POLISH PARASITOLOGICAL SOCIETY

ANNALS OF PARASITOLOGY

volume 68 · supplement 2 · 2022

eISSN 2300-6706

The 26th Congress of the Polish Parasitological Society will be held from 12 to 15 September 2022 at the **Conference and Training Center of the University of Warmia and Mazury in Olsztyn, Dybowskiego 11 Str., 10-719 Olsztyn, Poland.**

The Congress is organized by **Executive Board of the Polish Parasitological Society** and the **Olsztyn Branch of the Polish Parasitological Society**.

"XXVI Congress of the Polish Parasitological Society" paid from the funds of the Ministry of Education and Science under the agreement no. DNK/SP/ 563765/2022. Name of the project "Excellent Science", module "Support for scientific conferences"; the amount of funds is PLN 40,000. "

Ministry of Education
and Science

SCIENTIFIC COMMITTEE

ORGANIZING COMMITTEE

Małgorzata Dmitryjuk, DSc, PhD
General Chair
Department of Biochemistry, Faculty of Biology and Biotechnology,
University of Warmia and Mazury in Olsztyn

Katarzyna Kubiak, PhD
Vice-chair
Department of Medical Biology, Faculty of Health Sciences,
University of Warmia and Mazury in Olsztyn

Mirosław Mariusz Michalski, DSc, PhD
Treasurer
Department of Parasitology and Invasive Diseases, Faculty of Veterinary Medicine,
University of Warmia and Mazury in Olsztyn

Robert Stryiński, MSc
Secretary
Department of Biochemistry, Faculty of Biology and Biotechnology,
University of Warmia and Mazury in Olsztyn

Joanna Korycińska, PhD
Head of the Congress Office
Department of Medical Biology, Faculty of Health Sciences,
University of Warmia and Mazury in Olsztyn

Members of the Organizing Committee

Anna Cichy, PhD
Anna Stanicka, PhD
Department of Invertebrate Zoology and Parasitology,
Faculty of Biological and Veterinary Sciences,
Nicolaus Copernicus University in Toruń

Magdalena Szczotko, MSc
Department of Biochemistry, Faculty of Biology and Biotechnology,
University of Warmia and Mazury in Olsztyn

Iwona Jeleń, PhD †13 VIII 2022
Department of Zoology, Faculty of Biology and Biotechnology,
University of Warmia and Mazury in Olsztyn

Hanna Szymańska, PhD
Małgorzata Lepczyńska, PhD
Department of Medical Biology, Faculty of Health Sciences,
University of Warmia and Mazury in Olsztyn

Maria Michalczyk, PhD
Sylwia Koziatek-Sadłowska, PhD
Department of Parasitology and Invasive Diseases, Faculty of Veterinary Medicine,
University of Warmia and Mazury in Olsztyn

Remigiusz Gałęcki, DVM
Department of Veterinary Prevention and Feed Hygiene, Faculty of Veterinary Medicine,
University of Warmia and Mazury in Olsztyn

Aleksandra Czułowska, MSc
Department of Microbial Ecology and Akaroentomology,
Faculty of Biological Sciences, University of Wroclaw

Authors are responsible for supplied abstracts.

Editor: Rusłan Sałamatin

The co-organisers and honorary patronage:

Voivode of Warmia and Mazury
Artur Chojecki

Marshal of the Warmia and Masuria Voivodeship
Gustaw Marek Brzezin

President of City of Olsztyn
Piotr Grzymowicz

Rector of University of Warmia and Mazury in Olsztyn
dr hab. Jerzy A. Przyborowski, prof. UWM

Warmia and Mazury Medical Council

National Medical and Veterinary Council

Warmia and Mazury Medical and Veterinary Council

WOJEWODA
WARMIŃSKO-MAZURSKI
Artur Chojecki

PATRONAT HONOROWY
Marszałek
Województwa Warmińsko-Mazurskiego
Gustaw Marek Brzezin

PATRONAT HONOROWY
Prezydent Olsztyna
Piotr Grzymowicz

UNIWERSYTET
WARMIŃSKO-MAZURSKI
W OLSZTYNIE

JM Rektor UWM w Olsztynie
dr hab. Jerzy A. Przyborowski, prof. UWM

CONFERENCE SPONSORS
„GOLDEN SPONSORS”

IVC EVIDENSIA

Elanco™

CELiUS.

vetAgro

BIO-RAD

MSD Animal Health

DELTA® optical

Grupa Centrowet
Rozwiązania dla weterynarii

CONFERENCE SPONSORS

MEDIA PARTNERS

Annals of Parasitology
eISSN 2300-6706
ISSN 2299-0631
(formerly *Wiadomości Parazytologiczne*)
edited by the Polish Parasitological Society

Weterynaria w terenie

POLSKIE RADIO OLSZTYN

UWM 95,9 fm uwierz w muzykę

The XXVI Congress of the Polish Parasitological Society

12–15 September 2022, Olsztyn

Abstracts

Plenary session

Monogenea – past – present – future

Ewa Dzika

Department of Medical Biology, School of Public Health, Collegium Medicum, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

Monogenea are a group of about 6,000 – 7,000 species, which are mainly ectoparasitic on fishes, especially on the gills and skin, and occasionally other aquatic organisms, such as amphibians and tadpoles. A small number of species also occur as endoparasites. The majority of monogenean species are highly host–specific, usually being restricted to a single host species with which they have co-evolved. According to Gerasev et al. (2013), 74–78% of monogenea parasitizes only one species of host. According to Dzika (2008), the monogenea fauna of Poland consisted of 130 species. Over the last 14 years it has been enriched with 7 more species: *Gyrodactylus latus* (Bychowsky, 1933), *G. teuchis* (Lantraite, Blanc, Thiery, Daniel et Vigneulle, 1999), *G. proterorhini* (Ergens, 1967), *G. perccotti* (Ergens et Yukhimienko, 1973), *Dactylogyrus squameus* (Gusev, 1955), *Thaparocleidus caecus* (Mizelle et Kritsky, 1969), *Sciadicleithrum variabilum* (Mizelle et Kritsky, 1969). Mainly, these are species found in introduced or aquarium fish. The use of molecular biology methods and electron microscopy (SEM) has made it possible to confirm and verify many species of monogenea; as well as describe many new species in the world. In addition, the use of ultrastructural studies, confocal and electron microscopy has allowed for a deepening of the knowledge concerning the external structure of the parasite, the digestive and excretory systems, as well as the mechanisms of digestion and excretion in one of the monogenea groups from the Diplozoidae family. In the future, the above-mentioned studies of other monogenea groups will allow the verification of these systems and descriptions of new mechanisms in the parasite-host system for this group of parasites.

Freshwater snails in the transmission of parasitoses

Elżbieta Żbikowska, Anna Cichy, Anna Stanicka

Department of Invertebrate Zoology and Parasitology Nicolaus Copernicus University in Toruń, Toruń, Poland

The Digenea is perhaps the largest group of endoparasitic metazoan parasites and includes over 18000 nominal species. They choose representatives of all groups of vertebrates as final hosts, and the relatively low specificity to these hosts determines their high zoonotic potential. Completely different relationships connect Digenea representatives with first intermediate hosts, among which freshwater snail species play a dominant role. More than 90% of known Digenea species use molluscs, and in this group – freshwater snails – as the host of their larval stages. The uniqueness of the snail-fluke relationship is probably due to the very long shared history of both partners. In studies on Digenea, the role of invertebrate hosts is usually underestimated due to their low – except the certain parts of the world – commercial importance. Meanwhile, the low mobility of molluscs and the fact that in their body develop invasive stages for vertebrates should constitute important premises for the characterization of environmental conditions at risk of parasitoses of digenean etiology.

In host snails, parasites, at the expense of the host's reproductive energy, are able to produce invasive larvae of up to hundreds of thousands of individuals. The released cercariae can encapsulate in the environment or penetrate into other invertebrate or vertebrate hosts, including humans. The high productivity of cercariae and the long-lasting relationship of the snail–flukes at the individual level guarantee the success of parasites in the environment.

In order to illustrate the role of snails in spreading parasitoses, selected examples of diseases of digenean etiology are presented.

Ultrastructure of Extracellular Vesicles and Multivesicular Bodies in Cestoda and Trematoda

David B. Conn

Department of Biology and One Health Center, Berry College, and Department of Invertebrate Zoology, Museum of Comparative Zoology, Harvard University, Mount Berry, Georgia and Cambridge, Massachusetts, United States

The study of specialized secretions from diverse animal and protist cells involving extracellular vesicles and multivesicular bodies has become a major area of research on intercellular communication in recent years. This area of new exploration has high relevance to the interactions between parasites and their hosts, including modulation of the immune response and parasite-host signaling. As new information has emerged there is a need to reassess ideas about the nature of epithelial secretory processes and excretory/secretory products of neodermatan Platyhelminthes, especially endoparasitic Cestoda and Trematoda. For many years there have been many assumptions and speculations about the function of neodermatan epithelia based largely on ultrastructural studies that were not designed to test for specific function. Such studies include the tegument, excretory ducts and reproductive ducts of cestodes and trematodes, and the gastrovascular epithelium of trematodes. More recently, approaches such as proteomic and surfaceomic analysis have shed more light on the functional, physiological, and immunological aspects of these tissues and their secretory products.

To begin examining the potential formation of extracellular vesicles and multivesicular bodies within cestode and trematode epithelia, we conducted new examination of epithelia in two cyclophyllidean cestodes, *Mesocestoides lineatus* and *Oochoristica anolis*. Using transmission electron microscopy we report here for the first time numerous membrane-bound surface vesicles extending from the distal cytoplasm of the tegument. These occur in three forms: 1) moniliform strands of ovoid vesicles; 2) multivesicular bodies containing many single pedunculated vesicles; 3) individual surface vesicles.

Beyond these new data, we have begun a retrospective analysis of previously published ultrastructure studies of cestode and trematode epithelia to determine whether such secretory structures appear in published micrographs, but were not noted by earlier authors who were not yet aware of the widespread occurrence of these structures among animals. To date, we have located several examples, including multivesicular bodies in the tegument of *Ornithodiplostomum ptychocheilus diplostomula*, moniliform strand vesicles in *Ophryocotyly insignis* metacestodes, and individual vesicles in *Eubothrium salvelini*, *Taenia o*vis, and *Mosgovoyia ctenoides hexacanths*.

It will be necessary to complete more retrospective studies from the extensive literature on cestode and trematode epithelia. This must also include reexamination of structures previously reported tentatively or with insufficient evidence as having another origin or function, such as the purported viral particles in the excretory ducts of cestodes. In the future, all ultrastructural research on the epithelia of parasitic flatworms should consider the likely presence of these vesicular secretory products.

Omics approaches towards tick and parasite vaccine development

Ana Domingos, Sandra Antunes, Joana Couto

Institute of Hygiene and Tropical Medicine/NOVA University of Lisbon, Lisbon, Portugal

The undesirable impact of ticks and the (re)emergence of chief tick-borne diseases affecting both animal and human health is an issue that has been of growing concern to health organizations.

The major tick and disease control measure depend mostly on the use of acaricides showing, as largely noticed, unwanted effects reinforcing the need for alternatives, such as vaccination.

Research activities focusing pathogen-vector complex interactions is a good clue to identify critical molecules, potential vaccine candidates, that can be targeted to reduce, not only the vector competence, but also pathogen development.

The rationale of our studies is based on the evidence that tick salivary glands (SG) have a crucial role on hematophagous behaviour and on pathogen transmission and that omic tools, allied to gene silencing experiments, allow to correlate changes in gene expression with changes in the proteome and confirm the role of selected genes and proteins in the infection (or other condition) process.

As a first step, a *Rhipicephalus bursa* tick–*Babesia ovis* parasite model has been established in order to find new protective antigen candidates for vaccine design. R. bursa is widely distributed in the Mediterranean region, is found mainly on sheep and, occasionally on humans and is the principal vector of *Babesia ovis*, a highly pathogenic malaria-like parasite, showing a high mortality rate, in susceptible animals.

The *R. bursa* sialotranscriptome and sialoproteome were obtained and screened in order to point out which genes and proteins were involved in the feeding and infection processes.

The comparative analyses of the transcriptomes and proteomes datasets of *R. bursa* SG revealed that blood feeding induces the production of tick molecules, which was translated by the increased gene expression and protein synthesis. Data suggested a key role for stress response and apoptosis pathways in response to infection.

Gene silencing RNAi-mediated experiments were performed and the effect gene knockdown in tick weight, mortality and oviposition and parasite infection was evaluated.

The gene knockdown of some genes showed a significantly lower female weights after feeding and also in lower weight of laid eggs while the silencing of others presented a strong effect on feeding and parasite infection.

The analysis of imuno-response ability following an established reverse genetics immunoinformatics pipeline enable to choose the best vaccine candidates and a pilot vaccination study confirmed the antigens capability to inhibit tick feeding and pathogen transmission.

Impact of infection with *Hymenolepis diminuta* on exploratory behavior and cognitive processes in rats

Kamila Blecharz-Klin[1], Magdalena Świerczyńska[1], Agnieszka Piechal[1], Adriana Wawer[1], Ilona Joniec-Maciejak[1], Justyna Pyrzanowska[1], Ewa Wojnar[1], Anna Zawistowska-Deniziak[2], Anna Sulima-Celińska[3], Daniel Młocicki[3], Dagmara Mirowska-Guzel[3]

1 Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, Centre for Preclinical Research and Technology CePT, Warsaw, Poland
2 Witold Stefański Institute of Parasitology, Polish Academy of Sciences, Warsaw, Poland
3 Department of General Biology and Parasitology, Medical University of Warsaw, Warsaw, Poland

Parasites may significantly affect the functioning of the host organism, with some even being able to invade the brain and alter the host behavior. Recent advances in the research on parasitic manipulation and control of the nervous system of their host resulted in the development of neuro-parasitology, a new and emerging branch of science. However, still the neuro-parasitology of parasitic infections in vertebrate hosts remains unexplored. Especially the impact of intestinal worms on the host central nervous system (CNS) remains unexplored. Therefore in our study we evaluated the effect of intestinal infection by the tapeworm *Hymenolepis diminuta* on the behavior, cognitive processes and neurotransmission in rats. Our goal was to evaluate the effect of intestinal infection by the tapeworm on behavior and functions of the CNS in rats. The three months old animals were infected, and the effects on anxiety, exploration, sensorimotor skills and learning processes were assessed at 18 months in Open Field, Novel Object Recognition and the Water Maze tests. After completing the behavioral studies infected and non-infected rats were sacrificed, and the collected brain tissues were subjected to biochemical analysis. The levels of neurotransmitters, their metabolites and amino acids in selected CNS structures were determined. In addition, the gene expression profile of the pro- and anti-inflammatory cytokines was evaluated by Real-Time PCR to determine the immune response of the CNS to the infection. We observed that the parasites caused significant changes in exploratory behavior, most notably, a reduction of velocity and total distance moved in the OF test; the infected rats exhibited decreased frequency in the central zone, which may indicate a higher level of anxiety. This infection positively influences spatial memory assessed in the WM test, and new object recognition. At the same time, the infected animals develop a greater level of anxiety and move more slowly. These behavioral changes were related to the reduction in noradrenaline level in the CNS structures, and less pronounced changes in striatal serotonergic neurotransmission. H. diminuta infestation was also found to cause a significant reduction of hippocampal expression of IL-6. Our results provide new data for further research on brain function during parasitic infections especially in relation to helminths and diseases in which noradrenergic and serotonergic system may play an important role. In the near future may turn out that the role of the intestinal macrobiome in the CNS functioning may be just as significant as that of the microbiome.

Babesia microti confers macrophage-based cross-protective immunity against malaria in rodent model

Artemis Efstratiou, Eloiza Galon, Xuenan Xuan

National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan

Malaria and babesiosis, the two primary intraerythrocytic protozoan diseases of humans, have been reported in multiple cases of co-infection in endemic regions. As the geographic range and incidence of arthropod-borne infectious diseases is being affected by climate change, co-infection cases with Plasmodium and Babesia are likely to increase. The two parasites have been used in experimental settings, where prior infection with *Babesia microti* has been shown to protect against fatal malarial infections in mice and primates. However, the immunological mechanisms behind such phenomena of cross-protection remain unknown. Here, we investigated the effect of a primary *B. microti* infection on the outcome of a lethal P. chabaudi challenge infection using a murine model. Simultaneous infection with both pathogens led to high mortality rates in immunocompetent BALB/c mice, similar to control mice infected with *P. chabaudi* alone. On the other hand, mice with various stages of *B. microti* primary infection were thoroughly immune to a subsequent *P. chabaudi* challenge. Protected mice exhibited decreased levels of serum antibodies and pro-inflammatory cytokines during early stages of challenge infection. Mice repeatedly immunized with dead *B. microti* quickly succumbed to *P. chabaudi* infection, despite induction of high antibody responses. Notably, cross-protection was observed in mice lacking functional B and T lymphocytes. When the role of other innate immune effector cells was examined, NK cell-depleted mice with chronic *B. microti* infection were also found to be protected against *P. chabaudi*. Conversely, in vivo macrophage depletion rendered the mice vulnerable to *P. chabaudi*. The above results show that the mechanism of cross-protection conferred by *B. microti* against *P. chabaudi* is innate immunity-based, and suggest that it relies predominantly upon the function of macrophages. Further research is needed for elucidating the malaria-suppressing effects of babesiosis, with a vision toward development of novel tools to control malaria.

SESSION 1

Modern research tools in parasitology (molecular and omics studies, immunoparasitology)

Oral session

Preliminary evaluation of immunomodulatory effects of cystatin-like protein from *Trichinella britovi* on mouse splenocytes and bone marrow cells

Anna Stachyra

Witold Stefański Institute of Parasitology, Polish Academy of Sciences, Warsaw, Poland

Trichinella has a unique life cycle among parasitic nematodes, and is therefore a very interesting research object. The parasite does not have any free-living stages and is not present in the outer environment. The entire life cycle of Trichinella is completed within a single host. During the early stage of infection, parasite reproduce in the intestine, what results in new born larvae migration to the muscle tissue. Afterwards, nurse cells are formed and enable invasive larvae to remain metabolically active until they can be ingested by the next potential host. During this phase, immunological homeostasis ensures the survival of both parasite and host, as Trichinella occupies the muscle cells without killing them.

To successfully complete their life cycle, *Trichinella* have developed specific mechanisms, in a great part involved in immunomodulation. Studying them will provide a valuable insight into the functioning of the immune system. Also, Trichinella products may be used as potential therapeutic agents to treat immunological disorders or autoimmune diseases. However, limited number of *Trichinella* proteins have been studied so far. This study investigates the immunomodulatory potential of recombinant multi cystatin-like protein (CLP) derived from *T. britovi* to determine whether CLP has anti-inflammatory properties in vitro. CLP is a highly antigenic glycoprotein present in *Trichinella* excetory-secretory (ES) products; its biological function remains unknown. It consists of three type-two cystatin-like domains separated with oligopeptide linkers.

Mouse CD11b+ splenocytes, CD11b-depleted splenocytes, total splenocytes and bone marrow cells were stimulated in vitro with lipopolysaccharide (LPS) and with recombinant CLP for 24, 48 and 72 hours. The culture supernatants were collected and tested for secreted cytokine levels using ELISA. The panel of cytokines tested included IFNγ, TNFα, TGFβ, IL-2, IL-6 and IL-10. CLP was found to reduce LPS-induced TNFα and IL-6 secretion, both being important proinflammatory cytokines, in some experimental groups. On the contrary, in some experimental groups, co-stimulation with CLP resulted in increased secretion of the regulatory cytokine IL-10. The obtained results indicate that CLP has immunomodulatory and anti-inflammatory properties and future research on its function is advisable, specifically in the context of CLP use in the therapy of inflammatory disorders.

Financial support for this study was provided by the National Science Centre Poland (Grant MINIATURA 2020/04/X/NZ6/00084).

The *Trichinella britovi* recombinant proteins as potential diagnostic markers of trichinellosis in animals

Sylwia Grzelak, Anna Stachyra, Justyna Bień-Kalinowska

Witold Stefański Institute of Parasitology, Polish Academy of Sciences, Warsaw, Poland

Trichinellosis, a disease caused by infection with *Trichinella* spp., poses a recurrent health problem for animals and humans worldwide. It is ranked twelfth most common human zoonosis from European Union countries. It results from ingestion of raw or improperly-thermally processed meat infected with encysted muscle larvae (ML). The International Commission on Trichinellosis (ICT) recommend that ML excretory-secretory (ES) products of *T. spiralis* are appropriate for infection diagnosis. Nevertheless, ES-based serological tests have been found to demonstrate cross-reactivity with antigens shared with other parasites and pathogens. Discovering the new diagnostic tests may be achieved by identification and production of species- and stage-specific recombinant proteins of *Trichinella* genus which are recognized by the host antibodies after infection.

The aim of the study was to clone the protein-coding sequences identified earlier in *T. britovi* ES products: TbCTRL, TbES21 and TbHSP20, and to obtain recombinant proteins using a eukaryotic *Pichia pastoris* expression system. The subsequent goal was to verify immunodiagnostic potential of the produced proteins in sera samples of mice and pigs experimentally infected with *T. spiralis* or *T. britovi* species. To that end the abundances of specific IgG antibodies were measured in tested sera samples.

The rTbCTRL and the rTbES21 proteins were more effectively produced and stable than rTbHSP20. The results for mice sera indicated that rTbCTRL showed the highest IgG antibody level out of all antigens, whereas the IgG level for rTbES21 was significantly lower. Similarly, the CTRL occurred to be the most sensitive protein for serodiagnostic purposes in pig sera samples; anti-rTbCTRL IgG level increased at 41 days post infection (dpi) in animals infected with *T. britovi* and 45 dpi in those infected with T. spiralis. Following the multiple-antigen strategy in *T. britovi*-infected mice sera, the combination of rTbCTRL+rTbES21 antigens yielded a higher specific-IgG level than rTbES21 alone. The results were similar to protein which was used as a reference: rTbCLP. For pig sera the specific antibody response against combined proteins did not differ significantly from these against single antigens.

The multiple-antigen approach may be suitable for the development of Trichinella infection diagnostic tests. It may be concluded that rTbCTRL combined with different proteins is a promising approach, nevertheless further research is needed to approve its potential.

The usefulness of multiplex PCR for the detection of nematodes contaminations (*Ascaris, Toxocara, Trichuris*) in the sewage sludge

Katarzyna Goździk, Małgorzata Bednarska, Renata Welc-Falęciak

University of Warsaw, Department of Parasitology, Warsaw, Poland

In the concept of the circular economy, sewage sludge from municipal wastewater treatment plants should be reused, e. g. in agriculture and land reclamation, as organic fertilizers. Thus, shall not pose a threat to the environment nor human or animal health. According to Polish and EU legal regulations (Dz. U. Poz. 257, 2015; Directive 86/278/EEC) sewage sludge designed for agricultural use cannot contain live *Ascaris* spp., *Toxocara* spp. or *Trichuris* spp. parasite eggs.

The currently used reference method of analysis is the isolation of the parasites eggs from a representative sample of the sewage sludge and microscopic examination.

The aim of this study was to optimize the molecular method for the control of compounds utilized in the production of natural fertilizers from sewage sludge.

Multiplex PCR assay for the detection of *Ascaris* spp. *Toxocara* spp. and *Trichuris* spp. was developed based on primer pairs specific for three species.

Genomic DNA was isolated from adult worms and eggs of nematodes using QIAGEN DNeasy Kit. DNA concentration was measured by Qubit™ Fluorometer, Invitrogen.

The PCR reaction was performed in the total volume of 20 µl containing Thermo Scientific DreamTaq Green PCR Master Mix with DNA Polymerase and dNTPs and 1 µl of template DNA. To PCR reaction 0.5 µM of each primers were added. The optimal primer annealing temperature was empirically determined using gradient PCR.

To assess the specificity of the reaction, 1 ng of DNA from three species were applied in the PCR. Additionally, to determine the sensitivity of the assay serial dilutions of each parasite DNA from 1 ng to 10 pg were prepared and tested in the PCR.

The PCR products were separated on 1.5% agarose gel stained with GelRed. After the electrophoresis bands were visualized under UV light in ChemiDoc™ XR+ BIO-RAD and analysed in Image Lab 6.1.

The highest achieved sensitivity in heterologous DNA samples resulting in positive PCR runs was 10 pg genomic DNA.

Later on the method was tested on material obtained from two mechanical biological municipal water treatment plants.

Prior to molecular tests sewage sludge samples obtained from the wastewater treatment plant were prepared for microscopic examination according to the method recommended by Polish Standardization Committee PN-Z-19005:2018-10.

In conclusion, we have developed a molecular tool that provides identification of three species simultaneously. The achieved levels of specificity and sensitivity are adequate for epidemiological studies.

The project was funded by the University Technology Transfer Center.

Fasciola hepatica Fatty Acid Binding Protein 1 modulates T-cell polarization by promoting dendritic cell thrombospondin-1 secretion

Alicja Kalinowska[1], Joost M. Lambooij[2], Thiago A. Patente[2], Mateusz Pękacz[1], Maciej Łapiński[3], Katarzyna Basałaj[1], Bruno Guigas[4], Anna Zawistowska-Deniziak[1]

1 Witold Stefański Institute of Parasitology, Polish Academy of Sciences, Warsaw, Poland
2 Department of Parasitology, Leiden University Medical Center, Albinusdreef 2, 2333 ZA, Leiden, the Netherlands
3 International Institute of Molecular and Cell Biology in Warsaw, Warsaw, Poland
4 Department of Parasitology, Leiden University Medical Center, Albinusdreef 2, 2333 ZA, Leiden, the Netherlands

Infection with the liver fluke Fasciola hepatica is an emerging food-borne zoonosis. This parasitic trematode is extremely successful to evade the host immune defence through secretion of various immunomodulatory molecules. Fatty Acid Binding Protein (FABP) is among the main excreted/secreted proteins by F. hepatica and has been shown to display some anti-inflammatory properties. However, little is currently known on its impact on human dendritic cells (DCs) and their capacity to prime specific $CD4^+$ T cell subsets.

The immunomodulatory effects of *Pichia pastoris*-produced recombinant F. hepatica FABPs on monocyte-derived human DCs (moDCs) was assessed using a combination of experimental approaches, including DC-allogenic T cell co-culture and DC phenotyping through transcriptomic, proteomic and FACS analyses.

Following binding and internalization, FABP induced a tolerogenic-like phenotype in LPS-stimulated moDCs characterized by a dose-dependent increase in the cell-surface tolerogenic marker CD103 and IL-10 secretion while DC maturation markers were not affected. A decrease in DC secretion of IL-12p70 and IL-6 pro-inflammatory cytokines was also observed, also when stimulated with CD40L-expressing J558 cells. These effects were associated with an increase in both Th2/Th1 balance and IL-10 secretion by $CD4^+$ T cells following DC-T cell co-culture. However, the IL-10-expressing T cells neither had increased FOXP3 expression nor suppressive capacity, suggesting that FABP-conditioned DC did not prime classical regulatory T cells. Finally, RNA sequencing and targeted proteomic analyses allowed to identify thrombospontin-1 (TSP-1) as a non-canonical factor highly expressed and secreted by FABP-primed moDCs. Remarkably, the effects of FABP on T cell skewing was abolished when using aTSP-1 blocking antibody during DC-T cell co-culture. The TSP-1 is not induced by the other *F. hepatica* FABP isoforms and Sm14, the FABP *S. mansoni* homologue.

We showed that *Fasciola hepatica* FABP1 induces a tolerogenic-like phenotype in human moDCs and modulates T-cell polarization by promoting DC TSP-1 secretion. Further studies are required to explore the in vivo relevance of these findings in various models of inflammatory diseases.

Nematode-derived Galectin universal activity – influence of Teladorsagia circumcincta Galectin (Tc-Gal-1) on various species cells

Marta Maruszewska-Cheruiyot[1], Maja Machcińska[2], Katarzyna Krawczak-Wójcik[2], Ludmiła Szewczak[2], Magdalena Kierasińska[2], Michael Stear[3], Katarzyna Donskow-Łysoniewska[2]

1 Military Institute of Hygiene and Epidemiology, Warsaw, Poland
2 Laboratory of Parasitology, General Karol Kaczkowski Military Institute of Hygiene and Epidemiology, Warsaw, Poland
3 Agribio, Department of Animal, Plant and Soil Sciences, La Trobe University, Bundoora, Australia

Galectins are a group of proteins that bind β-galactosides and play key roles in inflammation, autoimmunity and cancer development. Parasitic nematodes produce galectins to modulate the host immune response and increase their survival. Parasite galectins show similarity in molecular structure to human proteins despite low overall sequence similarity. *Teladorsagia circumcincta*, the parasite of the sheep, produces galectin Tci-1-Gal that has a similar structure to human galectin-3 and -9.

Based on the similar structure of galectins, we hypothesised that Tci-1-Gal can affect tumor progression in mammals.

The aim of the study was to evaluate the influence of nematode galectin on EL4 – (a murine lymphoma cell line) and HeLa – (immortal human cells isolated from a cervical carcinoma).

Our preliminary experiments revealed that Tc-Gal-1 may influence tumor growth. We noted, increased proliferation of EL4 cells and sphere number and size in EL4 cells after exposure to Tc-Gal-1. However, apoptosis of EL4 cells slightly increased while Bcl-2 expression decreased.

Whether nematode-derived galectin promotes tumor progression needs more research, including investigation of the effect of Tc-Gal-1 on tumor cells. However, the effect of nematode galectins on cancer cells cannot be ignored.

This work was supported by grants: TEAM-TECH/2017-4/22 (Foundation for Polish Science), No.2016/23/B/NZ6/ (National Science Centre, Poland) and La Trobe University.

Vaccine potential of trivalent recombinant chimeric *Toxoplasma gondii* antigens

Maciej Chyb[1, 2], Bartłomiej Ferra[3], Malwina Kawka[4], Bożena Dziadek[4], Katarzyna Dzitko[4], Lucyna Holec-Gąsior[5], Justyna Gatkowska[4]

1 Department of Molecular Microbiology, Institute of Microbiology, Biotechnology and Immunology, Faculty of Biology and Environmental Protection, University of Lodz, Banacha 12/16, 90-237 Łódź, Poland
2 The Bio-Med-Chem Doctoral School of the University of Lodz, Łódź, Poland
3 Department of Molecular Biotechnology and Microbiology, Faculty of Chemistry, Gdańsk University of Technology, Gdańsk, Poland
4 Department of Molecular Microbiology, Institute of Microbiology, Biotechnology and Immunology, Faculty of Biology and Environmental Protection, University of Lodz, Łódź, Poland
5 Department of Molecular Biotechnology and Microbiology, Faculty of Chemistry, Gdańsk University of Technology, Gdańsk, Poland

Toxoplasmosis caused by the opportunistic, cosmopolitan protozoan *Toxoplasma gondii* is one of the most common parasitoses in the world. This parasite can pose a threat to people with immunodeficiency but also to the fetus, since the invasion can lead to miscarriages. Moreover this parasite can contribute to economic losses in livestock farming. There is currently no immunoprophylaxis for people against this parasite. These problems lead to the implementation of new, safe solutions for development of effective toxoplasmosis immunoprophylaxis, mainly thanks to the use of genetic engineering and *T. gondii* proteomics. The teams from the Department of Molecular Microbiology, University of Lodz and the Department of Molecular Biotechnology and Microbiology, Gdańsk University of Technology, have been working on obtaining new recombinant *T. gondii* antigens and testing them for serodiagnostics and immunoprophylaxis of this parasitosis. In this work, newly produced recombinant trivalent chimeric proteins of *T. gondii*, composed of SAG1-SAG2 antigens, and different terminal antigen; MAG1, MIC1, P35, ROP1, were tested in terms of their ability to induce an effective post-vaccination response, guaranteeing satisfactory immunoprotection. In the studies, mice of the C3H/HeOuJ strain were immunized with recombinant *T. gondii* chimeric proteins, which were administered after emulsification with incomplete Freud's adjuvant. Mice were vaccinated three times at two-week intervals, and then two weeks after the last dose mice were either sacrificed to assess selected parameters of the immune response or infected with *T. gondii* DX strain to determine the degree of protection one month later. The result of serological tests revealed high level of serum IgG antibodies specific for the native *T. gondii* TLA antigens. TLA-stimulated splenocytes produced cytokines important in inhibiting protozoal invasion. CD3 + CD4 + and CD3 + CD8 + T cell subpopulations of splenocytes were also analyzed by flow cytometry. One month after experimental infection mice were sacrificed, and their brains were isolated to count *T. gondii* tissue cyst. Reduction in cyst number is a marker of vaccination efficacy, which is used to calculate the percentage of protection. Immunization of mice with trivalent chimeric proteins of *T. gondii* resulted in protection rates reaching even 74%. The obtained results demonstrate strong immunogenicity of the studied proteins and will allow to select candidates for further research aimed at increasing the immunoprotective properties of experimental vaccines against toxoplasmosis based on *T. gondii* chimeric antigens.

The work was financed by the National Science Centre, Poland (grant UMO-2018/31/D/NZ6/02839).

Poster session

TLR4 receptor and their role in the expression during cutaneus acanthamoebiasis in immunosuppresssed mice

Agnieszka Wojtkowiak-Giera[1], Monika Derda[1], Joanna Walczykiewicz[1], Agnieszka Kolasa-Wołosiuk[2], Danuta Kosik-Bogacka[3], Piotr Solarczyk[1], Anna Słodkowicz-Kowalska[1]

1 Department of Biology and Medical Parasitology, Poznan University of Medical Sciences, Poznań, Poland
2 Department of Histology and Embryology, Pomeranian Medical University in Szczecin, Szczecin, Poland
3 Independent of Pharmaceutical Botany, Department of Biology and Medical Parasitology, Pomeranian Medical University in Szczecin, Szczecin, Poland

Free-living amoebae of the genus Acanthamoeba are cosmopolitan protozoans and pathogenic strain may cause infections in the brain, eye, lungs and skin. This infection are present in patient with immune disorders. This group includes patients infected with HIV and patients immunosuppressive therapy.

The aim of the study was to determine the expression of TLR4 receptor in the skin of BALB/c mice infected with Acanthamoeba strain AM55 isolated from a patient with acute myeloid leukemia (AML) and atypical pneumonia. The expression was assessed by real-time polymerase chain reaction (qPCR) and immunohistochemical reaction in the group of immunocompetent mice infected with Acanthamoeba and in the group of immunosuppressed mice treated 4 days earlier to infection with the immunosuppressive drug such as MPS (Methylprednisolone).

Our results presented that the expression of TLR4 receptor in the skin of immunocompetent mice infected with Acanthamoeba was statistical increased at 8 dpi compared to uninfected mice and also infected with Acanthamoeba and immunosuppressed mice.

In summary, increased the expression of TLR4 in skin of immunocompetent mice infected with Acanthamoeba suggested the involvement of this receptor during acanthamoebiasis.

However, in skin of immunosuppressed mice no observed statistically significant changes of the expression of TLR4. The above results provide new data about the expression of TLR 4 receptor in skin mice of the host's immune system during parasitic infection.

Gastric cancer – analysis of SMOX gene expression and connection with Helicobacter pylori infection

Magdalena Dzikowiec[1], Ewa Brzeziańska-Lasota[1], Dorota Pastuszak-Lewandoska[2]

1 Department of Biomedicine and Genetics, Medical University of Lodz, Łódź, Poland
2 Department of Microbiology and Laboratory Medical Immunology, Medical University of Lodz, Łódź, Poland

Carcinogenesis is a complex process with genetic and environmental factors involved. Increasingly, attention is paid to the participation of infectious agents in cancer formation and it is even estimated that they can cause up to 20% of all cancers. Understanding the mechanisms underlying the pathogenesis of gastric cancer is very important in the context of the search for new methods ensuring early diagnosis and treatment of gastric cancer.

We aimed to assess the expression levels of SMOX in the context of *Helicobacter pylori* infection in gastric cancer patients.

H. pylori is classified as a class 1 carcinogen, due to infection-based chronic inflammation that consequently lead to gastric cancer. The SMOX gene, induced by *H. pylori* infection, encodes the enzyme spermin oxidase, which is associated with oxidative damage to cell DNA.

The expression analysis of SMOX was performed by qRT-PCR method, in tissue samples obtained during gastrectomy from gastric cancer patients (n=32). Anti-H. pylori antibodies were assessed in the serum of cancer patients.

The results showed significantly increased expression of SMOX (RQ=2.87) in tumor tissues, slightly increased in the samples obtained 3 cm away from the tumor (RQ=1.2) and decreased in the tissue from the opposite to the tumor site (RQ= 0,84). Moreover, the level of SMOX expression was the highest in the tumor tissue of patients with the presence of anti-*H. pylori* IgG (RQ = 3.8), and the lowest in patients without previous infection in the tissue taken from the gastric wall opposite to the tumor site (RQ = 0.68). Also, the expression level of SMOX in tumor tissue was different depending on the history of *H. pylori* infection: in patients without infection the mean RQ was 1.52, and in patients with infection SMOX expression was increased almost 4 times (RQ = 3.8). The occurrence of anti-*H. pylori* IgG in the patients was compared with the stage of the tumor: in stage I group, 25% of patients had antibodies, in stage II – 77.8% and in stage III – 80%.

In conclusion, we found statistically significant differences in SMOX expression levels between different studied gastric tissue samples, negative correlation between SMOX expression and tumor stage, and positive correlations between the presence of anti-*H. pylori* IgG and the stage of tumor development as well as the size of the primary tumor.

Our results confirm the significance of *Helicobacter pylori* infection in gastric carcinogenesis, and also the role of spermin oxidase.

