to the market is also an important requirement to prevent infections with causative agents of human diseases, including parasitoses such as human anisakiasis. This currently emerging zoonosis is caused by larvae of marine nematodes of the genus *Anisakis*. In this work, we examined three marine fish species, *Clupea harengus*, *Scomber scombrus*, and *Pollachius virens*, from fish processing plants and ready-to-eat products from supermarkets in Slovakia and the Czech Republic for the presence of third-stage (L3) anisakid larvae. A total of 4,403 larvae were isolated by direct observation, UV diaphanoscopy, and artificial muscle digestion. All randomly selected specimens for genotyping were identified as *Anisakis simplex* s.s., the most common causative agent of human anisakiasis in the North Atlantic. All 100 partially gutted and frozen Atlantic herring (*C. harengus*) from fish processing plants were infected with anisakid larvae. The mean intensity of infection was 42 larvae/fish, with a range from 2 to 368 larvae per host. The vast majority of larvae (96.5%) were localised in the abdominal cavity (prevalence 100 %), 74 larvae were in the gonads (prevalence 21%), and, most importantly, 71 larvae were localised in the fish flesh (prevalence 33%). In six of 18 pickled herring purchased in a supermarket, 21 *Anisakis* larvae were found, with a range of 1 to 9 larvae/fish. In Atlantic mackerel *S. scombrus* from a supermarket, 219 anisakid larvae were found in six of eight fish, with a mean intensity of 219 larvae/fish (range 1–151). In this fish, anisakids were found in the muscles of one frozen (13 larvae) and two smoked (1 and 2 larvae, respectively) individuals. Fillets from two Saithe, *P. virens*, were negative. The risk of human infection with this marine parasite in a landlocked country is supported by the positive result of a cross-sectional pilot study of IgE sensitisation with the anisakid allergen t-Ani s7 in 91 volunteers regularly consuming fish or fish products, performed by indirect ELISA.

This study was supported by the Slovak Research and Development Agency (APVV SK-CZ-RD-21-0078) and the Ministry of Education, Youth and Sports of the Czech Republic (project LUASK22045).

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P6: *GAMMARUS BALCANICUS* AND *GAMMARUS FOSSARUM* (CRUSTACEA, AMPHIPODA) AS THE INTERMEDIATE HOSTS OF HELMINTH PARASITES IN EASTERN SLOVAKIA

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The study of native biodiversity is important for understanding the complex interactions between species and the impact of human activities and global climate change on the environment. Gammarids were sampled and examined for helminth parasites at 5 localities in eastern Slovakia. A total of 2075 specimens of *Gammarus balcanicus* and 110 specimens of *G. fossarum* were examined. Ten species of helminths were found: 3 species of trematodes (*Maritrema neomi*, *M. pyrenaica*, *Cephalotrema minuta*), 4 cestodes (*Coronacanthus integra*, *C. omissa*, *Triodontolepis hamanni*, *T. torrentis*) and 3 acanthocephalans (*Polymorphus minutus*, *P. cincli*, *Pomphorhynchus tereticollis*). The trematodes *M. neomi*, *M. pyrenaica*, and the acanthocephalan *P. cincli* are the first record for Slovakia. Amphipods are the intermediate hosts for all helminths reported. The definitive hosts for *P. tereticollis* are a broad range of fish, *P. cincli* is a specific parasite of the bird, *Cinclus cinclus*, while the remaining 7 helminth species are parasites of insectivorous mammals. The parasite prevalence (P) ranged from 0.14% for *C. omissa* to 14.1% for *P. tereticollis* from *G. balcanicus* and 2.2% for *T. torrentis* and *P. minutus* to 6.7% for *M. pygmaeus* from *G. fossarum*. The intensity of infection (I) was low, reaching the maximum value of 22 specimens for *M. pyrenaica*. The previous study performed in the small Morske Oko Lake (Dudiňák and Špakulová, 2001) showed significantly higher infection parameters for acanthocephalan *P. tereticollis* compared to our observation. Thus, the prevalence of this parasite species sharply decreased from 71.4% in 1999 to 4.7% in 2022. The reasons for these changes could be attributed to the dramatic depopulation of definitive hosts, *Phoxinus phoxinus* and *Carassius carassius*, the only fish occurring in the lake. The decline in fish populations could be related to global climate change resulting in intensive evaporation and the currently observed decline in water levels, as other human disturbances are unlikely as the small Morske Oko Lake is strictly protected.

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

WEDNESDAY, MAY 10

Session IV

Molecular Biology & Transcriptomics of Helminths

(Chairman: R. Leontovyč)

A GENE EXPRESSION ATLAS BY SINGLE-CELL AND SPATIAL TRANSCRIPTOMICS AS VALUABLE RESOURCE IN LIVER FLUKE RESEARCH

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Fasciolosis is a zoonotic disease caused by liver flukes of the genus *Fasciola* spp. Up to date, there is a significant lack of knowledge on the parasite's cell types and cell-specific gene expression repertoire. Novel developments in transcriptomic techniques like single-cell (sc) transcriptomics and spatial transcriptomics (st) allow for large scale analysis of the transcriptomics landscape also of non-model organisms. We planned to establish a comprehensive gene expression atlas for *F. hepatica.*

Both techniques utilize barcoded oligonucleotides that allow the retracing of sequencing data to their respective source. For scRNA-seq, this results in transcriptome data on a single cell level. The st method involves capturing and barcoding of transcripts *in situ* using oligonucleotide-coated glass slides. This way, data on gene expression can be spatially resolved and mapped into the tissue context.

