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Published by the Nature Research Centre  
Warm welcome to the 9th Conference of the Scandinavian-Baltic Society for Parasitology!

As always, our conference will offer interesting new research findings and a platform for networking. This time – for the first time in our society’s history – we will meet virtually. I am convinced that this conference will be brilliant, wonderful and one to remember.

Organizing this conference virtually is an example of what we as a scientific society can do, especially in times like this. We use this opportunity to make our conference even more accessible, enabling even wider attendance. Organizing a virtual conference also illustrates that we prioritize taking the best possible care of each other and ourselves.

This pandemic shall pass, and it is important that we continue our work and collaborations, even during these challenging times. The research presented will show how many parasites do the same, in this changing world that we share.

Dr. Pikka Jokelainen
President of Scandinavian-Baltic Society for Parasitology
On behalf of the Scientific and Local Organizing Committees, I am pleased to cordially welcome you to the 9th Conference of the Scandinavian-Baltic Society for Parasitology (CSBSP9), hosted by Nature Research Centre and Young Academy of the Lithuanian Academy of Sciences, in Vilnius, Lithuania.

This biennial event is an official conference of the Scandinavian-Baltic Society for Parasitology (SBSP). After unification of the Scandinavian and the Baltic Societies of Parasitologists in 2003 in Bergen, the first conference of the Scandinavian-Baltic Society for Parasitology took place in Vilnius in 2005. Fifteen years later, we are happy to host CSBSP9 in Vilnius again – this time virtually.

The theme of this year’s meeting is ‘Parasites in a changing world’. The vision of this conference is to create a great platform for experienced and early-career researchers in the fields of wildlife, veterinary and medical parasitology and to share interdisciplinary science between researchers from Northern Europe and other countries around the world. The CSBSP9 Scientific Committee has created an excellent program with focus on both global and regional issues in parasitology. The meeting will provide high scientific value and excellent opportunity to share ideas and strengthen scientific communication and cooperation.

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Chair of Scientific and Local Organizing Committees
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PARASITE
**PROGRAM**

**APRIL 21, 2021**

10:10 - 10:50  
**Morning coffee with dear Editor Pikka Jokelainen and Jean-Lou Justine, Editor-in-Chief of Parasite**  
Chair: Vaidas Palinauskas

11:00 - 11:15  
**Opening session: Welcome**  
SBSP President, NRC Director, CSBSP9 Organizing Committee

11:15 - 13:00  
**SESSION 1**  
Chairs: Olena Kudlai and Kurt Buchmann  

**Keynote lecture:**  
11:15 - 11:45  
**David Thieltges** “The risky rise of aquaculture: collateral disease impacts on aquatic wildlife”

**Oral presentations:**  
11:45 - 12:00  
**Albert Ros** “How does climate change affect PKD in Central European salmonid populations”

12:00 - 12:15  
**Signe Martin** “An investigation of the disease status of velvet crab (*Necora puber*) in Galway Bay, with a focus on microparasites”

12:15 - 12:30  
**Quinton Marco Dos Santos** “Infection of wels catfish (*Silurus glanis*) by an alien copepod *Sinergasilus major* in Danube River basin in Hungary”

12:30 - 12:45  
**Maarten P.M. Vanhove** “Collection-based parasitology in invasion biology: showcasing the integration of biodiversity infrastructure and disease research”

12:45 - 13:00  
**Louise von Gersdorff Jørgensen** “Infections in the skin with the fish parasite *Ichthyophthirius multifiliis* and zebrafish immune responses”

13:00 - 13:30  
**Break**

13:30 - 15:00  
**SESSION 2**  
Chairs: Gunita Deksne and Olena Kudlai  

**Keynote lecture:**  
13:30 - 14:00  
**Kurt Buchmann** “Dynamic interactions between Baltic fish communities and their parasites”

**Oral presentations:**  
14:00 - 14:15  
**Kirill Galaktionov** “Capturing boundaries in a group of Parvatrema (Digenea: Gymnophallidae) cryptic species”

14:15 - 14:30  
**Inga Martinek** “Parasite fauna of river lamprey (*Lampetra fluviatilis*) and black goby (*Gobius niger*) from Western Finland”

14:30 - 14:45  
**Nikol Kmentová** “The pelagic realm in Lake Tanganyika: phenotypic variation and geographically unrestricted gene flow of fish parasites”

14:45 - 15:00  
**Anja Vermaak** “Super klipfish, the super host: astonishing digenean diversity of *Clinus superciliosus* from the coast of South Africa”

15:00 - 16:00  
**Break + Posters**
16:00 - 17:30  **SESSION 3**  
Chairs: Carolina Chagas and Andrew Williams  
**Keynote lecture:**  
16:00 - 16:30  **Susanne Nylen** "Immune-regulation in Human Visceral Leishmaniasis"  
**Oral presentations:**  
16:30 - 16:45  **Gupse Kubra Karademir** “Molecular prevalence and phylogenetic characterization of *Leishmania infantum* in dogs in the Middle Black Sea Region of Turkey”  
16:45 - 17:00  **Alberto Cornet-Gómez** “Dual role of *Trypanosoma cruzi* exovesicles in cell apoptosis: evaluation of the molecular mechanism involving the effect in cell viability”  
17:00 - 17:15  **Anna Ganyukova** “*Zelonia* sp. (Kinetoplastea, Trypanosomatidae) from the ichneumon wasp *Alexeter* sp.: the trypanosomatid from the Arctic”  
17:15 - 17:30  **Anna Seetsi** “*In vitro* evaluation of novel nitrofurantoin derivatives against animal African trypanosome parasites”  

17:30 - 18:00  **Break**  

18:00 - 19:45  **SESSION 4**  
Chairs: Mark van der Giezen and Vaidas Palinauskas  
**Keynote lecture:**  
18:00 - 18:30  **Elin Videvall** “Dual transcriptomics of avian malaria”  
**Oral presentations:**  
18:30 - 18:45  **Christen Rune Stensvold** “Detection and differentiation of intestinal parasitic protists of zoonotic relevance in pigs by amplicon-based next-generation sequencing and real-time PCR”  
18:45 - 19:00  **Kokcu Delibasi** “Complete mitochondrial genome sequencing and phylogeny of the Malaria vector *Anopheles sacharovi*”  
19:00 - 19:15  **Mateusz Pękacz** “Selection of new diagnostic markers for *Dirofilaria repens* infections with the use of phage display technology”  
19:15 - 19:30  **Sadullah Uslug** “Molecular characterization, expression and functional analyses of tick vaccine candidate antigen enolase of the ixodid tick *Hyalomma marginatum*”  
19:30 - 19:45  **Justė Aželytė** “A new protocol to distinguish morphologically identical avian malaria parasites in co-infection”  

19:45 - 21:00  **Social Program**  

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**APRIL 22, 2021**  
10:10 - 10:50  **Morning coffee with the project leader: what makes a successful research project?** With Mark van der Giezen and Mikas Ilgūnas  

11:00 - 13:00  **SESSION 5**  
Chairs: Asghar Muhammad and Mikas Ilgūnas  
**Keynote lecture:**  
11:00 - 11:30  **Laryssa Howe** “Avian Malaria in New Zealand: How isolated are we?”
Oral presentations:

11:30 - 11:45  **Seraina Meister** “Fatal avian malaria in captive Atlantic puffins (*Fratercula arctica*) in Switzerland”

11:45 - 12:00  **Melanie Ludivine Duc** “Formerly neglected avian haemoproteosis: megalomeronts of *Haemoproteus majoris* develop in different bird species over different seasons”

12:00 - 12:15  **Daniela de Angeli Dutra** “Migrant birds disperse haemosporidian parasites and affect their transmission in avian communities”

12:15 - 12:30  **Elena Platonova** “Experimental study on development and virulence of tropical avian malaria parasite *Plasmodium collidatum* (genetic lineage pFANTAIL01) in a local European bird species”

12:30 - 12:45  **Carolina Hernández Lara** “Massive damage of lungs during natural *Haemoproteus attenuatus* (Haemosporida, Haemoproteidae) infection in European robins”

12:45 - 13:00  **Maria Erokhina** “How much does avian malaria cost?”

13:00 - 13:30  Break

13:30 - 15:00  **SESSION 6**
Chairs: Saulius Petkevičius and Rasa Bernotienė

Keynote lecture:
13:30 - 14:00  **Smaragda Sotiraki** “Vector borne diseases: an expanding health threat for animals and humans”

Oral presentations:

14:00 - 14:15  **Rita Žiegytė** “*Culicoides* biting midges involved in transmission of haemoproteids”

14:15 - 14:30  **Indrė Lipatova** “Prevalence of pathogens in fleas (Siphonaptera) in Lithuania”

14:30 - 14:45  **Margarita Kazak** “Trypanosomatids in wild-caught biting midges”

14:45 - 15:00  **Eliza Kondzior** “Multispecies reservoir of *Spirometra erinaceieuropaei* (Cestoda: Diphyllobothridae) in carnivore communities in north-eastern Poland”

15:00 - 16:00  Break + Posters

16:00 - 17:30  **SESSION 7**
Chairs: Laima Baltrūnaitė and Andrea Miller

Keynote lecture:
16:00 - 16:30  **Urmas Saarma** “*Echinococcus* parasites: from correct identification to global phylogeography”

Oral presentations:

16:30 - 16:45  **Nadine Closset** “Run to the hills! Reindeer (*Rangifer tarandus tarandus*) are safer from brainworm (*Elaphostrongylus rangiferi*) in the mountains”

16:45 - 17:00  **Mattanja Stuut** “Size does matter: age and gender differences in the prevalence and intensity of brainworm (*Elaphostrongylus rangiferi*) infections in semi-domesticated reindeer”

17:00 - 17:15  **Anna Ciezarek** “Modelling reindeer (*Rangifer tarandus* ssp.) brainworm (*Elaphostrongylus rangiferi*) transmission”

17:15 - 17:30  **Walter U. Basso** “*Toxoplasma gondii* and *Neospora caninum* infections in small ruminants in Switzerland”

17:30 - 18:00  Break
SESSION 8
Chairs: Pikka Jokelainen and Carolina Chagas

Oral presentations:

18:00 - 18:15  Armando Jairo Cruz-Laufer  "Many strings attached: Network analyses of host-parasite interactions of cichlid fishes and their gill parasites (Platyhelminthes: Monogenea, Dactylogyridae)"

18:15 - 18:30  Evelina Juozaitytė-Ngugu  "Investigations of Sarcocystis species in muscles of Laridae and Corvidae birds"

18:30 - 18:45  Kevin M. Santana Hernández  "On the identity of Sarcocystis (Apicomplexa: Sarcocystidae) from invasive California kingsnakes (Lampropeltis californiae) in the Canary Islands: Another biological invasion or an unknown native parasite?"

18:45 - 19:00  Gamze Yetismis  "First report and molecular characterization of Dientamoeba fragilis in budgerigars in Turkey with zoonotic concern"

19:00 - 19:15  Algimantas Paulauskas  "Long-term investigations (1995–2020) on ticks and tick-borne pathogens in Lithuania"

19:15 - 19:30  Jana Radzijevskaja  "Molecular detection and genetic diversity of Babesia canis in ticks and pet dogs in Lithuania"

19:30 - 19:45  Vesta Jonikė  "Prevalence of tick-borne pathogens in migratory birds and their ticks in Lithuania"

APRIL 23, 2021

10:10 - 10:50  Morning coffee with the Post-Doc: Olena Kudlai and Andrew Williams

11:00 - 13:00  SESSION 9
Chairs: Gediminas Valkiūnas and Angelika Krūmiņa

Keynote lecture:
11:00 - 11:30  Alexander Ryss  "Review of plant nematology challenges in Nordic and Eastern Europe"

Oral presentations:

11:30 - 11:45  Darya Krupenko  "Similar but not the same: new species of Derogenes (Digenea), with evidence from molecules, morphology and life cycles"

11:45 - 12:00  Julia Dabrowska  "The presence of hepatic intramitochondrial paracrystalline inclusions in liver parenchyma in BALB/c mice infected with Trichinella spiralis"

12:00 - 12:15  Joanna Nowicka  "Seroprevalence of Toxoplasma gondii among sylvatic rodents in Poland"

12:15 - 12:30  Maria Teresa Galán-Puchades  "A One Health perspective on rat-borne parasitic zoonoses in urban settlements"

12:30 - 12:45  Marius V. Fuentes  "Ecological analysis of the helminth community of the wood mouse, Apodemus sylvaticus, from a Mediterranean ecosystem in post-fire regeneration"

12:45 - 13:00  Anneke L. Schoeman  "A forgotten introduction reveals a hidden one: co-phylogeography of the African clawed frog Xenopus laevis (Anura: Pipidae) and its parasitic flatworm Propoletysoma xenopodis (Monogenea: Polystomatidae) sheds new light on their shared introduction history"

13:00 - 13:30  Break

13:30 - 13:45  Golden Flea Award
13:45 - 14:00  Young Scientist Award and Closing
14:00 - 15:30  SBSP General Assembly
Dynamic interactions between Baltic fish communities and their parasites

Kurt Buchmann

Laboratory of Aquatic Pathobiology, Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Denmark

The Baltic Sea is a semi-enclosed marine area with a marked salinity gradient from the western Baltic to the east. The inflowing North Sea water through the Danish straits become diluted by the freshwater run-off from the countries surrounding the sea but the slow water exchange of the represents a critical factor for the organisms. In the deeper zones (basins of Bornholm, Gdansk and Gotland) a sharp vertical halocline is found but it is also here that oxygen deficits may prevail. The marine fish species inhabiting the Baltic comprise local and stationary sub-populations of salmonids, gadids, pleuronectids and clupeids. In addition, several freshwater species (vertebrates, including fish, and invertebrates) interact with the marine organisms in this highly changing environment. They have through millennia adjusted genetically to the extreme conditions of the Baltic Sea and are all playing important but special roles in the Baltic food web system. The special biology of the Baltic fish communities is clearly reflected by the characteristic parasitofauna of each species which also demonstrates how the different species interact. Special focus should be placed on the metazoan parasites (helminths and crustaceans) of the salmon, trout, cod, herring, sprat, turbot, flounder and plaice and their connection to invertebrate intermediate hosts and vertebrate final hosts.
Avian Malaria in New Zealand: How isolated are we?

Laryssa Howe

Department of Infectious Disease, School of Veterinary Science, Massey University, Palmerston North, New Zealand

New Zealand is a geologically young country, which split from the supercontinent approximately 83 million years ago. This geographically isolated country has only two native land mammals, two species of bat, and is devoid of snakes and many reptilian groups such as iguanids or crocodiles. These features provided a spectacular habitat for the 245 species of endemic and native birds. Due to the distance to other land masses and challenging ocean environments, other than sea or shore birds, New Zealand uniquely has very few migratory bird populations with some introduced species even abandoning migration upon arrival. However, research is revealing the cosmopolitan species of *Plasmodium* are present and established in the native bird population and frequently causing mortality events. As most of the endemic avifauna already on the threatened, endangered or extinct list after human colonization, these infections and mortality events have the potential to dramatically limit the success of protection strategies. This presentation will briefly review the research on *Plasmodium* spp., native and introduced, in New Zealand avifauna, with comments on possible vectors, fitness effects, and pathogenicity. The impact on and mitigation strategies for endangered avifauna management plans and what unique opportunities New Zealand’s isolation can provide for avian malaria research will be discussed.
Immune-regulation in Human Visceral Leishmaniasis

Susanne Nylén

Department of Microbiology, Tumor and Cell Biology at Karolinska Institutet, Sweden

The leishmaniases are a group of protozoan diseases, transmitted by sandfly vectors, that manifest in a spectrum of disease ranging from slow healing cutaneous lesions to life-threatening visceral leishmaniasis (VL). Visceral leishmaniasis also known as kala-azar, is caused by *Leishmania donovani* and *Leishmania infantum* (*Leishmania chagasi* in the Americas). These *Leishmania* species infect macrophages throughout the viscera, and parasites are typically found in the spleen, liver, and bone marrow. VL affect the poorest and most vulnerable groups in the society. Why some individuals develop disease while most do not is still unclear. Host immune responses are involved, and generation of protective T cell responses are necessary to control *Leishmania* infections. In particular, CD4+ T helper cells skewed towards the type 1 (Th1) phenotype are needed for control of *Leishmania* parasites and protection against disease. Patients with VL typically exhibit marked immunosuppression and their peripheral blood mononuclear cells (PBMC) fail to respond when stimulated with leishmanial antigens in vitro. Understanding immune failure and the underlying immune mechanism that leads to disease as well as control of infection are key questions for research in this field. We have for many years investigated how T cell responses are affected in *L. donovani* caused VL in Bihar, India. I will in this talk discuss how T cells can be driven towards responses that contribute to immune failure in human VL and how we by targeting immune modulatory pathways can improve immune response and promote parasite clearance.
A review of the research and methods in Nematology based on the analysis of 100 publications (2010-2021) from 18 countries: Scandinavia (3 countries), Baltics (3), Central region (CE: 5), Black Sea region (BS: 4), Caucasus (Cau: 2) and Russia (Ru) is presented. The most economically important plant nematodes from 10 genera listed by the EPPO were identified by a global survey. Species of the genera *Bursaphelenchus* (conifers), *Meloidogyne* (vegetable crops), *Globodera* (potatoes), *Heterodera* (cereals, sugar beet), *Ditylenchus* (potato, bulbs and others) are considered as important for many European countries. Virus vectors *Longidorus*, *Trichodorus* (berries) and *Xiphinema* (grapevine). Endoparasites *Pratylenchus* spp. and *Hirschmaniella*, (BS, Cau, Ru), leaf and bud nematodes *Aphelenchoides* (Romania) are economically important for Southern European countries. PCR diagnostics are presently widely introduced in many labs. The Czech Republic is the leader in the development of Multiplex Real-Time PCR and this technique is also applied in other countries. Pest Risk Modeling for forest pests is applied in Norway and Russia. Control management includes: crop rotation (many countries), automated environmentally safe fumigation with EDN, HCN, and biocontrol application (Czech Republic), selection for nematode resistance of various crops (Russia); biocontrol in glasshouses using nematophagous fungi and vermicompost (6 countries; CE, BS, Ru) and nematicidal plants (Czech Republic, Slovakia, Russia). The topics of the regional fundamental studies include: biodiversity and bioindicators analysis, integrated taxonomy (10 countries) including molecular phylogeny (Poland, Belarus, Bulgaria, Georgia and Russia); phylolgeography with analysis of pest origin centers (Bulgaria and Russia); interspecific hybridization (Poland); relationships of virus-vector nematodes and viruses (Poland, Czech Republic, Romania, Bulgaria, Russia) and HPR resistance genetics (Russia). Bulgaria, Romania, Czech Republic, Slovakia, and Russia lead in the number of publications by international teams, whereas the Czech Republic leads in the development of new applied technologies. Support: RFBR project 20-04-00569A; literature database: State Assignment AAAA-A19-119020690109-2.
Tapeworms of the genus *Echinococcus* are important parasites of mammals, causing life-threatening diseases called echinococcoses. Human echinococcoses are zoonotic diseases that in Europe are caused by two parasite taxa: *E. multilocularis* and *E. granulosus sensulato* (Eg-sl), causing alveolar and cystic echinococcosis, respectively. A stable taxonomy of Eg-sl is essential to the medical and veterinary communities for correct diagnostics and accurate communication on the role of different species. Eg-sl displays high genetic diversity and has been divided into different species and genotypes. Despite several decades of research, the taxonomy of Eg-sl has remained controversial. In my presentation I will provide a short overview of the taxonomy and diagnostics of Eg-sl.

