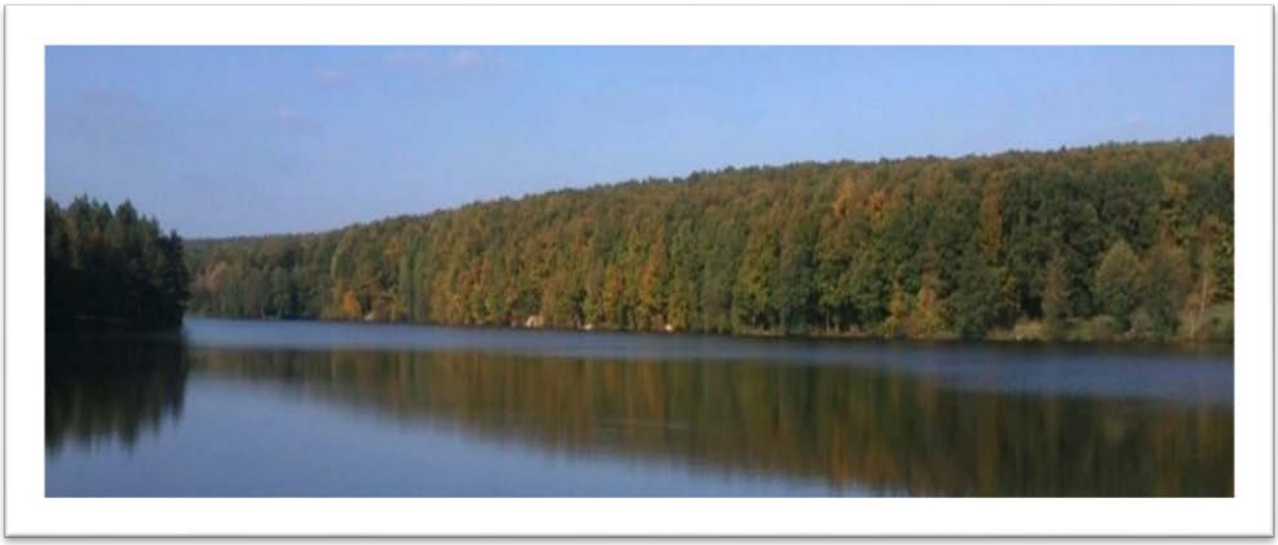
Helminthological Days 2017

Programme & Abstracts



Editors: Martina Orosová & Marta Špakulová

Duchonka, Pension Slniečko, Slovakia

8 – 12 May 2017

Conference organizer:	Institute of Parasitology, Slovak Academy of Sciences, Hlinkova 3, 040 01 Košice, Slovak Republic
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Conference date:	8 - 12 May 2017

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Contents

(Only the presenting author is listed in the Contents.)

Conference sponsors

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PROGRAMME OF THE OF 23RD HELMINTHOLOGICAL DAYS, 2017

MONDAY, MAY 8

16.00–18.00 **Arrival and registration of participants**
18.00–00.00 **Get-together-evening / Grill party (campfire place)**

***- competitive student presentation**
Invited talk: 25 min + 5 min discussion
Other presentation: 10 min + 5 min discussion

TUESDAY, MAY 9

07.30–08.30 **Breakfast**

09.00–09.10 **Opening ceremony (*Chairman: Mikuláš Oros*)**
09.10–9.40 **Invited talk:** František Stejskal, Lenka Richterová: IS STRONGYLOIDIASIS ENDEMIC IN THE CZECH AND SLOVAKIA?

Session I - Diversity and distribution of helmints, part 1 (*Chairman: Mikuláš Oros*)
09.45–10.00 Jana Bulantová, Roman Leontovyč, Jitka Aldhoun, Jiljí Sitko, Gerard Kanarek, Petr Horák: *PHYLLOSCOPUS COLLYBITA* (PHYLLOSCOPIDAE) AS AN UNUSUAL HOST FOR AVIAN SCHISTOSOMES OF THE GENUS *TRICHOBILHARZIA.*
10.00–10.15 *Juliana Moreira, Andrea Vetešníková Šimková, José Luque*:* *ANACANTHORUS* SPP. (MONOGENEA) PARASITIZING THE GILLS OF SERRASALMIDS (CHARACIFORMES) FROM TWO BRAZILIAN RIVER BASINS. **PhD**
10.15–10.30 *Kateřina Vyčítalová, Michal Benovics, Šárka Mašová, Anna Faltýnková, Tomáš Scholz, Yuriy Kvach, Simona Georgieva, Andrea Vetešníková Šimková: DIVERSITY OF METAZOAN PARASITE COMMUNITIES OF ENDEMIC CYPRINID SPECIES FROM THE SELECTED AREAS OF MEDITERRANEAN. **MSc**

10.30–11.00 **Coffee break**

Session II - Diversity and distribution of helmints, part 2 (*Chairman: Roman Kuchta*)
11.00–11.30 **Invited talk:** Algimantas Paulauskas, Dovilė Nugaraitė, Vytautas Mažeika: HELMINTH PARASITES OF WILD MUSTELIDS (FAMILY: MUSTELIDAE) IN LITHUANIA.
11.30–11.45 *Chahrazed Rahmouni, Maarten P.M. Vanhove, Andrea Vetešníková Šimková: *CICHLIDOGYRUS* SPP. (MONOGENEA: DACTYLOGYRIDAE) COMMUNITIES OF CICHLIDS FROM THE CONGOLESE LAKESHORE OF LAKE TANGANYIKA: NEW MONOGENEAN SPECIES, FISH HOST AND DISTRIBUTIONAL RECORDS. **PhD**

11.45–12.00 *Daniel Barčák, Mikuláš Oros, Vladimíra Hanzelová, Tomáš Scholz: A SYNOPTIC REVIEW OF THE GENUS *CARYOPHYLLAEUS* GMELIN, 1790, (CARYOPHYLLIDEA), TAPEWORMS OF CYPRINID FISHES. **PhD**

12.00–12.15 *Naraiana L . Taborda, José Luis Luque: BRAZILIAN FISH PARASITOLOGY. **PhD**

12.15–12.30 *Dovilė Nugaraitė, Algimantas Paulauskas, Vytautas Mažeika: FIRST DATA ON THE HELMINTH FAUNA OF THE EUROPEAN OTTER *LUTRA LUTRA* (LINNAEUS, 1758) IN LITHUANIA. **PhD**

12.30–13.30 **Lunch**

14.00–14.30 **Invited talk:** Ľubomír Šmiga, Lenka Koščová, Peter Košuth, Peter Lazar: AN OVERWIEV OF TREATMENTS OF ORNAMENTAL FISH HELMINTHOSES. **(*Chairman: Anna Faltýnková*)**

Session III - Host-parasite interactions (*Chairman: Anna Faltýnková*)

14.30–14.45 Miroslava Soldánová, Ana Born-Torrijos, Tereza Vyhlídalová, Rune Knudsen, Roar Kristoffersen, Per-Arne Amundsen: CONCOMITANT PREDATION ON PARASITES AND HOSTS AFFECTS TREMATODE TRANSMISSION SUCCESS.

14.45–15.00 *Terézia Mačák Kubašková, Gabriela Hrčková, Dagmar Mudroňová: THE EXPANSION OF MYELOID CD11B+ GR-1+ CELLS IN THE PERITONEAL CAVITY AND THE SPLEEN OF MICE INFECTED WITH LARVAL STAGES OF TAPEWORM. **PhD**

15.00–15.15 *Michal Babják, Alžbeta Königová, Michaela Urda Dolinská, Marián Várady, Štefánia Megyesiová, Ladislav Molnár, Edina Sesztáková: EXPERIMENTAL INFECTION WITH *HAEMONCHUS CONTORTUS* IN THREE SPECIES OF WILD RUMINANTS (*OVIS MUSIMON*, *CAPREOLUS CAPREOLUS* AND *DAMA DAMA*). **PhD**

15.15–15.30 *Tomáš Pakosta, Andrea Vetešníková Šimková: HOST-PARASITE COEVOLUTION IN THE DIPLOID-POLYPLOID COMPLEX OF *CARASSIUS AURATUS*. **MSc**

15.30–15.45 *Vadym Krasnovyd, Lukáš Vetešnik, Andrea Vetešníková Šimková: THE EFFECT OF FISH HYBRIDIZATION ON MONOGENEAN PARASITES: THE EXPERIMENTAL CROSS-BREEDING OF *ABRAMIS BRAMA* AND *BLICCA BJOERKNA*. **PhD**

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16.15–17.15 **Poster session P1 – P7, individual poster discussion**

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P2. Vojtech Boldiš, František Ondriska, Simona Lipková: Evaluation of immunoglobulin A for the immunodiagnosis of human toxocarosis.

P3. Oldřiška Hložková, Milan Jirků, Kateřina Sobotková, Laura Wegener Parfrey, Kateřina Jirků Pomajbíková: Introduction of model commensal helminth to the culture and characterization of the host immune response.
P4. Adriana Iglódyová, Ján Čurlík, Peter Lazar, Renáta Karolová, Ľubomír Šmiga: The endoparasites of selected cloven hoofed game living in environmental polluted area.
P5. Milan Jirků, Kateřina Sobotková, Jana Levá, Radek Šíma, Laura Wegener Parfrey, Kateřina Jirků Pomajbíková: Commensal tapeworm ameliorates DNBS-induced colitis in the rat model system.
P6. Veronika Konstanzová, Božena Koubková, Milan Gelnar: Ultrastructural observations on representatives from family Diplozoidae (Monogenea).
P7. Martina Matoušková, Magdaléna Bruňanská, Mikuláš Oros, Jana Nebesářová: Interesting ultrastructural organization of male gametes in caryophyllidean cestodes.

18.00–19.00 **Dinner**
19.00–00.00 **Free evening**

WEDNESDAY, MAY 10

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09.00–09.30 **Invited Talk:** Peter Šalamún, Vladimíra Hanzelová, Dana Miklisová, Oľga Šestinová, Lenka Findoráková, Peter Kováčik: NEMATODES AS BIOINDICATORS OF SOIL ECOSYSTEM CONTAMINATION. **(*Chairman: Jana Bulantová*)**

Session VII – Genomics, proteomics (*Chairman: Jana Bulantová*)
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09.45–10.00 *Nikol Reslová, Lucie Škorpíková, Karolína Snížková, Martin Kašný, Petr Králík: XMAP TECHNOLOGY: DETECTION OF PARASITIC AGENTS. **PhD**
10.00–10.15 Katarzyna Basałaj, Anna Zawistowska-Deniziak, Alicja Sielicka, Halina Wędrychowicz, Luke J. Norbury: PHAGE DISPLAY LIBRARY AS A USEFUL TOOL FOR *FASCIOLA HEPATICA* ANTIGEN CHARACTERIZATION.
10.15–10.30 *Lucie Škorpíková, Nikol Reslová, Michal Slaný, Edoardo Pozio, Martin Kašný: GENOTYPING OF EIGHT *TRICHINELLA* SPECIES USING HIGH RESOLUTION MELTING ANALYSIS. **PhD**

10.30–11.00 **Coffee break**

Session V – Immunology, Biochemistry and Diagnostics part 1 (*Chairman: Libor Mikeš*)
11.00–11.15 *Anna Zawistowska-Deniziak, Katarzyna Basałaj, Alicja Sielicka, Agnieszka Wesołowska, Peter M Smooker, Halina Wędrychowicz, Luke J Norbury: THE IMPACT OF RECOMBINANT *FASCIOLA HEPATICA* ANTIGENS ON HUMAN MACROPHAGES, IN VITRO STUDY. **PhD**

11.15–11.30 *Jan Novák, Lucie Panská, Tomáš Macháček, Libuše Kolářová, Petr Horák: HUMORAL RESPONSE OF MICE INFECTED WITH *TOXOCARA CANIS* FOLLOWING DIFFERENT INFECTION SCHEMES. **MSc**

11.30–11.45 *Adrian Leontovyč, Lenka Ulrychová, Anthony J. O'Donoghue, Lucie Marešová, Jiří Vondrášek, Michael Mareš, Martin Horn, Jan Dvořák: SECRETED SERINE PROTEASE SMSP2 OF THE BLOOD FLUKE *SCHISTOSOMA MANSONI*: BIOCHEMICAL CHARACTERIZATION, LOCALIZATION AND HOST PROTEIN PROCESSING. **PhD**

11.45–12.00 *Anna Sulima, Justyna Bień, Rusłan Sałamatin, Daniel Młocicki: MASS SPECTROMETRY IDENTIFICATION OF ANTIGENIC PROTEINS OF *HYMENOLEPIS DIMINUTA* (CESTODA, HYMENOLEPIDIDAE) CYSTICERCOID. **PhD**

12.00–12.15 *Linda Vlčková, Martin Majer, Tomáš Macháček, Lucie Panská, Petr Horák: NEW INSIGHTS INTO CELLULAR IMMUNE RESPONSE OF DUCKS AND MICE INFECTED BY *TRICHOBILHARZIA REGENTI*, A NEUROTROPIC SCHISTOSOME. **MSc**

12.30–13.30 **Lunch**

Session VI – Immunology, Biochemistry and Diagnostics part 2 (*Chairman: Martin Kašný*)

14.00–14.15 *Barbora Bucková, Emília Dvorožňáková, Andrea Lauková: DISTRIBUTION OF LYMPHOCYTE SUBPOPULATIONS IN THE SMALL INTESTINE OF MICE AFTER PROBIOTIC THERAPY AND *TRICHINELLA SPIRALIS* INFECTION. **PhD**

14.15–14.30 *Jana Ilgová, Jedličková Lucie, Hana Dvořáková, Libor Mikeš, Gabriela Sajlerová, Michal Benovics, Pavel Roudnický, Jiří Vorel, Libor Vojtek, Pavel Hyršl, Jiří Salát, Milan Gelnar, Martin Kašný: STEFIN OF *EUDIPLOZOON NIPPONICUM* (MONOGENEA): IMMUNOMODULATOR OR HOUSEKEEPING PROTEIN? **PhD**

14.30–14.45 *Jiří Vorel, Pavel Roudnický, Jana Ilgová, Hana Dvořáková, Lucie Jedličková, Libor Mikeš, Petr Brož, Dagmar Jirsová, Roman Leontovyč, Lukáš Vetešník, Pavel Jurajda, Marie Jankůjová, Jan Oppelt, Božena Koubková, Milan Gelnar, Martin Kašný: *EUDIPLOZOON NIPPONICUM* (MONOGENEA: DIPLOZOIDAE): COMPARATIVE STUDY OF TRANSCRIPTOME AND SECRETOME OF FISH PARASITE. **PhD**

14.45–15.00 *Petra Rubešová, Adéla Jílková, Pavla Fajtová, Martin Horn, Pavlína Řezáčová, Jiří Brynda, Conor R. Caffrey and Michael Mareš: STRUCTURAL BASIS FOR VINYL SULFONE INHIBITION OF THE SMCB1 DRUG TARGET FROM THE HUMAN BLOOD FLUKE. **PhD**

15.00–15.15 *Jan Pankrác, Tomáš Macháček, Martin Kašný, Petr Horák: EFFECT OF NITRIC OXIDE AND HYDROGEN PEROXIDE ON AVIAN SCHISTOSOMES *TRICHOBILHARZIA REGENTI* AND *TRICHOBILHARZIA SZIDATI*. **PhD**

15.15–15.30 *Pavel Roudnický, Jiří Vorel, Jana Ilgová, Libor Mikeš, Lucie Jedličková, John Dalton, Jan Dvořák, Lubomír Janda, Adam Norek, Milan Gelnar, Martin Kašný: SERPIN, A KEY MOLECULE IN THE LIFE OF *EUDIPLOZOON NIPPONICUM*?!

