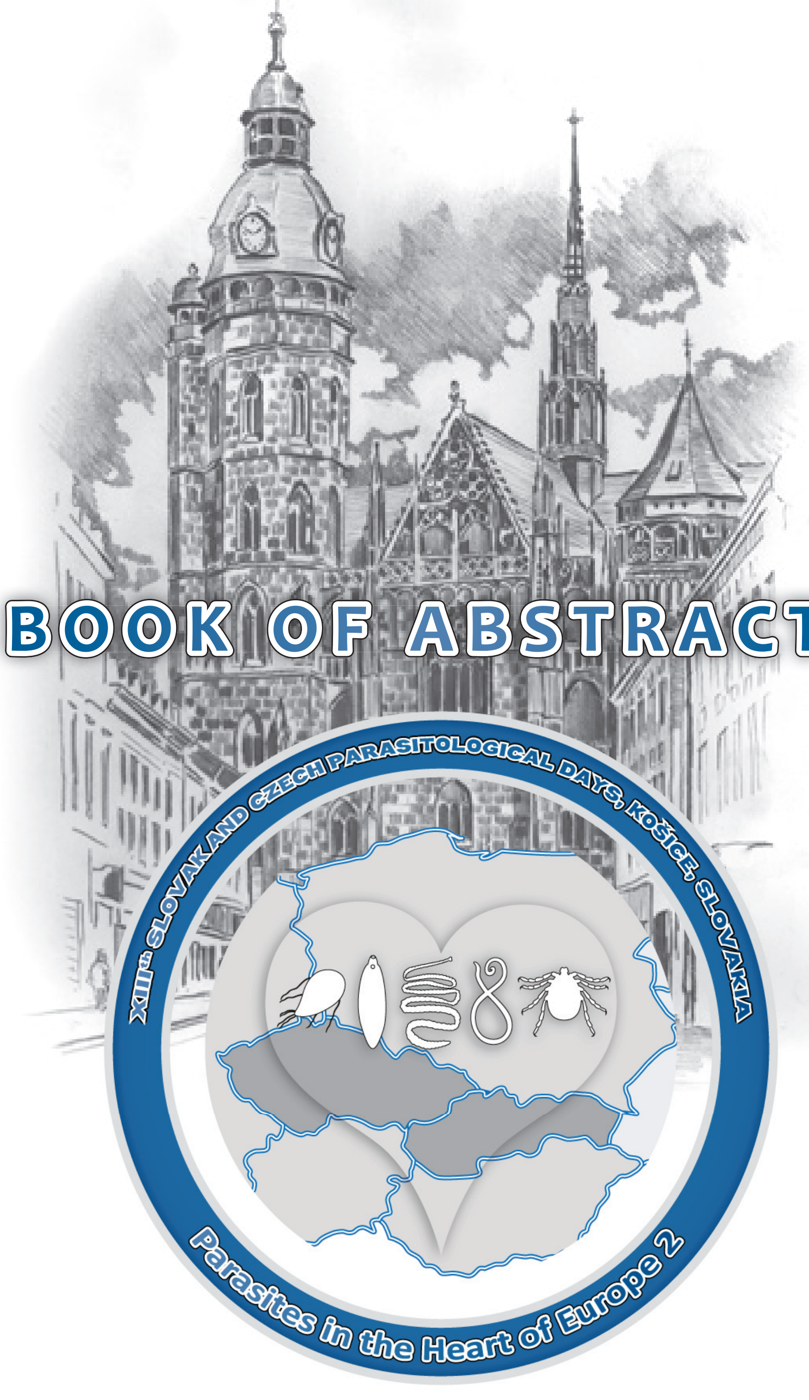
XIII^th^ SLOVAK AND CZECH PARASITOLOGICAL DAYS

XIII. SLOVENSKÉ A ČESKÉ PARAZITOLOGICKÉ DNI

Parasites in the Heart of Europe 2



BOOK OF ABSTRACTS

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”Omics” of monogeneans

Jiří Vorel[1], Jana Ilgová[1], Pavel Roudnický[1], Libor Mikeš[2], Hana Dvořáková[2], Lucie Jedličková[2], Dáša Jirsová[1], Hynek Strnad[3], Roman Leontovyč[2], Ewa Dzika[4], Božena Koubková[1], Lukáš Vetešník[5], David Potěšil[6], Zbyněk Zdráhal[6,7], Marie Jankůjová[7], Jan Oppelt[7], Pavel Jurajda[5], Milan Gelnar[1], Martin Kašný[1,2]

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The representatives of the group Monogenea are primarily ectoparasitic worms infecting the (semi-)aquatic vertebrates, predominantly fishes. Members of this group are not of direct human health concern, but a number of species are known to cause huge economic losses in the aquaculture- and fishing industries, e.g. the “salmon killer” *Gyrodactylus salaris*, in Norwegian economy ca 50 mil. US$/year. During last decade the number of flatworms, such as trematodes and cestodes, sampled at a proteome, transcriptome and genome level significantly increased. In contrast, the Monogenea are currently represented by only one published draft genome for *G. salaris*, one transcriptome dataset for *Neobenedenia melleni* and for one species from the list of the “50 Helminth genome project initiative”, *Protopolystoma xenopodis* the draft genome is available. In reaction to this situation we use the experimental organisms - *Eudiplozoon nipponicum* (Diplozoidae, Heteronchoinea); a blood-feeding monogenean inhabiting gills of common carp (*Cyprinus carpio*) and applied the high throughput sequencing methodology (454/Roche, MiSeq/HiSeq) enabled us to yield robust trancriptomic/genomic data and comprehensive mass spectrometry analysis (Orbitrap-Elite MS System) of excretory-secretory products isolated from the adult worms. To elucidate the key genes/proteins of *E. nipponicum* biology the bioinformatic pipelines were adopted, the corresponding sequences assembled and annotated. The selected functional protein molecules have been molecularly/biochemically/immunochemically characterized.

The research was financially supported by the Masaryk University, Brno (MUNI/A/1362/2016), CEITEC project (CZ.1.05/1.1.00/02.0068), Czech Science Foundation (GBP505/12/G112), the Charles University (PRVOUK P41, UNCE 204017 and SVV 260432/2017) and European Regional Development Fund (CZ.1·05/1·1·00/02·0068).

Genetic structure of the population of *Isthmiophora melis* (Schrank, 1788) Luhe, 1909 (Digenea, Echinostomatinae) in Poland

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Isthmiophora melis is widespread parasite in Europe, Asia and North America and uses numerous (more than 30 species, including humans) vertebrates as definitive hosts. In Poland this species has been reported from foxes, martens, badgers, hedgehogs and rodents. Our previous studies have shown that the morphology of this species is strictly host-dependent while observed genetic diversity was at very low level or even in some cases *I. melis* isolated from various host did not expressed any molecular variability. However, this result was based on a small number of individuals used for genetic studies. Thus, in this survey we would like to investigate, based on larger datasets, the level of genetic diversity of *I. melis* occurring in American mink (*N. vison*) and rodents in Poland.

A total of 138 individuals of *I. melis* were collected from 7 localities (Słowiński National Park [SPN], Biebrza National Park [BbPN], Ujście Warty National Park [PNUW], Narew National Park [NPN], “Wisła”, Drawno National Park [DPN] Nature reserve “Milicz Pond” [NRM]). Digeneans have been examined based on standard procedure, i.e. DNA was isolated from single specimens of *I. melis* and next the extract was used for amplification of nad1 partial sequence. All amplifications were successful and as the final result 402 bp long alignment was obtained. A median-joining (MJ) network was constructed using Network 4.6.1.3 to investigate phylogenetic relationships between *nd1*

haplotypes. Haplotype-based statistics (Hs and Hst) and nucleotide-based statistics (Ks, Kst, Z and Snn) were calculated using DnaSp 5.10. For 402 bp long *nd1* sequence of *I. melis* 14 variable sites were observed and 15 haplotypes was identified. We have observed that genetic diversity was depending on study sites and host species.
This study was supported by the Polish Ministry of Science and Higher Education grant IP2014 0536 73 and project no. 2012/05/B/NZ8/01247 funded by the National Science Center, Poland

Quo vadis Ashworthius sidemi? Current status of a non-native parasite in the Czech Republic

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Ashworthius sidemi is a hematophagous trichostrongylid nematode that occurs primarily in Asiatic cervids. During the last century, this parasite has been introduced into several European countries where it has colonized several new hosts. In the Czech Republic, *A. sidemi* was first recorded in the early 1970s in sika deer and subsequently detected in other native cloven-hoofed animals. The main objective of this study was to provide much needed information concerning the current spread of this invasive nematode within the territory of the Czech Republic, as well as to conduct diagnoses and pathological examinations of *Ashworthius* infections.

During the main hunting season of 2015, 2016 and 2017, as many as 80 individuals of various cloven-hoofed animal species were investigated for the presence of *Ashworthius sidemi*. The majority of these animals were acquired from fifteen game reserves across the Czech Republic; one of these specimens was a moose that was killed after being struck by a vehicle. The digestive tracts of the animals were examined using standard necropsy procedures, and all recovered nematodes were morphologically identified. Faecal samples from the recta of the surveyed animals were examined using standard flotation techniques, and tissue samples were collected from infected individuals for histopathological investigation.

In our survey, *A. sidemi* was recovered from red deer, sika deer, roe deer, fallow deer, European bison and moose, with an overall prevalence of 59 %. The occurrence of *A. sidemi* was confirmed in various game reserves across the entire country, a vast area extending from the Pilsen region in the West to Czech Silesia in the East. In the game reserves with animals that tested positive for *Ashworthius* infection (10/15), the prevalence of the nematode ranged from 67 % to 100 %. The abundance of *A. sidemi* varied significantly - from 1 to 11,500 nematodes. The mean infection intensity was somewhat lower in red deer (247 individuals) and sika deer (78 individuals) than in roe deer (440 individuals) and fallow deer (460 individuals). As far as we know, this is the first record of *A. sidemi* in fallow deer and moose in the Czech Republic. The vast majority of *Ashworthius* infections were associated with major pathological lesions in the abomasal mucosa. Infected individuals exhibited significant inflammatory tissue reaction, local necrosis and considerable changes in tissue morphology; all of these manifestations had significant influence digestive activity. Traditional faecal flotation methods were deemed inaccurate for *Ashworthius* infection detection and quantification; during the autumn and winter of 2016/2017 and 2017/2018, the quantity of recovered nematode eggs was negligible even in cases of large numbers of adult nematodes in the abomasum. Due to the high pathogenicity of *Ashworthius* infections in wild ruminants as well as the potential for livestock infection by this invasive parasite, future research should focus on accurate intravital diagnostic methods and control approaches.
This research was supported by the Technology Agency of the Czech Republic, Project No. TJ01000009.

Invasive parasite of common carp *Atractolytocestus huronensis* (Cestoda); a tour around four continents

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The monozoic tapeworm *Atractolytocestus huronensis* Anthony, 1958 (Caryophyllidea), an intestinal parasites of common carp (*Cyprinus carpio*), is characterized by a potential to colonize new territories. The parasite was described from common carp in the Huron River in Michigan, USA and since then it was reported from different US regions, European countries, China and recently from South Africa. Ribosomal (ITS2) and mitochondrial

data (*cox*1) were applied for phylogenetic analyses in order to assess the genetic interrelationships among *A. huronensis* populations worldwide. Newly obtained data from two Chinese localities (Danjiangkou and Poyang) and from South Africa (Limpopo province) were compared with previously published results on *cox*1 and ITS2 structure of *A. huronensis* from North America and Europe. The analysis of *cox*1 indicated that *A. huronensis* is a monophyletic lineage comprising two slightly differentiated clusters; one cluster was represented by samples from China, the USA and the UK, while second cluster was represented by tapeworms from South Africa and continental Europe (Slovakia, Hungary, Croatia, Romania). Divergent intragenomic copies of ribosomal ITS2 were detected in all newly analysed specimens what confirmed an assumption that ITS2 paralogues are species-specific feature. Molecular analyses provided useful pilot information about the interrelationships of *A. huronensis* on four continents and indicate that China (or the eastern Palaearctic), with the highest population diversity observed, served as the original source population for the global expansion of this invasive tapeworm. The results revealed a close genetic relationship among *A. huronensis* from China and those from the USA and the UK. Common carp is not autochthonous in North America, and cyprinids are rarely imported to Europe from the American continent; much more common is the import of cyprinids from the Far East. Therefore, *A. huronensis* was most likely introduced to the UK from China rather than from the USA. Concerning *A. huronensis* in USA, it could hypothetically be introduced either from China or from the UK, although there is also an alternative that the British and American populations were introduced independently from the same source of origin (China). The analyses revealed genetic similarity of tapeworms from South Africa with those from Slovakia, Hungary, Croatia and Romania, what suggested that *A. huronensis* was introduced to Africa from continental Europe. However, given the genetic uniformity of the regions thus far surveyed, the introductory population would most probably lay outside central or eastern Europe.
This study was supported by project VEGA 2/0134/17 and the Slovak Research and Development Agency APVV 0653-11.

Development of polymorphic microsatellite loci for broad fish tapeworm *Dibothriocephalus latus* (syn. *Diphyllobothrium latum*)

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The broad fish tapeworm *Dibothriocephalus latus* (syn. *Diphyllobothrium latum*) (Cestoda: Diphyllobothriidea) is one of the most common causative agents of human food-borne cestodosis, diphyllobothriosis. Parasite's life cycle includes two intermediate hosts (crustaceans and fish) and final host (fish-eating mammals, including human). The source of infection for human and other fish-eating mammals is consumption of raw or undercooked fish containing infective larvae, plerocercoids. In Europe, different fish species (e.g. *Perca fluviatilis*, *Esox lucius*, *Lota lota*) were found to be infected with *D. latus* mainly in the Alpine lakes region (Italy, Switzerland, France), Baltic countries (Estonia, Latvia, Lithuania), Finnland, Russia and USA. Medical importance, scattered geographical distribution and unknown origin of *D. latus* in Europe and USA make this species to be an interesting model in a field of population biology. The main aim is to find out whether diphyllobothriosis is native in Europe or it is a result of trans-continental import/introduction. For population genetic studies, microsatellites, or short tandem repeats (STR), are genetically informative and suitable tools. Therefore, development and characterization of polymorphic microsatellite loci in *D. latus* using next-generation sequencing (NGS) approach was performed. For NGS analysis and selection of suitable STR loci, DNA pool was prepared from *D. latus* plerocercoids from perch (Iseo Lake, Italy). In total 122 STR candidates generated after NGS were tested by PCR amplification. PCR products of expected size were detcted in 110 candidate loci, and the declared repetitive motif was identified by Sanger sequencing in 78 of them. After the fragment analysis, 6 polymorphic loci were tested statistically (determination of observed (Ho) and expected (He) heterozygosity, deviations from Hardy-Weinberg equilibrium (HWE), etc.). The outputs of statistical analyses indicated high level of monomorphic loci and rapid loss of heterozygosity what can be explained by the reproduction strategy of hermaphroditic *D. latus*. On the other hand, six loci were proved to be polymorphic but all of them revealed significantly higher proportion of heterozygotes than was expected under HWE. Such excess of heterozygotes may be due to natural selection against homozygotes at loci linked to the tested microsatellites. Finally, six microsatellite loci were proved to be statistically informative and may be applied in future studies on genetic interrelationships, origin and migratory routes of this medically important emerging tapeworm.
This work was supported by the projects APVV-15-0004 and VEGA 2/0134/17.

Spermiogenesis and ultrastructure of the spermatozoon of the caryophyllidean cestode *Caryophyllaeides fennica* (Schneider, 1902)

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Caryophyllaeides fennica (Schneider, 1902), a parasite of a wide spectrum of distantly related cyprinid fishes from Europe and Russia, was investigated by means of transmision electron microscopy, cytochemistry and electron tomography. Specimens were obtained from four cyprinid fish hosts cought in Tisa and Latorica rivers (southeastern Slovakia). Living worms were fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer (pH 7.2) and prepared routinely for TEM observations. The process of spermatozoon formation corresponds in the basic pattern to that described by Bruňanská and Kostič (2012). The beginning of spermiogenesis is marked by the formation of a zone of differentiation which contains the two centrioles, apical dense material, and cortical microtubules. Each centriole is jointed with one typical striated root. New is a finding of the two additional striated roots which are localised oppositely each other. These structures may be situated in two different positions to the typical striated root with respect to the stages of spermiogenesis. The intercentriolar body is composed of three electron-dense and two electron-lucent plates. Both the developing free flagelum and short flagellar bud rotate towards growing median cytoplasmic protrusion. Only a free flagellum fuses with median cytoplasmic protrusion during proximodistal fusion. The mature spermatozoon of *C. fennica* is composed of one axoneme with the 9 + “1” trepaxonematan structure, cortical microtubules and nucleus, all embedded in electron-lucent cytoplasm. Glycogen was detected in the cytoplasm of regions II, III and IV of the mature spermatozoon using periodic acid-thiosemicarbazide-silver proteinate (PA-TSC-SP) method. The two attachment zones were found under the plasma membrane of regions II. and III. of the male gametes. Application of electron tomography has revealed that peripheral nine doublet microtubules of the 9 + “1” trepaxonematan structure of one-axoneme mature spermatozoon of *C. fennica* are interconnected with the central core unit by regularly spaced spokes. The central core of the axoneme has spiral shape resembling a screw conveyor.

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Laser microdissection-based proteomics of helminth tissues

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For a thorough understanding of parasite-host interactions, the protein composition of selected parasite tissues/organs may clarify molecular mechanisms used for invasion, migration, and feeding.

The main aim of our research is to functionally characterize and compare proteins of two *Trichobilharzia* species, namely *T. regenti* and *T. szidati*, which have different feeding preferences and migratory routes in the vertebrate host. While schistosomula (migratory larvae) of *T. szidati* migrate through the blood system and digest blood cells, schistosomula of *T. regenti* occur in the nervous system and feed on the nervous tissue and immune cells. Therefore, we will focus on differences in schistosomulum intestinal proteins.

To identify the proteins properly, it is necessary to avoid possible contamination from the surrounding tissues. Unfortunately, small size and architecture of the parasite body represent a general problem. In addition, proteomics, unlike other molecular analyses, requires sufficient amount of material (proteins cannot be amplified prior to their analysis).

Nowadays, laser microdissection enables to isolate tissues or cells of interest for subsequent analysis. There are several steps to get the material for analysis: (a) microdissection of cryosections (using MMI CapLift technology),

(b) one-step reduction and alkylation followed by protein cleavage, and (c) analysis of samples by LC-MS/MS (Orbitrap Fusion system). Bioinformatics applied for the raw data should be followed by backtracking of selected proteins in histological sections (immunohistochemistry, *in situ* hybridization).

In order to optimize the method, we used *T. szidati* cercariae and tested (a) the impact of fixatives, (b) the effect of sample processing after microdissection, (c) the optimal amount/volume of the analyzed material, and (d) the time-dependent material degradation before LC-MS/MS analysis. The best results were obtained with the unfixed material (frozen in the optimal cutting temperature (OCT) compound) processed *via* the In-StageTips method with a minimal volume of 7.5 mils μm^3 of the microdissected material. Exposure of the microdissected material to room temperature up to 24 hours (simulating a long microdissection process) had no significant effect on the proteomic analysis.

It can be concluded that the method represents a fast and easy way how to perform protein analysis of cells/tissues of a broad spectrum of small-size organisms.

The study was financially supported by a grant from the Czech Science Foundation (Grant No. 18-11140S) and by the project „BIOCEV – Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University“ (CZ.1.05/1.1.00/02.0109), from the European Regional Development Fund.

New insights to the coevolutionary relationships of host-specific monogeneans and their peri-Mediterranean cyprinid hosts

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Diversity, distribution and evolutionary history of host-specific parasite taxa is usually intimately linked with historical dispersion, diversity and evolution of their hosts. Parasites belonging to genus *Dactylogyrus* (Monogenea) are known for their narrow host specificity and morphological adaptations to their respecitve hosts, freshwater fish belonging to family Cyprinidae. Each cyprinid species can serve as potential host for at least one *Dactylogyrus* species, resulting in a high species richness of this remarkable parasitic taxa. Recently, cophylogeny has become fundamental approach in the study of co-coevolutionary host-parasite associations. Therefore, we used highly host-specific *Dactylogyrus* parasites as the additional tool to shed new light into phylogenetic relationships between endemic cyprinids of peri-Mediterranean region.

Over years 2014 to 2018 we collected and parasitologically processed 113 cyprinid species from 7 European countries in the peri-Mediterranean region (Albania, Bosnia and Herzegovina, Croatia, Greece, Italy, Portugal and Spain). In total, 93 cyprinid species were parasitized by *Dactylogyrus* representing 69 species (including 18 potentially new species for science). Phylogenetic reconstruction revealed two lineages of endemic *Dactylogyrus* parasitizing cyprinids of Iberian Peninsula. While the first lineage was phylogenecally closely related to the clade of central European and Balkan species, the other one was more related to the north African endemic *Dactylogyrus*, which suggests the multiple origin of both *Dactylogyrus* and their cyprinid hosts in Iberian Peninsula. In regards of *Dactylogyrus* in Balkan and Apennine Peninsulas, our phylogenetic analyses revealed four well supported clades. These results outlined recent contacts between endemic cyprinids and species with the wide distribution range where the potential host-switch of *Dactylogyrus* occurred also between phylogenetically unrelated hosts. Applying cophylogenetic methods we found the significant coevolutionary signal between phylogenies of endemic Balkan and Apennine *Dactylogyrus* and cyprinids. The most significant coevolutionary links were detected between *Dactylogyrus* of Barbinae and their hosts belonging to the genera *Aulopyge*, *Barbus* and *Luciobarbus*. Additionally, strong coevolutioary link was also detected between *D. vistulae* and its respective hosts.