To date, numerous studies have explored the genetic diversity and population structure of Eg-sl in various geographic regions. Although most of these have been conducted at local scales, a series of global studies have been published recently and several are ongoing. In the presentation, I will highlight the most important aspects emerging from global phylogeographical studies.
Vector borne diseases: an expanding health threat for animals and humans

Smaragda Sotiraki

Veterinary Research Institute, Hellenic Agricultural Organization ELGO-DIMITRA, 57001 Thermi, Thessaloniki Greece

Vector-borne diseases (VBDs) are caused by a range of pathogens transmitted to animals and humans by blood-feeding arthropods, e.g., ticks, fleas, mosquitoes and sand flies. On top of threatening human health, they have a major impact on the health and welfare of companion animals and livestock affecting their productivity, resulting in great economic losses. VBDs represent a growing global threat, due to their constant spread from traditional geographical and temporal restraints to new areas, exposing new populations to previously unknown infectious agents and posing unprecedented challenges to practitioners. The constantly changing epidemiology of VBDs is being influenced by different factors, such as the impact of climatic change on vector distribution and pathogens development rates, the habitat change introduced by humans, e.g., wetland creation, the increased movement of goods, humans, livestock and companion animals worldwide and, for many of those, an increasing insecticide resistance. So far is the spread of VBZ, endemic in southern Europe to northern non-endemic regions of Europe, is well documented. However, their relative significance cannot be quantified, since for most of them there are no surveillance programs or accurate assessment of prevalence in place, combined with gaps in reporting and accurate diagnosis. Combating VBDs is likely to face an increasingly serious combination of challenges in the coming years. It is therefore important to adapt a collaborative, multinational, multidisciplinary and holistic approach based on the One Health principal. During this presentation lessons learned in Southern Europe will be presented, giving examples on leishmaniosis, tick-borne infections, dirofilariosis and bluetongue.
The risky rise of aquaculture: collateral disease impacts on aquatic wildlife

David Thielges

NIOZ Royal Netherlands Institute for Sea Research, The Netherlands

Aquaculture is an increasing and promising source of fish and other aquatic organisms to ensure human food security but it comes at the price of diverse environmental impacts. Among others, these include diseases which often thrive under the conditions in aquaculture settings and can cause high economic losses. These diseases may also affect wildlife, however, the impacts of aquaculture on disease dynamics in wild species in surrounding ecosystems are poorly understood. In this presentation, I provide a conceptual framework for studying the effects of aquaculture on wildlife diseases, and illustrate the different mechanisms identified with several examples. In addition, I highlight further research needs and provide recommendations for management and policy.
Malaria parasites engage in an evolutionary battle with the vertebrate immune system. The way avian hosts control and suppress malaria infections on a molecular level, both during acute and subsequent phases of infection, remains poorly understood. We experimentally infected birds (Eurasian siskins) with avian malaria parasites (Plasmodium spp.), and used high-throughput dual RNA-sequencing to measure the avian transcriptome in blood collected during multiple time points of the infection. Furthermore, we assembled the transcriptome of the malaria parasite and evaluated the expression of parasite genes during the course of the infection. By analysing genome-wide expression in the blood using RNA-sequencing, we gained a much more complete view of the host's molecular response to malaria and also the counter strategies simultaneously employed by the parasite.
ORAL PRESENTATIONS
A new protocol to distinguish morphologically identical avian malaria parasites in co-infection

Justė Aželytė1, Elena Platonova1,2, Staffan Bensch3, Olof Hellgren3, Vaidas Palinauskas1

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2 Biological Station Rybachy of Zoological Institute RAS, Rybachy 238535, Russia
3 Department of Biology, Lund University, Lund, Sweden

Co-infections and interactions of parasites are complex, however, information received from studies on avian Plasmodium spp. is scarce and fragmented. During co-infections both, the data about parasites impact on host health and the influence on the development of each parasite are important. To determine the intensity of parasitemia of each parasite in the host, the microscopy of blood smears and actual counting of Plasmodium blood stages is a standard method. However, this task is impossible when co-infections of morphologically identical parasites are present. In such cases, sensitive molecular methods become essential for diagnosing co-infections.

In the present study, we designed a new real-time PCR (qPCR) protocol to accurately quantify the intensities of blood stages of two closely related genetic lineages of Plasmodium relictum (GRW4 and SGS1). We tested the lineage-specific primer pairs for both mitochondrial and nuclear loci and selected the ones with the highest specificity and efficiency. New qPCR assays were tested using blood samples from experimentally infected Eurasian siskins (Carduelis spinus) to investigate the development of P. relictum (GRW4) during single and co-infection with lineage SGS1.

The assay was sensitive to low intensities of parasitemia and could amplify the target lineage in co-infection. Our results showed that the lineage GRW4 in co-infection with SGS1 was capable of developing only transient infection and disappeared when the acute increase of SGS1 was determined.

Sensitive molecular methods based on qPCR and primers targeting both mitochondrial and nuclear loci are relevant for analysis of co-infections with morphologically identical Plasmodium lineages.

The study was supported by the Research Council of Lithuania (No. S-MIP-20-25) and Russian Science Foundation (grant No. 20-14-00049).
Toxoplasma gondii and Neospora caninum infections in small ruminants in Switzerland

Walter U. Basso1, Fabienne Holenweger1, 2, Gereon Schares3, Norbert Müller1, Lucia M. Campero1,4, 5, Caroline F. Frey1, Patrik Zanolari2.

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2 Clinic for Ruminants, Vetsuisse-Faculty, University of Bern, Switzerland
3 Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Epidemiology, Greifswald-Insel Riems, Germany
4 Immunoparasitology Laboratory, Faculty of Veterinary Sciences, National University of La Plata, Argentina
5 National Scientific and Technical Research Council (CONICET), Argentina

Toxoplasma gondii and Neospora caninum infections are important causes of abortion in ruminants. Besides, meat from T. gondii infected animals represents a major infection source for humans. The occurrence of these protozoan parasites in Switzerland was investigated both, in aborted sheep and goat foetuses by molecular methods, and in a nationwide cross-sectional serological survey. A total of 653 sheep from 143 farms and 748 goats from 164 farms were tested by commercial ELISAs and inconclusive results were defined by immunoblot. Besides, a risk factor analysis for infection was performed. T. gondii and N. caninum DNA were detected in 6.1% and 2.4% (n=82), and in 6.8% and 1.4% (n=73) of ovine and caprine foetuses, respectively. The observed seroprevalences for T. gondii in sheep and goats were 66.3% and 50.5% at the animal level, and 90.9% and 81.1% at the farm level, respectively. For N. caninum, the detected seroprevalences in sheep and goats were 0.8% and 0.9% at the animal level, and 2.8% and 1.8% at the farm level, respectively. Elder animals and sheep had a higher risk of being seropositive to T. gondii. Alpine grazing in summer was identified as a protective factor for seropositivity to T. gondii in both animal species.

These results suggest the involvement of these parasites in abortions and reveal a high prevalence of T. gondii and lower prevalence of N. caninum infections in small ruminants in Switzerland. They also suggest that consumption of undercooked meat from sheep and goats may represent a risk for public health.
Modelling reindeer (Rangifer tarandus ssp.) brainworm (Elaphostrongylus rangiferi) transmission

Anna Ciezarek1, Rebecca K. Davidson2, Torill Mørk2, Geir Rune Rauset3, Diana J. Williams1 and Hannah Rose Vineer1

1 Department of Infection Biology and Microbiomes, Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom,
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Elaphostrongylus rangiferi, commonly known as the brainworm, is a nematode which causes neurological disorders (elaphostrongylosis) in reindeer (Rangifer tarandus ssp.). Favourable climatic conditions have been inferred as the cause of sporadic outbreaks of elaphostrongylosis in Norway, supported by positive associations between observed outbreaks/intensity of infection and summer temperatures in the previous years. Climate warming which results in increased transmission of E. rangiferi therefore presents a risk to the health of semi-domesticated and wild reindeer in Fennoscandia, the health of co-grazing small ruminants, and the livelihoods of indigenous Sámi herders. As a first step toward developing climate change impact assessments for E. rangiferi, a degree-day model was developed for E. rangiferi larval development in a range of gastropod hosts. Predictions were validated by comparison against historic parasitological and outbreak records. An overall increase in thermal suitability for E. rangiferi was predicted over recent decades. To improve the potential of the model to be used as a risk assessment tool for reindeer herders in Norway on a shorter timescale, the broad spatial model was refined and restricted to match the distribution of the hosts. Predictions will be validated with data currently being collected from affected herds in Norway, to incorporate seasonal migrations and realistic parasite shedding patterns. Further work will introduce mortality of intermediate hosts to allow risk to reduce as well as increase during the year.
Run to the hills!

Reindeer (*Rangifer tarandus tarandus*) are safer from Brainworm (*Elaphostrongylus rangiferi*) in the mountains

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*Elaphostrongylus rangiferi* is a nematode parasite in reindeer (*Rangifer tarandus*) with terrestrial gastropods acting as intermediate hosts. A normal lifecycle takes about two years, with the development of the parasite in the gastropods being heavily temperature-dependent. Summers in Norway are becoming hotter which is turning this two-year lifecycle into a one-year cycle. Common symptoms in reindeer include ataxia, paresis of the hindlimbs and general weakness. These could affect the survival chances of the last wild tundra reindeer. There is little known about the infection prevalence of *E. rangiferi* in wild reindeer. Previous research has shown that the infection rate is lower at high altitudes, but whether this difference in infection rate is connected to gastropod densities is unknown.

We hypothesized that *E. rangiferi* prevalence can be explained by the gastropod density in their summer grazing area. This was tested by collecting feces from wild reindeer from different areas and analyzing them for *E. rangiferi* prevalence using the Baermann technique. We used qualitative visual searching plots to estimate the gastropod density in different habitats within the summer grazing area of the reindeer. Statistical predictions models were made to map the predicted gastropod density in these areas.

Preliminary results indicate that prevalence of *E. rangiferi* appear to be higher for reindeer that graze in places with a high gastropod density. This is mainly in forested areas at low altitudes. These findings, in combination with previous research, could potentially be used for management strategies to prevent future outbreaks in reindeer herds.
The presence of hepatic intramitochondrial paracrystalline inclusions in liver parenchyma in BALB/c mice infected with *Trichinella spiralis*

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*Trichinella spiralis* had tissue dwelling larvae stage, which migrated through the soft tissues such as liver parenchyma of many hosts. Larvae of these nematodes only passed through the liver on their way from intestine to the skeletal muscles, but they induced many alterations in the liver parenchyma. That's why we decided to describe the pattern of changes in the liver parenchyma at the electron microscopic level of BALB/c mice experimentally infected with *T. spiralis*. Each mouse was orally infected with 400 or 800 *T. spiralis* larvae and at 12 or 21 days post infection, the fragments of liver, were obtained and standard processed for electron microscopy. The predominant cells of the liver parenchyma were the hepatocytes, in which we observed a lot of large fat droplets and irregular disposal of glycogen, it can be assumed that damaged liver cells indicate a pathological process. We investigated also that mitochondria of hepatocytes were often swollen and they had the reduced cristae, dilatation of mitochondrial cristae and para crystalline inclusions, which are frequently elongated along their longitudinal axis in the mitochondrial matrix. These hepatic inclusions were described in patients with cirrhosis.
Formerly neglected avian haemoproteosis: megalomeronts of *Haemoproteus majoris* develop in different bird species over different seasons

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Haemosporidian parasites infect hosts from various classes: mammals, reptiles and birds. Bird haemosporidians, genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon*, are found on every continent except for Antarctica. In genus *Haemoproteus*, over 150 species have been described morphologically and more than 1500 genetic lineages were identified. *Haemoproteus majoris*, with its five recognized lineages, is one of the species for which the blood life stages are relatively well investigated. Recently, the discovery of megalomeronts (exo-erythrocytic meronts up to 360 μm in diameter in bird organs) in case-reports of three *H. majoris* lineages, particularly often present in kidneys, have deepened the knowledge about megalomeronts, which were reported in spring, during the bird breeding season and when relapses occur. However, it remains unclear if similar megalomeronts are present in other bird species infected with *H. majoris* and if they occur in autumn when haemosporidian transmission interrupts. To answer those questions, samples were collected in Lithuania in July and September. Birds with parasitaemia were euthanised, dissected and processed for histological examination and PCR-based testing. Microscopic examination reported megalomeronts in two *Parus major* and one *Parus montanus*. In all examined birds, the megalomeronts were similar in regard of morphology and location (kidneys) as the ones previously reported in birds sampled in the spring season. This study shows that the same parasite species develop megalomeronts of similar morphology and location in different bird species during the entire spring-autumn period. Due to the development of huge megalomeronts, *Haemoproteus* parasites could be pathogenic for different bird species over different seasons.
Migrant birds disperse haemosporidian parasites and affect their transmission in avian communities

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Migration has an important impact on the transmission of pathogens. Migratory birds disperse parasites through their routes and may consequently introduce them to new areas and hosts. Hence, haemosporidian parasites, which are among the most prevalent, diverse and important bird pathogens, are potentially dispersed when infecting migrant hosts. Here, we hypothesize and aim to evaluate if migratory birds spread parasite lineages along their routes, and localities crossed by more migratory birds have greater prevalence and richness of haemosporidians. For the first hypothesis, we tested whether parasite lineages found in migrants and residents and only in residents, differ in their frequencies of occurrence among localities. For the second hypothesis, we tested for a relationship among localities between the overall local haemosporidian parasite richness and prevalence, and the proportion of migratory bird individuals present in a locality. We combined a dataset on 13200 bird samples with additional data from the MalAvi database (~2800 sequences comprising 675 distinct lineages, from 506 host species and 156 localities) from South America, and used Bayesian multi-level and mixed models to test our hypotheses. We demonstrate that parasites shared between resident and migratory species are the most spatially widespread. The presence of migrants in a locality was negatively related to local parasite richness. We confirm that migrants contribute to parasite dispersal and visiting migrants are present in regions with lower Plasmodium prevalence. We observed their presence might raise Haemoproteus community prevalence. Therefore, we demonstrate migrants enhance pathogens spread and their presence may influence parasite community transmission.
How much does avian malaria cost?

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After invading a vertebrate host, parasites elicit a shift in metabolic priorities within the host, forcing it to reallocate its energy resources between an immune response and other life-history traits. Malarial parasites (Plasmodium, Haemosporida) proliferate in red blood cells and destroy them after releasing merozoites from infected cells. These processes may cause oppositely directed results: an increase of metabolic rate due to the immune response demand or its decrease because of the lower oxygen-binding capacity of blood. It is well known that long host-parasite coevolution may alter the extent of damage to the host’s organism. In our study, two groups of juvenile siskins (Spinus spinus) were infected: one with locally transmitted Plasmodium relictum (lineage SGS1) and second with an African P. ashfordi (GRW2). We used flow-through respirometry to measure resting metabolic rate (RMR) of infected siskins. We found a significant relationship between RMR and number of days post infection in both groups. After one-week post infection, RMR of all experimental birds decreased. However, by the next measurement there was a sharp increase in RMR followed by a further slight increase, which continued until the end of the experiment. Birds from the group infected with P. ashfordi had higher RMR compared with the birds from P. relictum group. Birds having initially high RMR tended to reduce it, while individuals with low initial RMR tended to increase it. It seems that malarial parasite, new to the host, causes mobilization of energy recourses in naive birds, which may lead to a rapid depletion and even death. The study has been funded by Russian Science Foundation (20-14-00049).
Ecological analysis of the helminth community of the wood mouse, *Apodemus sylvaticus*, from a Mediterranean ecosystem in post-fire regeneration

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The role of helminths of the wood mouse, *Apodemus sylvaticus*, as biological tags of the post-fire regeneration process in Serra Calderona Natural Park, a Mediterranean forest ecosystem, located between the provinces of València and Castelló (Valencian Country, Spain), has been analysed during almost twenty years. As a part of this multidisciplinary project, the helminthecological analysis of 917 *A. sylvaticus* (675 originating from the burned areas and 242 originating from the control areas) has been carried out between the 2nd and the 18th post-fire years. The helminths found were morphologically identified at species level, and their data, together with zoological and environmental data, were included in a database, used as the basis of the study. The influence of various intrinsic (host population density, sex and age) and extrinsic (site, period and year of capture, climatological variables) factors on the post-fire evolution of the helminth community of the wood mouse (composed of 17 species), the helminth prevalence, abundance, diversity, species richness and the biological cycle of the helminth species was analysed, using parametric and non-parametric tests. Taking into account the most important results obtained, various aspects of the helminth community dynamics of the wood mouse are confirmed as biological tags of the post-fire regeneration process in Mediterranean ecosystems, such as: the higher diversity, species richness and prevalences in the burned areas; and the higher influence of climate and the seasonality, also in the areas in regeneration, as a consequence of their higher vulnerability to periodical changes in the ecosystem.
Capturing boundaries in a group of *Parvatrema* (Digenea:Gymnophallidae) cryptic species

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Within the digenean genus *Parvatrema* (Gymnophallidae) there is a group of species that share a unique feature: parthenogenetic metacercariae in the molluscan second intermediate host (2IH). We found that such species are common at the sea shores in the northern Holartic, and they use several gastropod species as 2IH. Species of *Parvatrema* differ in their interactions with 2IH: from commensalism in the extrapallial cavity to parasitism in the hepatopancreas and the gonad. Distinguishing between the metacercariae of different species is complicated because morphologically they are almost identical. rDNA-based genetic analysis also did not provide understanding of relationships between the *Parvatrema* isolates from different 2IH. We sequenced and annotated the mitochondrial genome of *Parvatrema* sp. (*Cercaria quadriramis*) from *Littorina saxatilis*, and used it for primer design. We then sequenced the *cox1* gene fragment for all available *Parvatrema* isolates (23). The resulting alignment was analyzed with ABGD (https://bioinfo.mnhn.fr/abi/public/abgd/), and distribution of pairwise distances indicated the barcode gap presence and delimitation of five species: *Cercaria quadriramis*, *Parvatrema homoeotecnum forma A*, *Parvatrema margaritense*, *Cercaria falsicingula*, *Parvatrema* sp. 1. Phylogenetic analysis showed strict geographic attribution of these species either to the North Atlantic (NA), or to the North Pacific (NP). Presumably, the expansion of *Parvatrema* spp. proceeded from NP to NA, with two independent transfer events. Morphological similarity of the studied *Parvatrema* spp. suggests that they can be considered cryptic species that diverged as they discovered new 2IH species and new geographic region (NA). The research was funded by the RSF grant #18-14-00170.
Zelonia sp. (Kinetoplastea, Trypanosomatidae) from the ichneumon wasp Alexeter sp.: the trypanosomatid from the Arctic

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Infrafamily Leishmaniinae (Kinetoplastea, Trypanosomatidae) (Kostygov&Yurchenko, 2017) unites both dixenous Leishmania, which are causative agents of humans and animals diseases, and monoxenous trypanosomatids of insects (gen. Crithidia, Leptomonas, Lothmaria, Novimonas, Zelonia, Borovskyia). The last three genera species are the closest relatives of leishmanians. Unfortunately, the flagellates Novimonas and Borovskyia are known only from a single findings and have never been re-isolated. The first species of the genus Zelonia – Z. costaricensis – was described from the redbug Ricolla simillima in Costa Rica (Yurchenko et al., 2006). Later, Z. australiensis was described from the Australian black fly Simulium dycei (Barratt et al., 2017). The third zelonian species was isolated from the Madagascar ligeid bug Stalagmostethus furcatus (Votýpka et al., 2020). However, it seems premature to associate the origin and distribution of the genus with tropical and subtropical regions.

Zelonia sp. isolate 06 (Trypanosomatidae, Leishmaniinae) was isolated in 2016 in the vicinity of the Sob’ river, Polar Ural (Russia) (67°06’ N, 65°61’ E). Flagellates colonized the intestines of the female ichneumon wasp Alexeter sp. (Ctenopelmatinae, Ichneumonidae). Parasites were identified using a fragment of the 18S rRNA gene. Axenic culture S06 deposited in the bank of cell cultures of the Zoological Institute RAS. Culture cells are represented by two morphotypes: promastigotes and elongated opistomastigotes. The organism corresponds to the northernmost finding of Zelonia species, which were previously noted only in equatorial latitudes. Infection of the ichneumonon wasp is also unusual among trypanosomatids.

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Trypanosoma cruzi (T. cruzi) is a flagellate protozoan parasite, the etiologic agent of Chagas disease. T. cruzi is endemic in Latin America, but due to globalization is becoming a potential public health problem nowadays, especially in countries with strong human migration from endemic countries. Chagas disease presents an acute phase which does not cause serious medical complications usually, but in the chronic phase patients could suffer cardiomyopathy, gastrointestinal manifestations or both. It is not clearly known the origin of this pathologies: autoimmunity, toxins, the parasite´s persistence; our hypothesis suggests that exovesicles (EVs) secreted by the parasite could play an important role in the pathogenesis, this hypothesis is based on the presence of immune complexes (EVs-IgG) in patients sera. As all cells, trypomastigotes of T. cruzi shed different EVs to the extracellular medium. In this study, our aim was to evaluate if exovesicles of tissue-culture cell-derived trypomastigotes could have a role in inducing apoptosis of the host cells, and to try to identify the genes of the different pathways involved in programmed cell death. The mechanisms involved in apoptosis have been studied by flow cytometry of VERO and RAW cells after the incubation with EVs and immune complexes EVs-IgGs anti T. cruzi, analyzing annexin V as a marker of apoptosis. To determine the molecular mechanisms involved, we analyzed the expression of different genes and proteins using rtPCR and Western blot.
On the identity of *Sarcocystis* (Apicomplexa: Sarcocystidae) from invasive California kingsnakes (*Lampropeltis californiae*) in the Canary Islands: Another biological invasion or an unknown native parasite?

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Invasive species not only pose a threat to biodiversity due to predation or competition with native fauna, but also by the pathogens they may carry such as *Sarcocystis* spp. Some species from snakes are zoonotic, and could also mean a threat to local biodiversity by causing fatal infections. Canary Islands are an Atlantic hot-spot of biodiversity, threaten by the increasing numbers of invasive species, and above all, a North American colubrid, California kingsnake (*Lampropeltis californiae*) in Gran Canaria.

During 2019-2020, seventy-seven snakes were inspected for *Sarcocystis* spp. (stool and intestine samples). To verify the snake as definitive host, standard histology was performed. The ssRFLP gene fragment was amplified using SarcoFext/SarcoRext primers. For comparison, *Sarcocystis stehlini* was sequenced from a tail musculature of the Gran Canaria giant lizard (*Gallotia stehlini*).