15.30–16.00 **Coffee break**

15.30–16.00 **Installation of posters P8 – P14**
16.00–17.00 **Poster session P8 – P14, individual poster discussion**

P8. Ingrid Papajová, Jana Pipiková, Naďa Sasáková, František Tóth: Helmiths eggs viability in the course of long-term storage of pig slurry.
P9. Jana Ondeková, Marek Renčo, Andrea Čerevková: The specific response of Nematoda community to different management of deciduous forest affected by windstorm.
P10. Dalibor Uhrovič, Mikuláš Oros, Tomáš Scholz: A new classification of *Glaridacris* Cooper, 1920 (Cestoda), parasites of suckers (Catostomidae) in North America.
P11. Marek Renčo, Franciszek Wojciech Kornobis, Krzysztof Domaradsky, Anna Jakubska-Busse, Jana Ondeková: Effects of alien plant invasion on behaviour of nematode community in different types of ecosystems.
P12. Povilas Sakalauskas, Vytautas Sabunas, Jana Radzijevskaja, Algimantas Paulauskas: Prevalence of *Dirofilaria repens* in dogs from Lithuania.
P13. Ľubomír Šmiga, Lenka Koščová, Ján Koščo, Peter Košuth, Jakub Fedorčák, Peter Lazar: Endoparasitic helminths of loaches of the genus *Cobitis* from Slovakia.
P14. Gabriela Štrkolcová, Mária Goldová: Occurence of negleted detectable soil-transmitted helminth in children and dogs in Eastern Slovakia.

18.00–19.00 **Dinner**
19.00–00.00 **Free evening**

THURSDAY, MAY 11

07.30–08.30 **Breakfast**

08.45–09.15 **Invited talk:** Mabel Ribicich: CURRENT SITUATION OF *TRICHINELLA* SPP. IN ARGENTINA AND SOUTH AMERICA: NEW SPECIES AND SCENARIOS. **(*Chairman: Milan Gelnar*)**

Session IV – Epidemiology, epizootology (*Chairman: Milan Gelnar*)
09.15–09.30 *Jana Pipiková, Ingrid Papajová: HELMINTH INFECTIONS IN TWO LOCALITIES WITH DIFFERENT SANITATION AND HYGIENE STANDARDS IN SLOVAKIA. **PhD**
09.30–09.45 *Natalia M Cardillo, Yanina Loiza, Ayelén Boboni, Fernando Fariña, Mariana Pasqualetti, German Martínez, Francisco Montalvo, Valentina Vera, Adriana Betti, Mabel Ribicich: SOIL TRANSMITTED HELMINTH ZOONOSES IN SQUARES FROM BUENOS AIRES CITY, ARGENTINA. **PhD**
09.45–10.00 *Tomáš Macháček, Libuše Turjanicová, Jana Bulantová, Martin Kašný, Petr Horák, Libor Mikeš: CERCARIAL DERMATITIS IN HUMANS: A SYSTEMATIC FOLLOW-UP STUDY OF TEN CASES. **PhD**
10.00–10.15 *Nicol Bernardová, Marta Chanová: MURINE CEREBRAL TOXOCARIASIS. **PhD**

10.15–10.30 Marina Winter, Sergio Abate, Diego Birochio, Mariana Pasqualetti, Fernando Fariña, Mariano Ercole, Bernardo Alonso, Andrea Marcos, Ricardo Veneroni, Marianela Castillo, Lais Pardini, Gastón Moré, Cecilia Venturini, Mabel Ribicich: TRICHINELLOSIS AND TOXOPLASMOSIS IN WILD BOARS (*SUS SCROFA*) OF A NORTHERN PATAGONIA, ARGENTINA.

10.30–11.00 **Coffee break**

11.30–12.30 **Lunch**

12.30–18.00 **Trip to National Stud Farm Topoľčianky & Bison game preserve**
(http://sk.nztopolcianky.sk/index.php?option=com_content&view=category&layout=blog&id=27&Itemid=100005&lang=sk)
(http://zubor.fotop.sk/)

19.00–00.00 **Closing ceremony**, **party with live music.**

FRIDAY, MAY 12

07.30–08.30 **Breakfast**
09.00–10.00 Departure of participants

Abstracts

Abstracts are listed alphabetically by the presenting author's last name.

EXPERIMENTAL INFECTION WITH *HAEMONCHUS CONTORTUS* IN THREE SPECIES OF WILD RUMINANTS (*OVIS MUSIMON, CAPREOLUS CAPREOLUS* AND *DAMA DAMA*)

Michal Babják[1], Alžbeta Königová[1], Michaela Urda Dolinská[1], Marián Várady[1], Štefánia Megyesiová[1], Ladislav Molnár[2], Edina Sesztáková[2]

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Anthelmintic resistance (AR) and gastrointestinal (GI) parasitism are well known phenomenon mainly in domestic ruminants. Less information is available about the status of AR and course of parasitic infection in wild ruminants. Knowledge about GI parasites of wild ruminants is important because they could serve as a source of infection and potential vector of resistant parasites between sheep and goat farms. Three species of wildlife ruminants: European mouflon (*Ovis musimon*), Roe deer (*Capreolus capreolus*) and Fallow deer (*Dama dama*) were selected for this study. Three animals from each species were infected with 8000 L_3 larvae of susceptible MHco3 strain and resistant MHco4 strain of abomasal blood-sucking nematode *Haemonchus contortus*. At the same time 6 lambs (3+3) were infected with identical strains. Faecal samples were collected on day (D) 0, D16, D37, D58, D77 and D99. Values of helminth eggs per gram (EPG) were quantified by a modified McMaster technique. *In vitro* egg hatch test (EHT) and larval development test (LDT) were used to identify ED_{50} values. Highest level of infection at mouflons was observed on D58 where EPG was 26 500 for resistant strain and 3 800 for susceptible strain. Roe and Fallow deer had low levels EPG (100-150) till the D58. After D58 helminth eggs in Roe and Fallow samples were not observed possibly due to phenomenon of self-cure. Values of ED_{50} for tiabendazole (TBZ) in EHT of susceptible MHco3 strain in mouflons and sheep varied in range from 0.043±0.005 to 0.064±0.008 and in LDT from 0.007±0.002 - 0.009±0.001 µg/ml TBZ which confirmed the same level of AR in sheep and mouflons. Values for resistant MHco4 strain were equal in both species from 0.091±0.001 to 0.111±0.004 µg /ml TBZ in EHT and 0.0213 ±0.002 – 0.0312±0.001µg/ml TBZ in LDT.

Acknowledgements
The study was supported by Grant Agency VEGA, Grant No. 2/0120/16 of the Scientific Agency of the Slovak Academy of Sciences and Slovak Research and Development Agency Project No. 14-0169.

A SYNOPTIC REVIEW OF THE GENUS *CARYOPHYLLAEUS* GMELIN, 1790, (CARYOPHYLLIDEA), TAPEWORMS OF CYPRINID FISHES

Daniel Barčák[1], Mikuláš Oros[1], Vladimíra Hanzelová[1], Tomáš Scholz[2]

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[2]*Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic*

Monozoic tapeworms of the genus *Caryophyllaeus* Gmelin, 1790 (Caryophyllidea: Caryophyllaeidae) are common parasites of cyprinid fishes in the Palaearctic Region including *C. laticeps* (Pallas, 1781), which served as a model for pioneer ecological and epidemiological studies in the late 1960's. So far, 42 nominal taxa have been placed in the genus during its long history. However, detailed morphological studies on specimens in museum collections and newly-collected material supported by molecular data has shown that only several species could be considered as valid. Moreover, phylogenetic analysis of the partial mitochondrial *cox*1 gene and large subunit of ribosomal gene (*lsu* rDNA) in relation to morphological data revealed unexpected phenotypic plasticity within two most widespread species of the genus.

Within the type species, *Caryophyllaeus laticeps,* five possibly host-specific morphotypes were recognised and morphologically characterised. In fact, some of them were initially considered as new species based on the morphology of the anterior part of the body, until molecular analyses showed their genetic similarity. Besides the morphological differences, they are sharing characters, i.e. form of the cirrus-sac, vas deferens and surrounding structures located on the posterior part, which were established as novel species-specific morphological traits.

Since its original description, *Caryophyllaeus brachycollis* Janiszewska, 1953 was considered as a parasite of barbels and chubs. Recent studies, however, revealed another morphotype, which morphologically partly resembles *C. laticeps* and also parasitizes same fishes, freshwater breams. However, second morphotype of *C. brachycollis* can be easily distinguished based on a massive, elongated cirrus-sac and vas deferens surrounded by vitelline follicles, in contrast to a subspherical, thin-walled cirrus-sac with internal seminal vesicle and conspicuous vas deferens of *C. laticeps* morphotype 1.

Taxonomic status of rare species of the genus *Caryophyllaeus*, such as *C. auriculatus, C. fimbriceps* and *C. syrdarjensis,* was assessed based on whole-mounts deposited in museum collections and records in the literature. Until new material suitable for genetic analyses will be collected, their validity remains rather unclear.

Acknowledgements

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PHAGE DISPLAY LIBRARY AS A USEFUL TOOL FOR *FASCIOLA HEPATICA* ANTIGEN CHARACTERIZATION

Katarzyna Basałaj[1], Anna Zawistowska-Deniziak[1], Alicja Sielicka[1], Halina Wędrychowicz[1], Luke J Norbury[1]

[1]*Witold Stefański Institute of Parasitology, Polish Academy of Sciences, Warsaw, Poland*

In order to understand and successfully treat parasite diseases a thorough characterization and analysis of parasite proteins needs to be undertaken. Phage display antibody libraries are powerful tools for obtaining monoclonal antibodies or antibody fragments that can facilitate research on parasite proteins. Appropriate antibodies obtained from these libraries can be used in a range of assays in such research.
In our project we constructed an immune single-chain variable fragment (scFv) library. Rats were exposed to two subsequent infections of *Fasciola hepatica*. RNA obtained from the spleens of infected rats was used to generate a library using a phagemid vector. As the result of panning against several *F. hepatica* antigens a number of scFv that bind to *F. hepatica* antigens have been selected for further evaluation. The specificity of binding of chosen scFv was confirmed by ELISA and immunoblotting, and an initial evaluation for use in antigen characterization undertaken.
The constructed rat library is a valid tool for parasite characterisation and the method is a promising alternative for research on other parasites.

Acknowledgements
Project funded by NCN grant no. 2012/05/B/NZ6/00777.

PHYLOGENETIC POSITION OF *AULOPYGE HUEGELII*, ENIGMATIC CYPRINID SPECIES OF BALKAN PENINSULA, REVEALED BY HOST SPECIFIC *DACTYLOGYRUS* PARASITES

Michal Benovics[1], Maria Lujza Kičinjaová[1], Petra Zahradníčková[1], Andrea Vetešníková Šimková[1]

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Host specificity of fish parasites is considered as one of the most important parasite characteristic helpful for understanding the biogeography of their fish hosts. Monogeneans of the genus *Dactylogyrus* with more than 900 nominal species are common parasites of the freshwater cyprinid fish exhibiting narrow host specificity. The historical dispersion and phylogenetic position of some cyprinid groups or species are still unclear. One of such enigmatic species is *Aulopyge huegelii* Heckel, 1843. Therefore, in the present study, we used the host specific *Dactylogyrus* parasites as the additional tool potentially shedding the light to the history and phylogenetic relationships of *A. huegelii* within Cyprininae lineage.

In total, 14 specimens of *A. huegelii* from Šujica River (Bosnia and Herzegovina) were investigated for the presence of metazoan parasites. Monogeneans were removed from gills, fins and body surface. Two different species of *Dactylogyrus* (1 described as new for science) and three species of *Gyrodactylus* (2 described as new for science, 1 undescribed species) were identified. The record of *D. vastator*, common parasite of *Cyprinus carpio* and *Carassius* species, on *A. huegelii* supports evolutionary proximity of this fish species to the species of Cyprininae. The other *Dactylogyrus* species described as *D. omenti* n. sp. in our study, is according to phylogenetic reconstruction, closely related to *Dactylogyrus* species parasitising European *Barbus* and *Luciobarbus* species. The morphological similarity between *D. omenti* n. sp. and *Dactylogyrus* species of Middle Eastern *Barbus*, such are *D. affinis* and *D. deziensioides*, suggest historical contact between cyprinids recently living in allopatry and the possible diversification of these two lineages from common ancestor in Middle East.

By computing the genetic distance between *D. vastator* of *A. huegelii* and *D. vastator* collected from other cyprinid host species from different localities across Eurasian supercontinent, we revealed that specimens from *A. huegelii* are genetically identical to *D. vastator* from *Barbus plebejus* (Italy) and *Carassius gibelio* (Croatia). Moreover, *D. vastator* of *A. huegelii* was also genetically more similar to *D. vastator* of *Cyprinus carpio* rather than that of *Carassius gibelio* collected in Czech Republic.

Acknowledgements

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MURINE CEREBRAL TOXOCARIASIS

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Toxocara canis is alimentally, transplacentally and transmammally transmitted parasite of canids distributed worldwide. In its natural life cycle also paratenic host may be used. In paratenic host, the parasite undergoes only larval development and infectious L3 stage larva persists in various tissues, including brain, for a long period. Various vertebrates may serve as paratenic hosts and accidentally also humans are infected. Seroprevalence of larval toxocariasis in human population is high (up to 36% in some locations), contrary to low number of clinically diagnosed cases. Despite of general opinion that the infections are mostly asymptomatic and thus harmless, some authors suppose the association of long-term persistence of larvae in human brain with the development of chronic neurodegenerative and neuropsychiatric diseases. The aim of our study was to evaluate the consequences of long term presence of *T. canis* larvae in central nervous system of mice. Experimentally infected mice were observed for the development of neurological signs, investigated for antibody response development, and sacrificed in desired period of acute, early chronic and late chronic phase for histological investigation of impaired tissues. The most severe signs (neuromotor disorders) were observed on the 9th week post infection for the first time, and with increasing frequency in later phases. Association between these signs and localization of larvae and/or pathologies found in brain tissue was in focus. Histopathological findings did not reveal any significant differences among mice in different phases of infection. We assume that histopathologic changes in the brain caused by parasite activity do not lead to neurologic signs directly, as they are present far prior their development. Potential effect of long-term chronic impact is discussed.