Clustering the scRNA-seq data based on transcriptional information identified 15 cell clusters for adult liver flukes, which represent biologically relevant cell types. We could validate these cell types including gastrodermal cells expressing cathepsins, neoblasts expressing nanos2, and neuronal cells. Eight different tissues could be resolved with st, such as intestine, tegument and reproductive organs. By differential expression analysis, marker genes for each sc and st cluster were identified and validated by RNA *in situ* hybridisation. Clusters were further functionally assessed by functional gene ontology (GO) enrichment analysis. This revealed characteristic biological processes and molecular functions associated for each cluster. Furthermore, this new atlas enabled us to identify several drug target genes (such as ß-tubulins, protein kinases and calcium channels), drug resistance genes, and transcription factors with cell type- or tissue type-specific expression.

Taken together, this work provides the first transcriptomes for the liver fluke *F. hepatica* on a single-cell and spatial resolution. Our expression atlas serves as novel tool for the unravelling of cell biological secrets in this parasite.

IN SILICO METABOLIC MODEL OF THE PARASITIC NEMATODE *HAEMONCHUS CONTORTUS*

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Parasitic nematodes threaten the health of the human population and represent a major burden on livestock and crop production. Pharmacotherapy, the mainstay in combat against these parasites, has become less and less efficient due to development of drug-resistance in nematodes. Therefore, only detailed knowledge of the metabolism of each nematode species will make prevention and treatment possible in the future. Great advances in "omics" technologies, bioinformatics, and computational biology can be used to understand the metabolism of nematodes properly. We used a computational approach to create a constraint-based model (CBM) for *Haemonchus contortus* additionally applying methods for the integration of transcriptomic and metabolomic data. CBMs were developed shortly after the first microbial genomes were sequenced and they rely directly on the genomic information and biological databases in order to predict metabolic functions through gene-protein-reaction mechanisms. These models allow us to simulate the growth of *H. contortus in silico* as well as test various hypothesis and provide an alternative to traditional experimental approaches. Furthermore, they function as a knowledge base of the organism and highlight gaps in our understanding of it's metabolism. The created metabolic model of *H. contortus* can be a building block for future research such as the identification of potential drug targets and mechanisms of drug-resistance.

WINNERS VS. LOSERS - COMPARATIVE TRANSCRIPTOMIC ANALYSIS OF *SCHISTOSOMA MANSONI* EGGS FROM GUT AND LIVER

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Schistosomiasis is a neglected tropical disease that affects more than 200 million people worldwide and causes significant morbidity and mortality. Eggs laid by adult *Schistosoma mansoni* worms are key players in the pathology and transmission of schistosomiasis, with eggs that end up in liver tissue causing chronic inflammation and fibrosis. However, research to date has largely focused on these "losers" eggs, which have limited impact on disease transmission, while the more biologically important "winners" eggs from the gut, which are essential for maintaining the parasite's life cycle, have been largely overlooked. In our study, we compared the gene expression and fitness of *S. mansoni* eggs isolated from the intestine and liver. Surprisingly, we found that the well-known and well-studied immunomodulatory egg molecules *IPSE* and *Omega-1* are almost exclusively expressed by liver-derived eggs. We also observed that genes encoded by the mitochondrial genome and pathways related to the ribosome, translation, and peptide biosynthesis were highly upregulated in intestine-derived eggs, suggesting that these eggs are more metabolically and developmentally active and "wired for success" compared to liver-derived eggs. In addition, gut-derived eggs showed higher fitness compared to liver-derived eggs in terms of hatching rate and viability. Our results provide compelling evidence that eggs actively respond to surrounding tissues and that liver-derived eggs have reduced protein synthesis and energy metabolism compared to gut-derived eggs. This has important implications for understanding the pathology and transmission of schistosomiasis. Overall, we provide new insight into how the winner and loser eggs differ and speculate that there is likely a reason why even loser eggs are evolutionarily advantageous to schistosomes.

The study was supported by Czech Science Foundation grant 23-06638S and ARES Trading S.A., an affiliate of Merck KGaA, Darmstadt, Germany (Schistosomiasis Research Grant FR4003935).

ROLE OF EGGS SPECIFIC VENOM ALLERGEN-LIKE PROTEINS DURING THE INFECTION WITH *SCHISTOSOMA MANSONI*

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The Venom allergen-like protein (VAL) family belongs to the SCP/TAPS superfamily, which shares a unique protein domain with a highly conserved α-β-α sandwich conformation. This strong conservation of the tertiary structure and specific residues within the domain suggest that all SCP/TAPS domain-containing proteins share a common but unelucidated biological activity. While excretory-secretory products of helminths reflect their diversity in lifestyle and hosts, members of the VALs superfamily are ubiquitous in these products, indicating a close association with parasitic lifestyle. Previous studies have identified 29 genes coding SmVAL proteins in the *Schistosoma mansoni* genome, but so far only 4 of these proteins have been associated with any functional data and the results are inconclusive. However, the quality of the parasite genome has significantly improved over the last decade and with the most recent version of it we were able to identify 6 previously undescribed SmVAL genes in the parasite's DNA. Moreover, through meta-analysis of freely available RNASeq data of all *S. mansoni* life stages, we revealed SmVALs expression profiles across the life cycle as well as all the other parasite's genes. We found that genes encoding SmVAL proteins are most highly expressed in the eggs of the parasite, which are the main pathogenic agent of schistosomiasis. Seven of these genes form a common phylogenetic branch and their expression is restricted exclusively to the egg stage. To determine whether these proteins can directly interact with host proteins, we generated recombinant forms of the most highly expressed egg-specific SmVALs and performed localization studies in various host tissues using specific antibodies. Next, we plan to perform pull-down assays to identify binding protein partners, thus approaching the possible function of these enigmatic proteins. Our data not only provide valuable insights into the egg-specific VAL proteins of a major human parasite, but also, the resulting atlas of *S. mansoni* gene expression across the life cycle will serve as a valuable resource for the entire community upon publication.