Ten out of seventy-seven snakes presented oocysts in their faeces consistent with *Sarcocystis*. Developmental stages were observed in sections from small intestines of three snakes. Identical sequences of unknown *Sarcocystis* were obtained from five different snakes (two from fecal sediment and three from small intestine tissue).

Phylogenetic analysis showed that *Sarcocystis* sp. from the invasive California kingsnakes is not related to *Sarcocystis stehlini* from the endemic Gran Canaria giant lizard. The dixenous coccidia are only rarely reported to invade new predator-prey systems. However, it is clear that the species is well established and circulates among the snakes and unknown intermediate hosts with undetermined consequences in the fragile ecosystem of Gran Canaria.
Prevalence of tick-borne pathogens in migratory birds and their ticks in Lithuania

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Some studies suggest that the spread of tick-borne pathogens in new locations is related with ticks carried by birds. However, there are still lack of information about the bird's infection with tick-borne pathogens and the influence of birds as a carrier of ticks and their pathogens. For this reason, the study to evaluate the presence of tick on migratory birds and their pathogens diversity in Lithuania were conducted. Ventės ragas ornithological station was selected as an important study area for North-West bird's flyway route migration stop oversite. For tick species identification morphological and molecular methods were used. Collected ticks were screened for the presence of epidemiologically important pathogens such as Borrelia spp., Anaplasma phagocytophilum and Rickettsia spp. A total 5099 birds belonging to 42 bird species were caught and 1262 ticks from 22 bird species were collected. The highest infestation of ticks in Lithuania were found on blackbirds (Turdus merula), redwings (Turdus iliacus), song thrushes (Turdus philomelos), dunnocks (Prunella modularis) and European robins (Erithacus rubecula). The infestation rate of ticks was higher in short migrant birds ($\chi^2 = 19.537$, df=1, $p<0.001$), which feeds on ground ($\chi^2 = 711.58$, df=2, $p<0.001$) and migrate during the night ($\chi^2 = 510.7$, df=2, $p<0.001$). The dominant tick species on birds was Ixodes ricinus. Besides, exotic Hyalomma marginatum nymphs were identified. For the first time Rickettsia helvetica, Rickettsia monacensis, Rickettsia aeschlimanii and Borrelia valaisiana was detected in ticks from migratory birds in Lithuania.
Infections in the skin with the fish parasite *Ichthyophthirius multifiliis* and zebrafish immune responses

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The fish parasite *Ichthyophthirius multifiliis* is the causative agent of white spot disease and a major problem for the aquaculture and ornamental fish industry. It infects skin and gills of freshwater fish and causes outbreaks with high morbidity and mortality. The zebrafish has become an important model for fish diseases, especially with regard to understanding immune reactions during infections. Here, the zebrafish is used as a model to study immunological responses during an infection with *I. multifiliis*. Using a zebrafish reporter line, with neutrophils tagged with Green Fluorescent Protein (GFP), the behaviour of these cells during a parasite assault was examined using confocal microscopy. The zebrafish model offers an unprecedented real-time view of the interactions between the parasites and the neutrophils at the single cell level. The neutrophil population dynamics were also investigated and within the first day of the parasite infection, the number of neutrophils in the infected tail fin increased four-fold. Subsequently, during the following two days, the number of neutrophils declined, despite an increase in the size of the parasites and a resulting escalation of the damage to the skin of the fish. Video-recordings of the interface between the parasites and the neutrophils revealed how the parasites evaded and fought the immune system of the host. Using zebrafish as a tool to investigate cellular immunity has expanded our knowledge on this host/parasite relationship and with the many accessible reporter lines, potential for new discoveries lies ahead.
Molecular prevalence and phylogenetic characterization of *Leishmania infantum* in dogs in the Middle Black Sea Region of Turkey

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Dogs are the main reservoir for *L. infantum*, the most common etiological agent of visceral leishmaniosis (VL). In Turkey, leishmaniosis is an emerging disease, with a growing number of human and canine clinical cases especially in the South East and Mediterranean regions of Turkey. This study was carried out in the Middle Black Sea Region in Turkey to investigate VL in dogs and reveal the responsible *Leishmania* species with phylogenetic characterizations. Conjunctival swabs from a total of 81 dogs including clinically leishmaniasis suspected, serologically positive with commercial rapid test and randomly selected were subjected to gDNA isolations. qPCR targeted the kinetoplast DNAs of *Leishmania* sp. were utilized on gDNA isolates and 5 out of 81 dogs (6.2%) were found positive for *Leishmania* sp. Four of the qPCR screened dogs were also positive with commercial rapid test and exhibited clinical form of VL. Sequence analyses of the mitochondrial *cytb* and *hsp70* gene regions identified *L. infantum* in all samples and phylogenetic analyses revealed close relationship with the isolates reported from humans and dogs in different regions of Turkey as well as other countries such as China, Brazil, Israel, and Spain.

In conclusion, we provide first data on the occurrence and genetic characterization of *L. infantum* in dogs in the Black Sea Region in Turkey. Our results provide evidence on the potential threat of VL for animal and human health in the region. This study has been supported by TUBITAK with the project code 119O732.
Trypanosomatids in wild-caught biting midges

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Trypanosomatids are protozoan parasites possessing a dixenous or monoxenous life cycle. Monoxenous parasites are mostly restricted to invertebrate hosts. Dixenous parasites are pathogenic to humans and animals, they have an invertebrate vector that facilitates their transmission. *Trypanosoma* infections in birds are not well studied, but these parasites do affect the growth and fitness of highly infected individuals causing such symptoms like lethargy, weakness, inability to fly, weight loss and death. The biology and life cycles of most avian *Trypanosoma* species remain unknown. Vectors of avian trypanosomes are mostly bloodsucking dipterans, however, knowledge about particular species of insects capable transmitting different trypanosomes remains scarce. Vectors of only a few avian *Trypanosoma* species have been determined.

The purpose of this study was to investigate the prevalence of trypanosomatids in wild-caught biting midges (*Ceratopogonidae, Culicoides*). We collected parous biting midge females using UV light trap in Juodkrantė (Lithuania) in June 2020. The midges were identified to species level and PCR-based method was applied to estimate the natural trypanosomatid infections.

In total 420 parous wild caught biting midge females were collected and investigated. We have detected trypanosomatids in 16 (5.7%) *Culicoides* females belonging to 6 species. *Trypanosoma avium* was detected in *Culicoides segnis* and *Trypanosoma everetti* was detected in *Culicoides festivipennis*. Both *Trypanosoma* species are known to infect birds. *Trypanosoma* spp. were detected in *C. pictipennis*. Monoxenous parasites *Herpetomonas ztiplika* and *Sergei apodlipaevi* were detected in several *Culicoides* females.

This research was funded by a grant (No. S-MIP-20-25) from the Research Council of Lithuania.
Despite its generally lower species richness compared to littoral zones, the global importance of the pelagic realm lies in the primary production supporting fisheries worldwide. Helminths were proposed as an alternative marker for identifying migration patterns of pelagic fishes, potentially offering a higher resolution compared to the host genetics: parasites as a “magnifying glass”. Lake Tanganyika is one of the biggest lakes worldwide providing exceptional conditions to study general evolutionary mechanisms. To explore the parasites’ potential as tags for lake-wide population connectivity of pelagic fish assemblages, we examined the monogenean fauna of three economically important fish groups (clupeids, latids and bathybatine cichlids). Parasites captured from several localities, including all three subbasins of the lake, were characterized by means of their morphometric and shape variation in the sclerotized structures. Gene flow restrictions between respective populations were evaluated using a range of nuclear and mitochondrial markers. Overall, our results show a lake-wide distribution of all monogenean species infecting the studied pelagic fish taxa. Morphometric and shape variation data revealed environmentally dependent phenotypes and incipient speciation related to host species for *Kapentagyrus tanganicanus* infecting both clupeid species in the lake. The population connectivity of all the studied parasite species mirrors the lack of behavioural and physical restrictions of gene flow in the pelagic realm as suggested for most of their host species. Differences in the level of genetic diversity and in demographic histories between the parasites are suggested to be linked to different host population densities and age of diversification.
Complete mitochondrial genome sequencing and phylogeny of the Malaria vector Anopheles sacharovi

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Anopheles sacharovi is one of the most important vectors for Plasmodium vivax malaria in the world. There are limited studies on its genetic structure and phylogenetic relationships. Moreover, there is no available mitochondrial genome of An. sacharovi. In this study, we aimed to characterize mitogenome of An. sacharovi and reconstruct phylogenetic tree. We collected An. sacharovi samples in Kayseri province in Turkey. The samples were identified as morphological characteristics and distinguished by species specific PCR targeting ribosomal ITS2. We amplified and sequenced mitogenome of An. sacharovi using a long-range PCR and next generation sequencing. One mitochondrial genome was assembled and annotated using available mitogenomes of Anopheles species in GenBank. The mitogenome of An. sacharovi is 15,456bp in length consisting of 13 protein-coding genes (PCGs), 22 tRNAs, two rRNAs and a non-coding region. The gene organization is consistent with known Anopheles mitogenomes. All genes, except ND5, ND4, ND4L, ND1, two rRNA, and nine tRNA genes, were encoded on minority strand (N strand) similar to mitogenomes of other insects. The nucleotide composition was A + T biased at 77.1%; A was the most abundant nucleotide and G the least abundant. Phylogenetics of An. sacharovi was constructed by maximum likelihood and Bayesian inference for concatenated PCG sequences. In conclusion, mitogenome of An. sacharovi was firstly characterized with this study. Our findings provide a resource for further studies on the phylogenetics and molecular epidemiology of mosquitoes. This work has been supported by TUBITAK with the project code 119O968.
Multispecies reservoir of *Spirometra erinaceieuropaei* (Cestoda: Diphyllobothriidae) in carnivore communities in north-eastern Poland

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*Spirometra erinaceieuropaei* is a tapeworm with a complex life-cycle including definitive, intermediate and paratenic (transport) hosts. Spargana (*Spirometra* larvae) cause sparganosis, a severe disease mainly found in Asia. In Poland, *Spirometra* sp. was reported in Białowieża Primeval Forest (BPF) for the first time in the 1940s and was recently confirmed as *S. erinaceieuropaei* in several animals using molecular methods. In total, 583 carcasses of 9 carnivore species from north-eastern (NE) Poland were necropsied. The spargana were isolated from subcutaneous tissue, counted, and preserved for genetic analyses. The prevalence and intensity of infection was calculated and spatial variation in *S. erinaceieuropaei* infection probability in NE Poland was assessed. To confirm the species affiliation of isolated larvae, a fragment of the 18S rRNA gene was amplified. Spargana were found in 172 animals of 7 species (raccoon dog, European badger, pine marten, red fox, European polecat, American mink, and river otter) and confirmed genetically as *S. erinaceieuropaei*. The overall prevalence in hosts was 29.5% with a mean infection intensity of 14.1 ± 33.8 larvae per individual. European badgers and raccoon dogs were characterized by the highest prevalence. An analysis of parasite spread showed a spatially diversified probability of infection with the highest values occurring in the BPF.

Our study revealed that various mammal species can serve as *S. erinaceieuropaei* reservoirs. The frequency and level of infection may differ between selected hosts and likely depend on host diversity and habitat structure in a given area.
Similar but not the same: new species of *Derogenes* (Digenea), with evidence from molecules, morphology and life cycles

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*Derogenes varicus* (Digenea, Derogenidae) is one of the most widespread digenean parasites of marine teleosts. It has exceptionally low specificity towards the definitive host, and thus may actually be a complex of cryptic species. To shed light on species of *Derogenus* from the White Sea, we used molecular phylogeny together with morphological observations and life-cycle studies. In addition to *D. varicus* we found one previously undescribed species of *Derogenus* in White Sea fish. These two species consistently differ by 17 substitutions in a 1123bp fragment of the 28S rRNA gene. So far, the only clear morphological difference between the adult worms is in the egg size: 56–64 µm in *D. varicus* and 46–54 µm in the new species. The life cycles of two *Derogenes* species are also different. Rediae and cystophorous cercariae from moonsnail *Cryptonatica affinis* which had been previously regarded as the life cycle stages of *D. varicus* were shown to belong to the new species of *Derogenes*. True intra molluscan stages of *D. varicus* were found in another species of naticid gastropods — *Amauropsis islandica*. The cercariae of *D. varicus* are two-times bigger than those of the new species, and they have different shape of delivery tube (structure providing the infection of the second intermediate host). The reported study was funded by Russian Science Foundation, the project No. 19-74-10029.
Massive damage of lungs during natural *Haemoproteus attenuatus* (Haemosporida, Haemoproteidae) infection in European robins

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*Haemoproteus (Parahaemoproteus)* species are cosmopolitan bird parasites belonging to the Haemosporida (Apicomplexa). The majority of the described species are transmitted by *Culicoides* biting midges, which inject sporozoites in birds during blood meals. The sporozoites initiate tissue merogony, resulting in numerous merozoites, part of which penetrate red blood cells and produce blood stages (gametocytes), which are infective for vectors. The blood stages of *Haemoproteus* parasites have been relatively well-investigated, though tissue stages and patterns of their development remain unidentified in the majority of these parasite species. However, recent histopathology studies show that these parasites markedly affect bird organs during tissue merogony. This study aimed to contribute to the better understanding of exo-erythrocytic development of *Haemoproteus attenuatus* (lineage hROBIN1), the common parasite of flycatchers (Muscicapidae). Naturally infected European robins (*Erithacus rubecula*) were caught in Lithuania during autumnal migration in September 2020. Parasite species and lineage were identified using morphological features of gametocytes and DNA sequencing. Organs of six infected birds (parasitemia 0.7 to 19.6 %) were collected and processed for histological examination. Meronts were seen in the lungs, but not in other organs of five individual birds. The parasites were of various maturity, shape and size (5 to 20 µm length) and usually located in groups. Megalomeronts were not observed. Mature meronts contained numerous roundish merozoites of approximately 0.8 µm in diameter. Presence of meronts in the lungs seems to be an important feature during haemoproteosis caused by *H. attenuatus* in European robins. This study was partly funded by RCL (grant 09.3.3-LMT-K-712-19-0005).
Many strings attached: Network analyses of host-parasite interactions of cichlid fishes and their gill parasites (Platyhelminthes: Monogenea, Dactylogyridae)

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Gill parasites belonging to the genus *Cichlidogyrus* and *Scuto gyrus* (Platyhelminthes: Monogenea, Dactylogyridae), are one of the most species-rich parasite genera in the tropics with 141 species and 478 host-parasite interactions reported mainly from the Africa. Their hosts, mostly cichlid fishes, are one of the best-known model systems for speciation research. Therefore, research on their gill parasites has focused on evolutionary aspects including phylogenetics and co-speciation research. Ecological aspects of the cichlid-*Cichlidogyrus* species network have been studied considerably less because of a lack of available field data. In an attempt to address this data deficiency, we assembled a dataset of cichlid-*Cichlidogyrus* interactions including more than 11,000 infected host specimens based on an extensive literature survey of approximately 170 peer-reviewed publications. We applied an array of network analytical methods to characterise the network structure and host specificity as well as to infer predictors for host-parasite interactions. First, we analysed the structure of the species network involving phylogenetic, ecological, and morphological data of hosts and parasites. Second, we assessed the predictive values of these parameters using network link prediction algorithms recently proposed for incompletely sampled ecological networks. Third, we evaluated the effects of sampling biases on these analyses and propose steps to increase data availability and to study species interactions in host-parasite networks. This study aims to highlight the use and limitations of link prediction algorithms in parasitological research. The implementation of an integrative network analysis could be a roadmap for future analyses in host-parasite systems and improve the knowledge on host-parasite interactions.
Fleas (Insecta: Siphonaptera) are common ectoparasites of mammals and birds in different habitats throughout the world. Negative effects of flea (Siphonaptera) parasitism on the host may be expressed in different ways include host blood loss, skin damage, irritating bites, response to saliva injected into the wound, and transmission of pathogens. Flea-borne pathogens (Bartonella sp., Rickettsia sp., Yersinia sp.) are widely distributed over the world, and these diseases could acquire an epidemic form due to changes in vector-host ecology. Human susceptibility to zoonotic infection is not clear due to changes in climatic conditions, increased human migration and animal transportation. In this study, we investigated flea-borne pathogens in fleas from different hosts in Lithuania. A total 13 fleas species were identified: Ctenophthalmus agyrtes, Ctenophthalmus assimilis, Ctenophthalmus uncinatus, Ceratophyllus sciurorum, Ctenocephalides felis, Ctenocephalides canis, Chaetopsylla globiceps, Hystrichopsylla orientalis, Megabothris turbidus, Megabothris walker, Palaeopsylla soricis, Peromyscopsylla bidentate, Nosopsyllus fasciatus. We used real-time PCR, nested-PCR, multiplex PCR and vector-borne bacteria flow chip for different pathogens (Bartonella, Rickettsia, Anaplasma and Borrelia) detection. Pathogens detected in fleas are causing zoonoses (Bartonella grahamii, Bartonella rochalimae, Bartonella henselae, Bartonella claridgeiae, Bartonella washoensis, Rickettsia helvetica, Rickettsia felis, Rickettsia monacensis).
An investigation of the disease status of velvet crab (*Necora puber*) in Galway Bay, with a focus on microparasites

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Various microparasites have been identified in the tissues of velvet crab, with a particularly high incidence of a protozoan parasite *Paramarteilia* sp., but little is known about the prevalence, abundance and potential impacts of these parasites on the crab hosts. Velvet crab are an important commercial species, with landings in Ireland worth €419K in 2015, but recent declines have been reported leading to the finding of several microparasites infecting these crabs. Due to the negative effects that parasites can have on commercial crustacean species and their associated fisheries further research is required. We investigated the presence and prevalence of microparasites in velvet crab in Galway Bay every month for one year. As part of this ongoing work twelve months of data has been collected. Crab size, sex ratio, and the proportion of females carrying eggs varied throughout the year. Overall, more males were sampled than females. Two protozoan microparasites have been identified following histological preparations of velvet crab tissues, namely *Paramarteilia* sp., a paramyxid, and *Hematodinium* sp., a dinoflagellate. Of the crabs analysed so far, there was a very low instance of *Hematodinium* sp., and prevalence was approximately 50% for the parasite *Paramarteilia* sp. infection rates varied by month as well as between males and females. Further work will involve investigating the pathobiome of these crabs to describe the microbial community associated with *Paramarteilia* sp. infection. Findings from this study will contribute to our understanding of host-parasite interactions and to the effective management of velvet crab fisheries in Ireland.
Parasite fauna of river lamprey (*Lampetra fluviatilis*) and black goby (*Gobius niger*) from Western Finland

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In this study we report the parasite fauna of 20 specimens of river lamprey (*Lampetra fluviatilis*) and 38 black goby (*Gobius niger*) from the Archipelago Sea and three rivers in Western Finland. Six parasitic taxa were found in lampreys (five lineages of malacosporean myxozoans and the acanthocephalan *Corynosoma semerme*) and eight in black gobies (the trematode *Diplostomum* sp., the cestode *Proteocephalus* cf. *gobiorum*, the nematodes *Contracaecum* sp., *Cucullanus* sp. and *Pseudoterranova decipiens* and the acanthocephalans *Neoechinorhynchus rutili*, *Corynosoma magdalen* and *C. semerme*). Gravid individuals were found only for *P. cf. gobiorum*, while other taxa were collected as larval stage (*Diplostomum* sp., *Contracaecum* sp., *P. decipiens* and *Corynosoma* spp.) or immature (*N. rutili*) forms. Malacosporeans were detected using molecular methods.

Malacosporea is the earliest branching lineage of Myxozoa with only a handful of nominal and twelve known yet undescribed species. Our findings show that there are altogether five lineages of malacosporeans present in Finnish rivers, three of them belonging to nominal species. Furthermore, all have been found in an agnathan host, a lineage of fish never identified as host for Malacosporea before. The parasite community of black gobies from the Archipelago Sea exhibited a similar species composition and diversity as reported from other localities in the Baltic. However, the great majority of the parasites reported in black gobies in our survey correspond to non-specific larvae. Adults of all these helminth taxa are parasites of fish-eating birds and seals.
Fatal avian malaria in captive Atlantic puffins (*Fratercula arctica*) in Switzerland

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Avian malaria is a vector-borne disease caused by *Plasmodium* species, which may affect a broad spectrum of bird families worldwide. In most endemic and migratory birds, *Plasmodium* infections seem not to cause severe harm; however, non-indigenous species kept in human care (e.g. penguins) may experience high morbidity and mortality rates. The aim of this study was to analyze seven cases of sudden death in captive Atlantic puffins (*Fratercula arctica*) at Berne Animal Park in Switzerland between 2010 and 2020, and to determine the involvement of haemosporidian parasites in the fatal outcome. In all cases, lymphoplasmacytic inflammation, necrotic lesions in several organs and presence of protozoan stages within tissues/erythrocytes or accumulation of iron-based pigment were observed histologically. A one-step multiplex PCR designed to simultaneously detect and discriminate haemosporidia belonging to the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon*, and a nested PCR detecting *Plasmodium* and *Haemoproteus* infections were performed on DNA extracted from formalin-fixed and paraffin-embedded (FFPE) or fresh liver and spleen tissues.