SESSION 2

Parasitological diagnostics, prevention, drugs and treatment

Oral session

Natural compounds in the control of *Dermanyssus gallinae*

Monika Roczeń-Karczmarz[1], Marta Demkowska-Kutrzepa[1], Paulina Leśniak[1], Leszek Guz[1], Krzysztof Puk[1], Klaudiusz Szczepaniak[1], Marta Studzińska[1], Krzysztof Tomczuk[1]

Department of Parasitology and Invasive Diseases, Department of Parasitology and Fish Diseases, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Lublin, Poland

Dermanyssus gallinae is one of the most important hematophage ectoparasites found in the laying hens industry. Dermanyssus infections are observed in many countries worldwide and they have a huge impact on the world economy bringing colossal economic losses. The presence of the infestation of *D. gallinae* in the staffing of the chicken coop causes a decrease in the production parameters of hens, the number and size of eggs laid decreases, and the amount of feed consumed by the birds increases. Additionally, hens peck and scratch each other, and there is self-mutilation and cannibalism.

Among the compounds used for the global control of *D. gallinae* population are chemical acaricides. New compounds containing diatomaceous earth, kaolin, silica as well as new acaricides belonging to the phoximes group are constantly appearing. There is growing concern about harmful residues found in eggs and chickens, as well as increased resistance of mites to several compounds. None of the chemical compounds is 100% effective and recurrent invasions are still observed. Products naturally occurring in nature have many advantages, including low toxicity to mammals, short environmental persistence and reduction of pest resistance development. Our in vitro tests have shown promising activity of essential oils against *D. galline*.

The aim of study was to find further effective natural compounds for the control of *D. gallinae*. The *in vitro* activity of ten water extracts on adult mites was assessed: *Allium sativum*, plant of *Eclipta alba*, *Dysphania ambrosioides*, *Artemisia absinthium, Cichorium intybus, Cannabis sativa, root of: Arctium lappa, Cichorium intybus, Coptidis chinensis*, herb with inflorescence of *Cannabis sativa*. The extracts were assessed by the method of Zdybel et al. For each plate containing the disc dripped with an extract a mortality rate of mites was calculated, with the correction taking the mortality in the control group into consideration (Abbott correction). An average constituted the final count from two repetitions.

Only the *Cannabis sativa* extract significantly decreased the survival rate of *D. galline*. The effectiveness of the operation in this case was 56.3%. Artemisia absinthium extract reduced the survival rate of mites and was effective in 41.7%. The remaining extracts slightly decreased the survival rate of *D. gallinae.*

The extracts used do not meet the expectations as substances combating *D. galline.* There is a need to find a compound that will be neutral, maintain its killer properties in the environment for a long time and be safe for animals.

The Influence of Proton Pump Inhibitors and Histamine Receptor 2 Antagonists on *Blastocystis* ST3 and Selected Microorganisms of Intestinal Microbiota *In Vitro*

Małgorzata Lepczyńska[1], Ewa Dzika[1], Wenchieh Chen[2], Chien-Yu Lu[3], Joanna Korycińska[1]

1 University of Warmia and Mazury, Olsztyn, Poland
2 Technical University of Munich, Munich, Germany
3 Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan

Proton pump inhibitors (PPIs) and histamine receptor 2 (H2) antagonists are commonly prescribed medications for gastroesophageal reflux disease, gastric and duodenal ulcers, dyspepsia or Helicobacter pylori infection. An association between PPIs and the alteration of the gut microbiota has been reported. Blastocystis, the most common intestinal protozoan worldwide, occurs in both healthy and symptomatic people with gastrointestinal or cutaneous disorders, with controversial pathogenicity. The current study aimed to investigate the influence of PPIs and H2 blockers on the *in vitro* proliferation of selected intestinal bacteria, fungi, and protozoa.

Cultures of *Lactobacillus rhamnosus, Escherichia coli, Enterococcus faecium, Candida albicans,* and *Blastocystis* subtype 3 with three other numbers of cells were treated with different concentrations of respective medications in vitro, and the numbers of microorganisms were quantified and compared. During the treatment, the number of Blastocystis cells was counted in a Neubauer chamber under x400 magnification. The numbers of each bacteria and fungus cells were likewise assessed every 12 hours by cultivation on agar plates and the counting of colonies. The inhibition rates caused by the added agents were determined by the ratios of the microbial numbers between the treated groups and the untreated controls. The pH value was measured daily.

Pantoprazole and esomeprazole exerted a significant inhibition on *Blastocystis* and *C. albicans*, especially at higher concentrations, which were even more effective than metronidazole and clotrimazole, respectively. On the other hand, treatment with both proton pump inhibitors caused an increase in the proliferation of *L. rhamnosus*. Additionally, pantoprazole promoted the proliferation of *E. coli*. There was no influence of H2 blockers on the examined microorganisms.

PPIs, such as pantoprazole, can be a potential treatment in the prophylaxis or eradication of *Blastocystis* and *C. albicans*. The mode of action may include: 1) direct antiproliferation, since benzimidazole derivatives (PPIs) resemble antiparasitic drugs in structure, and 2) indirect regulation of the intestinal probiotic bacteria via an increase of the pH value. Due to the high safety and tolerability of proton pump inhibitors, they can be considered for the clinical treatment of intestinal protozoan infections as well as to regulate the homeostasis of gastrointestinal microbiota. The results of the current study are the first step in researching the mechanism of action of PPIs against Blastocystis ST3 and *C. albicans*, and in consequence the modification of modern pharmacotherapy against *Blastocystis*.

New synthetic compounds with nematicidal activity

Anna Bogucka-Kocka[1], Przemysław Kołodziej[1], Monika Wujec[2]

1 Chair and Department of Biology and Genetics, Faculty of Pharmacy, Medical University of Lublin, Lublin, Poland
2 Department of Organic Chemistry, Faculty of Pharmacy, Medical University of Lublin, Lublin, Poland

Parasitic nematodes are the cause of many diseases among people and domestic and farm animals. Currently, there is a lack of effective vaccines on the market, and drugs play a major role in fighting parasitic diseases. Unfortunately, the research of recent years shows that nematodes are becoming more and more resistant to commonly used antiparasitic drugs. Parasite resistance to drugs is due to, inter alia, the limited pool of available drugs and inappropriate practices in the use of antiparasitic drugs to treat both animals and humans. Therefore, it is very important to develop new therapeutic strategies and find effective nematicides.

As part of the research, the nematicidal activity of the newly synthesized triazole derivatives was assessed. Research on the activity of compounds was carried out according to the procedure patented in the Patent Office No. 232918 (Bogucka-Kocka A., Kołodziej P. 2019).

Among the tested new triazole derivatives, compounds showing a very high nematicidal activity were identified. The tested derivatives may become candidates for new nematicides in the future

This research was funded by the Statutory Funds of the Medical University of Lublin provided by the Polish Ministry of Science and Higher Education, grant number DS43 (ABK) and DS15 (MW). The research was also funded by National Science Centre, Poland, No. 2017/01/X/NZ6/01001 (PK) and under the "Innovation Incubator 2.0" (ABK).

Alternative methods of controlling intestinal nematodes in horses: *In vitro* studies.

Maria Studzińska, Klaudiusz Szczepaniak, Marta Demkowska-Kutrzepa, Monika Roczeń-Karczmarz, Krzysztof Tomczuk

Department of Parasitology and Fish Diseases, University of Life Sciences in Lublin, Lublin, Poland

Equine parasites have been an important subject of research for years. In recent years, the literature has indicated a higher prevalence of drug resistance among Cyathostominae. Our research confirms the limited efficacy of IVM against *Parascaris* spp. Also, there is a technical problem inherent in controlling infestations connected with the application of preparations for deworming horses. It can apply to animals kept in pasture conditions for most of the year. Therefore, alternative methods of controlling parasites are being sought using methods of natural medicine.

The goal of this study is to evaluate *in vitro* effects of infusions obtained from herbs: *Artemisia absinthium, Humulus lupulus, Anethum graveolens, Salvia officinalis, Tanacetum vulgare, Inula helenium, Thymus vulgaris, Acorus calamus, Achillea millefolium, Populus tremula* – against nematodes of the family Strongylidae.

Eggs for testing were obtained from the faeces of horses, which had not been dewormed for 12 months. Eggs were isolated using the sedimentation-flotation method as well as via decanting to clean them more thoroughly. Herbs at a concentration of 2 g/10 ml were heat-treated to obtain infusions. Eggs, after cleaning and standardization (50 eggs in 10 µl), were placed 50 eggs each in the wells of culture plates. Next, each well was supplied with 100 µl of individual infusions. The control group had tap water supplied. The development of eggs was evaluated on the 0, 1, 2 and 4 day of incubation.

Of all herbs used, the best effect was achieved using Inula helenium infusion. The effect of ovicidine (11% destroyed eggs and 87% with the eggs containing a dead larva) was visible already after 24 hours of the infusion administration. Comparable results were obtained by means of Populus tremula infusion (8% destroyed eggs and 74% containing a dead larva). In the remaining cases, after 24 hours living larvae of Cyathostominae were observed. In the samples involving *Tanacetum vulgare, Thymus vulgari,* and *Artemisia absinthium*, after 48 hours a large proportion of larvae were dead (83.5%, 76%, 55%, respectively). In the majority of samples in which larvae hatched after 96 hours of incubation, dead larvae were observed. In the samples involving an infusion from yarrow, hops and dill, living larvae were found (15%, 5%, 3.25%, respectively). The larvae in the control sample showed traits of viability at 100%.

The results indicate that alternative methods do not eliminate infestations but can reduce and be an adjunctive and additional factor in the control of nematodes.

Current parasite problems in small exotic and wild animals in veterinary practice in the West Pomeranian Voivodeship (2020–2022)

Karolina Rozbiewska[1], Agata Stapf[2], Izabella Rząd[3]

1 Veterinary office VETMEDIC, Szczecin, Poland
2 Department of Biological Sciences, Faculty of Sport Science in Gorzów Wielkopolski, Poznan University of Physical Education, Gorzów Wielkopolski, Poland
3 Institute of Marine and Environmental Sciences and Molecular Biology and Biotechnology Centre, University of Szczecin, Szczecin, Poland

Parasitological testing was conducted in 786 exotic animals belonging to 36 species: amphibians (*Trachycephalus resinifictrix* and *Ranoidea caerulea*), reptiles (*Pogona vitticeps, Eublepharis macularius, Correlophus ciliatus, Chamaeleo calyptratus, Varanus acanthurus, Phelsuma madagascariensis, Salvator merianae, Agama agama, Iguana iguana, Boiga dendrophila, Python regius, Morelia bredli, Orthriophis taeniurus, Python reticulatus, Heterodon nasicus, Pantherophis guttatus, Testudo horsfieldii, Testudo hermanni, Centrochelys sulcata, Geochelone carbonaria, Geochelone elegans, Trachemys scripta* and *Melopsittacus undulatus*), birds (*Pavo cristatus, Nymphicus hollandicus,* and *Melopsittacus undulatus*), and mammals (domesticated *Rattus norvegicus, Mesocricetus auratus, Phodopus sungorus, Chinchilla lanigera, Oryctolagus cuniculus f. domesticus, Atelerix albiventris, Cavia porcellus* and *Octodon degus*). In addition, 47 wild animals belonging to 4 species were tested: birds (*Falco tinnunculus* and *Columba livia f. urbana*) and mammals (*Sciurus vulgaris* and *Erinaceus europaeus*). Faeces were tested by the flotation method and by direct faecal smears. In some cases antigen tests for giardiasis or cryptosporidiosis were conducted as well. Parasitological diagnosis was supported by morphological and morphometric descriptions of the parasites to determine their taxonomic affiliation.

Among the exotic animals, parasites recorded in amphibians and reptiles included Protozoa, i.e. flagellates *Monocercomonas* sp. and *Giardia* sp., coccidia *Eimeria* sp., *Isospora amphiboluri*, *Isospora jaracimrmani* and *Choleoeimeria* sp., amoeboids *Entamoeba* sp., and ciliates *Balantidium* sp., as well as nematodes (Metazoa, Nematoda): Rhabditida (*Rhabdias* spp./*Strongyloides* spp.), Oxyurida (Pharyngodonidae), and Ascaridida (*Ascaris* sp.). Among birds, Indian peafowl were infected with *Eimeria* sp., *Ascaris* sp. and *Capillaria* sp. Mammals were infected with protists – the genera *Eimeria*, *Entamoeba* and *Giardia* and ciliates; nematodes *Aspiculuris tetraptera, Trichosomoides nasalis, Syphacia obrelata, Passalurus ambiguus,* and Ascarididae*;* and cestodes *Rodentolepis nana*.

Among wild animals, birds were infected with protozoa – *Cryptosporidium* sp. and *Eimeria* sp. – and nematodes *Tetrameres* sp. and *Ascaris* sp. Larvae of nematodes of the family Strongyloididae were found in Eurasian red squirrel, and nematodes *Capillaria* sp. in European hedgehog.

The purchase of a 'friend' from a good breeder does not guarantee its health, which was confirmed in our study by cases of numerous coccidia in rabbits from recommended breeders or nematode infection in Bearded dragons or Amazon milk frogs born in the care of respected breeders. It is important to protect animals against parasites, systematically test the faeces, and implement treatment in the case of infection. It is also important to raise awareness among pet owners and encourage them to have the animals tested for parasites, even if they show no symptoms of infection.

Bioactivity screening of antiparasitic drugs on *Anisakis simplex* using metabolomic approach

Iwona Polak, Robert Stryiński, Elżbieta Łopieńska-Biernat

Department of Biochemistry, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

Anisakiasis is one of the parasitic diseases of the digestive tract of animal origin (zoonosis). Recent research has shown that the total number of global anisakidasis cases (almost all anisakiasis) caused by the Anisakidae family was over 76,000 as of December 2017. Humans become accidental hosts of the invasive larvae of *Anisakis simplex* L3 after eating infected, raw or undercooked fish. These parasites pose a very serious threat to human health, as they can penetrate the mucous membranes of the digestive tract and cause acute gastrointestinal problems and severe allergic reactions. One way to combat parasitic diseases is the use of drugs. There are three main groups of antiparasitic drugs: macrocyclic lactones, imidothiazoles, and benzimidazoles. Each group acts on the parasite in a different way. Nevertheless, the efficacy of the drugs is not always satisfactory, and the response of the parasite to the drug may differ from the known mechanisms of action. An emerging problem is drug resistance, which is often related to the way the drug molecule is metabolized. To understand how the drug affects the parasite and to learn about the drug's metabolism and efficacy, 'omics' research is needed. Metabolomic studies allow evaluation of the effects of an external stressor (drug) on biochemical changes. Metabolomics is concerned with the metabolite composition of biological systems, and their dynamic responses to endogenous and exogenous stimuli are particularly well suited for exploring the holistic metabolic responses to infection. In this study, we show for the first time the metabolites of antiparasitic drugs (ivermectin, pyrantel, albendazole) present in larvae and culture medium after drug treatment of larval stage L3 of *A. simplex* using untargeted UPLC-TOF-MS.

We identified metabolites from L3 of *A. simplex* using online databases such as the Human Metabolome Database using the data of exact masses and MS/MS fragments. Further confirmation was obtained by comparisons with standards, including identification of retention times and MS / MS fragmentation patterns. The metabolomics study showed that drug treatment of *A. simplex* triggered metabolic changes in a variety of metabolic pathways, including oxidation and oxidative metabolism. The metabolites could serve as clues for disease treatment and for studies in which metabolomics led to useful biomarkers for metabolic processes. Differences in biotransformation pathways following antiparasitic treatment and changes between antiparasitic drugs used indicate the differential effects of each drug on *A. simplex*.

This work was supported by National Science Centre of Poland, grant no.2020/37/N/NZ9/03312.

Encephalitozoon cuniculi and inflammation – another risk factor for infection?

Marta Kicia[1], Bohumil Sak[2], Martin Kváč[2, 3], Magdalena Szydłowicz[1], Andrzej B. Hendrich[1], Żaneta Zajączkowska[1]

1 Department of Biology and Medical Parasitology, Wroclaw Medical University, Wrocław, Poland
2 Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic
3 Faculty of Agriculture, University of South Bohemia in České Budějovice, České Budějovice, Czech Republic

Microsporidia of the genus Encephalitozoon are intracellular pathogens infecting a wide range of animal species. The best described microsporidian species is *Encephalitozoon cuniculi*, which resides mainly in the small-intestine epithelium, but dissemination and systemic infections are also well known. Since *E. cuniculi* is an opportunistic pathogen, extraintestinal infections and severe symptoms it causes are of concern in immunocompromised hosts. On the other hand, as slow acting pathogens, microsporidia are often overlooked and underdiagnosed, resulting in the increased possibility of hidden infections, especially in immunocompetent individuals. Recently, patients with chronic inflammatory process seem to emerge as a new possible group at risk of *E. cuniculi* dissemination. It is possible that *E. cuniculi* could be activated from latent phase and replicate inside resting macrophages, where it can evade the immune response and be transported throughout the host. Therefore, the potential influence of inflammatory process on *E. cuniculi* dissemination should be considered.

Funding: National Science Centre, Poland (grant number DEC-2012/05/D/NZ6/00615; grant number 2020/39/O/NZ6/02313) and Grant Agency of the Czech Republic (grant number 17-12871S).

Platlate endothelial cell adhesion molecule type 1 (PECAM-1) in complicated malaria patomechanism – role in the clinical course and prognosis of malaria imported to Poland

Sszymon Paweł Nowak, Małgorzata Paul

Department and Clinic of Tropical and Parasitic Diseases, Poznan University of Medical Sciences, Poznań, Poland

Malaria ia a vector-borne parasitic disease caused by protozoa of the genus *Plasmodium* that is widespread in tropical and subtropical areas of the world. Due to rapid nature of pathological changes with a potentially severe clinical course, it always has a serious prognosis with significant risk of death in the course of severe multiorgan dysfunction.

Platlate endothelial cell adhesion molecule type 1 plays important role in pathogenesis of malaria in humans. It is responsible among others for sequestration of red blood cells, which finally leads to blockage capillary circulation in different organs. This mechanism is responsible for impairment of capillary circulation in various tissues and leads to their irreversible damage. Clinically acute kidney injury, coma, pulmonary oedema may be observed.

The first aim of the study carried out in Department and Clinic of Tropical and Parasitic Diseases, Poznan was to determine level of PECAM-1 in peripheral blood of polish patients and immigrants infected by *Plasmodium* spp. during their stay in tropical and subtropical endemic areas

The second aim was to correlate between PECAM-1 levels and the occurrence of clinical and laboratory indicators of complicated malaria, which are defined by WHO. Also determination between PECAM-1 and risk of poor prognosis of malaria infection in polish patients was assessed.

For the study group of 68 patients was enrolled, everyone was admitted to Clinic of Tropical and Parasitic Diseases, Poznan University of Medical Sciences with confirmed *Plasmodium* infection, imported from different endemic regions. Each patient was checked using epidemiological interview, malaria was confirmed using Giemsa stained thick drop and thin smears of capillary blood, basic laboratory investigations were taken, then level of PECAM-1 was check using ELISA test, and corelate with clinical and laboratory indicator of complicated malaria.

As a result of conducted study we observed statistically significant correlation between high level of PECAM-1 in serum of patients with complicated malaria. Statistically significant was also correlation between level of PECAM-1 and occurrence of cerebral oedema and acute kidney injury.

Our observations prove that PECAM-1 may be good candidate as a new biomarker of clinical course and prognosis of Plasmodium sp. imported infection, in non-endemic patients.

Applying nanoparticles to contact lens solutions for reduction of amoebic adhesion in terms of prevention of *Acanthamoeba* eye infections

Marcin Padzik[1], Edyta Beata Hendiger[1], Lidia Chomicz[1], Marta Grodzik[2], Jarosław Grobelny[3], Jacob Lorenzo-Morales[4], Gabriela Olędzka[1]

1 Parasitology Laboratory, Department of Medical Biology, Faculty of Health Sciences, Medical University of Warsaw, Warsaw, Poland
2 Department of Nanobiotechnology and Experimental Ecology, Institute of Biology, Warsaw University of Life Sciences, Warsaw, Poland
3 Department of Materials Technology and Chemistry, Faculty of Chemistry, University of Lodz, Łódź, Poland
4 Instituto Universitario de Enfermedades Tropicales y Salud Pública de Canarias, Departamento de Obstetricia, Ginecología, Pediatría, Medicina Preventiva y Salud Pública, Toxicología, Medicina Legal y Forense y Parasitología, Universidad de La Laguna, La Laguna, Spain

Acanthamoeba spp. are pathogenic, cosmopolitan amoebae capable of causing *Acanthamoeba* keratitis *(*AK) that can pose a significant threat to human health. AK can lead to complete loss of vision. Current treatments are ineffective and toxic to corneal cells. More than 90% of AK cases are related to irresponsible wearing and management of contact lenses. Even proper lens care does not provide complete protection against AK as these amoebae were found in contact lens solutions and contact lens storage containers. Adhesion of amoebae to the contact lens surface is the first step in developing the eye infection. To limit incidences of AK infections, it is important to enhance anti-adhesive activity of the most popular contact lens solutions. Silver nanoparticles (AgNPs) are known to be active agents against bacteria, viruses, fungi and protozoa. Their effectiveness against *Acanthamoeba* spp. has been also recently confirmed, especially with addition of a plant metabolites such as tannic acid. We already proved the anti-amoebic and anti-adhesive activity of AgNPs and tannic acid-modified silver nanoparticles (AgTANPs) against Acanthamoeba castellanii. In our recent studies we confirmed anti-adhesion potential of AgNPs and AgTANPs in combination with selected contact lens solutions against *Acanthamoeba* spp. on four FDA groups of contact lenses. The obtained results showed increased anti-adhesion activity of contact lens solutions with limited cytotoxicity effect, compared to contact lens solutions acting alone. We conclude that low concentrations of tested nanoparticles in conjunction with contact lens solution may provide a benefit in improving prevention of amoebae eye infections. However, there is still need for further studies on different pathogenic strains of *Acanthamoeba* to assess the adhesion of the cysts to the contact lens surface, and to reveal more comprehensive picture of the nanoparticles and contact lens solutions activity.

Acanthamoeba spp. in oral microbiome of patients with impaired immunity and malformations treated due to masticatory system disorders

Konrad Perkowski[1], Monika Łazicka-Gałecka[2], Wanda Baltaza[3], David B. Conn[4], Paweł J. Zawadzki[5], Małgorzata Zadurska[1], Lidia Chomicz[6]

1 Department of Orthodontics, Medical University of Warsaw, Warsaw, Poland
2 Independent Public Clinical Ophthalmology Hospital, Department of Ophthalmology, Medical University of Warsaw, Warsaw, Poland
3 Department of Public Health, Medical University of Warsaw, Warsaw, Poland
4 Department of Invertebrate Zoology, Museum of Comparative Zoology, Harvard University; One Health Center, School of Mathematical and Natural Sciences, Berry College, Cambridge; Mount Berry, United States
5 Clinic of Cranio-Maxillo-Facial and Oral Surgery and Implantology, Medical University of Warsaw, Warsaw, Poland
6 Department of Medical Biology, Medical University of Warsaw, Warsaw, Poland

Amphizoic amoebae of *Acanthamoeba* sp., common in natural environments are detected also on accessories in health facilities, in a hospital environment contaminating surfaces of various equipment, contact lenses and lens boxes, dialyzers, surgical instruments and dental irrigation units. Some strains of *Acanthamoeba* are able to enter human bodies and exist as the endozoic forms; they are found on the surfaces of skin, in paranasal sinuses and lungs. These worldwide facultative parasites may cause serious health risk as etiological agents of vision-threatening *Acanthamoeba* keratitis. The amphizoic amoebae were found in the human oral cavities in our examination of oral microbiome. In this study, the occurrence and interactions between opportunistic, potentially pathogenic and parasitic protists, components of the oral cavity microbiome were determined. The retrospective interdisciplinary study pertained to the results of oral microbiota examination in the people with malformations treated due to various masticatory system disorders. Data of 100 young people from 6 to 13-year-old and 14 to 23-year-old were evaluated. These patients categorized into four groups: treated with removable orthodontic appliances, treated with fixed orthodontic appliances, patients of control groups without orthodontic needs treated conservatively. Data of clinical evaluation for patient health status of periodontium, presence of inflammatory processes and tooth decay were assessed. From each person, swabs of the dental plaques, the periodontium and from dental pockets for parasitological, bacteriological and mycological examinations by the microscopic and in vitro culture methods were taken. Oral protists, opportunistic facultative parasitic *Acanthamoeba* spp. and bacterial and fungal strains were identified in the superficial layer of the oral biofilm. Significantly higher prevalence of potentially pathogenic microorganisms occurred in young people treated with fixed orthodontic appliances in comparison to those without orthodontic needs. Oral microbiota form multilayer biofilm with complex, still insufficiently investigated interrelations between particular species and the human organism. Oral cavity can act like a reservoir of microorganisms that can induce medical complications. The use of orthodontic appliances can impact oral cavity colonization with different opportunistic/ pathogenic strains. In patients with disabilities and impaired immunity, pretreatment assessment of components of the oral microbiome, as well as monitoring of potentially pathogenic microorganisms are particularly desirable to decrease the risk of health complications.

Severe course of *Acanthamoeba* keratitis incidents in Polish patients: assessment of factors affecting clinical, diagnostic and treatment management

Lidia Chomicz[1], Beata Szostakowska[2], Jerzy Szaflik[3], Justyna Izdebska[4], Wanda Baltaza[5], David B. Conn[6], Jacek P. Szaflik[7]

1 Department of Medical Biology, Medical University of Warsaw, Warsaw, Poland
2 Department of Tropical Parasitology, Faculty of Health Sciences, Medical University of Gdańsk, Gdańsk, Poland
3 Laser Eye Microsurgery Centre Clinic of prof. Jerzy Szaflik, Brand Med Medical Research Centre, Warsaw, Poland
4 Independent Public Clinical Ophthalmology Hospital, Department of Ophthalmology, Medical University of Warsaw, Warsaw, Poland
5 Department of Public Health, Medical University of Warsaw, Warsaw, Poland
6 Department of Invertebrate Zoology, Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA; One Health Center, School of Mathematical and Natural Sciences, Berry College, Mount Berry, GA, USA, Cambridge, Mount Berry, United States
7 Independent Public Clinical Ophthalmology Hospital, Department of Ophthalmology, Medical University of Warsaw, Warsaw, Poland

Acanthamoeba keratitis (AK) the devastating, progressive ocular disease can result in loss of visual acuity and blindness. Recently, serious human health threat generated by pathogenic strains of *Acanthamoeba* is emerging medical problem for the public health worldwide. In Poland, the sight threatening illness is still considered a rare disease that is insufficiently known, however, during the last decades, incidents of infections caused by Acanthamoeba strains are reported with constantly increasing frequency. This interdisciplinary study was performed to determine factors hindering correct diagnosis and exacerbating complicated AK course. AK incidents detected among Polish patients referred to our Clinics during ten-years period were analyzed. These cases included in the study presented challenges in terms of AK diagnosis and treatment. Usefulness of in vivo confocal microscopy and in vitro cultivation of corneal isolates from affected eyes were assessed on clinical, microbiological, parasitological,

morphological and molecular diagnostics in relation to keratitis course. The cases were assessed in aspects risk factors for AK. Apart of contact lens (CL) wearing, there were incidents associated with a swimming in the pool and lake, a showering in the CL, washing in a tap water, eye injuries. Assessment of scrapings from corneal epithelia examined for the verification of initial diagnoses indicated amoebic concomitant bacterial and fungal infections. There was a clear correlation between *in vitro* dynamics of Acanthamoeba strains, an advancement of AK and variable response to the therapy. Effects of molecular tests were in line with the identification of corneal isolate based on the morpho-physiology of cysts and trophozoites. The retrospective analysis of data on corneal strains deriving from complicated, difficult to diagnose and treat, Acanthamoeba keratitis incidences, showed the variability in duration of symptoms until proper diagnosis was performed, different effects of applied diagnostics as well as various in vitro dynamics /viability of particular Acanthamoeba strains. An improvement in duration from first symptoms until suitable diagnosis is crucial for the efficacy of the therapeutic management. Early suitable diagnosis, confirmed by detection of live amoebic developmental forms, involving in vivo and in vitro techniques is necessary for therapeutic efficacy. Initial confusing symptoms and misdiagnoses delaying the appropriate treatment contributed to a complicated course of AK. A reduction in time from a symptom onset to proper diagnosis is essential for improved prognosis Early and continued monitoring of *in vitro* dynamics of corneal strains is useful for prediction of AK course.

Poster session

Preliminary studies on the effect of insect repellent IR3535 on the feeding behaviour of *Dermacentor reticulatus* (Ixodida: Ixodidae) ticks from the south-eastern Polish population

Weronika Buczek, Alicja M. Buczek, Tomasz Lenartowicz, Katarzyna Bartosik, Alicja Buczek

Chair of Pharmacology and Biology, Department of Biology and Parasitology, Faculty of Health Sciences, Medical University of Lublin, Lublin, Poland

The wide spread and important role of *Dermacentor reticulatus* in the epidemiology of human and animal tick-borne diseases indicate the need for investigation of the sensitivity of this species to chemicals used in anti-tick prophylaxis. Therefore, this study investigated the behaviour of adult *D. reticulatus* on hosts at exposure to repellent 3-(N-acetyl-N-butyl) aminopropionic acid ethyl ester (IR3535, EBAAP), which is a component of many commercial products.

The study involved 102 adult *D. reticulatus* ticks, including 62 specimens (40 females and 22 males) intended for testing the repellent and 40 specimens (30 females and 10 males) in the control group. The behavioural tests of *D. reticulatus* were carried out on New Zealand white rabbits *Oryctolagus cuniculus* at room temperature of ca. 20±3 ^{0}C and ca. 60±5% humidity. The spray formulation (10% IR3535) was applied at 40-cm^2 area on the skin of each animal host. After 20–25 minutes, unfed *D. reticulatus* females and males were placed on each rabbit. The effect of the IR3535 on the ticks was assessed by counting the attached and unattached females and males at 30-min intervals for the first 3 h and next after 4, 5, and 24 h after the application of the repellent on the host skin.

The results revealed that 11.3% of the tested specimens attached to the host skin already after 0.5 h (control 7.5%). The number of attached specimens increased with time after the application of the repellent. After 1, 1.5, 2, 2.5, 3, 3.5, 4.5, and 24 h, ticks attached to the host skin constituted 22.6%, 40.3%, 35.5%, 51.6%, 53.5%, 66.1%, 70.0%, and 82.3%, respectively. In the control group, 20.0%, 25.0%, 30.0%, 40.0%, 40.0%, 70.0%, and 87.5% of ticks started feeding, respectively. The statistical analysis did not show a significant difference between the number of specimens attached after the repellent application and in the control group at the same time points of the experiments. A statistically significant difference in both groups was noted only at 4 h after placing the *D. reticulatus* ticks on the hosts ($p = 0.0095$).

Our results indicate that the 10% IR3535 solution does not repel *D. reticulatus* ticks from a studied population. Determination of the effectiveness of the repellent effect of IR3535 on ticks requires further studies on other populations of *D. reticulatus* and other tick species and the use of different concentrations of the 3-(N-acetyl-N-butyl) aminopropionic acid ethyl ester.

Occurrence of pathogenic intestinal protozoan parasites in asymptomatic school children from Balkan region

Aldona Gajek[1], Anna Lass[2], Karolina Baranowicz[2], Krzysztof Korzeniewski[3]

1 Department of Tropical Parasitology, Institute of Maritime and Tropical Medicine in Gdynia, Medical University of Gdańsk, Gdynia, Poland
2 Department of Tropical Parasitology, Institute of Maritime and Tropical Medicine in Gdynia, Medical University of Gdańsk, Gdynia, Poland
3 Epidemiology and Tropical Medicine Department in Gdynia, Military Institute of Medicine in Warsaw, Gdynia, Poland

Giardia intestinalis, *Cryptosporidium* spp., and *Entamoeba histolytica* are protozoans considered as the most common and important parasites connected with intestinal infections. Knowledge about their prevalence in Balkan countries is fragmentary; population living in Kosovo region in particular is poorly investigated. The aim of the study was to estimate occurrence of these parasites in asymptomatic school children living in the area of eastern and southern part of Kosovo.

Stool samples were collected in 2017 and 2018 from 844 asymptomatic children aged 1–15 living in the area of Gjilan and Ferizaj District of Kosovo. After isolation of DNA, samples were investigated using multiplex real-time PCR targeting fragments of small subunit (SSU) rRNA gene of *G. intestinalis* and *E. histolytica* and Oocyst Wall Protein (COWP) of *Cryptosporidium* spp. Positive results were subsequently confirmed with nested PCR and sequencing.

Pathogenic protozoan parasites were detected in 123 (14.78%) samples investigated, including presence of DNA of *G. intestinalis, Cryptosporidium* spp. and *E. histolytica* in 111 (13.34%), 6 (0.72%), and 4 (0.48%) samples, respectively.

Results of studies showed high level of parasitic infections among investigated children, and significant disproportion in the rate of infections between investigated regions, with Ferizaj district being more affected.

Tenebrio molitor – new farm animal that might be the potential carrier of parasites

Remigiusz Gałęcki, Tadeusz Bakuła

Department of Veterinary Prevention and Feed Hygiene, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Oczapowski Street 13, 10-719 Olsztyn, Poland, Olsztyn, Poland

The so-called "six-legged livestock" is mentioned as one of the most innovative ideas in recent years in the field of human and animal nutrition. Insects have been hailed by the scientific community as a milestone in the diversification of the global food chain as an alternative protein source for conventional food and feed substrates. Originally the mealworm (*Tenebrio molitor*) was considered only as flour and grain pest. Currently, this insect is included in the "Novel Food" law, and its potential can be used for the production of food and feed. It is also worth noting that large-scale breeding of this insect is developing in all European countries. It should also be pointed out that this organism is a model organism in parasitological research. The aim of the meta-analysis was to assess the potential of the mealworm as a potential carrier of parasites.

The conducted research indicates that this insect is an intermediate host and a mechanical vector of some parasites. Studies on this insect have shown the presence of protozoa (eg. *Cryptosporidium* spp.), tapeworms (eg. *Hymenolepis* spp.), nematodes (eg. Rhabditida). The conducted meta-analysis indicates that it may also be a carrier of parasites with allergenic potential (gregarines, mites). The research also suggests that the probability of parasites occurrence in these insects depends mainly on: the methods of breeding, the origin of the insects, compliance with biosecurity rules, sanitary conditions and the feed used. The mealworm has the potential to be an accidental parasite. This insect caused canthariasis in humans, pigs and birds.

This study indicates that *T. molitor*, whether as a storage pest or as a food/feed additive in the form of unprocessed mealworm larvae may pose a threat to humans and animals. Our results warn against using live larvae. Processed and heat-treated *T. molitor* larvae will not pose a direct threat to the security of food chains. However, there is a need to carefully evaluate insect processing methods, including temperatures and cooking / freezing times, to prevent possible parasitic infections. With the current development of the insect breeding sector, there is a need to develop legal and biosecurity guidelines to avoid contamination of this new farm animals with parasite developmental forms.

Research supported by a project "Lider XII" entitled "Development of an insect protein food for companion animals with diet-dependent enteropathies"; financed by the National Centre for Research and Development (NCBiR) (LIDER/5/0029/L-12/20/NCBR/2021).

Cross-resistance of *Candida* spp. – search for potential mechanisms

Katarzyna Góralska[1], Małgorzata Szybka[2], Filip Karuga[3], Dorota Pastuszak-Lewandoska[4], Ewa Brzeziańska-Lasota[3]

1 Department of Biology and Parasitology, Chair of Biology and Medical Microbiology, Medical University of Lodz, Łódź, Poland
2 Department of Microbiology and Medical Laboratory Immunology, Chair of Biology and Medical Microbiology, Medical University of Lodz, Łódź, Poland
3 Department of Biomedicine and Genetics, Chair of Biology and Medical Microbiology, Medical University of Lodz, Łódź, Poland
4 Department of Microbiology and Medical Laboratory Immunology, Chair of Biology and Medical Microbiology, Medical University of Lodz, Łódź, Poland

Azoles, such as fluconazole and voriconazole, are among the most commonly used antimycotics in the treatment of invasive candidiasis. In recent years, the increasing drug resistance of Candida species, including multidrug resistance, has become a huge challenge in antifungal therapy. This phenomenon may be due to the interaction of other drugs, e.g., anti-cancer drugs with multi-drug pumps in the fungal cell membrane, which also leads to the secondary resistance to antifungal drugs, but this issue remains unexplored. Research focused on modulators of fungal multi-drug transporters may prove to be crucial in counteracting fungal multi-drug resistance.

The aim of the study was to analyze the selected molecular mechanisms potentially responsible for the occurrence of cross-resistance in *Candida* spp. as a result of fungal exposure to methotrexate. We evaluated the expression levels of genes related to azole resistance in fungi: ERG11, coding for cytochrome P450 lanosterol 14α-demethylase enzyme (CYP51), and MDR1 and CDR1 genes encoding proteins of membrane transporters.

Seven strains of *Candida albicans* and nine strains of *Candida parapsilos* were used in the study. Susceptibility to fluconazole and voriconazole was tested using micro-dilution method according to EUCAST and KORLD recommendations. Subsequently, they were stimulated twice with methotrexate and the antimycotic susceptibility was re-determined. Using qPCR method, the expression levels (RQ values) of ERG11, MDR1 and CDR1 genes were determined for all strains before and after stimulation with methotrexate.

The average sensitivity of the tested strains before stimulation with methotrexate was 4.52 μg/ml for fluconazole and 2.07 μg/ml for voriconazole. After stimulation with methotrexate twice, the MIC value was 64 μg/ml for fluconazole and 3.03 μg/ml for voriconazole. The expression level of the analyzed genes was on average: RQ ERG11 = 1.38, RQ MDR1 = 1.63 and CDR1 = 2.78 before stimulation and RQ ERG11 = 0.97, RQ MDR1 = 2.10 and RQ CDR1 = 3.46 after stimulation with methotrexate. Detailed results will be presented during the conference.

