10th Conference
of the
Scandinavian-Baltic Society for Parasitology
CSBSP10

(5.) 6.–7. June, 2023
Tartu, Estonia

Book of abstracts

LABRIS
Riigi Laborituuringute ja Riskihindamise Keskus
National Centre for Laboratory Research and Risk Assessment

Eesti Maaülikool
EMÜ
Estonian University of Life Sciences

UNIVERSITY OF TARTU
1632

REPUBLIC OF ESTONIA
MINISTRY OF RURAL AFFAIRS

WELCOME BY THE PRESIDENT OF SBSP

Dear friends and colleagues,

Our society, Scandinavian-Baltic Society for Parasitology (SBSP), turns 20 years young this year*, and we celebrate this in Tartu, Estonia, at our tenth conference! We hope to see many of you there, to share knowledge and inspiration, and to discuss parasites, hosts, and our shared environment.

Dr. Pikka Jokelainen
President of Scandinavian-Baltic Society for Parasitology
President of World Federation of Parasitologists

* Scandinavian-Baltic Society for Parasitology (SBSP) is an active international society that encourages and promotes parasitology in the Nordic and the Baltic countries. SBSP was founded in 2003 by the fusion of Scandinavian Society for Parasitology (est. 1967) and Baltic Society for Parasitology (est. 1993). The society organizes an international congress every second year, and hosted EMOP XII in 2016 in Finland and co-hosted ICOPA XV in 2022 in Denmark.

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PLENARY SPEAKERS

Dr. Pikka Jokelainen (Denmark)

Prof. Urmas Saarma (Estonia)

Dr. Adriano Casulli (Italy)

Prof. Tomáš Scholz (Czech Republic)

Dr. Lena Hulden (Finland)

PROGRAM

10th Conference of the Scandinavian-Baltic Society for Parasitology (CSBSP10)

Theme: Parasites and hosts in shared environment
(05.) 06.-07.06.2023 Tartu, Estonia

ON-SITE PROGRAM

Virtual participants can follow the main sessions online. Some parts, such as the workshops, are for on-site participation only.

Timezone: Eastern European Summer Time (GMT+3)

5th of June

PRE-CONFERENCE EVENTS

Venue: The Institute of Forestry and Engineering of the Estonian University of Life Sciences (Tehnikamaja), Fr. R. Kreutzwaldi 56, Tartu 51006, Estonia
Google Maps: https://goo.gl/maps/1bFDYsMTCv4r6N5h8

14:00–16:00	Organizer: Dominique J. Bicout	**Workshop** “The basic principles of modelling host-parasite interactions”
14:00–16:00	Organizers: Kristina Noreikiene Konrad Taube Alfonso Diaz-Suarez Anti Vasemägi	**Workshop** “Parasitology decoded: current and future potential of genomic technologies in parasitological research”
16:00–16:30	Break	
16:30–18:00	Organizers: Azzurra Santoro Presenters: Elisabeth Dorbek-Kolin Carolina Chagas Vaidas Palinauskas	Early career researchers’ event

20:00–22:00 Social program

6th of June

Venue: Conference Centre of University of Tartu Library (W. Struve 1, Tartu, Estonia).
Google Maps: https://goo.gl/maps/RQVVUZ9ZCPknTNfN6

9:00–9:30	Registration	
9:30–9:45	Conference opening	
Session 1. Moderator Epp Moks		
9:45–10:30	Pikka Jokelainen	Parasites from One Health point of view
10:30–10:50	Poster pitches. Posters P.1.–P.18.	
10:50–11:10	Break/poster session	
Session 2. Moderators Gunita Deksne and Elisabeth Dorbek-Kolin		
11:10–11:55	Urmas Saarma	Major parasitic zoonoses of the past: new insights with modern methods
11:55–12:10	Ants Tull	High overlap of zoonotic helminths between wild mammalian predators and rural dogs – an emerging One Health concern?
12:10–12:25	John Ellis	A state-of-the-art methodology for high-throughput in silico vaccine discovery against protozoan parasites and its application to *Toxoplasma gondii*
12:25–12:40	Ewa Bilska-Zając	Trich-tracker a new tool for epidemiological investigations in *Trichinella* outbreaks
12:40–13:00	Poster pitches. Posters P.20.–P.36.	
13:00–14:00	Lunch break	
Session 3. Moderator Urmas Saarma		
14:00–14:45	Adriano Casulli	Understanding transmission risk and unveiling the public health impact of CE and AE in Europe
14:45–15:00	Teivi Laurimäe	Exploring the genetic diversity of genotypes G8 and G10 of the *Echinococcus canadensis* cluster in Europe based on complete mitochondrial genomes
15:00–15:15	Tetiana Kuzmina	Horse strongylid community structure in the “ivermectin era”
15:15–15:30	Georg von Samson-Himmelstjerna	A long-term evolutionary ivermectin drug resistance study in the parasite model *Caenorhabditis elegans*
15:30–15:45	Mindaugas Šarkūnas	Comparative efficacy of injectable and oral moxidectin in treatment of cyathostomin infection in horses
15:45–16:00	Break/poster session	
16:00–18:00	Organizers: Mindaugas Šarkūnas Ants Tull Teivi Laurimäe Liina Kinkar	**Workshop** “A discussion panel on interdisciplinary challenges in the field of parasitology“

18:30-23:30 - Social program

7th of June

Venue: Conference Centre of University of Tartu Library (W. Struve 1, Tartu, Estonia).
Google Maps: https://goo.gl/maps/RQVVUZ9ZCPknTNfN6

Session 4. Moderator Kristina Noreikiene		
9:45–10:30	Tomáš Scholz	Gaps in parasitological research in the molecular & omics era
10:30–10:45	Alfonso Diaz-Suarez	Metabarcoding reveals spatial and temporal variation of fish eye fluke communities in lake ecosystems
10:45–11:05	Break/poster session	
Session 5. Moderator Lea Tummeleht		
11:05–11:50	Lena Hulden	The Northern range of malaria
11:50–12:05	Mélanie Duc	*Haemoproteus majoris* exo-erythrocytic stages across its different lineages and avian hosts
12:05–12:20	Anna Faltýnková	A unique species spectrum of larval trematodes (Trematoda, Digenea) in *Bithynia tentaculata* (Gastropoda, Littorinimorpha) in Europe
12:20–12:35	Kristina Noreikiene	Transcriptomic responses of perch to diplostomid cercariae exposure
12:35–12:50	Ivanna Dmytriieva	Helminth species co-occurrence in marsh frog *Pelophylax ridibundus* (Amphibia: Ranidae) in Ukraine
12:50–13:05	Katarzyna Tołkacz	From mummy, with love: vertical transmission of Candidatus *Neoehrlichia mikurensis* and *Hepatozoon* sp. in voles (*Microtus* spp.)
13:05–14:00	Lunch break	
Session 6. Moderators Teivi Laurimäe and Ants Tull		
14:00–14:15	Pernille Klein-Ipsen Rosalina Rotovnik	Occurrence of Endoparasites in Greenland Sled Dogs (*Canis lupus familiaris borealis*) of East Greenland (Daneborg and Ittoqqortoormiit)
14:15–14:30	Guðný Rut Pálsdóttir	*Strongyloides stercoralis*: 10 years of colonisation attempts through imported dogs in Iceland (2012-2022)
14:30–14:45	Carina Schneider	The forgotten natives: Species identification and the assessment of genetic diversity of *Mesocestoides* spp. from wild and domesticated carnivores in Europe
14:45–15:00	Valentina Oborina	Anticipating *Angiostrongylus vasorum* emergence in dogs in Estonia: add to list of differential diagnoses
15:00–15:15	Neringa Kitrytė	Diversity and host specificity of ectoparasites of small mammals in Lithuania
15:15–15:30	Laima Baltrūnaitė	Seasonal dynamics and factors shaping ectoparasite communities of small mammals in Lithuania
15:30–15:45	Closure of the conference	
15:45–16:00	Break	
16:00–16:45	SBSP General Assembly	

ABSTRACTS

* presenting author

PLENARY LECTURES

Parasites from One Health point of view

Jokelainen, P.

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One Health approach recognizes that the health of humans, animals, plants, ecosystems and environments are closely linked and interdependent. Using a One Health approach often requires multidisciplinary or cross-sector collaborations.

Parasitology provides brilliant opportunities to practice and develop One Health ways of thinking and working. Parasite life cycle illustrations typically show key connections between the parasites, their hosts and often also the environment. In addition to the characteristics of the parasites, the health of their hosts and the environment shape their story. We can look at that story from different point of views – including from a One Health point of view.

This talk aims to inspire discussions during the congress and beyond to include more One Health thinking and to encourage more One Health collaborations.

Major parasitic zoonoses of the past: new insights with modern methods

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The concept of One Health emphasizes the interdependence of human, animal and environmental health and is of growing significance, owing to the fact that majority of infections are zoonoses. I will provide an overview of recent advancements in studies of past parasitic zoonoses and bring an example of a recent genetic study that focuses on genetic analysis of a 46-year-old *Echinococcus* isolate in Iceland prior to the eradication of cystic echinococcosis (CE) in this remote country.

Cystic echinococcosis is considered the most severe parasitic disease ever affected the human population in Iceland. Eradication of CE from Iceland by 1979 was a huge success story and served as a leading example for other countries. Before the eradication campaign in the 1860s, Iceland was a country with very high prevalence of human CE, with approximately every fifth person infected. Why was the prevalence so high and did genetic background of the parasite play a role? However, there was no genetic information on *Echinococcus* parasites before eradication. Here I will present the main findings of the genetic analysis for one of the last *Echinococcus* isolates in Iceland, dating back nearly half a century ago, and discuss its significance.

Understanding transmission risk and unveiling the public health impact of CE and AE in Europe

Casulli, A.

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Echinococcosis is one of the current 20 disease, condition or syndrome groups prioritized by the World Health Organization (WHO) within the umbrella of the Neglected Tropical Diseases (NTDs). Within this disease group, cystic echinococcosis (CE) caused by *Echinococcus granulosus sensu lato* complex and alveolar echinococcosis (AE) caused by *Echinococcus multilocularis* are those of major human interest for their impact on global public health. The neglected zoonosis CE and AE affect mainly pastoral and rural communities in both low-income and upper-middle-income countries. In Europe, CE and AE should be regarded as an orphan and rare diseases. Although human CE and AE are notifiable parasitic infectious diseases in most European countries, in practice they are largely under-reported by national health systems.

This talk aims to unveil and compare some molecular and clinical characteristic of CE and AE, providing a wide European-scale epidemiological pictures and putting these diseases in a global public health context.

Gaps in parasitological research in the molecular & omics era

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We live in the age of molecular biology and -omics, and molecular methods have opened up unprecedented opportunities for biological research, including all areas of parasitology. However, there are two sides to every coin, and too one-sided a focus on new approaches can lead to major gaps as less “cool,” i.e., “old-fashioned,” topics are neglected. In recent decades, much emphasis has been placed on the study of parasites and their interactions with the host at the molecular level. This somewhat ignores the fact that parasitology is primarily an ecological discipline, as it deals with the interactions between parasites and their hosts and between parasites and their environment. Selected areas of parasitological research are briefly reviewed, highlighting the advantages (= benefits and achievements) and disadvantages (= problems and gaps) of the current focus on molecular and -omic methods. These areas include taxonomy and biodiversity studies, parasite classification and evolution, parasitological surveys and screening, routine parasite diagnostics, life cycles, epidemiology/epizootiology, and parasite ecology. It is recommended to combine both ‘classical’ methods and modern ones (molecular and ‘omics’ approaches), without ignoring the complexity of parasite-host-environment interactions (One Health concept), which is even more urgent now, in the rapidly changing world. The younger generation should be more attracted to participate in field investigations and multidisciplinary assessment of the parasites found in order to understand the complexity of parasitology.

The Northern range of malaria

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Malaria probably spread to Northern Europe with agriculture and permanent settlements. There are sporadic mentions of the disease in Danish, Swedish and Russian medieval sources. Endemic malaria had its widest distribution during the end of the 19th century. It was found in Scandinavia, Finland, the Baltic states, Russia, Siberia, Canada and the US. Northern malaria is usually attributed to *Plasmodium vivax*, but there are reports of *P. falciparum* as North as Arkhangelsk. The hypnozoites of *P. vivax* made it possible, for humans to carry the parasite with them to new settlements. It has been suggested that the hypnozoites were activated by the bites of uninfected anophelines, which would explain the epidemics. The number of malaria cases in the Holarctic area was highest in spring and then dropped radically during summer.