*Plasmodium* spp. DNA was detected in the tissues from six of seven birds by PCR. Direct sequencing of the amplification products allowed the molecular identification of *Plasmodium relictum* SGS1 and *Plasmodium matutinum* LINN1 as the involved species. In one bird, no haemosporidian DNA could be amplified from FFPE tissues despite of suggestive histopathological findings. These results indicate that avian malaria represents an important cause of death in captive puffins and it should be considered as a differential diagnosis in unclear or fatal cases in this threatened bird species.
Investigations of *Sarcocystis* species in muscles of Laridae and Corvidae birds

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Parasitic protists of the genus *Sarcocystis* are cyst-forming coccidians with an obligatory two-host life cycle. Up till now, 27 *Sarcocystis* species using birds as intermediate hosts have been described. There is a lack of studies on the diversity of *Sarcocystis* in birds of the families Laridae and Corvidae. Till 2020, barely two species, *S. lari* and *S. wobeseri* were detected in the muscles of gulls. It was shown that corvids act as intermediate hosts of *S. corvusi* and *S. cornixi*. In the period of 2015-2020, muscles tissues of 272 birds (165 of Laridae and 107 of Corvidae) collected from Lithuania were examined for *Sarcocystis*. Morphologically sarcocysts were characterised in fresh squashed samples under a light microscope. *Sarcocystis* species were identified by 18S rRNA, 28S rRNA and ITS1 sequence analysis. Microscopic sarcocysts were detected in muscles of 30 birds of the family Laridae and in 41 birds of the family Corvidae. New species, *S. kutkienae* were described from the common raven (*Corvus corax*), simultaneously *S. cornixi* was identified from the new host, the jackdaw (*Corvus monedula*). Four species, *S. columbae*, *S. halieti*, *S. lari* and *S. wobeseri* were identified in the herring gull (*Larus argentatus*). *Sarcocystis halieti* was also confirmed in corvids. This species demonstrates low specificity to intermediate host and can infect birds of different orders (Accipitriformes, Charadriformes, Passeriformes, Strigiformes and Suliformes). This is the first comprehensive report of greater *Sarcocystis* species diversity in corvids and larids.

Keywords: *Sarcocystis*, Corvidae, Laridae, host specificity, molecular identification.
Seroprevalence of *Toxoplasma gondii* among sylvatic rodents in Poland

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There is currently considerable interest in understanding the transmission of pathogens and the range of different variables that influence infection dynamics. Wild rodents pose a particular threat to human communities because they constitute the most abundant and diversified group of all living mammals. *Toxoplasma gondii* is an intracellular Apicomplexan parasite with a broad range of intermediate hosts, including humans and rodents. Rodents are considered to be reservoirs of infection for their predators that include cats, pigs and dogs. We conducted a multi-site, long-term study on *T. gondii* in northeastern Poland. Our objectives were to monitor the seroprevalence of *T. gondii* in the four abundant vole species found in the region (*Myodes glareolus*, *Microtus arvalis*, *Microtus agrestis*, *Alexandromys oeconomicus*) and to assess variation in seroprevalence attributable to both intrinsic and extrinsic factors that were quantified. A bespoke enzyme-linked immunosorbent assay was used to detect antibodies against *T. gondii*. We detected *T. gondii* antibodies in the sera of all four rodent species with an overall seroprevalence of 5.5% (3.6% for *M. glareolus* and 20% for other vole species). Seroprevalence in bank voles varied significantly between host age and sex. These results contribute to our understanding of the distribution and abundance of *T. gondii* in voles in Poland and confirm that *T. gondii* circulates also in *M. glareolus* and *Microtus*/*Alexandromys* spp. Therefore, they may potentially play a role as reservoirs of this parasite in the sylvatic environment.

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Long-term investigations (1995–2020) on ticks and tick-borne pathogens in Lithuania

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Tick-borne diseases constitute a major health problem in many parts of the world. In the past three decades, many tick-borne pathogens have emerged, creating new challenges for public and animal health in Europe. The factors that drive the emergence of tick-borne diseases are difficult to identify due to the complexity of the pathogen-vector-host triad. Long-term studies are important because they may improve our understanding of the ecological factors that shape the dynamics of tick-borne pathogens. In Lithuania, the first studies on tick and tick-borne pathogens began in 1995. Analysis based on long-term datasets (1995–2020) of the incidence of vector-borne diseases in humans and animals in Lithuania demonstrated that exposure to ticks was an important factor influencing tick-borne diseases incidences in human and animals in Lithuania. The geographical and spatial distributions of some European ticks have been changing in the last few decades, and new viral, bacterial and protozoan tick-borne pathogens have been detected in former non-endemic areas. Climate changes over recent decades have led to a wider spatial distribution of ticks, and an extension in their periods of activity in Lithuania. Climatic changes, the significant increase of tourism and travel of dogs across Europe have caused an increase in the geographical range of canine babesiosis. Currently, the Baltic countries are an endemic area for a number of vector-borne diseases such as Lyme borreliosis, tick-borne encephalitis, anaplasmosis, babesiosis, bartonellosis, rickettsiosis. The advances in molecular biology during the last two decades and using of molecular diagnostic techniques have allowed researchers to better diagnose, trace and genetically characterize the ticks and causative agents of important endemic tick-borne diseases and have led to the discovery of new emerging vector-borne pathogenic organisms in Lithuania.
Selection of new diagnostic markers for *Dirofilaria repens* infections with the use of phage display technology

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*Dirofilaria repens* is a parasitic nematode causing a vector-borne zoonotic infection (dirofilariosis), considered an emerging problem in human and veterinary medicine. Currently, diagnosis is based on the detection of the adult parasite and microfilariae in the host tissues. However, the efficacy of tests relying on microfilariae detection is limited by microfilariae periodic occurrence. Therefore, a new reliable and affordable serological diagnostic method is needed.

Our research used Ph.D.-12™ Phage Display Peptide Library to select highly immunogenic 12-mer peptides reacting with IgG antibodies from dogs infected with *Dirofilaria repens*. First, to eliminate cross-reactivity, we performed a prescreening step using serum from *Dirofilaria*-negative dogs and dogs infected with *Toxascaris leonina* and *Uncinaria stenocephala*. For final library biopanning, we used antibodies pooled from a few dogs infected with *Dirofilaria repens*. Based on phage ELISA screening, we selected three potentially specific peptides and used them for further analyses with positive and negative sera. All blood samples collected from dogs were classified as positive or negative based on detection of microfilariae in the bloodstream (Knott's method) or by adult *Dirofilaria repens* somatic antigen (DrSA) ELISA test.

Our method could be considered as a new specific diagnostic tool for subcutaneous dirofilariosis. Interestingly, our approach enables us to detect infections in dogs with no clear microfilariae presence in the bloodstream or showing a weak signal in the DrSA ELISA test, which might indicate occult/prepatent infection and were confirmed as positive with a molecular test.

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Experimental study on development and virulence of tropical avian malaria parasite *Plasmodium collidatum* (genetic lineage pFANTAIL01) in a local European bird species

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Avian malaria is a mosquito-borne disease caused by protozoan parasite *Plasmodium* spp. The development of the parasite is often accompanied by various pathologies in the host's organism and could lead to the death of an infected bird. Ecological studies indicate that after their Spring migration, the long-distance migrant birds in Europe are infected with tropical *Plasmodium* lineages. Most of those lineages are not transmitted in Europe yet, but the current rapid climate change and anthropogenic activities might create suitable conditions for exotic parasites. In the light of this fact, it is crucial to understand the possible negative effects of tropical malaria parasites on the non-migrating European birds.

In the present study, we aimed to experimentally determine the development of a tropical *Plasmodium collidatum* parasite in common European bird species and mosquitoes. The parasite was isolated on the Curonian spit of the Baltic Sea from the naturally infected Common rosefinch (*Carpodacus erythrinus*) and was inoculated into the Eurasian siskins, (*Carduelis spinus*). Parasitemia and health parameters of experimental birds were measured. To study sporogonic development of the parasite, experimental mosquitoes *Culex pipiens* form molestus and *Culex quinquefasciatus* were exposed to the infected bird.

The results of the study reveal that *P. collidatum* completes its development in siskins but not in experimentally exposed mosquitoes. This parasite is highly virulent for Eurasian siskins which can be considered as a potential vertebrate host if the transmission of the infection starts occurring in Europe. Competent vectors are, apparently, Asian mosquito species in the regions of natural transmission.

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A One Health perspective on rat-borne parasitic zoonoses in urban settlements

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The One Health approach is a concept that implies that human and animal health are interdependent and linked to the ecosystems in which they coexist. Rats play an active role in the transmission of a large number of zoonoses. *Rattus norvegicus*, the sewer rat, with a worldwide distribution, is the most commensal species since its main habitat is always linked to humans. Data related to parasitic zoonoses found in 100 captured specimens of *R. norvegicus* from Barcelona (Spain), 85 in the sewers, and 15 in parks, are presented. After dissection, the helminths found were extracted and specifically determined. The intestinal content was collected to determine protozoans. A concentration technique (Parasep®) was used to carry out the study of the samples, whilst a molecular analysis by multiplex PCR (AllplexTM®) was performed. The spleens were analysed to detect and quantify *Leishmania infantum*. A highly sensitive quantitative PCR (qPCR) technique was performed. Histological sections of the spleens were stained with Giemsa as well as with immunohistochemical staining. Ten zoonotic parasite species were found: the protozoans *L. infantum*, *Blastocystis*, *Giardia intestinalis*, *Dientamoeba fragilis* and *Cryptosporidium* spp.; the cestodes *Hymenolepis nana*, *H. diminuta*; the nematodes *Calodium hepaticum* and *Gongylonema neoplasticum*; and the acanthocephalan *Moniliformis moniliformis*. The obtained results confirm the populations of *R. norvegicus* in cities to be a source of a large number of zoonotic protozoans and helminths, with the inherent public health repercussions that may derive from this. Rats in cities, therefore, may serve as sentinel species to detect human zoonotic parasites.

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Molecular detection and genetic diversity of *Babesia canis* in ticks and pet dogs in Lithuania

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*Babesia canis*, a haemoprotozoan parasite, is the main cause of a tick-borne infection of dogs-canine babesiosis. During the last decade, a spread of canine babesiosis due to *B.canis* to the previously non-endemic areas has been reported in Europe. Expansion of *B.canis* in Baltic countries is associated mainly with the expanding range of the main vector – *Dermacentor reticulatus* ticks and international travel. Genetic variability and antigenic variation are important mechanisms for the survival of *Babesia* parasites in their vertebrate hosts. Differences in the virulence of *B.canis* are associated with observed genetic heterogeneity among *B.canis* strains. We have investigated the prevalence of *B.canis* in *D.reticulatus* ticks along its current distribution range in Lithuania and the genetic diversity of *B.canis* strains isolated from ticks and naturally infected dogs. Partial regions of the 18S rRNA and Bc28.1 gene of *B.canis* were amplified through PCR assays, followed by PCR-RFLP and sequence analysis for molecular characterization of strains. *B.canis* was detected in 1.2% (26/2259) of *D. reticulatus* ticks with the prevalence varied in different locations from 0% to 11%. The molecular analysis indicates the presence of genetically heterogenic *B.canis* strains in Lithuania: three genotypes were detected based on 18S rRNR gene, while thirteen Bc28.1 gene sequence variants which divided into four genetically distinct groups were identified. Our findings on the genetic diversity of *B. canis* strains in Lithuania can be useful for the development of a polyvalent vaccine, assessing its potential for use and for predicting the risk of complicated diseases.
How does climate change affect PKD in Central European salmonid populations

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Proliferative kidney disease caused by the myxozoan parasite *Tetracapsuloides bryosalmonae* (PKD) is an emerging salmonid disease implicated in recent temperature dependent declines in salmonid populations. In the current study both empirical data and ecological niche modelling is used to extrapolate the impact of future climate changes on the spread of PKD. The model data integrate the strong overlap in the areas suitable for *T. bryosalmonae* and its primary bryozoan hosts, and the strong link with temperature. Projections based on moderate to high emission-case climate change scenarios predict a 50 to 82% decrease in the area suitable for salmonids within this century. The strongest declines in populations are expected to be those that are currently linked to PKD. Thus, with ongoing climate change, both current and future populations are at severe risk to *T. bryosalmonae* and its associated disease PKD. Conservation efforts of salmonid stocks are dependent on climate mitigation and measures to help salmonids adapt to the disease.
The wells catfish *Silurus glanis*, known also as sheatfish or European catfish, is a silurid native to central, southern, and eastern Europe. Its biology, distribution, and parasitofauna have been well studied and documented. Nevertheless, in 2016 a mass infection of an unknown copepod was recorded from a water reservoir in the Danube River basin in Hungary. The infection resulted in severe pathogenic effects, including distinct emaciation of infected fish, and was subsequently described. Initial identification was inconclusive, but additional samples collected in 2017 and 2018 allowed for more thorough identification using microscopy and genetic characterisation. The external morphology of the collected parasite specimens was studied using both light microscopy and scanning electron microscopy (SEM). Two fragments of rDNA, 18S and 28S, were also used to assess the genetic identity of the copepods. Observed morphology of the copepods was consistent with published descriptions of the ergasilid copepod *Sinergasilus major* of Asiatic origin. Also, the extracted rDNA fragments were consistent with published sequence data designated as *S. major*. This constitutes the first record of *S. major* from *S. glanis* and the first record of this parasite from the Danube River system. Ergasilids are not host specific and therefore have the potential to infect new hosts and invade areas outside of their native range. It should be emphasized that current literature suggests alien parasites may compromise the health status of native fishes, leading to severe pathogenic effects, mortality and associated economic losses, indicating a need for programs intended to monitor such invasions.
A forgotten introduction reveals a hidden one: co-phylogeography of the African clawed frog *Xenopus laevis* (Anura: Pipidae) and its parasitic flatworm *Protopolystoma xenopodis* (Monogenea: Polystomatidae) sheds new light on their shared introduction history

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The management of biological invaders relies upon methods to trace their origins and expansion, also in the case of domestic invasions involving phylogeographic lineages within species. In South Africa, behavioural evidence and historical export records of the frog *Xenopus laevis* point towards the possibility of introduction of the southwestern lineages of the frog in the native range of the northeastern lineage. In an integrative approach, this study demonstrates the use of the monogenean parasite *Protopolystoma xenopodis* to trace the heretofore undetected translocation of the southwestern lineages of *X. laevis* across southern Africa. Using global fit analyses of co-phylogeny, we uncover a signal of congruent phylogeographic structuring among *X. laevis* and *P. xenopodis* lineages. Translocation of lineages is largely masked in the frog's phylogeography. However, incongruent links between host and parasite phylogeography, identified by ParaFitLink tests, indicate switches from southwestern to northwestern host lineages after they were brought into contact due to human-mediated translocation to the native range of the northwestern host lineage. Thus, past translocation of host lineages is revealed by the invasion success of its co-introduced parasite lineage. Based upon the distribution of these invasive parasite lineages, we infer that the widespread translocation of hosts is mainly facilitated by the use of *X. laevis* as live bait by the local angling communities and not via official export routes. This study demonstrates the concept that parasite data can serve as an independent line of evidence in invasion biology, also on the intraspecific level, shedding light on previously undetected invasion dynamics.
In vitro evaluation of novel nitrofurantoin derivatives against animal African trypanosome parasites

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Animal African trypanosomosis (AAT) is a threat to livestock production and food security in sub-Saharan Africa. Animal trypanosomosis caused by Trypanosoma brucei brucei, T. congolense, T. vivax and surra causing T. evansi. These trypanosome parasites are mostly transmitted by tsetse flies and it remains a concern in animals especially the impact it has on livestock. Application of effective trypanocidal drugs has long been used to reduce the impact of AAT, however, the currently used trypanocidal agents are expensive. Therefore, there is a need to develop new affordable, effective and safe trypanocidal agents, this highlights a need for continued effort to develop new affordable, safe and effective trypanocidal drugs. Nitrofurantoin derivatives have been reported to exhibit antimicrobial activity possibly against certain protozoan parasites. This study assessed anti-trypanosomal activity of 20 nitrofurantoin derivatives against animal trypanosomes (T. b. brucei, T. congolense and T. evansi) and cytotoxicity using Madin-Darby bovine kidney (MDBK) cells. Out of 20 tested nitrofurantoin derivatives only 1 compound showed anti-trypanosomal activity against T. b. brucei, 17 compounds against T. congolense and none of the compounds showed anti-trypanosomal activity against T. evansi comparable to reference compounds diminazene aceturate and pentamidine with IC50 values between 0.23 ± 0.02 and 0.33 ± 0.05 µM, respectively. There were no cytotoxic effects on MDBK cells with selectivity indices >100µM. The potential nitrofurantoin derivatives tested in this study have shown potential as antitypanocidal candidate compounds and require further in vivo evaluation to determine efficacy against, T. congolense.
Detection and differentiation of intestinal parasitic protists of zoonotic relevance in pigs by amplicon-based next-generation sequencing and real-time PCR

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Several parasite species are shared between humans and pigs. We applied next-generation sequencing (NGS)-based metabarcoding supplemented with real-time PCR to faecal DNAs from pigs to detect and differentiate single-celled intestinal parasites of zoonotic relevance. Excluding 14 samples due to low number of sequence reads, 259 samples from 116 pigs, collected from four farm in Denmark, were included in the study. Enterocytozoon bieneusi, Balantioides coli, and Giardia duodenalis were observed in 34/37 (92%), 148/259 (57%), and 86/259 (33%) samples, respectively. Entamoeba polecki ST1, E. polecki ST3, and Entamoeba hartmanni were detected in 104/259 (40%), 161/259 (62%), and 8/259 (3%) samples, respectively. Metabarcoding and real-time PCR detected Cryptosporidium in 90/259 (35%) and 239/259 (92%) of the samples, respectively, with Cryptosporidium suis and Cryptosporidium scrofarum observed in nearly equal proportions. Blastocystis subtypes 1, 3, 5, and 15 were found in 72 (28%), 6 (2%), 176 (68%), and 36 (14%) of 259 samples, respectively. Iodamoeba was seen in 1/259 samples (<1%), while none of 37 tested samples were positive for Dientamoeba fragilis or Encephalitozoon intestinalis.