As a result of methotrexate stimulation, all tested strains achieved complete resistance to fluconazole, and eight strains became resistant to voriconazole. The expression level of the ERG11 gene decreased due to the stimulation, and the expression levels of the MDR1 and CDR1 genes increased. The obtained data suggest that the mechanisms related to the MDR1 and CDR1 genes may be responsible for the occurrence of cross-resistance to azoles as a result of contact of *Candida* spp. with methotrexate.

Development of a chemiluminescent ELISA based on trivalent chimeric recombinant proteins for detection of specific *Toxoplasma gondii* antibodies in sera from different parasite hosts

Lucyna Holec-Gąsior, Bartłomiej Ferra, Karolina Sołowińska

Department of Molecular Biotechnology and Microbiology, Faculty of Chemistry, Gdańsk University of Technology, Gdańsk, Poland

Toxoplasmosis, caused by the protozoan parasite *Toxoplasma gondii*, is one of the most prevalent parasitic zoonosis worldwide. It has been estimated that nearly one third of the world human population has been exposed to this parasite. Moreover, toxoplasmosis is one of the most common parasitic diseases of farm animals and results in reproductive losses. Toxoplasmosis in humans is usually caused by eating raw or undercooked meat or consuming dairy products containing the parasite. Therefore, widely conducted diagnostics of this disease is very important in both human and livestock populations. Laboratory diagnosis of toxoplasmosis is mainly based on the results of serological methods detecting specific anti-*T. gondii* antibodies in sera of parasite hosts. Serodiagnosis is currently mainly based on the use of native antigens of parasite (TLA, *Toxoplasma* lysate antigen) obtained from peritoneal fluid of infected mice or from tissue cultures *in vitro*. Continuous development of scientific disciplines such as molecular biology and genetic engineering allows for the production of new chimeric recombinant proteins which are a very good alternative source of antigens used in serodiagnosis of toxoplasmosis.

In this study, we aimed to develop a chemiluminescent ELISA based on six trivalent chimeric proteins containing immunodominant regions of two surface antigens (SAG1 and SAG2) and six different dense granule proteins (GRA1, GRA2, GRA5, GRA6, GRA7, and GRA9) for detection of *T. gondii* specific antibodies in sera from different parasite hosts. The reactivity of individual chimeric antigens was analyzed in relation to the results obtained in IgG chemiluminescent ELISA based on TLA. All chimeric proteins were characterized by high specificity (between 95% to 100%), whereas the sensitivity of chemiluminescent tests was variable (between 87.5% and 100%). This data shows that the trivalent recombinant chimeric proteins have the ability to detect specific IgG antibodies in the sera of different parasite hosts with *T. gondii* infection and therefore could be an alternative to the TLA antigen used in serodiagnosis of toxoplasmosis. Moreover, application of a chemiluminescent detection method based on a luminol-H_2O_2-HRP system is rationalized by the high sensitivity, simplicity, low cost, and rapidity of light-emitting detection methods. The developed chemiluminescent ELISA based on trivalent chimeric proteins can be a promising serodiagnostic tool for the detection of *T. gondii* infection.

This research was funded by The National Centre for Research and Development, under the research project no. LIDER/34/0188/L-10/18/NCBR/2019.

The profile of gene expression related to the PIK3CA/AKT/mTOR pathway in pregnant women with seropositivity for IgG anti-*Toxoplasma gondii* antibodies

Przemysław Kołodziej[1], Janusz Kocki[2], Krzysztof Wiktor[3], Henryk Wiktor[3], Anna Bogucka-Kocka[1]

1 Chair and Department of Biology and Genetics, Faculty of Pharmacy, Medical University of Lublin, Lublin, Poland
2 Department of Clinical Genetics, Medical University of Lublin, Lublin, Poland
3 Chair of Obstetrics and Gynecology, Faculty of Health Sciences, Medical University of Lublin, Poland

Toxoplasmosis is a disease caused by the *Toxoplasma gondii* protozoan. This disease is particularly dangerous in pregnant women because during its course the parasite can cross the placenta and infect the fetus. As a result of invasion, there may be many complications and even death of the fetus. The diagnosis of toxoplasmosis is based mainly on immunoserological tests. Currently, there is a need to develop new diagnostic markers, including molecular markers.

The aim of the study was to analyse changes in the level of gene expression related to the PIK3CA/AKT/mTOR pathway in pregnant women with seropositivity for IgG anti-*Toxoplasma gondii* antibodies.

Blood was collected for the EDTA anticoagulant from pregnant women seropositivity to IgG antibodies against *Toxoplasma gondii* (the study group) and from seronegative pregnant women (control group). The mRNA was isolated from the lymphocytes obtained from whole blood using a column set according to the manufacturer's instructions. Thereafter, a reverse transcription reaction was performed to obtain cDNA. The relative method was used to determine changes in the expression level of genes. The RQ-PCR experiment was performed with the aid of an Applied Biosystems 7900.

The expression profile of the genes studied was obtained. The study of gene expression related to the PIK3CA/AKT/mTOR pathway showed differences in the level of gene expression between the studied groups

This research was funded by the Statutory Funds of the Medical University of Lublin provided by the Polish Ministry of Science and Higher Education, grant number DS43 (ABK) and MB15 (PK).

Endoparasites in selected species of birds of prey used for falconry

Maria Michalczyk[1], Agata Prątnicka[2], Rajmund Sokół[1]

1 University of Warmia and Mazury in Olsztyn, Faculty of Veterinary Medicine, Department of Parasitology and Invasive Diseases, Olsztyn, Poland
2 Scientific Circle of Parasitologists, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

For many years, people have been using birds of prey for hunting (falconry), and now for biological protection of airports, agricultural crops, for education and historical reconstruction. Falconers arrange birds for these tasks, keeping their innate hunting instincts and close contact with the environment. In addition, they use natural nutrition, mainly raw meat of various species of animals. This favors infecting birds with parasites, including endoparasites.

The aim of the study was to assess the occurrence of endoparasites in birds of prey used for falconry. The research material was collected in falconry aviaries in northern Poland. Fresh faeces samples (approx. 5–6 g) were collected from each bird immediately after defecation, 3 times with an interval of 2 days into plastic containers and stored in the refrigerator. A total of 21 samples were collected from 7 goshawks (*Accipiter gentilis*) (3 ♂ and 4 ♀ aged 1–4 years), 7 buzzards (*Parabuteo unicinctus*) (3 ♂, 3 ♀, one sex unknown, aged 1–5 years), 7 peregrine falcons (*Falco peregrinus*) (3 ♂, 4 ♀, aged 1–11). In the research were used the flotation method according to Fülleborn and the McMaster method (OPG / EPG). Each pooled sample was tested 5 times and the result was recorded as the mean. An interview with bird keepers was also conducted, in which they asked about the observed clinical symptoms. Results: in 8 (38%) of 21 (100%) samples tested, the developmental forms of parasites were detected: Eimeria spp. Oocysts in 2 samples (25%) (OPG 850 and 2300) in one falcon and ♀ hawk; *Ascaridia* spp. eggs in 4 samples (50%) in one ♀ hawk (EPG 3075) and one ♂ (EPG 375) and in 2 social buzzards, u ♂ (EPG 400) and ♀ (EPG 2950,as well as *Capillaria* spp eggs in 2 samples (25%) (EPG 2592) in 2 of the hawk (EPG 1875, 200). Developmental forms of parasites were more common in males (62.5%) of infected birds and in 1–2 years old young birds fed with fresh hunted by them victims. The interview shows that clinical symptoms were observed in 4 (50%) of the 8 infected birds, incl. nervousness, constant hunger, unusual fluctuations in weight, difficulty breathing, diarrhea, blood in the stool, and reluctance to contact the handler. The results of the research indicate that monitoring birds for endoparasite infestation is a necessary condition for maintaining good condition.

Parasites of domestic horses of Tori and Hutsul breeds from Western Ukraine and methods of their control

Alla Vyniarska[1], Tetiana Kuzmina[2]

1 S. Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv, Lviv, Ukraine
2 I. I. Schmalhausen Institute of Zoology NAS of Ukraine, Kyiv, Ukraine

Domestic horses are parasitized by more than 90 species of parasites. Despite of multi-year studies in parasite control methods, intestinal helminths still remain a significant problem for the horses health in Ukraine. The aim of our study was to investigate the distribution of different groups of parasites in horses of Tori and Hutsul breeds and to analyze their control methods in the Western Ukraine. During 2015–2019, 352 horses of Tori (n=103) and Hutsul (n=249) breeds from the the Lvivska and Zakarpatska regions of Western Ukraine were examined. Horses were kept under different horse-keeping conditions: Tori were kept in individual stables with access to pasture; Hutsuls were kept in stables with access to paddock. The level of horse infection was examined using the McMaster method with sensitivity of 25 EPG (eggs per gram of faeces). Information concerning parasite control methods and anthelminthics used was collected by interviewing of the owners. The efficacy of the anthelmintics was examined by the Faecal Egg Count Reduction Test (FECRT).

Only intestinal nematodes were found in horses of both Tori and Hutsul breeds; intestinal strongylids (Nematoda; Strongylidae) were dominant parasites. In Tori breed the level of infection by strongylids was from 25 to 1375 EPG; prevalence of infection varied from 70% (in 2016) to 94% (in 2017). Average level of horse infection was significantly higher in autumn than in spring. Level of the Tori horse infection with parascarids (presumably Parascaris equorum) varied from 25 to 1375 EPG; in 2019, extremely high level of 3700 EPG was detected in one young horse. Prevalence of *P. equorum* was from 7% (in 2015) to 47% (in 2018). In Hutsuls, the level of infection by strongylids was from 25 to 2650 EPG. Average prevalence of strongylid infection varied from 88% to 100%. Level of the Hutsul horse infection with parascarids varied from 25 to 5100 EPG with prevalence from 22% to 50%. The level of horse infection with intestinal nematodes depended on the horse-keeping conditions. In all horse farms, the Ukrainian anthelmintic drugs containing praziquantel and macrocyclic lactones (ivermectin, aversectin) were used. The efficacy of anthelmintics was high – from 95% (for praziquantel) and 98–100% (for macrocyclic lactones). No resistance in parasites to anthelminthics was detected in parasites of both horse breeds. The main method of horse parasite control in the western regions of Ukraine is the anthelmintic treatment; other parasite control methods are not used.

Prevalence of *Cryptosporidium* spp. among different groups of patients – are there alternative risk factors potentially predisposing them for *Cryptosporidium* infection?

Żaneta Zajączkowska[1], Martin Kváč[2, 3], Magdalena Szydłowicz[1], Andrzej B. Hendrich[1], Bohumil Sak[4], Marta Kicia[1]

1 Department of Biology and Medical Parasitology, Wroclaw Medical University, Wroclaw, Poland
2 Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic
3 Faculty of Agriculture, University of South Bohemia, České Budějovice, Czech Republic
4 Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic

In humans, *Cryptosporidium* is known mainly as the etiological agent of severe diarrhea and, less often, the cause of infection of the respiratory system among high-risk individuals, such as children from developing countries and immunosuppressed patients, particularly those with HIV infection. Currently, patients with pharmacologically induced immunosuppression, especially transplant recipients and oncologically treated individuals, are considered as a main group at risk of cryptosporidiosis. Since in immunocompetent people Cryptosporidium infection is usually limited to the gastrointestinal tract causing a self-limiting diarrhea or persisting asymptomatic, this group of patients is rarely in the field of interest.

Here we compare *Cryptosporidium* occurrence in different groups of patients, both immunocompetent and those receiving immunosuppressive treatment: (i) renal transplant recipients, (ii) cancer patients, (iii) patients with various pulmonary diseases, (iv) children with inflammatory bowel disease (IBD), (v) pre-school children. Samples from intestinal tract (stool or colon tissue) or respiratory specimens were screened using molecular methods, by nested-PCR amplifying *Cryptosporidium* SSU rRNA and 60 kDa glycoprotein genes, followed by sequencing and phylogenetic analyses. Microscopic methods were used to confirm the presence of the pathogen.

Cryptosporidium was identified in children with IBD (stool) and immunocompetent patients with neoplastic lesions: one suffering from colon adenocarcinoma (cancer tissue), and the second with lung hamartoma (bronchial washings). Microscopic observations revealed the presence of *Cryptosporidium* in all infected patients.

Our results emphasize that also other groups of patients, not only being under life-long immunosuppression, might be at risk of *Cryptosporidium* infections. Presence of pathogens in patients with neoplastic lesions and children with chronic inflammatory process suggests that pathologically changed tissue might be more susceptible for *Cryptosporidium* infection.

Low-Molecular-Weight Secondary Metabolites from Fungi: *Cerrena unicolor* as a New Proposal of an Effective Preparation against *Rhabditis* Nematodes

Marta Ziaja-Sołtys[1], Przemysław Kołodziej[1], Dawid Stefaniuk[2], Anna Matuszewska[2], Magdalena Jaszek[2], Anna Bogucka-Kocka[1]

1 Chair and Department of Biology with Genetics, Medical University of Lublin, Lublin, Poland
2 Department of Biochemistry, Maria Curie-Skłodowska University, Lublin, Poland

Infections of humans and animals with intestinal nematodes are a serious health and economic problem in the world. Depending on the species of the parasitic nematode, the course of the infection may differ. It is also common to be infected with several species of worms simultaneously, which increases the harmful effect on the host's organism.

Low cost of currently used anti-nematode drugs and easy access to them have resulted in the emergence of a growing phenomenon of nematode drug resistance, especially in those parasitizing farm animals. The remains of the massively used anthelmintics end up in food and the environment, posing a threat to human health. Therefore, new, natural, safe substances with nematocidal potential are sought. Ligninolytic fungus *Cerrena unicolor* is a rich source of biologically active substances.

The aim of the study was to investigate the nematocidal potential of extracellular, low molecular weight (< 10 kDa), secondary metabolite fractions isolated from idiophasic *C. unicolor* culture.

The studied mushroom fractions were prepared according to the procedure previously described by Jaszek *et al*. [Biomed. Res. Int. 2013, 2013, 497492] and Matuszewska *et al*. [Sci. Rep. 2019, 9, 1975].

Rhabditis sp. nematodes from the collection of the Department of Biology and Genetics were used as a research model. Nematode cultivation was carried out according to the method described in patent no. 232918 (Bogucka-Kocka and Kołodziej).

On the basis of the obtained results, it was found that the fraction containing metabolites with molecular weight < 10 kDa (CU-A) and the 0.02–1.5 kDa subfraction (CU-B) showed nematocidal activity. The tested substances caused the movement disorders of the nematodes caused by the paralysis of the back part of the nematodes body. The degree of body paralysis was proportional to the increase in the concentration of the mushroom fractions.

Summarizing, it can be considered that the low-molecular, extracellular fractions of the secondary metabolites of *C. unicolor* are a good research material for the search for new anti-nematode drugs.

The abstract was prepared on the basis of the article: Ziaja-Sołtys, M.; Kołodziej, P.; Stefaniuk, D.; Matuszewska, A.; Jaszek, M.; Bogucka-Kocka, A. Low-Molecular-Weight Secondary Metabolites from Fungi: *Cerrena unicolor* as a New Proposal of an Effective Preparation against Rhabditis Nematodes. Molecules 2022, 27, 1660.

SESSION 3

Animal parasitoses: parasitoses of domesticated animals; the role of wild animals in spreading parasitoses; parasites of aquatic environments

Oral session

Coprological survey of the wild ungulates in the Chornobyl Exclusion Zone

Kateryna Slivinska[1], Vitaliy Kharchenko[1], Denis Vishnevsky[2]

1 I.I. Schmalhausen Institute of Zoology of National Academy of Sciences of Ukraine, Kyiv, Ukraine
2 Chornobyl Radiation and Ecological Biosphere Reserve, Kyiv, Ukraine

In 2016 within the exclusion zone and the zone of unconditional (compulsory) settlement was established the Chornobyl Radiation and Ecological Biosphere Reserve. Through the density of ungulates and the threat of natural-focal diseases on the Reserve, parasitological studies are relevant.

There are 6 species of wild ungulates that live in the Reserve: elk (*Alces alces* Linnaeus, 1758), European roe deer (*Capreolus capreolus* Linnaeus, 1758), red deer (*Cervus elaphus* Linnaeus, 1758), wild boar (*Sus scrofa* Linnaeus, 1758); free population of the Przewalski's wild horses have lived since 1998 (*Equus ferrus przewalskii* Pol., 1881). There is non-press information about the activities of male bison from the adjacent territory of Belarus. All species of wild ungulates are characterized by an increase in the number of years after the accident, even with increasing numbers of wolves and other predators. Wild ungulates are the hallmark of the Chornobyl Reserve. Helminths are one of the main factors influencing their numbers.

Totally, 117 fecal samples from five species of wild ungulates were collected and examined to analyze available data on ungulate parasites in the Chornobyl Exclusion Zone during two field trips in August and October 2021. Using standard methods of parasitological examination (ovoscopy, sedimentation, and Baermann method), eggs/cysts of gastrointestinal parasites and larvae of pulmonary nematodes were detected during all study period – Ostertagia, Trichostrongylus, Haemonchus, Cooperia, Oesophagostomum, Trichuris, Bunostomum, Nematodirus, Ostertagia and Dictiocaulus spp. Only in August were recorded the „srongyle" type eggs, indicating parasitism of the roundworm Hyostrongylus /or Oesophagostom in wild boar and Dictiocaulus spp. In October, Coccidia was detected in red deer.

Trematode eggs were not recorded among the examined samples during the whole study period. It can be assumed that this is due to the dry period during August–October 2021, which is unfavorable for the development of trematodes.

Eggs of Strongylidae and *Parascaris equorum* were found in wild Przewalski's horses in August and October, and *Giardia coccidia* cysts in August.

The level of parasite infestation among the studied material was low. Due to the conditions of the gathering of the biological material in the field and the old (and dryness) of some collected samples, it should be assumed that some of the parasites could not be detected.

Consequently, there were identified 15 species/groups of parasites among the five species of wild ungulates in the Chornobyl Exclusion Zone. Further monitoring studies of the helminthological situation of the area are recommended.

The medical and epidemiological significance of the bloodsucking flies from the Tabanidae and Hippoboscidae families, and their potential role in the transmission of pathogens

Grzegorz Karbowiak, Joanna Werszko, Tomasz Szewczyk

Witold Stefański Institute of Parasitology, Polish Academy of Sciences, Warsaw, Poland

Studies on the infection of Hippoboscidae and Tabanidae flies by pathogens of transmission diseases were carried out in north-eastern Poland. Tabanidae and *Lipoptena* spp. were collected near the Research Station of the Institute of Parasitology of the Polish Academy of Sciences in Kosewo Górne (53°49'12" N 21°26'36" E) and forests in the Forest District of Strzałowo (53°46′ N, 21°27′ E). *M. ovinus* were collected from sheep in Podlaskie Voivodeship (Rzepiska 52°49′56″ N, 23°33′56″ E, Nowoberezowo 52°45′36″ N, 23°33′43″ E, Waliły 53°08′30″ N, 23°35′25″ E, Gródek 52°05′48″ N, 23°39′24″ E). The diagnosis of flies' infection with pathogens of transmission diseases was carried out using the PCR method, based on the amplification of specific gene fragments: for *Trypanosoma* spp. – 18S rDNA approx. 650 bp, primers TrypF 150 and TrypR 800 were used; *Bartonella* spp. – rpoB42, 850 bp, primers 1400F and 2300R; B. burgdorferi s. l. – fla B, 824 bp and 605 bp; *A. phagocytophilum* – 16S rDNA, 932 bp and 546 bp.

129 specimens of *M. ovinus* were collected. The presence of *Trypanosoma* spp., *Bartonella* spp. and *B. burgdorferi* s. l. were detected in 58.91%, 86.82% and 1.55% of the keds, respectively. *A. phagocytophilum* was not detected. The co-infections have been shown – *Trypanosoma* spp. + *Bartonella* spp. (50.39%), *Trypanosoma* spp. + *Bartonella* spp. + *B. burgdorferi* s. l. (55.0%). 118 individuals of *Lipoptena cervi* and 37 individuals of *L. fortisetosa* were collected and tested for infection with trypanosomes. The flies were collected from the hosts, and in the case of *L. cervi* 27 were winged, collected from the vegetation. Trypanosomes were detected in 42 (20.2%) of *L. cervi*, including 5 winged (18.5%) and in 18 (48.6%) of *L. fortisetosa*. 140 horse flies from the Tabanidae family were tested, belonging to 4 species: *Haematopota pluvialis*, *Tabanus distinguendus*, *T. bromius*, *T. maculicornis*. Infection with *A. phagocytophilum* was found in 19.0, 33.0, 33.0, 0.0%, respectively, and trypanosomes in 53.5, 16.7, 17.9, 60.0%, respectively.

The described result is the first in Europe confirmation of the presence of *A. phagocytophilum* in *H. pluvialis*, *T. bromius*, *T. distinguendus* (Tabanidae); it is the first confirmation in Poland of *Trypanosoma* spp., in *H. pluvialis*, *T. bromius*, *T. distinguendus*, *T. maculicornis* (Tabanidae), *Trypanosoma* spp. in *L. cervi* and *L. fortisetosa* (Hippoboscidae), and the presence of *Trypanosoma* spp., *Bartonella* spp. and *B. burgdorferi* s. l in sheep ked (*M. ovinus*). In the light of current knowledge, it is known that Tabanidae are a vector for *T. theileri* trypanosomes because they feed repeatedly. The role of Hippobioscidae is unclear. Detection of trypanosomes in winged forms suggests vertical transmission. Molecular analysis of *A. phagocytophilum* sequences detected in Tabanidae shows that they are pathogenic strains for humans and strains circulating among rodents and insectivores. At the present stage of knowledge, it is difficult to say whether Tabanidae are an effective vector of the pathogen or they are mechanically transmitted.

The role of ground beetles as intermediate hosts for *Mastophorus muris*

Joanna Nowicka[1], Daniela Antolová[2], Anna Bajer[3], Jerzy Behnke[4], Maciej Grzybek[1]

1 Medical University of Gdańsk, Gdańsk, Poland
2 Slovak Academy of Sciences, Košice, Slovakia
3 University of Warsaw, Warsaw, Poland
4 University of Nottingham, Nottingham, United Kingdom

Mastophorus muris known as cosmopolitan, gastric spiruid nematode requires an obligatory intermediate invertebrate host. Larvae can develop in stomach of Orthoptera, Dermaptera, Dictyoptera and Siphonaptera. Larvae of spirurid parasite may also develope in ground beetles (e.g. Geotrupidae). Bank voles are omnivorous rodents, especially herbivorous. However, their diet is occurred also with arthropodes and annelids. Natural sources of proteins from invertebrates are usually the most important during of breeding and lactation, when rodents have the greatest protein requirements. Beetles are considered to be reservoirs of infection for their occasional predators that include rodents.

Our objectives were to monitor the prevalence of *M. muris* in the ground beetles species (*Anoplotrupes stercorosus, Trypocopris vernalis*) found in three separated locations – Pilchy, Tałty and Urwirałt and to assess the potential role of ground beetles as intermediate hosts for *M. muris*. Beetles were dissected and then any parasites present were counted, recorded, transferred to a glass slide, and examined under light microscope. Found larvae were stored in 70% ethanol for molecular analysis – Polymerase Chain Reaction (PCR). Products of PCR reaction were observed by electrophoresis method. We detected *M. muris* larvae in 18 from 240 dissected beetles, with an overall prevalence of 7,5% (23,8% for Pilchy, 40,0% for Tałty and 26,3% for Urwitałt). We provided sequencing of *M. muris* genome and obtained 10 various sequences.

These results contribute to our understanding of the abundance of *M. muris* in ground beetles in Poland and confirm that *M. muris* circulates in *A. stercorosus* and *T. vernalis*. Therefore, they may potentially play a role as reservoirs of this parasite in the sylvatic environment.

This research was funded through the 2018–2019 BiodivERsA joint call for research proposals under the BiodivERsA3 ERA-Net COFOUND programme. JN and MG were supported by the National Science Centre, Poland, under the BiodivERsA3 programme (2019/31/Z/NZ8/04028).

Coprological study on endoparasites of alpacas kept in Poland; comparison of two centrifugal techniques

Anna Maria Pyziel-Serafin[1], Adrianna Raboszuk[1], Daniel Klich[1], Blanka Orłowska[1], Dagmara Sierociuk[2], Krzysztof Anusz[1]

1 Warsaw University of Life Sciences – SGGW, Warsaw, Poland
2 Specialised Veterinary Office "Alpawet", Jabłonna, Poland

Alpaca (*Vicugna pacos*) is a species of ruminants belonging to the order of the cloven-hoofed camelid family, native to South America. One of the main problems in their breeding is infestation with parasites. The aim of the study was to identify species of parasites in alpaca herds living in the farm conditions in Poland and estimate the parasite loads in alpacas faeces kept in Poland with the use of two centrifugal flotation methods. In 2021/2022, a parasitological study of 248 samples from alpacas kept in 43 farms located in 12 provinces in Poland was carried out. Modified Willis (WM) and Stoll methods (SM)were applied simultaneously on 59 samples. The most prevalent findings were Trichostrongylidae eggs in WM and Eimeria spp. oocysts in SM. The following four parasitic eggs found in both methods were *Nematodirus* spp., *Nematodirus battus*., *Capillaria* spp., and *Trichuris* spp. Oocysts of *E. macusaniensis* and eggs of *Moniezia* spp. tapeworms were the rarest under WM, as were the oocysts of *E. macusaniensis* under SM; the eggs of *Moniezia* spp. were absent in SM. The prevalence of *Eimeria* spp. oocysts was found to be significantly higher with the use of SM than WM ($p< 0.004$); however, the prevalence of *Nematodirus* spp. and Trichostrongylidae eggs was significantly higher with WM than SM ($p = 0.000$, and $p = 0.006$, respectively). No significant differences were observed between the two methods regarding the prevalence of the remaining parasitic eggs and oocysts ($p> 0.05$). The general prevalence of GINs was found to be 72.9% (43 positive samples; WM) and 52.5% (31 positive samples; SM).

Sever, fatal mange in the Huacaya alpaca (*Vicugnia pacos*) – a case report

Blanka Orłowska[1], Grzegorz Karbowiak[2], Joanna Werszko[2], Mateusz Pękacz[2], Anna Rodo[3], Wojciech Bielecki[3], Anna Pyziel-Serafin[4], Krzysztof Anusz[4]

1 Department of Food Hygiene and Public Health Protection, Institute of Veterinary Medicine, Warsaw University of Life Sciences (SGGW), Warsaw, Poland
2 Witold Stefański Institute of Parasitology, Polish Academy of Sciences, Warsaw, Poland
3 Department of Pathology and Veterinary Diagnostics, Institute of Veterinary Medicine, Warsaw University of Life Sciences (SGGW), Warsaw, Poland
4 Department of Food Hygiene and Public Health Protection, Institute of Veterinary Medicine, Warsaw University of Life Sciences (SGGW), Warsaw, Poland

Alpacas (*Vicugna pacos*), as well as llamas (*Lama glama*), guanacos (*Lama guanicoe*) and vicugnas (*Vicugna vicugna*) are members of the Camelidae family, originating from South America (the South American Camelids – SAC). In the last three decades, they have become increasingly popular in Europe due to their unique fiber, mild temper, and the possibility of using them in a therapy (alpacotherapy), among others. One of the most common health problems of alpacas are parasitic diseases. Mange is one of the most important skin diseases in alpacas. This disease can be caused by a range of mite species, such as the burrowing *Sarcoptes scabiei* and the non-burrowing *Chorioptes* spp. and *Psoroptes* spp.

This study aimed to present a severe, fatal case of mange in a Huacaya alpaca.

A 12-month-old female Huacaya alpaca, was sent to the veterinary clinic because of sudden weakness. The animal died during the transport and was sent to the Department of Pathology and Veterinary Diagnostics of Faculty of Veterinary Medicine of Warsaw University of Life Sciences for the post-mortem examination. The animal was emaciated and had severe skin lesions almost all over the skin. Hyperkeratosis of the skin, crusts and alopecia around the mouth, ears, eyes, on the abdomen, groin and around the hooves were observed. Samples for the parasitological and histopathological examination were taken. Skin scrapings and skin fragments were collected from the area of head, back, legs, and abdomen. This samples were treated with 10% KOH. Then samples underwent centrifugation-flotation technique in sucrose solution and were viewed under the microscope. Mites were morphologically identified. Sarcoptic and chorioptic mange were diagnosed.

Mange in alpacas can be a very serious problem. Treatment is challenging and can last a long time. Anti-mange pour-on drugs are poorly distributed, possibly due to the lack of lanolin in alpacas or the hyperkeratosis associated with the disease. Systemic treatments often require multiple administrations. Cure is not always achieved.

Grassland versus woodland dwelling rodents as indicators of environmental contamination with the zoonotic nematode *Toxocara canis*

Martyna Krupińska[1], Daniela Antolová[2], Katzarzyna Tołkacz[3], Aneta Strachecka[4], Aleksander Goll[1], Joanna Nowicka[1], Katarzyna Baranowicz[1], Jerzy M. Behnke[5], Maciej Grzybek[1]

1 Medical University of Gdańsk, Gdańsk, Poland
2 Institute of Parasitology of SAS, Košice, Slovakia
3 University of Warsaw, Warsaw, Poland
4 University of Life Sciences in Lublin, Lublin, Poland
5 University of Nottingham, Nottingham, United Kingdom

Toxocara canis is a cosmopolitan nematode parasite of carnivores, notably canids, both wild and domestic, which act as definitive hosts. Small mammals are suspected of contributing to the dissemination of *T. canis* and helping with the parasite's survival during periods when there is a temporary absence of suitable definitive hosts. They can also play a role as indicators of environmental contamination with Toxocara. While the primary aim of the current study was the assessment of seroprevalence of *Toxocara* spp. infections in wild rodents in Poland, we also explored the role of intrinsic (sex, age) and extrinsic factors (study site, year of study) influencing the dynamics of this infection to ascertain whether grassland versus woodland rodents play a greater role as indicators of environmental contamination with *T. canis*.

We trapped 577 rodents belonging to four species (*Myodes glareolus, Microtus arvalis, Microtus agrestis, Alexandromys oeconomus*) in northeastern Poland. Blood was collected during the parasitological examination, and serum was frozen at –72°C until further analyses. A bespoke enzyme-linked immunosorbent assay was used to detect antibodies against *T. canis*.

We found *T. canis* antibodies in the sera of all four rodent species with an overall seroprevalence of 2.8% [1.9–4.1]. There was a significant difference in seroprevalence between vole species ($\chi^2_3 = 28.6$; $P < 0.001$), with the grassland species (*M. arvalis, M. agrestis,* and *A. oeconomus*) showing a 16-fold higher seroprevalence (15.7% [8.7–25.9]) than the forest-dwelling, M. glareolus (0.98% [0.5–1.8]).

We hypothesis that the seroprevalence of *T. canis* differs between woodland and grassland rodents because of the higher contamination of grasslands by domestic dogs and wild canids. Our results underline the need for wide biomonitoring of both types of ecosystems to assess the role of rodents as indicators of environmental contamination with zoonotic pathogens.

Multispecies reservoir of *Spirometra erinaceieuropaei* (Diphyllobothridae) in carnivore community and factors affecting its spread in NE Poland

Marta Kołodziej-Sobocińska[1], Eliza Kondzior[1, 2], Tomasz Borowik[1], Rafał Kowalczyk[1]

1 Mammal Research Institute of the Polish Academy of Sciences, Białowieża, Poland
2 University of Bialystok, Bialystok, Poland

Sparganosis is a zoonotic infection caused by larvae (spargana) of *Spirometra* tapeworm with a complex life cycle including definitive, intermediate, and paratenic hosts. It's increasingly recognised as a dangerous parasitosis, also for humans. European studies on Spirometra were based so far on incidental reports. To investigate spread and rates of Spirometra infection in NE Poland, we necropsied 583 carcasses of nine carnivore species. *Spirometra* larvae were found in subcutaneous tissue of seven species including: raccoon dog, European badger, pine marten, red fox, American mink, European polecat, and river otter. The parasite was not found in Eurasian lynx and stone marten. It was genetically confirmed as *Spirometra erinaceieuropaei*. European badgers and raccoon dogs were characterized by the highest prevalence, and European badgers and American minks by the highest infection intensity. Native European badgers showed an increase in the probability of infection with age, while no such relationship was observed in invasive raccoon dogs. Spirometra spread analysis showed a spatially diversified probability of infection with the highest values in the biodiversity hot spot, Białowieża Primeval Forest. Our study revealed that various mammal species can serve as *S. erinaceieuropaei* reservoir. The frequency and level of infection may differ between selected hosts and likely depend on several factors including host diversity and habitat structure in a given area. It may also possibly be shaped by duration of host-parasite co-evolution.

The study was financed by the National Science Centre, Poland project no. 2016/21/B/NZ8/02429.

Neosporosis in cattle herds in the Lublin province

Krzysztof Tomczuk, Klaudiusz Szczepaniak, Maria Studzińska, Marta Demkowska-Kutrzepa, Monika Roczeń-Karczmarz

Department of Biology and Fish Diseases, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Lublin, Poland

Since its first detection in cows in the 1980s, the invasion of *Neospora caninum* has become a global parasitizes that significantly limits the reproductive potential in the dairy cattle stages.

The aim of the research was to obtain data on the occurrence, causes and effects of *N. caninum* invasion in a traditionally agricultural region of Poland characterized by a large variety of herd sizes and animal keeping systems.

In the period from March to July 2020, the sera of 360 cows over 2 years of age from 40 dairy herds located in the Lublin province were tested with the ELISA test.

The population represented by the research consisted of 1,057 cows and 535 heifers in total. During the research, an interview was made regarding the age of the animals, the occurrence of abortions, the presence of dogs on the farm, including cowsheds, methods of disposal of placenta after childbirth, purchase of animals, including imported ones, and the manner of keeping animals.

The *N. caninum* serum blocking version: P00510 / 02 Institute Pourquier test was performed strictly according to the manufacturer's recommendations.

Infection with N. caninum was confirmed in 68 out of 360 tested animals (18.9%). The infected animals were confirmed in 15 out of 40 herds (37.5%) inspected. The prevalence of invasion in herds with neosporosis ranged from 25 to 75% (mean 43%).

Based on the interview data, risk factors were identified.

They are the dynamic development of breeding related to the use of all heifers for the renovation of the herd.

Also purchase of animals from farms of unknown invasive status, including imported ones. Keeping dogs on the farm with access to animal housing. Postpartum placenta was not properly protected against carnivores.

In herds with a high prevalence of *N. caninun* infestation, abortions and births of "weak" calves were more frequent.

The presented research proves the problem of cattle neosporosis in the Lublin province, but with a lower intensity than in other (Podlaskie voivodeship – 32% prevalence, 75% of infected herds) typically dairy regions of the country.

The differences probably result from the different dynamics of breeding development.

Neosporosis can be a serious problem especially in herds with high development dynamics, focused on high efficiency shortening the exploitation period of animals.

The presented research is a selected part of the cycle concerning neosporosis in cattle in Poland.

References available from the authors of the report.

Comparison of methods for detecting *Echinococcus multilocularis* eggs from dog stools

Emilia Załugowicz, Anna Lass, Karolina Baranowicz, Paweł Gładysz

Department of Tropical Parasitology, Institute of Maritime and Tropical Medicine in Gdynia, Medical University of Gdańsk, Gdynia, Poland

Echinococcus multilocularis is currently the most dangerous parasite in the Northern Hemisphere. Despite an increasing amount of research into the genetic diversity of this parasite, the circulation of genotypes between hosts and their implications remains unknown. Our research project consists mainly in estimating the genetic profiles of *E. multilocularis* in the final host and casual host, i.e. a human in highly endemic and non-endemic areas of Poland. The key issue of the project is to assess the relationship between the genetic profiles of the parasite and its ability to infect humans. Additionally, we are investigating the role of dogs in the epidemiology of *E. multilocularis* infections. Dogs can participate in the circulation of parasites in the environment in Poland, especially in endemic areas, and dogs assisting trackers and hunters may show a higher infection rate than domestic dogs. Domestic dogs in cities may be less infected with *E. multilocularis* than freely roaming rural dogs. We recognize that infected dogs can pose a potential risk to owners and that dogs can become infected with certain specific genetic profiles of the parasite.

The first phase of the study included comparative trials of all available methods of collecting and detecting *E. multilocularis* eggs from the faeces of dogs in shelters and dogs living in private households, both hunters and non-participants. Both direct recovery and detection of eggs in stool samples were compared with molecular methods such as Nested PCR, semi-nested PCR and Real-Time PCR. Based on these comparative studies, the most effective and sensitive method was selected and used for further research in the project. Stool samples were collected in 2021 from 298 asymptomatic dogs, (213 dogs from shelters, 85 dogs from private household).

E. multilocularis were detected in 18 (6,04%) from 298 samples, including presence of parasitic DNA in 6 (7,06%) from private dogs, and 12 (5,63%) from shelters dogs, respectively.

Results of studies showed that Nested PCR was most effective method from all molecular methods to detected parasite DNA from dog stool. Other results of studied showed that domestic dogs may be the same infected with *E. multilocularis* than freely roaming rural dogs, and can pose a potential risk to owners.

This work was created thanks to funds from grant no. 2020/37 / B / NZ7 / 03934 of the National Science Center, Poland, obtained under the OPUS 19 project.