Our findings suggest that host-switch played important role in the evolutionary history of *Dactylogyrus* parazitizing Balkan and Apennine cyprinids. Moreover, genetic structuration and geographic distribution of these host-specific parasites has been strongly influcenced by recent introduction of the non-native cyprinid species into peri-Mediterranean area. Preliminary analysis based on molecular data also imply that several supposedly generalist species are in fact complexes of the cryptic species and therefore, the endemic *Dactylogyrus* fauna is probably far more diverse than was expected.

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Diplozoids (Monogenea) from the endemic cyprinid species from Mediterranean area: diversity and phylogeny

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The Mediterranean area is unique for the presence of many endemic fish species, especially cyprinids. However, the evidence and studies focussing on extensive research of diplozoid species of endemic cyprinids are rather exceptional. During 2014-2017 a total of 125 endemic cyprinid species were sampled in the selected areas of Mediterranean and examined for gill parasites. Identification of diplozoid species was based on the morphometric analyses of sclerotized parts of attachment organ and molecular analyses performed using ITS2 region of nuclear ribosomal DNA. We investigated the phylogenetic relations among species of *Paradiplozoon*. Over 1000 specimens of diplozoids were reported on endemic cyprinids and a total of five species of the genus *Paradiplozoon* were identified. *Paradiplozoon homoion*, a common parasite of several species of cyprinids in Palaearctic region, reached the highest abundance. This study reveals seventeen new host records of *P. homoion* representing 12 cyprinid species in Balkan Peninsula and 5 cyprinid species in Spain. We also identified *Paradiplozoon megan*, known as a parasite of cyprinids of the genera *Squalius* and *Leuciscus* in Palaearctic region. This study provides new host records of *P. megan* on endemic cyprinids, i.e. *Squalius zrmanjae* and *S. squalus* from Croatia and *S. lucumonis*, *S. squalus*, *Protochondrostoma genei* and *Rutilus rubilio* from Italy. Three new species of *Paradiplozoon* were reported on endemic cyprinids in Mediterranean. *Paradiplozoon* sp. 1 was recorded on *Scardinius acarnanicus* and *Tropidophoxinellus hellenicus* from Greece. On the base of phylogenetic analysis this species is closely related to *P. megan*. *Paradiplozoon* sp. 2 was discovered on five genera of cyprinids in Iberian Peninsula, i.e. *Luciobarbus*, *Squalius*, *Iberochondrostoma*, *Parachondrostoma* and *Pseudochondrostoma*. Low population variability was found between *Paradiplozoon* sp. 2 parasitizing Spanish and Portugal cyprinids. This species was phylogenetically closely related to *Paradiplozoon pavlovskii*. *Paradiplozoon* sp. 3 was identified on *Luciobarbus lepineyi* in Morocco. This species is phylogenetically related to *P. bingolensis* from *Garra rufa* (Turkey), and they are included in monophyletic group with African species *Afrodiplozoon polycotyleus* from *Labeobarbus marequensis*.

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Phylogenetic relationships among dactylogyrid species from African tetras

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Freshwaters of Afrotropical Region harbour more than 200 characiform fishes (Characiformes) currently arrayed in four families: Alestidae (118 species), Citharinidae (8 species), Distichodontidae (101 species), and Hepsetidae (5 species). Alestidae, often called African tetras, are known to be parasitized by ectoparasitic flatworms (Monogenea: Dactylogyridae) representing three genera: *Annulotrema* (41 species), *Characidotrema* (10 species), and *Afrocleidodiscus* (1 species, namely *A. hydrocynuous*).

During field trips (2007 – 2017) we sampled 11 species of African tetras (*Alestes baremoze, A. dentex, Arnoldichthys spilopterus, Brycinus imberi, B. leuciscus, B. nurse, Hydrocynus forskahlii, H. brevis, H. vittatus, Micralestes accutidens, M. elongatus*) from eight African countries (Cameroon, Democratic Republic of the Congo, Kenya, Nigeria, Senegal, South Africa, Sudan, and Zimbabwe).

Our investigation of parasite diversity of African tetras revealed the presence of monogeneans belonging to two genera within the Dactylogyridae: *Annulotrema* (19 species), and *Characidotrema* (6 species). Preliminary phylogenetic analyses supporting morphological data confirmed the monophyly of *Characidotrema* species, while species of *Afrocleidodiscus hydrocynuous* and representatives of *Annulotrema* clustered together. *Afrocleidodiscus paracleidodiscus* from *Distichodus rostratus* (Characiformes: Distichodontidae) forms separate cluster close to the *Characidotrema* spp. Division of both *Afrocleidodiscus* species highlights the necessity of revising the genus. The presence of haptoral bars with supporting membranes provides an additional supportive character for transfer of *A. hydrocynuous* to *Annulotrema*.

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A long temporal study of Red Queen dynamics in diploid-polyploid populations of *Carassius gibelio*

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Carassius gibelio, a member of *Carassius auratus* complex, is a specific cyprinid species exhibiting two forms of reproduction, sexual and asexual. It is a non-native, invasive fish species with more than fifty years of history on the European continent. We hypothesized that parasite selection is one of the potential mechanisms contributing to the coexistence of the two reproductive forms of *C. gibelio* living in the same habitat.

The Red Queen hypothesis describes host-parasite dynamics on the basis of negative parasite-mediated frequency dependent selection, which can represent one of the advantages of sexual reproduction over asexual reproduction. Following the Red Queen hypothesis, asexually reproducing organisms should be more susceptible to pathogen infection than sexually reproducing organisms, because they exhibit lower genetic variability in comparison to sexual form. We performed a four-year study to investigate the dynamics of parasite infection in *C. gibelio*. A total of 22 metazoan parasite species were found. However, our results showed similar levels of prevalence for all studied parasite species in both forms. Monogeneans represented the most frequently observed parasite group in both sexual and asexual forms. A total of 7 species of *Dactylogyrus*, 3 species of *Gyrodactylus* and *Paradiplozoon homoion* were identified in *C. gibelio*. The maximum prevalence and the highest intensity of infection were found for three monogenean species *Dactylogyrus dulkeiti*, *Dactylogyrus anchoratus*, *Gyrodactylus sprostonae* in both forms of gibel carp. We detected that both reproduction forms were more infected by parasite species in the last year of investigation. However, the sexual form was more parasitized by ectoparasites in the first and last years and less parasitized by nematodes in the last year when compared to the gynogenetic form. We concluded that the observed dynamics of parasite infection does not support the Red Queen hypothesis, because we failed to find a significantly higher level of parasite infection in the asexual form in any year of our investigation.

Our study was also focused on the analysis of genetic variability of functional MHC genes. A total of 47 original sequences MHC IIB DAB genes were revealed. Individuals of both forms expressed the alleles of one locus (DAB1 or DAB3) or the alleles of both loci (DAB1 and DAB3). We also revealed some alleles specific to sexual form and alleles specific to gynogenetic females. However, we confirmed that number of allelic types in gynogenetic females is reduced in comparison with sexual diploids.

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New perspectives on postlarval metamorphosis and juvenile development of *Mesocestoides* from Europe and the Americas

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Mesocestoides is a genus of cyclophyllidean cestodes that occur around the globe, ranging from equatorial to polar climates. Adults inhabit the intestine of diverse mammalian and avian hosts, including humans, with most infecting canids and other carnivores. These definitive hosts become infected by ingesting tetrathyridia juveniles, which occur in a wide range of vertebrate intermediate hosts, including many amphibian, reptilian, avian, and mammalian taxa. Oncospheres containing hexacanth larvae lack an outer eggshell and are passed through the host's feces en masse within the distinctive parauterine organ. Despite decades of intensive study by many researchers, infection of an intermediate host by hexacanths has never been demonstrated, so the full life cycle remains unknown.

We have collected and studied *Mesocestoides* tetrathyridia from diverse hosts around the world for several decades. We have recorded variations in development and morphology including neoplastic malignant transformation in some. The present report details our most recent findings, including the first report ever of naturally occurring early post-hexacanth stages, prior to scolex morphogenesis, and pre-tetrathyridial metamorphosis stages that possess a full tetra-acetabulate scolex with a muscular apical sucker occupying the rostellar region. These occurred in an individual field-collected lizard, *Scincella lateralis*, from North America. They appear very similar to metamorphosis stages developed in vitro from hexacanths of *Mesocestoides vogae* by earlier authors. However, ours showed no sign of asexual proliferation originating in the scolex. Thus, ours differ from the cases of *M. vogae* from western North America, which exhibit unique asexual proliferation from an apical massif that occupies the rostellar region. Ours also did not exhibit any sign of proliferation associated with malignant transformation in the hindbody, such as we have reported previously from some specimens collected across Europe, North America, and South America. As with our specimens reported here, most *Mesocestoides* from all parts of the world do not exhibit asexual proliferation.

We performed DNA-sequence analyses on our specimens based on mitochondrial 12S, cox1, and nad1 genes. Our results show that the developing juvenile worms from *S. lateralis* belong to a species distinct from any *Mesocestoides* previously sequenced from other locations in North America, including many from California near the area of historic collections of the unusual *M. vogae*. Our distinct species from *S. lateralis* also differs from those previously sequenced from Europe, including *Mesocestoides lineatus* and *Mesocestoides litteratus* from *Vulpes vulpes* from Slovakia.

These results extend our understanding of post-larval, pre-adult development and metamorphosis for this important cosmopolitan genus, but leave the life cycle still unresolved. However, the fact that we have shown a continuous range of early post-hexacanth stages and nearly fully formed tetrathyridia occurring alongside each other within a single host may suggest a two-host life cycle, despite earlier failures to infect hosts experimentally by feeding them oncospheres or gravid proglottids. The tendency for some *Mesocestoides* tetrathyridia isolates to proliferate asexually, including the frequent association with neoplastic malignant transformation, remains poorly understood.

Diversity of monoxenous trypanosomatids originated from the Philippines

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The dixenous (with two hosts) trypanosomatids are well known because they represent dangerous pathogens for human, domestic animals and plants. Their monoxenous (with one host) relatives have been effectively neglected for years, but recent studies have demonstrated their immense impact on insect communities, and importance for studies in evolution of parasitism. Here we summarize results of the biodiversirty assessment of monoxenous trypanosomatids in the Philippines. Our data revealed phylogenetic affinities of the isolates under study to the previously known trypanosomatid clades and allowed to select two potentially interesting groups for further investigation. The first one corresponds to the previously recognized (but not formally described) “Clade 2”, while the second group unites several putative species of the subfamily Paratrypanosomatinae, relatives of *Paratrypanosoma confusum*.

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The effect of selected micro RNA on the change of genes expression of the pathway Toll of *Apis mellifera carnica* infested with Varroa destructor

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Phenomenon of mass disappearance of honey bees (CCD-Colony Collapse Disorder) is the result of co-interaction of many factors, but especially large negative part in its essence has mite Varroa destructor. This is a parasitic mite feeding with hemolymph of capped brood and mature individuals, deprives bees of valuable nutrients but what is more dangerous, a vector of viruses, bacteria and fungi. Due to the lack in 100% effective means fighting that parasitic mite, molecular regulation recognition of the of the pathogenesis of disease caused by it is an important issue. MicroRNAs (miRNAs) are important post-transcriptional regulators of gene expression that have been implicated in fine-tuning a large variety of biological processes. miRNAs are essential in both adaptive and innate immunity. Post-transcriptional regulation of the immune system by miRNAs has also occur in the honey bee. The

aim of the research was to examine potential miRNAs as post-transcriptional regulators of immune system genes in honey bees infested by Varroa destructor.

The research material consisted imago of worker naturally infested with V. destructor. For the detection and quantification of miRNAs, cDNA was synthesized from total RNA using the TaqMan Advanced miRNA cDNA Synthesis Kit (Applied Biosystems, USA) following the manufacturer's protocol. Amplification reactions were performed in the FAST 7500 Light Cycler (Applied Biosystems, USA) using miRNA-specific primers. The primer sequences for each miRNA of A. mellifera corresponded to the mature miRNA sequences available at miRBase (http://www. mirbase.org). Amplification was carried out using SYBRGreen 2xPCR Master Mix (A&A Biotechnology, Poland), according to the manufacturer's instructions. Data were analyzed and normalized to reference gene U6 snRNA and endogenous control samples (imago not infested workers) using the 2-ΔΔCt method (Pfaffl 2001). All the tests were performed in three replicates. We assessed expression levels of the following of A. mellifera miRNAs: let-7, miR-2, miR-12, miR-13a, miR-278, miR-7, miR-278, miR-3769, miR-3776, miR-1006 and miR-3795. Let-7, mir-278 and miR-1006 have not amplified, indicating that those miRNAs are not expressed in newly emerged workers infested by V. destructor. All amplified miRNAs were up-regulated in bees infested by Varroa. The miRNAs: miR-2, miR-13a, miR-7, miR-278, miR-3769, miR-3776 and miR-3795 are candidate regulators of immune system genes in honey bees. We demonstrate that the honey bee immune system is responding to parasite infestation by regulating immune-related genes.

The differential expression of some miRNAs may help to provide new sights into gene regulation of the honey bee immune system during the parasite infestation.

Do the two loaches of genus *Cobitis* (Cobitidae, Teleostei) and their hybrids share the same helminths fauna in the hybrid zone?

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Cobitis taenia hybrid complex in Poland represent two species: spined loach *C. teania, and* Danubian loach *C. elongatoides* which are accompanied by various hybrid forms of three ploidy levels and different genome composition. In particular localities one of parental species coexists together with clonally reproducing hybrids. However, the most published parasitological data from Central and Eastern Europe concern *C. taenia* which, till 1996, was commonly regarded as the only widely distributed member of genus *Cobitis* in the area. The aim of the study was to find potential differences in helminths fauna occurring in loaches belonging to such complex populations within a hybrid zone in Oder River system. Studies on parasitic helminths of native loaches were conducted from 2013 to 2015. A total of 446 fishes including 251 *C. taenia* and 195 *C. elongatoides* were caught by electro-fishing at seven localities in south-western Poland. All individuals were subjected to standard helminthological dissection. A total of 1177 parasites representing 11 taxa were found in 151 individuals of *C. taenia* (prevalence 60,2%), while 530 parasites belonging to 12 taxa were found in 111 *C. elongatoides* (56,92%). Nine taxa of parasites occurred in both loach species and hybrids: *Gyrodactylus latus*, *Tylodelphys clavata*, *Metorchis xanthosomus*, *Holostephanus cobitidis*, *Apophallus muehlingi*, *Allocreadium transversale*, *Raphidascaris acus* and unidentified cysts of Nematoda. *Pseudocapillaria tomentosa*, *Caryophyllaeus laticeps* and *Diplostomum* spp. were detected only in populations where *C. elongatoides* present, while *Posthodiplostomum cuticola* and *Ligula colymbi* were found in loaches of populations with *C. taenia*. Some sources of detected differences, including genetic structure of host populations and/or site-specific environmental factors which can influence helminths assemblages were discussed.

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Monitoring potentially zoonotic metacercariae of common carp (*Cyprinus carpio*) in four Hungarian aquacultures

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The ParaFishControl project running since 2015 aims to research the parasites of the six most important fish species of European aquaculture. As a part of it, the possible occurrence of Opisthorchiid trematodes (Platyhelminthes: Digenea) in common carp (*Cyprinus carpio*) were monitored in four Hungarian aquacultures. The metacercariae of these trematodes are known parasitise the musculature of fish and may cause zoonotic diseases.

Four geographically distinct and methodologically different fish farms were appointed for monitoring. In the years 2016 and 2017, 258 one-summer-old fingerlings were investigated from each farm. In addition in 2017, 60 market sized specimens (30 two-summer-old and 30 three-summer-old) were examined from the most infected aquaculture sites. The fish were anaesthetised and decapitated. The left and right sections of their musculature were studied under stereomicroscope for the occurrence of metacercariae. For further morphological analysis muscle fillets were digested in pepsin solution to free metacercariae from the tissue. Some metacercariae were selected for molecular analysis and the ITS region was sequenced to supplement the morphological observations.

Opisthorchiid metacercariae have not been found up to this time, but in one of the farms other muscle parasite species were present in abundance. These metacercariae were supposed to be a *Holostephanus* sp. based on their morphological characteristics, which was confirmed by the sequences of the ITS region. The rate of infection proved to be remarkably different among the four fish farms. One of the farms was infected by large numbers of *Holostephanus* sp. metacercariae, while in the other three farms no infection was recorded. In the infected farm, fish showed heavier infections in ponds which were close to natural habitats, where water birds (final host) and freshwater snails (first intermediate host) were present in large numbers. There are no data about these parasites developing in mammals therefore an infection experiment in mice was conducted to obtain adult worms, however the mice proved to be inadequate host to *Holostephanus* trematodes.

ParaFishControl Horizon 2020 (634429), Hungarian Scientific Research Fund (OTKA PD108813), Bolyai Scholarship (BO/00417/15/4).

Experimental and molecular studies on *Metagonimus* infection of cyprinid fishes occuring in the river Danube

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Metagonimus Katsurada, 1912 (Digenea: Heterophyidae) species are well-known zoonotic parasites, which reach their mature stage in the small intestine of water birds, mammals and humans as well. Up to this time, ten *Metagonimus* species are known mostly from Eastern and Southern Asia, namely *M. yokogawai* Katsurada, 1912, *M. takahashii* Suzuki, 1930, *M. miyatai* Saito, Chai, Kim, Lee & Rim, 1997, *M. minutus* Katzuta, 1932, *M. katsuradai* Izumi, 1935, *M. otsurui* Shimazu & Urabe, 2002, *M. hakubaensis* Shimazu, 1999, *M. ovatus* Yokogawa, 1913, *M. suifunensis* Shumenko, Tatonova & Besprozvannykh 2017 and *M. pusillus* Tatonova, Shumenko & Besprozvannykh 2017. The representatives of the genus *Metagonimus* have been reported from Eastern Europe too, but only the species *M. yokogawai* was documented in Serbia, Bulgaria, Hungary, Czech Republic, in the Past Sovietunion and Spain. The identification of the European findings was based only on morphological characteristics. In the past decade sequence analysis of Digenean flukes became common and several studies of *Metagonimus* species involves molecular methods. Mostly the nuclear ITS region, 28S rDNA and the mitochondrian COI are the subject of these sequence analysis.

During a recent survey 150 fish specimens of bleak (*Alburnus alburnus*), common nase (*Chondrostoma nasus*), ide (*Leuciscus idus*) and chub (*Squalius cephalus*) were investigated for *Metagonimus* infection in the river Danube. Experimental infections in chicks were used to obtain adult specimens of the parasite, which were particularly important for correct species identification. The morphology (native microscopy and histology) of metacercariae and adult individuals was characterised and 14 specimens were analysed by sequencing the ITS region and the 28S rDNA. The molecular results strongly support that *Metagonimus* specimens collected in Hungary differ remarkably from the already described *Metagonimus* species known from the Far-East and may represent *Metagonimus romanicus* (Ciurea, 1915). Phylogenetic analysis of the sequences showed that the collected *Metagonimus* specimens form a well-differentiated clade with low variability within the group.

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G1 and G3 genotypes of *Echinococcus granulosus* sensu stricto – geographical and host distributions, cyst fertilities and anatomic locations

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High infectivity of *Echinococcus granulosus* sensu stricto tapeworm for humans is generally accepted phenomenon, with 88.4% of global burden of human cystic echinococcosis (CE) assessed as implicated by this species (Alvarez Rojas et al., 2014). According to the current concept, the species is subdivided into the genotype G1 ("common sheep strain") and the genotype G3 ("buffalo strain"). Based on our summarized global data from published reports, the most homogeneous structure of *E. granulosus* s.s. with a predominance of G1 was so far recorded in continents of Africa (97.7% frequency of G1) and America (96.6% of G1). A shared genetic structure in geographically isolated populations indicates the worldwide distribution of a single G1 lineage and that expansion occurred via anthropogenic movement of domesticated animal hosts. On the other hand, G3 genotype is being substantially distributed in Europe (24.7%) and Asia (8.7%). The likely origin of G3 is a territory of Indian subcontinent given that the form has been recorded as particularly abundant in India (69.8%) across five intermediate hosts (buffalo, cattle, human, pig, goat). A global predominance of G1 over G3 is especially pronounced in humans (G1 detected in 92.2% of patients infected with *E. granulosus* s.s.), goats (91.7%), cattle (90%), sheep (89.4%), and camelids (camel, alpaca; 86.3%). On the contrary, a highest prevalence of G3 variant was documented in water buffaloes (26.2% of animals infected with G3).