Three elements are crucial for endemic malaria: the vector, a human host and the necessary temperature needed for the sporogony. In the Holarctic the *Plasmodium* species have been able to use local *Anopheles* mosquitoes as vectors. In the subarctic area temperature hardly rises high enough for the sporogony to take place outdoors. Therefor the transmission took place indoor by activated hibernating Anophelines. Human houses then provided both the human hosts and the necessary temperature for the sporogony. In crowded conditions infective vectors could easily transmit the pathogen to several people. Malaria finally disappeared in the 20th century. The last area with indigenous malaria was the Yakutian Autonomous Republic, where it remained still in the 1960's.

ORAL PRESENTATIONS

O.1.

High overlap of zoonotic helminths between wild mammalian predators and rural dogs – an emerging One Health concern?

Tull, A.*, Valdmann, H., Tammeleht, E., Kaasiku, T., Rannap, R., Saarma, U.

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The concept of One Health emphasizes the interdependence of human, animal and environmental health and is of growing significance, in part owing to the problems related to emerging infectious diseases of wildlife origin. Wild mammalian predators are a potential risk factor for transmission of zoonotic pathogens to domesticated animals and humans. This is especially relevant in rural areas, where transmission of zoonotic pathogens can occur particularly efficiently when free-ranging dogs are present. The main aim of this study was to determine helminth infections among wild mammalian predators and evaluate the overlap between helminth faunas of wild mammals and dogs. Scat samples of predators were collected in coastal areas of Western Estonia and genetic methodology applied for the correct identification of predator species from their scat. Parasitic helminths of mammalian predators in the scat samples were analysed and compared with dog data from a previous study. High helminth prevalence (~90%) was found in dominant predator species in the area, namely the red fox (*Vulpes vulpes*) and golden jackal (*Canis aureus*). Moreover, the helminth fauna of both wild species, including potentially zoonotic helminths, overlapped largely with that of rural dogs in the same area. The results, together with the ones from earlier parasitological studies among humans in Estonia, emphasize the potential risk of pathogen transmission from wild mammalian predators to dogs and from dogs to humans, making parasitic diseases of wildlife a One Health concern.

O.2.

A state-of-the-art methodology for high-throughput *in silico* vaccine discovery against protozoan parasites and its application to *Toxoplasma gondii*

Goodswen, S.J.[1], Kennedy, P.J.[2], Ellis, J.T.[1]*

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Vaccine discovery against eukaryotic parasites is not trivial as highlighted by the limited number of known vaccines in comparison to the number of protozoal diseases that need one. Very few protozoal diseases of significance have commercial vaccines. Live and attenuated vaccines have proved to be substantially more effective than novel vaccine designs such as subunits. However, unacceptable risks posed by live and attenuated vaccines means that there remains a critical need to research alternative designs. One promising alternative is an *in silico* vaccine discovery approach, designed to identify protein vaccine candidates given thousands of target organism protein sequences. This approach, nonetheless, is simply an overarching concept with no standardised guidebook yet on how to implement the approach. No known subunit vaccines against protozoan parasites exist as a result of this approach, and consequently none to emulate. The study goal was to combine current *in silico* discovery knowledge specific to protozoan parasites and develop a workflow representing a state-of-the-art approach. This approach reflectively integrates a parasite's biology, a host's immune system defences, and importantly, bioinformatics programs needed to predict vaccine candidates. To demonstrate the pipeline effectiveness, every *Toxoplasma gondii* protein was ranked in its capacity to provide immunity.

O.3.

Trich-tracker a new tool for epidemiological investigations in Trichinella outbreaks

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When a *Trichinella* infection is found in pigs, it is particularly important to identify the source of the infection for the animals and stop further transmission of the parasite to next pigs and into surrounding areas. For this, an epidemiological investigation is used, which is a complex procedure including few stages: epidemiological interview, serological investigations, species identification of discovered *Trichinella* larvae, and differentiation of isolates of the same species. The last step is practically not applicable due to the lack of available methods to distinguish isolates of *T. spiralis* larvae. And thus, in most cases, it was not possible to obtain an answer as to what could have caused the infection of pigs in a given outbreak of trichinellosis.

Here, we propose a method based on ddRADseq and bioinformatics analysis called - Trich-tracker. The methodology applies the ddRADseq technique during which DNA libraries are created using restriction enzymes. Such DNA libraries contain the sequences of multiple random loci distributed throughout the genome under analysis. The obtained in NGS raw sequence data is used for finding single nucleotide polymorphisms (SNPs) and then for phylogenetic analysis or genetic structure analysis. Both of the analyses are useful to distinguish isolates one from another, what is the most important during epidemiological investigation in *Trichinella* outbreaks on pigs' farms. The discriminating power of this tool is tunable and scalable, allowing application in a variety of epidemiological contexts. The simplicity of the entire procedure, and the timeliness and cost effectiveness of Trich-tracker ensure the usefulness of its practical application in ongoing *Trichinella* outbreaks. Furthermore, Trich - tracker may be also used to track origin of cured meat containing *T. spiralis* which was a source of human infection.

O.4.

Exploring the genetic diversity of genotypes G8 and G10 of the *Echinococcus canadensis* cluster in Europe based on complete mitochondrial genomes

Laurimäe, T.[1*], Kinkar, L.[1], Moks, E.[2], Bagrade, G.[3], Saarma, U.[1*]

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Cystic echinococcosis (CE) is a zoonotic disease that is caused by a group of tapeworm species that are collectively referred to as *Echinococcus granulosus sensu lato* (*s.l.*). Among this species group, the *Echinococcus canadensis* cluster includes genotypes G8 and G10 that are primarily transmitted between wolves and wild cervids. Studies investigating the genetic variation of genotypes G8 and G10 are rather scarce and the extent of genetic variation of these two genotypes has so far not been explored at the complete mitochondrial (mt) genome level. The aim here was to investigate the genetic variation of genotypes G8 and G10 in Europe based on analysis of complete mtDNA sequences, as well as provide a high-quality reference dataset for future studies. Complete mt genomes (approx. 13 500 bp) were produced for a total of 29 G8/G10 samples collected from wolves, moose, reindeer, and roe deer, originating from Finland, Sweden, Russia, Poland, Latvia, and Estonia. Phylogenetic network analysis showed marked mitochondrial differences of over 400 mutations between G8 and G10, and more detailed patterns of intragenotypic variability within the two genotypes than previously observed. Understanding the patterns of mitochondrial genetic variation of a species provides baseline information for future studies aiming to explore whether this mt distinctiveness is reflected in the nuclear genome and subsequently whether it could possibly have any impact on any phenotypic traits and/or parasite transmission ecology.

O.5.

Horse strongylid community structure in the “ivermectin era”

Kuzmina, T.A.*[1,2], Viniarska, A.V.[3], Tomczuk, K.[4], Studzińska, M.[4], Burcakova, L.[1], Konigova, A.[1]

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Strongylids are the most pathogenic equine parasites worldwide. Nowadays due to development of anthelmintic resistance, macrocyclic lactones (ivermectins, IVM) are the main anthelmintics regularly used for horses. Our **aim** was to analyze changes in the species composition and structure of horses strongylid community after the long-term use of IVMs.

The study included horses from two Ukrainian farms: with long-term regular IVM treatments (Farm A) and with irregular deworming with different anthelmintics (Farm B). Strongylids were collected *in vivo* by the “diagnostic deworming” method. In addition, the strongylid community structure in horses from a slaughterhouse in southeastern Poland was analyzed. In total, 20,309 strongylid specimens of 26 species were collected and identified.

In Farm A, 12 cyathostomin species were found. Three species (*Cylicocyclus nassatus*, *Cyathostomum pateratum*, *Cylicostephanus goldi*) dominated in the community (prevalence>80%); the Berger-Parker dominance index for *C.nassatus* = 0.41–0.93, for *C.pateratum* – up to 0.57. In Farm B, 26 species were found; 21 species of Cyathostominae and 5 of Strongylinae. Twelve cyathostomin species dominated the community; their Berger-Parker indexes were 0.16–0.28. In Polish horses, 21 strongylid species were found; the Berger-Parker indexes for dominant species were 0.15–0.57.

A decrease in species diversity and an increase in the dominance indexes indicate the destruction of the strongylid community due to regular IVM deworming. Dominant cyathostomin species have sufficient genetic plasticity to keep their populations and potentially develop resistance to IVMs.

Funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V01-00015

O.6.

A long-term evolutionary ivermectin drug resistance study in the parasite model *Caenorhabditis elegans*

Hellinga, J.[1], Trubenova, B.[2], Wagner, J.[1], Piecyk, A.[3], Regoes, R.[2], Schulenburg H.[3], Krücken, J.[1], von Samson-Himmelstjerna, G.[1]*

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Caenorhabditis elegans (C. elegans) is a critical model for parasitic nematodes in working with antiparasitic drugs, from understanding current and new drugs' molecular mechanisms to uncovering more drug targets. These worms are free living nematodes that are also an appealing tool for experimental evolution given the easiness of working with them in cultured laboratory conditions. We present one of the first evolutionary experiments designed to look into the impact of intrinsic factors (i.e. population size and genetic diversity) on the rate of ivermectin resistance development in *C. elegans*. We used a step-wise ivermectin introduction experiment to evolve resistant strains which was accompanied with a mathematical model. The starting ancestor and final resistant populations were subjected to RNASeq and phenotypic analysis with ivermectin. We found that having a genetically diverse population shortened the time it took for the worms to evolve resistance to ivermectin. As well as having different population sizes directly correlated to the rate of ivermectin resistance during the evolutionary experiment. The experimental evolution of ivermectin resistance was accompanied by mathematical modelling resulting in nearly identical resistance development predictions as observed in the laboratory experiment. Our RNASeq has alluded to trends in genetic drift and patterns of genetic expression alterations that could be responsible for resistance against ivermectin. We concluded that the work done here is the first of many examples using evolution experiment guidelines to find answers into how anthelmintic resistance could be happening in the larger parasitic worm world.

O.7.

Comparative efficacy of injectable and oral moxidectin in treatment of cyathostomin infection in horses

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The aim of this study was to evaluate the anthelmintic efficacy of two moxidectin formulations of oral gel (licensed for horses) and an injectable solution (licensed for cattle) in horses on two horse farms in Rhineland-Palatinate, Germany. The horses were divided into two groups based on the number of cyathostomin EPG of faeces, age, sex and body weight. On day 0 of the study 5 horses were dewormed with moxidectin gel (Equest®, Zoetis) according to the manufacturer's instructions, and 5 horses were dewormed with injectable moxidectin (Cydectin 1%, Zoetis) at the dose of 0.2 mg/kg of bw. Stool samples were taken rectally twice a day on days 0, 17, 28, 35, and 42, and examined using a concentration McMaster technique (Vadlejch et al., 2011). The efficacy of oral gel (99.4%, 95%CI 97.84–101.01) and intramuscular treatment (99.0%, 95%CI 97.17–100.73) was comparable on 17 dpt and differed slightly (97.4%, 95%CI 92.78–101.92 vs 95.9%, 95%CI 87.71–103.99, respectively) on 42 dpt. Cyathostomin eggs did not reappear in the faeces by 42 dpt, except for one horse. The possible impact of bias due to under dosing of injectable moxidectin for this horse on the reduced efficacy is discussed. The efficacy of oral (99.4%) and injectable (99%) moxidectin on 17 dpt confirmed the absence of resistance in cyathostomins to moxidectin on farms with 10 years of moxidectin use history.

O.8.