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EVALUATION OF IMMUNOGLOBULIN A FOR THE IMMUNODIAGNOSIS OF HUMAN TOXOCAROSIS

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Human toxocarosis is considered a widely prevalent infection with a worldwide distribution, thanks to a large and increasing number of final hosts (dogs and cats). The study was focused on distinction of acute and chronic toxocarosis in humans following the assessment of specific IgA antibodies. Anti-*Toxocara* IgA antibodies were detected by excretory-secretory (ES)-enzyme-linked immunosorbent assay (ELISA). The presence of IgA in suspected patients for toxocarosis were evaluated in respect of some characteristics of examined persons. IgA antibodies were determined in 17 (32.7 %) cases of 52 persons who were IgG seropositive; in 75.0 %, 28.6 % and 28.0 % of above 41- year-old, 11 – 20-year-old, and up to 10- year-old age groups, respectively. The differences were statistically significant ($P = 0.026$). The occurrence of *Toxocara* spp. specific IgA antibodies in patients with increased total IgE (44.8 %) was significantly higher than in patients with normal level of IgE (17.4 %; $P = 0.036$). Low- and high-avidity IgG antibodies against *Toxocara* spp. were represented in 37.5 % and 28.6 % of patients who were seropositive for the specific IgA, respectively. The seroprevalence of specific IgA antibodies was higher in patients with clinical symptoms (44.4 %), patients with history of geophagia (37.5 %) and dog/cat owners (35.6 %). However, these associations were not statistically significant ($P > 0.05$). Also, no associations ($P > 0.05$) were found between the IgA seropositivity and gender, level of specific IgG antibodies, eosinophilia, domicile or travelling abroad. The sensitivity and specificity of ELISA IgA were 57.1 % and 100 %, respectively. Correlation analysis between the levels of anti-*Toxocara* IgA antibodies and age revealed mild positive correlation ($r = 0.302$). The values for eosinophils amounts versus IgA antibody levels showed higher correlation coefficient $r = 0.336$, also considered as mild positive correlation. The highest correlation was observed between the amounts of eosinophils and the values of IgG avidity ($r = - 0.417$), but considered as mild negative correlation. In conclusion, our results suggest that measurement of *Toxocara* spp. specific IgA, total IgE antibody levels, eosinophils, IgG avidity may be useful for the determination of acute toxocarosis.

DISTRIBUTION OF LYMPHOCYTE SUBPOPULATIONS IN THE SMALL INTESTINE OF MICE AFTER PROBIOTIC THERAPY AND *T. SPIRALIS* INFECTION

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Important components of the intestinal mucosal immunity are free intraepithelial and *lamina propria* lymphocytes involved in the regulation and activity of the immune response. This study detected the presence of helper CD4 and cytotoxic CD8 T lymphocytes, and B lymphocytes in the small intestine of mice treated with probiotic strains and infected with *T. spiralis*. Bacterial strains of different origin (*Enterococcus faecium* AL41= CCM8558, *Enterococcus durans* ED26E/7, *Lactobacillus fermentum* AD1 = CCM7421, *Lactobacillus plantarum* 17L/1) were administered daily in dose of 10^9CFU/ml in 100 µl and mice were infected with 400 larvae of *T. spiralis* on 7th day of treatment. *L. fermentum* AD1 = CCM7421 and *L. plantarum* 17L/1 increased numbers of helper CD4 T cells in the epithelium and cytotoxic CD8 T cells in the *lamina propria* on 7th day of administration (before parasitic infection). *T. spiralis* infection caused a significant inhibition of examined lymphocyte subpopulations from 5 to 25 days post infection (p.i.). Lactobacilli restored the CD4 T cell numbers in the epithelium and *lamina propria* on the level of healthy control from day 11 p.i. All strains stimulated the numbers of CD8 T cells in infected mice, but in comparison to control, CD8 T cells were reduced in the epithelium until day 25 p.i. and in the *lamina propria* only on day 5 p.i. An inhibition of B cells (CD19) in the small intestine after *T. spiralis* infection was not affected by probiotic therapy till day 25 p.i., but a stimulation of B cells was found after treatment with *E. durans* ED26E/7 and *L. fermentum* AD1 = CCM7421on day 32 p.i. The greatest anti-parasitic effect against adults was presented by *E. faecium* AL41=CCM8558 (65 % reduction). A significant decrease in the number of muscle larvae was detected in all treated groups (reduction 55 %). The obtained results confirmed the strain-specific immunomodulatory effect of probiotic bacteria. The greatest immunomodulatory potential on the gut CD4 and CD8 lymphocytes during *T. spiralis* infection was confirmed by *L. fermentum* AD1 and *L. plantarum*17L/1. It could be caused by a longer presence of *Trichinella* adults in the gut in contrast to mice treated with enterococci. The greatest protective effect against adults was presented by *E. faecium* AL41 and *E. durans* ED26E/7, strains producing enterocins that could influence mucin secretion. A significant decrease in the number of muscle larvae was detected in all treated groups. The CD8 T cell subpopulation was stimulated after probiotic treatment and it could prevent a migration of newborn larvae to the muscles.

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PHYLLOSCOPUS COLLYBITA (PHYLLOSCOPIDAE) AS AN UNUSUAL HOST FOR AVIAN SCHISTOSOMES OF THE GENUS *TRICHOBILHARZIA*

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Trematodes of the family Schistosomatidae are always connected with aquatic environment. Their two-host life cycles include marine or freshwater snails of various groups that release cercariae, larvae attacking vertebrate hosts. The infection of vertebrates is usually accomplished via penetration of the host skin, but experimentally the conjunctival sac or the upper part of alimentary tract also served as entry sites. As far as the avian schistosomes are concerned, the most common definitive hosts are representatives of the orders Anseriformes, Gruiformes and Charadriiformes, but occassionally also Ciconiiformes, Podicipediformes, Suliformes, Pelecaniformes, Gaviiformes, Sphenisciformes and Coraciiformes - i.e., birds with close relationship to the aquatic environment. Nevertheless, rare experimental and/or natural infections of orders Passeriformes, Columbiformes or Galliformes suggest, that avian schistosomes do not specifically depend only on water birds.

Recently, two fragments of a filiform avian schistosome (anterior part of a male and anterior part of a female) were found in the liver tissue of a bird which has no association with water environment - *Phylloscopus collybita* from Záhlinice (eastern region of Czechia). Morphologically, the worms bear the main characteristics of the genus *Trichobilharzia*; molecular analysis confirmed it is an unknown species.

The genus *Trichobilharzia* contains more than 40 species. As the members of *Trichobilharzia* are usually similar to each other in terms of morphological characteristics, molecular tools need to be applied for accurate identification. In Europe, the main definitive hosts of *Trichobilharzia* spp. belong to the order Anseriformes, but some worms were also found in passeriform birds - namely in the liver of one *Motacilla alba*, where fragments of adult *Trichobilharzia* males were discovered. In that case, material was identified based exclusively on morphological characteristics. In contrast to *P. collibyta*, *M. alba* is known to inhabit biotopes with water bodies at shores of which it forages for food. It remains, therefore, questionable if *P. collibyta* represents a regular or only occasional host for the new avian schistosome.

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SOIL TRANSMITTED HELMINTH ZOONOSES IN SQUARES FROM BUENOS AIRES CITY, ARGENTINA

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Soil can be an important source of human and animal pathogens; many of them are etiological agents of zoonotic helminthoses. Companion animals are potential reservoirs and transmitters of soil borne helminths, contaminating the environment through the faeces. Parasite contamination level of the cities would be a consequence of the animal density, of the lack of hygienic sanitary measures that control the presence of faeces in public spaces, and the absence of fences that avoid the access of canines and felines and efficiently delimit the zones intended for children recreation. The aimed of this study was to describe parasite contamination from public squares of the Autonomous City of Buenos Aires with emphasis on the study of zoonotic geohelminths and to identify the impact of fencing (perimetral fence, kennels and children´s sandboxes) as a preventive measure to the access of pets and their defecation. A total of 11 squares were studied during a follow up year, from which 216 fresh sample faeces were collected and individually analyzed by modified Bembrook´s technique and the Baermann´s technique. A general prevalence of parasitism was found of 27.31% (59/216), in which 31.67% (19/59) presented parasitic associations.

Parasite	N	Prevalence
***Ancylostoma* spp.**	42 (19,44%)	71,12 %
***Trichuris* spp.**	20 (9,26%)	33,89 %
***Sarcocystis* spp.**	8 (3,70%)	13,55 %
***Toxocara* spp.**	6 (2,78%)	10,17 %
***Coccidios* spp.**	5 (2,31%)	8,47 %
***Giardia* spp.**	1 (0,46%)	1,69 %
Total	59 (27,31 %)	

Ancylostoma spp. was the most prevalent genus, followed by *Trichuris* spp, coinciding with other reports from different Argentinean cities. This parasitic association was the most prevalent, which suggests that adult animals could be reservoirs and sources of infection for the environment as they are both genera frequently found in this age group. The prevalence of *Sarcocystis* spp. indicates that pets are fed raw or undercooked meat, which warns the potential risk of acquiring toxoplasmosis infection either. No *Strongyloides stercoralis* larvae were found by Baermann´s technique. The 79% of the squares studied had perimetral fencing. With one exception, all the children's sandboxes and all the kennels were fenced. General parasite prevalence in the three sectors sampled were for Kennels 4/21(19,05%); Children´s sandboxes 5/7 (83,33%) and general sector 50/188 (26,60%). There were significant differences in the prevalence of general parasitosis among the three sectors, with the general sector of the square being the most parasitized ($X2 = 7.6$, $p = 0.022$). Regarding the relationship in the parasites prevalence between squares with perimetral fencing and without fencing, no significant differences were found ($X2 = 0.027$, $p = 0.87$), since fencing does not prevent animals entry. It is concluded that the fencing of public squares is a relevant measure to prevent animal access and indirectly to reduce environmental contamination by parasites of companion animals.

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INTRODUCTION OF MODEL COMMENSAL HELMINTH TO THE CULTURE AND CHARACTERIZATION OF THE HOST IMMUNE RESPONSE

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The present study is the part of the larger project focused on the investigation of commensal tapeworm, *Hymenolepis diminuta*, as a candidate for helminth therapy (HT). HT is one of the approaches of biological therapies for immune-mediated diseases in the past decade. The principle of this therapy is the controlled infection with non- or mildly- pathogenic helminths. Here, we addressed two objectives: (i) to establish a culture of *H. diminuta* in laboratory conditions for further research on HT, and (ii) to observe an effect of *H. diminuta* on the mucosal immunity of the host intestine by detection of IgA antibodies. *Hymenolepis* has an indirect life cycle involving invertebrates as intermediate hosts (with larval stages – cysticercoids) and rats as definitive hosts (with adult tapeworms colonizing the small intestine). Human is an accidental host. We introduced the culture of *H. diminuta* under laboratory conditions according to the scheme of its natural life cycle - grain beetles (*Tenebrio molitor*) as an intermediate host and outbred Wistar rats as definitive hosts. The first rats were infected with cysticercoids of *H. diminuta* obtained from collaborative laboratory. When the rats began to shed the eggs in feces (means that the adults were developed in the rat intestine – patent period, 16-18 days post infection), the beetles were infected by feeding of these feces. After several experiments, we realized that to get the infective cysticercoids for further infections of the rats it is necessary to optimize the temperature for their breeding between 28-30°C. Under these conditions, we are able to get the infective cysticercoids after three weeks post infection of beetles. Further, we also use the rats as an experimental model system for testing of an effect of *H. diminuta* on the host organism. To meet the second aim of this study, we optimized indirect ELISA for detection of IgA antibodies from the rat feces. First, we performed several experiments focused on the preparation of an antigen from *H. diminuta*. The most optimal antigen for ELISA was prepared from the cysticercoids disrupted mechanically and by temperature change (liquid nitrogen: -196°C and thermoblock: 40°C) and sonication (0,5 impulse and 20% amplitude). Further, we performed experiment to reveal a fluctuation of IgA antibodies within prepatent and patent period of *H. diminuta* infection in the rat model system. We found that the level of IgA is significantly higher in infected animals in contrast to control ones in the patent period. Our results show that *H. diminuta* infection strongly stimulates the mucosal immunity of the host intestine. This suggests that *H. diminuta* has a strong immunomodulatory effect on the host organism.

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THE ENDOPARASITES OF SELECTED CLOVEN HOOFED GAME LIVING IN ENVIRONMENTAL POLLUTED AREAS

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In recent years the environmental pollution by anthropogenic activities is considered one of the limiting factors of its quality. Number of the pollutants, that can cause the serious health problems in overflow concentrations, is really high. Among other health problems, the immunosuppression causes increased sensibility to infectious agents. Wild ruminants are often infected by wide spectrum of parasites without impact on their health. The study was focused on monitoring cloven hoofed game living in polluted areas in order to provide information on the endoparasite prevalence, intensity of infection, clinical signs associated with these infections and the possible influence of environmental factors such as anthropogenic pollution. We examined 207 faecal samples from selected wild ruminants using coprological and serological methods. Faecal samples were collected from red deer (*Cervus elaphus*) (78), roe deer (*Capreolus capreolus*) (34) and mouflon (*Ovis musimon*) (95). Samples were taken from areas nearby Strážske. 92,28 % samples were positive for the presence of endoparasites, with the highest incidence of lungworms and gastrointestinal nematodes, then the presence of trematodes of the genus *Dicrocoelium* and *Paramphistomum*, tapeworms of the genus *Moniezia* and *Coccidia*. Clinical signs were recorded such as diarrhoea, weight loss, lethargy. The results show high prevalence of endoparasites in cloven hoofed game from polluted areas and significant clinical signs which may be related to the presence of endoparasites in these animals. Environmental pollution may have a marked influence not only on the increased prevalence of endoparasites, but also on their impact on health status of animals.

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STEFIN OF *EUDIPLOZOON NIPPONICUM* (MONOGENEA): IMMUNOMODULATOR OR HOUSEKEEPING PROTEIN?