Only fertile cysts can ensure the completion of the parasite life cycle, since sterile cysts are not capable of infecting definitive hosts of *E. granulosus*. To compare host susceptibilities for infection with G1 and G3 genotypes, we have compiled data involving both *E. granulosus* s.s. genotyping and information about cyst viabilities. Cysts with viable protoscolices were considered as fertile, whereas sterile cysts and cysts with degenerative modifications (cysts with caseous necrosis and/or calcified cysts) were ranked as non-fertile. For 1947 documented isolates, no significant difference ($p = 0.359$, Fisher's Exact Test) between G1 and G3 viabilities was found (G1 found in fertile cysts, n = 1174; G1 in non-fertile cysts, n = 551; G3 in fertile cysts, n = 141; G3 in non-fertile cysts, n = 63). High fertility rates of *E. granulosus* s.s. were measured especially in humans (80.4%), buffaloes (79.1%), and camels (87.6%).

The liver is the most commonly infected organ for CE in worldwide reports as a result of portal blood flow, followed by lungs. When anatomic locations of cysts were globally evaluated, no significant difference ($p = 0.938$) in numbers of G1 and G3 isolates with respect to their allocation to liver and lungs was found (G1 in liver, n = 625; G3 in liver, n = 87; G1 in lungs, n = 752; G3 in lungs, n = 103). Proportions of G1/G3 distributions in livers and lungs were similar (7.18 and 7.04, respectively). The G3 genotype thus slightly more prefers lungs (6.6% records within the overall G1/G3 sample) over liver (5.6%), but the difference is low. Interestingly, when only human hosts were considered, the p-value was significant, indicating a higher affinity of G3 samples to livers than to lungs ($p = 0.007$, $p < 0.05$).

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Genetic survey of causative agents of cystic and alveolar echinococcoses in central-eastern Europe

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Cystic echinococcosis (CE) and alveolar echinococcosis (AE) caused by *E. granulosus* sensu lato (s.l.) and *Echinococcus multilocularis* cestodes present substantial disease burden and public health economic problem worldwide. To extend knowledge of the genotype spectrum with fragmentary data available for central-eastern Europe, *Echinococcus* isolates derived from 19 pigs, 16 humans, and 1 cattle originating from five countries of central-eastern Europe (Slovakia, Romania, Ukraine, Poland, Hungary) were analyzed. Fragments of mitochondrial genes targeted for PCR amplifications were cytochrome *c* oxidase 1 (*cox1*, 789 bp), ATP synthase subunit 6 (*atp6*,

513 bp), NADH dehydrogenase 1 (*nad1*, 471 bp), and 12S rRNA (*rrnS*, 295 bp). In five *E. multilocularis* isolates from humans in north-eastern Romania, resulting *cox1* haplotypes of four Romanian isolates were identical to the E5 isolate (described by Nakao *et al.*, 2009), which represents the most common European species variant. For the R5 isolate derived from Vaslui county, three nucleotide substitutions were recorded. One of these mutations (411T/G) corresponded to previously described N1 and N2 haplotypes from North America. The peculiar genetic composition of this *E. multilocularis* isolate coupled with the discontinuous distribution of the parasite in recent European territory support a hypothesis that the European clade has been derived from isolated populations in glacial refugia. The data provide first molecular evidence of *E. multilocularis* in clinical samples in Romania. In analyses focused on CE causative agents, in 16 pigs and 2 humans from Slovakia exclusively *E. canadensis* (G7 genotype) was detected. In Hungary, *E. granulosus* s.s. (G1 genotype) was firstly documented in humans, being found in liver and lung cysts of patient from Békés county (south-eastern Hungary). In six CE samples originated from Romania, two human isolates from western part of the country were identified as *E. granulosus* s.s. (G1, G3 genotypes), and two as *E. canadensis* G7. In addition, both human isolates from eastern Romania possessed *E. granulosus* s.s. G1 characteristics. Pig and cattle isolates from western Romania were allocated to *E. canadensis* G7 and *E. granulosus* s.s. G1. In Ukraine, *E. canadensis* G7 was detected in two pig samples (Sumy oblast in north-eastern Ukraine) and in one human isolate recovered from Volyn oblast in north-western Ukraine. The results obtained support the circumstantial evidence that *E. canadensis*, which is regarded as species with low infectivity for humans, is highly prevalent (or exclusive) in Poland, Slovakia and the forest-steppe zone of Ukraine. On the other hand, highly infectious *E. granulosus* s.s. was indicated as the primary causative agent for CE in Romania.
The study was supported by the Grant Agency VEGA (project No. 2/0162/17).

Hybrid *de novo* assembly of the model tapeworm *Hymenolepis diminuta* genome

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The aim of this study was to isolate *Hymenolepis diminuta* (HD) genome, sequence and assemble it *de novo*. DNA was isolated from laboratory strain WMS-il1 underwent Next Generation Sequencing using the Illumina HiSeq 1500 platform. Additional sequencing was performed using a MinION sequencer from Oxford Nanopore Technologies (ONT) and mentioned earlier Illumina HiSeq 1500 system. For Illumina sequencer two types of data were obtained: paired-end tags (mean insert size c.a. 400 bp) and mate-pairs (mean insert size c.a. 7 kbp). The read length for both experiments carried out on Illumina system was equal to 100 bp, for ONT data the mean value of read length was greater than 7 kbp, the size of the longest DNA read reached almost 400 kbp.

Obtained reads were *de novo* assembled in three steps. Fistly, paired-end tags were assembled by ABySS, Velvet and dnaasm applications, the results from other applications were merged by GAM-NGS tool. The number of sequences (greater than 1000 bp) was equal to 6416, value of N50 – c.a. 70 kbp, sum – 166.120 Mbp. Secondly, obtained DNA sequences were scaffolded using mate-pairs by SSPACE application. The N50 increased to c.a. 844 kbp, the number of sequences greater than 1000 bp decreased to 2342 and the sum of DNA sequences increased to 170.838 Mbp. Lastly, sequences obtained from paired-end tags and mate-pairs were joined using ONT sequencing data by LINKS tool. Final value of N50 was equal to 2537 kbp, the number of sequences greater than 1000 bp decreased up to 719 and the sum of the sequences increased to 177.348 Mbp.In this study we showed, that merging data from another sequencing platforms could greatly improve the final results of *de novo* assembling. Especially, combining short DNA reads obtained from next-generation sequencing with long DNA reads obtained from third generation sequencing could significantly improve the length and the quality of the resultant DNA sequences. Moreover, the increasing of coverage of the each sequencing technology above the certain level did not increase assembly results significantly, what we also showed in this study.

Our study shows that combination of emerging genomic technologies is useful approach in producing accurate *de novo* assemblies of helminth genomes.

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Mitochondrial genomics of the tapeworms *Hymenolepis hibernia*

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Hymenolepis hibernia is a cestode parasitizing rodents and is considered as potentially invasive for humans. The aim of this study was to sequence and characterise the mitogenome of *H.-hibernia* and compare it with these available for *Hymenolepis diminuta*.

Adult stages of *H.-hibernia* were collected from naturally infected rodents occurring in Warsaw city region. DNA isolated from *H.-hibernia* underwent Next Generation Sequencing using the Illumina HiSeq 1500 platform. Sequence annotation and gene identification were performed by comparison with published flatworm mt genomes. Obtained whole mitochondrial genome sequences were compared with reference sequence of *H.-diminuta* (GenBank: NC002767).

The *H.-hibernia* mitogenome arrangement is comparable to that observed in *H.-diminuta*. All genes are transcribed in the same direction. Mitochondrial genome of *H. hibernia* is composed of c.-a.-13,8K-bp and consist of 36 genes, including 12 protein-coding genes, 2 rRNA-coding genes, and 22 tRNA-coding genes. The highest percent identity between mitogenomes of *H.-hibernia* and H.-diminuta was observed in genes encoding tRNA. For 11 from 22 tRNA-coding genes the percent identity for homologous genes was higher than 90% (maximum 98%). For the other 11 of tRNA genes observed percent identity varied between 75% and 89%. Whereas, the percent identity for homologous protein-coding genes was lower – the median for the percent identity was 82%, and the higher percent identity was only 88%. The percent identity of sequences for rRNA-coding genes (l-rRNA i s-rRNA) were respectively 86 and 91%.

According to our knowledge our results are the first to show the fully sequenced mitogenome of *H. hibernia*. Comparative mitogenomics of *H. hibernia* and *H. diminuta* indicated important differences between the sequences of the majority of homologous genes. Our results may be useful in the studies of population genetics and molecular systematics of *H. hibernia.* Further phylogenetic analyses should shed more light into the phylogenetic interrelationships among hymenolepidids and other eucestode families.

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Nematode responses towards environmental stress – is relationship the key?

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Free-living soil nematodes and plant parasites were previously repeatedly tested for their use as bioindicators of soil contamination. We investigated soil nematode communities from grasslands in four different industrial areas in Slovakia to estimate their responses to heavy metals. We considered differences or similarities in behavioural reactions of nematodes to different quantities of selected heavy metals in the soil. Using the CCA analysis and t-value biplot diagrams with van Dobben circles, we split individual nematode genera by their tolerance/intolerance to the particular heavy metal into several clusters. Our results showed that (a) representatives of nematode genera showed concurrently positive and negative associations with two or more heavy metals, (b) most nematode genera expressed a strong positive relation with Ni and to a lesser extend with Cr and Zn, (c) taxonomically closely related nematodes did not demonstrated similar responses to the same pollutant. We assume that nematode genera with a higher level of tolerance to trace elements should be prioritized in the heavy metal bioindication instead of sensitive species, due to their longer persistence in the stressed environment and better indicative ability. We pointed nematode genera, which according to received results may serve as suitable sentinels for specific soil pollutants.

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Environmental factors affecting the soil nematode community and microbial biomass

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Comprehensive studies are required to better understand relationships between soil microfauna groups under the influence of various biotic and abiotic factors drive and characterize ecosystems. We analyzed nematoda and microbial diversity and soil properties on three soil types to assess the effect of these environmental variables on biological diversity in natural (forest), semi-natural (meadow) and managed (agriculture) habitats of the Slovak We hypothesized that cultivation may reduce biological diversity and soil food webs will be more coherent in natural habitats; however, the rate of reduction can differ according to the soil types. Our results showed that the highest nematode species number and diversity, nematode functional guilds abundance, values of nematode soil food web descriptors, microbial biomass, microbial richness and diversity as well as values of soil properties was found in the forest grown on Chernozem soil type followed by meadows on Cambisols. The lowest biological diversity and worst properties were observed in agricultural Stagnosol soils. Several nematode species were found to be new for Slovak nematode fauna. The sampling date and the interaction of all three factors (ecosystem × soil × date) had minor or no effect on most of the parameters analyzed, except soil moisture, microbial richness, Nematode channel ratio, nematode Maturity index and Plant Parasitic Index. We conclude that in respect to both biological indicators and soil properties analyzed, natural forest grown on Chernozem soil type is the best habitat for ecological studies among studied ecosystem and soil types. However, depending on aims of the study, semi-natural or managed ecosystems are also valuable, but in combination with Chernozem soil type.

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Changes in nematodes and microbial communities in beech forest after windstorm

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The windstorm which appeared in Slovakia in May 2014 damaged the productive beech deciduous forests in the many areas throughout country. Our study has been carried out in the one of them where all fallen trees were removed from damaged area and as control the close standing vital beech forest not damaged by windstorm have been chosen.

The aim of the study was to investigate as soil nematode communities and microbes react to dramatic changes in their living environment (soil) caused by windstorm trees devastation and their removals. The soil samples to analysis were collected first and third years after windstorm event. Out of soil physical-chemical; soil acidity, N, C, S and K content were slightly lower at the windstorm affected plot, while Mg and Ca content increased in soil samples of clearing. 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 functional and feeding groups in comparison to control. Microbial soil characteristics showed variable trend. While basal respiration, catalase activity, species richness and functional guilds diversity were not significantly affected by trees removals, values of microbial biomass carbon and N-mineralization considerably decreased, indicated degraded living condition for microorganisms than that in the vital forest soil. The study showed that differences occur in the nematode communities, microbial activity and biomass at the disturbed plot in comparison to an intact forest. Future long-term observation could reveal how the changed of ground vegetation in the abundance, richness and diversity of functional guilds of soil nematodes and microorganisms in the ecosystem recovery processes.

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Session II – Parasitology in Genomic, Immunology and Biochemistry Era

Plenary lecture

Gabriela Hrčková, Terézia Mačák Kubašková, Oldřich Benada, Olga Kofroňová, David Biedermann (SK): Naturally Designed Molecular Mixture of Polyphenols in Silymarin and their Effects on Host and Cestodes: One Key for Many Locks

Oral presentations

Libor Mikeš, Lucie Jedličková, Martin Kašný, Ingrid Hrachovinová, Jan Dvořák, Lenka Ulrychová, Marketa Havelková, John Dalton (CZ): Kunitz-Domain Inhibitors in the Biology of a Haematophagous Helminth

Terézia Mačák Kubašková, Gabriela Hrčková, Dagmar Mudroňová (SK): Changes in Peritoneal Myeloid Populations after Combination Therapy with Low Molecular Weight Fraction of Leukocytes in *Mesocestoides vogae* Infected Mice

Robert Stryiński, Mónica Carrera, Jesús Mateos, Santiago Pascual, Ángel F. González, José M. Gallardo, Elżbieta Łopieńska-Biernat, Isabel Medina (PL): Proteomic Analysis and Characterization of Glucose Transport in L3 and L4 Development Stages of *Anisakis simplex* by TMT-Based Quantitative Proteomics

Marta Maruszewska-Cheruiyot, Katarzyna Donskow-Łysoniewska, Maria Doligalska (PL): Nematode Inhibit Maturation of Dendritic Cells Induced by Lipopolysaccharide

Tomáš Macháček, Jan Pankrác, Kerstin Immig, Petr Horák (CZ): *Trichobilharzia regenti* (Schistosomatidae) in Mice: Dynamics of Immune Cells in the CNS and Implications for Parasite Clearance

Bartłomiej Ferra, Lucyna Holec-Gąsior, Karolina Błaszkowska (PL): *Toxoplasma gondii* Tetravalent Recombinant Chimeric Antigens as Good Diagnostic Tools for Detecting IgG and IgM Antibodies in Human Sera

Natalia Kreshchenko (RU): Planarian as a Biological Model for Studying Localization and Physiological Function of Serotonin in Flatworms

Emília Dvorožňáková, Barbora Bucková, Zuzana Hurníková, Andrea Lauková (SK): Oxidative Metabolism of Macrophages in Mice after Probiotic Therapy and *Trichinella spiralis* Infection

Vyacheslav Yurchenko, Danyil Grybchuk, Diego Macedo, Alexei Kostygov (CZ): Viruses in Trypanosomatids

Poster presentations

Jana Ilgová, Jiří Salát, Milan Gelnar, Martin Kašný (CZ): Interactions of Blood-Feeding Monogenean Parasite with the Fish Host Immune System

Bohumil Sak, Michaela Kotková, Martin Kváč (CZ): Revision of Effect of Adaptive Immune Response to Control Microsporidiosis Induced by *Encephalitozoon cuniculi*

Małgorzata Dmitryjuk, Janina Dziekońska-Rynko, Katarzyna Mierzejewska (PL): Influence of Effectors on the Activity of Trehalose Synthesis Enzymes from Parasitic Nematode *Cystidicola farionis*

Elżbieta Łopieńska-Biernat, Robert Stryiński, Karol G. Makowczenko, Tomasz Borkowski, Iwona Polak (PL): Single-Nucleotide Polymorphism (SNP) Analysis in the ABC Transporter Genes of *Anisakis simplex* s.s.

Barbora Bucková, Gabriela Hrčková, Emília Dvorožňáková, Andrea Lauková (SK): Effects of Probiotic Strains on Cytokine Expression in Murine Trichinellosis

Natalia Kreshchenko, Nadejda Terenina, Semen Bondarenko (RU): Immunocytochemical Identification of Serotonin 5HTR7 Receptor in the Flatworms *Hymenolepis diminuta* and *Opisthorchis felineus*

Berezhko V.K., Novik T.S., Kamyshnikov O.Yu., Koveshnikova E.I., Danilova T.I, Napisanova L.A., Chukina S.I., Uspensky A.A., Thakahova A.A., Rudneva O.V. (RU): Effects of *Echinococcus multilocularis* Cyst Fluid on Proliferation of Burkitt's Lymphoma Cells (Raji) *in vitro*

Karolina Błaszkowska, Bartłomiej Ferra, Lucyna Holec-Gąsior (PL): New Trivalent SAG1-SAG2-GRA1 and SAG1-SAG2-GRA2 Recombinant Antigens Used for Detection of IgG Antibodies against *Toxoplasma gondii* in Human Sera

Kateřina Skulinová, Jan Novák, Libuše Kolářová, Martin Kašný (CZ): Characterization of Immunodominant Antigens in *Toxocara canis* L3 Larvae Excretory-Secretory Products

Lucie Škorpíková, Nikol Reslová, Edoardo Pozio, Martin Kašný (CZ): Differentiation of Eight *Trichinella* Species Using a High Resolution Melting Assay

Novik T.S., Berezhko V.K., Koveshnikova E.I., Danilova T.I, Napisanova L.A., Chukina S.I., Kamyshnikov O.Yu., Uspensky A.A., Thakahova A.A., Rudneva O.V. (RU): *Toxocara canis* as an Inhibitor of Mcf-7 and Caco-2 Tumor Cell Proliferation *in vitro*

Edina Szestáková, Alžbeta Königová, Ladislav Molnár, Martin Babják, Peter Major, Štefánia Megyesi, Michaela Urda Dolinská, Marián Várady (SK): Dynamics of Changing Hematological Indicatore in Experimental Parasite Infection *H. contortus* in Mouflons (*Ovis musimon*)

Gabriela Hrčková, Dominika Faixová, Terézia Mačák Kubašková, Dagmar Mudroňová, David Biedermann (SK): Flavonolignan Silybina/B as Adjunct to Albendazole Therapy of Model *Mesocestodes vogae* Infection in Mice

Ivana Nemčovičová (SK): Development of anti-HCMV agents by using the host-pathogen immune interaction

Naturally designed molecular mixture of polyphenols in silymarin and their effects on host and cestodes: One key for many locks.

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Proliferative larval stages of several cestode species cause serious diseases in humans and animals and therapy of chronic infections is complicated. *Mesocestoides vogae* (syn. *M. corti*) larvae possess an ability of asexual multiplication in various vertebrate hosts and can be easily maintained *in vitro* in axenic conditions. Higher plants are able to synthetize the specific compounds, identified as secondary metabolites. Among them a large group of flavonoids have multiple beneficial effects on vertebrate hosts and their antiparasitic activities are subject of intensive research. Silymarin (SIL) is a mixture of flavonolignans and flavonoids and has been used for the centuries in liver diseases. Due to the low oxygen supply in the tissues occupied by flatworm parasite in the hosts, we have investigated both aerobic and hypoxic conditions on *M. vogae* larvae. We found that larvae possess a very flexible mitochondrial metabolism, energy-generating biochemical pathways and antioxidant systems. These unique features of larvae make them a suitable model for pharmacological studies. We have employed *M. vogae* larval infection in mice, *in vitro* axenic/hypoxic larval cultures as well as several cell-based assays to examine the effect of SIL and its main components: silybin (SB), 2,3-dehydrosilybin (DHSB) and silychristin (SCH). Of these compounds, only 2,3-dehydrosilybin exhibited significant larvicidal activity *in vitro*. We have demonstrated that all three compounds markedly suppressed metabolism, respiration and energy reserves in the form of glycogen, glucose and neutral lipids in the order DHSB > SCH > SB, which was associated with changes of larval morphology. In addition, DHSB inhibited transport of glucose through the tegument of larvae, caused the damage to the surface and down-regulated enzymatic activity of glutathione S-transferase as well as GSH, suggesting a high affinity for membrane associated functional proteins. We showed that SB and SCH could cause changes in physiological homeostasis and starvation of larvae probably by interfering with certain intracellular enzymatic systems. 2,3-DHSB caused complete disruption of larval physiology. SIL showed dose-depended immunomodulatory effect on primary immunocytes *in vitro* what was demonstrated also in infected mice treated with SIL alone or more in combination with praziquantel (PZQ). In mice, the combined therapy resulted in the significantly higher efficacy due to the complex activities of individual SIL components and synergistic immune-dependent effect of SIL and PZQ on tegument. Direct antioxidant activity of SIL contributed to decreased liver fibrosis, pathology and attenuation of Th2/Treg biased immunosuppression. Our studies emphasized the high pharmacological potential of flavonoids and demonstrated that silymarin co-administration with anthelmintic drug is a more effective treatment option *vs* monotherapy due to multiple molecular targets of its individual components in the hosts and in the helminths.

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Kunitz-domain inhibitors in the biology of a haematophagous helminth

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Serine peptidases are involved in many physiological processes. They often build enzymatic cascades which are regulated by specific endogenous inhibitors balancing their function. Parasites possess analogous inhibitors which help them to modulate these proteolysis-based processes to their own benefit. Thus, blood-feeders regulate functioning of the coagulation cascade, complement, and immunity in the host by various inhibitors, often belonging to Kunitz type protein family. In the present study, we revealed potent inhibitory activities targetting proteolytic factors of coagulation cascade in homogenates of the blood-feeding monogenean *Eudiplozoon nipponicum* parasitizing common carp. Additionally, mining data from *E. nipponicum* transcriptome, we found several transcripts encoding Kunitz domain proteins. We further focused on EnKT1 that is structurally similar to textilinin-1, an antihaemorrhagic factor from the venom of the Australian brown snake *Pseudonaja textilis*. The protein was expressed *in vitro*, purified, and characterized biochemically. It has been shown as a potent inhibitor of factor Xa of the coagulation cascade, but had even lower IC_{50} values with plasmin and plasma kallikrein that participate in fibrinolysis, production of kinins, and in complement activation. Anticogulation properties of EnKT1, but no effect on fibrinolysis were confirmed by tromboelastography with whole human blood. Recombinant EnKT1 also blocked the cytolytic activity of fish complement. Localization within haematin digestive cells of the parasite, as shown by *in situ* hybridization and immunohistochemistry, suggests that it is likely involved in the process of blood digestion, where it may prolong the time needed for coagulation, thus providing a longer time frame for digestive peptidases to degrade soluble proteins including clotting factors and fibrinogen in bloodmeal. Moreover, since fXa and plasmin act as both C3 and C5 convertases, the inhibition of these two host peptidases may prevent complement activation inside parasite's gut, thus avoiding damage to the cells of gastrodermis.