Metabarcoding reveals spatial and temporal variation of fish eye fluke communities in lake ecosystems

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Diplostomids (Trematoda: Digenea) is a group of parasitic Platyhelminthes that are globally distributed and ubiquitous in marine and freshwater ecosystems. This group has a complex life cycle that involves snails and fish as intermediate hosts, while piscivorous birds serve as definitive hosts. Diplostomids are one of the most common pathogens in fish having negative effects on their fitness and survival. Within fish, diplostomids are found in the eye and occasionally in the brain, where they can form complex communities since coinfection of several species is frequent. However, the environmental factors and host-related characteristics that determine these communities' diversity, composition, and coexistence are poorly understood. Here, we applied a quadrupled-indexing COX1 region-specific metabarcoding approach to characterize the temporal and spatial variation of diplostomid communities in perch and roach sampled from seven lakes in Estonia, as well as, to characterize, for a first time, brain-infecting communities in perch. Our results demonstrate that diplostomid communities in temperate lakes are complex and dynamic systems influenced by various environmental factors, as well as by interactions between host and parasite species that show spatial and temporal heterogeneity. Furthermore, the comparison between eye and brain parasite communities suggested that brain infection by diplostomids may represent an opportunistic event in perch, as the genetic diversity found in the brain represents a fraction of the variation observed in the eye. In addition, this study demonstrated that quadrupled-indexing metabarcoding method is a sensitive and efficient tool for characterizing parasite communities, facilitating ecological and biodiversity research on cryptic parasite species.

O.9.

Haemoproteus majoris exo-erythrocytic stages across its different lineages and avian hosts

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Patterns of the exo-erythrocytic (tissue stages) development remain insufficiently understood in haemosporidian parasites. *Haemoproteus majoris* is a relatively well-studied species among avian haemoproteids (Apicomplexa, Haemoproteidae), with five recognized lineages (CCF5, PARUS1, PHSIB1, PHYBOR04, and WW2). The exo-erythrocytic stages are known for three of them, each found in one host species: PARUS1 in *Parus major*, PHSIB1 in *Phylloscopus sibilatrix*, and PHYBOR04 in *Turdus pilaris*. However, the exo-erythrocytic stages are unknown for the other lineages, including the lineage CWT4, which has long been associated with *H. majoris*, but was never proved to belong to this species.

Between 2016 and 2022, samples were collected from 14 species infected with *H. majoris*. Parasite species and lineages were determined by microscopic examination of blood films and DNA sequencing. Birds single-infected with one lineage or mixed-infected with different lineages of *H. majoris* were investigated for the presence of exo-erythrocytic stages by screening of histological sections stained with haematoxylin & eosin and sections processed with the chromogenic in-situ hybridization technique.

Based on the morphological characters of gametocytes, the lineage CWT4 was assigned to *H. majoris*. Megalomeronts were found in birds infected with the lineages CCF5, CWT4, PARUS1, PHSIB1 and/or WW2, revealing several affected organs: kidneys, lungs, gizzard, intestine, and liver. However, some organs were more often infected than others depending on the parasite lineage, and the morphology of the observed tissue stages vary between some lineages. These observations seemed consistent in different bird species infected with the same lineage, indicating the existence of a pattern.

O.10.

A unique species spectrum of larval trematodes (Trematoda, Digenea) in *Bithynia tentaculata* (Gastropoda, Littorinimorpha) in Europe

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The palaearctic *Bithyna tentaculata* are common snails, they are highly adaptable and can be found in almost any type of freshwater body. They are well known as hosts of larval trematodes and among the Heterobranchia they host the most diverse trematode species spectrum. Although a high number of trematode species was recorded in *B. tentaculata* in the past, they were mostly reported under provisional names. Recently, with aid of molecular methods, cercariae from *B. tentaculata* collected in waterbodies in Germany were molecularly characterised and some of them linked to adult trematodes, and thus correctly identified. Our aim was to examine the trematode diversity in *B. tentaculata* in lakes in Ireland using morphological and molecular analyses (ITS2, *cox*1, *nad*1, and 28S rDNA sequence data). We found eight species of trematodes from six families (Cyathocotylidae, Lecithodendriidae, Lissorchiidae, Opecoelidae, Opisthorchiidae and Psilostomidae), maturing in fishes or birds; seven species were newly recorded in Ireland. The species spectrum and identity well overlapped with that recorded in Germany, confirming that *B. tentaculata* hosts a specific spectrum of trematodes. Because of the versatility of *B. tentaculata* in habitat choice, and being a suitable host to trematodes, it could serve with its parasites as an indicator of the state of the environment. Further studies using methods of integrative taxonomy are needed to assess the trematode species spectrum of *B. tentaculata* over larger areas and to link trematode larval stages to adults.

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O.11.

Transcriptomic responses of perch to diplostomid cercariae exposure

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Immune privilege is the conundrum of host-parasite arms race as host immune defense has to be balanced against self-inflicted damage to vital tissues. Fish eye flukes (*Diplostomidae*) may serve as a model for studying such vertebrate responses because these parasites specialize in infecting different regions of the eye which is considered to be a classical immunologically privileged organ. Here, we conducted an experiment on parasite naive perch ($n = 40$) from two populations by experimentally exposing juvenile individuals (six months post-hatch) to *Diplostomum pseudosphataceum* cercariae. Subsequently, we used RNAseq for elucidating transcriptomic changes in the eye and spleen tissues at 48h post exposure. Results revealed that several hundred genes were significantly differentially expressed ($p_{adj}< 0.05$) in the eye and spleen tissues between the control and exposed individuals. In line with immune privilege, eye tissues did not show a strong enrichment in gene ontology (GO) terms related to immune system functioning, but rather in processes related to metabolism. On the other hand, multiple immune and stress related GO processes were triggered by diplostomid cercariae exposure in the spleen. Taken together, these results indicate that regulation of eye metabolism may be an important mechanism in response to parasite infection. We discuss to what extent this process may serve as an evolutionary conserved pathway allowing to regulated parasitosis in immunoprivileged tissues.

O.12.

Helminth species co-occurrence in marsh frog *Pelophylax ridibundus* (Amphibia: Ranidae) in Ukraine

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Study of helminth co-occurrence in amphibians gives an information about species common intake pathways in frogs and their interaction patterns (such as competition or facilitation). Besides, it can provide data regarding frogs' biology, behavior and connection with intermediate hosts. Current study aims to investigate helminths species co-occurrence in marsh frog, *Pelophylax ridibundus* in northern part of Ukraine, based on the interspecific associations and covariations and to find out possible biological reasons of co-occurrences. We examined 201 individuals of *P. ridibundus* from 14 localities in Ukraine. In the entire sample of hosts, we found 32 helminth species. Nine helminth species with the infection prevalence of 15% or higher were used in the analysis. The associations were revealed for seven pairs of species, while covariations were recorded between four pairs. Biological grounds of some species interactions are suggested. A negative covariation (r_s=-0.32; p<0,05) found between the nematode *Cosmocerca ornata* and the trematode *Diplodiscus subclavatus* can be explained by their common site in host (rectum), which causes space competition. Besides, the former species had a negative association (χ^2=7.54) with the nematode *Icosiella neglecta* which can be related to their occurrence in hosts of different age. We recorded more abundant infection of *C. ornata* in young (smaller) frogs, while *I. neglecta* was found more frequently in mature (larger) frogs (r_s=0.4; p=<0,01). Positive interspecific associations were found in trematode species which use the same intermediate hosts in their transmission: *Prosotocus confusus* and *Pleurogenoides medians*.

O.13.

From mummy, with love: vertical transmission of *Candidatus Neoehrlichia mikurensis* and *Hepatozoon* sp. in voles (*Microtus* spp.)

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Rodents are important hosts of vector borne parasites of humans and animals. *Candidatus* Neoehrlichia mikurensis (CNM) is a pathogen of humans, while *Hepatozoon* are parasites of terrestrial vertebrates, commonly found worldwide. We aimed to determine the prevalence of CNM and *Hepatozoon* spp. in three species of *Microtus* and to assess the occurrence of vertical transmission of these pathogens in naturally-infected voles.

Molecular and microscopic techniques were used to detect pathogen infection in blood and tissue samples. The prevalence of CNM in the vole community ranged 24–47% depending on *Microtus* species. The DNA of CNM was detected in 21% of pups from three litters of six infected *Microtus* dams (two *Microtus arvalis* and one *M. oeconomus*) and in 6.6% of embryos from two litters of eight CNM-infected pregnant females. We detected *Hepatozoon* infection in 14% of *M. arvalis* and 9% of *M. oeconomus* voles. *Hepatozoon* sp. DNA was detected in 48.7% of pups from seven litters (6 *M. arvalis* and 1 *M. oeconomus*) and in two embryos (14.3%) obtained from one *M. arvalis* litter.

The high prevalence of CNM infections may be a result of a relatively high rate of vertical transmission among naturally infected voles. Vertical transmission was also demonstrated for *Hepatozoon* sp. in *M. arvalis* and *M. oeconomus*. Our study underlines the significance of alternative routes of transmission of important vector-borne pathogens.

O.14.

Occurrence of Endoparasites in Greenland Sled Dogs (*Canis lupus familiaris borealis*) of East Greenland (Daneborg and Ittoqqortoormiit)

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The Greenland sled dog (GSD) (*Canis lupus familiaris borealis*) population has declined considerably during the past two decades. Research regarding the health of GSDs, e.g. parasite infections, is scarce. Our objective was to investigate the occurrence of endoparasites in GSDs from the East coast and discuss possible routes of transmission.

Thirty-eight faecal samples and 43 serum samples were collected from privately-owned GSDs in Ittoqqortoormiit. Forty-four faecal samples and 12 hearts were obtained from military-owned GSDs in Daneborg. Moreover, information regarding GSD husbandry was attained. Methods for direct parasite detection included concentration McMaster and immunofluorescence assay. Enzyme-linked immunosorbent assay kits were utilised for antibody detection. Taeniid eggs were analysed with PCR and Sanger sequencing.

Ittoqqortoormiit GSDs harboured *Toxascaris leonina* (31.6%), *Cryptosporidium* spp. (15.8%), *Sarcocystis* spp. (7.9%), *Taenia serialis* (2.6%) and *Giardia* spp. (2.6%) and were seropositive for *Trichinella* spp. (100%) and *Toxoplasma gondii* (39.5%) antibodies. In GSDs from Daneborg, *Cryptosporidium* spp. (8.3%), *Giardia* spp. (5.6%) and antibodies against *Trichinella* spp. (20%) were detected. Ittoqqortoormiit GSDs were mainly fed raw meat from wild game. This practice was rare in Daneborg. Both populations were at risk of faecal-oral parasite transmission from dogs and humans. Daneborg GSDs were treated with anthelmintics.

Several meat- or faeces borne parasites were present and they may impact the health of the GSDs. Regional differences in parasite occurrence may be explained by animal management and feeding patterns. Further investigation into local transmission pathways is needed, to reduce transmission of both dog specific- and zoonotic parasites.

O.15.

Strongyloides stercoralis: 10 years of colonisation attempts through imported dogs in Iceland (2012-2022)

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Iceland is an island in the N-Atlantic Ocean. With no bordering countries, strict conditions apply regarding importation of live animals to protect the local animal population from introduced pathogens. Since 1989, importation of dogs was permitted with a strict quarantine where faecal samples were primarily checked for helminth eggs and protozoans. In 2012, the Baermann technique was included in the routine parasitological diagnosis to test for nematode larvae such as those of *Strongyloides stercoralis*. This parasitic roundworm (Nematoda) has a complex life cycle that can alternate between free-living and parasitic cycles and involves autoinfection. It is a well-known parasite in humans, other primates, and dogs with a zoonotic potential, especially in tropical and subtropical regions. During 2012 and 2022, 2,608 dogs from 61 countries were imported to Iceland. Of those, 41 dogs (2%) were *S. stercoralis* positive. The breeds most frequently affected were French bulldogs (n=12) followed by Pomeranians (n=7) and Havanese (n=5). The areas where most of the positive dogs were imported from were Eastern Europe (n=19) and Scandinavia/Baltics (n=16). Although French bulldogs only represented 4% of imported dogs in this period, they amount to 30% of all *S. stercoralis* positive dogs. The data raises a series of questions regarding the emergence of this parasite in different countries, circumstances in kennels and a potentially higher susceptibility of certain breeds to infection or whether this nematode simply remained neglected.

O.16.

The forgotten natives: Species identification and the assessment of genetic diversity of *Mesocestoides* spp. from wild and domesticated carnivores in Europe

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Mesocestoides is a cosmopolitan cestode genus found in carnivorous wild animals throughout Europe. Especially in red foxes (*Vulpes vulpes*) high prevalence can be found. In southwestern Germany, *Mesocestoides* spp. is the most common helminth in red foxes with an average prevalence of 79% in the last decades. Although these parasites are ubiquitous, little is known about which species are actually present in different regions of Europe, let alone their intraspecific variability.