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Hematophagous fish parasite *Eudiplozoon nipponicum* (Monogenea: Diplozoidae) produces cysteine peptidase inhibitor (stefin) which has been detected in excretory-secretory products of the adult worms and thus might play a role in host-parasite interaction. Our study aims to reveal the biological function of the stefin of *E. nipponicum* (EnStef). Inhibitors of cysteine peptidases are synthetized by wide range of parasitic species. Besides regulation of endogenous processes in parasite bodies they play a substantial role e.g. in manipulation of the host immune system and/or blood digestion. We prepared recombinant form of EnStef (rEnStef) in *E. coli* BL21 host strain using pET19b expression plasmid vector. By adoption of fluorometric assay we observed efficient inhibition of cysteine peptidases (cathepsins L and B from *E. nipponicum* and mouse cathepsin L) via its conserved papain-binding domain as well as inhibition of asparaginyl endopeptidase (legumain) probably due to legumain-binding domain, untypical for stefins. rEnStef blocked proteolytic degradation of hemoglobin mediated by cysteine peptidases in the excretory-secretory products, soluble protein extracts from *E. nipponicum* and by recombinant cathepsins L3 and B of *E. nipponicum*, which manifests its role in blood meal digestion. In order to assess the immunomodulatory potential of EnStef we tested its effect on activation of complement in carp's plasma and oxidative burst in full blood using luminol-enhanced chemiluminescence. We performed series of experiments with stimulated porcine alveolar macrophages and rEnStef to examine its effect on cytokine production (IL-1β, TNF-α, IL-6 and IL-10). The immunohistochemistry techniques (using specific rabbit rEnStef antibodies) and *in situ* RNA hybridization (using DIG-labelled RNA probes) were adopted for localization of EnStef on paraffin sections of *E. nipponicum*.

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COMMENSAL TAPEWORM AMELIORATES DNBS-INDUCED COLITIS IN THE RAT MODEL SYSTEM

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The present study is focused on a testing of an additional helminth candidate for purposes of helminth therapy (HT) for Inflammatory Bowel Diseases (IBD). Accumulating epidemiological data provide compelling evidence for a vast increase in the incidence and prevalence of IBD across Western countries. The complex genesis of IBD includes chronic disabling gastrointestinal disorders such as Crohn`s disease (CD) and ulcerative colitis. HT has been identified as one of the promising approaches of biological therapy for IBD. Its principle is the controlled infection with non- or mildly-pathogenic helminths, which induce anti-inflammatory Th2-type immune response. We test commensal tapeworm, *Hymenolepis diminuta*, for amelioration of CD. Here, we addressed three objectives: (i) to characterize an effect of *H. diminuta* on commonly used model of acute colitis, (ii) to establish a model of chronic colitis, and (iii) to observe an effect of *H. diminuta* on chronic colitis. We use the rat model system for our experiments with two groups – control group and group infected with *H. diminuta*. We always tracked a gene expression of pro-inflammatory cytokine TNFα characteristic for colitis using qPCR. In the first experiment, we established the model of acute colitis induced by Dinitrobenzene Sulfonic Acid (DNBS), which phenotypes mimic Crohn`s disease (CD). We used this system to assess the ability of *H. diminuta* to prevent induced colitis in a rat model during in the patent period of infection. In case of acute colitis (persists for 4 days) induced in the beginning of patent period (i.e. 21 days post infection), analyses of TNFα show its faster decrease of in infected animal than in uninfected ones. The large intestine of infected rats showed signs of minimal inflammation based on the macroscopical pathological observation after colitis induction. Despite of this, the differences between levels of TNFα gene expression were not significant during acute colitis and, thus, we demonstrated its anti-inflammatory effect on chronic colitis. First, we optimized the model of chronic colitis induced by DNBS in the rat model. This chronic inflammation persisted for 12 days in total. Furthermore, we tested whether *H. diminuta* can ameliorate this chronic colitis. The colitis was again induced in the beginning of patent period (i.e. 21 days post infection). We revealed the significant differences in the intestinal inflammation between control group and rats infected with *H. diminuta*. It may be stated already that commensal tapeworm *H. diminuta*, despite it is localized in host small intestine, is able to ameliorate an acute and also chronic colitis, however, more research needs to be done on this topic.

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ULTRASTRUCTURAL OBSERVATIONS ON REPRESENTATIVES FROM FAMILY DIPLOZOIDAE (MONOGENEA)

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Diplozoons are blood-feeding ectoparasites with specific life-cycle, parasitizing mainly on gills of cyprinid fish, where they can cause mechanical damage to the gill filaments, initiating the development of secondary infections (bacterial, mycotic) and anemia. During the diplozoon life cycle two larvae, the diporpae, pair and subsequently fuse permanently producing the typical X-shaped diplozoon body arrangement, this is a unique character for Diplozoidae. Although these worms have been the subject of numerous taxonomical, phylogenetic, and ecological studies, the detailed studies of their inner systems have remained relatively neglected. This presentation is based in comprehensive study focusing on diplozoids morphology and ultrastructure, looking for phylogenetic and functional indicators of their specific life strategy. The samples of several diplozoon species were collected from the gills of different species of host fish *D. paradoxum* (*A. brama*), *E. nipponicum* (*C. carpio*), *P. homoion* (*A. alburnus*) and *P. bliccae* (*B. bjoerkna*), which were caught in the littoral zone of the Mušov lowland reservoir. The fish gills were extracted and checked for the presence of all diplozoid ontogenetic stages. Parasites intended for transmission electron microscopy (TEM) were washed in freshwater to remove any remaining mucus and then fixed in 2% osmium tetroxide and dehydrated through an ascending acetone series. The dehydrated samples were embedded in Spurr resin. In order to obtain a better orientation in basic diplozoon morphology several semi-thin sections (0.5 µm) of whole worm bodies were cut using a Leica EM UC6i. Sections were stained with toluidine blue. Ultrathin sections were contrasted with uranyl acetate, followed by citrate, and examined using a JEOL JEM-1010 TEM. The presentation focusses on the morphological and ultrastructural features of different body structures such as neodermal surface, gastrointestinal tract, excretory system and attachment clamps.

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THE EFFECT OF HYBRIDIZATION ON MONOGENEAN PARASITES: THE EXPERIMENTAL CROSS-BREEDING OF *ABRAMIS BRAMA* AND *BLICCA BJOERKNA*

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The hybridization in fish is a common phenomenon in nature even the frequency of cyprinid hybrids are often very low. The genetic disruption resulting from hybridization of divergent species may lead to the interruption of the system of the host-parasite co-adaptation. As a result, the hybrids suffer from high parasite load. Monogenean parasites due to their high host specificity and direct life cycle represent the suitable model to analyze the possible impacts of hybridization in fish. The aim of this study was to compare the composition of monogenean communities in pure parental lines and F1 hybrid lines. We focused on the effects of the hybridization and maternal origin of hybrids on the presence and abundance of monogenean parasites. The breed lines of pure *Abramis brama* and *Blicca bjoerkna* as well as F1 generation with different maternal position (either *A. brama* or *B. bjoerkna*) were prepared prior to this experimental study. Specimens of each line were submitted to experimental infection of monogeneans using both parental species as a source of infection. Fish were examined for the presence of monogenean parasites. All monogeneans were identified. Monogenean species richness was higher in hybrids when compared to common bream or silver bream. However, higher monogenean abundance was found in both parental species. The presence of the parental-species specific monogeneans in the hybrids might indicate the interruption of the host-parasite system of co-adapted genes. The monogenean communities in the hybrids with both maternal origins were more similar to the monogenean communities of the silver bream than those of common bream. The results of this study confirmed our previous hypothesis of the different degree of the host-parasite co-adaptation.

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SECRETED SERINE PROTEASE SmSP2 OF THE BLOOD FLUKE *SCHISTOSOMA MANSONI*: BIOCHEMICAL CHARACTERIZATION, LOCALIZATION AND HOST PROTEIN PROCESSING

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Schistosomiasis caused by parasitic blood flukes of the genus Schistosoma is the second most important parasitic infection after malaria with more than 240 million people infected. There is an urgent need to identify novel anti-schistosomal targets for therapeutic interventions. Our work is focused on *S. mansoni* serine protease 2 (SmSP2). It was localized in the tegument and esophageal glands, ovaries, testes and vitelaria of adult schistosomes by immunofluorescence microscopy and in situ RNA hybridization. Enzyme activity measurements and immunoblotting identified SmSP2 in the excretory/secretory products. Recombinant SmSP2 was produced in the *Pichia pastoris* expression system and its cleavage specificity was investigated using combinatorial substrate libraries and 3D model analysis. SmSP2 was found to activate plasmin, the key component of the fibrinolytic system, and releases vasoregulatory kinins from kininogen. Our results suggest that SmSP2 plays a role in host-parasite interactions and represents a potential target for inhibitory drugs.

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THE EXPANSION OF MYELOID CD11b+ Gr-1+ CELLS IN THE PERITONEAL CAVITY AND THE SPLEEN OF MICE INFECTED WITH LARVAL STAGES OF TAPEWORM

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Myeloid-derived suppressor cells (MDSCs) are heterogeneous population of precursors and immature cells in different stages of differentiation. One of the typical characteristics of these cells is the regulation of adaptive immune responses or the suppression of protective immune reaction and effector cells. They are also present in healthy individuals, but these cells rapidly turn into mature myeloid cells and lose the ability to inhibit effector mechanisms in hosts' defense. Currently, suppressive influence of MDSCs is mainly examined in different cancer diseases. However, the role of MDSC during parasitic infections is not fully reviewed. In mice, the population of immature myeloid cells is broadly characterized by the surface expression of CD11b and Gr1 molecules. In the current study, kinetic monitoring of the myeloid cells population in the peritoneal cavity and in the spleen during murine larval cestodiasis was performed. Flow cytometric analysis shows that the population of myeloid $CD11b^+$ $Gr\text{-}1^+$ cells expanded gradually in the course of *Mesocestoides vogae* infection. Further phenotypisation of the $CD11b^+$ $Gr\text{-}1^+$ myeloid cells revealed that they express low levels of F4/80 and MHC class II molecules. Moreover, enhanced concentration of the immunosuppressive cytokine TGF-β in the peritoneal cavity of infected mice has correlated with the accumulation of immature myeloid cells during the infection and with gradual decrease of NO production by these cells. Their suppressive function towards T lymphocytes was confirmed by highly decreased proportion of $CD4^+$ T lymphocytes in the peritoneal cavity and very low levels of IFN-γ as well as by suppressed proliferative index of T lymphocytes in the spleen. After oral infection larvae have been detected in the peritoneal cavity since day 3 post infection and their numbers rapidly elevated due to their asexual reproduction. It is possible that excretory-secretory molecules produced during early stage of infection contributed to rise of the population of immature myeloid cells with suppressive functions.

We assume that the heterogeneous population of immature myeloid cells may contribute to the reduction of immune response and inhibition of effector mechanisms in hosts' defense against metacestodes. Thus, this population of the regulatory cells became a promising target for immunotherapy.

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CERCARIAL DERMATITIS IN HUMANS: A SYSTEMATIC FOLLOW-UP STUDY OF 10 CASES

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Cercarial dermatitis (CD) is an emerging disease caused in temperate climate predominantly by larvae of avian schistosomes which penetrate the skin of mammals. As for humans, the usual clinical symptoms of this allergic reaction include prickling sensation, itching rash, erythema or oedema lasting for several days or weeks after the exposure. Although human CD may in some cases represent a serious health issue, literature data describing the continuous development of clinical symptoms are rather scarce. Hence we present data from a systematic follow-up study, in which we investigated 10 individuals (9 men and 1 woman) after exposure to cercariae of *Trichobilharzia szidati* (Schistosomatidae), a common causative agent of CD. We evaluated the skin symptoms at the cercaria-affected site daily for 3 weeks post exposure (wpe). Moreover, we used initial blood/serum samples and those from 1 and 3 wpe (a) to count the proportion of eosinophils in blood smears and (b) to test the serum reactivity with *T. szidati* cercarial antigens by ELISA and immunoblot. Preliminary data showed different dynamics of clinical symptoms in individuals exposed to the parasites for the first time or repeatedly. Considering eosinophils, a peak in their proportion among peripheral blood leukocytes was noticed 1 wpe in repeatedly exposed individuals. Cercarial antigen-specific IgG raised steadily in all cases, reaching the highest levels in repeatedly exposed individuals. However, no bands recognized specifically on immunoblots by sera from all (or at least a majority) of tested persons were identified. Altogether, we bring a unique dataset describing the development of human CD in terms of clinical symptoms, and basic immunological parameters. Nevertheless, further analyses and more probands are needed to get comprehensive results that would potentially be valuable in medical diagnostics.

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NEW INSIGHTS INTO CELLULAR IMMUNE RESPONSE OF DUCKS AND MICE INFECTED BY *TRICHOBILHARZIA REGENTI*, A NEUROTROPIC SCHISTOSOME

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Trichobilharzia regenti is an avian schistosome that uses anatid birds as definitive hosts. It migrates through their central nervous system (CNS) to the nasal area where it matures, copulates and lays eggs. Accidentally, *T. regenti* can infect mammals, such as mice, in which it can also undergo migration through CNS. However, the parasite development is incomplete, because it dies in CNS after several weeks. In our study, we monitored cellular immune response against *T. regenti* stages localized in the duck nasal mucosa and in the mouse spinal cord. In ducks (*Anas platyrhynchos* f. *domestica*), we focused on characterization of cellular infiltrates in the vicinity of adults, eggs, and miracidia of *T. regenti* in the soft tissue of *concha nasalis*, where these stages are detectable 14-25 days post infection (dpi). In particular, we mapped the presence of lymphocytes (CD4+ and CD8+ cells) and macrophages (KUL01+ cells) using immunohistochemistry (IHC). The nasal tissue started to be infiltrated by CD4+ and KUL01+ cells 16 dpi, but the cells did not accumulate around the adults or the already laid eggs. A similar situation was observed 22 dpi, when the infection reached a peak in terms of the amount of eggs deposited in the tissue; the tissue was seriously damaged and collagen fibers mainly around the clustered eggs were newly formed. However, no specific accumulation of leukocytes was noticed in these granuloma-like structures. In mice (strain C57Bl/6), astrocytes and microglia participate in parasite elimination in CNS. To assess their role in regulation of the immune processes ongoing in the affected nervous tissue, we monitored production of pro-inflammatory interleukin (IL)-17 and anti-inflammatory IL-4 by astrocytes and microglia using IHC. We analyzed the spinal cord samples from mice 7 dpi (early phase of infection), when a heavy inflammation around the migrating worms occurred. Preliminary data show the presence of IL-17 in the proximity of parasites. Localization of the signal correlates with hypertrophy of astrocytes in parallel sections. On the contrary, no IL-4 was seen in the affected sites which corresponds with the inflammatory status of the tissue. We assume that mice cellular immune response is one of the factors responsible for the parasite failure in accidental host CNS, because pro-inflammatory IL-17 and astrocytes accumulated around the parasites were detected. In contrast, in the duck nasal tissue, the immune cells (CD4+ and macrophages) were dispersed throughout the infected tissue. Further experiments clarifying the difference are required.