The occurrence of *Trichinella* spp. nematodes in free-living animals in Poland – a never ending story

Aleksandra Cybulska[1], Aleksandra Kornacka-Stackonis[1], Justyna Bień-Kalinowska[1], Katarzyna Goździk[1], Władysław Cabaj[1], Tomasz Szewczyk[1], Zdzisław Laskowski[1], Aleksander Demiaszkiewicz[1], Marcin Popiołek[2], Maciej Grzybek[3], Maciej Wierzchoń[4], Bożena Moskwa[1]

1 Witold Stefański Institute of Parasitology Polish Academy of Sciences, Warsaw, Poland
2 Department of Parasitology, Wrocław University, Wrocław, Poland
3 Department of Tropical Parasitology, Institute of Maritime and Tropical Medicine, Medical University of Gdańsk, Gdynia, Poland
4 Poviat Veterinary Inspectorate in Nowy Dwór Mazowiecki, Nowy Dwór Mazowiecki, Poland

Free-living animals are reservoirs of parasites, including parasitic nematodes of the genus Trichinella, which occur in many omnivorous and carnivorous animals and humans. In Poland, foxes, wild boars, wolves, raccoon dogs and martens are the main reservoir of Trichinella in wildlife. The presence of 4 species of *Trichinella* (*T. spiralis, T. britovi, T. nativa* and *T. pseudospiralis*) has been confirmed in Poland. The increase in the population of native species and the appearance of alien species, as well as animals migrations may cause the spreading of Trichinella nematodes in the forest environment. The research aims to monitor free-living animals for the presence of Trichinella nematodes.

The study was conducted in various regions of Poland between 2013–2022. A total of 2946 animals of different species (fox, wild boar, raccoon, raccoon dog, badger, marten, American mink, muskrat, domestic dog, otter, beaver, bear, lynx, wolf, field mouse, ermine, polecat, squirrel, shrew, bank vole) were tested for the presence of *Trichinella* spp. larvae. Trichinella larvae were obtained by digestion in artificial gastric juice 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.

The presence of *Trichinella* spp. larvae was confirmed in 7.37% of the tested animals, including: 13.70% among foxes, 1.68% among wild boars, 2.25% among raccoons, 26.88% among raccoon dogs, 4.85% among badgers, 12.37% among martens, 28,5% among wolves and 20% among lynxes. The dominant Trichinella species in wildlife is *T. britovi* (84%), followed by *T. spiralis* (15%) and *T. pseudospiralis* (0.5%). *T. britovi* isolates obtained from raccoon dogs are 100% identical within partial sequence of CO1 gene; *T. pseudospiralis* isolate obtained from raccoon showed 99.76% identity to reference strain (ISS013).

The research conducted by our team is important from an epidemiological point of view due to the constant occurrence of Trichinella infection in free-living animals. Epidemiological monitoring of the occurrence of *Trichinella* spp. in the environment should be continued due to the maintenance of Trichinella infections in wildlife, especially in species protected by law (wolf, lynx) and invasive species (raccoon, raccoon dog).

The research was partly carried out within the projects of the National Science Centre: 2017/25/N/NZ7/02625 and 2014/15/B/NZ8/00261.

Molecular assessment of the species diversity of hookworms in Poland

Marta Demkowska-Kutrzepa[1], Monika Roczeń-Karczmarz[1], Izabella Rząd[2], Alicja Laska[3], Klaudiusz Szczepaniak[1], Maria Studzińska[1], Krzysztof Tomczuk[1]

1 Department of Biology and Fish Diseases, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Lublin, Poland
2 Institute of Marine and Environmental Sciences, University of Szczecin, Szczecin, Poland
3 Vet Planet sp. z o. o., Łomianki, Poland

Within the Ancylostomatidae family (hookworms), in Europe the most common species in dogs are *Uncinaria stenocephala* and *Ancylostoma caninum*. The analysis of the canine parasitofauna carried out in Poland in recent years showed the common occurrence of these nematodes with a prevalence of 2–75%.

According to the literature, in the parasitological diagnosis of hookworm invasions in dogs, the flotation method is routinely used, followed by the determination of the nematode species based on the morphometric characteristics of the eggs. However, this method does not allow unequivocal differentiation between the *U. stenocephala* and *A. caninum* species, so they are often described as Ancylostomatidae, treating both nematode species as one invasion. This phenomenon is due to the high morphological similarity of those hookworms' eggs.

Despite the fact that the nematode species specification is often omitted in the diagnosis of hookworms, the medical and veterinary significance of *A. canium* and *U. stenocephala* is different. These species differ in their invasiveness and pathogenicity, which has consequences for the anti-parasitic prophylaxis system.

The range of *A. caninum* covers countries with a mild climate and relatively high temperatures. Hence, this invasion is more extensive in the southern part of the continent than in its northern part. In Poland, with a temperate climate, even representatives of the *U. stenocephala* species, despite their relative resistance to low temperatures, show seasonal variability in the occurrence of infestation.

The studies conducted so far on the species spread of hookworms in dogs in Poland are based on coproscopic methods. Initial molecular studies on the species diversity of hookworms in the area of the Lublin Voivodship indicated the presence of only *U. stenocephala* in dogs. Therefore, the purpose of this study was to analyze the species diversity of Ancylostomatidae invasion in dogs in Poland.

1435 feaces samples of dogs from urban, rural and dog shelter from the northern, southern, eastern and western regions of Poland were examined. The study was conducted from April 2021 till June 2022 using flotation method and molecular analysis (PCR).

The results of coproscopic tests show that 314 (21,9%) were positive for parasites. Among the owned dogs, *Trichuris vulpis* invasions were dominant (rural 11/72 (15,3%) and 38/456 (8,3%) urban areas), while hookworms invasions were the most common in samples from dog shelters 74/907 (8,2%). Molecular analysis clearly indicated one species of hookworm, which is *U. stenocephala*.

Comparative assessment of two tick species composition: *Ixodes ricinus* and *Dermacentor reticulatus* removed from dogs in the urban area of Olsztyn (from 2009 to 2021)

Mirosław Mariusz Michalski

Department of Parasitology and Invasive Diseases, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

In Poland there are 21 species of ticks from the order Ixodida recognized as permanent elements of Polish fauna. The most widespread species in the country is *Ixodes ricinus*. The less common species is *Dermacentor reticulatus*, which is traditionally found in the eastern belt of the country. The ticks present in urban parks and suburban areas, especially *I. ricinus*, are a potential vector of diseases for domestic animals and of zoonoses, including anaplasmosis, bartonellosis, borreliosis and rickettsiasis. Dogs and cats can be considered indicators of geographic and temporal (seasonal) prevalence of the ticks. The aim of our study was to assess the infestation of dogs in Olsztyn with particular tick species during the period of their greatest life activity in the long-term cycle. The choice of the city was due to the large percentage of open green areas, as Olsztyn's forests occupy 21.2% of the city, while numerous parks, squares and green recreation areas add another 6.5%.

The material for the tests were ticks removed from dog patients at selected veterinary clinics from the area of Olsztyn, from May to the end of June. The research was carried out on thirteen consecutive years (2009–2021). From the dogs' medical history it appeared that, on their walks, they only used areas within the city. Removed arachnids were fixed and stored in 70% ethyl alcohol, and then determined in the laboratory of the Department.

During the thirteen-year study, among the collected 6.711 ticks 58% were *I. ricinus*, and 42% *D. reticulatus*. The per-year number of collected ticks (from May to the end of June) ranged from 333 (in 2021) to 829 (in 2016). The fewest were obtained in the first three years of research (2009–2011) and in 2021, and the highest in 2014–2016. In the case of *I. ricinus*, adult females (29.25%) predominated, compared to 28.67% nymphs. In turn, in the case of *D. reticulatus,* nymphs prevailed (22.47%) in relation to mature females (19.52%). In conclusion, the dominant species was *I. ricinus* over *D. reticulatus* (however, with a gradual increase in prevalence of the latter) and the general environmental potential of ticks in the studied area was clearly on the rise, until 2016. Further research will show whether the sudden and marked drop in the number of ticks in 2017 and 2021 will continue in the following years

Risk of exposure of dogs and domestic cats to tick infestation and their epidemiological role in the transmission of tick-borne disease pathogens in southern Poland

Anna Kocoń[1], Marek Asman[2], Joanna Witecka[2], Magdalena Nowak-Chmura[1]

1 Institute of Biology, Department of Zoology, Pedagogical University of Cracow, Cracow, Poland
2 Department of Parasitology, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia, Sosnowiec, Poland

The ecology of ticks and hosts in urban and peri-urban environments is critical to quantifying the parameters necessary for an initial risk assessment and identifying public health strategies to control and prevent tick-borne disease. The aim of the study was to compare the occurence of species of ticks collected from dogs and cats from two provinces in southern Poland, tick identity to species level, investigate the potential exposure (prevalence) to ticks of domestic animals, determination of tick seasonal occurrence on dogs and cats and determining the role that domestic animals play in the transmission of selected pathogens of tick-borne diseases. In 2017–2018, 2,777 ticks were collected in selected areas of the Małopolska and Silesian provinces: 2,643 *Ixodes ricinus* (95.2%), 107 *Ixodes hexagonus* (3.9%), 23 *Ixodes crenulatus* (0.8%), 3 *Dermacentor reticulatus* (0.1%) and 1 *Ixodes apronophorus* (0.03%), from 1,209 dogs and 399 cats in cooperation with veterinary clinics, animal shelters and from own collections. The maximum intensity of infestation in domestic dogs was 41 ticks per host, in cats was 24. Ticks on the hosts were mostly located around the head, neck and nape in dogs and in cats. From 155 ticks collected from the area of Zakopane and the surrounding area, the presence of *Anaplasma phagocytophilum* in 3.4% of *I. ricinus*, *Babesia microti* in 24.3% of *I. ricinus* and in 22.32% of *I. hexagonus* and *Toxoplasma gondii* in 3.7% of *I. ricinus* and in 5.5% of *I. hexagonus* and also 2 co-infections. On the other hand, in studies conducted in selected recreational and urbanized areas of the Małopolska and Silesian provinces, out of 130 cats and 339 dogs, from the collected 909 ticks, 207 *I. ricinus* females and 2 I. hexagonus females were included for molecular testing, the presence of A. phagocytophilum was found in 4.3% of *I. ricinus, B. microti* in 35.0% of *I. ricinus* and in 2 females *I. hexagonus*, *Borrelia burgdorferi* sensu lato in 3.3% of *I. ricinus* and 3 co-infections. Current results indicate that domestic companion animals may contribute to the circulation of ticks and pathogens in both recreational and urban areas. Considering the steady growth and geographic spread of the tick population, the control and protection of animals against tick attacks should be applied.

Occurrence of *Toxoplasma gondii* infection among free-living small mammals in the Lublin Province

Jacek Sroka[1], Angelina Wójcik-Fatla[2], Violetta Zając[2], Jacek Karamon[1], Maciej Kochanowski[1], Joanna Dąbrowska[1], Weronika Korpysa-Dzirba[1], Jolanta Zdybel[1], Weronika Piotrowska[1], Tomasz Cencek[1]

1 Department of Parasitology and Invasive Diseases, National Veterinary Research Institute, Puławy, Poland
2 Department of Health Biohazards and Parasitology, Institute of Rural Health, Lublin, Poland

Small mammals, as intermediate hosts for *Toxoplasma gondii* parasite, play an important role in persistence of this protozoan in sylvatic environment. This is because these animals are often part of the diet of carnivores and omnivores, leading to further parasite transmission.

The aim of the study was to assess the occurrence of T. gondii infection in the free-living rodents and insectivores from the Lublin Province, and to determine their potential importance in spreading this infection in the environment. The research was based on the detection of parasite DNA in animal tissue samples.

Sixty small rodents and insectivores (dead animals), belonging to 7 species, were collected from the Lublin Province. In total, DNA was extracted from 180 samples of internal organs: liver, kidney and lung using a commercial kit (QIAGEN). To detect *T. gondii* DNA, nested PCR and Real-time PCR, based on the amplification of B1 fragment gene were performed. To determine the clonal type of the parasite, selected DNA isolates were tested by RFLP-PCR using 12 additional genetic markers (SAG1, 5'SAG2, 3'SAG2, SAG3, AltSAG2, GRA6, BTUB, C22–8, C29–8, PK-1, L358 i APICO). The obtained amplicons were digested with restriction enzymes and the products were imaged on an agarose gel under UV light. The amplicons were also sequenced and analyzed using the *T. gondii* reference strains sequences.

Overall, 10 of the 60 tested animals (16.7%) were positive in nested and/or Real time PCR for *T. gondii*. In most of the PCR-positive animals (9 out of 10) *T. gondii* DNA was found in only 1 of 3 organs tested; in one rodent, positive results were found in 2 out of 3 examined organs. RFLP-PCR and sequence analysis revealed the presence of *T. gondii* clonal types II and III in most of the samples tested. However, 6 samples positive for the B1 gene were negative using additional markers.

The results of the study showed a high percentage (16.7%) of *T. gondii* infection among free-living small mammals from the Lublin Province and confirmed the important role of these animals as a reservoir and vector of this parasite in the environment.

The importance of parasitic monitoring in the diagnostics and the course of bovine tuberculosis in ungulates – potential influence on the tuberculosis diagnostics in European bison

Marta Gałązka[1, 2], Anna Didkowska[3], Krzysztof Anusz[3], Anna Pyziel-Serafin[3]

1 Department of Food Hygiene and Public Health Protection, Institute of Veterinary Medicine, Warsaw University of Life Sciences (SGGW), Warsaw, Poland
2 Warsaw Zoological Garden, Warsaw, Poland
3 Department of Food Hygiene and Public Health Protection, Institute of Veterinary Medicine, Warsaw University of Life Sciences (SGGW), Warsaw, Poland

Bovine tuberculosis (BTB) is re-emerging zoonosis posing a severe threat for red-listed European bison (*Bison bonasus*) which seems particularly prone to MTBC (*Mycobacterium tuberculosis complex*) infections. It is known that BTB and parasites co-infections in humans can influence the efficiency of diagnostics of BTB. While co-infections of BTB and parasites have been widely described in humans, the most reports in veterinary medicine refer to Fasciola hepatica invasion in cattle. The aim was to review the publications concerning BTB and parasite co-infections in ungulates in order to collect data on potential impact of parasite infections on the diagnostics efficiency and the development of BTB, which could be related to diagnostics in E. bison. The species of ungulates were selected based on high occurrence of BTB in the populations, relatedness of species and similar habitat to E. bison. Only few species met the criteria which were cattle (*Bos taurus*), African buffalo (*Syncerus caffer*) and wild boar (*Sus scrofa*). The following aspects were taken under consideration: impact of parasite invasions and anthelmintic treatment on the diagnostics, course and control of BTB, condition and mortality of the host, variation of parasite composition.

The most frequently considered F. hepatica invasion may affect the immune response and result in false negative tuberculin skin test and gamma-interferon (IFN-γ) assay in the Bovidae. One case of Fasciola gigantica and BTB co-infection in cattle also reported a slightly lower IFN-γ response to testing. Variety of research was conducted on African buffalo analyzing the impact of parasitic invasion on the host immune response, and similarly to cattle, it was noted the Th2 response was strongly stimulated while the Th1 response was impaired. Furthermore, data on the influence of gastrointestinal nematodes and BTB co-infections on detectability and dynamics of BTB, host's mortality rates, potential correlation with shifts in microbiota composition and consequences of deworming were analyzed. Available literature on parasite and BTB co-infections in wild boar provided data on *Metastrongylus* spp. and helminths invasions and their influence on the severity and progression of BTB, efficacy of the vaccine against BTB and consequences of anthelminthic treatment.

Due to lack of corresponding data on E. bison the obtained results may be used as a reference to improve the BTB diagnostic algorithm in E. bison, therefore we suggest conducting parasitological monitoring and undertaking research in this field.

Molecular epidemiology of *Cryptosporidium* infections in pigs in Poland

Agnieszka Kaupke[1], Zbigniew Osiński[2], Artur Rzeżutka[1]

1 Department of Food and Environmental Virology, National Veterinary Research Institute, Puławy, Poland
2 Department of Hygiene of Animal Feedingstuffs, National Veterinary Research Institute, Puławy, Poland

Several species of livestock animals are susceptible to Cryptosporidium infections but in pigs infections are characterized by an asymptomatic course with a low number of faecally shed oocysts. Although different studies reported Cryptosporidium occurrence in farm animals in Poland, its presence in pigs has received little attention so far. In this five-year cross-sectional population study (2014–2018) the prevalence of Cryptosporidium infections in healthy pigs up to twelve weeks of age was investigated. In total, 1596 pig faecal samples were collected from 267 pig farms located across all Polish provinces. The detection and identification of Cryptosporidium species was performed at the 18 small subunit ribosomal RNA (SSU rRNA) locus by conducting PCR-RFLP and nucleotide sequence analysis of the amplified DNA fragments. The GP60 nested-PCR was used for identification of *Cryptosporidium parvum* subtype.

The prevalence of Cryptosporidium infections in pig population in Poland was estimated at 61.3%. It varied between age groups of pigs and animals housed in different Polish regions. In the group of post-weaned pigs it was higher (63.4%) then in suckling piglets (54.6%). Farms from Lubelskie province were characterised by the lowest prevalence of Cryptosporidium infections (47.2%) while the highest 72.2% prevalence was observed in Świętokrzyskie. The infected animals were housed in 245 (91.7%) out of 267 monitored farms. Based on the RFLP analysis of 18SSU rRNA locus the following parasite species were detected: *Cryptosporidium scofarum, Cryptosporidium suis* and *Cryptosporidium parvum* strain of not identified subtype. Their occurrence was associated with asymptomatic infections. In the pig population aged up to 3 months, 638 (65.2%) of Cryptosporidium – positive animals carried *C. scrofarum*. It was detected in 194 (72.65%) farms from all provinces. *C. suis* was found in 140 (52.4%) and C. parvum only in one farm (0.37%). Mixed infections (*C. scrofarum/ C. suis/ Cryptosporidium* sp.) were not common; however, they were observed in 51 (5.2%) of the positive animals.

This molecular-based population study showed a high prevalence of asymptomatic Cryptosporidium infections in pigs in Poland caused by a host specific *Cryptosporidium* species. Furthermore, the sporadic finding of *C. parvum* is subsequent evidence that pigs could serve as a reservoir for this zoonotic protozoan parasite.

Poster session

Parasites of marten (*Martes* sp.) from Beskid Sądecki mountains

Marta Basiaga[1], Marek Wajdzik[2], Maciej Lesiak[3], Jerzy Kowal[1]

1 Department of Zoology and Animal Welfare, Faculty of Animal Sciences, Univeristy of Agriculture in Kraków, Kraków, Poland
2 Department of Forest Biodiversity, Faculty of Forestry, Univeristy of Agriculture in Kraków,, Kraków, Poland
3 "Kaban" Company, Kraków, Poland

The aim of the preliminary study was to investigate diversity of helminths parasitizing two species of martens inhabiting Beskid Sądecki mountains.

Post mortem exminations were conducted on 11 specimens: 10 Beech marten (*Martes foina*) and one Pine marten (*Martes martes*). All animals were harvested during hunting season 2020/2021. Seven species of helminths were noted in Beech marten: cestodes – *Taenia serialis, T. hydatigena* and *Mesocestides* sp. (larvae), as well as nematodes – *Crenosoma vulpis, Eucoleus aerophilus, Aonchotheca putorii* and *Molineus patens.* In Pine marten only *Eucoleus aerophilus* was stated.

The results obtained suggest that Beech marten plays important role in circulation of cestodes in the studied area.

Toxoplasma gondii in red foxes (*Vulpes vulpes*) from northern Poland. Preliminary results

Beata Biernat, Karolina Baranowicz, Anna Lass

Department of Tropical Parasitology, Institute of Maritime and Tropical Medicine, Medical University of Gdańsk, Gdynia, Poland

Toxoplasma gondii is a coccidian parasite with a worldwide distribution that infects a whole range of warm-blooded vertebrates: mammals and birds. As predators and scavengers, foxes represent a potentially sensitive indicator of the circulation of *T. gondii* in environments where humans co-exist. In humans, a higher seroprevalence is found in Latin America, parts of Eastern/Central Europe, the Middle East, parts of southeast Asia, and Africa. *T. gondii* infection has been reported many times in Poland among farm animals; however, the current status of infection among wildlife is little known. Red fox (*Vulpes vulpes*) is one of the most abundant species of wild carnivores in Europe. The present study aimed to determine T. gondii prevalence in red foxes hunted in two sites: Rzucewo (Puck County and community) and Pażęce (Kartuzy County, Stężyca community) in Pomorskie voivodeship (northern Poland). Fox carcasses were kept frozen at − 70 °C (in a freezer) and thawed for necropsy. Samples of brain and diaphragm muscles were collected from a total of 179 red foxes (96 from Rzucewo and 83 from Pażęce) during hunting season 2022 in Poland. Before DNA extraction, the tissue samples were prepared using ten freeze-thaw cycles (using a –70°C freezer and a water bath) to destroy the tissue cysts and improve the efficiency of DNA extraction. Afterwards, DNA extraction was performed using a commercial DNeasy Blood & Tissue Kit, Qiagen (Germany) according to the manufacturer's instructions. For specific detection of Toxoplasma gondii DNA real-time PCR assay based on *T. gondii* B1 gene84 was used (primers: ToxB-41F, ToxB-169R and probe ToxB 69P).

Out of a total of 179 examined red foxes, Toxoplasma DNA was detected in 12 foxes (6.7 %, +/– 95% CL 3.3–12.9), 4 (4.2 %, +/– 95% CL 0.9–13.6) in Rzucewo and 8 (9.6 %, +/– 95% CL 4.2–19.8) in Pażęce. Positive tests were detected in 7 diaphragm muscles and 5 brain samples. Simultaneous positive samples from the brain and diaphragm muscles in the same individual were not detected.

The Toxoplasma infection in red foxes presumably is the result of eating infected prey or infected carcasses left on the hunting grounds after the evisceration of shot animals. In addition, contamination of the surroundings with *T. gondii* oocysts and their possible transfer to other animals are also probable.

Molecular identification of parasites in the stool samples of Svalbard reindeer (*Rangifer tarandus platyrhynchus*) from Spitsbergen

Katarzyna Buńkowska-Gawlik, Aleksandra Rygas, Patrycja Jaroszek, Sszymon Rawiak, Bartłomiej Dudek, Agnieszka Perec-Matysiak

Department of Parasitology, Faculty of Biological Sciences, University of Wrocław, Wrocław, Poland

The Svalbard reindeer (*Rangifer tarandus platyrhynchus*) is a small subspecies of reindeer found exclusive to Svalbard area. This study aims to investigate co-occurrence of microparasites (protozoa and microsporidia) and intestinal nematodes in stool samples of Svalbard reindeer by identifying parasite species based on molecular analysis. Fresh fecal samples were collected from adult Rangifer tarandus platyrhynchus individuals in the southwest part of the Wedel Jarlsberg Land, Spitsbergen, Svalbard. Samples were collected in the late summer of 2016, between July 15th and July 20th. The DNA was extracted from thirty five fecal samples using Stool DNA Purification Kit (EURx) according to manufacturer's recommendations. All samples were molecularly analyzed to identify the species of nematodes as well *Cryptosporidium* spp. *Eimeria* spp., and *Enterocytozoon bieneusi* using different markers: ITS rRNA and 18S rRNA. PCR products were sequenced. PCR analysis of fecal samples targeting a fragment of ITS2 rRNA gene revealed the presence of *Ostertagia gruehneri* DNA in 25 of 35 samples (71.4%). Sequence analysis of the identified pathogens revealed the occurrence of all of examined microparasites. Co-infections with *Ostertagia*, *Eimeria* and *E. bieneusi* were found in the stool samples. The results indicate that Svalbard reindeer is reservoir of micro- and macroparasites.

Research is carried on due to permission given by the Governor of Svalbard (project RIS-ID 10604, Microbiological Diversity of the Arctic Biosystems).

Role of red foxes as a zoonotic reservoir of *Cryptosporidium* and *Giardia* in Warsaw parks

Dorota Dwużnik-Szarek[1], Mateusz Jackowiak[2], Anna Bajer[1]

1 Department of Eco Epidemiology of Parasitic Diseases, Institute of Developmental Biology and Biomedical Sciences, Faculty of Biology, University of Warsaw, Warsaw, Poland
2 Central Laboratory for Environmental Analysis-CentLab, Institute of Environmental Protection, Warsaw, Poland

The intestinal protozoa of *Giardia* and *Cryptosporidium* species are parasites of medical significance worldwide. Main symptoms of infection by both parasites include watery and profuse diarrhea, sometimes with traces of blood, leading to rapid dehydration, anemia, abdominal pain and flatulence. Infection usually occurs through fecal–oral route, by direct or indirect transmission. An individual may get infected through direct contact with an infected person or animal or by contact with contaminated soil, food or water (indirect route). Free-living animals probably have the most significant role in contamination of environment (soil, water, ect.) with cysts/ oocysts. One of *Cryptosporidium*/*Giardia* hosts is synanthropic red fox (*Vulpes vulpes*). However, the role of the red fox as a reservoir for these protozoa is still poorly understood. The role of the zoonotic reservoir in contamination of water/soil/food with dispersive forms of protozoa, therefore in indirect transmission, is considered relevant and of wide impact.

The aim of the study was estimating prevalence and intensity of infection of *Cryptosporidium* and *Giardia* in foxes from the green areas of Warsaw.

Twenty-nine fecal samples were tested from foxes mainly from Kabaty and Natolin Forests. PCR amplification was conducted employing two genetic markers: 18S rDNA and 60kDa glycoprotein gene (GP60) fragment for *Cryptosporidium* detection. In case of *Giardia*, genetic markers 18S rDNA and fragment of the glutamate dehydrogenase (gdh) gene fragment were used. Samples were also examined by IFA (MeriFluor *Cryptosporidium*/*Giardia* and the modified Ziehl-Neelsen (ZN) staining method.

Differences in prevalence between *Giardia* and *Cryptosporidium* infection were detected in examined samples. More, there were significant discrepancy in the outcome of different applied methods for *Cryptosporidium* spp. detection, with ZN technique yielding the highest prevalence.

The obtained results contribute to expanding the knowledge of zoonotic reservoir of *Cryptosporidium* and *Giardia*, which might help to determine epidemiological threat posed by these protists.

The study was financially supported by National Science Centre (NCN) Preludium grant no 2019/35/N/NZ7/01772.

Molecular detection of *Trypanosoma* spp. in Eurasian moose (*Alces alces*) in Poland

Katarzyna Filip-Hutsch[1], Magdalena Świsłocka[2], Anna Myczka[3], Aleksander Demiaszkiewicz[3], Grzegorz Karbowiak[3], Joanna Werszko[3]

1 Department of Food Hygiene and Public Health Protection, Institute of Veterinary Medicine, Warsaw University of Life Sciences – SGGW, Warsaw, Poland
2 Faculty of Biology, University of Bialystok, Białystok, Poland
3 Witold Stefański Institute of Parasitology of the Polish Academy of Sciences, Warsaw, Poland

The genus *Trypanosoma* constitutes numerous worldwide distributed protozoan species of human and animal health importance. Great relevance shows trypanosomatids of the subgenus *Megatrypanum* (genus *Trypanosoma* Gruby, 1843), comprising a group of large trypanosomes, able to infect almost all mammalian orders, but typically diagnosed in ruminants. Different *Megatrypanum* species are commonly detected in cervids, which are an important component of zoonotic foci for many pathogens causing transmission of human and domestic animals diseases. Unlike the well-described red deer and roe deer, the role of the moose still requires recognition, also in regard to *Trypanosoma* spp. infection. As heat sensitive ungulates moose might be especially sensitive to the effects of climate change and thus growing pressure of vector borne pathogens.

Therefore the aim of our study was to detect the presence of *Trypanosoma* spp. DNA in the spleen samples of moose using molecular methods. To our best knowledge, it is the first molecular detection of trypanosomes in moose in central Europe.

Samples of spleen were collected from ten and three moose, found dead or killed in road accidents in Kampinos Forest and West Polesie respectively. Samples were examined for the *Trypanosoma* spp. 18S rDNA partial gene by nested PCR using two pair of primers (TRY927F, TRY927R and SSU561F, SSU561R).

Trypanosomes DNA was detected in six out of thirteen investigated moose spleen – two from West Polesie and four from the Kampinos Forest. Three infected male and three female moose were 3 to 12 years old. Four partial 18S rDNA nucleotide sequences *Trypanosoma* spp. were obtained.

DNA of *Trypanosoma* spp. was detected in almost half of moose, despite relatively small number of examined animals. As the number of moose in Central Europe has been recently growing, it is crucial to examine the current spread of *Trypanosma* spp. in their population and to determine the role of moose in the transmission of other vector borne pathogens in Poland.

Prevalence of *Cryptosporidium* spp. in dung beetles inhabiting cervid faeces in pastures

Remigiusz Gałęcki[1], Tadeusz Bakuła[1], David B. Conn[2]

1 Department of Veterinary Prevention and Feed Hygiene, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland
2 One Health Center, Berry College, Mt Berry, United States

Dung beetles belong to the Scarabaeoidea superfamily, which includes Geotrupinae, Scarabaeinae, Aphodiinae subfamilies. These insects play a vital role in dung management both in the animal husbandry and in the natural environment. They are also an important factor in nitrogen recirculation, improving nutrient recycling and soil structure. Due to the specific biology of these insects, they may also play a role in the dispersion of various parasites. Parasites may use these insects as intermediate hosts or mechanical vectors. The aim of this study was to assess the prevalence of *Cryptosporidium* spp. in dung beetles inhabiting faces of cervids in pastures.

The dung beetles were harvested from deer feces on the Berry College (USA) campus used as pasture by a large population of white-tailed deer, Odocoileus virginianus. The insects were rinsed with distilled water prior to parasitological testing. Insects were identified to genus level using i identification keys. Dung beetles were analyzed according to the method proposed by Kirkor with some modifications, by grinding body parts in a mortar with a corresponding amount of water and 0.5 ml of ether. The resulting suspensions were filtered into test tubes to separate large particles and were centrifuged at 3500x for 5 minutes. After loosening the debris plug, the top three layers of suspension were discarded. Three smears were obtained and stained with Ziehl-Neelsen method. *Cryptosporidium* spp. were identified based on morphological and morphometric parameters under a compound light microscope (at 1000x magnification).

A total of 325 insects were collected including 171 identified as *Onthophagus* spp. and 154 identified as *Aphodius* spp. Oocysts of *Cryptosporidium* spp. were found in 58 (33.9%) *Onthophagus* spp. samples and in 75 (48.7%) *Aphodius* spp. samples. The differences in the oocyst prevalence between the two insect species were statistically significant ($p < 0.05$). There was also a strong correlation between the presence of *Cryptosporidium* spp. in the faeces and in insects ($r = 0.59$; $p < 0.05$).

The differences in the prevalence of Cryptosporidium spp. between the *Onthophagus* spp. and *Aphodius* spp. are most likely due to different biological and behavioral mechanisms of these insects. The presented research suggests that dung beetles may play a role in the periodic occurrence of cryptosporidiosis due to their reproductive, feeding, and fecal burying behavior. However, their role in the circulation of *Cryptosporidium* spp. oocysts in the environment has not yet been defined. They may also act as a mechanical vector for these protozoa.

Prevalence of *Trypanosoma* (*Megatrypanum*) *theileri* in cattle in Lower Silesia (Poland)

Michał Gorczykowski[1], Wiesław Bielas[2], Marianna Gemra[3], Jolanta Piekarska[1]

1 Division of Parasitology, Department of Internal Medicine and Clinic of Diseases of Horses, Dogs and Cats, Wrocław University of Environmental and Life Sciences, Wroclaw, Poland
2 Department of Reproduction and Clinic of Farm Animals, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland
3 Department of Immunology, Pathophysiology and Veterinary Preventive Medicine, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland

Trypanosoma (*Megatrypanum*) *theileri* is a cosmopolitan, non-pathogenic, trypanosome of ruminants transmitted by blood-sucking arthropods. It is the only known member of the genus Trypanosoma in the blood of cattle in Central Europe.

The study was conducted on three farms of dairy cows (A-C) in Lower Silesia (Poland) between August 2020 and November 2021 as a supplement to routine veterinary animal health evaluation. A total of 66 grazing cows and heifers were examined.

Trypanosome detection was performed by in vitro culture method on liquid media at 37°C. The following media were used: Hank's solution with lactalbumin, Veal infusion broth with Hank's solution (1:1), and MEM. Prior to culture, blood was concentrated by centrifugation. Approximately 0.5ml of buffy coat of leukocytes fraction was taken as inoculum. Culture (5ml) was carried out in polystyrene tubes in oblique position. Control of media was carried out on day 5 and 9/10 of culture.

Trypanosome prevalence varied. On farm A, no parasites were found (0/21). On farm B the prevalence was 17.6% (3/17) and on farm C – 70% (14/20). The timing of survey on farm C (September 2020) was correlated with cows returning from the pasture. The high prevalence of Trypanosome on farm C was the reason for further observation. The cows examination in November 2020 resulted in persistence of *Trypanosoma* infection in 11 of 20 cows despite reduced pressure of intermediate hosts (Tabanidae). In 2021, herd C consisted of 22 cows and positive results obtained in March 2021 in 4 cows allowed to conclude on the possibility of persistent infection of Trypanosomes. Re-examination in October 2021 showed positive results in 12 of 23 cows (including 4 cows positive in March). Unfortunately, reorganization of the herd forced termination of observations. These results indicate that Megatrypanum infections can persist for longer periods > 1 year, and it is likely that cows are not resistant to reinfection.

All cows were subjected to parasitological examination of feaces and basic haematological tests. On farm A, single Trichostrongylidae eggs were found in 7 of 20 faecal samples. On farm B, only single oocysts of *Eimeria* sp. were found in one faecal sample. On farm C, few oocysts of coccidia and eggs of Trichostrongylidae were seen in four consecutive coproscopic tests. The results of haematological examinations were within normal range for the cattle, but in Trypanosoma infected animals lower leukocyte counts (WBC) and elevated platelet counts (PLT) was observed.

Cryptosporydium infections in cattle in Poland: A cross-sectional population study

Agnieszka Kaupke, Artur Rzeżutka

Department of Food and Environmental Virology, National Veterinary Research Institute, Puławy, Poland, Puławy, Poland

Cattle cryptosporidiosis is noted worldwide with varied frequency of infection prevalence depending on geographical, environmental and husbandry factors.

In this study, the prevalence of *Cryptosporidium* infections in cattle was determined on the basis of molecular results obtained by testing of 1601 faecal samples collected from cattle up to 4 months of age from 2014 to 2018. Healthy cattle of various breeds were kept in 267 farms representing all Polish provinces. Detection and identification of *Cryptosporidium* species was performed at the 18 small subunit ribosomal RNA (SSU rRNA) locus by conducting PCR-RFLP analysis of the amplified DNA fragments.

The prevalence of *Cryptosporidium* infections in cattle population was 45.3%. Parasites were detected in all age groups of animals with the following group prevalence 45.3% (CI95%: 36.4–54.2; > 1–4 weeks), 48.9% (CI95%: 40.4–58.2; ≥ 4–8 weeks), and 42.4% (CI95%: 33.2–51.0; > 8–16 weeks). There were no significant differences observed in frequency of infections between age groups of cattle. The infected animals were housed in 233 (87.3%) out of 267 monitored farms located across all 16 Polish provinces with a varied prevalence ranging from 27.8 to 62%. Restriction pattern of 18 SSU rRNA amplicons for positive samples was characteristic for *Cryptosporidium parvum*, *Cryptosporidium bovis*, *Cryptosporidium ryanae*, *Cryptosporidium andersoni*, and unexpectedly for *Cryptosporidium baileyi* and *Cryptosporidium suis*. Mixed infections caused by *C. bovis* and *C. ryanae* as well as *C. parvum* and *C. bovis* were detected. *C. bovis* (22.2%) and *C. ryanae* (12.5%) infections prevailed in all age groups of the studied cattle population and their occurrence differed significantly in compared to other *Cryptosporidium* species (F-test, $F = 40.31$; $p < 0.05$). Surprisingly, *C. parvum* with overall population prevalence at 6.2% was not the major parasite species in youngest cattle below the age of 1 month. Although all cattle breeds were found positive for *Cryptosporidium* DNA a relationship between the infection caused by a specific parasite species and animal breed was found (Chi-squared test, $\chi 2 = 154.6$, $p = 0.0002$).

Cryptosporidium infections in cattle from Poland are common and detected parasite species except *C. baylei* and *C. suis* were typical for bovine host. This is the first report describing nation-wide population study in the EU utilizing molecular methods for *Cryptosporidium* detection and identification of parasite species.

Research on the prevalence of *Theileria* spp. in ticks in Eastern Poland

Anna Kloc, Angelina Wójcik-Fatla, Anna Sawczyn-Domańska, Violetta Zając

Department of Health Biohazards and Parasitology, Institute of Rural Health, Lublin, Poland, Lublin, Poland

Protozoa of the genus *Theileria* cause severe disease in animals, mainly ruminants. The most pathogenic species is *Theileria parva*. The vectors of these pathogens are ticks, also native species occurring in Poland. The aim of the project was to investigate the occurrence of *Theileria* spp. in *Ixodes ricinus* and *Dermacentor reticulatus* ticks using molecular methods.

The research material was a group of 30 engorged female ticks. In laboratory conditions, the females laid eggs out of which some of the larvae emerged. In order to determine the occurrence and the possibility of transovarial transmission of protozoa of the genus Theileria, DNA was isolated from female ticks as well as eggs and larvae, which were then used to detect Theileria at the genus level. For this purpose, a polymerase chain reaction (PCR) was performed. A pair of primers complementary to the 18S rRNA gene sequences specific for the Theileria genus were used for the PCR reactions.

Of the 30 collected engorged female ticks, 15 females of the species I. ricinus and 15 females of the species *D. reticulatus* were identified. In total, females laid 6720 eggs, of which 2440 larvae hatched (36.31%). Both eggs and larvae were tested in pools of 20. Theileria spp. protozoa were not detected in the tested DNA isolates from *I. ricinus* and *D. reticulatus* females after the oviposition period, as well as in egg and larvae isolates.