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Changes in peritoneal myeloid populations after combination therapy with low molecular weight fraction of leukocytes in *Mesocestoides vogae* infected mice

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Metacestodes of tapeworm *Mesocestoides vogae* proliferate asexually in the liver and in peritoneal cavity of their intermediate hosts. In an experimental model of larval cestodiasis, infection of mice with metacestodes leads to the destruction of hepatic tissue and development of ascites in the peritoneal cavity. Generally, expansion of metacestodes in hosts' tissues induces a Th2-type immune response.

A potential immunomodulatory effect of low molecular weight leukocyte extracts (DLE) from human donors (IMMODIN, ImunaPharm, Slovakia) in combination with standard therapy (albendazole) was investigated in mice with experimental *Mesocestoides vogae* infection. In particular, this study has focused on the changes in myeloid lineage cells (peritoneal exudate cells) co-localized with larvae in the peritoneal cavity of mice by flow cytometry and by cytokine levels/gene expression produced by these cells in peritoneal exudates. Preliminary results showed that combination treatment with ABZ and DLE contributed to the reduction of $CD11b^{high}Gr\text{-}1^{+}$ myeloid cells in infected mice and this population of cells also expressed higher levels of F4/80 and MHC class II molecules (markers present on mature macrophages). Macrophages and eosinophils play a central role in the response to parasitic infection. Therefore, we investigated whether combination treatment has effect on the polarization of myeloid cells. We found that mRNA expression of genes for alternative activation markers (Fizz-1 and YM 1) was down-regulated within the peritoneal cells after administration of DLE alone and in combination with albendazole. On the other hand, the expression of pro-inflammatory markers (iNOS, IL-6, TNF-α) was up-regulated. Based on these results, we assume that combination treatment may modulate macrophage state from anti-inflammatory (M2) to a more pro-inflammatory phenotype (M1). We have previously found that this adjuvant treatment skew the lymphoid immune response of infected mice towards Th1 type. It seems that the conversion of macrophages phenotype could be response to the local signals, especially the higher level of IFN-γ in peritoneal cavities of treated mice. This hypothesis will be confirmed by an *in vitro* experimental model in a future study.

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Proteomic analysis and characterization of glucose transport in L3 and L4 development stages of *Anisakis simplex* by TMT-based Quantitative Proteomics

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Anisakis simplex (*A. simplex*) is a parasitic nematode that can causes anisakiosis and/or allergic reactions in humans. Presence of invasive L3 larvae was documented in 200 species of fish and 25 species of cephalopods around the world. World Health Organization and food safety authorities aim to control and prevent this emerging health problem. In this work, using the tandem proteomics with isobaric mass tags (tandem mass tags, TMT), the global proteome of L3 and L4 development stage of *A. simplex* was analyzed. The strategy was divided into four stages: (a) extraction of the L3 and L4 larvae proteins, (b) trypsin digestion assisted with high intensity focused ultrasound (HIFU), (c) high pH reverse phase fractionation (d) global proteome analysis (LC-MS/MS) of L3 and L4 *A. simplex* development stage using LTQ-Orbitrap Elite mass spectrometer. Total of 2443 different proteins were identified, where the results showed a high degree of overlap (1542 different proteins) between L3 and L4 of *A. simplex*. In addition, a high amount of proteins specific only for L3 (330) or L4 (571) were identified and quantified. The most complex nodes of the interaction network in the global proteome were those associated with energy metabolism, regulation of muscle contraction, protein catabolic processes, oxidative metabolism. Attention was given to proteins responsible for glucose transport and metabolism. 27 proteins were identified which taking a part in glucose transport and metabolic processes. Their participation in important for parasitic nematodes processes, could become a target in research on antiparasitic drugs. Using Geneious v.8.0.2, based on the phylogenetic relationships with *Brugia malayi*, *Toxocara canis* and *Ascaris suum*, partially sequenced genome of *A. simplex* allowed to determine the glucose transporter genes sequences (facilitated glucose transporters, FGT) FGT-1, FGT-3a, FGT-3b, FGT-5, FGT-9 and new class of glucose uniporters SWEET- sweet1. The presence of mRNAs of *fgt-1, fgt-3a, fgt-3b, fgt-5, fgt-9* and *sweet1* genes in L3 and L4 of *A. simplex* larvae was first identified using qRT-Real-time PCR. In the media with glucose at a concentration of 0.1, 0.5, 2, 10, 15 and 20 mg/mL were observed differences in genes expression between development stages and applied media, RPMI-1640 and 0.9% NaCl. In L3, higher mRNA expression of genes of glucose transporters was observed after 12h and at lower glucose concentrations (0.5 mg/mL) in NaCl than in RPMI-1640. In contrast, cultivation of L4 stage in NaCl only after 24h with higher glucose concentrations (10 mg/mL) affected the genes expression of glucose transporters. The presented research scheme is a relatively new applied strategy in parasitology, the use of which can provide a lot of valuable information and help in finding an effective treatment for anisakiosis and eradication of invasive L3 *A. simplex* larvae ensuring the safety to the consumers.

Nematode inhibit maturation of dendritic cells induced by lipopolysaccharide

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Helminths are strongly proposed as a source of therapeutic compounds against autoimmunological disorders and allergies. The main group of antigen presenting cells; a dendritic cell (DC) has significant influence in autoimmune diseases development as well as multicellular parasites immunoregulatory effect. Helminths including nematodes, can inhibit maturation of DC, hence results in decreased proinflammatory cytokine production and higher expression of costimulatory molecules on the surface. Toll-like receptors (TLR) play a significant role in maturation of DC which belong to transmembrane pattern recognition receptors (PRRs). Lipopolysaccharide (LPS) is a bacterial origin ligand for TLR-4 which induces maturation of DC.

The aim of this study was to evaluate the ability of live pre-male and pre-female *Heligmosomoides polygyrus* L4 stage to inhibit or reverse reaction of immature dendritic cells for stimulation with TLR4 ligand. Immature DC JAWSII were cultured with live *Heligmosomoides polygyrus* L4 stage isolated from intestine of BALB/c mice with colitis inducted by 3% DSS, for 2h and LPS (ligand for TLR-4) for next 46h or vice versa. Proinflammatory cytokines and chemokines: IL-6, MCP-1 and TNF-α level in cell culture medium were examined by ELISA method. Surface receptors expression: CD40, CD80 and MHCII on JAWSII cells were analyzed by flow cytometry.

Live *H. polygyrus* L4 stage inhibited production of proinflammatory cytokines, chemokines and costimulatory molecules expression induced by LPS in JAWSII cells.

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Trichobilharzia regenti (Schistosomatidae) in mice: dynamics of immune cells in the CNS and implications for parasite clearance

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The neuropathogenic schistosome *Trichobilharzia regenti* is reported from many European countries. It infects the central nervous system (CNS) of vertebrates, e.g. ducks or mice. While it matures and lays eggs in ducks, it is eliminated by host immune response in mice. Clearance of the parasite is observed within a few weeks and peripheral leukocytes infiltrate the CNS. In this study, we characterized the dynamics of immune cells present in the CNS of infected mice by flow cytometry. The influx of granulocytes and T-cells into the spinal cord peaked 14 days post infection (dpi). Microglia expanded during the disease, and they represented the major MHC II+ population. Interestingly, granulocytes and T-cells infiltrated also the brain, mainly 21 dpi, although the brain was mostly free from parasite DNA. Contrary to the spinal cord, expression of MHC II by brain microglia was inconspicuous, suggesting rather "a resting state". Additionally, we investigated the role of nitric oxide (NO) in clearance of the parasite because NO can be produced by granulocytes and activated microglia. Indeed, these cells account for the most prominent cell populations in the spinal cord during the period when the body of the parasite is damaged. However, no effect of NO on parasite mortality and surface or internal tissue pathology was noticed after 48-hour *in vitro* incubation with NO-donor. Taken together, granulocytes are the major population of cells infiltrating the spinal cord of mice infected by *T. regenti*, and their role in parasite clearance should be clarified. NO, as an antiparasitic agent possibly produced by granulocytes, is most likely not harmful to *T. regenti*.

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Toxoplasma gondii tetravalent recombinant chimeric antigens as good diagnostic tools for detecting IgG and IgM antibodies in human sera

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The intracellular parasite *Toxoplasma gondii* has the ability to infect a wide range of warm-blooded animals, including humans. From a medical point of view, the correct recognition of *T. gondii* invasion is very important in the case of pregnant women. This is associated with a significant risk of tachyzoites transmission *via* the placenta to the fetus, which may lead to miscarriages or to cause neonatal malformations, neurological damage, and blindness in newborns. Detection of parasite invasion is also significant for patients with immunodeficiency, for whom even the chronic phase of toxoplasmosis can be a serious threat. For immunocompromised patients, infection with *T. gondii* may cause serious problems, such as fever, headache, encephalitis, pneumonia, myocarditis, conjunctivitis, and nervous system damage

Currently, the diagnosis of toxoplasmosis is based mainly on the use of native antigens in enzyme immunoassay, which allows for the detection of IgG, IgM and IgA antibody classes. However, in some cases, the performed studies gives ambiguous results. For this reason, some research groups are currently working on a new diagnostic tools, which are mainly recombinant antigens. Compared to the native antigens their production is much easier, cheaper, faster and safer. An additional advantage of the recombinant antigens is easier way to standardize assays.

In this study, the efficient *Escherichia coli* expression systems for the production of different variants of recombinant chimeric antigens composed of the immunodominant regions of four selected *T. gondii* antigens (AMA1, GRA1, ROP1 and SAG2) has been constructed. In the next part of the work, the diagnostic usefulness of obtained proteins for detection of specific antibodies from human sera in the IgG and IgM ELISAs were evaluated. The results obtained for the tetravalent chimeric antigens were compared with those of IgG and IgM ELISAs based on *Toxoplasma* lysate antigens (TLA), and previously described trivalent recombinant chimeric protein SAG2-GRA1-ROP1 (Ferra et al. 2015). These studies showed that the newly produced tetravalent chimeric antigens could be an alternative to the TLA for the detection of anti-*T. gondii* antibodies found in the sera of patients with diagnosed toxoplasmosis (high sensitivity and specificity of ELISAs assays). Moreover, it was observed that during the construction of chimeric antigens in addition to a rational choice of proteins which exhibit strong immunogenic properties, particular attention should be also paid to the size of protein fragments and its amino acid sequences.

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Planarian as a biological model for studying localization and physiological function of serotonin in flatworms

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Planarians (Turbellaria, Platyhelminthes) are simple organisms in which the centralization of nervous system with cephalic ganglion and main nerve cords has firstly occurred in the evolution. In the absence of circulatory and true endocrine systems the nervous system plays an important role in the realization of nervous and humoral regulation of main vital functions of the organism. Being a well differentiated flatworms' nervous system produces a number of neuromediator molecules, such as acetylcholine, GABA, catecholamines, serotonin and various neuropeptides. Most of the cells in their bodies are connecting with each other and with environment by chemical messengers synthesizing by neurones. Turbellarians are also characterized by unprecedented regenerative ability. Planarians can restore any part of their body including pharynx, intestine, eyes and "brain" – the cephalic ganglion. They possess of pluripotent stem cells, which are the source for the restoration of morphological structure of tissue and organs after injury or everyday cell losses. To identify the endogenous and exogenous factors regulating the morphogenetic processes, including cell proliferation, differentiation, growth and reproduction the role of serotonin (5-HT) in planarians (Platyhelminthes, Turbellaria) has been studied. Physiological functions of serotonin remain poorly investigated due to the absence of adequate experimental models amongst parasitic flatworms. For detection of morphogenetic futures of serotonin the qualitative and quantitative models - head ganglion, pharynx and eyes regeneration, and a process of asexual division - were employed. Classical and modern methods of immunocytochemistry, cell biology, computer morphometry, confocal laser scanning microscopy were used.

The immunocytochemical study of localization of serotonin in planarians *Girardia tigrina* and adult parasitic trematodes *Opisthioglyphe ranae*, *Opisthorchis felineus* and *Gymnophallus deliciosus* has been performed. In parasitic worms the serotonin-immunoreactive nerve cells and fibers were revealed in the head ganglia, circular commissure, longitudinal nerve cords and their connective commissures, as well as around the oral and ventral suckers, oesophagus and genital pore. In planarians immunostainig to serotonin was identified in central and peripheral compartments of the nervous system, mostly in bipolar and multipolar neurons located in the vicinity of the brain arch formed by serotoninegric nerve fibres and comprising of 62-78 cell bodies, as well as in the nerve nods (containing 5-7 neurons) regularly distributed along the ventral nerve cords and located at the intersection of the nerve cords and transverse nerve commissures. The data support the idea that presence of serotoninergic elements in trematodes and planarians is a conservative trait characterizing the helminthes which belong to different taxa and have different hosts as well as localization inside them. Free-living planarians have also demonstrated a more developed serotoninergic system compare to parasitic species.

The dynamics of development of serotoninergic nerve components was for the first time described in details in dissected tail fragments of *G. tigrina* in course of its head ganglia regeneration. Observations indicated that differentiation of serotoninergic neurons in regenerating animals has occurred within 5-7 days post amputation. The gradual increase in the number, size, and intensity of neurotransmitter expression in newly differentiating serotoninergic neurons was observed from day 3 to day 7 of regeneration.

The role of serotonin in regulation of asexual reproduction and cell proliferative activity was investigated. Serotonin in concentrations of 0,1–10μM has produces the inhibiting effect on process of asexual division in two planarian species *G. tigrina* and *Schmidtea mediterranea*. Serotonin has also demonstrated an influence on daily dynamics of cells proliferation in planarian tissue estimated every 2 hrs during 24 hrs. On day 3 after serotonin treatment (1μM) the rhythmic mitotic activity with the period of about 4 hrs observed in control group of *S. mediterranea* has changed. In experimental group of animals the rhythmic fluctuations were smoothen and the amount of mitotic cells has decreased in dark time (82-98 cells per mm^2), and rose during daylight period (up to 112-145 cells per mm^2). The possible role of serotonin in the modulation of daily proliferative activity and/or formation of circadian rhythms in the organisms can be discussed.

Thus, new data will make progress in understanding role of serotonin in regulation of important biological functions in planarians, including morphogenetic. Planarians can be successfully used as an experimental model for the investigation of mechanisms that launch and implement the regeneration and reproduction processes in flatworms.

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Oxidative metabolism of macrophages in mice after probiotic therapy and *Trichinella spiralis* infection

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Probiotic bacteria confer a beneficial property to the host as immune stimulation, protection against pathogens; and have the capacity to control parasite infection, too. Their immunomodulatory activity of the non-specific immunity is expressed through inhibition of inflammatory response, stimulation of phagocytic activity and activation of antigen presenting cells. Macrophages are antigen presenting cells – essential in the process of phagocytosis, they release cytokines and regulate inflammation. After contact with the antigen, they stimulate and produce reactive oxygen species that are highly toxic to parasites. Superoxide anion O_2^- is the basic component of macrophage activity. This study was focused on the effect of probiotic and bacteriocin-producing bacterial strains on the production of superoxide anion in peritoneal macrophages of *Trichinella spiralis* infected mice.
The strains (*Enterococcus faecium* EF55, *Enterococcus faecium* 2019 = CCM7420, *Enterococcus faecium* AL41 = CCM8558, *Enterococcus durans* ED26E/7, *Lactobacillus fermentum* AD1 = CCM7421, *Lactobacillus plantarum* 17L/1) were administered to mice daily at the dose of 100μl (10^9 CFU/ml) and mice were infected with 400 *T. spiralis* larvae on the 7th day of probiotic therapy. Production of the superoxide anion in the peritoneal macrophages was detected *ex vivo*. The *E. faecium* EF55 and *E. faecium* CCM8558 strains increased the O_2^- production prior to parasitic infection, on the day 7 of application. The administration of lactobacilli and enterococci to mice significantly stimulated the metabolic activity of macrophages throughout the experiment, with the exception of the day 18 after infection (p.i.), when a significant inhibition of the superoxide anion formation occurred in the mice with application of *E. durans* ED26E/7. The most significant immunomodulatory effect on the peritoneal macrophage metabolic activity during the intestinal phase of *T. spiralis* infection showed the strains *E. faecium* CCM7420 and *E. faecium* CCM8558, and during the muscular phase of infection the strains *L. fermentum* CCM7421 and *L. plantarum* 17L / were also positively useful.
The increase in the metabolic activity of peritoneal macrophages induced by probiotic therapy indicates a positive anti-inflammatory modulation of the host immune response to *T. spiralis* infection. Therapeutic approaches with probiotics could help to reduce the risks of infestation by parasites or complement classical anti-parasite treatments.
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Viruses in trypanosomatids

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Viruses in general and RNA viruses in particular are capable of infecting virtually any cellular life form on Earth accounting for their immense diversity. Since present-day research is mostly focused on viruses pathogenic to vertebrates and economically relevant plants, the real diversity of viruses is unknown and largely underexplored. The study of RNA viruses in protists is an emerging field, with studies ranging from mere reports of the virus-like particles to full descriptions including nucleotide sequences. Virus-like particles in trypanosomatids were first documented over four decades ago in *Leishmania hertigi*, with their characterization mostly limited to ultrastructural studies. The first virus characterized in molecular terms was the *Leishmania RNA virus 1* (LRV1) of the family *Totiviridae*. Its host, *Leishmania guyanensis*, is a causative agent of the highly aggressive mucocutaneous form of leishmaniasis. The LRV1 contributes to the pathogenicity through the increased survival and metastatic potential of the parasite.
In this work we investigated diversity, stability, and evolution of RNA viruses in monoxenous trypanosomatids within the subfamily Leishmaniinae and *Phytomonas* spp. In addition to discovering a number of novel viruses belonging to different supergroups (*Narnaviridae*, *Bunyavirales* and uncharacterized family of tombus-like viruses), for the first time we documented the presence of the Endogenous Viral Element in trypanosomatids. We attempted to infer scenarios of evolution of all these divergent viruses and concluded that they could have independently originated from viruses of fungi, insects, and non-insect terrestrial invertebrates.
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Interactions of blood-feeding monogenean parasite with the fish host immune system

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Parasites use wide repertoire of molecules to manipulate the host's immune system. Such interaction ensures the increased chances for parasite survival and reproduction. Nevertheless, the molecular communication between the fish host and parasitic worms from the group Monogenea has been topic omitted by the research.

We chose experimental host-parasite system – blood-feeding, ectoparasitic *Eudiplozoon nipponicum* (Monogenea: Diplozoidae) and its typical host – common carp (*Cyprinus carpio*) and identified multiple parasite's molecules with immunomodulatory potential. We selected cysteine peptidase inhibitor, also called type I cystatin or stefin (EnStef), which we recognized as relatively highly expressed, for further immune assays.

After *in vitro* incubation of fish macrophages with recombinant EnStef we analysed the expression levels of pro- and anti-inflammatory cytokines (TNF-alpha, IL1-beta, IL-6, IL-8, IL-10) produced by the cells. Our preliminary results suggest that EnStef may cause down-regulation of studied cytokines.

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Revision of effect of adaptive immune response to control microsporidiosis induced by *Encephalitozoon cuniculi*

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This study determined the effect of Albendazole on Encephalitozoon cuniculi genotype II course of infection in immunocompetent BALB/c and C57Bl6 mice and immunodefficient SCID, CD4-/-, and CD8-/- mice. PCR and qPCR were used to detect and quantify E. cuniculi infection in tissues throughout the body.

Following oral inoculation, an acute infection, characterized by intense dissemination of microsporidia into most organs, developed in both immunocompetent and immunocompromised mice. Immunocompetent and CD4-/- mice subsequently developed a chronic, non-lethal infection, whereas CD8-/- and SCID mice developed a lethal infection. The administration of Abendazole during the acute phase of infection reduced microsporidia to non-detectable levels in immunocompetent and CD4-/- mice, and this effect persisted beyond the treatment course. Albendazole reduced the microsporidia infection in SCID and CD8-/- mice; however, in contrast to observations in immunocompetent and CD4-/- mice, microsporidia infection increased following the cessation of Albendazole treatment. The post-treatment infection in SCID mice was lethal, similar to that in untreated SCID mice. However, post-treatment, CD8-/- mice surprisingly survived untill the end of the experiment, despite a massive microsporidia burden (up to 109 spores per 1 g of tissue), suggesting a mechanism other than cell mediated cytotoxicity is responsible for survival following Albendazole treatment. Understanding how microsporidia survive in hosts despite a competent immune response and how infected hosts tolerate an enormous spore burden in their tissues without any clinical signs could significantly contribute to research related to human health.