This study received support from the Czech Science Foundation grant 23-06638S; Merck Schistosomiasis Research Grant FR4003935; Grant Agency of Charles University project No. 198722.

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Session V

Immunology III: Pathogenesis & Host Immune Response

(Chairman: L. Mikeš)

TRICHOBILHARZIA SPECIES AFFECT THE COURSE AND THE SEVERITY OF CERCARIAL DERMATITIS IN A SPECIES-SPECIFIC MANNER

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Clinical symptoms of cercarial dermatitis (CD), a skin allergic reaction caused by avian schistosomes, vary between infected individuals. Traditionally, the intensity of the immune reaction is linked to the number of exposures. However, other factors, such as the specific infection agent, are usually neglected. To investigate the link between parasite species and the symptoms of CD, we compared the immune response of mice exposed to the cercariae of *Trichobilharzia regenti* and *T. szidati*. In our experiments, infection by *T. regenti* led to stronger inflammation that was prominent early after cercarial penetration. Cercariae of *T.* regenti, exclusively, induced significant production of thymic stromal lymphopoietin, an alarmin that could drive the early type 2 inflammation. Additionally, the greatest influx of leukocytes into the *T. regenti*-infected skin was between 24 and 48 hours post infection, whereas cellular immune response towards *T. szidati* peaked at 7 days post infection. Moreover, *T. regenti*-infected skin was conspicuously erythematous. Furthermore, systemic immune response, evaluated by the levels of parasite-specific IgG antibodies and serum cytokines, was stronger in mice infected with *T. regenti*. Collectively, our screening study revealed the noteworthy impact of parasite species on the severity and the dynamics of the immune response while showing a higher immunopathogenic potential of *T. regenti*. Our results show variable CD-causing potential of the *Trichobilharzia* spp., which could explain differences in the intensity of the symptoms as well as the frequency of reported CD cases over different localities.

The study was supported by Czech Science Foundation (18-11140S), ERDF and Ministry of Education, Youth and Sports of the Czech Republic (CZ.02.1.01/0.0/0.0/16_019/ 0000759: Centre for Research of Pathogenicity and Virulence of Parasites), and Charles University institutional funding (Charles University COOPERATIO BIOLOGY (2022-2026), UNCE/SCI/012 - 204072/2018 (2018-2023), SVV 260563/2020).

BSEP/ABCB11 KNOCKOUT AMELIORATES *SCHISTOSOMA MANSONI* LIVER PATHOLOGY BY REDUCING PARASITE FECUNDITY

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Infection with *Schistosoma mansoni* is one of the worldwide leading causes of liver fibrosis and portal hypertension. Here we examined the disease outcome in mice lacking the bile salt export pump (*Bsep/Abcb11* KO mice; further referred to as "BSEP KO"), a transporter localized on the hepatocyte canalicular membranes where it facilitates biliary excretion of bile acids. BSEP KO mice accumulate polyhydroxylated bile acids that protect them from the development of cholestatic liver injury (including inflammation and fibrosis). Therefore, we infected WT and BSEP KO mice with *S. mansoni* and examined them eight weeks later. Specifically, we evaluated effects on liver histology, serum biochemistry, the gene expression profile of (pro-)inflammatory cytokines and fibrotic markers, and the hepatic collagen content. Also, the host immune response was analyzed by flow cytometry. The infected BSEP KO mice showed significantly less hepatic inflammation and tendentially less fibrosis than WT controls. Despite elevated ALT, AST, and AP levels in infected BSEP KO mice, inflammatory cells such as M2 macrophages and Mac-2/galectin-3+ cells were reduced in these animals. Accordingly, mRNA-expression levels of anti-inflammatory *Il4* and *Il13* were increased in infected BSEP KO mice. Furthermore, they exhibited decreased hepatic egg load and parasite fecundity, affecting the worm reproduction rate. These findings may, at least in part, be attributed to elevated serum bile acid levels and hence lower blood pH in infected BSEP KO mice. We conclude that the loss of BSEP and the resulting changes in bile acid composition and blood pH reduce parasite fecundity, thus attenuating the development of *S. mansoni*-induced hepatic inflammation and fibrosis.

SEARCHING FOR PROTEINS INVOLVED IN THE INFECTION OF THE ENTOMOPATHOGENIC NEMATODE *HETERORHABDITIS BACTERIOPHORA*

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Entomopathogenic nematodes (EPNs) are obligate lethal insect parasites used in the biocontrol of many insect pests. Their use as alternatives to synthetic insecticides reduces the accumulation of pesticides in the environment.

During infection, EPNs release excretory/secretory products (ESPs) containing molecules that facilitate the invasion and modulate the host immune system. EPNs involve two genera *Heterorhabditis* and *Steinernema*. We chose the less studied *H. bacteriophora* species to uncover the spectrum of molecules released during the invasion process by infective juveniles (IJs). It is believed that IJs start producing active compounds upon the contact with the molecules of host origin. Three types of homogenates derived from the insect host *Galleria mellonella* (the greater wax moth) are usually used to activate IJs of *H. bacteriophora*. Our aim was to evaluate the effect of these activation agents on the ESP protein content and to identify invasion-specific molecules by comparison with the ESP protein profiles of nonactivated IJs.

ESPs produced by IJs challenged with three types of homogenates prepared from *G. mellonella*, PBS or water were collected from the media, concentrated, and subjected to LC-MS/MS analysis using RSLCNano system on-line connected to Impact II Ultra-High Resolution Qq-Time-Of-Flight mass spectrometer. Protein searches were performed against a protein database derived from the *H. bacteriophora* reference genome.