The aim of this study is to obtain new data on the distribution and genetic diversity of *Mesocestoides* species in Europe. For this purpose, tapeworms are collected from various wild and domesticated carnivores from Germany, France, Slovakia, Finland, the Netherlands and Italy. The collected isolates are to be analyzed both genetically and morphologically. The mitochondrial *cox*1 and *nad*1 genes will be employed to investigate the phylogenetic relationship of the *Mesocestoides* species found and their genetic diversity.

Preliminary results from southern Germany confirm an ongoing high prevalence (77 %) of *Mesocestoides* spp. The only species detected so far was *Mesocestoides litteratus*. Our analyses, which are one of the first to investigate genetic diversity of *Mesocestoides* spp. using complete *cox*1 and *nad*1 gene sequences, indicate high intraspecific genetic variability with high haplotype diversity. Clusters can be observed, but they do not correlate with the geographic collection site.

In the course of this study, the *Mesocestoides* species of the other European regions will be identified, and the analysis of the genetic diversity will allow an insight into the population dynamics of *Mesocestoides*.

O.17.

Anticipating *Angiostrongylus vasorum* emergence in dogs in Estonia: add to list of differential diagnoses

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Angiostrongylus vasorum can cause life-threatening parasitic disease in dogs. In recent years, *A. vasorum* has been reported spreading towards north-eastern Europe. Despite autochthonous cases in dogs described in Finland and detected in wildlife in Estonia, no studies had focused on *A. vasorum* in domestic dogs in Estonia.

A multi-clinic study was carried out in 2018-2019: altogether 115 dogs with clinical signs suggestive of canine angiostrongylosis were enrolled for testing using a commercial *A. vasorum* antigen detection test. In addition, we collected information on potential risk behaviors for acquiring *A. vasorum* infection – eating potential intermediate hosts and paratenic hosts – among the dogs.

None (0.0%, 95% CI 0.0–2.6) of the 114 dogs included in the study tested *A. vasorum* antigen positive. Two (2.0%) of the 102 dogs included in the risk behavior analysis had been seen to eat slugs and/or snails, and this behavior was considered possible for further 17 (16.7%) of the dogs. Four (3.9%) of the dogs had been seen to eat frogs, and this behavior was considered possible for further 14 (13.7%) of the dogs.

Our results indicate that *A. vasorum* was either not established or not common in dogs in Estonia in 2018–2019, but this needs to be interpreted with caution due to several limitations of the study. Taken the presence of the parasite in local wildlife, it is important to include *A. vasorum* infection in the list of differential diagnoses for dogs with suggestive clinical signs of canine angiostrongylosis in Estonia.

O.18.

Diversity and host specificity of ectoparasites of small mammals in Lithuania

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Small mammals are well-known reservoir hosts of many agents of veterinary and medically important infectious diseases. Meanwhile, ectoparasites act as vectors of pathogens in small mammal communities. Data on small mammal ectoparasite fauna is fundamental knowledge necessary for the successful management of zoonotic infections. Our research took place in various habitats in Lithuania from 2016 to 2022. During this study, we examined 2151 small mammal specimens belonging to 14 species, and 34337 ectoparasites were collected. We identified 50 ectoparasite species: four chigger mites (Trombidiformes: Trombiculidae), 20 laelapid mites (Mesostigamata: Laelapidae), three ixodid ticks (Ixodida: Ixodidae), six lice (Phthiraptera: Hoplopleuridae, Polyplacidae) and 17 fleas (Siphonaptera: Ceratophyllidae, Ctenophthalmidae, Hystrichopsyllidae). Eight species of ectoparasites were recorded for the first time in Lithuania. Twelve ectoparasite species were identified as highly specific parasites for host species or genus, three species as moderately specific for host species or genus, and 15 species as generalist parasites. Parasite species of all three host specificity levels were found among laelapid mites and fleas. Lice were either generalists or highly specific parasites. Meanwhile, chigger mites and ticks were typical generalist parasites.

O.19.

Seasonal dynamics and factors shaping ectoparasite communities of small mammals in Lithuania

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Small mammals and their ectoparasites are known as reservoir hosts or vectors of various pathogens. Despite increasing number of studies related to host – vector – pathogen interactions, many questions still need to be investigated or clarified.

The aim of the study was to analyse seasonal changes in small mammal ectoparasite communities and to evaluate influence of environmental (habitat, temperature, precipitation), host (species, relative abundance, diversity, species richness) and co-infection factors on communities of ectoparasites. The research was performed from May 2019 till May 2020 in three different habitats in Lithuania. In total, 650 small mammal specimens of 10 species were trapped and 10,904 ectoparasites were collected. Ectoparasite communities of small mammals were represented by mites (Trombidiformes: Trombiculidae, Mesostigmata: Laelapidae), ticks (Ixodida: Ixodidae) and insects (Siphonaptera: Ceratophyllidae, Ctenophthalmidae, Hystrichopsyllidae; Phthiraptera: Polyplacidae, Hoplopleuridae). The prevalence and mean abundance varied significantly among months in all ectoparasite groups except Phthiraptera, whereas mean intensity showed significant variation only for Trombiculidae and Ixodidae parasites. Combinations of various environmental, host and co-infection factors influenced communities of ectoparasites.

POSTER PRESENTATIONS

P.1.

TOXOSOURCES – European One Health approach to source attribution of *Toxoplasma gondii* infections

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Toxoplasma gondii infection can be acquired by several ways: by ingesting oocysts (in food or feed, water, or the environment contaminated with feces of infected, shedding felids; environmental pathway) or by ingesting tissue cysts (in meat or other tissues of infected animals; meatborne pathway). The relative contributions of these transmission pathways to human infection and disease have been unknown, partly due to lack of appropriate methods. A large European One Health project TOXOSOURCES (2020-2022) developed new methods, conducted multicentre studies, and used several approaches to investigate the relative contributions of different potential sources of the zoonotic infection.

P.2.

Toxoplasma gondii and ovine abortions in Estonia: questionnaire survey

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The zoonotic protozoan *Toxoplasma gondii (T. gondii)* is an important parasite for sheep husbandry due to the potential for production losses caused by abortions. The seroprevalence of *T. gondii* in sheep in Estonia (42%) is noticeably higher than that in Finland (24%) or in the Nordic-Baltic region in northern Europe (23%).

The aim of this study was to contribute to the knowledge on spontaneous ovine abortions or stillbirths that might be related to *T. gondii* infection on sheep farms in Estonia. We designed an online questionnaire survey to investigate the occurrence of ovine abortions and related possible risk factors associated with *T. gondii* infections in sheep.

We received 84 full responses to the questionnaire. Preliminary results showed that *T. gondii* infections had been reportedly diagnosed in 2% of the farms, whereas 19% of the respondents did not know whether they have had *T. gondii* infections on the farm. Stillborn or weak lambs were reportedly been born on 32% of the farms and 26% of the farms reported abortions in ewes that occurred the previous year. Free-roaming cats were reportedly present on 79% of the farms, and 36% of the respondents indicated that cats have access to feed intended for the sheep. Our preliminary results demonstrate that there are abortions and stillbirths on sheep farms in Estonia, but often the causes remain unknown. Furthermore, there seems to be a lack of awareness among sheep farmers that feed sources should be protected from cats.

P.3.

The new therapeutic approach to human toxoplasmosis

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Human toxoplasmosis is a widespread disease. In search of an effective treatment, nitazoxanide (NTZ) was evaluated in the treatment of acute and chronic toxoplasmosis in the mice models and it has significantly reduced the number of brain cysts and reduced the mice mortality rate. NTZ significantly reduces inflammation caused by acute and chronic T. gondii infection and significantly increases the production of serum IFN-γ and enhanced iNOS production in brain tissue, suggesting an immunomodulatory role of NTZ. The link between T. gondii and schizophrenia, bipolar disorder (BP) and suicidal/aggressive behaviors in humans has been widely documented. Based on the findings of the mice studies, the link between T. gondii and mentioned psychiatric conditions and widely known safety of NTZ in humans we decided to use NTZ as medicine in patients diagnosed with schizophrenia, bipolar disorder (BP) and suicidal/aggressive behaviors with positive serological Toxoplasma gondii tests (IgA, IgM, IgG, avidity). Despite the fact that all our patients have got better and we have a promising initial serological tests evolution, this area remains controversial without the further cause-effect studies. The research is needed to investigate the role of T. gondii in larger samples of schisophrenia, BD patients and suicidal/aggressive behaviors including the different stages of the mentioned illnesses. Finally, it seems to be of importance to consider that the identification, prevention and treatment of toxoplasmosis contributing to the etiopathogenesis of mentioned above psychiatric conditions could have a significant impact on mentioned diseases course and perception. For this reason, since there is no expected anti-Toxoplasma vaccine, every attempt to prevent and treat safely toxoplasmosis should be encouraged.

P.4.

Cryptosporidium spp. and *Giardia* spp. infection in Humans in Latvia: underdiagnosed and underreported

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In the presents study we would like to highlight the hypothesis that the actual number of cryptosporidiosis and giardiasis human cases in Latvia is higher than that officially reported by comparing the data from retrospective study of the officially reported human cases with the preliminary data of prospective study in both children and adults in Latvia.

Retrospective data of officially reported cases of cryptosporidiosis and giardiasis were collected from the Centre for Disease Prevention and Control of Latvia, 2000-2020. While prospective data were collected from 2022-2023, a total of 1211 human fecal samples (age range: from 1 days to 110 years) were collected and analyzed using flotation-centrifugation method and stained with AquaGlo™ (Waterborne INC, USA) (Ethics committee (No. 6-1/13/2020/51).

Retrospective data shows that during 20-year period, 71 case of cryptosporidiosis and 1020 cases of giardiasis were reported, with significantly higher proportion of cryptosporidiosis reported in age group of 30-39 years (33.8%), but for giardia 1-6 (9.0%) and 7–14-year-olds (14.1%) ($p<0.05$). While the preliminary data from prospective study shows that *Cryptosporidium* spp. was found in 2.6% (N=32; 95%CI 1.9-3.9), but *G. duodenalis* in 0.4% (N=5; 95%CI 0.1-1.0) of the cases with significantly higher proportion of cryptosporidiosis reported in age group of 45-64 years (6.6%), but for giardiasis – 25-44 (1.2%).

The results highlights that both parasites are more prevalent in adult human population in Latvia.

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P.5.

Cryptosporidium spp. and *Giardia duodenalis* in untreated wastewater in Latvia: preliminary results

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Cryptosporidium spp. and *Giardia duodenalis* are zoonotic waterborne and foodborne parasites and may cause enteric disease for both animals and humans. The aim of this study is to assess prevalence of these parasites in wastewaters in Latvia from One Health perspective.

From 2021-2022, a total of 622 wastewater samples of untreated 24-hour composite material were obtained from eleven wastewater treatment plants. Samples were pre-treated with 0.01% Tween® 20 for 24 hours and afterwards the sedimentation and centrifugation steps were used to obtain 2 ml of purified material. Further, 100 µl of the thoroughly suspended and purified sample was analyzed by fluorescent microscopy using Aqua-Glo™ (Waterborne INC, USA), labelling *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts by specific antibodies.

The preliminary results shows that significantly higher prevalence in wastewater was observed for *Giardia* cysts (68.7%; n=427) comparing to that for *Cryptosporidium* spp. oocysts (7.4%; n=49), while prevalence of mix infection was observed in 6.3% (n=39). Accordingly, the number of observed *Giardia* cysts was higher (mean 3.3; range 1-38) than *Cryptosporidium* spp. oocysts (mean 1.5; range 1-5) per 100 µl sample. In most cases, both *Cryptosporidium* spp. oocysts and *Giardia* cysts were found to be potentially viable (with DAPI staining), 71.7% and 69.6%, accordingly.

Further studies will be based of molecular analyses to detect *Cryptosporidium* species and subtypes, as well as *G. duodenalis* assemblages from One Health perspective.

This research was funded by the Fundamental and applied research “Transmission of Foodborne Parasitic pathogen from animals to humans: TRANSPAR” (lzp-2021/1-0055).

P.6.

Cryptosporidium parvum gp60 subtype diversity in dairy calves in Finland

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Aim: Cryptosporidiosis, a diarrheal disease caused by the protozoan *Cryptosporidium parvum*, has increased over tenfold in Finnish cattle herds during the last decade. We conducted the epidemiological study as part of a research project “Cryptosporidiosis – an emerging zoonosis from cattle” to identify *C. parvum* subtypes in dairy farms by gp60 subtyping.