In Poland, there is little research on the prevalence of *Theileria* spp. in ticks, therefore the aim of the study was to assess the pathogen's occurrence and the role of ticks as vectors of protozoa. One route of pathogen transmission in ticks is transovarial transmission, well understood in protozoa of the genus *Babesia*. The presence of the studied pathogen was not found in the tested females, eggs, and larvae of both ticks species. The obtained results did not confirm the role of ticks belonging to the species ***I. ricinus*** and *D. reticulatus* in the transmission of *Theileria* spp. in the Lublin region as well as a transovarial transmission of protozoa. Further research on vector and reservoir of *Theileria* spp. and also the source of infection in the environment will provide more information about the health risk for animals.

The study was funded by the Institute of Rural Health, within a subsidy from the Ministry of Science and Higher Education in Warsaw (Project No. 22050).

A presence of *Toxoplasma gondii* in European grey wolf (*Canis lupus*) and brown bear (*Ursus arctos*) from Poland

Aleksandra Kornacka-Stackonis[1], Bożena Moskwa[1], Adam Olszewski[2], Krzysztof Anusz[3], Blanka Orłowska[3]

1 Witold Stefański Institute of Parasitology, Polish Academy of Sciences, Warsaw, Poland
2 Kampinos National Park, Poland, Izabelin, Poland
3 Department of Food Hygiene and Public Health Protection, Institute of Veterinary Medicine, Warsaw University of Life Sciences – SGGW, Warsaw, Poland

The European grey wolf (*Canis lupus*) and the brown bear (*Ursus arctos*) are two of the largest carnivores in Poland, and both are strictly protected from 1998 and 1952. Currently the population of grey wolf is growing, according to official data the population consists of 1900 individuals while the population of brown bear is estimated to be 100-120. Due to the limitations in obtaining the samples little is known about the exposure to zoonotic agents in grey wolves and brown bears. Due to the predator behaviour these animals are at risk of infection with *Toxoplasma gondii*, that tissue cysts may remain viable in the pray they hunt.

In the present study we examined samples collected from 8 wolves and two brown bears from the Bieszczady Mountains and one wolf from Kampinos Forest. For the detection of the presence of *T. gondii* antibodies in meat juice samples commercial ELISA kit has been used. The PCR reaction has been conducted on the DNA isolated from blood and liver of examined animals.

The presence of anti *T. gondii* antibodies has been detected in 77.8% of examined wolves and in one brown bear. The PCR reaction confirmed the presence of the DNA of T. gondii in blood samples of one wolf and one brown bear from the Bieszczady Mountains. Analysis of 2 sequenced products verified that our products belong to *T. gondii* VEG strain, which is predominant in wildlife of a moderate virulence. Sequences are available in GenBank with accession numbers: ON380664, ON380665.

Marshallagia sp. and unique Tatra Chamois (*Rupicapra rupicapra tatrica* Blahout 1972) – *M. marshalli* or neglected *M. brigantiaca*?

Jerzy Kowal[1], Elżbieta Łopieńska-Biernat[2], Gabriela Chovancova[3], Anna Wyrobisz-Papiewska[1]

1 Department of Zoology and Animal Welfare, Faculty of Animal Sciences, Univeristy of Agriculture in Kraków, Kraków, Poland
2 Department of Biochemistry, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland
3 Research Station and Museum of the Tatra National Park, Tatranská Lomnica, Slovakia

The species diversity among Ostertagiinae is currently a main issue in relation to perform valid species identification, as well as define their host specificity. Currently the genus *Marshallagia* complicates the taxonomy of parasitic, which indicates the need for further research in this area. The aim of the preliminary study was to investigate diversity of abomasal nematodes of the genus Marshallagia parasitizing Tatra chamois (*Rupicapra rupicapra tatrica* Blahout 1972).

Morphological and molecular analysis were performed on post-mortem isolated, pre-identified as *Marshallagia* sp. adult nematodes. Specimens (n = 60) were collected in Research Station and Museum of the Tatra National Park. Analyzed nematodes were morphologically similar to *Marshallagia marshalli* (shape of the spicules and bursal ray pattern), whereas morphometrically to *Marshallagia* (*Ostertagia*) *brigantiaca* (Blanchard in Railliet et Henry, 1909). Differences in ITS-2 sequences were also observed. Species *M.* (*O.*) *brigantiaca*, originally described in alpine chamois from France was then synonymized with *M. marshalli* by Gebauer in 1932. The results obtained suggest the distinctiveness of *Marshallagia* nematodes collected from Tatra chamois and therefore should now be considered to be a different species/strain that those derived from other ruminant host species. Moreover, analyzed specimens may be typical for Tatra chamois or whole genus *Rupicapra*.

Studied was financed from project Miniatura 2, National Science Centre (NCN) 2018/02/X/NZ5/03145

Recreational horses as hosts of protozoan and nematode parasites

Agnieszka Perec-Matysiak[1], Kamila Kot[1], Weronika Hildebrand[2], Joanna Hildebrand[1], Katarzyna Buńkowska-Gawlik[1]

1 Department of Parasitology, Faculty of Biological Sciences, University of Wrocław, Wrocław, Poland
2 Veterinary clinic "NeoVet" S.C., Wrocław, Poland

Recreational horses host many groups of parasites which in some cases can also be a threat to humans. The presented study focuses on intestinal protozoa and nematodes. The aim of this study was to determine the taxonomic variety of selected intestinal parasites found in recreational horses and their molecular identification in faecal samples. The stool samples were obtained from recreational horses from various studs in Lower Silesia. Molecular methods were used to detect intestinal parasites. After isolation of genetic material from stool samples, PCR reactions were performed using different molecular markers – 18S rRNA, gp60 and ITS rRNA. The products were sequenced. Among the 203 tested stool samples, 34 were found to contain the desired intestinal protozoa. Prevalence of *Cryptosporidium* spp. was 5.4%, and prevalence of Eimeriidae was 14.9%. This study allowed to identify Cryptosporidium horse genotype. The presence of *Eimeria* sp., *Isospora* sp. and *Cryptosporidium* spp. was also confirmed in the positive samples. Nematodes belonging to subfamily Cyathostominae were identified by DNA sequencing. Additionally among nematodes *Oxyuris* and *Parascaris* were noted. Co-infections of nematodes with microparasites were also recorded.

„Research project supported/partly supported by program „Excellence initiative – research university" for years 2020-2026 for University of Wrocław" (BPIDUB.4610.7.2021.KP.A)

Parasitofauna of the gastrointestinal tract of sheep occurring during the farming cycle of meat-type lambs

Jolanta Piekarska, Jarosław Pacoń, Michał Gorczykowski

Division of Parasitology, Department of Internal Medicine and Clinic of Diseases of Horses, Dogs and Cats, Wrocław University of Environmental and Life Sciences, Wrocław, Poland, Wrocław, Poland

Parasitic infections in sheep are one of the biggest threats to their productivity. The risk of infection affects sheep grazing on pasture and feeding on fresh fodder.

The aim of this study was to monitor parasite species in the gastrointestinal tract of sheep during the farming cycle of meat-type lambs.

Faecal samples were collected from the rectum of sheep of the Heather breed and Cameroon-Heather hybrids. A total of 67 animals from two flocks in the mountain and foothill areas of Lower Silesia (Poland) were tested. Faecal samples were also collected from grazing places, paddocks and areas adjacent to pastures. All samples were analyzed by flotation and sedimentation methods and nematode larvae were cultured. Intensity of infection was measured using the McMaster method. Coproscopic examinations were carried out in July/August and September/October in 2021.

In sheep flock 1 in July and August, oocysts of 3 species of the genus *Eimeria* were found in the examined faeces. The average excretion rate of *Eimeria* oocysts was 200 (100–450) in 1g of feces (OPG). Faecal flotation and morphometric examination of cultured larvae revealed the presence of individual nematodes of the families Trichostrongylidae, Mollineidae and Dictyocaulidae. In sheep flock 2, oocysts of 2 species of the genus *Eimeria* were found with an average excretion rate of 100 (50–300) OPG. Eggs of nematodes of the families Trichostrongylidae, Mollineidae, and Trichuris were found in fecal samples. The mean intensity of gastrointestinal nematode egg excretion was 300 (50–425) EPG. Morphometric examination of the larvae obtained from the culture revealed the presence of nematodes of the families Trichostrongylidae and Protostrongylidae.

Therefore, animals in herd 1 were dewormed with toltrazuril and eprinomectin in herd 2 with albendazole and eprinomectin. Results of faecal examination after deworming in herd 1 showed a reduction in the number of excreted oocysts of *Eimeria* to about 50 (0–100) OPG and single nematode eggs in several tested samples. In herd 2, the treatment reduced the number of excreted oocysts (single per microscopic slide) and excreted eggs to approx. 75 (0–150) EPG.

The applied treatment was not fully effective due to the constant presence of infective forms of parasites in pastures. The results obtained in this study confirmed the necessity of antiparasitic prophylaxis during the farming cycle of meat-type lambs as the only effective method to reduce the occurrence of dispersal forms of parasites in the environment.

Infections of gastrointestinal worms in Polish primitive horses (*Equus caballus gmelini* Ant.) in two breeding centers

Kateryna Slivinska[1, 2], Jakub Gawor[2], Marta Siemieniuch[3], Zbigniew Wróblewski[4]

1 I.I. Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, Kyiv, Ukraine
2 Witold Stefański Institute of Parasitology Polish Academy of Science, Warsaw, Poland
3 Department of Reproductive Immunology and Pathology, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences; The Research Station of the IARF PAS in Popielno, Olsztyn, Ruciane-Nida, Poland
4 Veterinary Private Practice, Pisz, Poland

Gastrointestinal worm infections of Polish primitive horses (*Equus caballus gmelini* Ant.) in two breeding centers, i.e. Roztocze National Park (RNP) and Popielno Research Station (PRS) are presented.

A total of 56 individual fecal samples were collected in May 2022 and analyzed using MacMaster technique with a detection limit of 25 eggs per gram (EPG).

Anthelmintic treatments were not carried out at the RNP before the study. In PRS stabled horses were treated in March 2022 with moxidectin. Horses included in the study were divided in three groups – "stabled" (20 horses), "forests group" (8 horses) in PRS, and "merged" (28 forests and stabled horses) in RNP.

Fecal samples with counts above 500 EPG (n = 13) were selected for larval culture. Following 8 days of incubation (at 28–30^{0}C), larvae were retrieved and 100 larvae from each sample were identified (with a limit of detection 1%) using an identification key (Norman D. Levine, 1980) to determine the proportions of *S. vulgaris*, *S. equinus*, *S. edentatus*, *Triodontophorus* and Cyathostominae infective larvae.

According to data from fecal egg counts (FEC) horses from RNP were infected with strongylids, parascarids and anoplocephalids. Total strongyle FEC ranged from 150 to 4400 (overall mean of 1742 ± 1473,3 EPG).

Parascaris spp. eggs were present in 17,9% (5/28) of samples, with FEC ranging from 25 to 275 EPG (mean 140 ± 114). The prevalence of *Anoplocephala* spp. eggs were low with the one (3,6%) of 28 samples testing positive for Anoplocephala spp. eggs (mean of 25 EPG).

Accordingly, to the results of fecal egg counts in PRS, 20% of stabled horses (4/20) and 100% of forests horses were infected with strongylids, and 5% of stabled (1/20) and 25% of forests horses (2/8) were infected with *Eimeria* spp. oocysts.

The mean egg output in the treated stabled horses in PRS was low – 31,25 ± 12,5 EPG (25–50 EPG). The mean egg output in forests horses never exposed to anthelmintics was 65 times higher than in stabled horses in PRS, i.e. 2031,3 ± 1491,5 EPG (50–4675 EPG).

Parascaris spp. and *Anoplocephala* spp. eggs were not recorded in horses examined in PRS.

The presence of S. vulgaris was confirmed by larval culture, with its infective larvae identified in 12/13 samples (92.3%). Out of 100 larvae from each sample, 1–40 (mean 10) were identified as *S. vulgaris*. Other larvae identified included *S. edentatus* in 5/13 samples (38.5%), *Triodontophorus* spp. in 3/13 samples (23.1%), and cyathostomes in all 13 samples.

Dispersion of *Cryptosporidium, Cyclospora, Giardia* and mircrosporidians by migrating waterfowl in Wielkopolska region, Poland

Piotr Solarczyk[1], Anna Sułek-Stankiewicz[1], Artur Trzebny[2], Mirosława Dabert[2], Agnieszka Wojtkowiak-Giera[1], Lukasz Skrzypczak[1], Anna Slodkowicz-Kowalska[1], Monika Derda[1]

1 Department of Biology and Medical Parasitology, Poznan University of Medical Sciences, Poznań, Poland
2 Molecular Biology Techniques Laboratory, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland

The seasonal migration of water birds is one of the most spectacular phenomena of nature. However, this involves dissemination of enteric agents via faecal droppings of birds. The presence of waterfowl flocks have been associated with the decreasing quality of the places they residue. Because of the transmission of the eukaryotic microorganisms by migrants, these contaminated places (e.g. ponds, fields) burden may be a source of infection for humans and other animals (e.g. farm and domestic) that share the same area. So, migrants play role as either as a potential reservoir hosts or carriers that mechanically disseminate gastrointestinal, unicellular eukaryotes. Study aims were: (1) detection of *Cryptosporisium* spp., *Cyclospora* sp., *Giardia* spp. and microsporidian (oo)cysts/spores direct from waterfowl's feacal droppings during migration period and (2) determination their host-specificity. In total, 100 ground-feacal samples were investigated from white fronted geese (*Anser albifrons* sensu lato) in Wielkopolska National Park over autumn migration time. Screening of pathogens were based on fresh and stained (Ziehl-Neelsen) microscopy examination followed by molecular methods. For specific detection of *Giardia*, microsporidians and *Cryptosporidium*, PCR and nested–PCR assays based on the species specific primers for beta-giardin, 16S rRNA and COWP genes were used. The pathogens were detected in 7/100 (7%) feacal samples using both methods; 4 (4%) were positive for *Cryptosporidium* sp., 1 (1%) was positive for *Cyclospora* sp. and 2 (2%) samples were positive for microsporidian *Encephalitozoon intestinalis*. All samples were *Giardia* negative. The results of the study indicate that wild water birds disseminate enteric and zoonotic pathogens during their migration in Wielkopolska region.

Chronic Sternostomosis in Amadine birds (*Peophila gouldiae*)

Zenon Sołtysiak, Natalia Osten-Socken, Jędrzej M. Jaśkowski, Marek Gehrke

Department of Diagnostics and Clinical Sciences, Institute of Veterinary Medicine, Nicolaus Copernicus University, Toruń, Poland

Sternostomosis in the amadine birds is a serious disease that always leads to the death of birds in larger mite infestations. Amadine birds are particularly fast and prone to Sternostoma infestation. The characteristic macroscopic and microscopic picture of this parasitism is due to the migration of mites in the lung tissue. Many factors such as high temperature and humidity in aviaries, where parasites find optimal conditions for development, but also inadequate adaptation of animals to our European climate contribute to it. The clinical and pathological-anatomical picture of sternostomosis in birds are described in the international literature by Kroneberg, Kummerfeld, Mathey, Neuhybel and Beranek and in Poland by Szeleszczuk and Kruszewicz.

Six amadine birds – (*Pephila gouldiae*) from Wrocław, who had been suffering from parasite invasions for about 10–12 weeks, were examined. Birds did not die during the invasion with *Sternostoma tracheocolum*, but were euthanized at different times. After sectioning, small organ samples were fixed in neutral 10% formalin, embedded in paraffin, and the histological slides were stained with hematoxylin-eosin.

The clinical course of the disease was typical, with the following symptoms being observed: loss of feathers, constant bouncing on the cage floor, permanent head shaking, beak opening, shortness of breath, sneezing, wheezing, apathy and paralysis. The section found that all birds were more or less emaciated, 2 of them even cachectic. All animals had dense yellow-gray mucus in the nasal and pharyngeal cavities. In the lush gryish-white mucus, many parasites got stuck, looking like small dark patches. 30 to 50 individuals of Sternostoma tracheocolum mostly female parasites, were isolated from each bird. Irregular and blotchy bleeding was visible on the lungs and the airbag walls. Numerous mature mites could be detected from the surface and from the incisions of the lung parenchyma. Often the parasites were surrounded by eggs and massive bleeding. Under the bronchial lung epithelium and the tubes in the smooth muscle layer, cell infiltrates consisted of lymphocytes, histiocytes, and acidophilic granulocytes were also observed. Very old changes caused by the mites were composed with inflammatory cells e.g. numerous neutrophils, macrophages, giant cells, with surrounding proliferating fibroblasts.

Six amadine birds were examined and the results of dissection showed a strong infestation with *Sternostoma tracheocolum*. Many lung sections that have been examined histopathologically show typical changes depending on the time of the resulting damage. The clinical course of the disease was typical.

Metastrongylosis and ascariasis in a wild boar weaner. A case report

Zenon Sołtysiak, Natalia Osten-Socken, Jędrzej M. Jaśkowski, Marek Gehrke

Department of Diagnostics and Clinical Sciences, Institute of Veterinary Medicine, Nicolaus Copernicus University, Toruń, Poland

Metastrongylosis and ascariasis are a very common parasitosis by swine and wild boar. *Metastrongylus apri* is a worldwide lungworm infecting domestic pigs, wild boars, and peccary. Adult nematodes are found in the small bronchi and bronchioles, particularly in the posterior lobes of the lung. *Ascaris suum* is a helminth parasite who modulates host immune and inflammatory responses, which may drive immune hyporesponsiveness during chronic infections. Pigs with a chronic *A. suum* infection may have a substantial suppression of inflammatory pathways in the intestinal mucosa, with a broad downregulation of genes encoding cytokines and antigen-processing and costimulatory molecules.

The histopathological examination of lungs and small intestine of 4 months old weaner was performed. The sections of lungs and intestines were fixed in 10% formalin.

The changes mainly concerned the lower parts of the basal lobes of both lungs. In the light of the dilated lumen of the bronchioles and bronchi numerous nematodes, causing obturation of the light, were found. The presence of mature nematodes caused inflammation of the bronchial tree, and then the inflammation spread to the peribronchial tissue and alveoli, causing bronchopneumonia. Mechanical damage healed by the scarring process with the formation of connective tissue scars, i.e. they were repaired, led to extensive pulmonary fibrosis. Catarrhal pneumonia was shown as a result of the migration of *Metastrongylus* larvae through the lung parenchyma, and secondary purulent infection was caused by Staphylococcus.

A strong invasion of 36 adult worms of *A. suum* was present in the section of gastrointestinal tract of wild boar weaner. In intestinal ascariasis caused by adult forms *A. suum*, chronic atrophic enteritis were noted. Together with atrophic changes in the intestinal submucosa and muscular membranes, it causes a significant thinning of the wall due to the chronic intestine atrophic inflammation and contributes to the formation of the parchment intestine (intestinum papyraceum). In the histological image of the small intestines, we can see villi shortening, atrophy of the intestinal glands and the presence of inflammatory infiltrates with eosinophils.

The cause of the death of the wild boar 4 months female was bronchitis and bronchopneumonia caused by the invasion of *Metastrongylus* spp. and gastric and intestinal ascariasis caused by *A. suum*. In foamy bronchial mucus *Metastrongylus* sp. was isolated. The bronchi and bronchioles showed catarrhal inflammation. Section of gastrointestinal tract of wild boar weaner strong invasion of *A. suum* was observed. The small intestines showed chronic atrophical inflammation.

First case of cysticercosis by *Taenia crassiceps* in a captive non-human primate (Lemur catta) in Poland

Małgorzata Samorek-Pieróg[1], Jacek Karamon[1], Adam Brzana[2], Lesław Sobieraj[3], Mariusz Włodarczyk[3], Jacek Sroka[1], Tomasz Cencek[1]

1 Department of Parasitology and Invasive Diseases, National Veterinary Research Institute, Puławy, Poland
2 Regional Veterinary Laboratory, Opole, Poland
3 Zoo Opole, Opole, Poland

Taenia crassiceps is a cosmopolitan tapeworm endemic to the northern hemisphere. It has indirect life cycle. The definitive hosts are carnivores, mainly red foxes, wolves and other canids, harbouring the adult tapeworms in their small intestines. The intermediate hosts are rodents and rabbits, harbouring in their body cavity, muscles or nervous system cyst-like larvae. Non-human primates in zoos appear to be highly susceptible for *T. crassiceps* cysticercosis, therefore they can be perceived as a sentinels for the zoonotic potential of cestodes parasite species. Factually, several cases of infection in a zoo primates have been reported. In Poland, such case of cysticercosis in a captive lemur has not been reported so far. This prompted us to conduct the research described in this paper. The aim of this study was to confirm the presence and the molecular characterization of *T. crassiceps* cysts, isolated from a ring-tailed lemur. Surgery revealed multifocal, transparent saccules containing several thin walled tapeworm cysticerci. Metacestodes was round to oval shape, with 1–4 mm in diameter and white to pale yellow color. Each cysticerci contained a single inverted scolex, one or more suckers, rostellum with two rows of hooks and numerous calcareous corpuscles. In some of the metacestodes single or multiple exogenous buds of daughter cysticerci were spotted. The molecular analysis, with two protocols to obtain partial sequences of NADH dehydrogenase subunit 1 (nad1) and cytochrome oxidase subunit 1 (cox1) of *Taenia* spp., was performed to confirm the morphological examinations. On the basis of morphological features and molecular analysis, the cysticerci were identified as *T. crassiceps* metacestodes. The products from the PCRs were sequenced. Interpretation of the sequencing results of the obtained amplicons, by comparing them with the GenBank database, proved that the causative agent, in this case, was *T. crassiceps*. The received data can be used to supplement the species description. To our knowledge, this is the first case of cysticercosis by *T. crassiceps* in non-human primate in Poland. Our case report and scarce data on *T. crassiceps* in Poland, encourage to the necessity of testing domestic and wild carnivores, especially red foxes, for the presence of this parasite of zoonotic threat. What is more, our case additionally emphasize the risk of spreading the parasites and parasitic diseases, especially zoonoses, when there is a probability of contact between zoo's and wild animals.

Zoonotic pathogens in shelter dogs as a potential source of human infection

Magdalena Szydłowicz[1], Marta Kicia[1], Żaneta Zajączkowska[1], Julia Rozmus[1], Mateusz Kamiński[1], Michał Maciejewski[1], Andrzej B. Hendrich[1], Bohumil Sak[2], Martin Kváč[2, 3]

1 Department of Biology and Medical Parasitology, Wroclaw Medical University, Wroclaw, Poland
2 Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic
3 Faculty of Agriculture, University of South Bohemia in České Budějovice, České Budějovice, Czech Republic

Domesticated animals may serve as a source of infection with various zoonotic pathogens. Many of these infectious agents reside in the intestine tract, therefore their dispersive forms are excreted with animal stool and may easily be spread to other hosts, including humans – especially to individuals with impaired immunity. These include unicellular opportunistic organisms such as Cryptosporidium spp., Giardia intestinalis or microsporidia.

The aim of the present study was to investigate the prevalence of these pathogens in dogs living in ten different animal shelters. In total, 187 fecal samples were collected: 101 in Poland and 86 in Czech Republic (five shelters in each country). Genomic DNA was extracted from samples after their homogenization with bead disruption, followed by genus-specific nested PCRs to detect pathogens' DNA. Specific primers were used to amplify the sequences of TPI (triosephosphate isomerase) and SSU (small subunit) rRNA loci for detection of *G. intestinalis* and *Cryptosporidium* spp., respectively, while partial sequence of the 16S rRNA gene, the entire ITS (internal transcribed spacer) region, and a partial sequence of the 5.8S rRNA gene amplification protocols were used in case of microsporidial DNA detection.

In Poland, the general prevalence was 29% (29/101), with the highest reported for *G. intestinalis* (21/101, 20.8%). *E. bieneusi*, *Encephalitozoon* spp. and *Cryptosporidium* spp. were observed in 6 (5.9 %), 2 (2%) and 2 (2%) cases, respectively. The general prevalence in Czech Republic was similar (22/86; 25.6%), although *E. bieneusi* was the most common pathogen detected (10/86, 11.6%), followed by *G. intestinalis* (7/86; 8.1%) and *Cryptosporidium* spp. (5/86; 5,8%). No co-infections were observed in any samples from both of these countries.

The high observed pathogens' prevalence in tested dogs, especially of *G. intestinalis*, confirms that domesticated animals are a significant reservoir of zoonotic infection. This should be borne in mind by people at risk of development of symptoms of these opportunistic diseases who consider adoption of homeless animals. Interestingly, the percentage distribution of the detected pathogens differed between both countries, and even between individual shelters within the country, suggesting that the risk of infection with a given species may be associated with the place of residence.

This work was financially supported by the Polish Ministry of Health subvention according to number STM.A060.20.093 from the IT Simple system of Wroclaw Medical University.

Distinct habitats as a determinant of roe deer abomasal nematode communities – a preliminary study

Anna Wyrobisz-Papiewska[1], Jerzy Kowal[1], Weronika Rynkiewicz[2], Marek Wajdzik[3], Maciej Lesiak[4], Paweł Nosal[1]

1 Department of Zoology and Animal Welfare, Faculty of Animal Sciences, Univeristy of Agriculture in Kraków, Kraków, Poland
2 University Centre of Veterinary Medicine UJ-UR, Kraków, Poland
3 Department of Forest Biodiversity, Faculty of Forestry, Univeristy of Agriculture in Kraków, Kraków, Poland
4 "Kaban" Company, Stare Wiślisko 48, 31-979 Kraków, Poland, Kraków, Poland

The impact of habitat and host co-occurrence on the composition of parasite communities has been so far poorly recognized. However, due to the ability to exist in distinct habitats, the roe deer appears to be a suitable model host species for such a research.

The aim of this study was to compare the abomasal nematode communities parasitizing roe deer from distinct habitats using mean abundance (MA) values. The post-mortem examinations were carried out on 55 roe deer harvested during hunting season or collected as roadkill. Hosts were categorized accordingly to their occupied habitats, i.e. forests (n = 41, Dulowska and Niepołomicka Primeval Forests occupied by moose, red deer and fallow deer), agricultural lands (n = 9, Miechów and Myślenice Counties without permanent existence of other cervids) and the urban areas (n = 5, Kraków agglomeration with red deer sympatry noted). All of the recovered abomasal nematodes were identified on the basis of the morphological features. Males of the Ostertagiinae were determined to species, females only to subfamily (the concept of polymorphism was included). Overall, eight abomasal nematode species were found (*Haemonchus contortus*, *Ashworthius sidemi*, *Ostertagia leptospicularis*, *O. antipini*, *Spiculopteragia boehmi*, *S. asymmetrica*, Mazamastrongylus dagestanica and *Trichostrongylus axei*). The most diverse nematode community was stated in roe deer from forest habitat. The MA value varied between analyzed habitats.

The results obtained suggest that the occupied habitat has an impact on the occurrence of particular nematode species of the subfamilies Haemonchiinae and Ostertagiinae. Moreover, the nematodes communities strictly depend on the presence of its principal host. *T. axei*, that has been stated, indicates that roe deer participate in parasite transmission between wild and domestic ruminants.

Gastrointestinal parasites of alpacas (*Vicugna pacos*) raised in Poland and their zoonotic potential

Katarzyna Szopieray, Julita Templin, Janusz Żbikowski, Elżbieta Żbikowska

Nicolaus Copernicus University in Toruń, Toruń, Poland

This study aimed to determine the prevalence of gastrointestinal parasites in alpacas (*Vicugna pacos*) in selected farms in Poland. In the period of July-August 2019, 97 individuals from six commercial farms were examined by coproscopic method. Faeces from individual animal were subjected to the flotation procedure in NaCl solution. In five studied farms, intestinal infections were found, caused by nematodes and protists. The initial analysis showed that distribution of the different parasites species was random. The statistically significant difference confirmed by the results of the R analysis concerned the number of parasite eggs in individuals of different sexes. Females had significantly more eggs of parasites per 1 g of faeces than males. The total percentage of alpacas infected with intestinal parasites was 57.7%. Eggs of *Nematodirus* sp. were found in 28.9%, *Trichostrongylus* sp. in 15.5%, *Strongyloides* sp. in 13.4%, *Camelostrongylus* sp. in 11.3%, other strongyle-type in 12.4%, *Trichuris* sp. in 3.1%, *Capillaria* spp. in 2.1%, *Oesophagostomum* sp. in 1.0% individuals. Oocysts of *Eimeria macusaniensis* were found in 8.2%, *Eimeria* sp. in 4.1%, and *Cryptosporidium* sp. in 3.1% of animals. Some of described parasites in tested alpacas have the zoonotic potential.

Parasites of newly introduced fish species and European perch decade post coexistence in Włocławek Reservoir on the Vistula River

Katarzyna Mierzejewska, Maciej Błażejewski

Department of Ichthyology and Aquaculture, Faculty of Environmental Sciences, University of Warmia and Mazury in Olsztyn

Presented study focus on parasites of chosen Perciformes living under the circumstances enabling the parasite exchange between exotic and native fish in Włocławek Reservoir. Previous research was conducted the decade before and parasites monitoring of alien fish as well as of european perch was recommended. At present, 80 fish were examined including Chinese sleeper, *Perccottus glenii*; western tubenose goby *Proterorhinus semilunaris*; European perch, *Perca fluviatilis* and 3 specimens of Monkey goby *Neogobius fluviatilis*, last one rare in fish catches during study period in 2018–2019.

Typical for Chinese sleeper were ciliates *Trichodina domerguei* recorded in 81,5 % of the fish at numerous or even mass occurrences of the parasite. Furthermore a specialist, the cestode *Nippotaenia mogurndae*, the larvae of nematode *Eustrongilides excisus* (prevalence 44,4 % mean intensity of infection 3,5), small metacercariae of *Echinochasmus* spp. encysted in gills (indices 25,9% and 8,1 respectively), eyeflukes *Diplostomum paracaudum* and glochidia of *Anodonta cygnea* (29,6% and 5,6). Other parasites were scarce.

Western tubenose goby was commonly infected with metacercariae of *Holostephanus luhei* and *H. cobitidis* encysted in muscles, with metacercariae of *Apathemon gracilis* – in body cavity. Particularly abundant on gills and fins were glochidia of *Anodonta cygnea* (74,1% and 11,5).

Parasite fauna of European perch was dominated by metacercariae of *Tylodelphys clavata*, *Diplostomum baeri*, *Ichthyocotylurus variegatus*, *A. gracilis*, by *Bunodera lucioperca*, *E. excisus* L. (43,5% and 7,4), *A. cygnea* gloch. and *Eimeria* sp., last one causing coccidiosis, at massive or very numerous spores occurence in scrapings from terminal intestine. Infection with eye flukes, *E. excisus* and coccidia are of concern of Perch health and condition.

Parasites exchange between alien Perciformes and native perch was thus limited and do not referred to parasites specific for alien fish. Though, the presence of Chinese sleeper supports populations of *E. excisus*, *T. domerguei* and *A. cygnea*, likewise the presence of Ponto Caspian gobies supports populations of *A. gracilis* and *A. cygnea*, the parasites infecting native perch too.

Further research should particularly approach: 1) molecular study of Echinochasmus genera detected in alien fish, to exclude presence of *E. japonicus* and *E. perfoliatus* potentially harmful for humans, 2) How effective are the introduced fish species as a hosts of *A. cygnea* gloch.

SESSION 4

Public health, zoonoses and anthropozoonoses, transmission diseases

Oral session

Zoonotic potential of *Blastocystis* infection – research on animals and their caregivers from Pomerania, Poland

Monika Rudzińska[1], Beata Kowalewska[1], Beata Szostakowska[2], Małgorzata Waleron[3], Maciej Grzybek[2], Mirosław Kalicki[4], Katarzyna Sikorska[1], Agnieszka Świątalska[5]

1 Department of Tropical Medicine and Epidemiology, Institute of Maritime and Tropical Medicine, Medical University of Gdańsk, Gdańsk, Poland
2 Department of Tropical Parasitology, Institute of Maritime and Tropical Medicine, Medical University of Gdańsk, Gdańsk, Poland
3 Laboratory of Plant Protection and Biotechnology, Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk, Gdańsk, Poland
5 Zoological Garden of Gdańsk, Gdańsk, Poland
6 Veterinary Hygiene Station, Gdańsk, Poland

Blastocystis is a unicellular enteric eukaryote most commonly found in human stool samples. Its presence in humans may cause ailments, mainly from the gastrointestinal tract. It is suggested that human *Blastocystis* infection may have zoonotic origin. Poland is the fifth pork producer in the European Union, and domestic animals are eagerly kept at home. The identification of animal reservoirs of this microorganism and its transmission between animals and humans is essential to prevent the spread of the infection to humans. Therefore the research for the presence of *Blastocystis* in selected groups of animals and their related humans (owners or caregivers) was undertaken.

Stool samples from 149 pigs (taken from 10 piggeriries in Pomerania District), 147 pet animals and 65 of their owners, as well as 201 zoo animals and 35 of their caregivers were investigated. The subtypes of *Blastocystis* were determined based on the nucleotide sequences of ribosomal RNA small subunit.

Blastocystis was detected in 46.97% of pig stool samples. The predominant subtype was ST5 (94.28%) while ST3 and ST1 were present only in individual animals from elder age groups and mostly in the form of mixed infections. Research on *Blastocystis* in pet animals shown no positives among dogs and cats, however, three stool samples from reptiles (lizards) included *Blastocystis*. Similarily, only three pet owners were positive for *Blastocystis* (identified as ST3, ST4, and ST7), however, they were not lizards 'owners but cats' owners. Lizards' *Blastocystis* did not belong to any of the known subtypes. Additionally, selected groups of zoo animals and their caregivers were examined. Among animals (26.86% positive) ST1, ST2, ST3, ST5, ST8, ST10, ST13, and ST14 were identified, while in humans (17.14% positive) ST1 and ST3. The two latter STs were also present in non-human primates. In one case, gens sequences in the 600 bp fragment of 18S rRNA of *Blastocystis* ST1 isolated from a human and three monkeys were identical.

The results of our studies showed that transmission of *Blastocystis* between animals and humans is possible in favorable conditions, although, it is difficult to establish the direction of tramsmission. Further research into *Blastocystis* harboring animals and their owners/keepers is essential to fully clarify the animal reservoirs and transmission dynamics of *Blastocystis* between the hosts.

Occurrence of *Blastocystis* in selected animals and environmental samples in northern Poland

Beata Szostakowska[1], Anna Lass[1], Maciej Grzybek[1], Emilia Załugowicz[1], Joanna Nowicka[1], Pawel Gladysz[1], Monika Rudzinska[2], Agnieszka Świątalska[3]

1 Department of Tropical Parasitology, Faculty of Health Sciences, Medical University of Gdańsk, Gdynia, Poland
2 Department of Tropical Medicine and Epidemiology, Faculty of Health Sciences, Medical University of Gdańsk, Gdynia, Poland
3 Veterinary Hygiene Station, 80-316 Gdańsk, Poland, Gdańsk, Poland

Blastocystis is a common, enteric protist harboring humans and numerous animal species. Its pathogenicity, as well as, the possible transmission routes, has not been established and are still the subject of many studies.

The aim of these preliminary studies was to assess whether and how often this protozoan occurs in the environment and in selected animals, and thus to expand the knowledge about possible sources of infection for human.

The study included stool samples of dogs from 6 shelters (n = 120), free-living rodents (7 species, n = 186), and environmental samples: sediments of water (n = 17), and soil (n = 13) as well as vegetable and fruit washings (n = 22). Most of the samples came from the Pomorskie Voivodeship, only rodent faeces samples were obtained from animals collected in the Warmińsko-Mazurskie Voivodeship.

The research was carried out using molecular methods: amplification of the SSU-rDNA region and sequencing of the obtained PCR products in order to define the Blastocystis subtype (so-called barcoding).

The presence of *Blastocystis*, confirmed by sequencing of the PCR product, was found in 6 dogs (7.2%). All dogs came from the same shelter, and all isolates represented the ST3 subtype.

For a few rodents and many environmental samples we obtained good quality PCR products similar in size to *Blastocystis*, but sequencing excluded this protozoan. On the other hand, in case of many environmental samples we received PCR products similar in size but of too poor quality to be sequenced, and it could neither be confirmed nor denied that they were *Blastocystis* isolates – for this reason, we cannot speculate about the presence or absence of *Blastocystis* in them. Environmental samples may contain many unidentified (unpredictable), not just faecal organisms, which can produce a variety of non-specific PCR products, similar in size to Blastocystis. Therefore, it seems that other research methodology should be used in the testing of such samples, for example, by preceding the molecular tests with an *in vitro* culture in a *Blastocystis* medium. The possibility of culturing *Blastocystis* from environmental samples in vitro was demonstrated by other researchers, but in our case, for logistical reasons, it could not be used.

This work was supported by grant number ST-02-0104/772 from the Medical University of Gdańsk and National Science Centre, Poland, under the BiodivERsA3 programme (grant numer 2019/31/Z/NZ8/04028)

Dirofilariosis – parasitic infection of increasing importance in Poland

Beata Szostakowska

Department of Tropical Parasitology, Faculty of Health Sciences, Medical University of Gdańsk, Gdynia, Poland

Dirofilariosis is a zoonosis of increasing medical importance, the etiological factor of which are Dirofilaria nematodes, mainly *D. repens* and *D. immitis*. The main definitive hosts and reservoirs of the parasite are dogs, and the intermediate hosts are female mosquitoes of different genera. Human is an accidental host in which the parasite usually does not reach maturity.

Over the last 20 years, there has been an increase in the range of occurrence of *Dirofilaria* spp. in Europe (including countries with a temperate climate), and an increase in the number of cases among people in these countries.