Metabarcoding exemplifies a one-fits-all approach to detecting intestinal single-celled parasites in faeces, and can be supplemented with real-time PCR for selected parasites. Combining metabarcoding with pathogen-specific assays, as done in this study, may prove useful for detecting emerging and previously underdetected pathogens.
Size does matter: age and gender differences in the prevalence and intensity of brainworm (*Elaphostrongylus rangiferi*) infections in semi-domesticated reindeer

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The increasing concern about brainworm (*Elaphostrongylus rangiferi*) in reindeer is driven by climate change. The development of the nematode in its intermediate host – gastropods – is strongly affected by temperature and accelerates with increasing temperatures. Warmer summers therefore result in an increased risk of large outbreaks. Whilst there have been a number of studies looking at wild tundra reindeer in southern Norway, there have been no systematic studies of brainworm infections and landscape use in semi-domesticated reindeer herds bordering these wild reindeer populations. More knowledge about brainworm and the involved environmental factors need to be integrated into a mathematical model to predict future outbreaks and allow herders to better manage infections. We hypothesized that brainworm infection prevalence and intensity is not only age- and sex-related, but will also be affected by landscape use of the reindeer. Data includes faecal samples of GPS-collared semi-domesticated reindeer in central Norway, from 2018-2021. Foeces were analysed using the Baermann method for the count of brainworm larvae. During autumn 2020, we conducted gastropod sampling by visually searching plots for gastropods inside the reindeer grazing areas. We integrated results of gastropod sampling and GPS data of collared reindeer into vegetation maps to analyse landscape use of the reindeer. Preliminary results of faecal analysis show patterns in which infections in all age-classes increase as the year progresses, which corresponds to literature describing the duration of the migratory life cycle of brainworm parasites. Further analysis will be conducted to correlate landscape use with prevalence and intensity of brainworm infections.
Molecular characterization, expression and functional analyses of tick vaccine candidate antigen enolase of the ixodid tick *Hyalomma marginatum*

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Enolase is known as one of the multifunctional proteins acting as a plasminogen receptor that contributes to fibrinolysis. This characteristic of the enolase has been emphasized for preventing the blood clot formation during blood feeding of ticks. Thus, enolase has been considered as a potential vaccine candidate antigen for tick control. Here, we aimed to characterize and recombinant expression of the enolase gene from *Hyalomma marginatum*, one of the most important and widespread tick species in Turkey. The open reading frame (ORF) of enolase in the cDNA library synthesized from total RNA extracts of nymph and adult stages of *H. marginatum* was amplified and sequenced after cloned to pJET 1.2 vector. The ORF of *H. marginatum* enolase was 1302 bp in length and encoded 433 amino acid residues. The *H. marginatum* enolase showed 85.5% amino acid similarity to that of the putative enolase of *Ixodes ricinus*, 90.1% to that of *Haemaphysalis flava* enolase and 81.8% to that of *Ornithodoros moubata* enolase. SDS-PAGE and Western Blot analysis showed the hexahistidine-tagged fusion recombinant enolase protein recognized a band of approximately 48 kDa. The recombinant *H. marginatum* enolase bound human plasminogen in a dose-dependent manner and increased plasminogen activation in the presence of host tissue plasminogen activator (t-PA). In conclusion, our findings contribute and highlight the importance of the enolase as one of the potential vaccine candidates that might be used in the livestock against tick infestations. This study was supported by Erciyes University Research Fund with the project code TSA-2019-9438.
Collection-based parasitology in invasion biology: showcasing the integration of biodiversity infrastructure and disease research

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Introduced alien species are a major cause of biodiversity decline. An underlying reason can be co-introduction and host-switching of parasites/pathogens. Nile tilapia and other tilapias are among the most popular aquaculture fishes globally; their range has tremendously increased in view of anthropogenic translocations, often to the detriment of local species and ecosystems. Potential co-introduction of infectious agents, however, is poorly monitored. To help fill this research gap, and to contribute to a better coverage of (Central) Africa in invasion biology and helminthology, we studied introduced tilapias in the Congo Basin, and the monogenean flatworms infecting their gills. We hypothesize that tilapia introductions were accompanied by co-introduced monogenean parasites, and that these subsequently altered the gill parasite community of native tilapia species. We inventoried the gill monogeneans of present-day native and introduced cichlid fishes throughout the Congo Basin. This flatworm fauna has hardly ever been studied before, rendering it difficult to recognize native and non-native parasite species. Therefore we established a baseline for the natural monogenean communities by scrutinizing historical (pre-introduction) collections of the relevant native tilapia species, throughout their distribution range, for their monogenean parasites. This allowed distinction of which co-introduced parasite species were new to the respective parts of the Congo Basin, some of which spilled over to native fishes. Likewise, some spill-back events were identified. This work contributed to media interest for using natural history collections in topical global change questions like biological invasions, and integrating biodiversity infrastructure into infectious disease research in a One Health context.
Super klipfish, the super host: astonishing digenean diversity of *Clinus superciliosus* from the coast of South Africa

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The coast of South Africa is known to be an area rich in biodiversity due to the unique conditions along the coastline – the meeting of the cold, nutrient-rich Benguela current from the west and the warm Agulhas current from the east. Previous studies have suggested that the diversity of marine fish parasites may be underestimated and understudied in this area. Thus, the aim of this study was to explore the diversity of digeneans from the Super klipfish, *Clinus superciliosus* L. 1758, along this unique coastline. These fish are common residents of rocky intertidal areas and are endemic to the coast of South Africa and neighbouring Namibia. Thus far, *C. superciliosus* has been reported as definitive host to two species of digeneans: *Coitocaecum capense* Bray, 1987 and *Helicometra fasciata* (Rudolphi, 1819). In total, 71 specimens of *C. superciliosus* were collected from Saldanha Bay, Cape Town harbour, Hermanus, Tsitsikamma National Park and Chintsa in South Africa. By using a combination of molecular (28S rDNA, ITS1-5.8S-ITS2 and *cox1* data) and morphological analyses, we were able to identify 14 digenean species belonging to seven families (Acanthocolpidae, Bucephalidae, Fellodistomidae, Hemiuridae, Opecoelidae, Strigeidae, Zoogonidae), from this fish host. Only one of these species, *Co. capense*, has previously been reported; thereby making this the first report for all other digeneans from this host. We also provide the first report of *C. superciliosus* as second intermediate host for four digenean species. This astonishing diversity highlights the potential for further investigative taxonomic studies from this biodiversity hotspot.
First report and molecular characterization of *Dientamoeba fragilis* in budgerigars in Turkey with zoonotic concern

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*Dientamoeba fragilis* is one of the most common parasites in the digestive system of humans and is widespread all over the world. Molecular epidemiological studies on *D. fragilis* are very limited and two different genotypes (1 and 2) have been identified to date. Genotype 1 was characterized from human as a common genotype and has also been reported from a restricted number of animal hosts including pig, dog and cat. In this study, the presence and prevalence of *D. fragilis* in budgerigars as pets living with humans in the Central Anatolia Region of Turkey were investigated. Faecal droplets from a total of 57 budgerigars were freshly collected. The gDNA extracted from faecal samples were analysed by TaqMan qPCR and nested PCR targeting the specific 5.8S and SSU rRNA gene regions, respectively. *D. fragilis* DNA was determined in 12 samples via both methods resulting in the prevalence of 21.1%. Among the 12 positive samples, cysts of *D. fragilis* were detected in five faecal samples by microscopic examination. Sequence analyses of SSU rRNA gene region revealed that 4 isolates belonged to the dominant zoonotic genotype 1 (GenBank accession: MW130447), and 8 isolates belonged to a new genotype (GenBank accession: MW130448). In conclusion, our results contribute to the molecular epidemiology of *D. fragilis*, whose transmission dynamics and zoonotic character have not yet been clarified. The host suitability of budgerigars for this parasite has been revealed for the first time in the literature. This study was supported by TUBITAK with the project code 119O005.
Culicoides biting midges involved in transmission of haemoproteids

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Culicoides biting midges (Ceratopogonidae) are known as vectors of avian Haemoproteus parasites, that cause diseases or even mortality in birds. The diversity of biting midges in Europe is great, but only four Culicoides species are known to be competent vectors of avian Haemoproteus parasites.

The aim of this study was to determine Culicoides species involved in the transmission of haemoproteids. Biting midges were collected using UV trap and sticky traps installed in bird nest boxes. Parous females were screened for the presence of haemoproteids using PCR and microscopy. We investigated more than 700 parous Culicoides females collected using UV trap, and 293 - from bird nest boxes. PCR screening showed that 6.3% of parous biting midges collected using UV trap and 4.4% of midges collected from nest boxes were infected with haemoproteids. Haemoproteus spp. DNA was detected in Culicoides kibunensis, C. pictipennis, C. punctatus, C. segnis, C. festivipennis, C. obsoletus and C. impunctatus females. Detection of haemosporidian parasite DNA allowed to indicate potential vectors of avian Haemoproteus parasites and required further confirmation of vector status. Sporozoites of Haemoproteus minutus were detected in the salivary glands of two C. kibunensis females and sporozoites of Haemoproteus spp. were detected in four C. pictipennis females using microscopy and PCR. Detection of both sporozoites and parasite DNA in the same insect allowed to add the information about new natural vectors of pathogens.

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First molecular detection of *Babesia divergens* in European bison in Europe

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Bovine babesiosis also known as redwater fever, one of the most significant tick-transmitted infectious diseases of cattle, is an emerging veterinary problem in the worldwide. *Babesia divergens* is recognised as the main causative agents of the bovine babesiosis in Europe. The clinical signs and outcome of bovine babesiosis depends upon the virulence of the infecting *B. divergens* strain (mild, severe, or fatal course) and host immunity. European bison (*Bison bonasus*) has been found to be exposed to pathogens endemic to domestic ruminants. European bison and domestic cattle are closely related, so bison may be susceptible to infectious diseases of domesticated livestock such as bovine babesiosis. The aim of this study was to investigate the presence of *Babesia* infection in European bison. A total of 37 European bison individuals (20 males and 17 females) were sampled during a period of 6 years (from 2014 to 2020) in Lithuania. The molecular characterization of the *Babesia* spp. was based on sequence analysis of the 18S ribosomal RNA gene. Sequence analysis of partial 18S rRNA gene indicated the presence of *B. divergens* and *Babesia capreoli* in European bison. This is the first molecular detection of *Babesia* spp. in European bison in Europe.

Keywords: *Bison bonasus, Babesia divergens, Babesia capreoli, Lithuania.*
Global distribution of the most common viruses of the honey bee

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Honey bees are important pollinators that plays a vital role in agriculture by pollinating on a wide variety of crops and flowers. Unfortunately, viruses are one of the major threats to the health and wellbeing of honeybees. Because of globalization in the past centuries viruses spread across the globe and increased honeybee colony losses. Honey bee colonies have been screened for the presence of bee viruses for several years in Lithuania by using uniplex PCR and RT-PCR. This study let us to see that viruses such as deformed wing virus (DWV), sacbrood virus (SBV), black queen cell virus (BQCV), acute bee paralysis virus (ABPV) and chronic bee paralysis virus (CBPV) detected in Lithuania over the past years are also the most widely distributed in European countries such as Austria, Germany, Belgium, Spain and others. Also, we were able to detect mixed virus infections (mono, dual, triple and tetra) in bee colonies during the investigation period. The aim of this study is to review and compare distribution of these most common (DWV, SBV, BQCV, ABPV and CBPV) honey bee viruses in Lithuania and other European countries as was noticed some significant differences of honey bee prevalence of viruses in past years.
Optimization of identification of *Sarcocystis* parasites from water samples

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Parasites of the genus *Sarcocystis* are protozoans infecting mammals, birds and reptiles, characterized by a mandatory life cycle of two hosts. *Sarcocystis* infection can cause illness for animals and humans. Until now, the prevalence of *Sarcocystis* parasites has been mainly studied by analyzing animal carcasses by morphological or molecular methods. To date, only one study on the diversity of *Sarcocystis* spp. in water has been conducted due to the ineffective methods for isolation of sporocysts and lack of molecular methods for parasites identification from environmental samples. The objective of the present work was to create and optimize molecular-based technique for identification of *Sarcocystis* spp. using farm animals as intermediate hosts in water samples.

During the study, water was collected in 200 ml, 1 L, and 3 L vessels. Studies have shown that the optimal volume of water sample is 1 L. At the beginning of the study, sucrose gradient method and the sediment collection from water were used for isolation of sporocysts. Parasites were more successfully identified in samples using the sucrose gradient method (8/30) than using sediment collection from water (5/45). Consequently, filtration of water samples was used to concentrate sporocysts. After selecting the appropriate PCR type and primers, *Sarcocystis* parasites were detected in the majority of water samples (89/114). This study provides the first insight into detection of *Sarcocystis* species in the water. The development and optimization of molecular-based method for the identification of *Sarcocystis* will allow the rapid and accurate detection of sporocysts in water samples.
Anthelmintic resistance and gastrointestinal nematodes control practices in sheep farms in Lithuania

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Anthelmintic resistance (AR) of gastrointestinal nematodes (GIN) in sheep is one of the main problem worldwide. In Lithuania this issue is under monitoring as well. Factors, such as the intensive and incorrect use of anthelmintics led to the development of AR. The aim of the study was to assess the GIN control practices used by Lithuanian sheep farmers and to evaluate the prevalence of AR to ivermectin (IVM), benzimidazoles (BZ), levamisole (LEV).

During 2019-2020 all members of the Lithuanian Sheep Breeders Association, representing in all territory of the country (193 members), were surveyed. The questionnaire was completed by 52 members (26.9% response rate). Based on results from the questionnaire sheep were treated on average 1.52 times per year. Only 5.8% of sheep breeders regularly monitor GIN infection by fecal egg count test. IVM was the most frequently used anthelmintic – 63.5%. Selected farms were invited for AR studies.

In vitro micro-agar larval development test was used to evaluate the prevalence of AR in 35 sheep farms. Results indicated a widespread resistance against IVM in 14 farms (40.0%) and BZ in 12 farms (34.3%). Resistance to LEV was not indicated. In 7 farms multidrug resistance was detected.

In conclusion, AR of GIN is common in Lithuania, especially resistance to IVM. IVM is also the most commonly used in Lithuania. This study provides information about prevalence of AR and GIN control practices which could help to develop sustainable GIN control practices in sheep farms in Lithuania.
African swine fever in Lithuania. Can insects play a role in the transmission?

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The current African Swine Fever (ASF) epidemic in Europe began in 2007 and spread from the Caucasus to the northern countries. First outbreak of ASF in Lithuania occurred in 2014 and since then it has spread among wild boar populations and domestic pigs. ASF outbreaks in pig farms begin in June and are detected until the end of October, suggesting that insects might be involved in the transmission of the virus.

Insects were collected in 2018-2019 from six farms with ASF outbreaks and from five farms without ASF outbreaks. Target insect species from families Muscidae, Calliphoridae, Tabanidae and Culicidae were analysed for the presence of ASF virus (ASFV) DNA. Data from meteorological stations located closest to the investigated farms were analysed.

Muscidae and Calliphoridae flies were detected to be PCR positive for the ASFV DNA inside (15.4% of all investigated insects) and outside (11.1%) the farm buildings during the ASF outbreaks. Culex mosquito, Lucilia fly, Musca domestica fly and Stomoxys calcitrans flies collected in farms without ASF outbreak were PCR positive for the ASFV DNA. All the tabanids screened during the investigation were PCR negative. Numbers of ASF outbreaks per week positively correlated with minimum temperature and with sums of mean temperatures for 14 and 7 days before ASF outbreaks but had no correlation with the seasonal activity of S. calcitrans.

Although there is no evidence that these insects can transmit virus, further investigation is required to find out virus viability and amount on insects.
Molecular and morphological characterization of the circulating microfilariae of filarioid nematodes in warbles

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Blood parasites have been the subject of much research, with numerous reports of microfilariae in the peripheral blood of birds belonging to many orders. However, there is still not much progress in studies of these parasite biology due to difficulties of their species identification. This study aimed to fill up this gap, with particular emphasis on morphological features and molecular identification of microfilariae, which are the most readily accessible stages of the filarioid nematodes (Filarioidea). Peripheral blood of birds was examined using buffy coat method for the presence of microfilariae. Blood films were stained with Giemsa and examined under microscope. Sequences of cytochrome c oxidase subunit I and nuclear 28S rDNA genes fragments were obtained and used for molecular characterization and Bayesian phylogenetic inferences. Six individuals of five warbler species were positive for microfilariae. This study shows that even though morphological identification of microfilariae is complicated due to the simple morphology and the similarity of different species, many microfilariae have readily distinguishable specific characters, which can be used during identification. The phylogenetic analysis indicates that each species of birds harboured different closely related species of filarioids. Available data suggest that species diversity of these avian parasites is markedly underestimated, and their species number might be bigger than number bird species. The linkage of the molecular and morphology data needs more attention in Filarioidea taxonomy, particularly because this approach provides new knowledge about species identity using only readily accessible blood stages avoiding host dissection and thus minimize harm to wildlife.
Patterns of *in vitro* oocinate development in *Haemoproteus* (Haemosporida, Haemoproteidae) species

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Species of *Haemoproteus* (Haemosporida, Haemoproteidae) are widespread blood parasites, which can cause disease in birds and kill their vectors. Knowledge about sporogonic development remains scarce for the majority of these parasite lineages. Preliminary observations indicate that phylogenetic trees based on partial cytochrome b sequences can be used to predict some features of oocinate development in these pathogens. The aim of this study was to test this hypothesis by investigation of development in vitro of *Haemoproteus palloris* WW1 and *Haemoproteus* sp. TUPHI1, which are closely related to a group of Haemoproteus species that possess rapidly developing (within 1-2 hour) tiny (< 10 µm) oocinates.

Wild birds naturally infected with parasite lineages WW1 and TUPHI1 were used as source of mature gametocytes for in vitro observation of gametes, zygotes and oocinates. The infected blood was collected, mixed with sodium citrate and exposed to air. Blood smears were prepared at intervals. Polymerase chain reaction and microscopy were used to identify parasite species. Bayesian phylogenetic tree was conducted to determine phylogenetic relationships between lineages.

Both parasite species developed in vitro. Gametes, zygotes and oocinates were seen and described. As predicted, *in vitro* oocinates developed rapidly (within 1-2 hours) and were tiny (< 10 µm).

This study supported the hypothesis that phylogenies based on partial cytochrome b gene sequences can be used for prediction of peculiarities of *Haemoproteus* parasite oocinate development. This finding is important for better understanding parasite biology by using simply phylogenetic information. This study was funded by RCL (09.3.3-LMT-K-712-19-0171).
Host feeding patterns of invasive *Aedes* mosquitoes in Europe

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*Aedes* invasive mosquitoes (AIMs) play a key role as vectors of several pathogens of ecological and public health relevance including autochthonous (e.g., heartworms, avian malaria) and imported ones (e.g., dengue virus). Four species are established in Europe, including *Aedes aegypti*, *Aedes albopictus*, *Aedes japonicus* and *Aedes koreicus*. In addition, *Aedes atropalpus* has been repeatedly recorded although it has not established populations yet. Here, we review the blood feeding patterns of these mosquito species in Europe, summarizing available information from their native and introduced distribution ranges. The feeding patterns of mosquitoes constitute a key parameter affecting the contact rates between infected and susceptible hosts, thus playing a central role in the epidemiology of mosquito-borne pathogens. Our results highlight that these mosquito species feed on the blood of different vertebrate groups from ectotherms to birds and mammals. However, humans represent the most important source of blood for theses mosquito species, accounting for 36% and 93% of hosts identified for *Ae. japonicus* and *Ae. aegypti*, respectively. In spite of that, important knowledge gaps have been identified for some particular species with studies restricted to a few particular areas in Europe. Given the high vector competence of these AIM species for the transmission of different emerging arboviruses such as dengue, Chikungunya, Zika or Yellow fever viruses and their high feeding rates on humans, these AIM species may have an important impact on the vectorial capacity for such pathogens on urban and periurban areas.
Blood meal source in mosquitoes (Diptera:Culicidae) from a Brazilian zoological park

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Zoological parks can host a huge diversity of animals from different regions in the world. Additionally, these zoos are located in areas with vegetation and water bodies. This creates the perfect conditions for the establishment of mosquitoes (Culicidae: Diptera) species, with available food, places to shelter, and breeding sites, allowing the transmission of vector-borne pathogens. Thus, identifying the source of blood meals of these mosquitoes is essential to understand which diseases might be transmitted and the risks that this would represent for animals and humans. The aim of this study was to investigate the source of blood meal in Culicidae collected at São Paulo Zoological Park Foundation, in Brazil. In March 2015, mosquitoes were collected by CDC light traps baited with CO2 and using Nasci aspirator. The traps were used from 6 pm to 7 am, one day per week, for four weeks, in eight different sites. Nasci aspirator was used in daytime collections, early in the morning, in four of these sites. Engorged females were selected, killed with chloroform steam, and morphologically identified to species. The source of blood meal was identified using PCR-based protocols. We were able to identify the blood meal in all 31 engorged females collected. Almost half of them had fed on mammals, the majority on humans, while the other half fed on birds (wild and captive). Nevertheless, our results confirm a potential risk for the transmission of vector-borne diseases in São Paulo Zoo and emphasize the need to closely monitor mosquito population.
The occurrence of *Trichinella* nematodes in wildlife in Poland – a never ending story?

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Free-living animals act as a host for many dangerous parasites eg. nematodes of genus *Trichinella*. Carnivores, as raccoon dogs or martens, are a reservoir for these nematodes, due to their lifestyle and wide spread throughout the country. The goals of the study were to assess the presence of *Trichinella* nematodes, and to determine the muscle distribution of this parasite in free-living animals from the Głęboki Bród Forest District (Poland). *Trichinella* larvae were obtained by digestion method from tongues, diaphragms, masseters, and limb muscles. DNA was extracted from single larvae, and multiplex PCR was performed to identify *Trichinella* larvae at species level. *Trichinella* larvae were detected in 140 out of 536 animals, with prevalence: 39.82% among raccoon dogs, 27.83% among foxes, 17.54% among martens, and 5.48% among badgers. All larvae were classified as *T. britovi*, with the exception of one fox infected by *T. spiralis*. The highest larval burdens were observed in the tongue and the lower forelimb among raccoon dogs, and in the tongue and the diaphragm among martens. Due to the small number of infected badgers predilection sites could not be described in that species. Animals from the Głęboki Bród Forest District are infected by *T. britovi* and *T. spiralis*, and they may be an important element in the sylvatic cycle of both nematode species in presented
Anthelmintic resistance of horse strongyle nematodes to ivermectin and pyrantel in Lithuania

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With intensive use of anthelmintic drugs in recent decades, anthelmintic resistance (AR) in horse nematodes is becoming a growing issue in many countries. However, there is little available information about the parasites, treatment practices or AR in the horse population in Lithuania. The aim of this study was to assess the current situation of AR on horse farms in Lithuania. The study was conducted in 25 stables on horses with a strongyle faecal egg count (FEC) of ≥200 eggs per gram. The efficacy of IVM was comparatively high, with 98.8% of 250 horses having a zero egg count 14 days after treatment. Two conditions were used to interpret the FECRT results for PYR: firstly, resistance was determined when FECR was <90% and the lower 95% confidence interval (LCL) was <80%, and secondly when in addition the upper confidence level (UCL) was <95%. Under the first condition, resistance against PYR was found in five stables (25% of all tested herds), while when considering the UCL as well, resistance was only detected in two stables (8%). The FEC showed a significant (P<0.01) difference between the treatment and control groups. Only cyathostomin larvae were detected in larval cultures derived from strongyle-positive faecal samples collected 14 days after treatment of a test group with PYR. This in vivo study showed that PYR resistance is prevalent on horse farms in Lithuania, while the efficacy of IVM still appears to be unaffected. However, further studies of ivermectin resistance are needed.
Diversity of fleas in mustelids from Romania

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The order Siphonaptera comprises 246 genera with over 2500 species, none of them being threatened or endangered. Fleas are highly specialized holometabolous insects which can infest a widerange of hosts and habitats. Several flea species are of medical and veterinary significance as they can act as vectors or intermediate hosts of various pathogenic agents, some of them being zoonotic. Carnivores represent an important group of hosts and they can be reservoirs for various flea-borne pathogens. In Romania, there is a wide diversity of carnivore fauna and their fleas have been poorly investigated. The aim of this study was to evaluate the presence and diversity of fleas among mustelids from Romania.