We quantified more than 300 proteins present in ESPs of *H. bacteriophora* and observed differences in ESP content related to each activation agent. Among ESPs, we identified metallopeptidases, ShTK domain proteins, fatty acid- and retinoid- binding protein, and other molecules with immunomodulatory potential.

The study was supported by the Czech Science Foundation, Project No. 23-06457S.

KUNITZ PROTEINS OF *EUDIPLOZOON NIPPONICUM*

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Kunitz domain-containing proteins are mostly 6-10 kDa inhibitors of serine proteases, but there are also rare examples of cysteine and aspartic protease inhibitors within this group. Their main characteristic is the presence of six cysteine residues forming three disulfide bridges shaping a typical active loop that fits the active site of various proteases. Their specificity is largely determined by a residue in P1 position. Their functions include the regulation of various proteolysis-based events, for example blood coagulation cascade or immune reactions. In addition, they are found in a wide range of animal toxins and serve as a powerful tool for many parasitic organisms to interact with their hosts.

Representatives of the class Monogenea have been a neglected group in terms of molecular parasite-host interactions, and only a few works have dealt with their biochemistry and the characterization of biologically active molecules. In this study, we provided a deeper insight into the spectrum of Kunitz inhibitors in *Eudiplozoon nipponicum*, a blood-sucking member of the class Monogenea that has become a fairly common parasite of the common carp (*Cyprinus carpio*), which is of high economic importance in Europe and Asia. Although *E. nipponicum* is not directly responsible for host death, its presence damages carp gills and may cause hypochromic microcytic anemia in the fish.

This work expands and complements the information on two Kunitz proteins (EnKT1 and EnKC1) from *E. nipponicum*, which are likely involved in blood uptake and defense against host immune response. For recombinant proteins obtained by expression in *E. coli*, the ability to inhibit selected proteases participating in the coagulation cascade or present in certain immune cells was confirmed, thus opening the assumption about the involvement of these proteins in interfering with the host's immunity. The ability of EnKT1 to inhibit the coagulation of fish blood and significantly slow down the coagulation of human blood was confirmed. Furthermore, the effect of both proteins on the production of cytokines from splenocytes and the effect on the overall activity of splenocytes and macrophages was studied in a mouse model using ELISA and the Griess reaction. Finally, an effort to localize EnKC1 in parasite's body by immunohistochemistry will be discussed.

Our findings lead to a better understanding of the monogenean-fish interactions on molecular and cellular basis.

The study was supported by ERD Fund and MEYS of the Czech Republic - Centre for Research of Pathogenicity and Virulence of Parasites" (no. CZ.02.1.01/0.0/0.0/16_019/0000759) and the Charles University COOPERATIO BIOLOGY (2022-2026), SVV 260563/2020.

THURSDAY, MAY 11

Session VI

Anthelmintic Resistance & Xenobiochemistry

(Chairman: L. Skálová)

WILL ANTHELMINTIC RESISTANCE THREATEN THE SUSTAINABILITY OF SMALL RUMINANT PRODUCTION IN THE CZECH REPUBLIC?

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Gastrointestinal nematodes (GINs) are ubiquitous on pastures grazed by ruminants. Although infections caused by these parasites are mostly subclinical, they can lower the production of meat, milk and wool in livestock. In intensive farming systems, control of GINs is traditionally achieved through regular treatment with anthelmintic drugs. Many farmers have relied on chemotherapeutics as the sole control strategy for decades, but intensive use of these drugs has led to the development of anthelmintic resistance (AR). AR has already been detected in all major GIN species in farmed ruminants and this phenomenon is now widespread in many countries all over the world and poses an important factor limiting ruminant production. Because the emergence of AR is inevitable at any farm applying chemotherapeutics continuously and reversion of GINs towards anthelmintic susceptibility is unrealistic, understanding the factors promoting AR development are essential to effectively control GIN infections in the future.

In comparison to other European countries, only limited data are available concerning AR and risk factors in both Czech sheep and goat farms, and information about this topic in cattle is still lacking in the Czech Republic (CR). Based on the available data it can be concluded that AR is well established amongst sheep flocks in the CR, and the current data suggest that the problem is not less pronounced in goat herds. Resistance to the two most commonly used drug classes (benzimidazoles and macrocyclic lactones) has been detected at both Czech sheep and goat farms. Multiple AR has yet to be identified in the CR; however, dual resistance in dairy goat herds has recently been published. The questionnaire surveys on putative risk factors for AR development administered with Czech farmers revealed the specific farmers' behaviour and farm management practices as the driving factors for AR.

Anthelmintic treatment highly probably will remain the cornerstone tool for controlling GIN infections in the near future, so measures are required to preserve the efficacy of anthelmintics. AR may strongly affect the profitability of small ruminant production in the CR and globally if the current strategies of parasite control continue. Dairy goat and sheep farms will presumably be the most severely affected due to the limited number of drugs applicable in lactating animals. Results of questionnaire surveys confirm the urgent need of implementing modern integrated parasite control measures in the farm management. However, dissemination of current knowledge about AR and sustainable parasite control amongst stakeholders will be challenging.