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Influence of effectors on the activity of trehalose synthesis enzymes from parasitic nematode *Cystidicola farionis*

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Cystidicola farionis Fischer, 1798 (Habronematoidea: Cysticolidae) is a nematode which infects fishes from Salmonidae and Osmeridae in Europe, Asia, and North America. *C. farionis* cause destruction of the highly vascularized swim bladder walls - physically during parasite movement as well as due to toxic metabolites secretion.

It can affect swimming performance and buoyancy control of infected fish.
In the available literature, there is no information about the trehalose metabolism of *C. farionis*.
Disaccharide trehalose (α-D-glucopyranosyl-1,1-α-D-glucopyranoside) is common in nematodes where has a number of key functions as: energy source, assisting glucose uptake and protecting against environmental stress. In most eukaryotes, including nematodes, trehalose synthesis is catalysed by two enzymes: trehalose-6-phosphate synthase (TPS, EC 2.4.1.15) and trehalose-6-phosphate phosphatase (TPP, EC 3.1.3.12). The end products of this pathway are trehalose and inorganic phosphate P_i.
The research material consisted of larvae and adults of *C. farionis* isolated from the swim bladder of European smelt from Vistula Lagoon (Poland). In the larvae and adults homogenates of nematode the influence of effectors on the activity of TPS and TPP were determined. To assess the influence of chemical compounds, 1, 2, 5, 10, 50 and 100 mM solutions of the KH_2PO_4, $MgCl_2$ and ethylenediaminetetra-acetic acid (EDTA) were used. The activity of the control sample, with water instead of the effector, was assumed as 100%.
The best activators of TPS and TPP from larvae and adult parasites were 5 and 10 mM solutions of $MgCl_2$. The strongest activator of TPS was 10 mM magnesium chloride (the increase to 165% in larvae and 208% in adults). 10 mM chloride raised TPP activity twice (202%) in larvae. In adults 5 mM and 10 mM $MgCl_2$ activated TPP more strongly – to 275% and 233%, respectively. Ethylenediaminetetra-acetic acid was inhibitor of the trehalose synthesis pathway from both sources (larvae and adults). Already 1 mM EDTA solution inhibited T6P synthase from both sources by about half. While 10 mM and 50 mM solutions of this component completely inhibited TPS in larvae and adults homogenates, respectivelly. We observed lack of TPP activity in larvae and adults at the addition of EDTA of 50 mM and 100 mM, respectively. KH_2PO_4 was also inhibitor of trehalose synthesis pathway. The effect was particularly pronounced in the case of T6P synthase. Additionally, both enzymes from adults parasites were more sensitive to the phosphoran than enzymes from larvae. KH_2PO_4 completely inhibited these enzymes in mature parasites at concentration 10 mM and 50 mM for TPS and TPP, respectively.
Acording to our data, the addition of magnesium ions activates the trehalose synthesis pathway. Chelating agent - EDTA inhibits trehalose synthesis reactions in *C. farionis* already at low concentrations. Inorganic phosphate, which is the final product of the sugar synthesis pathway, inhibits particullary the first enzyme of the trehalose synthesis pathway - TPS.

Single-nucleotide polymorphism (SNP) analysis in the ABC transporter genes of *Anisakis simplex* s.s.

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Nematodes belonging to the genus *Anisakis* are important parasites due to their abundance in seafood. Raw or inadequately thermally processed fishery products that are contaminated by third stage *Anisakis* larvae (L3), represent a risk factor for human, which are accidental hosts for this nematode. Most of the cases of anisakiasis have been reported in Japan where there is a great consumption of raw fish, but the number of cases is increasing worldwide, even in Europe and in the Mediterranean region due to the increased practice of raw fish consumption. The presence of *Anisakis simplex* is observed in herring and cod of the Baltic Sea. The anisakiasis is considered a rare disease even if the incidence is probably underestimated in many countries.
Members of the ABC transporter superfamily, particularly P-glycoproteins (PGPs) and multidrug resistance proteins (MRPs) have broad substrate specificity (e.g., antibiotics and chemotherapeutic agents). The diversity of nematode P-gps suggests that they may play a protective role in the efflux of a wide range of environmental toxins and internal metabolites. The effectiveness of combating anisakiasis is unsatisfactory and one of the reasons may also be drug resistance. Getting to know the ABC transporters genes of L3 *A. simplex* larvae may be the first step in finding an answer if the parasite is sensitive to the effects of drugs. The purpose of this work was to identify the sequence of ABC transporter superfamily (A-H) genes, as efflux pumps, influence the bioavailability and disposition of drugs through active efflux of compound out of cells, using Next-Gene Sequencing (NGS). In selected 24 genes, all potential nucleotide variations (SNVs) were identified, based on the results of mapping short paired-end sequences to their sequences. The largest polymorphism exhibiting amino acid substitutions was found in 2 genes: ATP-binding cassette sub-family E (member 1,3). The variation of the nucleotide sequence of the obtained PCR products was studied in 20 L3 *A. simplex* individuals, using the screening method SSCP (Single Strand Conformation Polymorphism). The purified PCR products were sequenced and the results were analyzed

using Geneious 11.1.2. Sequencing of the PCR product confirmed the occurrence of polymorphism in 20% of the tested nematodes. The analysis performed is a prelude to the knowledge of the properties, expression, and action of drugs on these transporters, which can be the basis for the diagnosis of anisakiasis.

Effects of probiotic strains on cytokine expression in murine trichinellosis

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The protective response developed against *Trichinella spiralis* infection provokes immune and inflammatory responses mediated by cytokines released from T helper cells. The *T. spiralis* infection evoked a mixed Th1/Th2 immune response, with initial predominance of Th1 response in the intestinal phase and subsequent Th2 response in the muscle phase. Probiotic bacteria modulate also cytokine release, which play a central role in maintaining the balance between essential and excessive activation of immune defense mechanisms. The aim of this study was to evaluate the effect of probiotic therapy on gene expression of Th1 (IFN-γ) and Th2 (IL-4, IL-5, IL-10) cytokines in the small intestine and spleen of mice experimentally infected with *T. spiralis*. Two bacterial strains of different origin (*Enterococcus faecium* CCM8558 and *Lactobacillus fermentum* CCM7421) were administered daily in dose of 10^9CFU/ml in 100 µl *per os* and mice were infected with 400 larvae of *T. spiralis* on 7th day of treatment. The expression of IFN-γ in the intestinal tissue of untreated infected mice had 6-fold increase on day 18 post infection (p.i.) and a high expression of regulatory cytokine IL-10 was detected on days 5 (6-fold) and 18 p.i. (5-fold). Both probiotic strains prevented the growth in IFN-γ and IL-10 expression. Also during the muscle phase of the infection, the expression of IL-10 was downregulated in the gut of treated mice. The stimulation of IL-5 expression in the intestine was observed in untreated infected mice during the intestinal phase, till day 18 p.i., but probiotic therapy shorted it till day 11 p.i. The IL-4 gene expression, a cytokine supporting larval growth in trichinellosis, peaked in the gut of untreated infected mice at day 18 p.i. and in mice treated with *L. fermentum* CCM7421 at day 11 p.i. and in next days, its expression was very low. The strain *E. faecium* CCM8558 did not support IL-4 gene expression in the gut. The IL-4 gene expression corresponds with the parasite presence in the host intestine. The immunomodulatory effect of probiotic therapy on gene expression of IFN-γ and IL-10 in the spleen of infected mice was demonstrated only by *E. faecium* CCM8558 with a significant increase in both cytokine mRNA levels on day 18 p.i. (Th1/Th2 immune response). The levels of IL-5 mRNA in the spleen of infected untreated mice were increased in the late intestinal and early muscular phases, but probiotic strains significantly inhibited IL-5 expression on day 25 p.i. A sharp increase of IL-4 mRNA level was detected on day 18 p.i. in untreated infected mice and mice treated with *E. faecium* CCM8558. Strain *L. fermentum* CCM7421 caused a significant reduction in gene expression of this cytokine in the spleen on days 18 and 25 p.i., and *E. faecium* CCM8558 only on day 25 p.i.

The obtained results suggest the anti-inflammatory effect of both probiotic strains during the intestinal phase of trichinellosis and *E. faecium* CCM8558 regulated the immunological balance in the early muscle phase. The gene expression of IL-5 was associated with the intestinal phase and was inhibited by probiotic therapy. The early muscle phase of trichinellosis in the spleen was accompanied with increased IL-5 expression which was stimulated by *L. fermentum* CCM7421. The IL-4 gene expression was reduced by probiotic strains during the muscle phase, but *L. fermentum* CCM7421 increase its expression in the intestinal phase. The probiotic strains used different modulating ways to protect the host against parasite infection and their modulating effect is strain-specific.

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Immunocytochemical identification of serotonin 5HTR7 receptor in the flatworms *Hymenolepis diminuta and Opisthorchis felineus*

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Flatworm's nervous system, in spite of the low evolutionary position of these organisms, is well differentiated and comprises a number of specific neurons synthesizing a variety of signal molecules including acetylcholine, GABA, FMRF- and NPF-like peptides and serotonin (5-HT). The localization of serotonin was shown in central and peripheral compartments of the worm's nervous system and its major function is thought to be the regulation of motor activity of parasites. A physiological function of serotonin in different animal phylum occurs by the activation of specific serotonin receptors, six out of seven types of which are G-protein coupled. In invertebrates the information are restricted to three 5HTR1, 5HTR2 and 5HTR7 serotonin receptor type's identification corresponding to the mammalian ones. The mechanism of serotonin action and existence of serotonin receptors in Platyhelminthes are poorly studied. The availability of serotonin receptors in parasitic flatworms *Hymenolepis diminuta* and *Opisthorchis felineus* is not known.

The presence and localization of serotonin 5HTR7 receptors in cestode's and trematode's larvae tissues has been studied by immunocytochemical method and confocal laser scanning microscopy. Cysticercoides of *Hymenolepis diminuta* were obtained from the abdominal body cavity of the experimentally infected flour beetle *Tenebrio molitor* (Insecta, Tenebrioniodae). Metacercariae of *Opisthorchis felineus* were removed from ides *Leuciscus idus* (L.) (Tobolsk, Russia). The metacercariae were excysted in alkaline-trypsin bile salt medium at room temperature. After fixation in 4% paraformaldehyde the wholemounts were incubated in primary antiserum to receptor 5HTR7 (ImmunoStar), than Alexa488-labelled secondary immunoglobulines were applied (Molecular Probes). The appropriate controls (omission of primary antibodies and using of non immune serum) were performed. The worms' musculature was stained by TRITC-phalloidin (Sigma). Samples were analyzed by Leica DM1000, LeicaDM6000 fluorescent and Leica TCS SP5 (Leica, Germany) confocal laser scanning microscopes. In cysticercoides of *H. diminuta* strong 5HTR7-immunoreactivity (IR) was found in flame cells (3 x 3 μm) of the excretory system, scattered through the larva's body. A weak staining to 5HTR7 was observed along the longitudinal and circular muscle fibers comprising the body wall, as well as in the nervous system – thin longitudinal nerve cords and transversal commissures. In metacercariae of *O. felineus* 5HTR7-IR was identified in main nerve cords, in head commissure connecting the head ganglia. It was also found in small and larger structures observing among the musculature of the oral and ventral suckers as well as in the round bodies (about 3-4 μm of size) located in the depth of the body trunk and near the tegument. 5HTR7-IR elements looked like small dots were noticed regularly distributed all along the longitudinal and diagonal muscle filaments of body wall. The staining was also evident along single muscle fibers in the depth of the body trunk, transecting its in lateral direction and bearing the supportive function for the abdominal organs (branches of intestine).

Comparative phylogenetic analysis of 5HTR7 receptor genes for several cestode (*Hymenolepis microstoma, Mesocestoides corti, Taenia solium, Echinococcus granulosus, Echinococcus multilocularis*) and trematode (*Fasciloa hepatica, Opisthorchis viverrini*) species and planarian *Schmidtea mediterranean* for which the full genome sequences have been annotated was performed using wormbase.org database. The phylogenic tree was constructed with PhyML and TreeDyn software (phylogeny.fr). The branch support analysis indicates that the 5HTR7 cestode's receptors are clustered together and separately from the trematode's ones. Planarian's 5HTR7 receptor is located outside of the cluster looking as outgrouped. The results suggest the cestode's 5HTR7 serotonin receptor is most ancient between the species.

Thus, the distribution of 5HTR7-immunoreactivity was for the first time investigated in tissues of cestodes *H. diminuta* and trematodes *O. felineus* larvae. The observations show the presence of specific serotonin-5HTR7-receptor's immunorena ruactivity in parasitic worms. It emphasizes the importance of the serotoninergic signal system for the fulfillment of the vital functions in representatives of Platyhelminthes. Results suggest the 5HTR7 type receptor can mediate the serotonin action in flatworms studied. Further researches are required.

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Effects of *Echinococcus multilocularis* cyst fluid on proliferation of Burkitt's lymphoma cells (Raji) in vitro

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One investigated the antiproliferative effects *of E. multilocularis* cyst fluid using Burkitt's lymphoma cells (Raji) in vitro. *E. multilocularis* cyst fluid was obtained from parasite larval cysts isolated from cotton rats experimentally infected by intraperitoneal injection of parasite scolices at dose level of 800 specimens/rat. The biological material was centrifuged at 15,000 rpm for 15 min on centrifuge Optima TLX (Beckman Coulter) and the protein concentration was determined to be 0.01 mg/ml using analyzer MICROLAB 600 (UNIMED, Russia) at 600 nm. Before experiment tumor cells were cultured in standard culture flasks (CORNING, 25cm^2) in RPMI-1640 medium (PanEco) supplemented with 10% fetal bovine serum (FBS) and gentamicin in a humidified CO_2 incubator (Binder, Germany) at 37°C and 5% CO_2. Burkitt's lymphoma cells were resuspended in sterile conditions of laminar flow hood and transferred to 12-well plates (SARSTEDT, Germany) in a RPMI-1640 culture medium with 10% FBS at 100,000 cells/well. *E. multilocularis* cyst fluid was sterilized by filtration through a Millipore filter membrane (0,45 μm) and added to the above plates at concentration of 2 μg/ml according to protein. Endoxane (Baxter Oncology (Germany); active ingredient cyclophosphamide) was used as a positive control and tested at the concentration of 2 mg/ml. The negative control was a sterile physiological solution. The experiment lasted 96 h and daily 50 μl of medium with cell suspension was taken to determine the cell counts in Goryaev chamber under a Leica DM 2000 microscope (Leica, Germany) on different time points. In control wells one noted a daily cell count increase by 1.5; 3; 2.5 and 2 fold post 24; 48; 72 and 96 h cultivation respectively. Following 24 h incubation of Burkitt's lymphoma cells with *E. multilocularis* cyst fluid the lymphoblast counts decreased by 2.5 fold compared to control and by 1.7 fold compared to the baseline level. On 48; 72 and 96 h any cell proliferation was not observed in wells with *E. multilocularis* cyst fluid. Following 48 h marked morphological changes in Burkitt's lymphoma cells were seen characteristic for necrobiotic processes: cell size reduction and cytoplasmic shrinkage, cells in culture medium did not form typical conglomerates observed in the control. Following 24 h in cell cultures with Endoxane no proliferation inhibition was observed (lymphoblast counts were at the control level). On 48; 72 and 96 h the cell counts decreased and remained reduced until the end of experiment but slight cell proliferation could be noted unlike cells post treatment by *E. multilocularis* cyst fluid. Morphological changes in cells subjected to Endoxane exposure were not observed. When Endoxane and the *E. multilocularis* cyst fluid were co-introduced to Burkitt's lymphoma cell culture medium (at concentration of 2 mg/ml and 2 μg/ml respectively) inhibition of cell division was observed similar to that value for *E. multilocularis* cyst fluid alone. The obtained data indicated that *E. multilocularis* cyst fluid had a cytotoxic effect on Burkitt's lymphoma cells in vitro and completely inhibited cell proliferation until to 96 h cultivation (whole observation period).

New trivalent SAG1-SAG2-GRA1 and SAG1-SAG2-GRA2 recombinant antigens used for detection of IgG antibodies against *Toxoplasma gondii* in human sera

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Toxoplasmosis, caused by the protozoan parasite *Toxoplasma gondii,* is one of the most prevalent parasitic zoonosis worldwide. It has been estimated that nearly one third of the world human population has been exposed to this parasite. Although the most of *T. gondii* infections remain asymptomatic or are benign, there is a high risk that in some cases, such as for immunodeficient patients and foetuses, may cause severe or fatal consequences. Accurate and early diagnosis is crucial for efficient treatment and prevent to development of severe toxoplasmosis in at-risk patients. Therefore, there are a number of research studies on improving or developing effective diagnostic tools.

The current diagnosis of toxoplasmosis is based mainly on the results of serological tests detecting anti-*T. gondii specific* antibodies in patient sera. The most of commercially available diagnostic kits use *Toxoplasma* lysate antigen (TLA), which has a significant drawbacks predominantly associated with non-standardized production and purification procedures and therefore its unspecified composition. It is commonly known that the sensitivity and specificity of serological tests are primarily dependent on the antigens used. Therefore, one of the research directions is developing new diagnostic tools capable of replacing TLA in serodiagnosis of toxoplasmosis, such as recombinant antigens. In comparison to native antigens of parasite, their production is much cheaper, faster and safer. Moreover, the use of recombinant antigens allows easier standardization of the diagnostic methods.

The aim of this study was to assess the diagnostic value of new recombinant chimeric antigens composed of the immunodominant regions of selected *T. gondii* antigens, such as SAG1, SAG2, GRA1 and GRA2. The chimeric antigens were obtained using the *Escherichia coli* expression systems, purified by affinity chromatography and then evaluated as potential diagnostic tools for the detection of IgG antibodies with the use of an enzyme-linked immunosorbent assay (IgG-ELISA) in human sera. The results of tests obtained for the chimeric antigens were compared with those from the IgG-ELISAs using the native antigens (TLA) and mixtures consisting of three adequate recombinant antigens.

The obtained results have shown that the trivalent recombinant chimeric antigens have ability to detect anti-*T. gondii* specific IgG antibodies in the sera of patients with diagnosed toxoplasmosis and therefore could be an alternative to the polyvalent *Toxoplasma* lysate antigen in the serodiagnosis of human toxoplasmosis.

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Characterization of immunodominant antigens in *Toxocara canis* L3 larvae excretory-secretory products

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Larval toxocarosis is a parasitic zoonosis caused by larvae of nematodes belonging to genus *Toxocara*, intestinal parasites of dogs and cats. Humans become infected by ingesting eggs with developed larva from contaminated soil or unwashed fruits and vegetables. For humans, the risk of obtaining infection is in our conditions relatively high, despite the effective treatment of animal hosts several times a year. The spectrum of diagnostic methods for human toxocarosis is still limited. Available commercial kits mostly rely on excretory-secretory products (pooled antigen) isolated from *in vitro* cultures of *T. canis* L3 larvae (TES), which are then used in routine serological diagnostics by ELISA. Our research is focused on the identification of specific proteins in TES, especially the suitable candidates for the development of recombinant form of protein antigens, which could improve the serodiagnostics of toxocarosis without necessity of L3 cultivation. Medium with TES was regularly collected and purified. Protein fractions were separated by 1-D SDS-PAGE electrophoresis, followed by Western Blot. PVDF membrane strips were incubated with negative/positive mice sera in order to detect the antigen-antibodies reaction. Appropriate protein bands were cut off from the polyacrylamide gel and analysed by mass spectrometry. The data analysis is in process and until now we have identified several promising immunodominant proteins.

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Differentiation of eight *Trichinella* species using a high resolution melting assay

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Zoonotic nematodes of the genus *Trichinella* are foodborne parasites with the high impact on public health and animal trade. They are causative agents of human trichinellosis, a serious human disease, which has been documented in 55 countries around the world. A broad range of carnivore and omnivore (mammals, birds, and reptiles) animals have also been reported to be hosts of these parasites.

Based on genetic, zoogeographical and epidemiological characters, 12 taxa are recognized in the genus *Trichinella*, which is separated in two clades. The “encapsulated clade” encompasses species with ability of muscle larvae to form a collagen capsule in host muscle tissues and the “non-encapsulated clade”. Despite the difference in capsule formation and some size differences, the species of all developmental stages of these parasites are morphologically indistinguishably.

In this study, we analyzed genomic DNA isolated from single muscle larva of eight *Trichinella* species (*T. spiralis*, *T. nativa*, *T. britovi*, *T. pseudospiralis*, *T. nelsoni*, *T. murrelli*, *T. papuae*, and *T. zimbabwensis*). For the species differentiation, we adopted a high resolution melting analysis (HRMA) as an additional step following a real-time quantitative PCR (qPCR). This single-tube method enables the distinguishing of genetic variation (down to single nucleotide polymorphisms) in amplified DNA fragments without necessity of subsequent sequencing.

Our qPCR-HRMA assay was based on polymorphisms of cytochrome c oxidase subunit I (COI) gene fragment on the same length (240 bp). This gene shows high sequence divergence even among closely allied species but exhibits conservation within a particular species. For the evaluation of melting curve data, a difference plot enabling the construction of *Trichinella* species-specific matrix curves were used.

We show that qPCR-HRMA based on the mitochondrial COI gene allows an unequivocal differentiation of single larvae of eight *Trichinella* taxa at the species level. In the future we envisage that the *Trichinella* species-specific matrix curves could be automatically evaluated using the computer application.