So far, 22 cases of human dirofilariosis have been described in Poland, all caused by *D. repens*, mostly imported or possibly imported, but also some certainly native. However, there are more cases, although the level of underestimation of the disease is unknown.

Among all described cases of dirofilariosis diagnosed in Polish patients, the subcutaneous form was the most common, although the majority of patients consulted at the Department of Tropical Parasitology Medical University of Gdańsk were patients with the ocular form (mainly subconjunctival). In the presentation we will show some photos documenting these cases.

In Poland, no cases of human infection with *D. immitis* have been reported so far, but infection of dogs with this parasite has already been described. Therefore, when this disease is detected in humans, the species identification of the parasite should be performed for epidemiological purposes. Moreover, also for epidemiological purposes, all cases of dirofilariosis in Poland should be obligatorily reported and registered.

Mathematical models used in the epidemiology of tick-borne diseases

Jacek Zwoliński[1], Violetta Zając[1], Anna Sawczyn-Domańska[1], Jacek Sroka[1, 2], Anna Kloc[1], Angelina Wójcik-Fatla[1]

1 Department of Health Biohazards and Parasitology, Institute of Rural Health, Lublin, Poland
2 Department of Parasitology and Invasive Diseases, National Veterinary Research Institute, Pulawy, Poland

Ticks may transmit many various pathogens to animals and humans, and therefore play an important veterinary, medical and economic role. Ticks carry a large number of various pathogens and more microorganisms than any other arthropod, which makes them one of the most important vectors of animal and human diseases.

Climate change may increase the range of individual tick species and their activity period. The spread of ticks and tick-borne diseases suggests the need to implement effective prevention programs.They should be based on epidemiological mathematical models. These models may help to bunderstand the epidemiology of ticks and tick-borne diseases and to develop appropriate methods.

There are many different types of models, which differ mainly in the degree of complexity, ranging from simple deterministic mathematical models to complex, spatial stochastic simulation models. The result of the model operation is dependent on many factors, such as the degree of understanding of the epidemiology of a specific disease, the quantity and quality of available data, and the experience of people implementing the model. Evaluating model performance its sensitivity, specificity and accuracy should be calculated.

Models can be used for retrospective analysis, planning of remedial actions, planning of resources necessary to perform these actions, training (threat simulation), finding markers of future events,and decision support in real time.

In particular, one can distinguish models examining the risk of disease, dependence on various risk factors, disease development in the population, population dynamics, economic analysis of epidemic effects and the effects of measures taken, and special models targeting specific aspects (e.g. transmission by birds).

The epidemiology of tick-borne diseases is closely related to the life cycle of ticks and is dependent on many environmental factors due to the variability of particular stages of ticks. The richness of host species also affects the diversity of pathogens transmitted. Special environmental requirements are the most important factors determining the distribution of ticks in nature.

This presentation will show examples of various existing models of ticks and tick-borne diseases, as well as the author's attempt to use a mathematical model relating to the occurrence of Lyme disease and tick-borne encephalitis in humans in Poland.

This study was supported by the Institute of Rural Medicine as part of the research project number 21050.

Detection of *Toxoplasma gonidii* in Women from Different Iraqi Regions Suffering from Continuous Abortions

Reem Turki Idan Rabeeah, Mahmood Almoalm

Al-Nisour University/Baghdad/mansor, Baghdad, Iraq

Nearly all warm-blooded animals are infected with the obligatory intracellular parasite *Toxoplasma gondii*, which plays a significant role in human infections. With about 30% of the world's human populations infected with *T. gondii*, toxoplasmosis is a widely frequent illness that is regarded as a severe public health issue. Toxoplasmosis, a disease caused by the obligate intracellular parasite T. gondii, a member of the phylum Sporozoa, is one of the causes of miscarriage. It affects humans and the majority of warm-blooded animals worldwide. In general, there are three main ways that a human can contract the disease: through drinking contaminated water, eating contaminated food (such as meat that has been partially cooked and contains tissue cysts, or food that has been contaminated with oocysts from cat feces), and congenital transition (transmission from an infected mother to her fetus – through the placenta). Pregnancy-related congenital toxoplasmosis can result in multiple miscarriages, stillbirths, and varying degrees of mental or physical impairment, hydrocephalus, blindness, and deafness.

This study included 300 samples of blood that have been collected from women suffering continuous abortions from different Iraqi cities. All pregnant subjects of this study have been informed for enrollment to the study. The subjects also have been asked to fill a questioner that contain the following informations: prior exposure to cats, signs of illness, level of education, line of work, and age; number of previous miscarriage.

The blood samples have been subjected to DNA extraction by following the instructions of the commercial kit Quick-DNA™ Miniprep Plus. Eluted DNA have been used to detect the *T. gondii* by adding 3 µl of this eluted DNA to a new sterile PCR tube, then 10 µl of the KAPA Sybr Fast Universal kit, 1µl of reverse primer and 1 µl of forward primer, then the volume were completed to 20 µl by adding 5 µl of nuclease free water. Real-Time PCR carried out in the apparatus Sa-cycler96/ SACACE under the following conditions: denaturation at 94°C for 5 min, then 35 cycles first at 94°C for 35 s, then at 60°C for 1 min, then the final step is 72°C for 7 min.

Preliminary studies have shown that higher percentage of infection has shown in the group of patients from Erbil province (31, p-value = 0.034). The women with higher age showed a higher percentage of infections (p-value = 0.001).

Babesia sp. '*venatorum*' in Polish Blood Donor: The First Recorded

Adam Kaczmarek[1, 3], Adrian Syta[2], Aleksandra Matras[3], Agata Stasiak[3], Rusłan Sałamatin[1, 4]

1 Department of Microbiology and Parasitology, Faculty of Medicine. Collegium Medicum, Cardinal Stefan Wyszyński University in Warsaw, Warsaw, Poland
2 Independent scientist, Warsaw, Poland
3 Department of Parasitology and Vector-Borne Diseases, National Institute of Public Health NIH – National Research Institute, Warsaw, Poland
4 Department of General Biology and Parasitology, Medical University of Warsaw, Warsaw, Poland

The *Babesia* spp. species are intra-erythrocyte parasites. Vertebrates, including humans, (intermediate host), become infected while being fed on by ticks, mainly those belonging to the family Ixodidae (definitive host).

A healthy person will generally pass the infection asymptomatically, but a immunocompromised person may experience symptoms such as general malaise and fatigue. As the disease progresses, flu-like symptoms may develop: fever, chills, sweating, and muscle and joint pain. Hemolytic anemia, intravascular coagulopathy, and enlargement of the liver and spleen may also develop. Where the disease is complicated, respiratory distress syndrome, cardiovascular failure, and inflammation of the central nervous system may occur – and in extreme cases it may cause death.

Pilot studies of blood donors have been carried out in, inter alia, the USA, where cases of blood transfusions from donors infected with Babesia have been reported.

The aim of our work was to test samples from blood donors for the presence of *Babesia* spp. DNA.

A total of 200 venous blood samples from blood donors from a Regional Blood Donation and Treatment Center were tested. DNA isolation was performed using a commercial kit for DNA isolation from blood. Real-time multiplex PCR studies were carried out according to Rożej-Bielicka *et al*. (2017).

A positive result was obtained in one case. After sequencing the PCR product, we obtained the sequence 333 bp, which corresponded to the *Babesia* sp. 'venatorum' deposited in the GenBank database.

To the best of our knowledge, this is the first time that *Babesia* has been detected in blood from donors in Poland.

Risk of Lyme borreliosis in human following *Ixodes ricinus* tick bite in Poland

Renata Welc-Falęciak[1], Julia Koczwarska[1], Olga Zdzienicka[1], Agnieszka Pawełczyk[2]

1 Department of Parasitology, Faculty of Biology, University of Warsaw, Warsaw, Poland
2 Department of Immunopathology of Infectious and Parasitic Diseases, Medical University of Warsaw, Warsaw, Poland

Lyme borreliosis (LB) is the most commonly diagnosed tick-borne disease in Europe, where almost 85,000 cases are reported annually. The estimated incidence of LB in Poland increased dramatically from 20.3 per 100,000 inhabitants in 2007 to 53.7.0 per 100,000 inhabitants in 2019. It is generally known that the risk of acquiring Lyme disease increases with the length of tick feeding, especially during the first 24 h. However, the risk of developing LB after the tick bite of *Borrelia*-infected tick in Poland is still unclear.

In 2021, 657 tick-bitten persons in Poland removed 840 Ixodes ricinus ticks. The participants were asked to complete the first on-line questionnaire concerning, among others, the site of the tick attachment, the number of tick(s) detached, where the tick(s) was/were encountered (urban/rural), the estimated time of tick exposure, the use of chemoprophylaxis as well as previous diagnosis and treatment of Lyme borreliosis. Eight weeks later the participants were asked to complete a second on-line questionnaire which included questions about new tick bites and the person's general health condition during the past two months, reddening at the bite site, symptoms possibly associated with tick-borne diseases, including erythema migrans (EM). In addition, medical records were obtained from participants who attended GP appointments for symptoms possibly associated with LB, including EM. The questionnaires included as well questions about the results of serological test for LB (if such were performed) as well as antibiotic treatment within the last 8 weeks. The duration of feeding time of nymphs and females removed from skin was estimated from scutal and coxal index. Genomic DNA from individual ticks was isolated and used for molecular screening for spirochetes through amplification of the flagellin gene (flaB) marker. Restriction fragment length polymorphism (RFLP) was used to differentiate *Borrelia*-positive isolates at the species level.

Overall, the prevalence of Borrelia infection in the I. ricinus ticks was 19.8%. The ticks were predominantly infected with B. afzelii (60%). Almost 70% of nymphs and female ticks were removed from skin more than 24 hours after the attachment. The majority of participants (78%) admitted non-using repellents. Non-specific, flu-like symptoms after tick bite were declared by 16 % of participants. Erythema migrans was reported in 14 tick-bitten persons (2.5%). Overall, the risk of LB In human after tick bite was < 5% in this study population.

The study was funded by the National Science Centre (NCN) OPUS grant no. 2020/37/B/NZ6/01587.

Occurrence of *Borrelia burgdorferi* s. l. in ticks removed from humans

Angelina Wójcik-Fatla[1], Anna Kloc[1], Anna Sawczyn-Domańska[1], Violetta Zając[1], Jacek Sroka[1, 2], Robert Chmura[1], Teresa Kłapeć[1]

1 Department of Health Biohazards and Parasitology, Institute of Rural Health, Lublin, Poland
2 Department of Parasitology, National Veterinary Research Institute, Lublin, Poland

The increased interest in testing ticks removed from the skin for the presence of *Borrelia burgdorferi* s. l. was observed over the 4 years of the e implementation of the National Health Programme (from 364 in 2017 to 1121 in 2020).

Ticks submitted by participants from 2017 to 2020 were identified to species and life stage and examined for the presence of fragment of fla gene of *Borrelia burgdorferi* sensu lato by PCR. The positive results of amplification were confirmed by sequencing. Additionally, the participants answered the questionnaire, including questions concerned tick attachment site on the human body, methods of tick removal, duration of feeding time, type of area where the bite occurred.

A total of 2945 ticks from humans were received of which over 95% were *Ixodes ricinus* (2083), followed by *Dermacentor reticulatus* (142). Humans were most commonly bitten by *I. ricinus* nymphs (1722 cases). The presence of *Borrelia burgdorferi* s. l. was confirmed only among *I. ricinus* population and the percentages of female, male and nymph infection rates were almost on the same level: 14.8%, 14.1% and 13.9%, respectively. The most common tick bites occurred in forested areas (35.5%). Over 1/4 of the cases concerned farms (27.8%), followed by playgrounds, parks (20.8%) and meadows (15.9%). Among the ticks supplied for the study, 155 individuals (5.3%) were classified as fully engorged. The vast majority were ticks engorged on blood with a medium degree (59.1%). Most tick bites occurred on the legs (42.4%) and torso (33.8%). Rarely, ticks were removed from the head/neck (13.2%) and the arms (10.6%). More than half of the participants (52.7%) confirmed the occurrence of skin lesions at the tick bite site. In cases where erythema migrans appeared, the tick result was positive. The participants asked for help family members/friends (40.1%) or medical staff (32.7%) in removing tick from their skin. Almost 25% decided to remove the tick on their own. The ticks were removed with tweezers (46.1%) or specialized devices such as tick traps and hooks (29.1%). In more than 20% of cases, ticks were removed with the fingers, also by medical workers.

The tick result cannot be the final confirmation or exclusion of spirochete infection, but it can arouse interest in health and take preventive measures in the form of diagnostic tests and medical consultations.

The research was conducted within the National Health Programme for 2016-2020, financed by the Ministry of Health (6/4/6/NPZ/FRPH/2018/793/209).

Toxoplasma gondii and *Borrelia burgdorferi* s. l. in patients with encephalitis

Agnieszka Pawełczyk[1], Karol Perlejewski[1], Monika Kurowicka[1], Marcin Makowiecki[2], Marcin Paciorek[2], Marek Radkowski[1], Tomasz Laskus[2], Renata Welc-Falęciak[3]

1 Department of Immunopathology of Infectious and Parasitic Diseases, Faculty of Medicine, Medical University of Warsaw, Warsaw, Poland
2 Department of Adults' Infectious Diseases, Faculty of Medicine, Medical University of Warsaw, Warsaw, Poland
3 Department of Parasitology, Faculty of Biology, University of Warsaw, Warsaw, Poland

Encephalitis is a result of a focal or disseminated brain inflammation caused by viruses, bacteria, parasites including *B. burgdorferi* s. l. and T.gondii or may be an immunological consequence of previous infections. Diagnosis of encephalitis is based on the analysis cerebrospinal fluid (CSF), serological and molecular testing and neuroimaging.

In the majority of the immunocompetent patients, infections of the central nervous system (CNS) with T.gondii are asymptomatic. Life-threatening reactivations of infection usually occur in immunocompromised patients and are triggered by a rupture of *Toxoplasma* cysts in the brain.

In CNS infections with *B. burgdorferi* s. l., the clinical course depends on the bacterial strain. Pathogens enter the CNS via the bloodstream or peripheral nervous system and their presence in the CSF is a key factor in the development of neuroborreliosis.

The aim of the study was to analyse the prevalence of serological and molecular markers of *T. gondii* and *B. burgdorferi* s. l. infection in patients with encephalitis of undefined aetiology.

Blood serum and CSF samples have been collected from 31 patients with diagnosed encephalitis, hospitalized in the Department of Adults' Infectious Diseases Medical University of Warsaw with diagnosed encephalitis. The presence of anti-Borrelia and anti-*Toxoplasma* antibodies has been tested by ELISA (Biomedica Laboratories, Vienna, Austria and NovaTec, Frankfurt, Germany respectively) method and DNA was detected using RT PCR.

Anti-*B. burgdorferi* s. l. IgM or IgG antibodies have been found in two (6.4%) serum samples, whereas in one case (3.2%) both IgM and IgG antibodies have been identified. No antibodies have been detected in the CSF. In 3 (9.6%) cases high-avidity IgG anti *T. gondii* were present in both serum and CSF, indicating that the patient had a previous contact/infection with the pathpgen. In 3 (9.6%) samples low-avidity IgG antibodies were found in the absence of the IgM antibodies. In one (3.2%) case, concurrent presence of IgM and IgG anti-*B. burgdorferi* s. l. antibodies and high-avidity IgG anti-*T. gondii* antibodies was reported. This may indicate past *T. gondii* infection with a possible risk of reactivation. Pathogen's DNA was not detected in any sample.

The presence of serological markers of *T. gondii* and *B. burgdorferi* s. l. infection in blood serum and/or CSF of encephalitis patients suggest possible relationship between the pathogens and the disease and requires further investigation.

This study was funded by grant 2017/25/B/NZ6/01463 from the National Science Center, Poland.

Ixodes ricinus as a potential vector of *Bartonella henselae* – *in vitro* study

Violetta Zając[1], Anna Sawczyn-Domańska[1], Jacek Sroka[1, 2], Anna Kloc[1], Jacek Zwoliński[1], Angelina Wójcik-Fatla[1]

1 Department of Health Biohazards and Parasitology, Institute of Rural Health, Lublin, Poland, Lublin, Poland
2 Department of Parasitology and Invasive Diseases, National Veterinary Research Institute, Pulawy, Poland

Bartonella henselae is a zoonotic Alphaproteobacteria that causes cat scratch disease. Cats are the main reservoir of *B. henselae*, and these bacteria are transmitted to cats by cat fleas. However, new potential vectors are suspected, in particular ticks. *Ixodes ricinus* is the most common species in Poland and vector of pathogens causing diseases in human and animals. The high seroprevalence of *B. henselae* in individuals occupationally exposed to tick bite may suggest the role of ticks in transmission of bacteria.

A preliminary study used the tick cell line to evaluate the ability of B. henselae to persist and multiply in tick cell. The IRE/CTVM19 line (Tick Cell Biobank) and *B. henselae* str. Houston-1 (ATCC) were used in experiments. The tick culture in multi-well plate was infected with *B. henselae* and evaluation was made from 1 to 9 days post infection. The experimental culture was maintained in the same conditions as for tick cell line. The material was collected from supernatant and monolayer separately and each probe (day) was performed in triplicate. RNA from assembled material was isolated immediately after collection. In next step the reverse-transcription was performed to obtain cDNA from the samples. cDNA was utilized to detect ribC gen as marker of *B. henselae* with use of Sybr Green in Real-time PCR method. The observations of viable cells were carried out daily under an inverted microscope.

Bartonella infection did not affect the morphology and proliferation of tick cells throughout the entire culture period compared to not-infected tick line. With use of molecular methods the live B. henselae were not detected from 1 to 8 day (p. i.), both for supernatant and for monolayer samples. Only for 9 day post infection B. henselae was detected in samples from monolayer cells.

Results of experiment indicated that live *B. henselae* may persist in tick cell line but at very low level. The grow of bacteria in the presence of tick cells is very slow and research should be extended over a longer period of time. The cultivation of *B. henselae* in tick cells in vitro may indicate the possibility of bacteria to grow in ticks in natural condition but the level of bacteria may be very low and transmission to human or animal may not be possible. The tick cell lines can be a useful tool in pathogen-vector relationship studies.

This study was supported by the National Science Centre, project MINIATURA (No. 2019/03/X/NZ7/02079).

Vertical transmission of *Babesia microti* – molecular studies in BALB/c mice and their offspring

Małgorzata Bednarska[1], Katarzyna Tołkacz[2], Patrycja Antos[1], Renata Welc-Falęciak[1]

1 Department of Parasitology, Faculty of Biology, University of Warsaw, Warsaw, Poland
2 Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

Babesia microti is a protozoan that belongs to Apicomplexa, causing babesiosis. Rodents are the zoonotic reservoir for this parasite. It is a species that infects only ticks of the genus Ixodes and replicates in red blood cells of intermediate hosts. There are three known pathways of Babesia infection: tick feeding, blood transfusion/organ transplantation, and vertical transmission. The aim of the study was to determine the intensity of *B. microti* infection in females and F1 progeny of BALB/c mice in blood and selected organs in 4 experimental groups. The intensity and extent of infection in individual groups was determined by RT-PCR. *B. microti* vertical transmission was confirmed in each research group. The offspring were born in two groups. Which numerous pathologies in the group of pregnant mice infected in late pregnancy and totally healthy in the group of mice which became pregnant in the phase of the chronic invasion. Pregnancy did not develop in the other two groups. Differences in pregnancy and fetal development are related to the intensity of the invasion in the blood and organs themselves. The protozoan was most often located in the liver, while the least often in the kidneys.

Poster session

The first statement of *Haemaphysalis concinna* in Świnoujście (North-West Poland)

Marek Asman[1], Justyna Jakubas-Zawalska[2], Joanna Witecka[1], Krzysztof Solarz[3]

1 Department of Parasitology, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia in Katowice, Sosnowiec, Poland
2 Sanitary-Epidemiological Station in Świnoujście, Świnoujście, Poland
3 Department of Parasitology, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia in Katowice, Sosnowiec, Poland

There are about 70 species of ticks in Poland. Nineteen of them belong to the constant fauna, while the others are transported to our country by their hosts, for example small, medium and big mammals and birds. One of them is *Haemaphysalis concinna*. The first official locality of this tick species in Poland was in Troszyn (North-West Poland) in 1956. Then, a few decades ago *H. concinna* was noted in a few new localities in Poland i.a. near Wolsztyn (West Poland), Lower Silesia (South-West Poland) and near Nowa Dęba (East Poland) in 2018. Dynamic climate changes and progressive urbanization are factors contributing to the colonization of new areas by such imported species like H. concinna. The aim of study was determination of the risk of human exposure to ticks and tick-borne pathogens in the selected recreational areas of Świnoujście. Ticks were collected by flaging from vegetation in the spring peak of their activity in 2019 from the 4 selected recreational areas to the tubes with 70% ethyl alcohol. Then they were determined to the species and developmental stage under the light microscope according to the key by Nowak-Chmura (2013). DNA from ticks were isolated by ammonia method. *Borrelia burgdoroferi* sensu lato, *Anaplasma phagocytophilum, Babesia microti* were detected in ticks by PCR. To examine of these patogens the primers specific to the flagelline gene fragment, 16S rRNA gene and 18S rRNA gene were used respectively. The amplification products were separated in 2% ethidium bromide stained gels and visualized under the ultra violet light. During the faunistic studies the two individuals of *H. concinna* (1 nymph and 1 female) were collected from the two various sites. The presence of nymph was noted in the edge of the forest along a residental road in an estate of single-family houses. In turn, the female was noted in the other place-forested area along a shared-use path in a coastal belt. None of the studied pathogens were not identified in the examined *H. concinna* individuals. The conducted studies showed the first statement of *H. concinna* in Świnoujście (North-West Poland).

Occurrence of *Blastocystis* in school children living in Kosovo

Karolina Baranowicz[1], Anna Lass[1], Krzysztof Korzeniewski[2]

1 Department of Tropical Parasitology, Institute of Maritime and Tropical Medicine, Medical University of Gdańsk, Gdynia, Poland
2 Epidemiology and Tropical Medicine Department in Gdynia, Military Institute of Medicine in Warsaw, Gdynia, Poland

Blastocystis is a unicellular anaerobic protozoan commonly found in the digestive tract of both humans and animals. Despite a lot of research, the role of *Blastocystis* as a commensal or parasitic factor has not been clearly defined. The most common symptoms of blastocystosis are diarrhea, abdominal pain, nausea or vomiting. Infection has been also considered as possible factor initiating the development of irritable bowel syndrome (IBS) or inflammatory bowel disease (IBD). However, some patients do not show any symptoms of infection, suggesting a commensal relationship with the host. Further scientific reports link the variable pathogenicity of *Blastocystis* with the presence of as many as 28 protozoan subtypes, showing considerable variability in the gene encoding the small ribosomal RNA subunit (SSU-r RNA). The prevalence of *Blastocystis* differs between developed and developing countries. The incidence of Blastocystis in individual European countries is estimated at 15–25%. In developing countries, inadequate hygienic conditions, consumption of contaminated water or food results in up to 50% of the population infected. So far, there are no data on prevalence of Blastocystis among populations of Balcan countries. Therefore, the aim of this study was to estimate the degree of the occurrence of the *Blastocystis* among children living in Kosovo being considered as developing country and to determine their genetic diversity.

In total, 478 samples fixed in 70% ethyl alcohol were collected from school children in age 6–15 in the community of Kaçanik, southern part of Kosovo in 2016. Next, the samples were investigated using molecular methods for specific detection of DNA of *Blastocystis*. DNA isolation was performed using A&A Biotechnology's commercial Genomic Mini AX Stool kit. The obtained DNA isolates were then analyzed for the presence of the specific SSU-rRNA Blastocystis subunit by PCR amplification using primers RD5StenF and BhRDr. Products of amplification were sequenced in order to estimate genotypes of the parastite.

Out of 478 tested trials, 126 (26.4%) positive results were obtained. Subsequently, the obtained PCR reaction products from 48 positive samples were sequenced and based on the conducted research, it was found that the isolated Blastocystis show genetic diversity. The most common subtype was *Blastocystis* ST3 (22 trials, 45.7%), followed by *Blastocystis* ST1 (15 trials, 31.3%). The remaining samples tested were classified as *Blastocystis* ST2 (11 trials, 22.9%).

Molecular studies confirmed high level of contamination of investigated school children with *Blastocystis* that corresponds most probably with low economic status of Kosovo and poor sanitary conditions.

Wild living mesocarnivores as competent or reservoir hosts for vector-borne pathogens

Joanna Hildebrand, Katarzyna Buńkowska-Gawlik, Izabella Myśliwy, Kacper Jurczyk, Klaudia Wojtków, Aleksandra Grzesiak, Marcin Popiołek, Agnieszka Perec-Matysiak

Department of Parasitology, University of Wrocław, Wrocław, Poland

The incidence, diversity and geographic range of the pathogens harboured by wild living mesocarnivores have increased due to several factors in recent years. These mainly include destruction of wildlife ecosystems, urbanization and climate changes as well as increased interaction of wild and domestic animals, as well as the spread of vectors of selected parasites and pathogens, including those with zoonotic potential. As the dynamics and epidemiology of vector-borne diseases are still not well understood, there is possible risk of vectore-borne pathogens transmission to another wildlife species as well as from wildlife to domestic animals.

The aim of the study was to identify and characterize the taxonomic diversity of VBP pathogens in wild living carnivores both invasive and domestic ones. The tissue samples (skin and spleen) were obtained form red fox, raccoon dog, raccoon, badger, pine and beech martens. The study material was collected from Ruszów Forestry, the Lower Silesian District in Poland during the predator control operation conducted as a part of the program to re-introduce the *Tetrao urogallus* (project LIFE11 NAT/PL/428).

To confirm the occurrence of vector-borne pathogens, nearly 700 isolates obtained from mesocarnivore specimens have been used for molecular analyses. The presence of DNA of following genera: *Babesia*, *Theileria*, *Hepatozoon*, *Anaplasma*, *Ehrlichia*, *Candidatus Neoehrlichia*, *Bartonella*, *Borrelia* and *Rickettsia* was confirmed. The detailed analysis of nucleotide sequences allowed for a taxonomic identification, and for determining the specificity of the pathogen-host relationships. These results combined with quantitative data such as the prevalence, type of tissue, pathogen coexistence, allowed for the determination of the role of individual carnivore species in circulation of these VBPs. Additionally, we analyzed the extent of interspecific transmission of identified pathogens between examined species of sympatric carnivores.

Neoehrlichia mikurensis, an emerging tick-borne pathogen in the north-eastern Poland: a preliminary screening

Magdalena Szczotko[1], Katarzyna Kubiak[2], Leonardo Moreira[3], Sandra Antunes[3, 4], Ana Domingos[3, 4], Małgorzata Dmitryjuk[5]

1 Students' Parasitology "Vermis" Science Club, Department of Medical Biology, Collegium Medicum, School of Public Health, University of Warmia and Mazury in Olsztyn, Poland, Olsztyn, Poland
2 Department of Medical Biology, Collegium Medicum, School of Public Health, University of Warmia and Mazury in Olsztyn, Poland, Olsztyn, Poland
3 1. Global Health and Tropical Medicine – Institute of Hygiene and Tropical Medicine – NOVA University of Lisbon, Lisbon, Portugal
4 Institute of Hygiene and Tropical Medicine – NOVA University of Lisbon, Lisbon, Portugal
5 Department of Biochemistry, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Poland, Olsztyn, Poland

Neoehrlichia mikurensis is an emerging tick-borne intracellular pathogen, etiological agent of neoehrlichiosis. Initially, it was assumed that neoehrlichiosis mainly affects people with immune deficiencies, however it was confirmed that the disease can affect immunocompetent patients as well. In 2004, the bacterium was classified as a member of the Anaplasmataceae family and named as *Candidatus Neoehrlichia mikurensis*. Recently, in 2019, isolation of the bacterium in pure culture has been reported and its name lost the prefix "*Candidatus*". In Europe, *Ixodes ricinus* is the main vector of tick-borne pathogens affecting humans, including *N. mikurensis*. The aim of the present research was to detect N. mikurensis in questing *I. ricinus* ticks collected in the Olsztyn districts in order to determine the risk of spreading the pathogen in north-eastern Poland.

Questing ticks were collected during the daytime using the standard flagging method and preserved in stay-RNA™ buffer. Collection of ticks was conducted from May to September of 2021. Tick`s RNA was extracted from the whole individuals using the NZol reagent according to manufacturer's instructions. The amount of obtained RNA was determined by Qubit Fluorometric Quantitation. To amplify a 107 bp long amplicon from the bacterium 16S rRNA gene, the primers NEO_16S_F and NEO_16S_R, previously described, were used. Synthetized gBlocks® Gene Fragment based in the 16S ribosomal gene was used for standard curves and relative quantitation. Reactions were run on a CFX Connect Real-Time PCR Detection System equipped with FAM and HEX filters sets and further analyzed with CFX Manager software. The prevalence of pathogens was calculated with 95% confidence intervals (95% CI) using the "exact" interval by Clopper and Pearson (Prism 6 program, GraphPad Software, San Diego, CA, USA). A Chi-square test was used to check whether there was a relationship between variables groups of tested tick. Values of $p < 0.05$ were considered statistically significant.

Preliminary screening have shown that 16.1% (20/124; 95% CI: 10.1-23.8) of the analyzed *I. ricinus* ticks were positive for the cDNA of *N. mikurensis*. Adult ticks were more infected (11/43, 11.1%, 95% CI: 13.5–41.2) than nymphs (9/81, 11.1%, 95% CI: 5.2–20.0). The differences between the analyzed groups were statistically significant ($Chi^2 = 4.35$, $p = 0.0371$).

Our preliminary results confirm that *N. mikurensis* is widespread in Olsztyn district with an average prevalence of 16.1%, making it a significant tick-borne pathogen, suggesting that North-eastern Poland can be considered as neoerlichiosis high risk region.

Ixodes ricinus from Portugal mainland: Tick-borne pathogens and an insight of its microbiota

Leonardo Moerbeck[1], Rita Velez[2], Gonçalo Seixas[1], Ana Sofia Santos[2], Ana Domingos[1], Sandra Antunes[1]

1 Global Health and Tropical Medicine, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa (GHTM-IHMT-UNL), Rua da Junqueira, 100, 1349-008 Lisboa, Portugal.
2 Instituto Nacional de Saúde Doutor Ricardo Jorge, Centro de Estudos de Vectores e Doenças Infecciosas Dr. Francisco Cambournac (CEVDI/INSA), Águas de Moura, Portugal

In Europe, *Ixodes ricinus* is the most important tick species medically, being its characterization fundamental to assess disease risk and improve control measures. These ectoparasites harbor a complex variety of symbiotic, commensal and pathogenic organisms, named microbiota. This microorganism network directly impacts in many physiological processes on ticks such as, reproduction, nutrition, and fitness, for example. Thus, the present work aims first to identify tick-borne pathogens (TBP) in *I. ricinus* collected from Portugal mainland; and secondly to describe their microbiota in females collected from dissimilar regions in Portugal mainland. From February 2019 until May 2021, questing *I. ricinus* ticks (females, males, and nymphs) were collected from the vegetation by dragging method at different geographical regions. For the first objective, after morphological identification adult ticks were individually submitted to extraction, while samples of immature stages (nymphs) were set up by pools of five specimens. All samples were tested for the presence of *Babesia* spp., *Anaplasma* spp., *Rickettsia* spp., and *Coxiella burnetii*, as described in the literature. Obtained sequences will be later used for phylogenetic characterization of these agents. Collected I. ricinus female ticks from Gerês and Mafra were selected for the second objective. These specimens were washed and DNA extracted. The conserved bacterial 16S hypervariable regions (V3–V4) was sequenced by MiSeq Illumina 2 × 300 bp in a pairwise alignment sequence dissimilarity approach. The Minimum Entropy Decomposition (MED) algorithm was used for operational taxonomic unit (OTUs) identification and further assignments was performed. Regarding the TBP screening, results have shown that the most frequent pathogen identified in *I. ricinus* was *Rickettsia* spp.. Preliminary results from questing I. ricinus microbiota profiling suggest that most abundant OTU was the novel family of the order Rickettsiales: Candidatus Midichloriacea. In addition, two more genera were identified: *Rickettsia* and *Borrelia*, thus constituting the main internal microbiota of *I. ricinus* in the two collection sites. The results here reported contribute for a better understanding of TBP prevalence and tick microbiota from questing ticks collected in Portugal mainland.

Acknowledgements: FCT projects PTDC/CVT-CVT/29073/2017 and PTDC/SAU-PAR/28947/2017.

Molecular identification of *Taenia* spp. and *Echinococcus* spp. larvae isolated from pigs

Monika Dybicz, Marta Tokaj, Albert Gandurski, Michał Jerzak

Department of General Biology and Parasitology, Medical University of Warsaw, Warsaw, Poland

A number of cestode species can develop in the liver of pigs. Pigs are intermediate hosts of the larval stage of the genus *Echinococcus* maintaining the parasite cycle on farms and consequently infecting dogs. Dogs are able to disperse the parasite eggs and cause a risk of human invasion. The other species of tapeworms found in the liver of pigs are *Taenia hydatigena* and *T. solium*. The larval form of *T. hydatigena* is a common species that causes cysts which are similar to *E. granulosus*. Both *E. granulosus* sensu lato and *T. hydatigena* have the same intermediate hosts including sheep, cattle and pigs. When these animals are slaughtered in abattoirs with sanitary inspection, they are subjected to rigorous examination in order to identify the cysts in viscera. The method to detect parasite is based on visual macroscopic observation in tissues. This method usually does not allow to distinguish the etiology of the lesion. The diagnosis of these parasitosis is very important since these infections generate economic losses and cystic echinococcosis is a problem for public health.

The aim of the study was to identify the parasite species in visceral cysts collected from swine slaughtered under veterinarian inspection located in eastern/northern regions of Poland.

A total of 74 cysts were isolated from liver fragments and indicated morphological features similar to hydatid cysts. The cysts ranged in size from 0.8 to 3.2 cm in diameter, most of them was over 1 cm in diameter. Total DNA was extracted and used for amplification of NADH dehydrogenase 1 gene fragment. PCR products were purified and sequenced. The nad1 sequences were compared using NCBI GenBank.

Forty eight out of 74 DNA samples used as the template in separate PCRs yielded a single product. The sequences of 39 isolates (54.17%) were highly similar to *T. hydatigena*, 9 (9.72%) were identical to *E. canadensis* G7 which is probably the main genotype infecting pigs in many European countries. Twenty six samples (36.11%) provided no PCR product indicating no parasitic origin. This study demonstrates that the most common species causing liver lesions in pigs was *T. hydatigena* (over 50% of cases) and then *E. canadensis* (about 10%). The final host of both species is the dog and other canids, therefore it suggests that the main pig invasion source are the farm dogs which do not undergo regular antihelminthic treatment.

The prevalence of *Rickettsia* spp. in questing *Dermacentor reticulatus* (Fabricius, 1794) in the newly emerging population of Lower Silesia, SW Poland

Aleksandra Czułowska, Dorota Kiewra

Department of Microbial Ecology and Acaroentomology, University of Wrocław, Wrocław, Poland

In Europe *Dermacentor reticulatus* ticks are among the most significant vectors of tick-borne pathogens, including *Babesia canis*, *Francisella tularensis*, *Coxiella burnetti*, and several *Rickettsia* species. The increasing range of *D. reticulatus* may cause a potential new medical and veterinary threats in the newly inhabited areas. This study aimed to estimate the infection level of *D. reticulatus* with *Rickettsia* spp. in the newly emerging population of Lower Silesia. Questing ticks were collected using standard flagging method from February to March 2020. The collection was carried out on both sides of the Oder river in Wroclaw and its surroundings, in sites where the presence of D. reticulatus had previously been confirmed. Only ticks identified as *D. reticulatus* were used for *Rickettsia* spp. detection. Totally, 160 randomly selected ticks, including 40 females and 40 males collected on both sides of the Oder river were used for *Rickettsia* spp. detection. The nested PCR based on amplification of the citrate synthase gene gltA showed 58,7% prevalence of *Rickettsia* spp. There were no statistical differences in the level of infection between males and females ($\chi^2 = 0.413$, $p = 0.5205$), nor between the right and left side of Oder river ($\chi^2 = 0.103$, $p = 0.7483$). Sequencing of randomly selected samples confirmed the presence of *Rickettsia raoultii*. The occurrence of *D. reticulatus* ticks infected with *Rickettsia raoultii*, on both sides of the Oder river indicates a potential risk of infection with rickettsia from the spotted fever group in areas newly inhabited by *D. reticulatus*.