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INTERESTING ULTRASTRUCTURAL ORGANIZATION OF MALE GAMETES AND THEIR DIFFERENTIATION IN CARYOPHYLLIDEAN CESTODES

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The order Caryophyllidea is presumably the most basal group of tapeworms (Eucestoda). It includes unique tapeworms with respect to their morphology, e.g. absence of body segmentation, one set of reproductive organs. Although many multidisciplinary studies have been done, the key questions regarding the evolutionary history of these cestodes are still a matter of speculation. Ultrastructural data on spermiogenesis and sperm morphology have the potential to contribute significantly to understanding the phylogenetic relationships between cestode parasites. To date, spermiogenesis and spermatozoon ultrastructure have been studied in 9 species of 3 of all 4 existing families of the Caryophyllidea. Cytodifferentiation of spermatids is characterized by the formation of a differentiation zone which contains an apical dense material, the two centrioles associated with striated rootlets and an intercentriolar body between them. Only one of the two centrioles gives rise to a free flagellum that grows externally to median cytoplasmic protrusion. The second centriole is a basis of a lateral flagellar bud. Later on, asynchronic flagellar rotation of both the free flagellum and flagellar bud is followed by proximodistal fusion of the free flagellum and median cytoplasmic protrusion. It is noteworthy that the presence of additional striated root situated in the opposite direction to the typical root occurs in some caryophyllids (*Caryophyllaeus laticeps, Khawia rossittensis*). A similar feature has been reported only in *Diphyllobothrium latum* and may support a presumed sister relationship of Caryophyllidea and Diphyllobothriidea. Another interesting feature of spermiogenesis in caryophyllideans is variability of the number of electrondense plates detected in the intercentriolar body among the examined species (e.g. one in *Khawia armeniaca*, three in *Wenyonia virilis*). The mature spermatozoa of caryophyllideans are filliform cells characterized by one axoneme of the 9+"1" trepaxonematan structure, parallel cortical microtubules and parallel nucleus. Knowledge about the fine structure of male gametes of cestodes is still far from to be complete. Only future ultrastructural studies combined with new techniques like electron tomography may help to elucidate specific questions of reproductive biology and evolution of these fascinating parasites.

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ANACANTHORUS SPP. (MONOGENEA) PARASITIZING THE GILLS OF SERRASALMIDS (CHARACIFORMES) FROM TWO BRAZILIAN RIVER BASINS

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Neotropical region is a highly diverse area for both fishes and their respective parasites, still the extent of its diversity it is far from being well known. *Anacanthorus* Mizelle and Price, 1965 is a highly diversified genus of monogenean parasites in Neotropical fishes, mainly characins. Parasitological surveys of serrasalmid fish (*Myleus schomburgkii*, *Serrasalmus maculatus* and *S. marginatus*) in the Xingu River, a tributary of the Amazon River, Pará, and in the Miranda River, Mato Grosso do Sul revealed the presence of two undescribed species of *Anacanthorus*. Both species of *Anacanthorus* were compared with the previously described species parasitizing serrasalmids and both can be distinguished from their congeners mainly by the copulatory complex. *Anacanthorus* genus is characterized by having anchors modified as 4A hooks (incipient) and a J-shaped male copulatory organ. Currently this genus includes 73 valid species, 37 described within serrasalmids. The records of two new species of this well studied group of monogeneans suggests a high level of speciation and diversification within this genus but further studies are necessary to reinforce this hypothesis. The data obtained herein increases our knowledge on the diversity, distribution and host specificity of the members of this genus.

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HUMORAL RESPONSE OF MICE INFECTED WITH *TOXOCARA CANIS* FOLLOWING DIFFERENT INFECTION SCHEMES

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The study was focused on the dynamics of humoral response to *Toxocara canis* excretory-secretory antigens (TES) antibodies in mice experimentally infected by *T. canis* L3 larvae in different ways. In particular, we compared the effect of infection with two doses of 1000 larvae vs. repeated infections with a low number of larvae (daily infection with 10 larvae, and weekly infection with 100 larvae in the course of 22 weeks). In ELISA, all infections, including both schemes with lower larval doses, elicited significant antibody response. Elevated levels of total IgE and TES-specific IgM were detected on day 12 after the first infection, followed by IgG and IgG1, and later by IgG3, IgG2a and IgG2b; specific IgE response was not detected. It seems that the high levels of IgM and IgG1 represent the best markers of infection. In addition, gradual increase of IgG2a and IgG2b could help in determination of the infection course. As a byproduct of our work, a new method of mouse infection by repeated drinking of larvae was introduced; it minimizes the pain and discomfort for the experimental mice.

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FIRST DATA ON THE HELMINTH FAUNA OF THE EUROPEAN OTTER *LUTRA LUTRA* (LINNAEUS, 1758) IN LITHUANIA

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The European otter *Lutra lutra* (Linnaeus, 1758) is a semi-aquatic mustelid belonging to subfamily Lutrinae. During the last century otter populations sharply declined all over the world. Since 2004 otter is listed as "Near Threatened" by the IUCN Red List. It could be the main reason why parasitofauna of wild otter populations are poorly understood. From the parasitological aspect, otters also play an important role in the life cycles of parasites associated with aquatic environment.

Currently European otter population is widely distributed across Lithuania (covered 95 % territory); however, no helminths were previously reported. The aim of this study was to explore helminth fauna of road-killed otters in Lithuania for the first time. Helminth fauna was compared with results from other European countries.

Trematodes *Pseudamphistomum truncatum, Isthmiophora melis, Phyllodistomum folium* (fish parasite), and one unidentified nematode species were found.

SPECIFIC RESPONSE OF NEMATODA COMMUNITY TO DIFFERENT MANAGEMENT OF DECIDUOUS FOREST AFFECTED BY WINDSTORM

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Windstorm is significant and key disturbance factor in most natural (unmanaged) forests, affecting not only trees and stands but also changing soil biota and initiating ground-layer successions. Ecologists have thus long studied the effects of windstorms of different extents, durations, intensities, or sizes on forest ecosystems, their dynamics, and rates of recovery to their original structure solely by natural succession. The windstorm that occurred in Slovakia in May 2014 damaged productive beech deciduous forests in many localities throughout the country. Our study was carried out at two localities, one with all fallen trees removed from the damaged area (Opátka), second within the protected landscape area (Vysoký vrch) with all fallen trees left for their natural succession. At both localities, the control plots have been chosen in the vital beech forest undamaged by the windstorm. The main aim of the study was to investigate which management practice would be better in the long-term ecosystem regeneration, using analysis of soil nematode communities. Nematodes, small soil inhabitants, are frequently used as indicators of ecosystem health as well as changes in the ecosystems caused by natural or anthropogenic disturbances. The soil samples for nematode faunal analysis were collected a year after windstorm event from all investigated plots (two damaged and two controls). The nematode communities negatively answered to fallen trees removing (clearing) on the windstorm damaged plot by decreasing of their total abundance as well as population densities of all feeding groups in comparison to control. Contrary to that, on the windstorm targeted plots where damaged beech trees were left for their natural succession, the nematode abundance increased markedly. It was reflected by higher population densities of bacterivores, obligate and facultative plant parasites or fungivores. Omnivores and predators, which are considered to be the most sensitive nematodes to ecosystem changes, answered differently; while predators decreased, the omnivore remained unchanged on the damaged plots in comparison to control. Moreover, changes in the nematode communities leads to changes of values of nematode ecological and functional indices, confirming that soil nematode communities are applicable indicators when ecosystems are injured by various natural or anthropogenic factors.

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HOST-PARASITE COEVOLUTION IN THE DIPLOID-POLYPLOID COMPLEX OF *CARASSIUS AURATUS*

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Carassius auratus complex represent the complex of forms (or subspecies) non-indigenous for the waters of Czech Republic. Based on the mtDNA genotyping, this complex is composed of four forms (or subspecies): *Carassius auratus auratus*, *Carassius auratus gibelio*, *Carassius auratus langsdorfii* and *Carassius auratus* M-line. Among them, *Carassius auratus gibelio* represent the most common form occurring recently in Czech Republic. The combination of gynogenetic and sexual reproduction in this species represent very successful life strategy. This invasive species exhibits a high degree of ecological tolerance and adaptability. The members of *Carassius auratus* complex entered to the waters of the Czech Republic from the Danube River through the Slovak-Austrian part of the Morava River in 1976. In the present study, we focused on metazoan parasite communities of *Carassius auratus gibelio*. During a long-termed study (four-year investigation of metazoan parasite communities) we analysed the dynamics of host-parasite interactions in diploid and triploid form of gibel carp. Our analyses revealed similar values for parasite infection in gynogenetic females and sexual individuals. The maximum prevalence and the highest intensity of infection were found for the following helminth species: *Dactylogyrus dulkeiti*, *Dactylogyrus anchoratus*, *Gyrodactylus sprostonae* (Monogenea), and *Schulmanela petruschewskii* (Nematoda) in both forms of gibel carp. However, our study revealed the temporal variation in parasite load in both forms which could be potentially explained by the dynamics of host-parasite interactions predicted by Red Queen hypothesis.

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EFFECT OF NITRIC OXIDE AND HYDROGEN PEROXIDE ON AVIAN SCHISTOSOMES *TRICHOBILHARZIA REGENTI* AND *TRICHOBILHARZIA SZIDATI*

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Production of reactive oxygen and nitrogen species by vertebrate immune cells is an important mechanism in elimination of pathogens. In helminth infections, the particular role of these molecules remains unclear or conflicting. Thus, we assessed the effect of NO donors (molecules releasing nitric oxide) and hydrogen peroxide (H_2O_2) on two closely related avian schistosomes: neurotropic *Trichobilharzia regenti* and viscerotropic *T. szidati*. First, we evaluated the dynamics of nitric oxide (NO) production by two NO donors (DETA NONOate and NOR-5) using Griess reaction. Steady release of NO was observed only in case of NOR-5. Afterwards, *in vitro* transformed schistosomula of *T. regenti* and *T. szidati* were incubated in schistosome culture medium (SCM) supplemented by 0.1 mM and 0.5 mM NOR-5 for 24 h and 48 h. No significant effect of the released NO on parasite mortality (examined by methylene blue staining) was observed. Furthermore, scanning electron microscopy (SEM) revealed no evident alterations in surface morphology when compared to untreated controls. Second, *in vitro* transformed *T. regenti* schistosomula were exposed to 1.5 mM solution of H_2O_2 for 15 minutes, and then cultivated in SCM. The exposure resulted in average mortality rate of 42 % in 24 h. One hour post-exposure (p.e.), undamaged schistosomula, schistosomula with blebs on the surface and schistosomula with uneven surface and no spines were observed by SEM. Oxidatively damaged proteins were localized immunohistochemically in muscle fibers by antibody against dinitrophenol 1 h p.e. Immobilization and shrinkage of schistosomula body were detected 4 h p.e. One day p.e., no ultrastructural alterations were detected on the surface (SEM), in the tegument, mitochondria, and muscles (transmission electron microscopy) of surviving schistosomula. These results suggest that NO itself is not toxic to the *in vitro* transformed schistosomula of *T. regenti* and *T. szidati*. On the contrary, H_2O_2 causes morphological changes under *in vitro* conditions that are usually fatal for *T. regenti* schistosomula.

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HELMITHS EGGS VIABILITY IN THE COURSE OF LONG-TERM STORAGE OF PIG SLURRY

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The model bacterial strains (*Salmonella typhimurium*) and helminths eggs (*Ascaris suum*) survival in the course of long-term storage of pig slurry was investigated. The experiments were carried out on the raw pig slurry stored for 115 days in closed plastic containers at the temperatures 4°C, 20°C and 42°C. The initial concentration of the tested *S. typhimurium* strain (3.6×10^5 CFU.ml^{-1}) in pig slurry stored at 4°C decreased by day 90 by three orders of magnitude and on day 115 of storage the test strain was no more recovered. The tested strain survived in slurry for less than 115 days at 20°C. The most marked decrease in plate counts of bacteria was recorded in pig slurry stored at 42°C. Our results showed decreased survival of *S. typhimurium* in pig slurry during storage at 20° and 42°C. This indicated that viability of bacteria in stored pig slurry was affected first of all by the temperature during the storage. The number of devitalised *Ascaris* eggs generally increased with the length of storage and the temperature. Considerable number of *Ascaris* eggs remained viable even after 115 days of storage at 4° and 20°C. Only at 42°C more than 90 % of eggs were devitalised after 12 days of storage. However, such temperature can only rarely be reached in animal slurries, thus the risk of persistence of this parasite is really high. This hazard increases when raw slurry is used for fertilization of soil or pastures.

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HELMINTH PARASITES OF WILD MUSTELIDS (FAMILY: MUSTELIDAE) IN LITHUANIA.

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The present study analyses the helminth fauna found in members of family Mustelidae. A total of 132 mustelids: 26 stone martens (*Martes foina*), 20 pine martens (*Martes martes*), 53 American minks (*Neovison vison*), 26 European polecat (*Mustela putorius*), 5 European otters (*Lutra lutra*) and 2 Eurasian badgers (*Meles meles*) were examined using a total helmintological examination of individual organs. Morhological and molecular analysis have been used for identification of helminth species. More than 12 taxa of parasitic worms belonging to phyla Nematoda and Platyhelmithes (classes Trematoda and Cestoda) were found.

A checklist of helminth species detected in mustelids worlwide, routes of infection, pathogenicity, geographic distribution, and distribution in host species of helminths is discused.

HELMINTH INFECTIONS IN TWO LOCALITIES WITH DIFFERENT SANITATION AND HYGIENE STANDARDS IN SLOVAKIA

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The aim of this study was to determine and compare the occurrence of helminth infections among dogs, children and soil found in two neighboring localities, based on different levels of sanitation and hygiene standards and socio-economic conditions of the population. Helminth infections were present in both study areas, but in the village with a higher standard of living of the population, better personal and communal hygiene levels and greater care of the dogs, a lower occurrence of helminth eggs among dogs, children and soil was recorded. Out of 199 examined dogs' feces samples 52.76 % of them contained parasitic germs. In 127 samples from the village A with low sanitation and hygiene standards 12 different species of intestinal parasites were detected. In particular the eggs from family Ancylostomatidae (50.39 %), *Ascaris* spp. eggs (40.94 %), *Toxocara canis* eggs (11.02 %), eggs of *Toxascaris leonina* (9.45 %), *Giardia duodenalis* cysts (9.45 %), oocysts of *Isospora* spp. (6.30 %), *Capillaria aerophila* (3.94 %), *Trichuris vulpis* eggs (2.36 %), *Taenia* type eggs (0.79 %), *Dipylidium caninum* (0.79 %), *Angiostrongylus vasorum* larvae (0.79 %) and *Sarcocystis* spp. (0.79 %) were present. In village B with high sanitation and hygiene standards the only 4 species of parasites in 72 dogs' excrements were documented – the eggs of *T. leonina* (8.33%), *T.canis* (8.33 %), *T. vulpis* (1.39 %) and *Sarcocystis* spp. oo/sporocysts (2.78 %). High prevalence of the endoparasitic developmental stages in dogs' feces can present a risk factor for the soil contamination, and consequently to the population living in such locality. For this reason, the incidence of helminths eggs in the soil was also examined. The soil samples from the village A were found to be more contaminated than samples from village B. In village A, 65.63 % prevalence of the parasites in soil were recorded, compared with village B in which 18.18 % positivity was observed. The overall infection prevalence in children in village A was 39.35%. The most prevalent parasite was *Ascaris lumbricoides*. No children from the village B were positive for the presence of parasite.