For five years, 146 carcasses of mustelids from 19 counties of Romania were examined for the presence of fleas. The bodies were combed with a fine-tooth fleas’ comb and the fur was systematically checked from detection of fleas. The identified specimens were collected in 2 ml 70% ethanol tubes and morphologically identified.

Overall, 70 (47.9 %) mustelids were found positive for infestation with fleas. Among these, fleas of genera *Pulex, Paraceras, Chaetopsylla,* and *Monopsyllus* were identified in badgers (*Meles meles*) and martens (*Martes foina, Martes martes*). Interestingly, there were no fleas found on polecats (*Mustela putorius*) or (*Lutra lutra*) or other small mustelids. However, this may be due to the low number of specimens examined.

Investigations on flea diversity among wildlife should be of interest and more studies are needed to describe the situation in Romania.

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When did *Dirofilaria repense* merge in domestic dogs and humans in the Baltic countries?

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Dirofilariosis, a vector-borne disease caused by nematode parasites of the genus *Dirofilaria*, is an emerging zoonosis in northern parts of Europe. It has been reported from the Baltic countries, but an overview on when it emerged, applying a One Health point of view, was lacking. We reviewed the available literature and reports of *Dirofilaria repens* infections in domestic dogs and humans in the three Baltic countries Estonia, Latvia and Lithuania. Based on the available literature and reports, the first finding in the Baltic countries was made in Latvia in 2008. This was followed by the first in Lithuania in 2010, and the first in Estonia in 2012. More findings were reported after the first reports, and by the end of 2019, autochthonous canine *D. repens* infections had been described from all three countries, and autochthonous human *D. repens* infections had been described from Latvia and Lithuania. Awareness about this zoonotic parasite should be increased not only among veterinarians and medical doctors, but also among the general public, including people living and visiting the region. Managing this zoonotic disease requires a One Health approach.

Investigating *Trichinella* spp. and *Alaria* spp. infections in wild boars in Greece by classical and novel methods – preliminary results

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Trichinellosis is endemic in Greece, reported in sporadic human cases, while the information regarding animal infections is scant. Conversely, no human alariosis case has ever been reported, albeit the parasite is prevalent in wild carnivores. Given the importance of both infections in human health, our aim was to investigate the occurrence of *Trichinella* spp. and *Alaria* spp. in Greece, in wild boars, considered the main source for human infection. A collaboration network with hunting associations was developed, and samples from 128 hunted wild boars were collected. Diaphragm tissue (DT), mixed tissues (under the ear, MT) and blood samples were collected. For *Trichinella* spp. detection, DT were examined by compressorium, artificial digestion (AD), and a real-time PCR, detecting all known species in wildlife. For *Alaria* spp. detection, fresh MT were examined by *Alaria* Migration Technique (AMT) and AD. A sensitive and specific real-time PCR was developed for *Alaria* spp., targeting a large subunit ribosomal RNA gene (lsrDNA) region using a LNA modified TaqMan probe. All samples were negative for both parasites in AD and AMT. Real-time PCR was negative for *Trichinella* spp. in 65 DT and 55 MT samples examined so far and the novel real-time PCR was also negative for 37 MT samples tested to date. Serological examination of the blood samples (ELISA) for IgG detection for both parasites and the molecular examinations for the rest of the collected samples are pending. A side result of the compressorium method was the detection of *Sarcocystis* spp. in 31% of the animals.
Molecular detection of *Rickettsia* spp. in ticks collected from domestic cats in Lithuania

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Climate change affects the transmission dynamics and geographic spread of vector-borne diseases and creates new risks for human and animal exposure to these diseases. *Rickettsia* spp. are gram-negative zoonotic bacteria, which are spread worldwide. *Rickettsia* spp. can be the cause of various fevers and typhus. Ticks, especially Ixodidae, are important in the maintenance and transmission of the rickettsiae. Domestic cats may be infected with vector-borne pathogens and hosted infected ectoparasites such as ticks, fleas or lice. Pet owners who have a close contact with their pets are also at risk. The epidemiology of feline vector-borne diseases is generally less investigated in cats than in dogs, and vector-borne pathogens in cats and their ectoparasites remain poorly defined. The aim of this study was to investigate the prevalence of *Rickettsia* spp. in ticks collected from domestic cats in Lithuania. A total of 167 *Ixodes ricinus* and *Dermacentor reticulatus* ticks collected from cats were tested for the presence of *Rickettsia* spp. by using nested PCR targeting gltA gene. *Rickettsia* spp. DNA was detected in 52 out of 167 ticks (31.1%). Both species of ticks harbored *Rickettsia*: 27.5% of *I. ricinus* and 3.6% of *D. reticulatus*. Our results showed a high prevalence of *Rickettsia* spp. in ticks infesting cats and possible risk of rickettsial infection for these pets.
Trematode diversity reflecting the community structure of Danish freshwater systems: molecular clues

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“Background: Digenean trematodes are parasitic platyhelminths that use several hosts in their life cycles and are thereby embedded in various ecosystems affected by local environmental conditions. Their presence in a habitat will reflect the presence of different host species and, as such, they can serve as ecological indicators. Only limited information on the occurrence of trematodes and their link to other trophic levels in the Danish freshwater ecosystems is currently available. Therefore, the main aim of the present study was to increase our knowledge in this field. Methods: Snails were sampled from 21 freshwater lakes in Denmark, following which shedding procedures were performed, cercariae were recovered and the released parasites were identified using molecular tools (PCR and sequencing). Results: A total of 5657 snail hosts belonging to ten species were identified, revealing a highly diverse parasite fauna comprising 22 trematode species. The overall trematode prevalence was 12.6%, but large variations occurred between host species. The snail host Lymnaea stagnalis showed the highest prevalence and also exhibited the highest diversity, accounting for 47.6% of the species richness. Conclusions: This survey contributes updated information on parasite–host relations and compatibility and may assist in describing the ecological structure of the investigated Danish freshwater ecosystems.”

This work has been published on Parasites & Vectors (https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-020-04536-x)
Cystic echinococcosis (CE) and alveolar echinococcosis (AE) are emerging zoonotic diseases caused by the larval stage of the *Echinococcus granulosus* complex and *E. multilocularis*, respectively. Humans can become accidental intermediate hosts while ingesting eggs. Larvae in man develop mainly in the liver (70%), lungs (20%), and other organs. The aim was to characterize species of *Echinococcus* spp. using molecular methods. Fragments of larvae were collected from 74 patients postoperatively. The mitochondrial region of NADH dehydrogenase 1 gene was amplified by PCR and sequenced. 59 samples were diagnosed positive by amplification of nad1 fragment (~500 bp). The sequences of 41 isolates showed identity to the pig strain G7, designated *E. canadensis*, 3 isolates were identical to the sheep strain *E. granulosus* G1 and 1 isolate to the cattle strain *E. ortleppi* G5. 14 isolates showed identity to *E. multilocularis*. 15 cysts were of non-parasitic origin. Molecular methods are a reliable tool for detecting the parasite and distinguishing between species and strains. The identification of the *E. canadensis* G7 strain in most cases confirms its role as the main aetiological agent of human cystic echinococcosis in Poland.
Trematodes in snails in sub-Arctic fresh water lakes in Iceland

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In recent years, a substantial diversity of trematodes completing their life-cycles in fresh waters in the sub-Arctic has been discovered. Iceland, which is an oceanic island with high densities of nesting bird populations of worldwide importance, is a sentinel environment for monitoring the impact of global change. Via an integrative taxonomic approach, we studied the diversity and distribution of trematodes in snails in the sub-Arctic freshwater ecosystems in SW and N Iceland. Novel sequences (for cox1, nad, 1 ITS and, 28S rRNA gene) were generated for cercarial isolates collected from a total of 2,260 *Radix balthica*, 699 *Physella acuta*, and 418 *Gyraulus cf. parvus* in 2018–2019. A total of 22 species/species-level lineages belonging to six major trematode families were revealed: Diplostomidae, Echinostomatidae, Notocotylidae, Plagiorchiidae, Schistosomatidae, Strigeidae. It is remarkable, that only three fresh water snail species are being used as first intermediate hosts by such a wide spectrum of trematodes. None of the species/lineages found in Iceland are endemic, all of them were found elsewhere, i.e. in Norway, Ireland, Central Europe or North America. We infer that the distribution patterns for the trematodes are constrained by snail host ranges and by migratory birds nesting or staging in Iceland or further in Greenland or Canada, and overwintering on the British Isles and Norway. Still, more research is needed to find out if the species’ geographical ranges of some of the trematode groups are restricted to high latitude ecosystems and if some of them might have circumpolar distribution.
Light-microscopic observations and multigene phylogenetic analysis of a new metchnikovellid microsporidium

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Metchnikovellids are a group of hyperparasitic microsporidia that have deviant invasion apparatus and possess two types of sporogony (free and sac-bound). They represent a sister lineage to the “core” microsporidia. There are about 30 known species that are classified by the morphology of the spore sacs into three-four genera. The genus Metchnikovella is the most numerous and diverse. In 2014 Larsson proposed to divide it into two genera: Metchnikovella having oval spore sacs with two polar plugs and Caulleryetta with oval and bottle-shaped spore sacs with one polar plug. Recent works on the molecular phylogeny of metchnikovellids supported the presumed heterogeneity of the genus Metchnikovella. Here, we present the results of light-microscopic observations and phylogenetic studies of a new Caulleryetta-like metchnikovellid from the archigregarine Selenidium pygospionis, a parasite of the polychaete Pygospio elegans. Oval spore sacs measured 3.29–5 x 5.45–9.15 µm had one polar plug. Each spore sac contained about ten spores. Sac-bound spores measured 0.85–1.56 x 1.29–2.42 µm, while free spores were slightly bigger (1.2–1.71 x 1.1–3.1 µm). Both free spores and sac-bound spores resided in parasitophorous vacuoles. We applied single-cell genomics methods with whole-genome amplification to perform next-generation sequencing of the new isolate. The phylogenetic analysis based on the SSU rRNA gene and multigene phylogeny based on the single-copy structural domains confirmed the phylogenetic heterogeneity of the genus Metchnikovella. This claims that the genus is an artificial assemblage. Supported by RSF No 19-74-20136.
Toxoplasma gondii seroprevalence among domestic cats in Ukraine

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Toxoplasma gondii is a cosmopolitan unicellular parasite of numerous warm-blooded species, including domestic cats and humans. Cats are important in the life cycle and epidemiology of T. gondii, as they are able to shed the oocysts of the parasite into environment, if infected. The main objective of this work was to estimate the seroprevalence of T. gondii among domestic cats in Ukraine. Blood samples of 367 domestic cats (age range from 2 months to 22 years; age was unknown for 93 cats) from Kyiv, Odessa, Poltava and Kryvyi Rih cities of Ukraine, were collected during 2020. The samples were investigated using a commercial enzyme-linked immunosorbent assay in accordance with the manufacturer's instructions. Of the samples, 145 were positive, yielding an apparent seroprevalence of 39.5%. The seroprevalence was 12.5% among young cats aged <12 months, and 39.8% in cats aged ≥12 months. Since cats are important for the epidemiology of T. gondii, the results are of considerable interest.
First molecular epidemiological study of *Kudoa thyrsites* (Cnidaria, Kudoidae) in Atlantic Mackerel, *Scomber scombrus* L., from the NE Atlantic Ocean

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*Kudoa* spp. are myxosporean fish parasites of concern to the fishery and aquaculture industries as they are associated with *postmortem* myoliquefaction of the fish muscle, commonly referred to as ‘soft flesh’. *Kudoa thyrsites* represents a serious economic risk for the Atlantic salmon aquaculture industry in Pacific North America, as well as for the NE Atlantic mackerel fisheries. Long-term monitoring (2005-2019) of *postmortem* soft flesh in Atlantic mackerel from the North Sea revealed 0.8-1.0% annual occurrence. However, to assess the “true” *K. thyrsites* prevalence, a molecular epidemiological investigation of 600 mackerel caught in the northern North Sea during 2020, was conducted. Parasite prevalence and ‘soft flesh’ occurrence was determined by *K. thyrsites* specific qPCR targeting the 18S rRNA gene, and by texture testing, respectively. ‘Soft flesh’ occurred in 22 of 600 mackerels (4%), while qPCR analysis revealed *K. thyrsites* presence in 140 of 600 fish (P = 23%). The findings suggest that only a small percentage of the infected fish develop ‘soft flesh’, and that the “true” parasite prevalence can only be assessed by highly sensitive molecular methods.

To the best of our knowledge, this study represents the first molecular epidemiological investigation of *K. thyrsites* in NE Atlantic mackerel. The apparent increase of *K. thyrsites* induced ‘soft flesh’ in Atlantic mackerel recorded here, along with the ongoing climate change and extended northerly distribution range of mackerel, highlights a risk of spread of the parasite further into the NE Atlantic Ocean, that may have severe consequences for the Norwegian pelagic fishery industry.
First data of helminthofauna of the larvae of tailed amphibians
*Lissotriton vulgaris* and *Triturus cristatus* in Latvia

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The tailed amphibians *Lissotriton vulgaris* and *Triturus cristatus* are reproducing in water bodies, often sympatrically. The growth and development of the larvae also is in water. After metamorphosis, both species lead a terrestrial life, feeding mainly on small insects, molluscs and worms, and are involved in numerous food chains.

The helminthological examination of larvae of *L. vulgaris* (n=250) and *T. cristatus* (n=18) was carried out in 2018-2019. The larvae were collected in Natura 2000 territories in Latvia from June till September.

Totally eight parasite species was found. Six species of trematodes were detected in *L. vulgaris* host: *Diplodiscus subclaviatus*, *Echinoparyhium recurvatum* mtc., *Opisthioglyphe ranae* ad., mtc, *Paralepoderma cloacicola* mtc., *Pleurogenoides medians* and *Strigea sphaerula*; and two species of nematodes: *Hedruris androphora* and *Oswaldocruzia filiformis*. In *T. cristatus* were detected two Trematoda species – *E. recurvatum* mtc. and *O. ranae* ad, and nematode *H. androphora*. The highest prevalence of *O. ranae* infection was 21,2% and the intensity of infection varied from 1 to 48 metaceraria. *O. ranae* adults were detected in the host intestines (E=4%), with maximal intensity 32 parasites.

The collection and euthanasia of the animals were performed in accordance with the Latvian legislation according to the permissions No 14/2018-E and No 21/2019-E issued by the responsible authority the Nature Conservation Agency of Latvia and according to EU requirements (Directive 2010/63/EU) and under supervising of C category specialist accredited by Federation of Laboratory Animal Science Associations (FELASA). We thank for cooperation the project "Pond aquaculture production and ecosystem service innovative research with modelling of the climate impact to tackle horizontal challenges and improve aquaculture sustainability governance in Latvia" (Izp-2020/2-0070) financed by Fundamental and Applied Research Projects (FLPP).
Parasitic infections have a negative impact on the fecundity and survival of wild ruminants, particularly moose, which despite being more susceptible to parasitic diseases than other wild cervids, remain poorly examined in this regard. Therefore, the aim of the present study was to determine the species composition in the gastrointestinal tract and liver of the moose population in central Europe, assess the factors contributing to any extremely high infection intensities and examine their impact on moose health. We examined parasite fauna of the abomasum, small intestine, caecum and liver, collected from overall 46 moose in Poland and performed histopathological examinations of organs. Additionally, 289 moose fecal samples were analyzed for the presence of eggs, oocysts and larvae of parasites. In total, 24 species and groups of parasites were found. The most prevalent were Mazamastrongylus dagestanica and Ostertagia antipini, which are typical nematodes of moose, together with Spiculopteragia boehmi and O. leptospicularis, characteristic also of other cervids. Parasite species diversity and abomasal parasitic infection intensity were higher in adult moose than in yearlings and calves. Histopathological lesions depended on the intensity of parasitic infections, and were most severe in the livers of moose infected with Parafasciolopsis fasciolaemorpha. Our findings indicate an accumulation of parasite infections over time in moose, which may be related to high environmental parasite pressure, possibly connected with high moose density and the presence of wetlands; they also serve as the most comprehensive study of moose parasites in central Europe to date.
Development of two avian *Trypanosoma* species in experimentally infected bloodsucking insects

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*Trypanosoma* spp. (Kinetoplastea) are flagellated blood parasites, which often cause trypanosomiasis in humans and domestic animals. Trypanosomes of wildlife vertebrates, particularly birds, are insufficiently studied, but some species might be pathogenic and affect the fitness of parasitized individuals. Avian trypanosomes are cosmopolitans and often prevalent in many bird populations, but their vectors remain insufficiently investigated. This study aimed to test experimentally whether *Culicoides* (Ceratopogonidae) biting midges and mosquitoes (Culicidae) are susceptible to infection of two widespread avian *Trypanosoma* species.

Wild passeriform birds of five species naturally infected with *Trypanosoma everetti*, *T. culicavium* and the mixed *T. everetti/T. culicavium* infection were caught in the wild. Laboratory reared *Culicoides nubeculosus*, *Culex pipiens* and *Aedes aegypti* as well as wild-caught *Culicoides impunctatus* and *Ochlerotatus* sp. were exposed by allowing them to take blood meals from infected birds. The experimentally infected insects were maintained in the laboratory and dissected at intervals to follow the development of the parasites. Infections were determined using microscopic examination and PCR-based testing.

*Trypanosoma everetti* developed and produced metacyclic trypomastigotes only in *C. nubeculosus* and *C. impunctatus* biting midges. *Trypanosoma culicavium* developed only in *Culex, Aedes* and *Ochlerotatus* mosquitoes. In case of mixed infections, *T. culicavium* was found to develop only in mosquitoes and *T. everetti* - only in biting midges. *Culicoides* biting midges and mosquitoes likely naturally transmit avian trypanosomes, but different parasite species use different vectors, which should be targeted in further epidemiology research of avian trypanosomiasis. Grant S–MIP–17–27 is acknowledged.
First report of *Haemoproteus majoris* megalomeronts in naturally infected wild birds

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Parasites of the genus *Haemoproteus* (Haemosporida, Haemoproteidae) are cosmopolitan in birds. Molecular studies indicated an enormous diversity of these pathogens. Despite the huge variety, life cycles remain largely unknown for the majority *Haemoproteus* species. Exoerythrocytic stage of the cycle is particularly fragmentally researched. The aim of this study was to gain knowledge on the exoerythrocytic development of the widespread blood parasite *Haemoproteus majoris*.

Single *Turdus pilaris* and *Parus major* individuals, infected with lineages hPHYBOR04 and hPARUS1 of *H. majoris*, respectively, were caught during fieldwork. The parasites were identified using microscopic examination on site and PCR-based testing in the laboratory. Both individuals were euthanized and processed for histological analysis. Exoerythrocytic stages were reported, and laser microdissection was applied for genetic confirmation of their identity.

Similar megalomeronts were reported in the organs of both avian hosts. These were big roundish bodies surrounded by a thick capsule-like wall and containing irregularly shaped cytomeres, where numerous merozoites developed. DNA sequences of laser-dissected megalomeronts confirmed the identification of *H. majoris*.

Phylogenetic analysis identified a group of closely related *H. majoris* lineages, two of which are characterized by identical blood stages, similar sporogonic and exoerythrocytic developments. It is probable that other lineages belonging to the same group would bear similar characters and phylogenies based on partial cytb gene could be used to predict life cycle features in avian haemoproteids. Present study reports morphologically unique megalomeronts in naturally infected birds and calls for research on exoerythrocytic development of haemoproteids to better understand pathologies caused in avian hosts.
TOXOSOURCES-project looks into the relative contributions of different sources of *Toxoplasma gondii* infection across Europe

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*Toxoplasma gondii* is a highly prioritized zoonotic foodborne pathogen in Europe and globally: the disease burden of toxoplasmosis is substantial. The infection can be acquired by ingesting oocysts (food, water, or the environment contaminated with faeces of infected, shedding felids; environmental pathway) or tissue cysts (tissues of infected animals; meatborne pathway). The relative contributions of the transmission pathways remain unknown, and the lack of information on the attribution to specific sources, partly due to lack of appropriate methods, has hampered the development of effective intervention strategies. To establish One Health interventions, transdisciplinarity, integrative research and capacity building are needed, but this has been challenging because research groups working on the parasite are scattered across sectors. In January 2020, we started TOXOSOURCES, a 2.5-year Joint Research Project within the One Health EJP. The consortium has more than 20 partner institutes across Europe, with complementary expertise from different fields. The research question - What are the relative contributions of the different sources of *Toxoplasma gondii* infection? - is being addressed using multidisciplinary approaches and novel and improved methods. The outcomes of the work include quantitative estimates of the contribution of the main sources and transmission routes and new data regarding role of fresh produce as a potential source. Moreover, approaches for detecting infections caused by oocysts using serology and for tracing the infection sources in outbreaks are explored. TOXOSOURCES has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830.
Blastocystis isolated from wild mammals inhabiting Białowieża Primeval Forest

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Blastocystis is a common unicellular protozoan inhabiting intestinal tracts of humans and animals. To the best of our knowledge, there is lack of data on the occurrence of Blastocystis in wild animals living in Poland, and the data from Europe is sparse. The aim of the study was to investigate the occurrence and genetic diversity of Blastocystis in selected species of mammals inhabiting Białowieża Primeval Forest (BPF). The material for this study consisted of 113 fecal samples obtained between 2018 and 2020 from seven species of wild mammals occurring within the Polish part of BPF and adjacent area.