The work was financially supported by Masaryk University institutional support (MUNI/A/0816/2017) and grants from the Ministry of Agriculture (RO0517) and the Ministry of Education, Youth and Sport (LH12096, LO1218 and LD15056).

Toxocara canis as an inhibitor of MCF-7 and Caco-2 tumor cell proliferation *in vitro*

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One investigated the antiproliferative effects of mature *T. canis* protein extract using MCF-7 (adenocarcinoma of mammary gland) and Caco-2 (adenocarcinoma of colon) human tumor cell lines in vitro. Before experiment tumor cells were cultured in standard culture flasks (CORNING, 25cm^2) in DMEM GlutaMAX medium (GIBCO) supplemented with 10% FBS and antibiotic/actinomycotic (Gibco, × 100) in a humidified CO_2 incubator at 37°C and 5% CO_2. Freshly prepared tumor cell suspension was plated at 35×10^3 MCF-7 cell and 30×10^3 Caco-2 cell density on 24-well plates (SARSTEDT, Germany). Following sterilization through a Millipore filter membrane (0,22 μm) one prepared *T. canis* extract serial dilutions (2 mg/ml to 12,5 µg/ml) and added to plates at the above concentrations. Daily light-optical observations of cells were performed assessing changes in cell morphology in test wells compared with control ones on inverted microscope (Olympus CK 40 (Japan)). Proliferation response of tumor cells to *T. canis* extract exposure was assessed by the formed monolayer density and viability post 96 h incubation (at staining with 0.4% trypan blue solution and determination of cell counts in Goryaev chamber) on different time points. On 24 and 48 h the density of colonies was comparable to control for both models. Following 72 h, one noted a measurable delay of MCF-7 and Caco-2 cell growth at *T. canis* extract concentration of 2 mg/ml. The size of cell colonies was smaller than in control; also there was a notable decrease of mitotically dividing cell counts. Also the slight growth delay was observed in wells with MCF-7 and Caco-2 cells at lower concentration levels (1 mg/ml and 500 µg/ml). Following 96 h cultivation one observed fewer proliferating colonies of MCF-7 and Caco-2 cells at extract levels of 2; 1 mg/ml and 500 µg/ml. Morphological changes were noted only in MCF-7 cells at the maximum concentration of 2 mg/ml. In this experimental group cells without clear and distinct contours with loss of a characteristic structure were seen. The number of mitotically dividing cells was reduced. The viable MCF-7 cell counts gradually decreased from 95.2% (at the minimum level of 12.5 µg/ml) to 79.5% (at the maximum level of 2 mg/ml) against 96.8% in control. On that model inhibitory effects of *T. canis* extract increased proportionally to its concentration and reached the maximum values at 2 mg/ml, where the total cell counts decreased threefold and appeared to be 60.0×10^3 cells/well. But the insignificant inhibitory effect of *T. canis* extract on MCF-7 cell proliferative activity occurred even at concentration of 50 µg/ml where the total number of cells was 151.9×10^3 cells/well against 181.3×10^3 cells/well in control. The total Caco-2 cell counts in control group post 96 hour culture was 116.7×10^3 cells/well compared with 72.0×10^3; 63.3×10^3 and 65.9×10^3 cells/well at 500 µg/ml; 1 mg/ml and 2 mg/ml respectively. Thus, *T. canis* extract inhibited the proliferation activity of Caco-2 tumor cells at the higher levels (500 µg/ml to 2 mg/ml) compared with MCF-7 cells (50 μg to 2 mg/ml). The results obtained indicated that *T. canis* extract had a pronounced antiproliferative effect on MCF-7 cell and Caco-2 cell lines. But in Caco-2 cell culture that inhibitory effect of *T. canis* extract manifested at a lesser degree and at higher concentrations. Thus, in vitro MCF-7 cell culture had a higher susceptibility to antiproliferative effects of *T. canis* extract compared to Caco-2 cells post 96 h cultivation period.

Dynamics of changing hematological indicatore in experimental parasite infection *H. contortus* in mouflons (*Ovis musimon*)

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This study describes changes in hematological markers in wild ruminants under parasitic infection. Six forest mouflons (*Ovis musimon*) were experimentally infected with 8000 L3 *Haemonchus contortus* gastrointestinal nematodes, of which 3 were infected with the MHco3 sensitive strain and 3 with the MHco4 resistant strain. Blood was taken on days 0, 16, 37, 58, 77 and 99 of the experiment. In red blood count, decrease in cell parameters (CPE, Ht, Hg) was recorded on days 37, 58 and 77 in both groups, with the most pronounced drop recorded on day 58 of the experiment. The white blood count showed the increase in eosinophils and monocytes. The observed changes in the dynamics of hematological parameters were not statistically significant. Our results show more changes in the red blood count of the group infected with the resistant strain. Changes in the white blood count can be attributed to the degree of infection and the response of the organism to the high intensity of the *H. contortus* parasitic infection and its subsequent local inflammatory response with the presence of hemorrhage.

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Flavonolignan silybinA/B as adjunct to albendazole therapy of model *Mesocestoides vogae* infection in mice

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Natural products are important source of structural motifs in drug discovery and secondary metabolites of higher plants are the most intensively studied for their multiple beneficial effects on vertebrates. Flavonoids and polyphenols are very rich group of molecules and among them silymarin, the mixture of phenolic molecules, is widely accepted in human medicine as hepatoprotective compound. In the present study, the effects of flavonolignan silybin A/B, which is the most abundant component of silymarin have been examined on model flatworm infection induced by larvae of *Mesocestoides vogae* in Balb/c mice. We have compared the responsiveness of female and male mice, which were infected with 65 larvae orally. On day 15 post infection, groups of mice were treated daily with anthelmintic drug albendazole alone, silybin (SB) alone and their combination for 15 consecutive days. The effect of treatment was evaluated next day after termination of therapy by several criteria. We found that female mice harboured lower numbers of larvae in the peritoneal cavity and the livers than males and co-administration of high doses of SB (200mg/kg/day) only slightly increased efficacy in comparison with albendazole-treated mice. SB has been proven to exert antioxidant and immunomodulatory effects and in this study we examined the changes in composition of lymphoid and myeloid cell populations in the peritoneal cavity, spleen and abdominal lymph nodes of mice. We also demonstrated that SB co-administration modulated intensity of proliferation of T cells in the spleens and production of IL-10 and IFN-γ cytokines. In addition, we found the significant antioxidant and cytoprotective activities of SB on myeloid cell populations as well as stimulation of phagocytic index. In summary, our results showed that therapy with very high doses of silybin had no larvicidal effect but it reduced oxidative stress in immune cells in the peritoneal cavity. The adjuvant therapy with SB and albendazole seems to be the suitable alternative due to host-protective immunomodulatory and cytoprotective activities of flavonolignan silybin. Data indicated that the immune system of female and male mice is differentially affected by polyphenol when was co-administered with albendazole.

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Development of anti-HCMV agents by using the host-pathogen immune interaction

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Human cytomegalovirus (HCMV) infection is associated with severe morbidity and mortality in immunocompromised individuals, mainly transplant recipients and HIV–AIDS patients, and is the most frequent cause of congenital malformations in newborns. Despite inducing the strongest immune responses observed for any human pathogen, HCMV evades host defenses and persists for life. The large size of the HCMV dsDNA genome (as large as 230 kb), allows this virus to dedicate many genes to viral fitness; a number of these genes thwart the host inflammatory, innate, and adaptive immune responses. Herein, we report viral stealth strategy to evade immune recognition and how this knowledge can be further used for the development of anti-HCMV agents.

HCMV UL141 reduces surface expression of a key endogenous cell molecules like TRAIL death receptors and CD155, impairing the ability of NK and T cells to be activated by HCMV-infected cells. Our analysis of the structure, binding, and function of the HCMV glycoproteins in their interaction with the host cell receptors shows that the Ig-like protein fold, apparently purloined from the host, has evolved to benefit the virus and also offers a snapshot of the ongoing mutual evolution by which the host counters viral infection and the virus then acquires new functions to resist the host's immunity. Our findings highlight a role for these molecules in recognition of HCMV-infected cells and may be relevant for development of future antiviral therapies.

To date, few drugs are licensed for the treatment of HCMV infections, most of which target the viral DNA polymerase and suffer from many drawbacks, including long-term toxicity, low potency, and poor bioavailability. Moreover, the emergence of drug-resistant viral strains is becoming an increasing problem for disease management. Finally, none of the current anti-HCMV drugs have been approved for the treatment of congenital infections. For all these reasons, there is still a strong need for new anti-HCMV drugs with novel mechanisms of action. Indeed, the successful development of HIV entry inhibitors has provided the impetus for designing antiviral compounds targeting one or more essential steps occurring early and throughout the virus life cycle. Moreover, the availability of compounds targeting other viral events beside genome replication could allow the design of more active therapeutic protocols based on the combined use of multiple anti-HCMV drugs, like the highly active antiretroviral therapy that has changed the lives of HIV-infected patients. Although many pieces of the function, structure, and evolution of the HCMV/immunoevasin puzzle remain unknown, we expect that additional components soon will be discovered and put in their appropriate places.

Research efforts to dissect further the molecular interactions and mechanisms involved in the events of HCMV life cycle are clearly necessary to identify other novel drug targets and to develop new inhibitors against these targets.

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Session III – Vectors and Vector-Borne Diseases

Plenary lectures

Markéta Derdáková, Zuzana Mtierová, Yuliya Didyk, Michal Chvostáč, Diana Selyemová, Jasna Kraljik, Radovan Václav (SK): *Borrelia* Diversity in Ticks and Hosts, Lesson Learned from Central Europe

David Bruce Conn (US): Insect Vectors in Temperate Continental Regions of North America and Central Europe: New Data and Comparative One-Health Analysis

Sara Savic, Suzana Otasevic, Marina Zekic, Aleksandar Potkonjak, Cornelia Silaghi (RS): Prevalence of *Dirofilaria* spp. in the Balkan Region

Oral presentations

Viktoria Levytska, Andryi Mushynskyi (UA): Comparison of the Efficiency of Classical Methods and Express Method for Carbon Marking of Bovine Babesiosis

Blažena Vargová, Juraj Kurimský, Roman Cimbala, Michal Kosterec, Igor Majláth, Viktória Majláthová (SK): The Behavior of *Dermacentor reticulatus* in Radiation/Shielded Mini Arenas under the Influence of 900 MHz Frequency

Algimantas Paulauskas, Vesta Matulaitytė, Jana Radzijevskaja (LT): Investigation of Tick-Borne Pathogens in Ticks from Migratory Birds in Baltic Region

Dorota Dwużnik, Ewa Julia Mierzejewska, Mohammed Alsarraf, Maciej Kowalec, Gzegorz Karbowiak, Łukasz Stańczak, Patrycja Opalińska, Małgorzata Krokowska-Paluszak, Grzegorz Górecki, Anna Bajer (PL): Ectoparasites of Red Fox (*Vulpes vulpes*) from Poland and the Phenomenon of Ticks in Subcutaneous Tissue

Jana Radzijevskaja, Algimantas Paulauskas, Evelina Kaminskienė, Dalytė Mardosaitė-Busaitienė, Indrė Lipatova, Michal Stanko (LT): *Rickettsia* Species and their Associations with Rodents and their Ectoparasites in Lithuania

Bronislava Víchová, Martina Miterpáková, Martina Koneval, Martin Bona, Jasna Kraljik, Viktória Čabanová, Lucia Blaňarová, Zuzana Hurníková (SK): Neglected Intravascular Pathogens of European Red Foxes (*Vulpes vulpes*) and the Role of Arthropod Ectoparasites in their Transmission

Witold Tarkowski, Joanna, Moneta-Wielgoś, Daniel Młocicki (PL): *Demodex* Mites as Potential Factor in Development of Selected Eye Disorders – Current Data and Research Perspectives

Ellen Schoener, Carina Zittra, Jan Votýpka, Hans-Peter Fuehrer (AT): Protozoan Parasites in *Culex pipiens* Mosquitoes from Vienna

Viktória Čabanová, Martina Miterpáková, Zuzana Hurníková, Hana Blažejová, Ivo Rudolf (SK): The Impact of Urbanization on Mosquitoes and their Disease-Transmission Potential

Remigiusz Gałęcki (PL): Food Insects as a Vector of Potential Zoonotic Parasitoses

Poster presentations

Małgorzata Dmitryjuk, Marta Chajęcka, Magdalena Szczotko, Mirosław M. Michalski (PL): Preliminary Studies of *Borrelia burgdorferi* sensu lato Detection in *Ixodes ricinus* Ticks Collected from City-Aglomeration Dogs

Yuliya M Didyk, Markéta Derdáková (SK): *Borrelia burgdorferi* s.l. Infection in *Ixodes ricinus* Ticks from Ukrainian Urban Parks

Diana Selyemová, Veronika Rusňáková Tarageľová, Michal Chvostáč, Jasna Kraljik, Tatiana Vaculová, Yuliya Didyk, Markéta Derdáková (SK): *Borrelia* in Questing and Host Feeding Ticks in Western Slovakia

Gabriela Hrkľová, Jozef Macko, Dana Blahútová, Ján Machava, Eduard Bublinec (SK): The Distribution of the *Ixodes ricinus* along Altitudinal Gradients in Two Different Regions in the Great Fatra Mountains

Dana Zubriková, Maria Wittmann, Václav Hönig, Pavel Švec, Sandra Essbauer, Gerhard Dobler, Kurt Pfister (SK): The Prevalence of Tick-Borne Encephalitis Virus in *Ixodes ricinus* Ticks from Two Regions of Bavaria, Germany

Bronislava Víchová, Monika Sláviková, Lucia Blaňarová, Dana Zubríková, Markéta Derdáková, Diana Selyemová, Martina Ličková (SK): Prevalence of Anti-TBEV Antibodies in Selected Farms of Slovakia - Preliminary Results

Ewa Pyrka, Anna Wojtoń, Grzegorz Zaleśny, Gerard Kanarek, Joanna Hildebrand (PL): Leeches (Hirudinea) as the Intermediate Hosts of the Trematodes – a Case Study from Poland

Kamilė Klepeckienė, Jana Radzijevskaja, Algimantas Paulauskas, Irma Ražanskė, Laura Songailaitė (LT): Distribution of Deer Keds among Different Species of Cervids and their Infection with *Bartonella* spp. in Lithuania

Alica Kočišová, Miloš Halán, Eva Bocková, Petronela Komorová, Zuzana Kasičová (SK): The Occurrence of the Blood Sucking Dipterans in the City of Košice

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Viktória Čabanová, Martina Miterpáková, Zuzana Hurníková (SK): *Thelazia callipaeda* is Spreading across Europe

Borrelia diversity in ticks and hosts, lesson learned from Central Europe

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Over the past decades, the causative agent of Lyme borreliosis has attracted a lot of scientific attention. At the time of its discovery about 30 years ago, it was thought to be a single species. Currently, *B. burgdorferi* s.l. forms a complex of at least 21 genospecies out of which 8 are present in Europe. The close link of LB spirochetes with the environment and the impact that reservoir hosts have on spread of the disease agents renders knowledge on host associations a crucial factor for understanding their circulation in natural foci. The ecological diversity observed in this species complex, not only at species level, makes it an ideal model to study the impact of biological mechanisms on the spread of tick borne pathogens. All the genospecies of *B. burgdorferi* s.l. are maintained via zoonotic transmission cycles involving vertebrate reservoir hosts and ixodid ticks. The importance of avian hosts for the maintenance of borrelial infection, especially the role of ground-foraging passerines, is now indisputable. It is also proposed that migratory birds contribute to the wide-spreading of *B. burgdorferi* s.l. spirochetes along their migration routes. In total, during more than a decade of monitoring the borrelial prevalence, 1355 out of 7739 (18%) questing *I. ricinus* ticks were infected. Variations between the years as well as habitats were observed. *B. afzelii*, *B. garinii*, and *B. valaisiana* were detected at each studied site as the most prevalent with the few exceptions. In sub-mountain area of central Slovakia, *B. lusitaniae* predominated. *B. burgdorferi* s.s. was found in urban parks from both western and eastern Slovakia. At these sites, *B. bavariensis* and *B. spielmanii* were detected. *Borrelia*-positive ticks in urban habitats harbored mostly *B. garinii* and *B. valaisiana* assigning blackbirds population an essential role for circulation of borrelia in towns. The lowest prevalence of *Borrelia* was found at urban area with very low density of rodents where deer supplemented the blood meal for the *I. ricinus* larvae. *B. myiamotoi* was detected in 23 out of 2969 (0.8%) tested questing ticks from various sites in Slovakia. 18.1% (41/226) ticks removed from patients were positive for *B. burgdorferi* s.l. *B. afzelii* predominated, followed by *B. garinii*, *B. valaisiana*, *B. lusitaniae* and *B. spielmanii*. Out of 365 ticks collected from birds during migration period 44% of ticks were *Borrelia* positive. *B. garinii* and *B. valaisiana* predominated. MLST analysis of *Borrelia* positive samples revealed presence of an unique sequence types among *B. garinii* and *B. lusitaniae*. Profound understanding of the eco-epidemiology of Lyme borreliosis is crucial part of accurate diagnostics, treatment and estimation of risk of infection. We have confirmed spread of *Borrelia* infected ticks in towns as well as in mountains and high genetic variability affected by local habitats. Recent evidence of *B. miyamotoi* in questing and human feeding ticks points out on possible risk of the infection in Slovakia. All this knowledge is necessary for building more precise theoretical models on the spread of novel Borrelial strains across the Europe.

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Insect vectors in temperate continental regions of North America and Central Europe: new data and comparative One-Health analysis

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The recent expansion of insect-borne pathogens around the world has prompted a growing interest in understanding the distribution of the vector populations across global, regional, and local scales. Understanding the recent widespread emergence of such pathogens as Zika virus, Chikungunya virus, and *Dirofilaria repens*, and the constantly changing distribution of such diseases as malaria, leishmaniasis, Chagas disease, yellow fever and others requires study of vector dynamics.

We have initiated a comprehensive biosurveillance program for mosquitoes and phlebotomine sand flies at selected sites in Georgia, Tennessee, and Alabama in the southeastern United States, with long-term plans for expansion into additional areas of these and other states. Adult insects were trapped using CDC light traps, BG-Sentinel traps, Mosquito Magnet traps, all baited with CO_2 and chemical lures.

Our initial year of sampling during spring, summer, and fall yielded one species of phlebotomine fly, *Lutzomyia* *shannoni*, which is a potential vector for leishmaniasis and some viral pathogens. We also collected 24 species of the mosquito genera *Aedes*, *Anopheles*, *Culex*, *Psorophora*, *Toxorhynchites*, and *Uranotaenia*. The exotic invasive mosquito, *Aedes albopictus*, was the most common species, and is a competent vector of several pathogens infective to humans, domestic animals, and wildlife. The most common native species in our samples included *Aedes vexans*, *Anopheles quadrimaculatus*, *Anopheles punctipennis* and *Culex erraticus*, which collectively can transmit several viruses and parasites that cause dirofilariosis, malaria, and other diseases. Each of these pathogens either: 1) occurs currently in this region, 2) was present historically with potential for re-emergence, or 3) has not been recorded in the region but has the potential for new introduction and spread. Thus, under appropriate conditions and with immigration of infected human or animal hosts, there is a potential for episodic or sustained local transmission.

Considering the potential for re-emergence or emergence of parasites and other pathogens into the region, it is useful to compare regions in other parts of the world that share similar climate, topography, prevailing weather patterns, and other environmental conditions. Similarity of animal agriculture practices, wildlife populations, and socioeconomic conditions and lifestyles of human populations might also be predictive of comparable circumstances that might influence the potential for emergence of vector populations, and therefore the potential for disease emergence. Combining these considerations in a One-Health approach (i.e., integrating human, animal, and environmental health) offers promise for better predicting and responding to potential disease occurrence or outbreaks under suitable conditions.

While distinctive in some ways, the southeastern United States and Central Europe possess many important similarities that make such comparative analysis beneficial for scientists working in either or both regions. Thus, this paper constitutes a call for increased joint cooperative action, including collaborative research, and advice to regional, national and local authorities responsible for establishing health policy.

Prevalence of *Dirofilaria* spp. in the Balkan Region

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Dirofilariosis is a parasitic mosquito borne disease in carnivores. During the last 20 years the disease has spread over the Balkan peninsula and some regions became endemic for dirofialriosis. The disease is spread and transferred by the mosquitoes and dogs are considered as reservoirs. Mosquitoes that can carry microfilariae and transfer the disease in the region are mostly *Culex (C.) pipiens* biotype molestus or *Aedes (A.) vexans*. In a study on vector competence for *D.immitis*, it has been concluded that *Culex pipiens* biotype molestus, the most prevalent mosquitoe in the region, are not the most competent vectors due to their size and inability to bear high number of microfilariae. *Aedes* mosquitoes are more tolerant to the presence of microfilaria, which makes them more competent vectors. So far, the presence of *Dirofilaria* in mosquitoes has been confirmed in several Balkan countries so far, but the prevalence of the causative agent in different mosquito species is not yet known.