Methods: We analyzed a total of 101 *C. parvum* positive faecal samples from calves from 101 different Finnish dairy herds. These samples were tested positive for *C. parvum* with a real-time PCR method. The majority of the samples (n=76) were obtained between 2017 to 2020 from farms with clinical cryptosporidiosis. In 2020, we received 25 faecal samples from dairy farms without a recent calf diarrhoea outbreak (control farms). *C. parvum* was subtyped by gp60 gene sequencing.

Results: We found nine gp60 subtypes, of which seven occurred in farms with clinical cryptosporidiosis and eight in control farms. The *C. parvum* subtype IIaA15G2R1 was found in 68% of samples from farms with clinical cryptosporidiosis and 40% of samples from control farms. Another commonly found subtype was IIaA13G2R1, found in 17% and 20% of farm samples, respectively.

Conclusions: Mostly, the same gp60 subtypes were found in farms with clinical cryptosporidiosis and control farms. The most common subtype was IIaA15G2R1, and the second most common subtype was IIaA13G2R1. These subtypes have been previously found in both calves and humans in different countries. Based on previous reports on human cryptosporidiosis, humans and dairy calves share the most common *C. parvum* gp60 subtypes in Finland.

P.7.

Observations on the transmission of *Dientamoeba fragilis*: Ambiguity of the life cycle and the cyst stage

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Little is known about the life cycle and mode of transmission of *Dientamoeba fragilis*. Recently it was suggested that fecal–oral transmission of cysts may play a role in transmission of *D. fragilis.* In order to establish an infection, *D. fragilis* is required to remain viable when exposed to the pH of the stomach. In this study we investigated the ability of cultured trophozoites to withstand extremes of pH. We provide evidence that trophozoites of *D. fragilis* are vulnerable to highly acidic conditions. We also investigated further the ultrastructure of *D. fragilis* cysts obtained from mice and rats by transmission electron microscopy. These studies of cysts showed a clear cyst wall surrounding an encysted parasite. The cyst wall was double layered with an outer fibrillar layer and an inner layer enclosing the parasite. Hydrogenosomes, endoplasmic reticulum and nuclei were present in the cysts. Pelta-axostyle structures, costa and axonemes were identifiable and internal flagellar axonemes were present. This study therefore provides additional novel details and knowledge of the ultrastructure of the cyst stage of *D. fragilis*.

P.8.

Prevalence of the infective larvae of *Anisakis* in *Trichiurus lepturus* from the Taiwan Strait of the North Pacific Ocean

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Anisakiasis is caused by the genus of *Anisakis* infective larvae and has been recognized as an important fish borne zoonotic disease. *Trichiurus lepturus* is one of the commonly found marine fishes in the commercial fish markets, and is the most abundant with *Anisakis* infective larvae in Taiwan. Our study aim to assess the infection rate of *Anisakis* infective larvae, and the different species of *Anisakis* infective larvae in *T. lepturus* caught from the Taiwan Strait of the North Pacific Ocean. A total of 1242 *T. lepturus* were examined for the presence of *Anisakis* infective larvae from May 2018 to April 2022. The genus of *Anisakis* infective larvae was recognized by microscopy, and their species were differentiated by Polymerase chain reaction with Restriction fragment length polymorphism assay. The results show that the infection rate of *Anisakis* infective larvae in *T. lepturus* is 82.7% in the recent 5 years. The most abundant *Anisakis* species found in *T. lepturus* is *A. pegreffii*, the second abundant species is *A. typica*, and the third abundant species is *A. physteris*. During these years, the highest season of *Anisakis* infestated *T. lepturus* is between July and September, and the lowest season of *Anisakis* infestated *T. lepturus* is between January and March.

P.9.

Anisakidae nematode infection in Baltic cod (*Gadus morhua*)

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Nematodes of the Anisakidae family are important parasites that can cause anisakiasis — human parasitic infection of gastrointestinal tract, which, in most cases, is caused by consumption of fish which are infected with the larvae. Therefore, Anisakidae is ranked as 7th most important foodborne parasite in Europe. The aim of the study was to analyze the prevalence of Anisakidae nematodes in Baltic cod and potential factors associated with the fish infection.

The Baltic cod (whole fish or liver) was collected in Baltic Sea's ICES coastal subdivisions 26 and 28 in winter and spring seasons from 2018 to 2022. In total 1,912 samples were collected and analyzed for Anisakidae prevalence. Nematode genus/species was identified through morphological characteristics and further molecular analysis. Additional data on fish was collected: sex, age, weight-length relationship, ect.

All detected nematodes in Baltic cod were identified as *Contracoecum osculatum* and the overall prevalence was 31.4% (n=600) with the median infection intensity of 3 nematodes (range 1 – 113) per fish. Cod age distribution ranged from 0-7 years old, with majority of fish being 3 years old (32.5%) and 2 years old (26.2%). A moderate positive correlation was observed between the infection intensity and age of fish ($r=0.442$, $p<0.05$).

The observed results indicate that the infection of *C. osculatum* may affect Baltic cod health condition, especially in older fishes.

This research was funded by the Fundamental and applied research "Baltic cod (*Gadus morhua*) condition and health status in the changing ecosystem of Eastern Baltic: CODHEALTH" (lzp-2021/1-0024).

P.10.

Babesia spp. in *Ixodes ricinus* in Lithuanian public parks

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Babesia is a protozoan parasite from the phylum Apicomplexa that is well-known as a causative agent of babesiosis in humans and domestic animals. In Europe, *Babesia* spp. are transmitted mainly by *Dermacentor reticulatus* and *Ixodes ricinus* ticks. *I. ricinus* is the most prevalent tick species in urban parks in Lithuania and is involved in the transmission cycles of *Babesia divergens*, *B. microti*, *B. venatorum*, and *B. capreoli*. The first three species are causative agents of human babesiosis. Due to the lack of data about the prevalence of this tick-borne pathogen in urban areas, this study aimed to investigate the prevalence of *Babesia* spp. in questing ticks collected in urban parks of Lithuania.

Questing *I. ricinus* ticks (n=536) were collected by flagging at twelve parks located in different Lithuanian cities in 2021. DNA from each tick was extracted, and DNA samples were pooled (n=144) into groups by developmental stage, sex, and location. *Babesia* spp. was screened by real-time PCR assay amplifying *18S rRNA* gene and internal transcribed spacer (*ITS*) region, which detect *Babesia* species from clade X and clade I, respectively. Amplification and sequence analysis of 382 bp fragment of 18S rRNA was performed to identify *Babesia* strains. The minimum infection rate (MIR) of *Babesia* spp. in *I. ricinus* was 3.6 % (18/536). *Babesia*-infected ticks were found in six parks, with infection rates ranging from 1.5% to 7.3%. Two *Babesia* species, *Babesia venatorum* and *Babesia microti* were identified. This study is the first report on the prevalence of *Babesia* spp. in urbanized areas in Lithuania.

P.11.

Prevalence and control of canine babesiosis in Lithuania

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Babesiosis is a vector-borne parasitic disease which is prevalent in many regions of the world, affecting various species of animals, including dogs and humans. It can be cured by early detection and diagnosis, but the disease often leaves irreversible changes in the body, causes complications and can be fatal.

A retrospective study was conducted - a survey on cases of babesiosis diagnosed in 2019 in dogs in veterinary clinics in Lithuania. The survey consisted of 7 questions, its electronic form was answered by 28 respondents, 170 were interviewed by telephone (122 provided answers). A total of 150 Lithuanian small animal clinics located in ten regions of Lithuania provided answers to the survey questions.

After analyzing the answers, the prevalence of babesiosis in the Lithuanian dog population was 1.18%. This size in different countries varied from 0.34% up to 1.64%. Babesiosis control in Lithuania is performed using preventive measures – chewable tablets, spot-on solutions and collars containing active substances that act against ectoparasites. However, the study showed that 71.84% of patients do not use or misuse measures. In addition, it was shown that the most popular group of preventive measures used in Lithuania are chewable tablets (45.33%).

The distribution of babesiosis on the landscape of Lithuania also revealed that the forest cover of Lithuanian counties, amount of water bodies and the type of preventive measures used do not have a statistically significant effect on the occurrence of the disease. However, it showed that the time of the year effect is statistically significant for the occurrence of babesiosis in the Lithuanian dog population.

P.12.

Specific antibody response of the host with experimental trichinellosis and Enterococci/enterocins treatment

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Enterocins from beneficial bacteria represent a new strategy to combat or prevent various parasite infections. Humoral immunity has a key role in Th2 responses in trichinellosis, participates in the rapid expulsion of infectious larvae from the intestine, the reduction of female fertility and the destruction of newborn larvae (NBL). 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 *Trichinella spiralis* (400 larvae) on 7th day of treatment.

We recorded a high level of serum anti-*T.spiralis* IgM already on the 5th day post infection (dpi.) and IgM level remained high until the end of the experiment. Enterococci/enterocins therapy did not alter the IgM response. Hypergammaglobulinemia IgA appeared on 11 dpi in untreated mice, but Enterococcii/enterocins therapy induced low IgA production. The level of total IgG showed an increasing trend from 11 to 32 dpi., and IgG_1 production highly increased during the muscle phase and confirmed the Th2 predominance. Enterococci/enterocins therapy did not change the kinetics of total IgG and IgG_1. IgG_{2a} and IgG_{2b} production developed during NBL migration (11-25 dpi). Treatment with E.faecium CCM8558 and Enterocin M increased IgG_{2a} level at 18 and 32 dpi. A significant IgG_{2b} rise from 11 to 25 dpi. initiated E.faecium CCM8558 and E.durans ED26E/7 strains. Enterococci/enterocins therapy altered IgG_2 production, thereby interfered with the Th1 modulation in muscle trichinellosis and participated in larval destruction.

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P.13.

Investigations of Muscle Parasites in Wolves (*Canis lupus*) from Lithuania

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The nematodes of the genus *Trichinella* and protozoans of the genus *Sarcocystis* are hazardous cosmopolitan parasites commonly found in wild carnivores, omnivores, and scavengers. The gray wolf (*Canis lupus*) is one of the largest predators in Europe and may be involved in the epidemiology of zoonotic pathogens such as *Trichinella* spp. and *Sarcocystis* spp.

In the period of 2020-2023, the muscles of 13 grey wolves, were collected from various locations in Lithuania. The active method of artificial digestion has been used to study the prevalence and intensity of *Trichinella* infection in tested samples. The infection intensity was estimated by counting number of larvae per gram of sample (*lpg*). Morphologically *Sarcocystis* were characterized in fresh squashed samples under a light microscope (LM). *Trichinella* species were identified using multiplex-PCR, while *Sarcocystis* were identified by nuclear *18S* rRNA, *28S* rRNA, *ITS1* mitochondrial *cox1*, and apicoplast *rpoB* sequence analysis.

Of 13 animals tested, nine (69.2 %) were positive for *Trichinella*. The invasion intensity of these parasites varied from 0.7 to 17.6 lpg, the mean intensity was 3.9 lpg. Of the 130 larvae 100 % were identified as *T. britovi* which is the dominant *Trichinella* species in wild animals in Lithuania. Microscopic sarcocysts were detected in the muscles of one grey wolf. By LM, sarcocysts were ribbon-shaped, with thin (up to 1.0 µm) and apparently smooth cyst wall and contained 7.5–8.5 µm long banana-shaped bradyzoites. Based on molecular analysis, this is the first report of *S. svanai* in the grey wolf.

P.14.

Mosgovoyia pectinata in Faroese mountain hares (*Lepus timidus*)

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The mountain hare (*Lepus timidus*) populates 15 of the 18 islands comprising Faroe Islands. We studied tapeworms collected from four mountain hares from four geographic areas of Faroe Islands by molecular identification using the nuclear ribosomal DNA (28S), mitochondrial cytochrome oxidase subunit 1 (*cox1*) and NADH dehydrogenase subunit 1 (*nad1*) genes. The results indicated that the tapeworms were *Mosgovoyia pectinata* (Spasskii, 1951) (Cestoda: Anoplocephalidae *sensu stricto*). Taken the parasite is quite common in Norway, from where the mountain hares were introduced in 1855, it is conceivable that co-introduction of *M. pectinata* from Norway to the Faroe Islands took place. The phylogenetic analyses showed high similarity across the *M. pectinata* sequences from three regions, and the position of the Faroese isolate as the sister lineage of isolates from Finland and East Siberia.