FLUBENDAZOLE-INDUCED CHANGES IN THE EXPRESSION OF SELECTED SDR GENES THROUGHOUT THE DEVELOPMENTAL STAGES OF *HAEMONCHUS CONTORTUS*

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Anthelmintic therapy in the agriculture industry is complicated by reduced drug efficacy. Long-term exposure to sub-lethal doses of drugs can lead to changes in the expression of specific detoxification enzymes and drug resistance development. Previous studies have shown that increased anthelmintic inactivation via biotransformation enzymes, such as cytochromes P450 or UDP-glycosyltransferases, belongs to a significant mechanism of drug resistance in Haemonchus contortus. However, the genome of H. contortus also contains genes from the short-chain dehydrogenases/reductases family (SDRs), catalysing the main metabolic transformation of carbonyl-containing anthelmintics (e.g. flubendazole, mebendazole). High variability in the constitutive expression of SDR genes in a drug-susceptible and drug-resistant strain of H. contortus indicates the importance of studying these enzymes in view of their potential participation in anthelmintic resistance. In the present study, the effect of sub-lethal doses of flubendazole (FLU) on the relative expression of 23 SDR genes throughout various developmental stages (eggs, larvae, adults) in H. contortus was evaluated.

Adult nematodes were incubated ex vivo with or without FLU (0.01 µM, 1 µM, and 5 µM) in a culture medium for 4 or 12 hours. Based on the gene expression results of all 23 SDRs in adults, only one concentration (1 µM) and one time (12 h) were selected for incubation and subsequent measurement of expression in younger developmental stages.

Contact of adult nematodes with sub-lethal doses of FLU resulted in significant induction of several genes at higher concentrations, particularly SDR3, SDR12, SDR16, and SDR19, while only minor changes were observed in the FLU-exposed developmental stages. Evaluating the ability of different developmental stages to reduce FLU, which is almost complete, will be a guide for the next steps.

The study was supported by Charles University, project GA UK No. 194421.

HETEROLOGOUS EXPRESSION OF SDR GENES FROM *HAEMONCHUS CONTORTUS*

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Haemonchosis is a serious parasitic disease of small ruminants, that causes the death of livestock which leads to socio-economic losses worldwide. Therapy of haemonchosis is becoming increasingly difficult due to growing resistance to all classes of anthelmintics, which are the treatment of choice alongside pasture management and nutritional supplements. One of the proven mechanisms of deactivation of anthelmintics (e.g., flubendazole) is the reduction of the carbonyl group by carbonyl-reducing enzymes (such as SDR and AKR). Carbonyl-reducing enzymes catalyze the first phase of xenobiotics biotransformation and thus participate in drug metabolism. Increased elimination leads to decreased toxicity and reduced efficacy of drugs in Barber´s pole worm and makes treatment more difficult. In addition, previous studies also confirmed, that resistant strain (IRE) is more efficient in the elimination of anthelmintic than sensitive strain (ISE). This project is focused on the heterologous expression of SDR genes from Barber´s pole worm in a suitable host. This process allows us to produce enzymes on a larger scale so that we can characterise them, determine their enzyme activity and even test new potential inhibitors in the future. The genome of *H. contortus* contains approximately 70 SDR genes and 24 AKR genes; however, information about expression and function is not yet known. In addition, previous quantitative gene expression analyses in *H. contortus* showed that the most highly expressed genes were SDR1, SDR3, SDR12, SDR16 and SDR18. Also, SDR12 expression was significantly higher in all life stages (eggs, larvae and adults) of the resistant strain. All of these genes were already subcloned into vectors for sequencing, SDR12 was also successfully cloned into expression vector pET-22b(+) and expressed in *E.coli* BL21. Production of protein in *E. coli* was induced by IPTG and confirmed by western blotting. A similar approach will be followed for the other SDR genes.

This project was supported by Czech Science Foundation (Grant No. 20-14581Y).

IN VITRO INHIBITION OF ENZYMES REDUCING THE ANTHELMINTICS FLUBENDAZOLE IN *HAEMONCHUS CONTORTUS*

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Haemonchus contortus is a gastrointestinal nematode of small ruminants with a high level of anthelmintic resistance. *H. contortus* causes a significant parasitic disease called haemonchosis, which affects small ruminants and spread worldwide, leading to livestock mortality and economic losses. The administration of anthelmintics is one of the most widely used forms of treatment for this disease, but there are only a limited number of approved anthelmintics. One of the proven mechanisms of anthelmintic resistance in Barber´s pole worm is increased deactivation and elimination of anthelmintics (e.g., flubendazole, mebendazole) by reduction of carbonyl group by carbonyl-reducing enzymes. Carbonyl-reducing enzymes (CBR) catalyze the first phase of xenobiotics biotransformation and participate in drug metabolism. The previous metabolism analysis also demonstrated a higher ability of the resistant strain of *H. contortus* to reduce flubendazole more effectively than the sensitive strain. This project aimed to study *in vitro* NADPH-dependent enzymatic reactions with the use of cytosolic fractions from a homogenate of *H. contortus* strain ISE and IRE and observed a reduction of flubendazole. The formation of reduced flubendazole (FLU-R) in response to selected inhibitors (mebendazole, glycyrrhetinic acid, naringenin, silybin, luteolin, glyceraldehyde and menadione) was investigated and then detected by LC-MS. The results were statistically evaluated by unpaired multiple T-test with Welch's correction ($n = 3$), $^*P < 0.05$. The administration of anthelmintics is one of the most widely used forms of treatment for this disease, but there are only a limited number of approved anthelmintics. In addition, declining efficacy due to the development of drug resistance makes the treatment of helminthiasis very difficult. The increase in resistance is due to several factors such as the overuse of prophylactic drugs. The discovery of new inhibitors that could enhance the effect of anthelmintics could help in the future in the treatment of helminthiasis.