Total prevalence of Blastocystis infection was 8.9%. The protozoan was detected in fecal samples from: wolf (2), wild boar (1), red deer (2) and European bison (5).

Our preliminary studies have shown that Blastocystis is present in at least 4 species of wild mammals in the area; the analysis of the genetic diversity of the pathogen is in progress. The limited number of both tested mammal species and collected fecal samples did not allow for obtaining comprehensive data on the Blastocystis infection prevalence in wildlife of BPF.
**Bartonella** infections in laelapid mites from small rodents in Lithuania

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The genus **Bartonella** contains facultative Gram-negative intracellular bacteria from the family Bartonellaceae which can cause bacteremia in humans and in a wide variety of animals. Hematophagous arthropods such as ticks and fleas parasitizing small rodents are considered potential vectors for various **Bartonella** species. However, the role of other ectoparasites - laelapid mites (Mesostigmata: Laelapidae) in transmission of **Bartonella** among rodent hosts needs to be confirmed. The aim of this study was to investigate the prevalence and diversity of **Bartonella** infections in laelapid mites parasitizing small rodents in Lithuania. A total of 670 small rodents of seven species (*Apodemus flavicollis*, *A. agrarius*, *Myodes glareolus*, *Micromys minutus*, *Microtus oeconomus*, *M. arvalis* and *M. agrestis*) were trapped during 2015-2016. Laelapidmites (n= 271) representing five different species (*Laelaps agilis*, *Haemogamasus nidi*, *Eulaelaps stabularis*, *Myonyssus gigas* and *Hyperlaelaps microti*) were collected from rodents. Bacteria DNA in mites was detected using a real-time PCR targeting a 124 bp fragment of ssrA gene. All **Bartonella**-positive samples were analysed further by nested PCR and sequence analysis of 16S-23S rRNA ITS region. The overall prevalence of **Bartonella** spp. in mites was 14.4% (39/271). **Bartonella** infection was detected in three mite species *L. agilis*, *H. nidi* and *M. gigas*. Sequence analysis of 16S-23S rRNA ITS region revealed the presence of *B. taylorii* in *L. agilis*, *H. nidi* and *M. gigas* mites, while **Bartonella grahamii** – only in *L. agilis*. Our results suggest a possible role for laelapid mites in the transmission of **Bartonella** spp. among rodents.

Keywords: **Bartonella**, Laelapidae, mites, rodents, Lithuania.
Trichinella spp. in lynxes (Lynx lynx) in Estonia

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Trichinella spp. are endemic in Estonian wildlife, including lynx (Lynx lynx), which is hunted as a game animal. Here we summarize the findings from primary Trichinella testing (i.e., not confirmatory analyses) of lynx samples at the Estonian Veterinary and Food Laboratory during 2007–2020. Ninety samples were collected in 2007–2014 (Kärssin et al., 2021), and five samples during 2015–2020. One lynx had been found dead, while 94 had been hunted. The animals originated from 13 of the 15 counties. All samples were tested using the artificial digestion method, and the observed larvae were counted and stored in ethanol. Trichinella larvae were found from samples of 63 (66.3%) of the lynxes, originating from 12 of the counties. The mean larval burden was 4.80 larvae per gram of muscle tissue, the range was 0.02–28.00. The Trichinella species were successfully identified using molecular methods in 52 cases out of the 63 for which it was attempted. Most infections were mono-infections: Trichinella britovi (55.8%), Trichinella nativa (7.7%) or Trichinella spiralis (5.8%). Both T. britovi and T. nativa were found in 28.8%, and T. britovi and T. spiralis in 2.0% of the lynxes. The results add to the knowledge of epidemiology of Trichinella spp. in Estonia and remind of importance of zoonotic risks related to uncommon food sources, such as lynx meat.

Parasitic infections, especially gastrointestinal nematodoses, are widespread and one of the main problems in sheep farms. For this reason, sheep are dewormed more often than other animal species, often several times a year. Sheep owners talk about anthelmintic resistance. In some cases this situation could be true, because some groups of antiparasitic drugs are used for very long time. The aim of this study was to find out which parasitic infections are diagnosed more often and the effectiveness of the anthelmintics. The study includes 25 sheep farms from different regions of Latvia. A total of 1154 sheep faeces were examined by flotation and Baermann method to determine parasito fauna. Of these, 578 sheep were tested for the efficacy of anthelmintics using a Faecal egg count reduction test. The study showed that sheep were most commonly infected with Eimeria spp. 97% (invasion intensity 767), Trichostrongylidae 91% (664) and Strongyloides spp. 80% (27). Less frequently Moniezia spp. 76% (61), Trichuris spp. 0.5% (83), Capillaria spp. 0.1% (50) and Protostrongylus spp. 0.1% were diagnosed(2). The most commonly used anthelmintics were ivermectin, albendazole, levamisole, monepantel, closantel, and the combination of ivermectin + albendazole. Work on medicaments resistance is still ongoing, but preliminary results have shown that around 50% is observed in some sheep farms following the use of ivermectin and albendazole.
Host and habitat preferences of chigger mites (Trombiculidae) on small mammals in Lithuania

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Trombiculidae is a cosmopolitan and diverse family of small mites. The larval stage of these mites, also known as chiggers, is the only parasitic life stage of Trombiculidae mites. Chiggers are found on a wide range of hosts including small mammals. Some chigger mites' species are vectors of *Orientia tsutsugamushi*, causative agent of Scrub typhus, the others can cause human trombiculosis. The study of ectoparasitic chigger mites found on small mammals was carried out in 2017–2020 in different habitats in Lithuania. In total, 1265 small mammals belonging to twelve species were captured. We collected 10614 chigger mites from infested hosts. Prevalence and intensity of infestation were calculated. Chigger mites showed preferences to some host species and habitats.

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Most common antiparasitic drug usage mistakes in sheep farms in Latvia

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Antiparasitic drug resistance is a recognized as a global threat and correct drug usage is one of the factors influencing the resistance. The aim of this study was to determine the most common inappropriate drug usage factors. A interview was conducted with the farm owner to gather information on farm's demographics, health, productivity and used drugs, to discover the most common drug usage mistakes.

In total, 20 farms breeding different sheep breeds (80% Latvian dark-head, 30% Texel or Merino, 15% Oxford, Romanov, less than 10% Suffolk, II-de-France or Estonian dark-head) were included in the study. Research showed that in 75% sheep are not scaled, weight was calculated by owners’ visual measures experience (possibility to scale animals was in 50% of farms (45% electronic and 5% mechanic scales)). Anthelmintic treatment was chosen without any faecal examination (65%) and in 70% decision was made by veterinarian. Drug vial in 25% were kept open till the next treatment time but in 35% farmers use open vial drugs repeatedly till finished. Subcutaneous injection in 30% are made by veterinarian (owner in 55%, 10% by the animal keeper, 19,4% couldn't specify) in those 57%, are using an automatically injection machines, but calibration is made only in 10% of farms. 50% of farms ruinously use singe use syringes, but two owners admitted that needle is not changed after every animal. Many inappropriate drug usage factors were found in farms, additional educational work on the appropriate drug usage should be done for the farmers and also veterinarians.
Molecular identification of *Sarcocystis* spp. from digested muscles of European mouflon (*Ovis aries musimon*)

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Members of the genus *Sarcocystis* are protozoan parasites of mammals, birds and reptiles. They are characterised by two-host prey-predator life cycle. Farm animals as intermediate hosts may suffer from clinical sarcocystosis, resulting in economic losses. Results of previous morphological studies suggest that mouflons may have sarcocysts similar to those in sheep. However, to date, there is no molecular evidence to demonstrate which *Sarcocystis* species use mouflons as intermediate hosts. The objective of the present work was to identify *Sarcocystis* species in diaphragm and cardiac muscle samples of 20 European mouflon from a population in Austria (Pitztal, North Tyrol). High prevalence of infection (95 %) in mouflons was determined. Molecular identification was performed from both isolated sarcocysts and digested muscle samples from the same individuals. Species were identified using *cox1* sequence analysis. According to the molecular analysis of isolated sarcocysts, the vast majority was determined to *S. tenella* which is known to form sarcocysts in sheep, and one sarcocyst was assigned to *S. capracanis* which previously was characterized in muscles of goats. Therefore, molecular analysis of digested tissue samples was performed in search for *Sarcocystis* species which use sheep as well as goats as intermediate hosts. A third species, *S. arieticanis*, was detected in the digested samples. Thus, digestion is more appropriate than analysis of individual sarcocysts if the species are known. The results of this study confirmed that the mouflon is intermediate host to species of *Sarcocystis* that are found in the muscles of sheep and goats.
Anthelmintic efficacy of gold nanoparticles biosynthesized from the phytopathogenic fungus *Alternaria alternata*

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Over the past few decades, green synthesis of nanoparticles has gained importance for their therapeutic efficacy and eco-friendly nature. Helminth infections are one of the most prevalent diseases in developing and developed countries. The cestode parasite *Raillietina echinobothrida*, highly prevalent in the common domestic fowl *Gallus gallus domesticus* causes a devastating loss for the livestock production industry in the tropical countries like India. The common control strategy adopted against such parasites is the usage of broad spectrum synthetic anthelmintic drugs whose frequent and long term usage has shown several instances of side effects and drug resistance. The integration of green chemistry principles into multidisciplinary nanoscience research has made scientists from different specializations, concerned about the need for developing environmentally benign and sustainable methods for synthesizing gold and silver nanoparticles. In the present study, *Alternaria alternata*, a fungus belonging to the family Pleosporaceae has been used for biosynthesis of gold nanoparticles to evaluate the anthelmintic potential. The drug has shown significant effect on the normal physiological functions of the cestode causing flaccid paralysis and subsequent death of the parasite. The ultra structural and enzymatic alterations revealed pronounced effects, thus establishing its anthelmintic potential. The histochemical localization studies of the tegument associated enzymes viz. AcPase, AlkPase, ATPase and 5’-NU, exposed to the drug showed a pronounced decline in activities. A considerable loss of activity nervous component enzymes like NSE and ChE ascertain the role of the mycogenic Au-NP as anthelmintic.
Prevalence of *Giardia duodenalis* and *Cryptosporidium* spp. in cattle in Latvia

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Dairy farming is one of the largest agricultural sectors in Latvia. The livestock generate large volumes of fecal waste, which can contaminate the environment with foodborne and waterborne parasites such as *Cryptosporidium* spp. and *Giardia duodenalis*. Both parasites cause gastrointestinal diseases in both humans and animals characterized by diarrhea and weight loss. The aim of the present study was to determinate the prevalence of both parasites in cattle and factors associated with the infection.

A total of 907 feces from cattle of different ages (one day till 12.2 years old) were collected from 29 dairy farms during March – December, 2020. Additional information about age, sex, and presence of diarrhea was collected. The fluorescent microscopy for antibody labeled cyst/oocyst detection was used (AquaGlo kit, Waterborne INC, USA).

*Cryptosporidium* spp. was found in 96.5% and *G. duodenalis* - in 82.7% of visited farms. While overall prevalence in animals was 22.6% and 7.6%, respectively. Significantly higher (p>0.05) prevalence of *Cryptosporidium* spp. (44.1%) and *G. duodenalis* (15.4%) was found in calves younger than three months than in cattle aged 3-24 months (19.3% and 5.9%) and cattle older than 24 months (8.3% and 2.8%), respectively. Diarrhea was observed in 25.9% of calves aged under three months infected with *Cryptosporidium* spp. and in 15.9% of calves infected with *G. duodenalis*, but not in older cattle.

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Sarcoptic mange in felines: does *Sarcoptes scabiei* var. *felis* exist? A preliminary molecular study

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Domestic and wild felines are considered suitable hosts for the parasitic mite *Sarcoptes scabiei*, and sarcoptic mange is reported in several felid species in the scientific literature. However, the traditional classification of host-specific varieties of *Sarcoptes* mites does not include *S. scabiei* var *felis* and it is not clear whether the transmission of sarcoptic mange in felines actually involves canids, other sympatric species, or exclusively felines. The aim of this study was to characterize the genetic structure of *S. scabiei* mites from domestic cats (*Felis catus*) and Eurasian lynx (*Lynx lynx*) comparing them with *Sarcoptes* mites from sympatric domestic and wild carnivores. Ten *Sarcoptes* microsatellite markers were used to genotype 81 mites obtained from skin scrapings of 36 carnivores: 4 domestic cats, one dog (*Canis lupus familiaris*), 4 lynx, 23 red foxes (*Vulpes vulpes*) and 4 grey wolves (*Canis lupus lupus*) from Italy, Switzerland and France. Results show the existence of two genetic clusters of *S. scabiei* with a geographical distribution-pattern: mites from Central Italian cats clustered with those from sympatric wolves, while all the other mites from Switzerland, France and Northern Italy clustered together. These results strengthen the previously advanced hypothesis that genetic strains of *S. scabiei* have a predominant geographic-related distribution, and that the “host-taxon law” is the prevalent underpinning transmission pattern. Geographical implementation of the *Sarcoptes* mite population from felids would be a desirable future step for further genetic analysis.
The digenean complex life cycle: the phylostatigraphy analysis of the molecular signatures

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The digenean complex life cycle is characterized by the alternation of parthenogenetic and amphimictic generations with specific ontogenies. From the molecular point of view, a single genome regulates all variety of stages and their characteristics within the life cycle is regulated by the activity of the one genome. The molecular basis for the functioning of the stage is the activation of the certain set of genes of the shared genome. These unique sets of genes, called molecular signatures, include genes that are expressed at several stages of the cycle, and possess stage-specific activity as well. Given the stages are “coupled” by genes with persistent activity, the question about the evolutionary origin of molecular signatures is raised. One of the most promising and powerful approaches to trace evolutionary innovations using genomic data is called “phylostratigraphy”. It allows identifying the phylogenetic origin of genes. Using the results of both phylostratigraphy and expression quantification, we analyzed the expression dynamics in groups of genes with the various phylogenetic origins of Fasciola gigantica, F. hepatica, Psilotremasimillimum, Schistosoma mansoni, Trichobilharziaregenti, and T. szidati to compare the phylogenetic ages of different life cycle stages. In general, phylogenetically “younger” genes are expressed at metacercaria and juvenile stages of fasciolids, cercaria of P. simillimum, as well as schistosomula stages of schistosomatids. We suggest that obtained results may reflect genetic novelties used by amphimictic generation stages to adapt to the wide list of hosts, and, consequently, a decrease in the “coupling” between stages in terms of genes used.
Angiostrongylus vasorum in Estonia: multi-center study in dogs with clinical signs suggestive of canine angiostrongylosis and survey of potential risk behaviors among the dogs

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In recent years, Angiostrongylus vasorum has been spreading to new areas, in particular in northeastern Europe. The parasite has been detected in wildlife in Estonia, but there are no published reports of canine angiostrongylosis in domestic dogs in the country. We conducted a multi-center study in 2018-2019: altogether 115 dogs with clinical signs suggestive of canine angiostrongylosis were tested at eight veterinary clinics, using a commercial A. vasorum antigen detection test. In addition, owner-reported information on potential risk behaviors of the dogs for acquiring A. vasorum infection was collected. None (0.0%, 95% confidence interval 0.0–2.6) of the dogs tested positive. Two (2.0%) of the dogs had been reportedly seen to eat slugs or snails, and four (3.9%) to eat frogs. Based on the results, interpreted carefully due to the sensitivity of the method and the limited sample size, it appeared that A. vasorum was either not established or at least not very common in domestic dogs in Estonia in 2018-2019.
Dipylidiasis as cause of medical misdiagnosis of „standard treatment-resistant enterobiasis” and „infantile onanism”

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A human infection with *D. caninum* (dipylidiasis) is rare, but if an infection does occur, it is more likely to occur in young children and toddlers that are at a greater risk. Most infections are asymptomatic, but sometimes these symptoms may be identified in an infected individual: mild diarrhea, abdominal colic, anorexia, restlessness, constipation, rectal itching, and pain due to emerging proglottids through the anal cavity. We refer the group of 8 patients that were misdiagnosed with „standard treatment resistant enterobiasis” (3 patients) and „infantile onanism” (5 cases). In 3 out of 8 cases the emerged proglottids have been considered by physicians as non-parasite related findings. After coproscopy has confirmed dipylidasis the differential diagnosis has been used to eliminate other conditions that present similar clinical features. We used praziquantel in standard doses and achieved 100% efficacy - all symptoms resolved. In the last year due to COVID-19 pandemics both the number of bought and adopted dogs and cats from animal shelters increased dramatically. Due to lockdown the domestic human-pets interactions became more intense. At the same time the pandemics related true and social fear caused limitations in veterinary care resulted in irregular deworming of pets and growing number of giardiasis, toxocarosis, dipylidosis (according to our statistics) and presumably as well the other zoonoses. And since there is a noticeable tendency to miss and/or to ignore the signs of parasitic infestations in humans- it may lead to the growing failure in meeting the standards and quality of medical care.
Efficacy of various medicines for metronidazole resistant blastocystosis. A comparative study. Initial results

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*Blastocystis* sp. is a frequent yet underestimated cause of an illness known as blastocystosis. Symptoms associated with the infection are: diarrhea, constipation, nausea, abdominal cramps, bloating, excessive gas, and anal itching. There have been only a few reviews of therapy for this infection and no definitive treatment protocols for metronidazole resistant cases have been published. When evaluating the clinical efficacy of agents used against *Blastocystis* sp., it is difficult to compare studies. They vary as to entry methodology (whether randomization was done and if treatment was blinded or open), population studied (children, adults, symptomatic and/or asymptomatic patients), outcome measures (clinical efficacy and/or stool negativity), and duration of follow-up. Nevertheless, conclusions may be drawn from the studies when viewed as a whole, and statements can be made about the relative efficacy of the agents. We decided to review the efficacy of agents currently used for the treatment of metronidazole resistant blastocystosis in our medical center. The differential diagnosis has been used to eliminate other diseases or conditions that present similar clinical features.

The total of 50 patients with metronidazole resistant blastocystosis were treated with 4 medicines used for this infection. The cure rates achieved with albendazole, nifuratelum, nitazoxanide and tinidazole were, respectively, 82%, 67%, 95% and 88%, while in a control group (given no medication) stools of 21% patients only became negative. Side effects were of minor importance in patients treated with albendazole and nitazoxanide and were somewhat more frequent and severe in those treated with tinidazole and nifuratelum.
Alaria alata prevalence in wild boar and a potential risk of human alariosis in Latvia

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Alaria alata trematode is widely distributed throughout Europe and has a complex life cycle where wild boar (Sus scrofa) as reservoir host seems to play an important role in their transmission. The aims of the study were to establish the occurrence of A. alata mesocercariae in naturally infected wild boar in Latvia and to assess the risk for humans to acquire A. alata infection via consumption of wild boar meat. By summarizing long-term data using the Trichinella inspection method from 2014 to 2019, the overall A. alata prevalence was 8.3%, of which significantly higher A. alata prevalence was observed during the summer seasons. Whereas the results obtained using Alaria mesocercariae migration showed significantly higher prevalence (43.9%, n=485) of A. alata in wild boar in Latvia. Our study suggested that the probability for humans to acquire A. alata mesocercariae infection through wild boar meat consumption is possible, yet improbable and varies from 0.2% to 2.2%. Most likely, it depends on frequency of parasite presence in wild boar using the mesocercariae inactivation technique during meat preparation for consumption.