P.15.

Molecular based identification of *Accipiter* hawks as possible definitive hosts of numerous *Sarcocystis* spp.

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The genus *Sarcocystis* (Apicomplexa: Sarcocystidae) consist of worldwide distributed abundant cysts-forming parasites prevalent in mammals, birds and reptiles. Currently, 28 *Sarcocystis* species are known to form sarcocysts in muscles or CNS of birds. In Europe *S. calchasi* is well investigated pathogenic species infecting birds of different orders; however, this species previously has not been detected in Lithuania. The aim of the study was to examine intestinal samples of Northern goshawk (*Accipiter gentilis*) and Eurasian sparrowhawk (*Accipiter nisus*) collected in Lithuania for the presence of *S. calchasi* and other species distinguished by bird-bird life cycle. In the period of 2018-2022, 16 northern goshawks and Eurasian sparrowhawks each were analysed for *Sarcocystis* spp. oocysts/sporocysts. Parasites were identified by species-specific nested PGR targeting *ITS1* region and Sanger sequencing.

Under light microscope all analysed northern goshawk intestinal samples and 13 samples of sparrowhawks were positive for sporocysts, oocysts and sporulating oocysts of *Sarcocystis*. Overall, 49 and 14 sequences of *Sarcocystis* spp. were obtained examining northern goshawks and sparrowhawks, respectively. Eight known *Sarcocystis* species (*S. calchasi*, *S. columbae*, *S. cornixi*, *S. halieti*, *S. kutkienae*, *S. lari*, *S. turdusi* and *S. wobeseri*) were identified including first report of *S. calchasi* in Lithuania. Furthermore, *Sarcocystis* sp. phylogenetically closely related to *S. calchasi* and *S. wobeseri* was determined. Also, this study represents first confirmation of *S. kutkienae* and *S. wobeseri* in *Accipiter* haws. The different distribution pattern of identified *Sarcocystis* spp. in northern goshawk and sparrowhawk can be explained by the diets of two examined *Accipiter* species.

P.16.

Prevalence of vector borne pathogens in feline ectoparasites in Lithuania

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Transmission of zoonotic pathogens occurs through direct human–animal contact and/or arthropod vectors, such as ticks, fleas, and mosquitoes. Climate change and human migration with companion animals have an impact on the spreading of vectors and associated pathogens, which causes a growing risk of zoonoses via spreading and subsequent establishment of disease vectors from endemic to non-endemic areas. *Bartonella* spp., *Mycoplasma* spp. and *Rickettsia* spp. are abundant zoonotic vector-borne pathogens that are found in cats and can be transmitted by ticks and fleas. In this study, 321 ticks and 102 fleas were collected from 59 cats and screened for pathogens by using multiplex real-time PCR, conventional and nested PCRs targeting particular region of pathogen genome. *Bartonella* spp. were detected in 10 (3.1%) out of 321 ticks and 21 (20.6%) out of 102 fleas. One of the 321 (0.3%) tick samples and six of the 153 (3.9%) flea samples were found to be positive for *Mycoplasma* spp. *Rickettsia* spp. were detected with high prevalence – 103 out of 321 (32%) tested ticks and 21 out of 102 (20.6%) flea samples were positive. The results of our study demonstrated the presence of multiple vector-borne pathogens in feline ectoparasites in Lithuania, covering a relevant gap in knowledge and showing the necessity of systematic ectoparasite control in cats with a further molecular diagnosis of pathogens.

P.17.

In - vitro anthelmintic activity of the *Artemisia absinthium* L. and *Artemisia vulgaris* L. extracts against Trichostrongylidae nematodes in sheep

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In ruminants, especially sheep and goats, gastrointestinal nematode infections and their control are relevant. Due to the anthelmintic resistance in the world, the principles of parasite control have changed. New alternatives have been sought and one of the most researched alternatives is phytotherapy. The aim of the study is to find the ovicidal and larvicidal activity of wormwood and mugwort against Trichostrongylidae in sheep.

The aerial parts of the plants were extracted separately in 70%, 50%, and 30% ethanol and acetone solution. Six different extracts were obtained. Six dilutions were prepared from each extract – 500 mg/mL, 200 mg/mL, 100mg/mL, 50 mg/mL, 20 mg/mL, and 10 mg/mL. To detect the anthelmintic activity of extracts, an Egg hatch test and Micro - agar larval development test was performed.

Artemisia absinthium L. has stronger ovicidal but *Artemisia vulgaris* L. - has larvicidal activity. The highest percentage of egg inhibition is 500 mg/mL dilution of wormwood 30% ethanol extract – 100%, then 98% are 500 mg/mL and 10mg/mL dilutions of wormwood 50% and 30% acetone extracts. 100% of larvae inhibition are all dilutions of wormwood and mugwort 30% and 70% acetone extracts. Ethanol extracts have better ovicidal activity but larvicidal – acetone. Wormwood and mugwort have anthelmintic activity against the Trichostrongylidae. Future studies are required to determine the anthelmintic activity of these extracts *in vivo*.

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P.18.

Efficacy of albendazole against *Dicrocoelium dendriticum* in naturally infected sheep

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Dicrocoeliosis is a significant disease of grazing small ruminants with often ineffective therapy in Slovakia. The aim of our study was to determine the efficacy of albendazole (ABZ) against *Dicrocoelium dendriticum* hepatic trematode using percentage of reduction (PR%) in an *in vivo* faecal egg reduction test (FECR) and percentage of efficacy (PE%) at necropsy in sheep naturally infected with *D. dendriticum* at day 14 and day 30 after therapy (dpt).

The study included 24 sheep divided into four groups. The first group T1 was treated with ABZ and necropsied on 14 dpt (n = 6). The second group of sheep T2 (n = 6) was ABZ treated and necropsied on 30 dpt. Two control groups were necropsied on 14 dpt (C1, n = 6) and 30 dpt (C2, n = 6). Faecal samples were examined by the MiniFLOTAC method with a sensitivity of 10 eggs per gram faeces EPG (eggs per gram).

Therapeutic effect of a single oral dose of 15 mg/kg b.w of ABZ in sheep with *D. dendriticum* was confirmed in the FECR test (PR 92.4%, $p < 0.01$) as well as post mortem by the necropsy (PE 92.97%, $p = 0.002$) on 14 dpt. On 30 dpt, the lower PR of 88.5% ($p < 0.01$) in the *in vivo* FECR assay and lower PE (PE 91.76%, $p < 0.001$) at necropsy was determined.

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P.19.

Genome-wide exploration reveals distinctive northern and southern variants of *Clonorchis sinensis* in the Far East

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Clonorchis sinensis is a carcinogenic liver fluke that causes clonorchiasis—a neglected tropical disease (NTD) affecting ~35 million people worldwide. No vaccine is available, and chemotherapy relies on one anthelmintic, praziquantel. This parasite has a complex life history and is known to infect a range of species of intermediate (freshwater snails and fish) and definitive (piscivorous) hosts. Despite this biological complexity and the impact of this biocarcinogenic pathogen, there has been no previous study of molecular variation in this parasite on a genome-wide scale. Here, we conducted the first extensive nuclear genomic exploration of *C. sinensis* individuals (n = 152) representing five distinct populations from mainland China, and one from Far East Russia, and revealed marked genetic variation within this species between “northern” and “southern” geographical regions. The discovery of this variation indicates the existence of biologically distinct variants within *C. sinensis*, which may have distinct epidemiology, pathogenicity and/or chemotherapic responsiveness. The detection of high heterozygosity within *C. sinensis* specimens suggests that this parasite has developed mechanisms to readily adapt to changing environments and/or host species during its life history/evolution. From an applied perspective, the identification of invariable genes could assist in finding new intervention targets in this parasite, given the major clinical relevance of clonorchiasis. From a technical perspective, the genomic-informatic workflow established herein will be readily applicable to a wide range of other parasites that cause NTDs.

P.20.

Reviving fish parasitology in Lithuania: Assessment of current diversity of helminth parasites of fishes in freshwater ecosystems

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Lithuania is enriched with natural and artificial freshwater ecosystems which harbour a rich local biodiversity. After intensive studies in the 1950−1980s, interest in fish parasites in Lithuania has dwindled and since then, Lithuanian freshwater ecosystems have been impacted, either directly through continual anthropogenic perturbation or indirectly through global environmental change. Further, introductions of non-native fish species have likely facilitated the colonisation of new parasite species which could potentially spill-over into native fish stocks. Thus, the aim of our study was to assess the current diversity of helminth parasites in native and non-native fish in Lithuanian freshwaters. For reliable estimates of helminth diversity, we applied morphological and molecular (nuclear and mitochondrial genes) methods for species identification. In 2022, a total of 350 fish individuals of 29 native species as well as two non-native species (*Neogobius fluviatilis* and *N. melanostomus*) from nine waterbodies were examined. Out of 350 fish, 89% were infected with helminth parasites, including: acanthocephalans, cestodes, digeneans, monogeneans, and nematodes. Parasite prevalence was distinctly uneven, with digeneans reported from 93% of infected fish, whereas the prevalence of other helminth groups ranged from 3 to 31%. With over 40 species recorded, digeneans were the most diverse group of parasites. Although most of these species were reported prior to our study, there are records of several new species in the region that use freshwater fish either as intermediate or definitive hosts. We discuss the taxonomic and ecological implications of this data.

The study was funded by the Research Council of Lithuania (S-MIP-22-53).

P.21.

Increasing complexity in the nervous system and behavior between stages corresponds to the transition in neuronal genes expression from redia to cercaria in trematode *Cryptocotyle lingua*

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Trematodes have a highly complex life cycle that few animal models can match. The transformation from the intramolluscan redia to the freely swimming cercaria involves extensive anatomical and behavioral metamorphoses, enabling the larva to locate and infect the next host in the complex water environment. However, the functional changes that occur in the nervous system during this transition are not well understood.

In this study, we characterized the behavior of two stages, redia and cercaria, of potetial model species and an economically important fish parasite *Cryptocotyle lingua* using comptuter vision. We utilized the *de novo* transcriptome to identify and characterize genes essential for nervous system function. By performing differential expression analysis, we identified several neuronal genes upregulated in the freely swimming cercariae, including genes involved in synaptic vesicle trafficking and release, two transmembrane channels that may be involved in sensory perception, two receptors, and notably, the nitric oxide receptor soluble guanylate cyclase. We validated our findings using antibody staining of neuronal markers and *in situ* hybridization. Our data on the localization of their expression in the nervous system of the two stages studied allowed us to hypothesize their function in relation to the adaptations, host-finding strategy, and behavior of the freely swimming cercaria.

Our results provide a foundation for future research aimed at better understanding the behavior and physiology of parasitic flatworms that can be beneficial for the development of antiparasitic measures.

P.22.

Helminth community in *Notothenia coriiceps* in Argentine Islands, West Antarctica: infection patterns and indicator species

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Parasites are known as sensitive indicators of the marine ecosystem state due to the complexity of their life cycles. Our study **aimed** to analyze the patterns of helminth infection of rockcod *Notothenia coriiceps* to understand the dynamics of parasite communities over time and highlight the helminth species – “indicators” of ecological changes in marine ecosystems.

Helminth samples were collected from 183 specimens of *N. coriiceps* in 2014–2015 and 2020–2021 in the vicinity of the Ukrainian Antarctic station “Akademik Vernadsky”, Argentine Islands, West Antarctica. Of 31 helminth species recorded, 25 taxa (9 trematodes, 4 cestodes, 5 nematodes, 7 acanthocephalans) were subjected to analysis.

Notothenia coriiceps was the definitive host of 19 helminth species; 12 species were larvae. The proportion of larvae was lower in 2014–2015 (73.4%) than in 2020–2021 (81.4%). The number of “dominant” species (prevalence >50%) increased from seven in 2014–2015 to nine in 2020–2021. At infracommunity level, the species richness was similar between samples; however, the abundance was significantly higher in 2020–2021. Seven species (*Pseudoterranova* sp., *Contracaecum* sp., *Ascarophis nototheniae*, monolocular metacestodes, bilocular metacestodes, *Metacanthocephalus rennicki*, *Diphyllobothrium* sp.) had a significant impact on the differences between helminth infracommunities. In the component community, the diversity indices (Shannon, Simpson, Pielou, Berger-Parker) evidenced higher evenness and lower domination in 2014–2015. Several biological factors caused slight, but significant changes in the *N. coriiceps* helminth infection patterns, particularly changes in the populations of intermediate, paratenic, and definitive hosts involved in the helminth transmission in Antarctic ecosystems.