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CICHLIDOGYRUS SPP. (MONOGENEA: DACTYLOGYRIDAE) COMMUNITIES OF CICHLIDS FROM THE CONGOLESE LAKESHORE OF LAKE TANGANYIKA: NEW MONOGENEAN SPECIES, FISH HOST AND DISTRIBUTIONAL RECORDS

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In summer 2013 and 2016, a total of 148 cichlid individuals belonging to 28 Tanganyikan species were collected from the Congolese lakeshore and examined for gill ectoparasites. Morphological and molecular identifications of the gill monogeneans were performed, and phylogenetic analyses were carried out by employing different molecular markers (28S rDNA, 18S rDNA and ITS-1). The examination of fish gills revealed the presence of 191 monogenean specimens and allowed us to recognize 24 Congolese *Cichlidogyrus* species, of which 14 are new for science. Our investigation represents the first parasitological examination of representatives belonging to the Tanganyikan cichlid genera *Callochromis*, *Cyphotilapia*, and *Xenotilapia*. Likewise, a few *Cichlidogyrus* species have already been recognized on the gills of *Petrochromis* spp., but no formal description has been provided yet. Our study allowed us to record 7 new *Cichlidogyrus* species on specimens of *Petrochromis*. Recent studies on the parasite diversity of *Ophthalmotilapia* spp. illustrated a remarkable biogeographical diversity among their monogenean communities along the lake. Four *Cichlidogyrus* species previously recognized on various species of *Ophthalmotilapia* inhabiting several Tanganyikan localities were found on the gills of the representative host studied. They are three new currently undescribed Burundese *Cichlidogyrus* species in addition to *C. sturmbaueri* previously found on Zambian *O. ventralis* and Tanzanian *O. nasuta*. Further, Congolese *Aulonocranus dewindti* hosted two new *Cichlidogyrus* species exhibiting similar morphotypes with two other species found on the same host from the Burundese lakeshore. Contrariwise, a new locality record is reported for a new undescribed *Cichlidogyrus* identified on *Cyprichromis microlepidotus*. *Cichlidogyrus* specimens sampled from the gills of *Tylochromis polylepis* represent *C. mulimbwai*, *C. muzumanii* and *C.* sp. "*T. polylepis 3*". We provide a morphological characterization of the hard parts of haptor and reproductive organs of this last species. Finally, *Pseudosimochromis babaulti*, *P. curvifrons* and *Simochromis marginatus* were parasitized by *C. frankwillemsi*, *C. franswittei* and *C. georgesmertensi* as previously reported. However, *P. babaulti*, in addition to *C. georgesmertensi*, hosted also *C. franswittei*, a species known to infect the gills of *P. curvifrons* and *S. marginatus*. Morphologically similar new *Cichlidogyrus* species mirror the relatedness among their cichlid hosts. The geographic intraspecific variation of Tanganyikan cichlid hosts plays probably a role in their *Cichlidogyrus* speciation and distribution.

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EFFECTS OF ALIEN PLANT INVASION ON BEHAVIOUR OF NEMATODA COMMUNITY IN DIFFERENT TYPES OF ECOSYSTEMS

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Abstract

Invasive plant species threaten biodiversity and ecosystem processes and can change community compositions and abundance of native species of both plants and animals including soil-dwelling taxa. Knowledge of the main effects of plant invasions on soil microfauna is essential for a better understanding of the invasion processes. One of the most invasive plants in Europe belongs into the genus *Heracleum.* Two of them, *H. mantegazzianum* and *H. sosnowskyi* are considered to be the most tricky and dangerous invasive plant species nowadays. In our study we analyzed how the soil nematode communities answered to *H. sosnowskyi (Hs*) invasion in four different habitats (wet damp ground depression, line between two agricultural fields, roadside edges inside agricultural farm; abandoned alluvial meadow) without human interference. *Hs* invaded and uninvaded (control) plots have been chosen for soil sample collection and nematode analysis in all habitats. We hypothesized that changes in nematode communities would depend not only on *Hs* invasion, but also on type of habitat where *Hs* community has developed. The results confirmed our hypothesis. In the wet damp ground depression *Hs* did not expand to become dominant species, despite the fact that the first specimen occurred there ten years ago, probably due to presence of a stronger competitor *Phragmites australis*. No differences in the nematode community structures, abundance, diversity and nematode feeding strategy between *Hs* invaded and uninvaded plots have been found. Contrary to that, in all other habitats *Hs* created monoculture, became dominant and negatively correlated with native plant species during 15-35 years of its existence. The changes in the soil nematode communities are apparent, but only fungivores (Fu_2) and plant parasites (Pp_3) decreased significantly under *Hs* invasion in all three habitats ($P<0.01$; $P<0.05$). Population densities of bacterivores decreased in the *Hs* invaded route edges, increased in the *Hs* wet alluvial meadow but remained similar in the *Hs* line between fields in comparison to related uninvaded controls. The stress sensitive omnivores significantly decreased in the *Hs* invaded route edge and line between fields ($P<0.05$). Factorial analysis of variance confirmed significant main interaction among habitat type (four types), invasion status (*Hs* invaded, uninvaded) and values of nematode community indices and nematode abundance. Our results indicated that significant changes in the herbaceous layer after *Hs* invasion at habitats where *Hs* become dominant have had important impact on soil-nematode communities and that habitat characteristics did affect *Hs* establishment.

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xMAP TECHNOLOGY: DETECTION OF PARASITIC AGENTS

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Parasitic zoonoses are recorded worldwide and some of them have endemic character. Parasitic agents may pass from animals to humans in several ways, e.g., by direct contact, vector, consumption of raw or undercooked foodstuffs containing the infective stages or by infective stages released into environment. Although in the last decades a number of novel diagnostic methodological approaches have been developed, the current diagnosis of some parasitic diseases is still based only on a combination of clinical signs, anamnesis, and direct visual identification of parasitological objects. The most common conventional diagnostic methods, such as microscopic examination, biochemical assays, ELISA or PCR, are available, but they are laborious, time-consuming and in many cases not reliable. With regard to the fact that parasites might exhibit very strictly confined localization within the host's body – intracellular/extracellular or tissue/organ, sampling can be very problematic and it often leads to a false negative results. Therefore, there is niche for the development of more sensitive diagnostic assays.

Microsphere-based assays utilizing xMAP technology are applicable for high-throughput, multiplex and simultaneous detection of different analytes within a single complex sample. xMAP multiplex assays are currently available in various nucleic acid and immunoassay formats, enabling simultaneous detection and typing of pathogenic agents and also antigen or antibody interception. As an open architecture platform, the xMAP technology is beneficial to end users and therefore it is used in various pharmaceutical, clinical and research laboratories.

The aim of our research is to exploit a potential of this approach to develop a multiplex oligonucleotide ligation PCR assay (MOL-PCR) determined for detection of nucleic acids in diagnosis of intestinal parasites in areas where co-infections are common and to upgrade conventional singleplex ELISA into the multiplex level in case of parasites with the ability to migrate through the tissues of the host's body.

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CURRENT SITUATION OF *TRICHINELLA* SPP. IN ARGENTINA AND SOUTH AMERICA. NEW SPECIES AND SCENARIOS.

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Trichinellosis is a meat borne zoonotic disease in humans caused by nematodes of the genus *Trichinella*. *T. spiralis* is typically found in the domestic cycle that includes pigs, humans and rodents. *Trichinella* spp. from a sylvatic cycle has also caused human outbreaks resulting from the consumption of meat from puma, armadillo and wild boar in South America. *Trichinella* infection has been reported in both humans and animals in USA, Canada, Greenland, Argentina, Chile, Bolivia, Ecuador and Mexico. Seven sources of infection have been identified in America: *T. spiralis, T. nativa, T.* T6, *T. murrelli, T. pseudospiralis, T. patagoniensis* and *T. britovi.* This parasitic infection is still a public health problem and affected people present different unspecific signs: fever, facial and periorbital edema, headache, muscle pain, eosinophilia and in some cases a macro-papular rash and subungal hemorraghes. *T. patagoniensis* is widespread in Argentina, with the cougar (*Puma concolor*) serving as a natural reservoir. Recently, it was shown that cats and mice are more susceptible to the infection of *T. patagoniensis* than rats and pigs. The transmammary route of infection in BALB/c mice does not play an important role in the transmission of *T. patagoniensis*. Moreover, it was demonstrated that, although this parasite can reach the adult stage, it is not able to complete its life cycle in chickens (*Gallus gallus domesticus*). *T. patagoniensis* is able to complete its life cycle in guinea pigs (*Cavia porcellus*), and thus, they could act as potential hosts for this parasite. Although *Trichinella* genus produce human disease and outbreaks, nowadays, the zoonotic role of *T. patagoniensis* has not been confirmed by laboratory diagnosis. At the domestic cycle, many pigs are raised outdoors and *Trichinella* infection is endemic. Based on the reports available, it is clear that there is a need to carry out better controlled epidemiological studies to determine the prevalence of *Trichinella* spp in domestic and wild animals in this region of the world. For these reasons it is necessary to have available effective detection methods for *Trichinella* infection in pigs to avoid transmission to humans. With the objective of controlling the increase in the incidence of human trichinellosis, methods for detection in pigs at slaughterhouses have undergone important changes. Recently, there was an interest to improve programs that provides documentation of pork production management practices that minimize the risk of exposure of pigs to the parasite. These programs are based on a complete knowledge of risk factors associated with pig infection. The principles of food safety risk analysis should be incorporated wherever possible and appropriate in the design and implementation of meat hygiene programmes.

SERPIN, A KEY MOLECULE IN THE LIFE OF *EUDIPLOZOON NIPPONICUM*?!

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Eudiplozoon nipponicum (family Diplozoidae, Polyopisthocotylea) is hematophagous ectoparasite from the gills of common carp (*Cyprinus carpio*). The properties of proteins (e.g. functions) of the members from the family Monogenea are among the less investigated in whole phylum Platyhelminthes. During the previous experimental work of our colleagues, we obtained data concerning the important functional molecules produced by *E. nipponicum* (e.g. endopeptidases cathepsins L, B and D). Some of them occurred in excretory-secretory products, which indicates their possible participation in host-parasite interaction and some of them were proved to be part of feeding mechanisms. The main aim of our current work is to understand the regulation related to peptidase activity; the peptidase protein inhibitors, such as also serpins, could play a key role in this process. Serpins of vertebrates are known as important regulators of the coagulation cascade, complement, fibrinolysis, angiogenesis, inflammation etc. They possess typical conserved domains and a reactive central loop binding to the active site of peptidase. The inhibition, mediated by serpins, is typically irreversible, comprising conformational changes in serpin molecule leading to distortion of peptidase tertiary structure. Except the inhibition, some of them, may have other functions like e.g. protein transporters or chaperones and they are relatively abundant also in secretions, body lysates and genomes/transcriptomes of helminths. We identified serpin gene/protein in transcriptome/proteome of *E. nipponicum*, subsequently it was prepared in recombinant form and in regard to reveal its functions molecular and biochemical characterization was performed (e.g. prediction of tertiary structure, antigenic properties evaluation, measurement of inhibitory effect).

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STRUCTURAL BASIS FOR VINYL SULFONE INHIBITION OF THE SmCB1 DRUG TARGET FROM THE HUMAN BLOOD FLUKE

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Schistosomiasis caused by parasitic blood flukes of the genus Schistosoma afflicts over 240 million people worldwide. *Schistosoma mansoni* cathepsin B1 (SmCB1) is a gut-associated peptidase that digests host blood proteins as a source of nutrients. In our recent work we demonstrated that SmCB1 is a drug target for vinyl sulfone peptidomimetic inhibitors. Now we performed a detailed analysis with a unique set of 30 vinyl sulfone derivatives with diverse substituents. The inhibitors were screened *in vitro* against recombinant SmCB1 and *ex vivo* against *S. mansoni*. Two most effective inhibitors in terms of IC_{50} values and parasite suppression were complexed with SmCB1, and high resolution crystal structures were determined. Analysis of 3D structures and inhibition profiling identify key binding interactions and provide insight into SmCB1 inhibition specificity. Our work provides a footing for the rational design of anti-schistosomal chemotherapeutics.

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PREVALENCE OF *DIROFILARIA REPENS* IN DOGS FROM LITHUANIA

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Canine dirofilariasis cased by *Dirofilaria repens* and D. *immitis* during the past decade has become recognized worldwide as an emerging parasitic disease. The climate change, extensive movement of dogs across countries and continents has contributed to the expanding of distribution range of *D. repens* to the areas they had never been found before.

In this study blood samples of 2180 randomly selected dogs presented in small animal clinic during 2014-2015 were investigated for filarial parasites. The microfilariae were determined on the basis of their morphometrical characteristics and the Diff-quick staining technique applied to blood smears and using Modified Knott's test. For accurate identification of the filarial species we also used PCR with pan-filarial primers that amplify fragments of ITS-2 region of rDNA from six different filariae species and species specific primers for amplification of COI gene of *D. repens*. Microfilariae were detected in blood smears of 42 (1.9%) dogs. In four of dogs the adult worms were removed by using surgery technique. Adults worm were identified as *D. repens* based on the morphological and morphometric characteristics and PCR analysis. Sequence analysis confirmed *D. repens* in all PCR positive samples. Additionally, blood samples collected from 101 shelter dogs were investigated for filarial parasites using PCR. *D. repens* was detected in 18.9% (19/101) of tested dogs.