New outbreaks of selected zoonotic tick-borne diseases in mountainous areas of southern Poland

Anna Kocoń[1], Dominika Maron[1], Marek Asman[2], Magdalena Nowak-Chmura[1], Joanna Witecka[2], Sylwia Koczanowicz[1]

1 Institute of Biology, Department of Zoology, Pedagogical University of Cracow, Kraków, Poland
2 Department of Parasitology, Faculty of Pharmaceutical Sciences in Sosnowiec, Sosnowiec, Poland

The Małopolska Province are one of the largest tourist areas in Poland, they are also cultural, economic and academic centres with a rich flora and fauna, with a large number of tourist routes and leisure places, which makes them attractive for tourists from all over Poland and Europe. There is a great interest for people in traveling to these regions of the country with their accompanying animals, which can be carried and attacked by ticks (Acari: Ixodida). The aim of the study was to verify the occurrence of *Ixodes ricinus* ticks in the selected areas of Czarny Dunajec, Piekielnik, Podszkle (Nowy Targ district), Ząb and Murzasichle (Tatra district) and to evaluate ticks in domestic dogs and cats from the Susz district: Zawoja, Maków Podhalański, Sucha Beskidzka, Białka, Juszczyn, Skawica i Wieprzec. A flagging method was used to harvest ticks from vegetation from five selected sites within 100 m^2. Ticks from 21 dogs and 7 domestic cats were collected thanks to cooperation with a veterinary clinic. Molecular testing was undertaken to evaluate the presence of selected tick-borne disease pathogens including *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Babesia microti*, *Rickettsia* spp. and *Toxoplasma gondii* in the DNA of ticks. The study involved the isolation of genetic material of ticks using the non-organic method and the pathogens were detected by PCR and nested PCR reactions. The field investigations revealed the presence of 41 *Ixodes ricinus* ticks: 26 nymphs, 6 females and 9 males in 4 study areas; no ticks were found in the Murzasichle area. In molecular studies, the presence of *Rickettsia* spp. was detected in 7.9% of I. ricinus. In contrast, 28 I. ricinus ticks including 25 females and 3 males were colled from domestic dogs and cats. Among them, *A. phagocytophilum* was detected in 7.14% of *I. ricinus* Pathogens were detected in adult *I. ricinus*. Analyzing the results of the study, it can be concluded that foothill and mountain areas are not free from ticks and potential tick-borne diseases. Many factors influence the occurrence and survival of ticks in inconvenient mountain conditions, including constant changes in weather, availability of a host, population density of the area, vegetation, frequent travel. Confirmation of new outbreaks of zoonotic tick-borne diseases and new locations of tick infestations serve to better understand the tick fauna and to take important preventive measures to protect the residents and tourists from these dangerous vectors of tick-borne diseases.

Trichobilharzia species in recreational water in north-eastern Poland

Joanna Korycińska, Małgorzata Lepczyńska, Ewa Dzika

Department of Medical Biology, School of Public Health, Collegium Medicum, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

In Europe, the most common agents involved in human cercarial dermatitis (HCD) are members of the genus *Trichobilharzia*. Swimmer's itch or human cercarial dermatitis (HCD) appears as a skin rash caused by an allergic reaction to larval (cercariae) flatworm parasites of the family Schistosomatidae. In the life cycle of this parasite, humans play the role of an accidental host. Water snails are the intermediate host and waterfowl are the final host.

The aim of the research was the molecular and phylogenetic analysis of *Trichobilharzia* species in recreational water.

The research involved three recreational water bodies (Lake Skanda, Lake Ukiel, Lake Tyrsko) during the summer season of 2021. The total of 961 pulmonate freshwather snails (Radix sp., Lymnaea stagnalis, Planorbarius corneus) have been collected. For cercariae, the whole region including ITS, 5.8S, and ITS2 of the ribosomal DNA and additionally ITS1 alone was amplified. Lymnaea stagnalis was infected with Trichobilharzia szidati, while Trichobilharzia franki cercariae were found in Radix sp. The phylogenetic analysis was based on the partial sequence of the ITS region.

Clinically, the diagnosis of swimmer's itch in people is made primarily basing on the history taken from the patient and assessment of skin lesions. From epidemiological perspective it is of great importance to identify the underlying factor in swimmer's itch on the species level, which makes it possible to estimate the scale of the problem in a particular area.

Ricketssia spp. in adult *Dermacentor reticulatus* ticks in urban and natural biotopes of north-eastern Poland

Katarzyna Kubiak[1], Hanna Szymańska[1], Janina Dziekońska-Rynko[2], Agnieszka Tylkowska[3], Ewa Dzika[1]

1 Department of Medical Biology, School of Public Health, Collegium Medicum, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland
2 Department of Zoology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland
3 Department of Biology of Animal Environment, Institute of Animal Science, Warsaw University of Life Sciences, Warsaw, Poland

The genus *Rickettsia* includes numerous obligate intracellular gram-negative bacteria that are transmitted to mammals by blood-sucking arthropods. The greatest medical importance in this group of bacteria is attributed to the rickettsiae of the spotted fever group (SFGR). In European countries, *Dermacentor* ticks are important vectors of *Rickettsia* spp. responsible for the group of symptoms known as TIBOLA/DEBONEL (Tick-Borne Lymphadenopathy/Dermacentor-Borne-Necrosis-Erythema-Lymphadenopathy). The aim of the study was to determine the prevalence of the Rickettsia spp. and to evaluate species diversity in adult *D. reticulatus* ticks collected in urban and natural biotopes of north-eastern Poland.

Questing *D. reticulatus* ticks (n = 886, 587 females, 299 males) were collected between March–June in 2016–2017 in urbanized areas (city of Olsztyn) (n = 395) and in natural biotopes of the central part of the Warmia and Mazury region (n = 87) as well as in the Biebrza National Park (n = 404). The presence of *Rickettsia* spp. in tick genomic DNA samples was confirmed by the PCR method using two sets of primers (CS409/Rp1258) specific to the citrate synthase (gltA) gene. The identification *Rickettsia* species were based on sequencing of PCR products. The obtained nucleotide sequences were compared with data registered in the GenBank database. A chi-square test was used to compare the infection rate between sex of ticks, regions, habitats and years of study.

In north-eastern Poland the overall Rickettsia infection rate in *D. reticulatus* ticks was 27.1% (240/886). Prevalence did not differ between females (27,8%) and males (25.8%). The percentage of *Rickettsia*-positive ticks was significantly higher in 2016 (32.0%) than in 2017 (20.2%). The significant difference was noted between ticks collected in natural biotopes (35.6%) and in urban areas (16.5%). The DNA of *Rickettsia* spp. was the most frequently confirmed in ticks collected in the Biebrza National Park (38.1%), followed by central part of Warmia-Mazury region (24.1%) and city of Olsztyn (16.5%).

Sequence analysis of partial gltA gene indicated the presence only *R. raoultii*. All obtained nucleotide sequences (n = 81) were similar and showed 100% identity to *R. raoultii* isolated from blood of patient (GenBank: KY474581) and dog (GenBank: MT019635) in China.

In conclusion, prevalence of Rickettsia spp. in *D. reticulatus* ticks in the north-eastern Poland is generally high and it differs between years, habitats and regions. However the species diversity is low. The presence of pathogenic *R. raoultii* indicates that *D. reticulatus* ticks play significant role as a vector of that pathogen to humans and animals.

Molecular detection of *Plasmodium* spp. in population of symptomatic BaAka Pygmies Living in the rural Dzanga Sangha region of the Central African Republic

Anna Lass[1], Emilia Bylicka-Szczepanowska[2], Karolina Baranowicz[1], Beata Szostakowska[1], Krzysztof Korzeniewski[3]

1 Department of Tropical Parasitology, Institute of Maritime and Tropical Medicine, Medical University of Gdańsk, Gdynia, Poland
2 4th Department of Infectious Diseases, Provincial Hospital for Infectious Diseases, Warsaw, Poland
3 Epidemiology and Tropical Medicine Department in Gdynia, Military Institute of Medicine in Warsaw, Gdynia, Poland

Malaria remains a diagnostic and therapeutic challenge in many endemic regions of sub-Saharan Africa. It is one of the most important causes of morbidity and mortality, especially in children < 5 years. *Plasmodium falciparum* is responsible for the majority of severe malaria cases in sub-Saharan Africa, but is not the exclusive one. The objective of the study was to assess the prevalence of *Plasmodium* spp. in BaAka Pygmies with clinical symptoms of malaria, and define the percentage distribution of infections caused by species other than *P. falciparum* in order to assess the need for diversification of malaria treatment protocols.

The study was conducted during the dry and rainy seasons in 2018 and involved a group of 540 symptomatic BaAka Pygmies, patients of both genders, aged 1–75 years-old. The most common clinical symptoms reported by the sick individuals were fever, shivers and fatigue. Blood samples were collected using the FTA® cards (Whatman Bio-Sciences Ltd.). Next, 2 mm in diameter discs were punched from each card, placed in sterile tubes, and processed with the specific purification reagents. DNA extraction from 2 mm in diameter discs punched from each card and washed with specific purification reagents was performed using a modified version of the Sherlock AX Kit (A&A Biotechnology, Gdynia, Poland). In order to detect and differentiate *Plasmodium* species a multiplex real-time PCR assay (FTD Malaria Differentiation kit, Fast Track Diagnostics) was used. PCR results were compared with RDTs tests targeting HRP2-protein specific for P. falciparum.

Molecular tests confirmed *P. falciparum* in 94.8% of the samples investigated. Additionally, DNA of *P. malariae*, P. *ovale*, and *P. vivax* was detected in (11.1%), (9.8%), and (0.7%), respectively. There were also noted coinfections with two or three different species of *Plasmodium*. In contrary, only 40.5% of symptomatic patients tested with RDTs for *P. falciparum* infections were positive. BaAka Pygmies aged < 5 years of age dominated in patients with positive results.

Results of performed study suggests the need for introducing accurate diagnostic methods for the diagnosis of malaria and the revision of malaria treatment protocols. Assessment of the Pfhrp2/Pfhrp3 deletions is necessary for evaluating malaria epidemiology in Central Africa.

This work was supported by statutory work of the Epidemiology and Tropical Medicine Department in Gdynia, Military Institute of Medicine in Warsaw and Department of Tropical Parasitology.

Preliminary studies on the prevalence of the selected pathogens in ticks collected from pets living in households and in shelter

Magdalena Szczotko, Małgorzata Dmitryjuk

Department of Biochemistry, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Poland, Olsztyn, Poland

Ticks are blood-feeding ectoparasites of vertebrates with relevant medical importance. The most frequent species in Poland are *Ixodes ricinus* and *Dermacentor reticulatus*. The main significance of ticks depend on the ability to spread various pathogenic bacteria, viruses and protozoa. Ticks are reckon to transmit the broadest spectrum of infectious agents than any other blood-feeding vector. Tick-borne diseases afflicting humans include Lyme disease, babesiosis, tick-borne encephalitis, anaplasmosis or rickettsiosis. The ticks present in urban parks and suburban areas, are a potential vectors of diseases for domestic animals. The occurrence of pathogens in ticks feeding on dogs living in close contact with man poses another threat to human health. The aim of this study was to identify *Borrelia burgdorferi* s. l., *Rickettsia* spp. and protozoan *Babesia* spp. in *I. ricinus* and *D. reticulatus* ticks isolated from pets in two areas – Stawiguda (ticks collected by owners) and Tomaryny (ticks collected by shelter workers).

Ticks attached to pet`s skin were collected and preserved in stayRNA TM buffer. DNA from the individuals was extracted using DNA Gravity Extraction Kit (A&A Biotechnology, Gdynia, Poland) according to the manufacturer's instructions. Spectrophotometric method and PCR reaction targeting the fragment of the 16S rDNA tick gene was used to determine the quality of the genetic material. Pathogens were detected using PCR method. The targets for PCR amplification were the: B. burgdorferi s.l., fla gene, Rickettsia spp. gltA gene and *Babesia* spp. 18S rRNA gene. PCR products were visualized by electrophoresis on 1.5% agarose gel.

The prevalence of Borrelia s.l., Rickettsia spp. and Babesia spp. in ticks was 11.9% (7/59, 95% CI: 4.9-22.9), 42.4 % (25/59, 95% CI: 29.6-55.9) and 3.4% (2/59, 95% CI: 0.4-11.7), respectively. *Borrelia* spp. was more frequently detected in *D. reticulatus* (3/18, 16.7%, 95% CI: 3.6-41.4) than in I. ricinus (4/41, 9,8%, 95% CI: 2.7-23.1). Infection frequency with Rickettsia spp. was similar (approximately 42–46%) in both tick species. Babesia spp. was found only in *I. ricinus* ticks (2/41, 4,9%, 95% CI: 0.6-16.5). Only one coinfection *Borrelia* spp./*Rickettsia* spp. was detected in *D. reticulatus* male collected from dog living in Tomaryny shelter. None of analysed pathogen was observed in 3 ticks collected from cats.

Our preliminary results confirm that ticks feeding on dogs are a reservoir of all tested significant tick-borne pathogens. Suggesting that dogs living in close contact with the owners can increase the risk of tick-borne diseases human infection.

Sharing cosmetics as a route of *Demodex* mites infection

Aleksandra Sędzikowska[1], Renata Przydatek-Tyrajska[2, 3], Katarzyna Bartosik[2]

1 Department of General Biology and Parasitology, Medical University of Warsaw, Warsaw, Poland
2 Department of Biology and Parasitology, Medical University of Lublin, Lublin, Poland
3 Reno-Med Non-public Health Care, Warsaw, Poland

Demodex mites are permanent residents of pilosebacious units in humans and other mammalian species. Two species, i.e., *Demodex folliculorum* and *Demodex brevis*, have been found to infest humans. The predilection sites mainly include the face, scalp, and chest, but the mites can also colonise other parts of the body. Direct contact or eggs present in dust as well as contact with infected towels, blankets, or sponges are the possible routes of infection.

The aim of the study was to determine the length of survival of *Demodex* mites in commonly used makeup cosmetics, i.e., powder cream (fluid), mascara, and lipstick, and to find out whether shared cosmetics could be a source of *D. folliculorum* infection.

The mites were collected from the patients with a method of lash sampling with sterile tweezers. Analyses were performed on positive slides with the presence of adults mites. The survival of *Demodex* mites was examined in basic makeup cosmetics, i.e., mascara, lipstick, and powder cream. The *Demodex* spp. specimens were observed under a light microscope at various intervals.

In total, the survival of the mites was examined in 77 samples (28 samples of the lipstick, 25 samples of the powder cream, 14 samples of the mascara, and 10 control samples). The survival time was the longest in the case of *D. folliculorum* in the lipstick – 69 h and 260 h for the fully and partially immersed mites, respectively. In the mascara, the mites were able to stay alive for as long as 56 h. In the powder cream samples, the overall survival time of *D. folliculorum* was the shortest, i.e., maximum 2.3 h and 4.5 h.

Facial cosmetics shared at a short interval may contribute to Demodex transfer between the users. Therefore, cosmetics available to many customers should be tested with the use of disposable spatulas and makeup cosmetics should only serve for personal use.

Urban population of Norway rats, *Rattus norvegicus*, as a reservoir of *Blastocystis*, in Wrocław, Poland

Maria Wesołowska[1], Marcin Suszczyński[2], Adam Kaczmarek[3], Rusłan Sałamatin[3,4]

1 Department of Biology and Medical Parasitology, Wrocław Medical University, Wrocław, Poland
2 Bogel Elżbieta Suszczyńska Pest Control, Wrocław, Poland
3 Department of Microbiology and Parasitology, Faculty of Medicine. Collegium Medicum, Cardinal Stefan Wyszyński University in Warsaw, Warsaw, Poland
4 Department of General Biology and Parasitology, Medical University of Warsaw, Warsaw, Poland

Blastocystis sp. is a common protozoan that has often been reported in humans and animals worldwide. Molecular analysis showed high genetic heterogeneity. 28 subtypes were identified (ST1–ST32), including 10 subtypes of *Blastocystis* (ST1–ST9 and ST12) in humans. Several studies reveal that *Blastocystis* is a potentially zoonotic parasite, as all genotypes have been shown to occur in both humans and animals, and over 90% of human *Blastocystis* subtypes belong to ST1–ST4. The transmission routes and reservoirs of this parasite are still little known, especially through zoonotic transmission in urban conditions. Rats (*Rattus norvegicus*) in the local population caught our attention. Rats live in cellars, sewage systems, garbage cans and warehouses. They are the hosts for a number of parasites, as well as a zoonotic reservoir for numerous zoonoses. In Poland, there was no extensive research on the frequency of infection with zoonotic parasites in wild rats population. In our study, we undertook the assessment of the occurrence and phylogenetic analysis of *Blastocystis* occurring in rats in the urban population of Wrocław. Seventy-four samples of migratory rat faeces were studied, using xenic in vitro culture (XIVC) with a modified Jones' medium and molecular techniques (PCR). Gene fragment of SSU-rRNA was amplified with forward primer RD5 (5′-ATCTGGTTGATCCTGCCAGT-3′) and reverse primer BhRDr (5′-GAGCTTTTTAACTGCAACAACG-3′).

Blastocystis was found in 10/74 (13.5 %) of study samples. Preliminary molecular analysis revealed the presence of ST3 *Blastocystis*, the subtype considered to be the most anthroponotic.

These data may indicate that the problem of *Blastocystis* infection in rats should be further investigated, and that rats in the urban population are a potential reservoir of the parasite. To our knowledge, this is the first study of the presence of *Blastocystis* in the wild rats population in Poland.

Funding: Wroclaw Medical University subvention according to number: SUBZ.A060.22.054

SESSION 5

Influence of climate change and globalization on the occurrence of parasites and parasitic diseases

Oral session

Helminths of teleost fishes from the area of the Ukrainian Antarctic station "Akademik Vernadsky": species diversity and parasite community structure

Tetiana Kuzmina[1], Oleksander O. Salganskiy[2], Igor Dykyy[3], Olga Lisitsyna[1], Eleonora Korol[4], Yuriy Kuzmin[1]

1 I. I. Schmalhausen Institute of Zoology NAS of Ukraine, Kyiv, Ukraine
2 State Institution National Antarctic Scientific Center, Kyiv, Ukraine
3 Ivan Franko Lviv National University, Lviv, Ukraine
4 National Museum of Natural History NAS of Ukraine, Kyiv, Ukraine

Monitoring studies of the species diversity in Antarctic marine ecosystems provide important data on the current state of these ecosystems and reveal the ecological changes caused by global warming and anthropogenic influence. This work was aimed to analyze the modern state the helminth species diversity in main teleost fish species inhabiting the area near the Ukrainian Antarctic Station "Akademik Vernadsky", Argentine Islands, West Antarctica.

During April–January of 2014–2015 and 2019–2021, 236 specimens of six teleost fish species (*Notothenia coriiceps, N. rossii, Chaenocephalus aceratus, Parachaenichthys charcoti, Trematomus bernacchii, Harpagifer antarcticus*) were examined. In total, 29,092 specimens of 31 helminth species belonging to five taxonomic groups: Monogenea (1 species), Digenea (9), Nematoda (5), Cestoda (4), and Acanthocephala (12) were collected. Species diversity in *N. coriiceps* was the highest (29 species); 11 species (aniskid nematodes, cestodes and acantocephalans of the genus *Corynosoma*) were on larval stages. In *N. rossii*, 14 helminth species were found: in *P. charcoti* – 27 species, in *Ch. aceratus* – 23, in *T. bernacchii* – 16 and in *H. antarcticus* – 6 species. Three groups of endohelminth species were separated in the parasite community of *N. coriiceps*: dominant (prevalence=70–100%), background (prev.= 20–70%) and rare (prev. < 20%) species. Significant changes in the species richness in helminth infracommunities and component communities of *N. coriiceps* during the last two decades were identified. These changes are related to ecological changes in marine ecosystem of the region and are supposed to be caused mainly by biotic factors. Six species were identified as "indicators species" of the changes in parasite communities: *Pseudoterranova* sp., *A. nototheniae*, *N. georgiensis, Diphyllobotrium* sp., *M. rennicki*, and *Corynosoma* spp. These "indicator species" are presumably most sensitive to the influences of climatic and anthropogenic factors and may be used in future assessment of ecological changes in marine ecosystems of Western Antarctic.

This study was supported by the National Research Foundation of Ukraine (2020.02/0074).

The New Zealand mudsnail, *Potamopyrgus antipodarum* (Grey, 1843) (Gastropoda: Tateidae), dilutes the prevalence of native lymnaeids with echinostome trematodes

Anna Cichy, Anna Stanicka, Elżbieta Żbikowska

Nicolaus Copernicus University in Toruń, Toruń, Poland

Biological invasions in freshwater ecosystems due to natural or anthropogenic processes raise the question of exotic species' impact on local communities. Although most studies indicate a negative effect because of threats to native ecosystems, public health, and the economy, the positive one is often more difficult to discern. The experimental work aimed to check whether exotic molluscs, *P. antipodarum* dilute the prevalence of echinostomes in native hosts, *Radix* sp. snails, and how water temperature and the biomass of alien invaders affect this phenomenon. We tested i) the lifespan of *Echinoparyphium aconiatum* Dietz, 1909 cercariae and their infectivity towards hosts in different thermal variants, and ii) the impact of different biomass of *P. antipoadrum* on the prevalence of parasites in lymnaeid snails. We found that cercarial lifespan decreased with increasing temperature, contrary to cercarial infectivity. Both exotic and native host species were successfully infected with *E. aconiatum* cercariae. The prevalence of metacercariae (stages formed in snails after cercarial invasion) in native Radix sp. snails decreased with increasing biomass of the New Zealand mudsnails. Our results indicate that the presence of compatible, exotic hosts may cause a 'dilution effect' and presumably protect, at least to some degree, from invasion of echinostome cercariae.

The research was financed by the National Science Center 2018/02/X/NZ8/02716 and partially 2017/25/N/NZ8/01345.

Human and animal Babesiosis as emerging and re-emerging tick-borne disease in Central, North and North-eastern Europe

Anna Bajer[1], Ana Beck[2], Relja Beck[3], Jeży M. Behnke[4], Dorota Dwużnik-Szarek[1], Ramon M. Eichenberger[5], Robert Farkas[6], Hans-Peter Fuehrer[7], Mike Heddergott[8], Pikka Jokelainen[9], Michael Leschnik[10], Valentina Oborina[11], Algimantas Paulauskas[12], Jana Radzijevskaja[12], Renate Ranka[13], Manuela Schnyder[5], Andrea Springer[14], Christina Strube[14], Katarzyna Tołkacz[15], Julia Walochnik[16]

1 Department of Eco-Epidemiology of Parasitic Diseases, Institute of Developmental Biology and Biomedical Sciences, Faculty of Biology, University of Warsaw, Warsaw, Poland
2 Ribnjak 8, 10 000 Zagreb, Zagreb, Croatia
3 Department for Bacteriology and Parasitology, Croatian Veterinary Institute, Zagreb, Croatia
4 School of Life Sciences, University Park, University of Nottingham, Nottingham, United Kingdom
5 Institute of Parasitology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
6 Department of Parasitology and Zoology, University of Veterinary Medicine, Budapest, Hungary
7 Institute of Parasitology, Department of Pathobiology, University of Veterinary Medicine, Vienna, Austria
8 Department of Zoology, Musée National d'Historire Naturelle, Luxembourg, Luxembourg
9 Infectious Disease Prepardness, Statens Serum Institut, Copenhagen, Denmark
10 Clinical Unit of Internal Medicine Small Animals, Department/Universitätsklinik für Kleintiere und Pferde, University of Veterinary Medicine, Vienna, Austria
11 Small Animal Clinic of Estonian University of Life Sciences, Tartu, Estonia
12 Faculty of Natural Sciences, Vytautas Magnus University, Kaunas, Lithuania
13 Latvian Biomedical Research and Study Centre, Riga, Latvia
14 Institute for Parasitology, Centre for Infection Medicine, University of Veterinary Medicine, Hannover, Germany
15 Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
16 Institute for Specific Prophylaxis and Tropical Medicine, Medical University of Vienna, Vienna, Austria

Babesiosis is an important, life threatening disease for humans and animals. There is now considerable evidence that in Europe babesiosis is an emerging infectious disease, some of the causative species spreading as a consequence of the increasing range of their tick vector hosts. In this review, we summarize both historic records and recent findings on the occurrence and incidence of babesiosis in 20 European countries located in South-Eastern (Bosnia and Hercegovina, Croatia, Serbia), Central (Austria, Czechia, Germany, Hungary, Luxembourg, Poland, Slovakia, Slovenia, Switzerland) and Northern/North-eastern (Lithuania, Latvia, Estonia, Iceland, Denmark, Finland, Sweden and Norway) parts of Europe, from humans and selected species of domesticated animals (cats, dogs, horses and cattle). This review covers the seven species of *Babesia* (*B. microt*i, *B. divergens*, *B. venatorum*, *B. canis*, *B. gibsoni*, *B. vogeli* and *B. caballi*) and includes also the related piroplasm *Theileria equi* from horses.

Recorded cases of human babesiosis are still rare but are expected to rise in the coming years because of the widespread and longer seasonal activity of Ixodes ricinus as a result of climate change and because of

more extensive use of the better molecular diagnostic methods. Bovine babesiosis has re-emerging potential because of the likely loss of herd immunity. Canine babesiosis is rapidly expanding in Central and NE Europe, its prevalence correlating with the rapid, successful expansion of the ornate dog tick (Dermacentor reticulatus) population in Europe. Taken together our analysis of the available reports shows clear evidence of an increasing annual incidence of babesiosis across Europe in both humans and animals that is changing in line with similar increases in the incidence of other tick-borne diseases. This situation is of major concern, and we recommend more extensive, standardised monitoring in future, in association with a One Health approach.

Migration of deer keds to urban agglomerations – a case study

Remigiusz Gałęcki[1], Xuenan Xuan[2], Tadeusz Bakuła[1]

1 Department of Veterinary Prevention and Feed Hygiene, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Oczapowski Street 13, 10-719 Olsztyn, Poland, Olsztyn, Poland
2 National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan

Deer keds (*Lipoptena* spp.) are obligate hematophagous ectoparasites of birds and mammals. So far, stable populations of *Lipoptena cervi* and *Lipoptena fortisetosa* have been observed in Poland. Deer keds are ectoparasites with a narrow host-specific range and are directly related to cervids. Populations of *L. cervi* have been identified in mainland Europe, Algeria, Great Britain, northern China and the east coast of the United States. *Lipoptena fortisetosa* was first identified in 1965 in Japan. This species is native to the Eastern Palearctic, in particular the Far East and Eastern Siberia. In Central Europe, *L. fortisetosa* is increasingly classified as an invasive species. Increasingly, the dispersion of these insects in the environment is observed. The aim of this studies was to monitor the occurrence and migration of Lipoptena spp. to the cities on the example of Olsztyn. Monitoring was carried out in forests to the south of the city and in the Kortowo district in 2018–2022. The location of the observed insects, date, number of acquired insects and the collection method were recorded. Insects were identified to the species level on the basis of their morphological and morphometric features under the stereoscopic microscope.

In the years 2018–2022, numerous occurrences of both species were observed in forests. The number of specimens varied depending on the years and weather conditions. Since 2019, an upward trend in the number of insects has been observed in the forests adjacent to the city, including Stary Dwór, Zazdrość, and Kupydy. In the years 2018–2019, no deer keds were observed in the Kortowo district. In 2020, one specimen of *L. fortisetosa* (July) and *L. cervi* (September) were obtained in the mentioned district. In 2021, numerous occurrences of both species were observed in the southern part of the district. In 2022 (June), several *L. fortisetosa* specimens were observed in the southern and central parts of the district.

In the described case, potential promoters of Lipoptena spp. migration to the city are: expansion of the agglomeration (in particular the city beltway), which led to the reduction and parcellization of the forest biotope, the intersection of animal migration routes, intensification of transport, intensive forest management around the city, increasing the number of deer, migration of wild animals to urban areas, climate changes favoring the development of the population of these ectoparasites. These insects are considered as potential vectors of infectious diseases, therefore their presence in anthropogenic areas should be monitored.

Poster session

The unexpected record of the ornate dog ticks parasitism on an indigenous dog from *Dermacentor reticulatus*-free zone in Poland

Olga Pawełczyk[1], Paulina Bubel[2], Marek Asman[1], Joanna Witecka[1], Agnieszka Bugaj-Nawrocka[3], Damian Kotela[2], Krzysztof Solarz[1]

1 Department of Parasitology, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia in Katowice, Sosnowiec, Poland
2 Veterinary Clinic for Small Animals "Dawet", Zawiercie, Poland
3 Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia in Katowice, Katowice, Poland

Dermacentor reticulatus (Fabricius, 1794) is a hard-bodied tick of significant medical and veterinary importance, which belongs to a group of vectors transmitting various pathogenic agents, e.g. *Babesia canis*, *Francisella tularensis*, *Coxiella burnetii*, *Rickettsia slovaca* and *Anaplasma marginale*. This tick species commonly occurs in the Eastern, Western and Central regions of Poland. The scientific reports confirmed the presence of *D. reticulatus*-free zone in this country, from West Pomerania and Pomerania Voivodeships in Northern Poland, to Opole, Silesian, Lesser Poland and Subcarpatia Voivodeships in Southern Poland, where only D. reticulatus-negative sites were recorded. In Poland, the seasonal activity of *D. reticulatus* (adults) has two peaks, the first between March and April and the second between September and October. In March 2021, a 2.5-years old male English Cocker Spaniel from Zawiercie (Silesia, Poland) was presented to the veterinary clinic with canine babesiosis symptoms (e.g. apathy, malaise, fever, vomiting and diarrhoea). Two ticks were observed and removed from the dog during a clinical examination by tick-tweezers. They were placed separately in plastic tubes with 70% ethanol and transported to the Department of Parasitology (Medical University of Silesia in Katowice) for identification to species and life stages, as well as for molecular analysing of tick-borne pathogens. Ticks were identified under the stereo microscope (Zeiss Stemi 2000C) using the identification keys. Then, DNA was isolated from ticks by the ammonia method. Ticks were analysed by PCR for the detection of *Babesia* sp., (18S rRNA gene), *Anaplasma phagocytophilum* (16S rRNA gene), *Borrelia burgdorferi* sensu lato (fla gene), *Rickettsia* sp. (gltA gene) and *Bartonella* sp. (rpoB gene) using the specific primers.

Two adults D. reticulatus ticks (female and male) were collected from an indigenous adult dog with acute canine babesiosis at the veterinary clinic. Both examined ticks were fully engorged. None of *D. reticulatus* ticks was positive for *Babesia* sp., *A. phagocytophilum*, *B. burgdorferi* s. l., *Rickettsia* sp. and *Bartonella* sp.

This is the first record of *D. reticulatus* ticks in an indigenous dog from the Zawiercie District, the location where this tick species has not been noted in the environment yet. The presented results are an introduction to the screening study of companion animals from the Silesian Voivodeship for tick- and flea-borne pathogens, which gives a new scientific perspective to epidemiological data from this area.

Parasites of the stone moroko *Pseudorasbora parva* (Temminck and Schlegel, 1846) in the River Wardynka (north-western Poland)

Izabella Rząd[1, 7], Przemysław Czerniejewski[2], Remigiusz Panicz[3], Ewa Dzika[4], Piotr Eljasik[3], Adam Brysiewicz[5], Lucyna Kirczuk[6, 7], Katarzyna Dziewulska[6, 7], Agata Stapf[8]

1 Institute of Marine and Environmental Sciences, University of Szczecin, Szczecin, Poland
2 Department of Commodity, Quality Assessment, Process Engineering and Human Nutrition Westpomeranian University of Technology in Szczecin, Szczecin, Poland
3 Department of Meat Science, Faculty of Food Science and Fisheries, West Pomeranian University of Technology, Szczecin, Poland
4 Department of Medical Biology, Collegium Medicum, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland
5 Institute of Technology and Life Sciences – National Research Institute, Raszyn, Poland
6 Department of Hydrobiology, Institute of Biology, University of Szczecin, Szczecin, Poland
7 Molecular Biology and Biotechnology Centre, University of Szczecin, Szczecin, Poland
8 Department of Biological Sciences, Faculty of Sport Science in Gorzów Wielkopolski, Poznan University of Physical Education, Gorzów Wielkopolski, Poland

The stone moroko *Pseudorasbora parva* (Temminck and Schlegel, 1846) is an invasive species in European water bodies, introduced to Europe together with stocking material of herbivorous fish species. It was first recorded in Europe in 1961 in southern Romania and in Albania, and from there it spread over nearly the entire continent. The stone moroko spreads very rapidly, adversely affecting native hydrobionts and transmitting infectious diseases (S*phaerothecum destruens*, Protista: Mezomycetozoea), and therefore it is considered an 'international pest species' in Europe. The status of infection of stone moroko by the pathogenic species *S. destruens* and other parasites in Europe is not fully known, and the stone moroko, as a host of *S. destruens*, poses a potential threat to indigenous species of both wild and farmed fish.

The aim of the study was a parasitological analysis of the stone moroko population in the River Wardynka, which belongs to the catchment of the Oder in north-western Poland. Parasitological studies were carried out in 2019–2020 in spring, summer and autumn (a total of 124 stone moroko individuals were examined).

PCR analysis used to detect S. destruens in stone moroko did not reveal the presence of this pathogen. This was the first attempt to identify S. destruens in Poland, and further genetic analyses of other populations of this species will be necessary. The parasite community of stone moroko is represented by protists *Trichodinella* sp. (Protista, Ciliophora), monogeans *Dactylogyrus squameus* Gussev, 1955 (Monogenea), digenean trematodes *Phyllodistomum elongatum* Nybelin, 1926, *P. folium* (Olfers, 1816) and *Posthodiplostomum cuticola* (von Nordmann, 1832) metacercaria (Digenea), nematodes *Pseudocapillaria* (*Pseudocapillaria*) *tomenosa* (Dujardin, 1845) Moravec, 1987 (Nematoda), nematode larvae (Nematoda gen. sp.), and glochidia (parasitic larvae of bivalves, Mollusca, Bivalvia). *D. squameus*, *P. elongatum*, *P. folium*, *P. cuticola* and glochidia were recorded in stone moroko for the first time in Poland. Prevalence over the entire study period was 59%, and the average intensity of infection was 3 parasites per fish (1–30 parasites). In Poland, parasites of stone moroko which had been recorded in indigenous species of fish were previously known (e.g. *Trichodinella subtilis*, *Diplozoon*

paradoxum, *Apatemon gracilis* and *Caryophyllaeus laticeps*). The role of stone moroko in European aquatic ecosystems new to the species consists in broadening the spectrum of hosts for the above-mentioned parasite species, which may increase their populations in both natural water bodies and fish ponds.

SESSION 6

Taxonomy, biology and ecology of parasites

Oral session

Walking with dinosaurs: phylogeny, biogeography and host associations of the Proterodiplostomidae

Vasyl V. Tkach[1], Tyler J. Achatz[2]

1 University of North Dakota, USA
2 Middle Georgia State University, USA

Crocodilians are an ancient group of reptiles that evolved at least 225 million years ago and inhabited the supercontinent Pangea prior to its breakup. The geographic range of early crocodilians was fragmented and their descendants were separated from each other by continental drift. Subsequent speciation and extinction events have shaped today's fauna and distribution of crocodilians. The same likely happened to their parasites. Although parasites of crocodilians have been studied insufficiently, published research revealed rich, mostly specific fauna of parasites in these reptiles. One of the most common helminths found in crocodilians are digeneans of the family Proterodiplostomidae Dubois, 1936 (superfamily Diplostomidea). Members of the Proterodiplostomidae intestinal parasites of their reptile definitive hosts, mostly in tropical and subtropical environments around the world. Although a few proterodiplostomid taxa are known from turtles and snakes, the overwhelming majority of these digeneans are found in crocodilians. The group is unique due to the presence of a paraprostate organ associated with the terminal reproductive organs and ducts. Until very recently, very little was known about interrelationships among the proterodiplostomids which prevented exploring their historical biogeography and evolutionary host associations. Almost no DNA sequence data were available from this interesting group of digeneans. The situation has changed dramatically in recent years with publication of several phylogenetic studies based on significant amount of novel sequence data, as well as descriptions of several new genera and species from the Americas, Africa and Australia. The system of the family has been revised and new keys for identifications of genera were proposed. We provide an overview of the current state of knowledge in proterodiplostomid phylogenetics and biogeography and host associations with a focus on their evolutionary associations with crocodilians. The results based on specimens from 3 continents and 20 proterodiplostomid genera have demonstrated the monophyly of the group. They also provided evidence that at least some of today's proterodiplostomid lineages are very ancient and likely evolved before the break-up of supercontinents, thus reflecting long co-evolutionary history between these parasites and crocodilians. Phylogenetic analyses also demonstrated several evolutionary host switching events among the Proterodiplostomidae. Molecular data helped to re-assess of certain morphological characters used in proterodiplostomid taxonomy which resulted in several systematic changes including descriptions of new genera as well as synonymizations.

This study was supported by the National Science Foundation (grants DEB-0515492, DEB-1120734) to VVT and the University of North Dakota and the Midwestern Association of Parasitologists to TJA.

First report on *Haemonchus contortus* (Nematoda: Trichostrongylidae) in ground beetle, *Carabus granulatus* (Coleoptera: Carabidae)

Joanna Werszko[1], Konrad Wilamowski[2], Sławomir Bakier[2], Anna Maria Pyziel-Serafin[3]

1 Witold Stefański Institute of Parasitology, Polish Academy of Sciences, Warsaw, Poland
2 Institute of Forest Sciences Faculty of Civil Engineering and Environmental Sciences Białystok University of Technology, Białystok, Poland
3 Institute of Veterinary Medicine, Warsaw University of Life Sciences – SGGW, Warsaw, Poland

Gastrointestinal nematode, *Haemonchus contortus* (subfamily Haemonchinae) is considered highly pathogenic as it causes haemorrhagic anaemia in small domestic ruminants and wild ruminants in Europe, including red-listed European bison. Moreover, H. contortus has shown a remarkable propensity to develop resistance to different anthelmintics used in its control. Carabus granulatus is one of the most prevalent species of ground beetles inhabiting vast variety of ecosystems in Poland. Up to date, ground beetles from the family Carabidae were proved to host exclusively protozoan parasites, mainly eugregarine. The study was conducted on 26 insects collected in Białowieża Primeval Forest. To detect the presence of helminths' DNA in the investigated beetles, the PCR with helminths' general primers was done. The presence of the DNA of Haemonchus contortus was detected in 6 of 26 insects. Four partial ITS-2 and LSU nucleotide sequences of H. contortus were obtained (GenBank accession number: ON171228, ON171226, ON152377, ON152397).

Our study provides the first molecular evidence of the presence of Haemonchus contortus in Carabus granulatus. The finding suggests that ground beetles may play a role of paratenic hosts of this blood-sucking parasite in the environment. This is a field of research clearly requiring further examination.