P.23.

Acanthocephalans from marine and freshwater fishes in Taiwan

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During the ichtyoparasitological survey in 2017–2020, acanthocephalans were found in Taiwan's freshwater (Cypriniformes, Xenocyprididae) and marine fishes (Scombriformes: Scombriidae, Trichiuridae): *Micracanthorhynchina dakusuiensis*, *Rhadinorhynchus laterospinosus*, *Pallisentis rexus*, *Longicollum* sp., *Micracanthorhynchina* sp., *Gorgorhynchus* sp. All specimens were morphologically characterized and illustrated based on light and scanning electron microscopy. *Micracanthorhynchina* sp. and *Gorgorhynchus* sp. were morphologically distinct from all existing species and will be described as new to science. The geographic range of *R. laterospinosus* and *P. rexus* has been expanded. Phylogenetic analyses using SSU rDNA and COI datasets of *Gorgorhynchus* sp. showed that it is grouped with *Bolbosoma* spp. (Polymorphida) in a well-supported clade and is a sister species to members of two other genera of the family *Polymorphidae*, *Corynosoma* and *Andracantha*. The genus *Gorgorhynchus* is currently classified in the family Rhadiorhynchidae (Echinorhynchida) based on its morphology, due to the presence of four cement glands in males and tegumental spines in the anterior part of the body in both sexes. The same morphological characters are also characteristic of the family Polymorphidae. Until recently, the order Polymorphida was believed to include acanthocephalans of birds, rarely of mammals, but not of fishes. The recent transfer of the family Istmosacanthidae, parasites of fish, to the order Polymorphida has overturned these ideas. Based on morphological and molecular data, we propose to consider the genus *Gorgorhynchus* as part of the family Polymorphidae.
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P.24.

Metal sheets (copper, zinc or brass) in fish tanks control *Ichthyophthirius multifiliis* infection of rainbow trout

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Infections with the parasitic ciliate *Ichthyophthirius multifiliis* challenge health of freshwater fishes worldwide by eliciting white spot disease ichthyophthiriosis. The present work documents that brass, and each of its pure constituents (copper and zinc), have *in vivo* and *in vitro* antiparasitic effects in freshwater. We used an experimental model with *I. multifiliis* infected rainbow trout (O*ncorhynchus mykiss*). In the *in vivo* study metal sheets of either brass (alloy of copper and zinc), pure copper or pure zinc, were placed in fish tanks with trout experimentally infected with *I. multifiliis* (duplicated trials). All three metals (pure or combined) effectively blocked the life cycle of *I. multifiliis* and inhibited reinfection and morbidity. In control tanks with infected trout, but no metal sheets, we observed significantly elevated infection levels and development of disease. *In vitro* assays, exposing three life cycle stages (tomont, tomocyst and theront) exposed to the same metal sheets in Petri dishes, confirmed the antiparasitic effects. Tomonts were sensitive to pure copper and brass (100% and 66 % mortality within 15 min, respectively) and zinc (100 % mortality within 30 min). Tomocysts, with enclosed tomites, were less sensitive, as even 12 h exposure to brass, pure copper and zinc merely elicited mortality rates of 44%, 50% and 60%, respectively. Theronts were killed within 75 min when exposed to brass and pure copper plates, whereas zinc plate exposure eliminated all theronts within 60 min. The use of stationary metal sheets in aquaculture settings may be a possible way to control ichthyophthiriosis.

P.25.

Prevalence of blood parasites in their potential *Culicoides* vectors

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Haemoproteus parasites are common blood parasites and are distributed worldwide. Infection of these parasites is mainly studied in their avian hosts, while infection in wild *Culicoides* (biting midges) is poorly understood. The aim of this study is to determine the prevalence of haemosporidian parasites in their potential biting midges vectors at different locations in Lithuania.

Biting midges were collected at 4 different locations in Lithuania using UV-light traps. The insects with burgundy pigment in the abdomen were identified and separated, indicating at least one gonotrophic cycle, meaning that there is a greater chance that the insect had, at least, one blood meal. The separated insects were dissected, and the remains were fixed in 96% ETOH for molecular and phylogenetic analyses.

A total of 1498 biting midges were dissected. Here we report that the prevalence of haemosporidian parasites was 8.1%. Seventeen different parasite cytochrome *b* lineages were found and identified in biting midges. In addition, three new lineages were found and one biting midge had a co-infection (two different parasites). The most common lineages were *Haemoproteus asymmetricus* TUPHI01 (55.7%) and *Haemoproteus minutus* TURDUS2 (18.9%), which are parasites of Turdidae birds. The phylogenetic relationships between these lineages were determined.

These results indicate the prevalence of haemosporidian parasites in biting midges. These findings are important for a better understanding of natural prevalence of haemosporidian parasites of wild birds and the role of biting midges as their potential vectors in nature.

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P.26.

Trypanosomatid infections of biting midges (*Culicoides*, Ceratopogonidae) in Lithuania

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Trypanosomatids are protozoan parasites that develop in one (monoxenous trypanosomatids) or two (dixenous trypanosomatids) different hosts – vertebrates and invertebrates, which serve as vectors. It is already known that trypanosomes are transmitted mostly by bloodsucking dipterans, however only a few avian trypanosome vector species have been determined so far.

This study aimed to compare the occurrence of trypanosomatids in wild-caught biting midges (Ceratopogonidae, *Culicoides*) at two different sites in Lithuania. Parous *Culicoides* females were collected using UV light traps at different heights: 2 m height above the ground and in the canopy of the trees (~12 m above the ground). PCR-based methods were used to estimate the prevalence of trypanosomatids in biting midges.

In total 2067 parous wild-caught *Culicoides* females belonging to 15 species were investigated. The infections with trypanosomatids accounted for 10.2 % in the canopy, compared to only 3.1 % at ground level. A difference in the species composition of trypanosomatids was observed: 124 biting midges (out of 1257) collected in the canopy were infected with *Trypanosoma* parasites, while at the ground level there were only 9 (out of 810); monoxenous parasites *Sergeia podlipaevi* and *Herpetomonas ztiplika* were found only at ground level, meanwhile *Crithidia brevicula* and *Obscuromonas* sp. were found in biting midges collected in the canopy.

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P.27.

Molecular detection of African swine fever virus and trypanosomes in horseflies (Diptera: Tabanidae)

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Tabanidae family consist of haematophagous insects, that due to their lifestyle play a role as vectors in the mechanical and biological transmission of large variety of disease agents. Due to diferences in biology and host preferences, distinct Tabanidae species can act as vectors of different pathogens. The main aim of this work was to determine the role of different horsefly species in the transmission of African swine fever (ASF) virus and *Trypanosoma* protists. Knowledge of the pathogens that can be transmitted by specific vector species is important for predicting and managing outbreaks of vector born diseases.

Insects were collected by Nzi traps in 2018-2019 in six farms with ASF outbreaks and in five farms without ASF outbreaks. Fourteen different Tabanidae species were identified from six areas in Lithuania. Insects were tested individually by PCR using ASF virus specific and Trypanosomatid specific primers.

Out of 101 investigated Tabanidae flies no cases of ASF virus DNA were detected. Meanwhile, 51 individuals (50,5 %) out of 101 tested flies had been infected with *Trypanosoma*. These parasites were detected in ten Tabanidae species. Nine different *Trypanosoma* sp. lineages were identified. Most of the sequences showed identity to *Trypanosoma theileri,* with more similarity to *T. theileri* complex TthI. This is parasite of herbivorous mammals. Identical sequences were associated with different Tabanidae species, so different lineages of *Trypanosoma* could be more related to the vertebrate host, but not the vector species. The research was partly funded by a grant (S-MIP-22-50) from the Research Council of Lithuania.

P.28.

Haemosporidae parasites in wild-caught *Coquillettidia* and *Culex* mosquitoes

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Bloodsucking mosquitoes (Culicidae) while feeding on blood cause not only a nuisance but poses a real threat by transmitting a variety of blood parasites. These parasites (Haemosporidae) are spread worldwide and can infect various hosts. Moreover – haemosporidian parasites are transmitted by vectors, which makes investigations and understanding of their life cycles more complicated. In this research, we studied the prevalence of Haemosporidae parasites naturally infecting wild-caught *Coquillettidia* and *Culex* mosquitoes.

Mosquito adults were collected in May-September 2022 in four localities in Lithuania. CDC mosquito traps baited with CO_2 were used twice a month. Caught mosquitoes were sorted, *Coquillettidia* and *Culex* mosquito females were identified morphologically. Mosquitoes were screened for the presence of haemosporidian parasites using PCR-based methods.

Overall 495 adult *Coquillettidia* and *Culex* mosquitoes were caught, and 300 mosquitoes (150 *Coquillettidia* and 150 *Culex*) were tested for the presence of haemosporidian parasites. *Coquillettidia* adult mosquitoes were abundant in June-August, the majority (61,4%) were caught in July, while *Culex* adults were abundant in May-September and the majority of them (48,2%) were caught in August. 3 (2,0%) *Coquillettidia* mosquito females and 6 (4,0%) *Culex* females were naturally infected with haemosporidian parasites. *Plasmodium* and *Haemoproteus* parasites have been detected in investigated mosquitoes.

These results contribute to the knowledge of Haemosporidae infection rates naturally occurring in two quite common genera of mosquitoes found in Lithuania.

P.29.

Co-infections of avian malaria in experimentally exposed vectors

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Although parasite co-infections are common in nature, the information about their interactions within vectors and possible impact on the development of sporogonic stages after a blood meal is scarce and not fully understood.

The aim of this study was to investigate and compare the sporogony, transmission and virulence of single *Plasmodium relictum* (pSGS1), *P. elongatum* (pERIRUB1) infections and co-infections of these two parasites in *Culex pipiens* form *molestus* mosquitoes.
Mosquitoes were infected by allowing them to take a blood meal on canaries infected with single *P. relictum* and single *P. elongatum.* Some mosquitoes were fed on two canaries infected with different parasites, thus gaining two parasite species. After blood meals, insects were collected and maintained until the development of sporozoites.

Our results revealed that sporogonic development of the single *P. relictum* infection does not cause mortality of exposed mosquitoes. In contrast, single *P. elongatum* infection and co-infection with both parasite species cause significantly higher mosquito mortality compared to the controls. Sporozoites of parasites were detected in salivary glands 15–20 days post infection in both single and co-infected mosquitoes. The presence of sporozoites in the salivary glands of two avian malaria parasites at the same time reveals that parasites can be transmitted simultaneously by the same mosquito. This result was confirmed as blood stages of *P. relictum* and *P. elongatum* were detected in the recipient canary after its exposure to a mosquito, co-infected with both malaria species.

The information about development and parasite interactions during co-infections in vectors helps to better understand parasite biology, transmission and epizootiology of avian malarial parasites.

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P.30.

Changes in bird gut microbiome during avian malaria infection

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Parasites are known to influence various aspects of their host biology. Recent evidence shows that host microbiome modulation by infection is one of the strategies of parasites to affect host immunity and physiology. Although avian malaria is a common and, in some instances, a lethal disease affecting wild birds, very little information is available about the impact of avian *Plasmodium* parasites on the gut microbiome of wild birds. Here, we tested whether experimental infection with avian malaria parasite, *Plasmodium homocircumflexum*, cause changes in the bird host gut microbiome from acute and chronic stages of infection to the beginning of the latent stage. To this aim, faecal samples were collected at different time points covering major infection stages and after 16S rRNA amplicon sequencing we characterized the microbial communities in infected and control birds.

The results showed that avian malaria infection did not have a significant impact on the bacterial diversity of infected birds. However, comparisons of taxonomic and functional profiling of microbiome in infected and control birds revealed an emergence of infection-specific taxa and pathways at the later stages of infection. The differential abundance and co-occurrence network analysis showed the changes in microbiome assembly of infected birds, affecting its structure and robustness.

Presented results show that infection with avian malaria parasite modulates the gut microbiome of birds, causing deviation from normal development with potential consequences for host health.

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P.31.