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POTENTIAL ANTHELMINTIC EFFECT OF SILYBINS

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Silybin, which belongs to the group of flavonolignans, is the main component of silymarin, an extract from the seeds of milk thistle (*Silybum marianum*), which has been used for its therapeutic effects since ancient times. In addition, current studies suggest that silybins may have hepatoprotective effects, neuroprotective effects in neurodegenerative diseases, and stimulate cardioprotective activity. Silybins have modulatory activity in multidrug resistance, flavonolignans can modulate transporters, and silybins have been shown to positively affect doxorubicin treatment. Not only for these reasons, silybin has received increasing research attention, which has led to the preparation of a large number of semi-synthetic derivatives that modulate and better target the biological activities of silybin and increase the bioavailability of silybin for more effective treatment.

This study aims to determine whether any of the nine semi-synthetic silybins (all prepared at the Institute of Microbiology, Centre for Biocatalysis and Biotransformation) will affect the efficacy of the anthelmintic Ivermectin on *H. contortus*. and if so, how the efficacy will vary depending on the type of strain. *Haemonchus contortus*, which belongs to the group of gastrointestinal parasitic nematodes, is well known in most of the world because it causes significant economic losses in livestock production. Moreover, resistance to all classes of anthelmintics has been reported. Three different strains of *H. contortus* will be used in this study: the benzimidazole susceptible strain ISE (Inbreed susceptible Edinburgh), the benzimidazole-resistant strain IRE (Inbreed resistant Edinburgh), and the multiresistant strain WR (White River).

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Session VII

Taxonomy, Diversity

& Phylogeny of Helminths

(Chairman: M. Oros)

NEW SCENARIO OF THE EVOLUTION OF NEODERMATA BASED ON TRANSCRIPTOMIC DATA

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Within flatworms, the vast majority of parasitism is represented by the Neodermata clade, which is traditionally divided into four major lineages that maintain different combinations of life strategies. These include both parasites that feed externally (ecto-) and internally (endo-). Some lineages complete their life cycles directly by infecting single hosts, while others are successful only when they infect a number of different hosts from various groups of vertebrates and invertebrates. Food sources and digestive methods add further combinatorial layers to the often incompletely understood mosaic of neodermatan life histories. Due to conflicting evolutionary inferences and lack of genomic data, their evolutionary development at the molecular level remains to be elucidated. Here, we generated transcriptomes for nine early branching representatives of the Neodermata and performed detailed phylogenomic analyses to fill these critical gaps. **Monopisthocotylea**, the ectoparasitic epithelial feeders, evolved earliest, followed by the endoparasitic and highly specialised **Cestoda**, which lack the gut **Polyopisthocotylea**, mostly hematophagous ectoparasites, together with the mostly hematophagous, but endoparasitic **Trematoda**, form the crown group of Neodermata. This phylogenetic scenario provides an unconventional perspective on the evolution of platyhelminth parasitism by rejecting a common origin for the endoparasitic lifestyle of cestodes and trematodes. Instead, our data suggest that complex life cycles and invasion of the vertebrate intestinal lumen, the characteristic features of these parasites, evolved independently within the Neodermata. We propose the demise of the traditionally recognised class Monogenea and the promotion of its two subclasses.

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DIVERSITY OF BIRD SCHISTOSOMES IN EUROPE WITH THREE NEW LINEAGES

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Bird schistosomes are the main causative agent of human cercarial dermatitis, an allergic disease causing skin eruption. This disease is re-emerging, with increasing cases reported worldwide. Despite the vast diversity of bird schistosomes, these parasites still remain undiscovered. In this study, bird schistosome samples were collected from snails in various freshwater ecosystems in Europe over a 10-year period. Cercariae released by the snails were molecularly characterized. Out of 184 infected snails from 20,000 snails examined, a total of 11 species of bird schistosomes were found in seven species of planorbid and lymnaeid snails collected from 18 localities in the Czech Republic, Germany, Norway, and Poland. Eight previously reported species were identified, namely *Anserobilharzia brantae*, *Trichobilharzia anseri*, *T. franki*, *T. mergi*, *T. regenti*, *T. szidati*, *Trichobilharzia* sp. haplotype '*peregra*', and Avian schistosomatid sp. l4. Three new lineages were discovered, namely (i) Schistosomatidae gen sp. I (ii) Schistosomatidae gen sp. II and (iii) Schistosomatidae gen sp. III. In addition, our study revealed the first report of *A. brantae* in the intermediate snail host in Europe, previously reported from North America, and the first report of *T. anseri* and *T. mergi* in the Czech Republic. This study contributes to the data about diversity and geographical distribuition of bird schistosomes in Europe. However, despite the high diversity of bird schistosomes detected, taxonomic classification still needs to be resolved.

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SPECIES RICHNESS, MORPHOLOGICAL DIVERSITY, AND MOLECULAR PHYLOGENY OF *GYRODACTYLUS* COMMUNITIES (MONOGENEA: GYRODACTYLIDAE) FROM NEARCTIC CYPRINIFORM FISH HOSTS

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Despite the known tremendous diversity of freshwater fishes in the Nearctic region, little is known about the community composition of viviparous monogeneans of genus *Gyrodactylus* and their phylogenetic position within congeners worldwide. Herein, we morphologically and genetically studied *Gyrodactylus* spp. found to parasitize highly diversified cypriniform fish representatives occurring in Noth American watersheds in the USA and Canada. Combination of taxonomically important haptoral features and sequences of the ITS regions and the 18S rDNA revealed 25 *Gyrodactylus* spp. parasitizing members of Catostomidae and Leuscicidae, of which ten monogenean species showed to be new to science. Phylogenetic inference based on sequences of the ITS regions demonstrated the paraphyletic origin of Nearctic *Gyrodactylus* lineages and their relatedness to their Palearctic congeners, supporting their dispersal scenarios across continents. In the Nearctic region, haptoral structures in members of *Gyrodactylus* have apparently evolved from a very simple form to a more complex one, as in many other monogenean taxa. In this system, morphotypes of haptoral sclerites of *Gyrodactylus* spp. ranged from structures with a "standard" morphology typical for many *Gyrodactylus* communities with a worldwide distribution, to the forms of attachment configurations predominantly restricted to Nearctic *Gyrodactylus* lineages. The characteristic features of "Neartic" morphotypes of haptoral sclerites are the median knob in the ventral bar and the plate-like membrane, or the additional filament attached to the handles of marginal hooks. The integrative taxonomic approach further evidenced possible ongoing gene flow, host-switching in generalist *Gyrodactylus* spp., and regional translocation of monogenean fauna through fish introductions.