Digenean trematodes of marine fishes from Brazil: A synoptic review and prospects

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Brazil holds a considerable portion of the world's biodiversity. Its coastal waters host a large variety of fish species (over 1200) with many of them being endemic. Recent studies suggested that our knowledge of the marine fish diversity in this region is far from being completed and even less is known about the diversity of parasites they harbour. Currently, the known diversity of trematodes in marine fishes from Brazil includes 142 species from 27 families reported from 167 species of fish. If each marine fish species would host a single unique species of trematode, the diversity of these parasites in Brazil would be much higher and is yet to be explored. The aim of the present study was therefore to investigate the diversity of trematodes in their marine fish hosts. A total of 152 specimens of 65 species of marine fishes belonging to 28 families were collected from two localities (Pará and Rio de Janeiro States) off the Brazilian coast in January 2021. Trematodes were collected from the intestine and stomach of 38 fish individuals (prevalence, 25%). All of the trematode species recorded were morphologically and genetically characterised based on multiple molecular markers. The results of the present study contribute to our knowledge of trematodes from marine fishes in Brazil and highlight a need for further dedicated research in this field.
Occurrence and diversity of vector-borne pathogens in rodents (Muridae, Cricetidae) from Lithuania

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Rodents represent an important group of mammalian hosts as considered reservoirs for a large number of vector-borne pathogens. However, there still is a lack of knowledge on the occurrence and diversity of vector-borne pathogens of zoonotic importance in rodents from Baltic countries. The aim of the research was to investigate Bartonella spp., Rickettsia spp. and Babesia spp. prevalence and genetic diversity in various species of rodents captured in Lithuania. Rodents representing eight species – Apodemus flavicollis, Apodemus agrarius, Mus musculus, Micromys minutus, Myodes glareolus, Microtus oeconomus, Microtus agrestis and Microtus arvalis were trapped with live-traps or snap traps in 19 locations of Lithuania during 2013–2016. DNA was extracted from the spleen samples of rodents. Conventional, nested and real-time PCR were used to amplify different genome regions of Bartonella (ssrA, rpoB, groEL and 16S-23S ITS), Rickettsia (gltA and 17kDa) and Babesia (18S rRNA) with subsequent sequence analysis. The present study demonstrates that Lithuanian rodents are frequently infected with Bartonella (54.8%), and Rickettsia (28.3%) pathogens and rarely infected with Babesia parasites (2.8%).

Sequence analysis of Babesia, Rickettsia and Bartonella isolates showed the presence of Babesia microti, Rickettsia helvetica and multiple Bartonella species belonging to six clades associated with human pathogenic Bartonella grahamii, Bartonella rochalimae and Bartonella tribocorum species, and other species Bartonella taylorii, Bartonella coopersplainsensis and Bartonella doshiae which pathogenicity to humans is still unknown. The findings of our study demonstrated the importance of rodents as considered reservoirs of these vector-borne zoonotic pathogens in Lithuania.

Keywords: rodents, Bartonella spp., Rickettsia helvetica, Babesia microti, Lithuania.
Culicoides biting midges: species composition, flying activity and intraspecific diversity

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Culicoides (Diptera: Ceratopogonidae) biting midges can transmit various parasites and viruses of humans and animals. Detailed knowledge of the Culicoides species composition and seasonal activity is essential to assess the risk of the introduction and transmission of pathogens. Since morphological identification of Culicoides is complicated, the sequencing of mitochondrial oxidase subunit I (COI) is one of the methods used for identification of Culicoides species.

Biting midges were collected in different localities in Lithuania using an Onderstepoort trap from April to October in 2018 – 2020. Both morphology and barcoding were used for the identification.

We identified 23 Culicoides species. The number of specimens trapped was the highest for the Culicoides obsoletus, followed by Culicoides kibunensis and Culicoides impunctatus. The highest relative abundance and diversity of biting midges were in May and June. COI fragments of 350 Culicoides individuals were sequenced and 76 haplotypes were obtained. High intraspecific diversities were detected for C. obsoletus and C. kibunensis with genetic distances being up to 12,04% (average 6,90%) and 8,25% (average 3,50%) respectively. A phylogenetic tree showed three distinct clades within C. obsoletus and three clades within C. kibunensis. Genetic distances between haplotypes within other Culicoides species were up to 2,71% (average – 0,76%). Although C. impunctatus was one of the most abundant species – intraspecific diversity was low: five haplotypes were detected, with an average of the genetic distance – 0,46%.

These results contributes to the total collection of DNA barcodes for Culicoides species found in Lithuania and Europe. Grant S–MIP–17–27 is acknowledged.
Parasites in a changing world: Transmission among three invasive species of *Mastophorus muris* in Gran Canaria, Spain.

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Invasive species represent one of the most important hazards for biodiversity loss, especially on isolated and unique ecosystems, such as islands. This threat is not solely through competition for resources or predation, but invasive species can act as ‘Trojan horses’ for important pathogens and parasites transmissible to wildlife, domestic animals and humans. Understandably, most reports focus on interactions between invasive and native species or humans, however interactions amongst invasive species, for example *Mastophorus muris*, a common nematode of rodents, are seldom studied.

Twenty-two brown rats (*Rattus norvegicus*), 108 California Kingsnakes (*Lampropeltis californiae*), 15 geckoes (*Tarentola boettgeri*) and 87 millipedes (*Ommatoiulus moreletii*) collected in Gran Canaria, were examined for parasites. Millipedes were digested with pepsin in pools of 2g. Where parasites were identified from these pools, DNA was extracted and a fragment of the *ITS1* region amplified by PCR and sequenced.

Larval nematodes consistent with *M. muris* were found in 3/6 pools of millipedes, 5/15 geckoes and 81/108 snakes. Adult *M. muris* were found in 3/22 rats. *ITS1* gene sequences from millipedes revealed 100% homology with *M. muris* (accession number MK829005). These findings suggest a model of transmission using three invasive and one endemic species. Empiric data describes *M. muris* as a generalist parasite in terms of intermediate hosts, thus, rats not only pose a threat to vertebrates (including humans) by harbouring dangerous pathogens, but also to invertebrate conservation. This is the first description of larval spirurids in Spanish geckoes and *O. moreletii* as an intermediate host of *M. muris*. 
Genetic diversity in Cryptocotyle lingua (Digenea: Heterophyidae): expanding a dataset to its geographical limits

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Intraspecific genetic diversity in digeneans was shown to follow several patterns, largely depending on the number and vagility of hosts in their life cycle. Better understanding of these patterns requires information on more species, and larger sample sizes. We contributed to this topic with research on Cryptocotyle lingua (Heterophyidae) from its first intermediate hosts, Littorina littorea. This topic has some important background.

In a 2008 study, April Blakeslee and her colleagues used information on the genetic structure of C. lingua as one of the sources of evidence to resolve the origin of L. littorea in North America. In this study, a large cox1 dataset for C. lingua was collected, covering 20 North American and 16 European locations. In Europe, sampling spanned from the Atlantic coast of France in the south to the Oslo region in the north. High genetic diversity was detected, and even more expected, if sampling effort increased.

In our study, we aimed to expand the available European cox1 dataset for C. lingua, covering the north-eastern limits of its distribution range. In summer 2020, we collected 37 isolates of C. lingua from periwinkles at the White and Barents Sea shores, and sequenced a cox1 gene fragment for them. These new data and 196 sequences from GenBank were aligned, and analyzed together in PopART and DnaSP6. In our material, we detected both new unique haplotypes and those that were shown to dominate in a 2008 study. These and other results of the current study prove and expand previous findings.
Molecular identification of *Sarcocystis* species in the intestines of mustelids from Lithuania

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Representatives of the genus *Sarcocystis* (Apicomplexa: Sarcocystidae) are worldwide distributed parasites of reptiles, birds, and mammals, including humans. These protozoans have an obligatory two-host prey-predator life cycle. Sarcocysts are formed in muscles and CNS of intermediate host, while oocysts/sporocysts develop in the small intestine of the definitive host. *Sarcocystis* species are described and morphologically characterized in the intermediate host. Whereas definitive hosts are known for less than half of *Sarcocystis* species. The ethical issues and complexity of methodology limit the use of transmission experiments for the disclose of the life cycle of *Sarcocystis* spp. On the contrary, there is a growing need to develop molecular methods for identifying definitive hosts under natural conditions.

In the present work the intestine samples of 84 mustelids belonging to five species were examined for *Sarcocystis* oocysts/sporocysts. Species-specific nested PCR of mitochondrial *cox1* and subsequent sequencing were applied for the identification of *Sarcocystis* species. The presence of *Sarcocystis* species using cattle and cervids as intermediate hosts were tested. In the analysed samples nine *Sarcocystis* species were confirmed. A high infection rate of examined species was recorded. Oocysts/sporocysts of *Sarcocystis* spp. employing cervids as intermediate host were determined in 80% of American minks. Also, the prevalence of *S. bovifelis* (89.3%) and *S. cruzi* (73.8%) from cattle was very high. The obtained results cause concern about food safety issues. Future research on the role of mustelids in the transmission of *Sarcocystis* are needed.
Molecular survey of feline pathogens in cats in Lithuania

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Feline pathogens have been increasingly investigated for their impact on cat health and their zoonotic potential. The aim of the present study was to assess the prevalence of *Toxoplasma gondii*, *Bartonella* spp. and haemoplasma infections in cats in Lithuania. Serological and molecular methods were used to investigate the occurrence of these zoonotic pathogens. A chromatographic immunoassay „Anigen Rapid Toxoplasma Ab Test Kit“ was used for the qualitative detection of antibody against *Toxoplasma gondii*. Bartonella spp. DNA was detected using a nested-PCR targeting the 16S-23S rRNA gene intergenic transcribed spacer (ITS) region followed by sequencing for species identification. PCR and sequence analysis of partial 16S rRNA gene was used for detection and characterization *Mycoplasma* spp. by. A total of 163 blood samples were collected from domestic cats presented at veterinary clinics and animal shelter in Kaunas (central part of Lithuania). The combined results of serological test and PCR revealed that 35 of 163 (21.4%) cats were positive for one or more pathogens. Antibodies against *T. gondii* were detected in 38.4% (15/39) of examined cats. *Bartonella* species were detected in 8 (4.9%) out of 163 cats. The sequence analysis revealed the presence of *Bartonella henselae* (n=4), *Bartonella clarridgeiae* (n=2) and *Bartonella* sp. (n=2). Haemoplasmas were detected in blood samples of 19 cats (11.7%): 2.5% were positive for *Mycoplasma haemofelis* and 9.2% for *Candidatus Mycoplasma haemominutum*.

Keywords: Domestic cat, *Toxoplasma gondii*, *Bartonella*, Haemoplasma.
Conspecific migration and environmental setting determine parasite infracommunities of non-migratory individual fish

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The mechanisms shaping parasite infracommunities are complex and involve many factors, among which host traits and environmental characteristics, such as the type of water source in streams may play a major role. We examined for the first time the effect of water source and the adult fish migratory behaviour on the metazoan parasite infracommunities of conspecific juvenile brown trout, *Salmo trutta* L. among streams flowing into Lake Lucerne (Switzerland). Thirteen parasite taxa were identified using morphological and molecular analyses. The similarity in the parasite infracommunity composition (zero-adjusted Bray-Curtis dissimilarity measures) of juvenile trout across streams was explained by the interaction between the type of water source and adult migration rates. Compositional diversity in species richness among infracommunities of juvenile fish was higher in groundwater-fed than in surface water-fed streams, whereas the rate of fish migration did not affect parasite richness. As hypothesised, autogenic parasite species with complex life-cycles were more common in groundwater-fed streams with high and medium rates of trout migration. In contrast, infracommunities of trout parasites in surface water-fed streams and streams with low rates of fish migration were dominated by one monoxenous parasite species or lacked infections. Our results demonstrate that similarity in the parasite infracommunity composition of resident freshwater hosts can be predicted by the local environmental factors and conspecific host migratory behaviour, whereas their richness is mainly influenced by the environmental characteristics.
Molecular detection of vector-borne pathogens from bats in Lithuania

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Bats (Chiroptera) are distributed worldwide and the second largest order of mammals (next to rodents) recognized as reservoirs or carriers of numerous species of viruses, bacteria and protozoan parasites, some of them with zoonotic potential to infect other animals or humans. They also host a range of ectoparasites (e.g. ticks, mites, bat flies, fleas) that might play a role in the transmission of pathogenic organisms. To date, much research on bats as a host of viruses has been done, while interest of vector-borne bacteria in bats has increased in recent years. In this study bat corpses were collected in different regions of Lithuania, from hibernacula sites, buildings or after wind turbine collisions. A total of 6 insectivorous bat species (Pipistrellus nathusi, P. pipistrelus, Nyctalus noctule, Vespertilio murinus, Eptesicus serotinus, Myotis daubentonii) were found. During necropsy heart, lung, liver, kidney, and spleen if possible due to disintegration were collected and used for further analysis. To examine living bats, blood samples were taken. Detection of Rickettsia, Anaplasma, Bartonella, Borellia and Babesia were done using real time PCR (screening), as well as conventional PCR and sequencing (for pathogen identification).
Molecular Characterization of *E. granulosus* s.s. isolates from hydatid cysts from Sardinian (Italy) Patients Clinically and Immunologically Evaluated

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Cystic echinococcosis (CE) is a zoonotic global public health problem, caused by the larval form of a tapeworm belonging to Taeniidae family, the *Echinococcus granulosus* sensu lato (s.l.). Life cycle comprises definitive hosts (canids) and intermediate hosts (ruminants) only occasionally represented by humans. Based on molecular studies, *E. granulosus* complex clustered into five species: *E. granulosus* sensu stricto (s.s.) (G1-G3), *E. equinus* (G4), *E. ortleppi* (G5), *E. canadensis* (G6–G8, G10), and *E. felidis*. The *E. granulosus* s.s. G1-G3 genotypes has high prevalence and are endemic in all Mediterranean countries.

The main aim of our study was to confirm the clinical diagnosis of 14 patients suspected of CE as by immunological tests as by molecular examination of the cysts, moreover, the latter analysis lead to identify specie, genotype and haplotype.

The 14 subjects were firstly investigated by ultrasound (US) and immunological analysis of the sera, then, after a pericystectomy needed for several complications, the cysts had been subjected to parasitological, histopathological and molecular biology examinations.

US results evidenced two CE1, one CE2, eight CE3b, one CE4, and two CE5, while immunological tests showed nine positive and five negative sera. Parasitology and histopathology confirmed 12 CE cysts, of which, 9 fertile displayed protoscoleces, conversely, 2 neoformations presented a non-parasitic source.

A neighbour-joining (NJ) phylogenetic tree was built including the DNA sequences isolated from the 9 fertile cysts that were identified as *E. granulosus* s.s. G1 (n=5) and G3 genotype (n=4). Haplotype network evidenced new haplotypes.
Shared makeup cosmetics as a route of *Demodex folliculorum* infections

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The aim of the study was to examine *Demodex folliculorum* survival in makeup cosmetics, i.e.,
powder cream, mascara, and lipstick, and to determine whether cosmetics shared with
others can be a source of *Demodex* mites infection. Live *D. folliculorum* adults were placed in
cosmetic samples and their motility was observed under a microscope. The mites were fully
or partially immersed in the powder cream and lipstick, and only partially immersed in the
mascara. Partial immersion means that only the opisthosoma was covered by the cosmetic,
whereas the gnathosoma and podosoma had no contact with the cosmetic. Cessation of
motility was regarded as a sign of death. In the control (mites placed on a microscope slide
with no cosmetics), the survival time was 41.2 hours. *Demodex* mites that were immersed
fully or partially in the lipstick substrate were viable for 38.5 hours and 148 hours,
respectively. The survival time of the mites at full and partial immersion in the powder cream
was 0.78 hours and 2.16 hours, respectively. The average survival time in the mascara was 21
hours. Makeup cosmetics used by different individuals at short intervals (from several hours
to several days) can be a source of transmission of *Demodex* mites.
Diversity of tick-borne pathogens in *Ixodes ricinus* ticks parasitizing small rodents in commercial gardens in Lithuania

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*Ixodes ricinus* tick is the main vector for the causative agents of various zoonotic diseases, including Lyme borreliosis and tick-borne encephalitis. Different species of rodents are the main hosts of immature stages of *Ixode* sticks and recognized as reservoirs of several tick-borne pathogens, such *Borrelia burgdorferi sensulato, Borrelia miyamotoi, Babesia microti* and *Rickettsia* species. Populations of rodents and their ectoparasites that have close contact with humans can influence public health due to the potential risk for diseases transmission. Also, there is little known about density of ticks in gardens and the diversity of tick-borne pathogens that they carry. The aim of this study was to investigate the presence and prevalence of pathogens in ticks parasitizing different species of small rodents in commercial orchards and berry plantations in Lithuania. Ticks were collected from six different species of rodents (*Apodemus flavicollis, A. agrarius, Microtus arvalis, M. agrestis, M. oeconomus* and *Myodes glareolus*) caught in apple orchards, and currant and raspberry plantations in Lithuania in 2018 and 2020. Rodents were infected with *Ixodes ricinus* larvae and nymphs. After DNA extraction, ticks were further analysed for the presence of *Borrelia* spp, *Rickettsia* spp. and *Babesia* spp. pathogens by real-time PCR followed by sequencing for species identification. Our investigations provide new information on detection of multiple pathogens in *I. Ricinus* ticks parasitizing small rodents in commercial gardens in Lithuania.
Seroprevalence of *Toxoplasma gondii* in cats in Kaunas, Lithuania

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A disease induced by *Toxoplasma gondii* is called toxoplasmosis. Most of all warm blooded vertebrates could be affected as well as humans. Most important place in disease epidemiology is taken by cats, because they are definitive host of *T. gondii*. Therefore cats could have toxoplasmosis with clinical signs and also transmit disease by faecal-oral route to humans. There is no reliable diagnostic protocol for cats with suspected toxoplasmosis in our country. The aim of our research was to determine the population of cats which are seropositive for *T. gondii*. A total of 90 cat serum or plasma samples collected from veterinary clinic of Kaunas, Lithuania in 2018 from January to June. For IgG and IgM detection in cat's serum or plasma we used indirect ELISA in house kit “ID Screen Toxoplasmosis Indirect Multi-species” (*ID.VET Innovative Diagnostics, France*). In parallel with the sample collections, the owners answered an epidemiological questionnaire that investigated the following variables: age group, gender, lifestyle (indoor or free-living animals), feeding (raw meat consumption). Results showed that 40% of research group cats were seropositive for *T. gondii* antigens. Also results showed that risk factor, such as possibility to go outside, is statistically significant (p<0,005) and have a positive relationship with cat's seropositivity.


Hepatozoon spp., Trypanosoma spp. and Babesia spp. in murine rodents of the Chernobyl Zone of Radioactive Contamination

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A study of 51 murine rodents of the species Myodes glareolus, Apodemus flavicollis and Apodemus agrarius of both sexes was conducted for the presence of agents of blood-borne parasitic diseases. Animals were carried out during August-October, 2020 on the landfills of the drained bed of the Chernobyl Nuclear Power Plant cooling pond and other places of Chernobyl zone of radioactive contamination according to the scientific radiobiology and radioecology monitoring program. Of the samples, in four (7.8%) with the help of blood film examination were identified Trypanosoma spp., in two (3.9%) samples Hepatozoon spp. and in 16 (31.4%) samples Babesia spp. Mixed infection with Trypanosoma spp. and Hepatozoon spp. was registered in one (2.0%) animal. Our research firstly identified Trypanosoma spp. and Hepatozoon spp. in blood films of murine rodents from the territory of Ukraine and it is important in our further studies to conduct the phylogenetic analysis of the received isolates and also the host-parasite interactions.
Toxocara cati infection in Eurasian lynx (Lynx lynx) in Finland: worm burden by sex and age

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Toxocara cati is a zoonotic parasite, whose definitive hosts include several feline species. In Finland, Eurasian lynx (Lynx lynx) are annually hunted, and carcasses are submitted for research purposes. Here, we summarize the T. cati burden observed in intestinal tracks of 2837 lynx hunted in 1999-2015. Altogether 2361 (84%) of the lynx had T. cati worms. The median number of worms per infected individual across all age groups and sexes was 12 (range 1-218). The median number of worms was 11 (range 1-218) in the 645 lynx aged about 6 months, 15.5 (range 1-213) worms in the 1278 lynx aged 1-2 years, and 8 worms (range 1-183) in the 836 lynx aged 3 years or over. Females harbored a median of 13 worms (range 1-213) and males 12 (range 1-218). Old females had significantly higher worm burden than old males: among the 67 lynx aged 9-15 years, the median was 11.5 in the 40 females (range 1-162) and 3 in the 27 males (range 1-42). We found that T. cati infection was common in lynx of all ages, which seems to contrast the results reported for domestic cats, in which the infection is more common in younger individuals.
Survival of *Blastocystis* in long-term *in vitro* culture

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*Blastocystis* *cf.* *hominis* is the most common unicellular eukaryote infecting humans and is associated with a variety of gastrointestinal disorders. *Blastocystis* is considered a fragile organism which is easily degraded, but there is little research to determine the survival rate of morphological forms of this parasite.

The aim of the study was to assess the survival of *Blastocystis* in xenic culture, stored at 37°C for 2–3 years at the Department of Biology and Medical Parasitology, Wrocław Medical University, Poland. Apart from the assessment of the survival rate of cultures, the aim was also molecular determination of the subtypes of the *Blastocystis*, subcultured in this study. The material for the research was 53 *Blastocystis* cultures stored at 37°C, obtained from patients’ stool samples in 2017–2018.

The cultures were subcultured on modified Jones’ medium supplemented with 10% horse serum under xenic conditions at 37°C and examined for morphological forms of *Blastocystis* after 48 hours, using light microscopy. Subsequently positive cultures were subjected to PCR analysis followed by Sanger sequencing to determine the *Blastocystis* subtyp. Different survival rates of subcultured *Blastocystis* were observed from 7 positive cultures.

The results of survival analysis revealed that ST1 was the most resistant to adverse environmental conditions *Blastocystis* subtype in this study, followed by ST2, ST3 and ST7. In-depth analysis of *Blastocystis* subtype survival requires observation on a larger number of cultures, however this study has shown that *Blastocystis* is more resistant than previously assumed.