GENOTYPING OF EIGHT *TRICHINELLA* SPECIES USING HIGH RESOLUTION MELTING ANALYSIS

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Nematodes of the genus *Trichinella* are worldwide distributed foodborne parasites with the high impact on public health and animal trade. They invade small intestine cells and skeletal muscles of wide range of mammals, birds and reptiles. *Trichinella* spp. are causative agents of human trichinellosis, a serious disease which has been documented in 55 countries with an average incidence of approximately ten-thousand cases per year. According to ability of muscle larvae to form a collagen capsule, within the genus *Trichinella* two main clades are recognized. The "encapsulated clade" contains six species (T1 - *Trichinella spiralis*, T2 - *T. nativa*, T3 - *T. britovi*, T5 - *T. murrelli*, T7 - *T. nelsoni*, T12 - *T. patagoniensis*) and three additional taxonomically undefined genotypes (T6, T8 and T9). "Non-encapsulated clade" includes three species (T4 - *T. pseudospiralis*, T10 - *T. papuae* and T11 - *T. zimbabwensis*). Despite the difference in capsule formation and some size differences, the species and genotypes of all developmental stages of these parasites are morphologically indistinguishable. Therefore, we have developed a high resolution melting analysis (HRMA) for the unequivocal differentiation of eight *Trichinella* species (T1, T2, T3, T4, T5, T7, T10 and T11). The assay was based on the mitochondrial cytochrome c oxidase subunit I (COI) gene, representing the variable part of DNA sequence which shows high sequence divergence even among closely allied species. The genomic DNA of a single muscle larva was used as a template in qPCR, the polymorphic region of the COI gene was amplified and the obtained amplicons were analysed for species-specific HRMA curves.

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CONCOMITANT PREDATION ON PARASITES AND HOSTS AFFECT TREMATODE TRANSMISSION SUCCESS

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Predation on trematode parasites has important consequences for food web structure as it reduces parasite biomass. In particular, concomitant predation, which occurs when parasites are consumed and digested along with their hosts, is suggested to be the most significant source of parasite mortality and reduction of population size as it prevents the successful completion of parasite life cycle. However, the significance of such predator-prey/parasite relationship remains to be tested. The main aim of this study was to evaluate the possible effect of concomitant predation on trematode populations and transmission success based on field data from the examination of "free-living" snails (*Radix balthica*), i.e. taken from the lake, and "predated" snails, i.e. consumed by the predator fishes arctic charr (*Salvelinus alpinus*) and brown trout (*Salmo trutta*). Altogether, 632 "free-living" snails were collected from the freshwater subarctic lake Takvatn in Norway and 323 "predated" snails with mostly intact shells, were retrieved from stomach and intestine contents of 12 snail-eating fishes (five charr and seven trout) during two sampling periods in August and October 2016. All snails were measured and examined for trematode infections. To elucidate the trematode biomass loss via predation by fish upon snail hosts, we examined snail densities in fish and infection levels in "free-living" and "predated" snails, respectively. This showed that the overall trematode prevalence in "predated" snails was almost three times higher than in "free-living" snails in August (64% *vs* 24%), whereas much smaller in October (2% *vs* 21%). Furthermore, our data show that larger fish prey upon larger snails and infected snails are larger than uninfected in both snail groups. This indicates that under certain circumstances an important proportion of trematode population might be excluded from the ecosystem, and concomitant predation hence significantly constrains the principal role of intermediate parasite stages, i.e. to infect the next target host in the life cycle. Together, this study contributes to our understanding of parasite transmission and the role of non-host predator species for trematode infection patterns, biomass loss and thus energy flow in aquatic food webs.

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IS STRONGYLOIDIASIS ENDEMIC IN THE CZECH REPUBLIC AND SLOVAKIA?

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Strongyloidiasis is caused by the rounwormes *Strongyloides stercoralis* and *S. fuelleborni* living in the duodenum and jejunum of humans and some animals (dogs, cats, primates). The females are excreting microscopic larvae with stool. In soil in a warm climate, the cycle of the freely living stages of the parasite may take place. Humans are infected either percutaneously with invasive filariform larvae in soil, or by the alimentary route after ingestion of contaminated food or water. The infection is highly endemic in tropics and around 100 million people may by infected worldwide. Almost one half of infections of immunocompetent persons proceed asymptomatically. In untreated patients, infection may last for decades by autoinfection. The larvae which hatch in the intestine are invasive, so they penetrate either through the intestinal wall, or through the skin in the perianal region into blood, where they migrate through the lungs back to the digestive tract and mature into adult worms. Immunosupression (treatment with corticosteroids, cytostatics etc.) may lead to hyperinfection and severe complications. Disseminated strongyloidiasis is manifested by gastrointestinal (abdominal pain, diarrhoea, malabsorption, weight loss), respiratory (dry coughing, wheezing, infiltrates or nodules on the chest X-ray), cutaneous (larva currens), liver and brain lesions. Fever and eosinophilia are presented.
In Europe, sporadic transmission was observed in some temperate countries (Italy, Spain, France) but in Central Europe it occurs very rarely. There were only a few case reports on autochthonous infections originated from Switzerland, Germany and eastern Slovakia in the literature during last three decades.
There were treated five patients with strongyloidiasis in the Department of Infectious Diseases, Hospital Na Bulovce in Prague since 2008. Two infections were imported from tropics (Africa, Thailand) but three cases originated in Central Europe and all three patients never travelled to tropics or subtropics. 8-year-old girl from Subcarpathian Ruthenia (Ukraine) but living in Prague had uncomplicated intestinal strongyloidiasis. Two male immunocompromised patients presented with disseminated infection. A 67-year-old man born in eastern Slovakia but living in the Central Bohemia was investigated for diarrhoea. Due to signs of colitis gastroenterologists initiated treatment with sulfasalazine. However, in the stool samples, there were later detected *Strongyloides* larvae. Treatment with a single dose of 12-mg ivermectine was successful. Further 84-year-old immunocompromised patient with lymphoma living in Prague was diagnosed with disseminated strongyloidiasis from duodenal biopsy and stool microscopy. One-week treatment with albendazole (400 mg/day) was unsuccessful, but after two 12-mg doses of ivermectine larvae from stool disappeared. Strongyloidiasis represents a neglected soil-transmitted helminthic infection that can be rarely diagnosed in the Central Europe. More detailed information on parasite distribution and risks of zoonotic transmission are needed.

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MASS SPECTROMETRY IDENTIFICATION OF ANTIGENIC PROTEINS OF *HYMENOLEPIS DIMINUTA* (CESTODA, HYMENOLEPIDIDAE) CYSTICERCOID

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Hymenolepis diminuta is a parasite of rodents small intestine (mostly mice and rats) but occasionally may infect humans. Cysticercoid, an invasive stage of this tapeworm, gets into final host body through the consumption of infected insects or water/food contaminated with infected stage. The cysticercoid is therefore an important model in understanding the adaptations and mechanisms involved at the early stage of invasion. Proteomic methods help in finding such adaptations to a parasitic lifestyle and explaining complex interrelationships between parasite and its host. In our study, we present the first proteomic analysis of the *H. diminuta* antigenic proteins of cysticercoid by using two-dimensional gel electrophoresis (2 DE) combined with 2D-immunoblotting and LC-MS/MS (liquid chromatography mass spectrometry).

H. diminuta cysticercoids were isolated from *Tribolium castaneum* beeatles. To identify immunoreactive proteins specifically recognized by anti-*Hymenolepis* antibodies, protein extract was subject to SDS-PAGE and immunoblot with serum samples collected from rats infected with *H. diminuta*. LC-MS/MS analysis enabled identification of the cysticercoid proteins presents in immunoreactive spots. The identified proteins were categorized according to their functions with the use of the UniProtKB database.

Forty-two spots were selected for final identification by LC-MS/MS. We identified 70 proteins with antigenic potential, all these proteins were identified by their homologies with the proteins of other Cestode species as there are no genome nor proteome of *H. diminuta* available. Several proteins were identified from multiple spots. Numerous spots contained more than one protein. Proteins that appeared most frequently in spots are: actin cytoplasmic 2 (14 spots), hypothetical transcript (12 spots), procollagen lysine 2 oxoglutarate 5 dioxygenase (8 spots) and type II collagen B (16 spots). Among the identified molecules we noticed also these known from their immunomodulatory properties (HSP60, HSP70, sHSP, paramyosin, calpain). Selected proteins are engaged in key metabolic processes. The number of identified proteins were reported for the first time as potential antigens in cestode larval stage. These proteins were classified according to their Gene Ontology (GO) to molecular function, cellular components and biological processes.

Identification of antigenic proteins of *Hymenolepis diminuta* cysticercoid stage is important for better understanding of the tapeworms biology, development, their adaptations to the parasitic way of life and host-parasite interactions. Our results indicate molecules that may help to establish successful invasion and play a crucial role in host – parasite interplay.

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NEMATODES AS BIOINDICATORS OF SOIL ECOSYSTEM CONTAMINATION

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Better understanding of interactions among belowground and aboveground components in biotopes may improve our knowledge about soil ecosystem, and is necessary in environment assessment using indigenous soil organisms. In this study, we proposed that in disturbed biotopes, vegetation play important role in the buffering of contamination impact on soil communities and decrease the ecological pressure on soil biota. To assess the effects of these interactions we compared nematode communities, known for their bioindication abilities, from four types of disturbed and undisturbed biotopes (coniferous forest, permanent grassland, agricultural field, clearings), where the main stress agent was represented by long-term acidic industrial emissions containing heavy metals (As, Cd, Cu, and Pb). To understand the ecological interactions taking place in studied biotopes, we studied abiotic factors (soil properties) and biotic factors (vegetation, nematode communities). Except significant increase in metals total and mobile concentrations in disturbed biotopes soil, we found acidification of soil horizon, mainly in the clearings (pH = 3.68), due to SO_2 precipitation from emissions. These factors has caused in clearings degradation of native phytocoenoses and decrease in decomposition rate characterized by high amount of organic matter (C_{ox} = 4.29%). Nematodes reacts to these conditions by shifts in trophic structure (bacteriovores to fungal feeders), increase in c-p 2 genera (e.g. *Aphelenchoides*, *Acrobeloides*, and *Cephalobus*), absence of sensitive groups (c-p 3–5, omnivores, predators), and decrease in ecological indices (SI, MI, MI2–5, H′). Similar contamination was found in forest biotope, but the nematodes composition indicates more suitable conditions; more complex community structure (presence of sensitive trophic and higher c-p groups), higher abundance and indices values, comparable with less stressed field biotope. As showed our results, the vegetation undoubtedly plays an important role not only as a resource of services indispensable for the ecosystem, but also as a significant buffer of negative impacts acting within.

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ENDOPARASITIC HELMINTHS OF LOACHES OF THE GENUS *COBITIS* FROM SLOVAKIA

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Parasitofauna of fishes from genus *Cobitis*, which is not commercial fish species, is little known. However, are very interesting for zoologist and ecologist, because the complex character of genotypes in different biotypes of *Cobitis* makes possible to test The Red Queen hypothesis. In Central Europe, approximately 17 endohelmith species as adults or larval stages in genus *Cobitis* were found.
Our study was focused to the endohelminths and their relationships with loaches biology. Altogether, 213 specimens were sampled at the localities of the rivers Latorica, Okna and Ondava by point sampling method within the years 2015-2016. General epizootological indices of found parasites were assessed. In all investigated localities, poor parasite diversity was detected. Prevalence of *Allocreadium transversale* Rudolphi, 1802 (Digenea, Allocreadiidae) was from 23.8 to 79.4 % at different localities. Range of mean intensities from different localities of *A. transversale* was 1-2.6. Metacercariae of *Clinostomum complanatum*, nematodes and acanthocephalans were satelite species. At only one locality on Okna river in september 2016, metacercariae of *C. complanatum* Rudolphi, 1814 (Digenea, Clinostomidae) were observed in high prevalence (68,4%) with intensity of infection 1-13. The relation between intensity of dominant parasites and standard length of fish at all localities was statistically significant. The number of worms increased with the length of loaches. Intensity of infection was the same in fish with different ploidy, despite the differences in morphology and physiology recorded. A more comprehensive survey is necessary to determine the actual threat of the parasitoses to endangered species of the Cobitidae family.

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AN OVERVIEW OF TREATMENTS OF ORNAMENTAL FISH HELMINTHOSES

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Certainly one of the most commonly asked questions concerning the practice of ornamental fish medicine is, "what drug should I use and what's the dose?". An uncomplicated question with a complex and frequently ambiguous answer. The problem, of course, lies in the lack of sound pharmacokinetic data available and the overwhelming number of species involved. Very little research related to pharmacology has been reported in aquarium fishes. The majority of the current information on chemotherapeutics used in aquarium fish has been extrapolated from the finfish aquaculture literature. When environmental differences such as temperature, pH and water hardness are tossed into the equation, selecting a drug and dosing regimen becomes even less objective. In aquarium conditions, the most common are monoxenous parasites, as monogeneans or nematodes with direct life cycles. But last years, heteroxenous parasites are often present, too. The highest prevalence of parasite infections are observed in commercial very attractive species, as are *Symphysodon discus* (Perciformes, Cichlidae) and *Poecilia reticulata* (Cyprinodontiformes, Poecilidae). The breeders themselves present the most severe problems in fish medicine as: 1. They do not see the veterinarians in case of occurrence of diseases; 2. They use medicaments without diagnosis; 3. When using the medicaments, they overdose or lower-dose which affects either toxic or pathogen resistantly; 4. They use insufficient or noneffective commercial treatments for certain diseases; 5. They assist the spread of pathogens which considers a global problem.

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OCCURENCE OF A NEGLETED DETECTABLE SOIL-TRANSMITTED HELMINTH IN CHILDREN AND DOGS IN EASTERN SLOVAKIA

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In the present work, we report the presence of *Strongyloides stercoralis* in children, dogs, and soil inside and outside the segregated settlement in the town of Medzev situated in Eastern Slovakia. We focused on the presence of S. stercoralis larvae by direct microscopy evidence in the faces of children, dogs living close those children, and in soil samples collected surrounding their dwellings, applying the larvoscopy and cultivation method. Indirect immunoenzymatic assay (ELISA) was used to prove IgG antibodies against *S. stercoralis* in the sera of children and dogs. Our findings confirm that the occurrence of this helminth must also be expected recently in the temperate zone, and the disease persists in the Slovak population from its first report 88 years ago (Dziuban 1928).

We examined faeces of Roma children and non-Roma children from preschool and school facilities in Medzev and excrements of dogs in the Roma settlement in Medzev and dogs from the shelter maintained by the Union of Mutual Aid of Humans and Dogs in Haniska near Košice. For the purpose of detection of *Strongyloides stercoralis* infection in children and dogs, we used a direct method – microscopy of stool samples and an indirect method – serological examination of blood using the ELISA method. Stool samples of children and dogs were subjected to a Koga agar plate (KAP) culture and Baermann technique.

The samples examined for the qualitative evidence of specific IgG antibodies against *Strongyloides stercoralis* applying the indirect immunological and enzymatic reaction (ELISA) showed the 33.3 % serum prevalence in Roma children and 23.8 % in the majority group. Seroprevalence was 38.1 % among preschool children aged less than 5 years, 26.3 % among school-age children aged 6–9 years, and 35.0 % among older pupils aged 10–14 years. We confirmed rhabditiform larvae in 13.3 % of dogs from the settlement and in 10 % of dogs from the shelter applying the KAP method. Microscopy and Baermann method of stool samples from children from the Roma settlement in Medzev and children from the majority population did not detect *Strongyloides stercoralis* larvae in any of the groups of children.