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MORPHOLOGY AND PHYLOGENY OF *DACTYLOGYRUS* IN THE MIDDLE EAST REVEALING UNEXPECTED DIVERSITY

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Despite several studies focusing on the diversity of *Dactylogyrus* species (Monogenea) in the Middle East, some knowledge gaps still need to be addressed, especially considering missing molecular data. Considering the Middle East's role as a crossroad for cyprinoid fish species to disperse from East Asia to Europe and North Africa, we aimed to investigate the diversity, phylogeny, and host specificity of *Dactylogyrus* in the Middle East with an emphasis to explore cyprinoid historical dispersion through phylogenetic relationships of their *Dactylogyrus* species.

In 2022, cyprinoid fish were sampled in Turkey and Iran, and the *Dactylogyrus* specimens were collected. Sixty-five *Dactylogyrus* species were identified on 55 cyprinoid species. To assess the *Dactylogyrus* diversity and phylogeny, partial 18S rDNA, 28S rDNA, and complete ITS1 region were sequenced. Performing phylogenetic analyses using the Middle East *Dactylogyrus* spp. and selected *Dactylogyrus* spp. retrieved from GenBank we revealed seven major clades, with the representatives of the Middle Eastern species positioned in four clades. The Middle East-specific *Dactylogyrus* species were grouped with European, North African, and East Asian species, confirming the role of the Middle East in *Dactylogyrus* diversification and cyprinoid historical dispersion. Four potentially new species for science were revealed in Turkey. *Dactylogyrus goktschaicus* showed unexpected genetic intraspecific variability despite the similarity in morphological characteristics. Further, morphological and molecular intraspecific variability was found in *D. lenkorani*. Surprisingly, the intraspecific variability in *D. goktschaicus* at the host species level and *D. lenkorani* at the geographical level indicated unexplored *Dactylogyrus* diversity on cyprinoids in the Middle East.

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DIVERSITY OF FRESHWATER FISH PARASITES IN IRAQ

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Several recent checklists reported extremely diverse helminth fauna of freshwater fish in Iraq. Nonetheless, from the molecular phylogenetic standpoint, this region is largely unexplored, as almost no DNA sequence data for parasites of endemic fish are available. As the Middle East played an important role in the colonization of Europe and North Africa by cyprinoid fish, we expect close phylogenetic relationships among congeneric helminths from these continents.

Our research aimed to investigate the parasite diversity of cyprinoid fish in Iraq using an integrative approach, combining morphology with genetics. A total of 15 cyprinoid species were examined in 2021 for the presence of ectoparasitic and endoparasitic helminths. The highest species diversity was recorded among monogeneans with 17 species of *Dactylogyrus* (5 species were recognized as new for science and described), 12 species of *Gyrodactylus* (all new for science), 4 species of *Dogielius* (2 new for science), and 2 species of *Paradiplozoon*. The diversity of endoparasitic helminth was low, with the highest species richness within trematodes. The endemic cyprinoids harboured the representatives of *Allocreadium* and *Asymphylodora* genera. In addition, the larval stages (metacercariae) of *Clinostomum complanatum* were collected from the fish surface. The potentially new species for science was also recorded for Acanthocephala.

Phylogenetic reconstructions based on the commonly used genetic markers revealed paraphyletic relationships among endemic congeneric helminth taxa (i.e., *Dactylogyrus* and *Gyrodactylus*). The phylogenetic proximity of the endemic species to African and European congeners supports the assumption that the historical diversification of freshwater species occurred in the Middle Eastern region.

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Session VIII

Host-Parasite Interactions:

Ecology & Evolution

(Chairman: R. Kuchta)

FISH-PARASITE SENTINEL SYSTEM AS EMERGING TOOL FOR ENVIRONMENTAL HEALTH BIOMONITORING: WELS CATFISH AND ITS CESTODES

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Pollution of water sources is of great concern as they are the habitat of fish and other aquatic organisms and affect the health of the resident human population directly or through the food chain. Fish are often used as bioindicators to monitor environmental pollution because they are close to the top of the food chain in aquatic environment with a relatively long life span, which allows them to store high amounts of contaminants. The aim of the present study was to determine the concentrations of the six indicator congeners of polychlorinated biphenyls (PCBs) in Wels catfish and its specific parasite (*Glanitaenia osculata*), in order to assess the reliability of this parasite-host model as a suitable bioindicator of organic pollution in the Zemplínska Šírava water reservoir and adjacent tributaries in the Bodrog River Basin. The analysis of these contaminants in catfish matrices showed the highest concentrations in the abdominal muscle, followed by the dorsal muscle, liver and intestine. Concentrations of ∑PCBs exceeding the limits for food set by European regulations were measured in the muscle tissue of catfish at all sites, even in the Bodrog River, 60 km away from the primary source of contamination, posing a significant risk to humans in the Zemplín Region. For the first time, the ability of *G. osculata* to accumulate higher amounts of PCBs compared to fish matrices has been demonstrated. Due to the enormous ability of the parasites to accumulate PCBs, we recommend this approach for alternative biomonitoring of PCBs in contaminated aquatic environments.