Host specificity or life strategy – what drives the successful infection? A story tale of two little snails

Grzegorz Zaleśny, Elżbieta Kowalska

Wrocław University of Environmental and Life Sciences, Wrocław, Poland

Host and parasite specificities affect the ecological and evolutionary dynamics of a host-parasite relationship, while the ecological and physiological mismatches can be identified among the reasons for the uneven distribution of parasite species within congeneric host taxa. It is possible to track the occurrence of parasites within a specific habitat occupied by closely related host species and thus to find an answer to the question, of whether they are infected with the same parasitic species. In case of negative answer is negative, it is necessary to ask, whether the reason for such a state may depend on distinct life strategies of the host species?

In our study, two species of snails of the genus *Monachoides* that live in the same locality and represent different life strategies were observed. The first species – *Monachoides vicinus*, that occurs mostly in mountain and foothill regions. It lives in damp deciduous forests near streams and forages and shelters in the litter, under the logs, and often climbs the lower undergrowth plants. The second species – *Monachoides incarnatu*s, that occurs in various types of forests or parks, in the mountains up to 1500 m a. s. l. It forages and shelters mainly in the litter, under logs, and less frequently climbs the lower undergrowth plants.

The material for the research (both of *M. vicinus* and *M. incarnatus*) was collected in the buffer zone of the Muszkowicki Beech Forest nature reserve. The snails were subjected to parasitological dissection. In both host species, we found metacercariae; the molecular analysis (based on 28S rDNA and COI mtDNA) confirmed the presence of flukes of Brachylaimidae and Panopistidae. Here we have discussed the possible reasons for discrepancies in the infection of both snail species with the metacercariae.

Genetic diversity of the larval stages of strigeid trematodes – taxonomical and evolutionary consequences

Joanna Hildebrand[1], Ewa Pyrka[1], Julia Gabrysiak[1], Witold Jeżewski[2], Anna Cichy[3], Anna Stanicka[3], Elżbieta Żbikowska[3], Grzegorz Zaleśny[4], Gerard Kanarek[5]

1 Department of Parasitology, University of Wrocław, Wrocław, Poland
2 Witold Stefański Institute of Parasitology, Polish Academy of Sciences, Warsaw, Poland
3 Department of Invertebrate Zoology and Parasitology, Nicolaus Copernicus University in Toruń, Toruń, Poland
4 Systematic and Ecology of Invertebrates, Wrocław University of Environmental and Life Sciences, Wrocław, Poland
5 Ornithological Station, Museum and Institute of Zoology, Polish Academy of Sciences, Gdańsk, Poland

Strigeidae Railliet, 1919 parasitize the gastrointestinal tract and bursa of Fabricius of birds, rarely mammals and reptiles. They are characteristic by cup-shaped forebody and bilobed holdfast organ, and three-host life cycle (in some species occur fourth host), in which they use different group of invertebrates and vertebrates as second intermediate host. Unfortunately, so far, species richness and molecular diversity of metacercariae of tetracotyle type, characteristic for strigeid digeneas, remain poorly understood, also with reference to parasite-host relationship.

In present study, we have performed the parasitological and taxonomical examination of tetracotyles from freshwater snails and leeches from three specific localities of Poland, supplemented with adult Strigeidae specimens collected from birds. In this study we report our use of recently obtained sequences of a few molecular markers: 28S rDNA, ITS2 region, and CO1 mtDNA, supplemented by results of a method of species delimitation (GMYC) to analyse some aspects of the ecology, taxonomy and phylogeny of members of the genus *Australapatemon* Sudarikov, 1959 and *Cotylurus* Szidat, 1928.

The taxonomic generic affiliation of tetracotyle derived from the leeches revealed the occurrence of two strigeid genera *Australapatemon* and *Cotylurus*, with their separate positions in obtained phylogenies. Metacercariae of *Cotylurus detected* in leeches were identified as two species: *C. strigeoides* Dubois, 1958 and *C. syrius* Dubois, 1934, while tetracotyle collected from snails were identified only as *C. cornutus* (Rudolphi, 1809) Szidat, 1928. Moreover, obtained results revealed also unexpectedly high molecular diversity within *Cotylurus* occurring in snails, with clearly expressed evidence of cryptic diversity and the existence of several novel-species lineages. Acquired data demonstrated the polyphyletic character of *C. syrius* Dubois, 1934 (with three separate molecular species-level lineages) and *C. cornutus* (with four separate molecular species-level lineages).

We demonstrated (1) the specificity and consistency of representatives of the genus *Australapatemon* in using leeches as a second intermediate host, and (2) the existence of two divergent phylogenetical and ecological lineages within *Cotylurus* (one using leeches and other snails as second intermediate hosts), differing significantly in their life history strategies, with potential serious evolutionary consequences for host–parasite relationships.

Phylogenetic Analysis of Chewing Lice Infested Pigeons (*Columba livia domestica*) with New Records in Kurdistan of Iraq

Mohammed Adnan Al-Badrani, Shamal Abdullah Al-Muffti

Department of Biology, College of Sciences, University of Duhok, Iraq

Columba livia is a species that descends from wild rock pigeons; many parasite species may affect birds when they were breeding. Lice are obligate ectoparasitic insects that infest birds. The aim of this study is to investigate the lice and create the phylogenetic tree among the sequenced lice, using both mitochondrial DNA (COI gene) and nuclear gene (18S rRNA gene). Sequencing data were aligned with the sequences available in the NCBI GenBank. From October 2017 until July 2018, two hundred outdoor domestic pigeons from three governorates (Duhok, Erbil, and Sulaymaniyah) in the Kurdistan region of Iraq, were examined for the presence of lice. After the morphological identification of the lice, the total genomic DNA of each louse was used to amplify the DNA of the mitochondrial gene COI (Cytochrome Oxidase Subunit I), and nuclear gene 18S rRNA, using universal primer and designed specific primers, respectively. Sequencing DNA results were submitted to the GenBank, as nine species of lice that were recorded in Iraq for the first time with accession numbers MN521453; MN521454; MN521455; MN521456; MN593304, and MN593305, belonged to the species of *Campanulotes compar*, as well as, MN588093; MN588094, and MN531683 belonged to *Columbicola columbae*, and *Hohorstiella lata*, respectively.

Poster session

Preoviposition and oviposition of female *Ixodes ricinus* (L., 1758) (Ixodida: Ixodidae) at different levels of humidity under laboratory conditions

Alicja M. Buczek[1], Ewa Barańska[1], Weronika Buczek[1], Katarzyna Bartosik[1], Dariusz Ciura[2], Alicja Buczek[1]

1 Chair of Farmacology and Biology, Department of Biology and Parasitology, Faculty of Health Sciences, Medical University of Lublin, Lublin, Poland
2 Chair of Pathophysiology, Department of Health Promotion and Treatment of Obesity, Medical University of Silesia, Katowice, Poland

The study was carried out to investigate the course of preoviposition and oviposition in females of *Ixodes ricinus*, which is the most common species in urban and non-urban areas in Europe, in various relative humidity (R.H.) levels.

In laboratory conditions, 29 fertilized I. ricinus females from the Upper Silesian population were fed on rabbits at room temperature of 20 ± 3°C and 60 ± 5% R.H. Each fully engorged female detached from the host was deposited in a separate rearing chamber at 25°C and 90% R.H. After the onset of oviposition, the *I. ricinus* females were placed at 30%, 50%, 75%, 80%, and 90% R.H. At the same time of each day, the females were gently transferred to another chamber with the same humidity.

The preoviposition period in the *I. ricinus* females at w 25°C and 90% R.H. lasted from 17 to 25 days (mean: 18.52 ± 1.74). As many as 34.48% and 24.14% of the females started laying eggs on days 17 and 18 after the end of feeding, respectively. At the analysed humidity levels, the oviposition period lasted from 3 to 9 days (mean: 3.18; at 90% R.H.) and from 7 to 13 days (mean: 5.41; at 50% R.H.). The statistical analysis showed no significant linear correlation between the humidity and the oviposition length (Pearson correlation coefficient – 0.025). The number of eggs laid by one female during the lifetime at 30%-80% R.H. ranged from 542–2269 (mean: 1345) to 799–3317 (mean: 1760.25). The lowest number of eggs was laid by females kept at 90% R.H. (303–2092 eggs; mean: 615.94), which may have been caused by fungi (molds) growing in these experimental conditions.

In all the experiments conducted at 25°C, the highest number of *I. ricinus* eggs was recorded on the first day of oviposition, regardless of the humidity level. The number of newly laid eggs declined on the subsequent days. The statistical analysis revealed a significant relationship between the oviposition length and the number of egg batches (Pearson correlation coefficient 0.719) and a relationship between the length of oviposition and the number of eggs (0.340).

In contrast, there was no correlation between the oviposition length and the mean number of eggs per batch (0.018).

The investigations indicate that lower humidity levels during oviposition do not disturb this process in *I. ricinus*. In turn, high humidity (90% R.H.) may promote the growth of fungi, which may inhibit egg development and even cause death of females.

Hyperparasitism among Digenea – new aspects of this phenomenon

Julia Gabrysiak[1], Witold Jeżewski[2], Anna Stanicka[3], Anna Cichy[3], Elżbieta Żbikowska[3], Gerard Kanarek[4], Joanna Hildebrand[1]

1 Department of Parasitology, University of Wrocław, Wrocław, Poland
2 Witold Stefański Institute of Parasitology, Polish Academy of Sciences, Warsaw, Poland
3 Department of Invertebrate Zoology and Parasitology, Nicolaus Copernicus University in Toruń, Toruń, Poland
4 Ornithological Station, Museum and Institute of Zoology, Polish Academy of Sciences, Gdańsk, Poland

The digenean trematodes are parasites characterized by complex life cycles with alternation of sexual and asexual generations using several hosts. Digeneas developed numerous strategies to close their complex life cycles. One of them is hyperparasitism, when a parasite is parasitic on another parasite. In case of digeneans, this phenomenon lies in the fact that larval stages (e.g. metacercariae) parasitize on another larval stages (sporocysts or rediae). So far, this rare phenomenon were widely described in literature data, unfortunately without profound taxonomic, ecological and evolutionary analysis.

In presented study we describe and analyse cases of hyperparasitsim, revealed during our works concerning parasite-host relationships within genus *Cotylurus* Szidat, 1928 (Diplostomoidea: Strigeidae). Species of *Cotylurus* Szidat, 1928 (Diplostomoidea: Strigeidae) are highly specialized digeneans that parasitize the gastrointestinal tract and bursa of Fabricius of water and wading birds. They have a three-host life cycle; the role of first intermediate host is played by pulmonate snails, while a wide range of water snails and leeches are reported as second intermediate hosts (host of invasive stage – metacercariae of tetracotyle type).

So far, we analyzed 1.123 freshwater snails from thirteen species of five families (Bithyniidae, Lymnaeidae, Physidae, Planorbidae, Viviparidae) from several sites located in three provinces of Poland (dolnośląskie, lubelskie, pomorskie). The tetracotyle were decteted in 249 examined snails (22.17%). What is important, in some dissected snails (13 specimens (5.22%) belonging of three species: *Lymnaea stagnalis*, *Radix auricularia* and *Planorbarius corneus*) tetracotyle were found inside sporocysts or rediae of other trematode species. To establish precise taxonomical position of these larval stages, we use three molecular markers commonly used in the taxonomy and phylogeny of Digenea (28S, ITS and COI). Obtained results clearly revealed, that all tetracotyle represented the genus *Cotylurus*, while their host-larval stages were identified as six species of the genera *Metaleptophallus*, *Tylodelphys*, *Notocotylus*, *Echinoparyphium* and *Hypoderaeum*. It is the first finding of this type of parasite-parasite antagonistic interaction in Poland and the first in the world supported by molecular data.

Progress and perspectives of research on skin and tissue mites of the family Demodecidae (Acariformes, Prostigmata)

Joanna N. Izdebska, Leszek Rolbiecki

Department of Invertebrate Zoology and Parasitology, University of Gdańsk, Gdańsk, Poland

The Demodecidae are skin and tissue parasites of mammals. Some species are of great pathogenic importance, causing demodecosis in humans or domestic animals. They have been found in mammals of most orders, and the geographical distribution probably coincides with that of their hosts; however, as most species are known from only a few or even individual publications (species description), this is often not confirmed by records. The body of evidence concerning the Demodecidae is therefore unsatisfactory, although recent years have yielded a number of important new discoveries concerning their systematic diversity, distribution and host-parasite relationships.

Currently, 128 species in nine genera are known, of which about 20% (23 species and two genera) have been described in the last ten years. Most species (60) have been reported from Poland so far, followed by the USA (23), the Czech Republic (18) and the UK (18); however, these numbers are most likely more indicative of the intensity of research than their true biodiversity.

The Demodecidae demonstrate host specificity, with almost all known species being monoxenic and only a few have been found in several, closely-related hosts. However, this issue still needs more research. They usually also show topical specificity (i.e. an association with specific skin structures or organs/tissues) and specificity or at least topographical preference (i.e. association with a specific body region). It is typical for a single host species to present perhaps several species of Demodecidae in different locations. The most synhospital species (7) were found in the house mouse. Among domesticated animals, four species are known in the domestic dog and another four in the domestic cat; these can give rise to demodecosis canina and demodecosis felina, two troublesome and often difficult to treat parasitoses.

The co-occurrence of several specific species in these hosts raises new questions regarding demodecosis etiology, because different species may be associated with different symptoms or disease course, and may require different therapeutic approaches. It also requires a change in the diagnostic approach to demodecosis, insofar that there is a need to identify the demodecid mite to the species level on the basis of taxonomic characteristics, not only presumed host specificity, and to include density criteria in the diagnosis. It is now known that despite the comparative rarity of disease symptoms, many species of Demodecidae are common in host populations, and often demonstrate very high infection intensity due to poor host condition or reduced host immunity.

Furcocercariae of aquatic snails (Lymnaeidae and Planorbidae) from selected locations in Central Poland

Witold Jeżewski[1], Julia Gabrysiak[2], Gerard Kanarek[3], Joanna Hildebrand[2]

1 Witold Stefański Institute of Parasitology, Polish Academy of Sciences, Warsaw, Poland
2 Department of Parasitology, Faculty of Biological Sciences, University of Wrocław, Wrocław, Poland
3 Ornithological Station, Museum and Institute of Zoology, Polish Academy of Sciences, Gdańsk, Poland

Several species of aquatic snails play role as first intermediate host for many taxa of digenetic trematodes, in which developed larval stages of these parasites, such as sporocysts, rediae, and cercariae. Aquatic snails are also widely recognized as second intermediate hosts, in which develop metacercariae, invasive stage for definitive hosts.

In the presented study, we analyse and describe the taxonomic identity and diversity of furcocercariae occurring in wide range of Lymnaeidae and Planorbidae freshwater snails from Central Poland. Snails were collected from extensive fish pond and small rivers with slow-flowing waters and related drainage canals with rich vegetation. Each snail was transferred to a separate plastic cup with water, transported to the laboratory, stored in the fridge, and fed ad libitum with lettuce. Emission of cercariae were induced by exposing snails to direct light. After emission of cercariae, snails were dissected according to generally adopted protocols or in the absence of cercariae emissions, the snails were released into the environment.

As a result of the conducted in-vivo study, furcocercariae were detected in 6,8 % of the obtained snail specimens and identified as representatives of the following genera: Alaria, Australapatemon, Cotylurus, Diplostomum, Tylodelphys and Trichobilharzia. Additionally, parasitological dissection of snails revealed occurrence of developmental stages of digeneas: sporocysts – mother and daughter as well as metacercariae. The determinations based on morphological criteria were verified and completed using molecular methods. Furthermore, the analysis of ecological and biological aspects of recorded parasite-host relationship was provided, with special emphasis on family Strigeidae.

The co-occurrence of *Hoplopleura* and *Polyplax* lice – analysis of the causes of the phenomenon

Paulina Kozina, Joanna N. Izdebska

Department of Invertebrate Zoology and Parasitology, Faculty of Biology, University of Gdańsk, Gdańsk, Poland

Sucking lice (Phthiraptera, Anoplura) are obligate, blood-sucking parasites of mammals. In Poland, the most numerous and abundant genera are *Hoplopleura* and *Polyplax*. They parasitize rodents and soricomorphs, the former being the most numerous and diverse group of mammals. It has been observed that in 12 cases, the two lice genera exhibit oligoxenicity and co-occur in the same host. The aim of this study was to determine the reasons for such cohabitation in the same host and identify any possible mutual influences.

The study analyses various factors that may influence host specificity or topographical preferences.

The lice do not differ significantly from each other; however, some divergence has been observed in the morphological structure of the first pair. In *Polyplax*, the grasping system is present on all three pairs of legs, while in *Hoplopleura*, it is absent on the first pair. Although this does not necessarily affect grasping ability, as *Hoplopleura affinis* has been observed grasping hair with the first pair, the system may improve adhesion to hair by providing a longer and stronger hold that can resist mechanical removal by the host.

Polyplax serrata has been found in *Rattus norvegicus*, *R. rattus* and *Apodemus agrarius*; while *H. affinis* was found only in *A. agrarius*. Co-occurrence was recorded in only 16 *A. agrarius*. The infestation parameters differed from each other. The highest intensity of infestation was observed for *H. affinis* in *A. agrarius* and the lowest for *P. serrata* in *R. rattus*. The highest intensity and range of intensities was observed for *P. serrata* in *R. norvegicus* and the lowest – for *P. serrata* in *A. agrarius*.

Regarding infestation, *P. serrata* demonstrates different parameters when cohabiting with *Hoplopleura* compared to when it is present alone. Higher parameters were recorded for lice on *R. norvegicus* than *A. agrarius*. This may indicate that cohabitation with other louse species does not influence the success of parasitism. Unfortunately, as no cases of parasitism by *H. affinis* have been recorded in other hosts, it is not possible to compare whether such differences occur in this genus.

The topographical preferences are similar. *H. affinis* more readily inhabits the head and the abdominal part of the rodent body than *P. serrata*, with only a few of the latter being found in these areas. Both louse genera lay eggs in the same places: on the neck, along the back of the animal, and in strips along the sides.

Repetitive host-seeking activity of adult *Dermacentor reticulatus* ticks under natural conditions

Aneta Woźniak, Zbigniew Zając, Joanna Kulisz, Katarzyna Bartosik

Zaklad Biologii i Parazytologii Uniwersytet Medyczny w Lublinie, Lublin, Poland

Ticks are the most important vectors of infectious diseases in Europe. In Poland, the ticks of the greatest epidemic importance are Ixodes ricinus, common throughout the country, and *Dermacentor reticulatus*, the range of which has been changing dynamically in recent years. The aim of our study was to investigate, for the first time, the rhythms of host-seeking activity of adult *D. reticulatus* ticks in natural conditions. For this purpose, 3 sites, differing in terms of ecological types, were selected. Recognized as preferred by *D. reticulatus*, the habitat of an open, unused agricultural meadow, meadow in the vicinity of a forest and a meadow constituting an enclave within the city were selected. Then, at each site, the activity of ticks was monitored at 7-day intervals. Individuals that showed activity were marked with a colored permanent oil marker. Each time a different color was used. Ambient temperature and humidity were also measured. The results of our research show that adult *D. reticulatus* ticks, regardless of the habitat, undertake multiple host-seeking activity and that they are able to survive in the habitats for many seasons (including unfavorable periods of winter) waiting for a host. Among all examined specimens, 70% undertook activity at least twice. The maximum observed number of attempts to host-seeking activity was 7. Rhythms of tick activity at the site within the city differed significantly from other sites. This is most likely due to limited access to medium and large size ungulates, which constitute the major host spectrum for adult *D. reticulatus*. In addition, the results of our research show that environmental conditions, primarily temperature, affect host-seeking activity.

Do lipid compounds influence host seeking behavior in ticks?

Joanna Kulisz, Zbigniew Zając, Aneta Woźniak, Katarzyna Bartosik

Department of Biology and Parasitology, Medical University of Lublin, Lublin, Poland

Ixodes ricinus and *Dermacentor reticulatus* ticks are important vectors and reservoir of many harmful pathogens belonging to divergent groups of bacteria, rickettsia, viruses, and protozoa. Because of their biology and ecology, and especially the adaptive flexibility allowing them to survive in changing environmental conditions, ticks are of great medical and epidemiological importance, particularly in the northern hemisphere.

The aim of the current research is to summarize the knowledge based on our own research and previously published data on the role and function of lipid compounds in the mechanisms of the host seeking behavior in ticks.

As a large and diverse family of organic compounds, lipids constitute one of the main groups of compounds found in the tick body. Given the specific biology of these arthropods, appropriate content of lipid substances is highly important for the ability of ticks to survive long periods of starvation and diapause (behavioral and developmental), as they serve as the basic source of stored energy. The composition of lipids present in ticks and the rate of their utilization depend on the tick species, sex, and life stage. Our research confirmed that lipid level have an impact on the determination and aggressiveness of these arthropods during questing activity and, thus, on the risk of transmission of pathogens and tick-borne diseases to humans and animals. According to available data, regulation of the expression of genes responsible for the host seeking (e.g. chemoreceptors) engages mechanisms based on lipid compounds. Therefore, elucidation of these mechanisms is extremely important from the anti-tick prophylaxis point of view and for research on new agents with repellent or acaricidal properties.

Young Researchers Competition

Genetic variation in *Dirofilaria repens* haplotypes from Poland and selected countries of Central and North-Eastern Europe and Middle East

Mustafa Al-Sarraf, Anna Bajer

Department of Eco-Epidemiology of Parasitic Diseases, Institute of Developmental Biology and Biomedical Sciences, Faculty of Biology, University of Warsaw, Warsaw, Poland

Subcutaneous dirofilariosis is a fast-spreading disease of dogs, occasionally other carnivores and humans. Several factors contribute to its spread, including climate change which facilitates development and survival of *Dirofilaria repens* in the mosquito vector. Moving infected definitive hosts (dogs) from endemic regions to non-endemic regions is another possible cause of local emergence and possibly abundant wild reservoirs of the parasite.

The main aim of our study was to evaluate the genetic diversity of *D. repens* from different regions of Europe to evaluate the spread of identified haplotypes and their geographic origin.

A total of 95 D. repens isolates were obtained from Central and Eastern Europe (Poland, Belarus, Ukraine, Austria, Romania), North-eastern Europe (Lithuania, Latvia, Estonia), Italy and Israel. All but one positive sample were obtained from dogs while one positive sample was obtained from an adult worm from a human case from the Lublin area in south-eastern Poland.

Genetic diversity in *D. repens* isolates was evaluated by PCR amplification and sequencing of three genetic markers, including 2 mitochondrial (mt) genes: the cytochrome c oxidase subunit I (COI) and dehydrogenase subunit I (NADH). Additionally, the genomic marker internal transcribed spacer 1 (ITS-1) was amplified and sequenced.

Haplotypes were differentiated based on sequence alignments by identifying Single Nucleotide Polymorphism (SNPs) using DnaSP and Mega X.

Both mt gene sequences (COI and NADH) were combined together for phylogenetic and network analyses.

Altogether 18 haplotypes (DR1-DR18) were identified in mt markers among 95 analysed samples.

Haplotype DR1 was the most common encompassing 66 isolates: 42 isolates from Poland (41 from dogs and one from a human), 13 from Lithuania, 4 from Latvia, 2 from Ukraine and 5 from Romania. All other haplotypes grouped around haplotype DR1 separated by 1-5 SNPs, forming star-like shape. Haplotype DR2 was the second most common haplotype, formed by 6 isolates from Romania. Interestingly, haplotype DR3 was represented only by four isolates from Israel. The remaining 15 haplotypes were represented by 1-4 isolates of different origins; thus, no distinct geographical segregation of haplotypes was observed.

Our study showed that only minor genetic diversity was found since all isolates appear to have clustered in or branched out from haplotype DR1 with 1-3 SNP differences. The genetic diversity appears to be governed by geographic origin since isolates from neighbouring populations (countries) appear to share certain haplotypes while other populations that are geographically distant form individual haplotypes.

Identification of immunodominant fragments of BmpA and BBK32 *Borrelia burgdorferi* sensu stricto antigens with the use of peptide microarray-based immunoassay

Weronika Grąźlewska[1], Lucyna Holec-Gąsior[1], Marinela Contreras Rojo[2]

1 Department of Molecular Biotechnology and Microbiology, Faculty of Chemistry, Gdańsk University of Technology, Gdańsk, Poland
2 SaBio, Instituto de Investigación en Recursos Cinegéticos IREC-CSIC-UCLM-JCCM, Ciudad Real, Spain

Borreliosis is the most common vector-borne disease in the northern hemisphere. It is transmitted by hard-bodied ticks of the Ixodes genus. The causative agent is gram-negative bacteria belonging to the complex Borrelia burgdorferi sensu lato (s.l.). Currently, this group consists of about 20 genospecies, 5 of which have been found to be pathogenic for humans. B. burgdorferi s.l. is characterized by a very diversified antigenic structure, both between different genospecies but also during different stages of its life cycle. Additionally, *B. burgdorferi* s. l. shares common amino acid sequences with other pathogens and even with sequences found in the human body. This causes the serodiagnosis of Lyme disease to have many limitations. These problems may be solved by recombinant proteins obtained by genetic engineering. Although many *B. burgdorferi* s. l. antigens are known to exhibit strong interactions with specific antibodies, individually they are not useful diagnostic tools. Therefore, in order to increase the sensitivity and specificity of novel serodiagnostic tests, it may be advisable to use only carefully selected fragments of antigens or chimeric proteins containing immunodominant sequences obtained from different antigens. For this purpose, B-cell linear epitope mapping of *B. burgdorferi* s. l. antigens is essential.

Peptide microarray analysis is a novel method that provides information on the distribution of linear epitopes in the protein sequence. In the above study, the epitopes of two *B. burgdorferi* sensu stricto (s. s.) BmpA and BBK32 surface lipoproteins were mapped. Both are described in literature as highly immunogenic proteins with diagnostic utility. In order to select epitopes recognized by both IgM and IgG antibodies, a microarray immunoassay was performed using 4 groups of human sera. The first group was a pool of mixed sera containing only immunoglobulin G (IgG), the second was positive for immunoglobulin M (IgM), and the third was a mix of sera positive for both IgG and IgM. Sera from healthy individuals were used as a control to detect epitopes responsible for non-specific interactions. The test allowed the identification of several peptides that show strong and specific interactions with the different isotypes of anti-*Borrelia* antibodies. These sequences can potentially be used in the development of more effective serodiagnostic tests as single peptides or be included in the construction of chimeric proteins.

Borrelia and other tick-borne pathogens co-occurrence in *Ixodes ricinus* ticks collected from humans

Julia Koczwarska, Julia Żórańska, Renata Welc-Falęciak

Department of Parasitology, Faculty of Biology, University of Warsaw, Warsaw, Poland

Ixodes ricinus is the major vector of *Borrelia* spirochetes and a broad spectrum of other tick-borne pathogens in Europe. Multi-species infections are common among ticks which may be highly relevant to epidemiology of these pathogens as well as public health. Co-infection in humans and animals might enhance disease severity and have significant consequences in terms of tick-borne disease treatment and diagnosis. Although numerous cases of multi-species infections and their health impact on the vertebrate host have been described, still little is known about what kind of interactions occur between different pathogen species in the arthropod vector. Coinfecting pathogens can interact among themselves, which can result in cooperation or competition. The mechanism by which Borrelia co-exist with other microbial pathogens within the tick remains still unexplored. Furthermore, it is as yet unclear the extent to which Borrelia engage in interactions or how multi-species infections might influence on spirochete loads in ticks and, consequently, on transmission to humans and pathogenicity.

The aim of this study was to investigate coexistence of *Borrelia* spp. with different tick-borne pathogens and to evaluate their impact on Borrelia occurrence in I. ricinus ticks. From June 1 to November, 2021, we collected 847 I. ricinus from tick-bitten individuals and analyzed individually for the presence of Borrelia spp., *Babesia* spp., *Rickettsia* spp. and Anaplasmataceae genus using molecular analisis.

Presence of fla gene specific to *Borrelia* spp. was found in 166/847 ticks which constituted 19.6% of all analyzed samples. 31,3% of Borrelia-positive ticks were co-infected with at least one different tick-borne pathogen. The most frequent multi-species infection was *Borrelia* with *Rickettsia* (21,2%). Co-infection of *Borrelia* and *Babesia* was the least common, however prevalence of *Babesia* was almost 4 times higher in *Borrelia* positive ticks (1,7% and 6,9%, respectively). A similar positive correlation was found between Borrelia and "Candidatus Neoehrlichia mikurensis" with higher infection rate of this bacteria in presence of Borrelia in tested ticks (18,5% vs. 30,4%). Our results suggest the possibility of positive interactions between Borrelia and different tick borne pathogens, which may be a highly important factor for the epidemiology of tick-borne diseases.

The study was supported by the National Science Center (NCN) grant no. 2020/37/B/NZ6/01587

Prevalence and genotyping of *Anaplasma phagocytophilum* strains from ungulates

Anna Myczka, Zdzisław Laskowski

Witold Stefański Institute of Parasitology of the Polish Academy of Sciences, Warsaw, Poland

Anaplasma phagocytophilum is a common intracellular bacterial parasite of the order Rickettsiales. This Gram-negative bacterium, demonstrating tropism to neutrophils causes pathological conditions in humans and animals. These microorganisms can be transferred between hosts by vector transmission, usually ticks from Ixodes genus. The constant presence and circulation of *A. phagocytophilum* in the natural environment is provide by natural reservoirs of these bacteria, which include e.g. wild ungulates.

305 individuals of ungulates from 6 species: red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*), wild boar (*Sus scrofa*), Eurasian moose (*Alces alces*) and European bison (*Bison bonasus*) were examined. To detection Anaplasma spp. DNA two types of peripheral tissue form spleen and liver were used. Three genetic markers: 16S rDNA, ankA and groEL were used to detection and genotyping of *A. phagocytophilum*.

Anaplasma spp. was detected in 112 individuals and the prevalence was 36.7% (112/305). Genotyping of a red deer strains showed that the *A. phagocytophilum* bacteria present in these hosts belong to the ecotype and cluster I. More various were strains isolated from roe deer individuals. These strains were assigned to ecotypes I and II and to clusters I and II. Analysis of amplified genetic markers of *A. phagocytophilum* isolates from European bison characterized these strains as ecotype I and cluster IV. Isolates of *A. phagocytophilum* from Eurasian moose to two ecotypes – I and II and to cluster I. Genotyping and phylogenetic analysis of *A. phagocytophilum* isolated from wild boars shown that these strains belong to ecotype and cluster I. All three genetic markers: groEL, ankA and 16S rDNA, from red deer, wild boar, moose and European bison, are identical or grouped with HGA strains, means that detected *Anaplasma phagocytophilum* strains may be potentially pathogenic to humans.

All bacteria detected by molecular methods belonged to one bacterial species – *Anaplasma phagocytophilum*. Sequence analysis of the 16S rDNA gene and phylogenetic analysis of the ankA and groEL partial genes allowed to confirm that ungulates in Poland are natural reservoirs of *A. phagocytophilum* and there are strains potentially zoonotic for humans among them.

Subcutaneous diroflariosis : evaluation of new diagnostic directions

Mateusz Pękacz, Katarzyna Basałaj, Anna Zawistowska-Deniziak

Department of Host Parasites Molecular Interactions, Witold Stefański Institute of Parasitology, Polish Academy of Sciences, Warsaw, Poland

Dirofilaria repens is a parasitic nematode causing vector-borne disease (dirofilariasis), considered an emerging problem in veterinary and human medicine. Due to climate changes and human activities, the disease spread throughout Europe. Currently, diagnostic methods are based on the occurrence of microfilariae in the bloodstream, ineffective in the case of amicrofilaremic infections. Therefore our study aimed to develop a new sensitive and specific diagnostic method. Our approach is based on both serological and molecular techniques.

First, almost ~700 sera samples collected from dogs living in Poland were classified as infected or non-infected based on three methods: Knott's method, *Dirofilaria repens S*omatic Antigen (DrSA) ELISA, and multiplex Real-Time PCR (qPCR). Sera were utilized to estimate the diagnostic potential of D.repens recombinant protein ES20/22 and synthetic peptide LH10 selected using phage display technology in our previous study. Additionally, we designed species-specific primers and MGB-Eclipse probes which enabled the development of a new multiplex qPCR with the use of genomic and cell-free DNA of *D. repens*.

The LH10 synthetic peptide allowed the detection of infected dogs with active microfilaremia and dogs with no clear presence of microfilariae in the bloodstream. Remarkably, results showed a similar pattern to DrSA ELISA and were confirmed by multiplex qPCR. Our molecular method enables the detection of fewer than 10 copies of the gene of interest and fast single-tube differentiation between *Dirofilaria repens* and *Dirofilaria immitis* and co-infections of these two species.

Our serological method is the first step to developing a new diagnostic tool that may be used for the early diagnosis of dirofilariosis. In addition, the multiplex qPCR may be an alternative for quick confirmation and differentiation of infection.

Financial support for this study was provided by The National Centre for Research and development, Poland (grant LIDER IX 0106/L-9/2017).

Novel *Toxoplasma gondii* chimeric antigens – development and assesment of diagnostic value

Karolina Sołowińska, Lucyna Holec-Gąsior, Bartłomiej Ferra

Gdańsk University of Technology, Gdańsk, Poland

Toxoplasma gondii, the etiologic agent of toxoplasmosis, is a zoonotic protozoan capable of infecting all warm blooded animals including humans. The main routes of transmission have been identified as oral intake of food and water contaminated with oocysts, as well as consumption of meat or other animal products containing parasitic tissue cysts. Considering that livestock serves as a direct source of infection for humans but also a possible reservoir for *T. gondii*, it is imperative to implement control measures to break the chain of infection. Furthermore, toxoplasmosis is recognised as one of the primary causes of reproductive failure in small ruminants such as goats and sheep. It has been reported that in some regions, this disease is responsible for up to 54% of ovine abortions – a phenomenon leading to considerable economic losses.

Progress in monitoring, prevention, and management of toxoplasmosis among farm animals has been impeded by the high cost of commercially available tests. What is more, serodiagnosis is currently mainly based on the use of lysed *T. gondii* native antigens (TLA) obtained from peritoneal fluid of infected mice or from tissue cultures *in vitro* – this approach not only generates high costs of antigen production but also yields inconsistent results as proteins obtained in this manner may be tainted by nonparasitic material. Considerable effort has been directed towards the development of more reliable, sensitive, and inexpensive diagnostic tools. One widely studied approach is the use of recombinant antigens, which are not only a cheaper and safer alternative to the use of native antigens in immunoassays, but also enable easy test standardization.

In this research, *Escherichia coli* expression systems were employed for the production of thirteen new, recombinant, trivalent *T. gondii* proteins, comprising of immunodominant regions of SAG1 and SAG2 proteins, coupled with; GRA1, GRA2, GRA5, GRA6, GRA7, GRA9, MAG1, MIC1, MIC3, AMA1, ROP1, P35, and LDH2 respectively. Their diagnostic value was assessed by IgG ELISA using ovine sera. The obtained results show that the produced chimeric antigens are a viable alternative to TLA in the detection of specific anti-T. gondii antibodies in animals.

This research was funded by The National Centre for Research and Development, under the research project no. LIDER/34/0188/L-10/18/NCBR/2019

Effect of *Dirofilaria repens* infection on blood parameters and cytokine expression level in healthy dogs and dogs infected with *Babesia canis*

Dagmara Wężyk

Department of Eco-epidemiology of Parasitic Diseases, University of Warsaw, Warsaw, Poland

Co-infection of *D. repens* and *B. canis* are rarely reported in the literature and there is very limited knowledge of their impact on canine health. Central Poland is endemic for both which poses a risk of co-infection of these parasites in dogs.

To evaluate the impact of co-infection of *B. canis* and *D. repens* on canine health, four groups of dogs were examined: healthy dogs, dogs infected with B. canis, dogs infected with *D. repens* and dogs co-infected. Blood parameters indicative of anaemia, kidney and liver damage were statistically analysed. Additionally, expression levels of immune response genes were determined and compared to assess the type of immune response in single- and co-infections.

In dogs infected with *D. repens*, no major alterations in blood parameters were observed. Dogs infected with the *B. canis* suffered from anaemia, kidney and liver insufficiency. In contrast, dogs with co-infection with *D. repens* and *B. canis* showed milder alternation in blood biochemical parameters associated with liver (AST, ALT, AP activity) and kidney (serum urea and creatinine levels) dysfunction compared to dogs infected only with *B. canis*.

The expression of genes associated with cellular (Th1) (STAT4 and IFN-g) and humoral (Th2) (STAT6, GATA3, IL-10, IL-13) response and SOCS3 gene was determined. For this analysis, dogs infected with *B. canis* were divided into 2 groups – '*Babesia* 1' (mild babesiosis), '*Babesia* 2' (severe babesiosis). In dogs infected with *D. repens*, expression of all tested factors except INF-γ was observed. In '*Babesia* 1' dogs the highest expression of GATA3 and Il-10 was detected, while in '*Babesia* 2' – INF-γ, IL-10 and SOCS3. The expression of IL-13 was predominant in dogs infected with *D. repens*, and the expression of STAT6 and IL-10 in dogs with co-infection.

In conclusion, no major deviations in blood parameters were found in dogs infected with *D. repens*. Interestingly, the values of biochemical parameters in dogs with co-infection were closer to these of healthy dogs than those solely infected with *B. canis* suggesting milder course of babesiosis in these animals. Dogs infected with *B. canis* showed expression of humoral and cellular immune response factors, whereas dogs infected with *D. repens* expressed humoral response factors. Features of Th1 or Th2 response were observed in *B. canis* and D. repens co-infected dogs. However, a humoral (Th2) response appears to predominate in co-infection.

Acknowledgements. The study was funded by the National Science Centre (NCN) OPUS grant no. 2017/27/B/NZ6/01691.