The disease has been discovered primeraly in dogs, becasue of the most obvieus clinical symptoms. Canine dirofilariosis can be found in 2 forms – subcutaneous dirofilariosis cause by *Dirofialria repens* and cardiopulmonal dirofialriosis cause by *Dirofilaria immitis* („heart worm"). In skin form there are hardly any symptoms, while in heart form of the disease, cardiopulmonal symptoms can be found which could potencially lead to a fatal outcome. Prevalence of *D.immitis* and *D.repens* in infected dogs in the Balkan region is from 3-20% and from 2-40% respectively, depending on the region and on the investigated group (with or without clinical symptoms).

During the past several years, dirofilariosis in humans is more and more diagnosed and reported. Until today, mostly subcutaneous and subconjunctival localization of *Dirofilaria* is found caused only by *Dirofilaria repens*. Prevalence of *D.repens* infection in humans from the Balkan region (with the exception of Greece which had higher prevalence) was from 1-13% before 2000. and from 1-35% in 2000-2014 period.

Diagnostics of dirofilariosis due to *D.immitis* infections in dogs is simple, known, and comercial tests are accessible to the vets. However, for *D. repens* there are no comercial kits, neither for dogs or humans, which gives the disease the opportunity for „undetected" disease to spread and this could have an imact to public health in the future.

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Comparison of the efficiency of classical methods and express method for carbon marking of bovine babesiosis

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Bovine babesiosis (BB) is a tick-borne disease of cattle caused by the protozoan parasites of the genus *Babesia*, order Piroplasmida, phylum Apicomplexa, which occure mainly erythrocytes, but can occur in the cytoplasm of cells of the reticulo-endothelial system and temporarily in the plasma of blood. This agent does not survive outside its hosts and can only be transmitted through a tick vector. During the life of animals critical to the confirmed diagnosis of babesiosis of cattle are laboratory studies, which include serological, molecular genetics methods and microscopy of stained blood smears.

The traditional and most commonly used method is the microscopic examination of thick and thin blood smears using an immiscible microscope system painted by various methods. For staining smears in veterinary practice, several methods are used: Romanovsky-Gimza, Pappenheim, Wright, Fold, Leishman, etc., and in the presence of a fluorescence microscope, the method is stained with acridine orange. The essential disadvantage of these methods is the complexity and duration of coloring, so now it is necessary to test and introduce modern rapid methods of staining smears for rapid diagnosis of babesiosis. One such method is the Diff-Quik Express Method with the help of the LDF® 200 set consisting of three solutions for rapid staining of blood strokes and tablets for the preparation of a flushing buffer solution.

The research was carried out in the farm, blood was collected from the peripheral vessels of the ear of cattle of different age groups and different breeds in the morning to prepare smears. To conduct these studies, 30 blood smears from an animal with a parasitemia level of 4% were prepared.

Comparing the methods of staining smears by Romanovskyi-Gimza, Pappenheim and the express-Diff-Quik method, they considered their specificity and efficiency as microscopic diagnostic methods for cattle. Since, with microscopy of smears, painted with different methods, almost the same degree of parasitemia was found, which is about 4%, and clearly visible morphological structures of babesia, it can be concluded that all methods of dyeing are effective and have a diagnostic value. However, while conducting research on the comparison of methods of staining smears blood revealed a number of drawbacks of traditional methods.

Diff-Quik color fast-staining with the help of the LDF®200 kit does not require special technical skills, long-term expectations and laboratory dishes. According to researchers, in the case of this method of painting the granules of heterophyles, basophils and eosinophils differentiate worse than in the case of staining by traditional methods. It should also be noted that the morphological structures of intraperitocytic and interperitrophytic forms of babesis are more clearly visualized than other methods of dyeing and are the basis for a final diagnosis.

The behavior *of Dermacentor reticulatus* in radiation/shielded mini arenas under the influence of 900 MHz frequency

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The concern about the environmental exposure to electromagnetic fields is increasing in relation to increasing requirements of human society for more sophisticated electronic devices, technologies and services in the last decades. Humans and animals are permanently exposed to a complex of the whole EMF spectrum of frequencies and intensities, often referred to as smog. The distribution and density of ticks in natural habitat is influenced by complex of abiotic and biotic factors. Exposure to radio - frequency electromagnetic field (RF-EMF) constitutes a potential cause altering the presence and distribution of ticks in the environment. Our main objective was to determine the affinity of *D. reticulatus* ticks towards RF-EMF exposure for frequencies of 900 and 5000 MHz and compare with control group. Originally designed and constructed Radiation-Shielded Tube (RST) test was used to test the affinity of ticks under controlled laboratory conditions. All tests were performed in an electromagnetic compatibility laboratory in an anechoic chamber. Ticks were irradiated using a Double-Ridged Waveguide Horn Antenna to RF-EMF at 900 and 5000MHz, 0 MHz was used as control. The RF-EMF exposure at 900 MHz induced higher concentration of ticks on irradiated arm of RST as opposed to the RF–EMF at 5000 MHz caused an escape of ticks to the shielded arm. We demonstrated different reaction of D. reticulatus ticks to RF-EMF of two different frequencies. This study represents the first experimental evidence of a D. reticulatus with the use of RF-EMF preference. The projection of obtained results to the natural environment could help assess the risk of tick borne diseases and could be a tool of preventive medicine.

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Investigation of tick-borne pathogens in ticks from migratory birds in Baltic region

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Migratory passerine birds are increasingly considered to be important in the global dispersal of tick-borne pathogens. Every spring and autumn, myriads of birds migrate over Baltic region from nortwest to southeast and from northeast to southwest flyways. In Baltic countries ticks and tick-borne pathogens were investigated in Estonia (Kabli and Pulgoja ornithological stations), Latvia (Kolka and Pape ornithological stations), Lithuania (Ventės ragas ornithological station) and Kaliningrad district (Rybachy ornithological station). To define the role of migratory birds as hosts and disseminators of infected ticks we examined tick-borne pathogens in ticks collected form birds and bird nests. The birds were caught in every country ornithological stations in 2005-2017 years and sand martin nests were collected from central part of Lithuania. *Ixodes ricinus*, *I. frontalis*, *I. persulcatus* and *Hyalomma marginatum* were removed from birds. The most predominant species was *I. ricinus*. From sand martin nests were collected *I. lividus*. The prevalence of *Borrelia* spp ranged from 4.4% to 18%, *Rickettsia* spp. from 11 to 12 %, *Anaplasma phagocytohylum* from 0.4 to 2%, *Babesia* spp. from 0,4 to 4,5%. Sequence analysis of positive samples revealed the presence of *Rickettsia helvetica*, *R.monacensis*, *R.vini, R.aeshlimannii, A. phagocytophilum*, *Borrelia garinii, B.afzelii, B.miyamotoi, Babesia divergens, B.venatorum* and *B.microti* in collected ticks. Infected ticks were found on *Turdus* spp., *Erithacus rubecula*, *Parus major*, *Tronglodytes tronglodytes*, *Coccochtrauses.coccochtrauses*, *Phoenicurus phoenicurus*, *Regulus regulus* and *Fringilla montifringilla*. These findings suggest that migratory birds may support the circulation and spread of medically important zoonoses in Baltic region.

Ectoparasites of red fox (*Vulpes vulpes*) from Poland and the phenomenon of ticks in subscutaneous tissue

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Red fox (*Vulpes vulpes*) is the most widespread carnivore in the world. In Poland it is the most abundant carnivore with high population level. Foxes occur in different types of habitat, from forests and meadows, to suburban and even highly urbanized areas like city centres. Because of this wide variety of used habitats and good adaptation to many environmental factors, red fox can be a host of various groups of ectoparasites, including fleas, ticks, mites and scabies.The aim of this study was to determine the ectoparasites fauna of the red fox populations in Poland. To achieve this, 228 carcasses from 5 voivodeships were examined:12 from warmińsko-mazurskie, 63 from dolnośląskie, 84 from kujawsko-pomorskie, 10 from wielkopolskie and 59 from mazowieckie. From mazowieckie voivodeship (the most urbanized area), specimens were acquired also from unique environment - Warsaw city center (10 carcasses). After freezing over the period of two weeks at the temperature -80°C, foxes were weighed, sexed and measured. Carcasses were examined for ectoparasites presence. Then foxes were skinned in order to detect parasites in subcutaneous tissue. Collected ectoparasites were preserved in 70% ethanol, selected representative specimens of fleas were additionally fixed in 10% NaOH solution. Ectoparasites were indentified accordingly to keys.

In total 619 ectoparasites were collected (123 ticks, 488 fleas, 5 mites, 3 deer ked). We identified 4 tick species: *Dermacentor reticulatus*, *Ixodes ricinus*, *I. hexagonus*, *I. canisuga*, five flea species including antropophil *Pulex irritans*. Additionally, 8 foxes were highly infested with *Sarcoptes scabiei*. Differences in prevalence of ectoparasites were detected in foxes from different voivodeships. The highest prevalence was in mazowieckie (54.2%), kujawsko-pomorskie (52.4%), the lowest in wielkopolskie voivodeship (10.0%). Furthermore, high prevalence of ticks in subscutaneous tissues was recorded in examined foxes. Possibly routes of this kind of infestation were observed and involved tick specimens were identified by molecular techniques.

Red fox constitutes a host of many ectoparasites of ecological significance and plays an important role in spreading parasites amongst various habitats, including urban areas.

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Rickettsia species and their associations with rodents and their ectoparasites in Lithuania

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Rickettsiae are emerging pathogens causing public health problems in many countries around the world. *Rickettsia* spp. are found in association with a wide range of arthropods which feed on different species of animals. However, the distribution, natural cycle of *Rickettsia* species and their association with different arthropod vectors are not fully established. In this study, we report the occurrence and the molecular characterization of *Rickettsia* pathogens in eleven species of ectoparasites parasitizing small rodents in the Curonian Spit, West Lithuania. Altogether, 238 small rodents representing six species *Apodemus flavicollis*, *Myodes glareolus, Micromys minutus, Microtus oeconomus, M. agrestis* and *M. arvalis* were trapped during 2013–2014. A total of 1261 ectoparasites (596 *Ixodes ricinus* ticks, 550 mites of five species and 115 fleas of eight species) were collected from these rodents. The overall prevalence of *Rickettsia* spp. in rodents was 27.6 %, with a higher prevalence detected in *M. minutus* (45.9%), followed by *A. flavicollis* (32.8%), and *M. glareolus* (14.3%). The frequency of *Rickettsia* spp. infection in rodent-derived ectoparasites varied among species and was found highest in fleas 43.5%, followed by *I. ricinus* ticks (MLE= 26.5%; CI 22.2-31.3), and mites (MLE=9.3%; CI 7.0-12.2). Sequence analysis of partial *gltA* and *17kDa* genes revealed the presence of *R. helvetica* in rodents and *R. helvetica , R. felis, R. monacensis, Rickettsia* sp. and rickettsial endosymbiont in rodents ectoparasites. In fleas four *Rickettsia* spp. were identified, while in Laelapidae mites – three *Rickettsia* spp. In *I. ricinus* ticks only *R. helvetica* was found. This is the first investigation of *Rickettsia* spp. in rodents and their ectoparasites in Baltic countries. Up to date, *R. felis* has never been identified in Lithuania. To our knowledge, this is the first report of *R. felis* in *Laelaps agilis* and *Hyperlaelaps microti* mites and in *Ctenophthalmus agyrtes* and *Hystrichopsylla talpae* fleas, as well as the first detection of *R. monacensis* in *Ct. agyrtes* fleas.

Neglected intravascular pathogens of European red foxes (*Vulpes vulpes*) and the role of arthropod ectoparasites in their transmission

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Canids are important reservoir hosts of many vector-borne pathogens, transmitted by the ticks and other blood-sucking arthropods. Red foxes (*Vulpes vulpes*) are among the most widespread and most significant wildlife species, from the perspective of epidemiology of many infectious diseases. The vaccination program aimed at the elimination of rabies has led to the significant increase in the red fox population in Central Europe and Slovakia.

Blood and tissue samples of red foxes (*V. vulpes*) from eastern and northern Slovakia, were screened for the presence of vector-borne pathogens by PCR. *Babesia vulpes*, *Hepatozoon canis* and haemotropic *Mycoplasma* species were identified by amplification and sequencing of 18S rDNA and 16S rDNA gene fragments, respectively. Investigated foxes carried DNA of *B. vulpes* and distinct haemoplasma species (*Mycoplasma haemocanis*, *Mycoplasma haemofelis*, a.o.). More than 17 % of foxes tested positive for *H. canis*.

This study brings the first information on the presence of *B. vulpes* in red foxes in Slovakia, and the first data on prevalence and diversity of haemotropic Mycoplasma spp. in European red fox population. Moreover, co-infections with two pathogens, B. vulpes and Mycoplasma spp. were confirmed in tested samples.

Despite an apparent absence of the vector tick R. sanguineus sensu lato in Slovakia, autochthonous infections caused by *H. canis* are reported in previously non-affected areas. The present study confirmed an existence of canine hepatozoonosis foci in Slovakia.

From 90 red foxes, altogether 512 ectoparasites were removed. Seven flea-species (*Chaetopsylla globiceps*, *Pulex irritans*, *Archaeopsylla erinacei*, *Chaetopsylla rothschildi*, *Chaetopsylla trichosa*, *Ctenocephalides canis*, *Ctenopthalmus assimilis*) and three species of hard ticks (*Ixodes ricinus*, *Ixodes hexagonus*, *Haemaphysalis concinna*) were recorded on sampled animals. Consequently, the DNA of five different pathogen taxa was confirmed in collected arthropod vectors: *Bartonella* spp., *Rickettsia* spp., *Anaplasma phagocytophilum*, *Theileria* sp. and *Hepatozoon canis*. *Mycoplasma* spp., *Dipylidium caninum*, and *Acanthocheilonema reconditum* were not found in fleas and/or ticks in this study.

The wide diversity of arthropod ectoparasites and relatively high rates of blood pathogens' prevalence and species diversity in wild foxes indicate the role of the fox population in the maintenance of parasites in sylvatic cycles and strengthen the assumption that foxes play important role in spreading of infectious agents within and outside the natural foci.

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Demodex mites as potential factor in development of selected eye disorders – current data and research perspectives.

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Demodex mites are highly specialised organisms, characterised by an obligate dependence on their host. Currently, there are two known species specific for humans: *Demodex folliculorum* and *D. brevis*. A growing number of recent studies suggests these parasites mites may contribute to the pathogenesis of human diseases, including ocular conditions. The aetiology of and pathogenesis of numerous ocular diseases remain unclear. Also, the causes of contact lens (CL)-associated discomfort and discontinuation of contact lens use among wearers who previously reported no tolerance issues, have yet to be explained. Taking into account the growing number of contact lens wearers, the identification of such causes could significantly impact the number of contact lens dropouts and reduce associated losses incurred by the industry. It is suggested that the discomfort experienced by these patients is related to dry eye syndrome (DES), a condition which is currently the subject of much research. There is a reason to believe that infestation with *Demodex* mites could be one of the potential aetiological factors behind the selected ocular conditions as well as soft contact lens intolerance among habitual users, and tear film instability. The goal of the present study was to evaluate if the presence of selected conditions may be associated with the presence of *Demodex* spp.

The study enrolled 710 subjects with following conditions: chalazion, pterygium, blepharitis, keratoconus, and soft contact lenses intolerance. All subjects underwent random sampling of at least 10 eyelashes. The sampled material was assessed by light microscopy. We also computed descriptive statistics of the datasets of the NIBUT (Non-Invasive Break-Up Time) measurements for healthy and mite-infested subjects with the use of Keratograph Oculus 5M.

Based on the results of our study, we propose the following conclusions: (1) Infestation with *Demodex spp.* mites can be an underlying aetiological factor of ocular diseases such as: chalazion, pterygium, chronic blepharitis. (2) The presence of mites on eyelid margins leads to tear-film instability. (3) Most probably *Demodex* mite infestation does not directly lead to the development of keratoconus but may play an indirect aetiological role, *inter alia* through an increased frequency of eye-rubbing in patients with eyelid demodicosis. (4) Discomfort reported by contact lens users should trigger appropriate testing to rule out or confirm demodicosis as a potential cause of discomfort experienced with contact lens use. (5) NIBUT results points to eyelid demodicosis as a possible cause of DES associated with instable tear-film and its excessive evaporation. Therefore, patients with the above mentioned conditions or symptoms should be examined for demodicosis as this may lead to more effective treatment.

Protozoan parasites in *Culex pipiens* mosquitoes from Vienna

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Insect vectors, namely mosquitoes (Diptera: Culicidae), are compulsory for some protozoan parasites to complete their life cycle. Despite this, little is known about vector competence of different mosquito species for the transmission of these parasites. The taxon *Culex pipiens* belongs to a species complex and is seen in Central and Western Europe in two forms, *molestus* and *pipiens*, which frequently hybridize and cannot be distinguished morphologically. Another species, *Culex torrentium*, while not part of this species complex, is also very difficult to distinguish morphologically. During several projects in Vienna and surrounding areas, we examined mosquitoes of the *Culex pipiens* complex for protozoan parasites in more detail. The mosquitoes were caught using a variety of sampling methods, identified morphologically and DNA was extracted to screen for parasite DNA and to also genetically identify mosquito form and taxon.

Nearly 5000 mosquito individuals were caught in the past years and a range of different protozoans were found. These parasites include monoxenous trypanosomids of the genus *Crithida* which parasitize insects, dixenous trypanosomids which parasitize vertebrates and avian malaria parasites of the genus *Plasmodium*. The most frequent trypanosomids in mosquitoes of the *Culex pipiens* complex were *Trypanosoma culicavium* and *T. avium*, both of which parasitize birds and are transmitted by insects. Of the avian malaria parasites, the most commonly found were the common European species lineages *Plasmodium vaughani* SYAT05, *Plasmodium matutinum* Linn1 and *Plasmodium relictum* SGS1.

This is the first large-scale study of protozoan parasites in Austrian mosquitoes. These results are of special interest, because molecular identification of the taxa of the *Cx. pipiens* complex and *Cx. torrentium* enabled the determination of parasite prevalence and diversity in the different mosquito taxa and hybrids of this complex in Austria.

We would like to thank all our citizen scientists for their invaluable help with mosquito sampling. Parts of this research were funded by the ERA-Net BiodivERsA, with the national funders FWF I-1437, ANR-13-EBID-0007-01 and DFG BiodivERsA KL 2087/6-1 as part of the 2012–13 BiodivERsA call for research proposals, the grant OP VVV 02_16_019/0000759 and the “Bright Spark” award for young scientists of the Veterinary University of Vienna. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of this manuscript.

The impact of urbanization on mosquitoes and their disease-transmission potential

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In recent years, increasing attention has been focused on mosquitoes due to outbreaks of several dangerous mosquito-borne diseases. In Slovakia, extensive research on dirofilariosis was performed, but only a little attention has thus far been paid to *Dirofilaria* vectors. For better understanding of dirofilariosis epidemiology, xenomonitoring for filarial parasites was performed in this study.

Almost 7000 female mosquitoes were trapped in two different habitats of Bratislava: the urban habitat of city centre and urban-fringe zone of outskirt part of Bratislava. Mosquitoes were identified by morphological features, and molecular methods were also used for determination of selected individuals belonging to cryptic groups of *Anopheles maculipennis* and *Culex pipiens*. The presence of filarioid DNA (*Dirofilaria repens, Dirofilaria immitis* and *Setaria* spp.) was detected using conventional PCR method and sequencing.

Within the study, twelve different mosquito taxa were distinguished (*Aedes vexans, Anopheles hyrcanus, Anopheles messeae, Anopheles daciae, Anopheles plumbeus, Coquillettidia richiardii, Culex modestus, Cx. pipiens* biotype *pipiens*, Cx. *pipiens* biotype *molestus*, *Culiseta annulata, Ochlerotatus caspius, Ochlerotatus sticticus*).The most abundant mosquito species was *Ae. vexans*, closely followed by members of the *Cx. pipiens* complex. Further investigation of mosquito pools (*n*=451) revealed DNA of *Dirofilaria* spp. in 26 pools with relative prevalence of 4.26%. Infected mosquitoes belong to various mosquito taxa (*Ae. vexans, An. messeae, Cq. richiardii, Cx. pipiens* biotype *pipiens, Och. sticticus* and unidentified members of *An. maculipennis* and *Cx. pipiens* complexes. *D. repens* were found to be the predominant species (3.61%). During the study, also mixed infection (0.33%) and *D. immitis* mono-infection (0.33%) were detected. *Setaria* spp. was not investigated in examined specimens. The majority of infected mosquitoes came from more natural environment of outskirt part of the city, from the urban-fringe zone Devin lake (*n*=24). In same area also the nine-time higher number of mosquitoes are captured. On the contrary, only two positive pools were found in urban habitat of city centre, but mono-infection by *D. immitis* was investigated only in this locations. In highly urbanized area were also observed co-occurrence of both *pipiens* biotypes (*pipiens* and *molestus*).

According our results, it should be clear that the more natural habitat of Bratislava creates better condition for mosquitoes and dirofilaria life. However, occurrence of sole *D. immitis* infection in city center in addition with interesting vector taxa captured in this area creates a serious risk for citiziens and their pets.

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Food insects as a vector of potential zoonotic parasitoses

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From 1 January 2018, the Regulation of the European Parliament and the Council (EU) of 25 November 2015 applies the concept of “novel food”, which includes insects and their parts. This allows the development of a new branch of the food industry, so-called insect farming - insect breeding for the use of diverse and readily available nutrients. Insects can be used not only for direct consumption, but also indirectly for the production of food, including animal feed. Moreover, the great interest in breeding exotic animals significantly increased the demand for this type of food. The role of food insects in the transmission of invasive diseases that pose a threat to humans has not been fully understood.