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DIPLOSTOMUM SPATHACEUM INFECTION INDUCING DIFFERENTIAL IMMUNE GENE EXPRESSION IN SEXUAL AND GYNOGENS OF GIBEL CARP (*CARASSIUS GIBELIO*): PARASITES AS POTENTIAL MECHANISM FACILITATING THE COEXISTENCE OF TWO REPRODUCTIVE FORMS

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Biological invasion by the alien species is one of the major drivers of ecological changes and declination of biodiversity. The invasion success of the invasive fish largely depends on their ability to deal with the pathogens encountered in the new environment. Gibel carp is one of the successful invaders due to its rapid reproductive strategy achieved by gynogenesis. During the early invasion of aquatic habitats in the Czech Republic, gibel carp populations were mostly composed of triploid females with gynogenetic reproduction. Subsequently, mixed populations composed of sexual diploids and gynogenetic triploid females with very rare presence of triploid males and tetraploid individuals) have been recorded. Differences in the effectiveness of the immune system and contrasting susceptibility to parasite infection may represent a potential mechanism facilitating the coexistence of asexual and sexual forms of gibel carp, thereby contributing to its invasive ability. In an effort to understand the mechanisms of the coexistence of two reproductive forms of gibel carp in nature, following the Red Queen hypothesis we investigated whether the the most common genotype expressed as gynogenetic genotype is infected more by the parasites than sexual diploid form. Hence, gynogenetic gibel carp is supposed to be more infected because genetic recombination can enable the sexual gibel carp to escape from the infection. In this study, the differential expression of genes related to immunity, activated by a digenean trematode *Diplostomum spathaceum*, was evaluated in gynogenetic females and sexual gibel carp. Gynogenetic females were more infected than sexual specimens. Based on the transcriptome profile analyses and extensive literature review, immunity-related genes were selected, and their expression was quantified using RT-qPCR. We revealed, for the first time, the changes of immune gene expression profile involved in digenean infection in gibel carp. The contribution of different immune genes expression was highlighted as potential mechanism promoting the coexistence of sexual and asexual forms in nature.

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CERCARIAL EMERGENCE PATTERNS AND IMPACT ON SNAIL HOST LONGEVITY: TRANSMISSION STRATEGIES OF LARVAL TREMATODES

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Digenetic trematodes possess a variety of transmission strategies to increase chances of encountering a suitable next host. One of these strategies is the synchronized emergence of large numbers of free-living larvae, cercariae, from their snail hosts. Cercariae are non-feeding stages that have a short window of 24–72 hours to infect their next host. In addition, intramolluscan larval stages can inflict damage on the snail host by overexploiting its resources, which can result in shortened snail lifespan. In the present study, we investigated cercarial emergence patterns and *per capita* output rates of three model species with different life cycles by counting cercariae during main day-time intervals (sunrise, day, sunset, and night) under natural light conditions. The number of emerged cercariae was recalculated to 1 hour due to the distinct duration of day-time periods. We also compared the survival rate of infected and control uninfected snails under laboratory conditions. Snails used for all experiments were sampled in four lakes in Northern Bohemia (Czech Republic) in 2021 and 2022. All model species showed primarily nocturnal emergence, with *Tylodelphys clavata* (eye fluke) peaking at sunset, and *Sanguinicola inermis* (blood fluke) and *Plagiorchis* sp. (intestinal fluke) peaking at night. Mean daily output rates of model cercariae species ranged from 207–4,205 cercariae $snail^{-1}day^{-1}$. The observed nocturnal pattern of emergence corresponds with the highest activity of the most common second intermediate host of *T. clavata*, i.e., perch, and the most common definitive host of *S. inermis*, i.e., carp. *Plagiorchis* sp. is a generalist species that infects a wide range of invertebrate second intermediate hosts, so its nocturnal emergence is probably not coordinated with the activity of its hosts but may be an adaptation to avoid dilution by many diurnal predators lurking in the environment. Snails without trematode infection survived longer (16.1±26.6 days) than snails infected with *Plagiorchis* sp. (7.3±4.9 days). Trematodes can shorten their host's lifespan by 45%, thus limiting the transmission duration. However, it seems they can compensate for host losses by large cercarial outputs into the environment.

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THE ROLE OF THE INVASIVE ZEBRA MUSSEL IN THE DILUTION OF TREMATODE CERCARIAE

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The zebra mussel (*Dreissena polymorpha*) is a highly invasive bivalve that poses a serious threat to freshwater ecosystems worldwide. Cercariae are a crucial stage in the trematode life cycle, as their main function is to disperse in the external environment and infect as many hosts as possible to increase the probability of completing the life cycle. However, numerous abiotic and biotic environmental factors can interfere with and prevent transmission of these free-living stages. We investigated the role of *D. polymorpha* in diluting trematode cercariae of *Diplostomum mergi* (Diplostomidae). The zebra mussel represents a non-host organism that does not serve as a suitable host, and eye flukes of the genus *Diplostomum* are common pathogens of fish. A series of laboratory experiments were conducted at two temperatures (18 °C and 22 °C) using 20 mussel individuals of similar size that were offered 115 freshly emerged cercariae from *Radix* spp. snail hosts for 30 minutes. Among mussel individuals, the removal of *D. mergi* cercariae varied at each temperature, but the presence of *D. polymorpha* resulted in a significant reduction in the number of cercariae by almost half, with the number of cercariae removed being significantly higher at 22 °C. Our results provide evidence that bivalves can play an important ecological role in trematode population dynamics by efficiently diluting their free-living stages, thereby limiting their transmission success. Despite the often deleterious effects of zebra mussels on native biota, their high filtration capacity could be important in modulating disease dynamics in nature.

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