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BRAZILIAN FISH PARASITOLOGY

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South America is a region where parasite biodiversity is clearly underestimated. Although this region shows high ichthyological diversity, only less than 5% of fish fauna has been examined for parasites. Brazil is promising in this respect as it has the longest coastline in South America being included in three ecoregions, i.e. North Brazil Shelf, Tropical Southwestern Atlantic and Warm Temperature Southwestern Atlantic, and it has an extensive continental shelf characterized by multiplicity of ecosystems. Currently, Brazil includes the majority of the research groups in South America which have been expanding networks in partnerships with research groups of South America, e.g. Chile, Argentina and from other continents, e.g. Czech Republic. The most serious obstacles for the advancement of Brazilian research on fish parasites are the lack of sufficient financial resources, small number of research groups with excellence and significant volume of unindexed publications. For years, the main focus of fish parasitology research was taxonomy, later the number of descriptions of fish parasite communities increased and currently the numerous papers on a wide variety of topics are published. With the advances of the research and increasing collaboration of different research groups, it is expected that our knowledge of parasite diversity will be much increased.

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A NEW CLASSIFICATION OF *GLARIDACRIS* COOPER, 1920 (CESTODA), PARASITES OF SUCKERS (CATOSTOMIDAE) IN NORTH AMERICA

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A taxonomic study of monozoic cestodes of the genus *Glaridacris* Cooper, 1920 (Cestoda: Caryophyllidea), parasites of catostomid fishes in North America, confirmed artificial character of the genus which is split to 2 different, morphologically distinct and not closely related genera. The two morphologically distinct groups are called herein, in accordance with Mackiewicz (1976), as '*catostomi*-group' and '*laruei*-group'. *Glaridacris* is newly circumscribed to include only 3 species, *G. catostomi* Cooper, 1920 (type species), *G. terebrans* (Linton, 1893) and *G. vogei* Mackiewicz, 1976, which are characterized by an elongate body, a cuneiloculate or wedge-shaped scolex with six shallow loculi, male and female gonopores at a distance from each other, follicular ovary and circum-medullary vitelline follicles (lateral and median). The remaining 3 species ('*laruei*-group') differ from the species of *Glaridacris* listed above by the possession of a bothrioloculodiscate scolex with a terminal disc at the scolex apex, with a pair of deeper median and two shallower lateral loculi, male and female gonopores close together, a compact, non-follicular ovary, and vitelline follicles lateral, i.e. missing medially. For species of this group, a new genus will be erected. An annotated list of all species of both genera with data on their hosts and distribution, and keys to their identification are provided.

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MICRODISSECTION AND SUBSEQUENT PROTEOMIC ANALYSIS OF SELECTED ORGANS/TISSUES OF HELMINTHS

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Excretory-secretory products of parasitic helminths play a key role in parasite-host interactions, and peptidases represent a dominant component of interest. Total secretom can be obtained during incubation of helminths under in vitro conditions, but isolation of separated products of particular organs represents a problem. This could be easily solved by laser-assisted microdissection of target cells or whole tissues/organs of interest from cryo/histological sections, as it was already used in cancer research.

Thereafter, the microdissected material (using e.g. MMI Caplift technology) can be analyzed by a wide spectrum of methods. During our pilot experiments with cryosections of Fascioloides magna, the presence of abundant protease cathepsin L was identified in the microdissected worm intestine by mass spectrometry (MS) with Orbitrap system; the finding was consistent with our previous results gained with immunolocalization analysis. Thus, we have an effective tool for analysis of wide spectrum of parasite products up to the cellular level. Currently, selected cells/tissues/organs of Trichobilharzia specimens are analyzed. We aimed to solve two distinct issues: 1. Identify the exact role of two distinct types of secretory glands used by larvae (cercariae) for penetration into the vertebrate host, by separate analysis of their content. 2. Specify qualitative differences in products secreted into the intestine of two species of avian schistosomes in the stage of schistosomulum, i.e., the stage that (depending on species) is neurotropic or viscerotropic. While T. szidati schistosomula digest predominantly blood cells as expected for all schistosomes, T. regenti seems to prefer nervous tissue components and/or immune cells infiltrating the tissue via blood-brain barrier. Microdissection and mass spectrometry analysis of dominant secretory components will be followed by in situ localization of selected proteins.

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EUDIPLOZOON NIPPONICUM (MONOGENEA: DIPLOZOIDAE): COMPARATIVE STUDY OF TRANSCRIPTOME AND SECRETOME OF FISH PARASITE

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Ectoparasitic flatworms from the family Diplozoidae (Platyhelminthes: Monogenea) represent a serious hematophagous fish pathogens. Information related to the biochemical and molecular nature of the physiological processes is rather sporadic, as well as the knowledge of the molecules produced by monogeneans and their role in host-parasite interaction. Therefore, we performed complex transcriptomic and proteomic (secretomic) analysis of monogenean representative *Eudiplozoon nipponicum* Goto, 1891 (Polyopisthocotylea: Diplozoidae), which was realized by two sequencing strategies (Roche 454 and MiSeq Illumina) and mass spectrometry (HPLC MS/MS). Total RNA from a few *E. nipponicum* adults, in the form of cDNA was sequenced (454/Roche and MiSeq Illumina) and raw reads were processed in order to get high quality transcriptomics sequences for further annotation. Specific bioinformatics tools were used for filtering and removing of low quality bases as well as contaminants – reads related to host organisms (common carp) and bacteria. Sequencing errors and mismatches were corrected too and finally the processed reads were assembled into the form individual transcripts. 454/Roche and Illumina sequences were pooled into ~39.000 nucleotide sequences with open reading frame and several further bioinformatics approaches (especially searching for the closest homologous in non-redundant databases and prediction of proteins key properties) were used for annotation. We annotated ~19.000 nucleotide sequences and for ~13.000 of them a source organism from phylum Platyhelminthes was assigned. ~5.800 sequences were determined as peptidases and ~1.400 as their inhibitors. Most abundant peptidases belong to metallo (~1.800), serine (~1.500) and cysteine (~1.400) catalytic type. Some of these peptidases may be related to hematophagy, e.g. cathepsins C, D, H, K, L1, L3, S (40 unique entries), their inhibitors (e.g. kunitz inhibitors; 11 unique entries) and anticoagulant agents (e.g. heparan sulfate; 12 unique entries). For secretomic study, the living specimens of *E. nipponicum* were continuously collected from the gills of naturally infected and freshly netted carp, excretory-secretory products (ESP) were isolated from ~100 adults and characterized by mass spectrometric methods (LC MS/MS). Data related to ESP identifications were compared with annotated transcriptome.

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DIVERSITY OF METAZOAN PARASITE COMMUNITIES OF ENDEMIC CYPRINID SPECIES FROM THE SELECTED AREAS OF MEDITERRANEAN

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The Mediterranean area is distinguished from most of the other parts of Europe in occurrence of many unique endemic fish species (especially cyprinids). Evidence and studies focussing on extensive research of parasitofauna of endemic freshwater fishes are rather exceptional**.** In the present study, we analysed the metazoan parasite communities in endemic cyprinid species of a large part of the Balkan Peninsula. A total of 577 specimens belonging to 57 cyprinid species were sampled in Albania, Bosnia and Herzegovina and Greece and examined for the presence of metazoan parasites. This study revealed over 100 species of metazoan parasites. The highest species diversity was reported within Monogenea, i.e. we reported 38 species of *Dactylogyrus*, from which 11 were identified as new, about 30 spp. of *Gyrodactylus* recognized on the basis of morphology (for confirmation of new species, molecular analysis is currently in progress), and *Paradiplozoon homoion*. Concerning Trematoda, 9 intestinal species – 7 species of Digenea in adult form (*Allocreadium isoporum*, *A. markewitschi*, *Asymphylodora demeli*, *Sphaerostomum globiporum*, *Nicolla skrjabini*, *Plagioporus stefanski*, *Palaeorchis* sp.), *Aspidogaster* cf. *limacoides* (Aspidogastrea) and *Clinostomum* sp. represented as metacercariae were found. In addition, *Diplostomum spathaceum* in the eye lens, and *Tylodelphys clavata* and *Posthodiplostomum* cf. *brevicaudatum* in eye vitreous humour were recorded. The adult tapeworms (Cestoda) *Caryophyllaeus brachycollis*, *Proteocephalus torulosus* and *Schyzocotyle acheilognathi* and larvae of *Paradilepis scolecina* and Proteocephalidae gen. sp. were found. Four adult nematode species, *Rhabdochona hellichi*, *R. denudata*, *Schulmanella petruschewski* and *Hysterothylacium* cf. *fabri*, and larvae of 6 species (*Raphidascaris acus*, *Anguillicoloides crassus*, *Cosmocephalus obvelatus*, *Spiroxys* sp., *Contracaecum* sp., *Pseudocapillaria* sp.) were recognized. Two species of Acanthocephala (*Pomphorhynchus laevis* and *Acanthocephalus* sp.) were recorded in intestine. From other ectoparasitic groups, 5 species of copepods of the genera *Ergasilus*, *Neoergasilus*, *Paraergasilus* and *Lamproglena*, *Argulus* sp. (Branchiura) and *Hydrozetes* cf. *lemnae* (Acari) (this last one is possibly an accidental presence of non-parasitic species or the species exploiting facultative strategy of parasitism), were reported. While the majority of species of *Dactylogyrus* and *Gyrodactylus* are characterised by a high degree of host specificity and include taxa endemic to the Mediterranean area, some monogeneans reported in endemic cyprinid species are commonly distributed in Europe. The remaining ectoparasites and all endoparasites are common parasites of cyprinids widely distributed in Palaearctic Region. We concluded that species diversity of parasites and their distribution in endemic cyprinid hosts are influenced by cyprinid biogeographical history (especially concerning the monogeneans), range of distribution of fish hosts and other abiotic and biotic factors. The study of parasite diversity in endemic cyprinids of the Balkan Peninsula (especially parasites exhibiting high host specificity) may represent a helpful tool for analysing biogeographical scenarios proposed for the historical distribution of Mediterranean cyprinids.

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TRICHINELLOSIS AND TOXOPLASMOSIS IN WILD BOARS (*SUS SCROFA*) FROM NORTHERN PATAGONIA, ARGENTINA

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Trichinellosis is a zoonotic disease transmitted by the consumption of raw or undercooked meat with infective forms of nematodes of the genus *Trichinella*. Currently, the genus is composed of three genotypes, six encapsulated species, and three non-encapsulated species. The wide range of hosts of the genus includes more than 100 species of mammals, birds and reptiles, with wild carnivores and omnivores holding the parasite's biomass. In Argentina, human outbreaks are generally related to the domestic pig cycle, however, in recent years there have been reports of the consumption of wild animals around the world. Toxoplasmosis is one of the major parasitic zoonosis worldwide, produced by the intracellular protozoan *Toxoplasma gondii*. Felines are their definitive hosts and different species of birds and mammals, domestic and wild, including man act as intermediary hosts. Among them, the parasite has developed several transmission routes, emphasizing the ingestion of cysts contained in muscle and animal viscera as a horizontal transmission route. The wild boar (*Sus scrofa*) is an exotic ungulate introduced in Argentina as a major game in the early twentieth century, which represents the second source of human trichinellosis infection worldwide. Likewise, there are studies that show the presence of toxoplasmosis in wild boars of different countries. The aim of the present work was to evaluate the circulation of *Trichinella* spp. and *Toxoplasma gondii* in wild boars from an area of the northern Patagonia of Argentina. Samples were obtained from specimens derived from hunting activity. For the diagnosis of trichinellosis, 150 diaphragm samples were analysed by Artificial Digestion (AD) and 83 serum samples by ELISA using the commercial IDvet excretory-secretory (E/S) antigen kit. For *T. gondii* antibody detection, 36 serum samples were evaluated by Indirect Fluorescent Antibody Test (IFAT), using as antigen tachyzoites of the RH reference strain. From the sera analysed, for trichinellosis, 2.41% (n=2) was positive. The diaphragm samples were negative. Antibodies to *T. gondii* were detected in 5/36 serum (13.51%), two were positive at the dilution 1:50 and three at ≥1:200. The presence of positive animals for *Toxoplasma gondii* and *Trichinella* spp in northern Patagonia, emphasizes the importance of implementing an acute diagnosis method before consumption. Besides it is very important to deepen health education actions in the exposed population and maintain epidemiological monitoring in wildlife.

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THE IMPACT OF RECOMBINANT *FASCIOLA HEPATICA* ANTIGENS ON HUMAN MACROPHAGES, *IN VITRO* STUDY

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Due to a worldwide increase in the incidence of auto-immune diseases and allergies, it is necessary to search for new strategies to prevent and treat these disorders. These diseases are not only an important health problem, but also impact economic and social development of the modern world. Despite the currently available drug therapies, new strategies to combat these types of diseases are needed to improve the health of patients. There is a lot of evidence that parasite proteins can lead to the inhibition of autoimmune diseases and allergies. This effect is due to the immunomodulatory properties of certain parasite proteins and their direct influence on the polarization of macrophages. There is strong evidence for the beneficial effects of immunosuppressive therapy targeted to macrophages in the treatment of renal diseases, diabetes, inflammatory diseases, and transplant rejection. Parasite proteins may be a great source of unique molecules that directly manipulate immune processes or inducing tissue-specific immune responses.

Here, our aim was to study the effects of the recombinant *F.hepatica* cathepsin B2 (FhCB2) and cathepsin L5 (FhCL5) on the human macrophage cell line THP-1, *in vitro*. Monocytes were differentiated into macrophages and cultured with recombinant proteins. We assessed the impact of FhCB2 and FhCL5 on the profiles of cytokines and analyzed the differences in kinase phosporylation profiles.

Our results revealed changes in cytokine and phosphokinase profiles in a macrophage cell line after stimulation with parasite antigens with or without LPS. A reduction in expression of the cytokines (i.a. IL-1β, TNF-α, IL-6, IL-11, IL-17A, IL-15, IL-22) and chemokines (i.a. IL-8, MIP-3α) was observed with cells cultured with recombinant proteins. We observed reduction in CD14, CD30, CD40 and ICAM-1 levels after culture with FhCB2 and FhCL5. Besides the changes in chemokine and cytokine levels after stimulation with recombinant parasite cathepsins, we noticed the influence of these proteins on factors involved in angiogenesis, like: Angiopoietin-1,2; Cripto-1, EGF, HGF, Endoglin, IGFBP-2 and others. Our results show that helminth-derived molecules can be used as tools to identify the underlying mechanisms of immune regulation or to determine new anti-inflammatory therapeutics.

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