New insights into *Lankesterella* genus in birds: parasite genetic diversity in passerines from Europe

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The number of *Lankesterella* species in birds has increased significantly due to the use of molecular analysis for their diagnosis. Recently, it was shown that several *Hepatozoon*, species in fact, belong to genus *Lankesterella*. However, the genus *Lankesterella* remains poorly studied and little is known about its diversity and specificity. This study aims to identify the genetic diversity of *Lankesterella* parasites infecting different species of passerine birds and test if they are species specific.

Blood samples were analyzed using PCR-based methods that amplify a fragment of the *18S* gene of *Lankesterella* and *Isospora* paraistes. The amplified sequences were used to construct phylogenetic hypotheses. Blood smears were also analyzed microscopically for the morphological identification of these parasites.

Lankesterella sequences were obtained from eight different bird species. Sequences from *Turdus merula* associated with the parasite species *Lankesterella bivacuolata*. Parasite from *Parus cristatus* was associated with the species *Lankesterella macrovacuolata.* Sequences recovered from *Parus caeruleus, Parus major* and *Parus ater* grouped with *Lankesterella valsainensis*. Parasite sequences from *Eritachus rubecula* were associated with *Lankesterella vacuolata*. Sequences obtained from *Acrocephalus schoenobaenus* grouped within the *Lankesterella kabeeni* clade. Parasites from *Troglodytes troglodytes* were not associated with any other reported species but were placed within the clade of avian *Lankesterella*.

This work shows that avian *Lankesterella* parasites have a big diversity and seem to be species specific at *18S* gene level. Detailed morphological analysis is required to confirm if they belong to different species, as well as the evolutionary relationships of Adeleorina parasites.

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P.32.

Comparative analysis of changes in bird gut microbiome after experimental infection with *Plasmodium relictum* and *Plasmodium homocircumflexum* parasites

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Empirical evidence shows that parasite infection status may account for changes in host microbiota composition. Experimental studies on murine malarial parasites suggest that *Plasmodium* infections affect the composition of commensal bacteria populations in the host. Avian malaria parasites vary in their specificity and impact on the vertebrate host. Such inter-species differences may also affect the gut microbiota of the vertebrate host.

In our recent studies, bird gut microbiota was characterized at different time points after experimental infection of canaries *Serinus canaria* with *Plasmodium homocircumflexum* and *Plasmodium relictum*. Fecal microbiota modulation was evaluated and compared between experimental birds.

None of the avian *Plasmodium* species caused significant changes in the alpha-diversity of the microbiota of infected birds compared to their respective control. However, changes in the composition and abundance of several bacterial taxa were found to be *Plasmodium* species-specific. Co-occurrence networks were used to characterize the impact of *Plasmodium* infection on the microbiota structure, which were found to have different patterns of bacterial taxa co-occurrence between infected and uninfected groups and between different *Plasmodium* species. Prediction of functional profiles in bacterial communities showed that infection by either *P. homocircumflexum* or *P. relictum* is associated with unique metabolic pathways. The results showed that both avian malaria parasites affect bacterial community assembly in the host gut microbiota.

Microbiota modulation by malaria parasites could have deleterious consequences for the hosts. Knowing the intricacies of bird-malaria-microbiota interactions may prove helpful in determining critical microbial players and the processes involved in antimicrobial manipulation.

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P.33.

Host cells inhabited by *Leucocytozoon* parasites: are they phylogenetically predictable?

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Leucocytozoon species (Haemosporida, Leucocytozoidae) are still poorly investigated haemosporidian parasites. Blood cells inhabited by their blood stages (gametocytes) remain insufficiently known. The aim of this study was to investigate the origin of host cells inhabited by *Leucocytozoon* gametocytes in different Passeriformes species, to test if this feature has a phylogenetic importance and can be predictable by phylogenetic analysis.

Giemsa-stained blood films were extensively examined under microscope and samples containing *Leucocytozoon* infections with young parasites were selected. Infected blood cells were compared with the same non-infected blood cells. PCR-based methods were used to amplify a fragment of the cytochrome *b* gene (*cytb*) to identify the parasite lineages. Obtained sequences were used to construct a phylogenetic tree.

Leucocytozoon parasites from six bird species and individuals were used in this study. The parasites were seen to inhabit erythrocytes in song thrush *Turdus philomelos* (*cytb* lineage STUR1), black bird *Turdus merula* (unknown lineage), garden warbler *Sylvia borin* (unknown lineage), and blue tit *Parus caeruleus* (PARUS4). The parasites found in wood warbler *Phylloscopus sibilatrix* (WW6) and common chiffchaff *Phylloscopus collybita* (AFR205) were found inhabiting thrombocytes.

All studied parasites form roundish host cell-parasite complexes. *Leucocytozoon* lineages infecting thrombocytes were closely related, while the parasites infecting erythrocytes were placed in different clades in the tree. This study shows that host cells inhabited by *Leucocytozoon* parasites can be phylogenetically important and should be considered in future taxonomic studies during species description. Noteworthy, phylogenetic analysis might be useful for prediction of possible host cells certain parasite lineages might inhabit.

P.34.

Identification, frequency, phylogeny and risk factors of Haemosporida infection of birds of prey in Greece

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Haemosporidians belong to the group of parasites that infect a wide range of organisms - including birds - and use blood-sucking dipteran insects as vectors. Most species of wildlife importance belong to the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon*. Despite the fact that, for the time being, the role of haemosporidiosis in avian pathology cannot be fully estimated, there are several cases of birds that fall ill and die of haemosporidiosis globally. Studies on avian haemosporidians focus mainly on passerines, whilst in other avian groups, such as raptors, data is limited. The aim of this study is to determine, for the first time, the prevalence of haemosporidian infection of birds of prey in Greecein correlation with some risk factors, including sex, age, biology, region of origin and the clinical condition of the birds. In total, more than 450 birds belonging to 15 common European raptor species are going to take part in this study. These birds are admitted to the biggest wildlife rehabilitation centre in Greece, as injured, exhausted, ill or even orphans. Morphological and molecular methods for detection of parasites are used. In these preliminary results of the study, mainly of the Common buzzard (*Buteo buteo*), the most common raptor species in Greece, the prevalence of haemosporidian parasitesexceed 50%, indicating that they are very common in raptor birds in Greece. Our study contributes to a better understanding of the prevalence and diversity of haemosporidian parasites in raptors, a group of birds that is underrepresented in studies of haemosporidians.

P.35.

Seabird ticks (*Ixodes uriae*) distribution in Antarctica

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Seabird tick *Ixodes uriae* is wildly distributed throughout circumpolar regions. It is associated with seabird colonies and it may affect host species negatively, due to heavy infestation as well as because of the pathogens that might be transmitted by these ectoparasite (e.g. *Borrelia burgdorferi*, flavivirus, orbivirus, phlebovirus, and nairovirus). For this reason, monitoring tick distribution in Antarctica is important due to possible negative effect of ticks infestation on Antarctic wildlife.

Several rockeries of Adélie, Chinstrap and Gentoo penguins located in the South Shetlands and along the Antarctic Peninsula were visited between December 2022 and February 2023. Ticks were searched for beneath stones located within the penguin colonies. Stones (between 50 and 100 stones in every location surveyed) were chosen by its proximity to the penguin colonies and by its size. Relative abundance was calculated as the number of stones with ticks divided by the total number of stones prospected. The approximated number of ticks under each stone was also recorded.

Ticks were found beneath stones close to penguin rookeries of Adélie, Chinstrap and Gentoo penguins. However, no ticks were found on the birds. Ticks were found on South Shetland Islands, and along the Antarctic Peninsula up to Palmer Archipelago. Three new locations where ticks are present were identified during this survey with relative abundances of ticks ranging from 1 to over thousand individuals per stone and from 4 to 40% of the stones inspected. Further studies will be carried out to investigate genetic diversity of the tick population in Antarctica.

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P.36.

Description and circadian rhythms of *Chandlerella sinensis* Li, 1933, with notes on the impact of microfilariae on host health

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Twenty-three common linnets (*Linaria cannabina*) were caught at Ventės Ragas Ornithological Station, Lithuania (2021 May). Examination of blood using the buffy coat method indicated one bird highly infected with microfilariae. An adult nematode was obtained from the vessels of the liver and was morphologically identified as *Chandlerella sinensis* Li, 1933. Common linnet is a new host for *C. sinensis*. The investigation of circadian rhythm indicated that *C. sinensis* was statistically more numerous at midnight and in the morning. Microscopic examination of histological preparations of organs revealed that microfilariae were located in the lamina of blood vessels and the largest numbers of microfilariae were found in the lungs, which vessels were full of microfilariae. There were no inflammations in organs, only parabronchial walls were thickened, which could disrupt air-blood gas exchange, and the walls of pulmonary vessels were thinned and fragile. Common linnet with high parasitaemia and one uninfected common linnet were used to feed wild mosquitoes. The survival rate of mosquitoes fed on uninfected blood and on the bird with the microfilaria differed significantly. Of 30 mosquitoes fed on infected blood 22 died within 24 hours, while first mosquito fed on an uninfected bird died on the fourth day.

Conclusions: *C. sinensis* exhibit nocturnal subperiodisity; possible disturbance of gas exchange in the lungs adversely affects the ability of the bird to exercise and makes breathing difficult at rest; blood heavily contaminated with microfilariae is lethal to mosquitoes.

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e-POSTERs

E.1.

Can benzanthrone luminophores show organization of somatic muscle elements in Trematoda?

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Confocal laser scanning microscopy (CLSM) is considered as significant tool in biology field. The microscopic instrument allows to investigate not only freshy collected samples but also museum samples stored for a years. Moreover, sample layer by layer examination results in high-resolution three-dimensional images that give a better understanding of the helminth's morphological characterization. Previously developed staining protocols with benzanthrone luminophore use allow noteworthy reduce time required for specimen preparation. Benzanthrone luminophores are luminescent dyes that had shown qualitative helminth imaging possibilities. In present work using synthesized benzanthrone luminophores AZPP, AZP5, P7 and CLSM the organization of muscle elements were investigated in various Digenea species: *Parafasciolopsis fasciolaemorpha*, *Prostocus confusus* and Monogenea: *Diplozoon* sp. Obtained results confirmed well-developed muscular system including three main muscle layers in species body wall: longitudinal, diagonal and circular. These layers were also observed in visceral organs such as reproductive, digestive and excretory system. In regions among suckers transverse muscles were observed as well as dense muscle bundles within acetabulum. Radial and meridional muscle elements were observed in attachment organs. The study results confirmed the images captured from the samples gave the opportunity to better visualize muscle elements in each species, therefore, it confirms that benzanthrone luminophores can be used to show somatic muscle elements in Trematoda. This work is part of complex study dedicated to the investigation of benzanthrone luminophore use in biological sample staining protocols.

E.2.

Helminths species in larvae *Lissotriton vulgaris* and *Triturus cristatus* in Latvia

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The aim of the research was to investigate the helminth fauna of the newt's larvae. The research was carried out in Laboratory of the Parasitology and Histology of Daugavpils University during June – September, from 2018 to 2022.

In total, 295 animals including 227 *Lissotriton vulgaris* and 18 *Triturus cristatus* were manually collected and examined for the presence of helminths. The method of full parasitological examination was carried out using Скрябин, 1928, Pessier and Mendelson (2010) methods.

Nine helminth species use smooth newt and crested newt as hosts. In general, seven trematodes species (metacercariae or adults): *Astriotrema monticelli* mtc., *Diplodiscus subclaviatus* ad., *Echinoparyhium recurvatum* mtc., *Opisthioglyphe ranae* mtc., ad., *Paralepoderma cloacicola* mtc., *Pleurogenoides medians* and *Strigea sphaerula* mtc., and 2 nematodes species: *Hedruris androphora* and *Oswaldocruzia filiformis* were detected in *L. vulgaris.* While in *T. cristatus* were found four helminth species - trematodes mtc. (*A. monticelli*, *E. recurvatum*, *O. ranae*) and one nematode species (*H. androphora*). Four common helminth species for both newt's species were detected.

The prevalence of the infection of the examined newts reaches 24.7%. In newts, most commonly found metacercaria of *O. ranae* (P 19.3%) and *P. cloacicola* (P 12.5%); the rarest were *E. recurvatum* (P 4.7%), *S. sphaerula* (P 4.4%) metacercariae and *Hedruris androphora* was observed in 3.4% of animals.

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