The aim of the research was to detect developmental forms of parasites in husbandry of mealworm beetles, house crickets, madagascar hissing cockroaches and migratory locusts from various farms in Central Europe (Czechia, Germany, Lithuania, Poland, Slovakia, Ukraine).

Material for the study (live insects) were collected from 50 cultures of mealworm beetles (*Tenebrio molitor*), 50 cultures of house crickets (Acheta domesticus), 50 cultures of madagascar hissing cockroaches (*Gromphadorhina portentosa*) and 50 cultures of migratory locusts (*Locusta migratoria*). Insects were collected at the same development stage. From the mealworms beetles and house crickets cultures, 40 insects were collected, out of which 4 collective samples with 10 insects were created, and from the cultures of cockroaches and locusts 10 insects were collected, which were tested individually. Prepared gastrointestinal tracts were examined by flotation using Darling’s liquid. The remaining elements of the insect body were examined under a stereoscopic microscope (×40 magnification) for developmental forms of larval parasites, and then modified Kirkor method was used. In 55 (27.5%) out of 200 insect cultures, developmental forms of parasites potentially pathogenic to humans have been found. In the mealworm cultures were found in 6 (3.00%) samples *Entamoeba* spp., in 9 (4.5%) *Balantidium* spp., in 8 (4.00%) cysticercoids, in 7 (3.5%) *Physaloptera* spp., in 2 (1.00%) *Spirocerca* spp. and in 2 (1.00%) Acanthocephala. In house crickets cultures were found in 2 (1%) samples *Physaloptera* spp. and in 2 (1%) samples cysticercoids. In cockroaches cultures were found, in 31 (6.2%) samples *Entamoeba* spp., in 28 (5.6%) *Balantidium* spp., in 2 (0.53%) cysticercoids, in 12 (2.4%) *Pharyngodon* spp., in 22 (4.4%) *Physaloptera* spp., in 4 (0.8%) *Spirocerca* spp., in 3 (0.6%) Acanthocephala and in 1 (0.2%) Pentastomida. In migratory locusts cultures were found in 7 (1.4%) samples of *Entamoeba* spp., 13 (2.6%) samples *Balantidium* spp., in 3 (0.6%) cysticercoids and in 6 (1.2%) *Physaloptera* spp.

Studies have shown a fairly common occurrence of parasites that may be a potential sources of infection to humans and animals. They also indicate the need of monitoring the presence of parasites in cultures of food insects intended for feeding.

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Preliminary studies of *Borrelia burgdorferi* sensu lato prevalence in *Ixodes ricinus* ticks collected from city-agglomeration dogs

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The spirochetes of *Borrelia burgdorferi* sensu lato complex are an etiological agent of the most common zoonotic disease, Lyme borreliosis. The main vector of pathogens are *Ixodes ricinus* ticks, the most numerous species of hard ticks in Poland. Nine different species were identified within *B. burgdorferi* s. l. complex in Europe. The most important from the viewpoint of human medicine are 3 species: *B. burgdorferi* s. s. associated mainly with Lyme arthritis, *B. garinii* associated primarily with neuroborreliosis, and *B. afzelii* linked mostly to skin afflictions.

This study investigated the prevalence and types of dangerous to humans geno-species of *B. burgdorferi* sensu lato in *I. ricinus* ticks removed from city-agglomeration dogs. In 2016-2017, ticks were collected from dogs visiting veterinary clinics within the urbanized areas of Olsztyn. DNA was isolated individually from 220 blood-filled tick females, and PCR was applied to detect the presence of the spirochetes, using primers in conservative regions of the flagelline gene of *B. burgdorferii* s. l. Subsequently, amplified positive DNA was digested by the *TasI* restriction enzyme (*FastDigest Tsp509I*). Restrictive analysis distinguished three geno-species: *B. garinii*, *B. afzelii*, and *B. burgdorferi* sensu stricto.

Positive PCR results were obtained in 85 of individuals. This accounted for over 38% of the examined ticks. RFLP analysis of these 85 samples revealed 72 (85%) mono-infections and 13 (15%) co-infections. Unidentified geno-species were present in 8 of the samples, one of these cases was a mono-infection, the other 7 showed co-infection with *B. garinii.* There were 3 dual infections of *B. garinii* with *B. afzelii* and 3 of *B. garinii* with *B. burgdorferii s. s.* The predominant species in infected ticks was by far *B. garinii*, present in 83 out of 85 of positive samples (70 mono-infections and 13 co-infections). *B. afzelii* was identified in a single sample, while *B. burgdorferi* s. s. was detected only in co-infections with *B. garinii*.

From this preliminary study it is concluded that the prevalence of *B. burgdorferi* s. l. in ticks isolated from dogs from the urban agglomeration of Olsztyn is high, as more than 1/3 of ticks collected from dogs are infected with this pathogen. The dominating species is *B. garini* responsible for neuroborreliosis in humans. Dogs are reservoir animals mainly of this geno-species. Perhaps the transmission mechanisms for this pathogen between dogs and ticks that feed on them are the most efficient. The proximity of dogs and people may be the reason for the rapid spread of Lyme disease in the urban population.

Borrelia burgdorferi s.l. infection in *Ixodes ricinus* ticks from Ukrainian urban parks

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Borrelia burgdorferi sensu lato complex currently are represented by 21 species. This complex comprises parasitic bacteria that are maintained in natural transmission cycles between vector ticks of the genus *Ixodes* and numerous vertebrate reservoir hosts (Piesman & Gern, 2004). In Europe, several genospecies are pathogenic for humans (*B. afzelii, B. garinii, B. burgdorferi* sensu stricto, *B. bavariensis* and *B. spielmanii*) causing Lyme disease or Lyme borreliosis. In Ukraine, more than 1.600 clinical cases of human borreliosis have been recorded in 2011 and 1200 – in 2012. The largest group of patients (80%) consists of urban residents.

The aim of our study was to investigate the prevalence and genetic diversity of *Borrelia* species in questing *I. ricinus* ticks collected in the Ukrainian urban parks.

In 2015 and 2016, altogether 1 045 *I. ricinus* ticks were collected by flagging the vegetation in 5 Ukrainian big cities: L'viv, Rivne, Zhytomyr, Kyiv and Poltava.

In total, 19% of examined ticks were infected with spirochetes from *B. burgdorferi* s.l. complex. Prevalence of *B. burgdorferi* s.l. infection was: 23% in L'viv, 17% in Rivne, 11% in Zhytomyr, 24% in Kyiv and 13% in Poltava urban parks.

The most prevalent genospecies identified in ticks was *B. afzelii* 89%. *B. garinii* was found in 7% of infected *I. ricinus* ticks. *B. burgdorferi* s.s. in 1.25%, *B. valaisiana* in1.25%, *B. bavariensis* –in 0.50%, *B. spielmanii* in 0.50%,

and *B. lusitaniae* in 0.50% of infected ticks.
We performed PCR amplification and sequencing of partial sequences of 4 chromosomal genes used for multilocus sequence typing (MLST): *clpX*, *pyrG*, *recG* and *uvrA* (Margos et al.,2008). Sequences were compared with strains deposited in the MLST database. In the most cases, we found a close phylogenetic relationship between the Ukrainian strains and European strains. The results demonstrate that the Ukrainian isolates of *B. afzelii* are more closely related to the Norwegian and Latvian *Borrelia* genotypes. *B. garinii* are most similar to French and Latvian, *B. bavariensis* are the most similar to Serbian and *B. burgdorferi s.s.* – to Canadian and Latvian *Borrelia* genotypes. This is the first multilocus sequence analysis of *Borrelia* strains identified from *I. ricinus* ticks from Ukraine. That comparative study between Ukrainian and European strains will create a better understanding of the *B.* burgdorferi s.l. population genetics in different geographic locations in Europe.

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Borrelia in questing and host-feeding ticks in Western Slovakia

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The most important vector of *Borrelia burgdorferi* s.l. is *Ixodes ricinus*, which is the most abundant tick species in Slovakia. *Borrelia miyamotoi* is zoonotic pathogen transmitted by same tick species (*Ixodes*) that is vector of the causative agents of Lyme borreliosis spirochetes from the *Borrelia burgdorferi* s.l. complex. *B. miyamotoi* causes acute febrile illness (relapsing fever).
The aim of this study was to study the prevalence and heterogeneity of borrelia in feeding ticks removed from human patients, reservoir hosts and in questing *I. ricinus* ticks from Western Slovakia to assess the potential of various hosts for borrelia transmission and its role in the epidemiology.
Questing and feeding ticks were collected by flagging the vegetation from suburban forest Železná studnička (1) in Bratislava and urban park Malacky (2). Moreover ticks were collected from birds traped in Bratislava and feeding ticks collected from human and dog patients presented in clinics in Bratislava. The highest prevalence in questing ticks was recorded in Železná studnička (n = 119; 28.6%) as compared to Malacky (n = 222; 19.4%). *B. miyamotoi* occurred in 0.8% and 1.8% of tested questing ticks, respectively.
370 ticks from people were investigated and 16.5% of them were borrelia-positive. A total of 792 dog-fed ticks and 295 bird-fed ticks were tested for presence of *B. burgdorferi* s.l. The overall prevalence was 9.7% in dogs and 12.5% in birds. *Borrelia afzelii* was the most dominant genospecies in dogs (58%) and *B. garinii* in birds (97%).
Profound understanding of the eco-epidemiology of borrelioses is crucial part of accurate diagnostics, treatment and estimation of risk of infection. In our study questing ticks and feeding ticks from dogs, humans and birds were used as a sentinels to express the epidemiological risk of being bitten by infected tick in urban area in Western Slovakia.

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The distribution of the *Ixodes ricinus*, along altitudinal gradients in two different regions in the Great Fatra Mountains

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From March to October in two year study 2016, 2017 at two vertical transect of the different regions in the Great Fatra Mountains data on microclimate, geology, pedology and the tick density were collected. The host-seeking adult and nymphal ticks were collected by flagging vegetation once a month. Air temperature and relative humidity were measured 5cm above the soil surface every day by external dataloggers. The temperatue and relative soil moisture were recorded during a collecting of ticks by trime sono TDR probe. Details about the soil type for all sites were studied. For futher characteristis pH of the soil were measured in a soil-water suspension (1:2,5) using a respective pH-meter.

The first vertical transect was located in Smrekovica. A total of seven sampling areas from 650 to 1460 m asl.The geological substrate of the site is granite and soil type podzol. The second vertical transect was located in Malinô Brdo, well-known as touristic place. A total of six sampling areas from 600 to 1200 m asl.The geological substrate of the site is limestone and soil type cambisol.

A total of 2137 (adults and nymphs) host-seeking *Ixodes ricinus*, were collected. The spring peak of ticks occurs in June 2016 respectively in April 2017. In terms of vertical distribution of ticks, we recorded the highest relative density of ixodid ticks in two sampling site: Malinô Brdo 600m asl. and Smrekovica 680 m asl. At a height of 1460m asl (Smrekovica) and 1200 m asl. (Malinô Brdo) we recorded all the developmental stages of *Ixodes ricinus* ticks with a relative density 0,83 and 3,4 respectively. Therefore the risk for visitors in the National Great Fatra Mountains of being exposed to ticks is real through the all transect.

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The prevalence of tick-borne encephalitis virus in *I. ricinus* ticks from two regions of Bavaria, Germany.

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I. ricinus tick is important vector of various virus species including tick-borne encephalitis virus (TBEV), the most significant human arboviral virus in Europe. Two regions of Bavaria, Germany (Lower Bavaria and the Upper Palatinate) with high TBE incidence were selected for the study of the prevalence of TBEV in *I.ricinus* ticks. 20 tick collection sites were selected using GIS tool. The criteria for the selection of collection localities were: the number of human tick-borne encephalitis cases in past 9 years (from 2001 to 2009), the altitude up to 750 m above the sea level, the tourism attractivity and the appropriate vegetation cover for ticks. *I. ricinus* ticks were collected by flagging an area of approximately 600 m^2 three times per year 2010. The presence of TBEV in *I.ricinus* ticks was determined by real-time RT-PCR method. The overall TBEV prevalence (calculated as minimum infection rate) was 0.26% (23 positive pools, 1006 negative pools, N=8805 ticks examined). The TBEV prevalence for nymphs was 0.24% (20 positive pools, 838 negative pools, N=8203 nymphs examined) and for adults 0.5% (3 positive pools, 168 negative pools, N=602 adults tested). Ticks positive for TBEV were found also directly in the park of the city Passau.

The research was financed by the European Development Fund of the European Union (project title: "Ticks and tick-borne diseases in the conditions of South Bohemia and Bavaria"). The presentation of data at the conference XIIIth Slovak and Czech Parasitological Days, Košice, Slovakia was supported by the Science Grant Agency VEGA project no.2/0126/16.

Prevalence of anti-TBEV antibodies in selected farms of Slovakia - preliminary results

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Tick-borne encephalitis virus (TBEV, family *Flaviviridae*) is the causative agent of serious disease, tick borne encephalitis (TBE). TBE manifestations vary from asymptomatic infections and fevers, with complete recovery of patients, to debilitating or fatal encephalitis. In the last 30 years, the incidence of tick-borne encephalitis has dramatically increased in Europe and Asia, but not only in known endemic areas. New foci have emerged in areas,

where they have not been determined before. The ticks (especially *Ixodes ricinus*) play a role of the vectors as well as reservoirs of the virus in the transmission cycle, however the most important reservoir hosts are small mammals. Human infection can be acquired not only by infected tick bite but there is also an alternative alimentary route of transmission, via the consumption of the infected raw goat and/or sheep milk. Here we present the preliminary results of the occurrence and prevalence of anti-TBEV antibodies in small ruminants from selected farms in Slovakia. We used enzyme-linked immunosorbent assay (ELISA) for detection of antibodies in goat and sheep blood sera. Based on the serological screening, we would determine localities with the increased risk of TBEV infection.

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Leeches (Hirudinea) as the intermediate hosts of the trematodes – a case study from Poland

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The representatives of Digenea are known from multi host, complex life cycle which include molluscs as first intermediate host and vertebrate as a definitive host. However, the vast majority of the species extending their life cycle by using additional intermediate hosts in order to complete transmission. Many of digenetic flukes carry out their life cycle in aquatic environment; in that case the role of the definitive host plays vertebrates, which commonly prey on aquatic organisms. Among them, an interesting group of organisms are leeches (Hirudinea). Some species of leeches serve as the second intermediate host for digeneans; for example, the strigeid trematodes use as the intermediate host the representatives of the genera *Erpobdella* or *Helobdella*. However, the information on the role of Hirudinea in the transmission of digeneans are still very limited because such studies are very demanding and time consuming. The aim of our study was to evaluate the role of the representatives of the Hirudinea in the life cycles of digeneans.

Lechees (n=87) represented by species: *Haemopis sanguisuga*, *Theromyzon tessulatum*, *Glossiphonia complanata*, *Erpobdella octoculata* were captured in the Podkarpackie Voivodeship and on the border of the Lower Silesia and Opole Voivodships during 2017. Parasitological section was performed on each specimen. All collected parasites were preserved in 70% alcohol for further research. DNA was extracted with the use of commercial kit (EurX) according to the manufacturer’s protocol. Next a partial sequence of the large ribosomal subunit (28S rDNA) was amplified. Newly generated sequences of 28S rDNA were identified with the use of BLAST (Basic Local Alignment Search Tool) and by comparison with our own sequences derived from adult trematodes. Metacercariae were found in two species of leeches, i.e., *Haemopis sanguisuga* and *Erpobdella octoculata*. The overall prevalence of infection with digeneans amounted 11.5%, i.e. 10 individuals from both localities were infected with flukes belonging to the family Strigeidae.

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Distribution of deer keds among different species of cervids and their infection with *Bartonella* spp. in Lithuania

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Deer keds (genus *Lipoptena*), are hematophagous ectoparasites which typically infest wild ruminants. However, the information on the distribution and abundance of deer keds on their mammalian hosts is still scarce, especially in Lithuania. Recently, *Lipoptena cervi* has been confirmed as vector of some *Bartonella* species. *Bartonella* pathogens are highly adapted to their mammalian hosts, in which they cause chronic relapsing intraerythrocytic bacteraemia. This study represents the first record of deer keds from cervids with the presence of *Bartonella* pathogens in Lithuania. The furs of cervids (roe deer, red deer and moose) harvested through the hunting during 2016-2017 were examined for deer keds. Taxonomic identification of deer keds was based on description of morphological characteristics of pupae and adults and sequence analysis of mitochondrial COI gene. Our investigation demonstrates that cervids in Lithuania are infested with two different species of deer keds *Lipoptena cervi* and *L. fortisetosa*. The highest infestation rate was detected in moose (*Alces alces*). PCR assay and sequence analysis based on *rpoB* gene and 16S-23S rRNA intergenic spacer region (ITS) were used for detection and molecular characterization of *Bartonella* spp. in deer keds. *Bartonella* DNA was detected in both species of deer keds with high prevalence. *Bartonella* strains detected in deer keds were heterogenic. Obtained *Bartonella* ITS and *rpoB* sequences derived from *L. cervi* and *L. fortisetosa* showed 96-99 % similarity with *B.schoenbuchensis, B.chomelii* and *B.caprioli* species. The present study is the first molecular identification of *L. cervi* and *L. fortisetosa* in Lithuania and the first detection of *Bartonella spp. in L. fortisetosa.*

The occurrence of the blood sucking Dipterans in the city of Košice

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The city's leisure and relax zones for residents in Kosice include places like the zoological and botanical gardens, the riding area and the Anička recreation area, where there are potentially different types of blood sucking insects. These are areas of different animal species (ruminants, ungulates, birds, reptiles, etc.) as well as environments suitable for the development of Dipterans, and thus conditions for pathogen transmission by insects are created. These places are visited by a number of tourists and visitors who are annoyed by mosquitoes, biting midges and horse flies, and there is a potential danger of infecting not only viruses but also parasites that are transmitted by Dipterans. The aim of the present work was to find out what species and prevalence of Dipterans are the most often these relax zones of the city of Košice.

In 2016, we trapped 7,113 of Dipterans, of which 7039 were biting midges (99%), 58 mosquitoes (0.8%) and 16 horse flies (0.2%). The mosquitoes were most commonly diagnosed with *Culex pipiens/C. torrentium*, which are considered to be ornithophilic species, but they have been adapted to suck blood in warm-blooded animals, especially horses and humans, and are considered to be major vectors of West Nile virus (Savini et al., 2012). The second most frequent species was *Aedes vexans*, which was confirmed by our previous research as a vector of zoonotic *Dirofilaria immitis and D. repens* (Bocková et al., 2015). Culicoides accounted for 99% of the fauna of Dipterans, most often captured at localities with animal breedings and increased population movements in the zoo and botanical gardens and at the UVLF riding area. These insects except animals are also humans plague and cause allergic skin inflammation (Schaffartzik et al., 2008 and 2012).

In 2017, 11 867 of insects were captured in 5 locations, 9 of which were Culicoides (9799/82.6%), 4 species of mosquitoes (1398/11.8%) and 4 species of horse flies (670/5.6%). The most abundant were *Culicoides obsoletus*/*C. scoticus* (76.2%) and *C. pulicaris*/*C. lupicaris* (12.6%). We also diagnosed *C. circumscriptus, C. kibunensis, C. dewulfi, C. tauricus/C. slovakus, C. deltus, C. punctatus* and *C. newstedi*. Of the horse flies, we recorded more than 55% of the species *Tabanus bovinus* and 33.6% of *T. bromius*, the pain-like biting species and sucking blood especially on the ungulates. As for mosquitoes, we recorded the species of *Anopheles maculipennis s.l.* (7.4%), species that are sucking on both birds and animals, *Culex pipiens/C. torrentium* (34.3%) and *Aedes vexans* in the ZOO Košice (60%).

The results show that in the urban environment of the city of Košice, in the locations with higher human visiting and animal breeding we have confirmed the occurrence of potential Dipterans vectors. However, their abundance and species composition have been greatly influenced by climatic conditions in particular years.

The survey was carried out within the grant project VEGA 1/0080/15.

Current status of mosquitoes (Diptera, Culicidae) in the Třeboň Basin (Bohemia, Czech Republic)

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The mosquito fauna has been monitored in a fish-pond country of the Třeboň basin. In 2012 - 2017, different collection methods were used (catching larvae, sweeping newly hatched adults around breeding places or rearing field collected pupae in the laboratory, and female mosquito trapping using EVS traps with CO_2 as attractant). The monitoring of the invasive mosquito *Aedes albopictus* was done by placing ovitraps on parking lots next to motorways. Flower vases in cemeteries were inspected for the presence of larvae of an invasive species, *Aedes japonicus*. Native larvae collections were carried out in approx. 30 various natural biotopes in the inundation zones of the New and the Old Lužnice Rivers and adjacent wetlands and flood plain forests, in numerous coniferous forests, peat bogs, and vast inundated meadows near the Třeboň city (49°N, 14°46´E). EVS CO_2 traps were placed mostly on the edges of fish ponds, especially in reed (*Phragmites australis*) zones. Among almost 50,000 collected specimens, 28 native mosquito species have been identified in the study area since 2012. After the catastrophic floods in 2013, larvae of the floodwater mosquito species, such as *Aedes vexans, Ae. sticticus,* and *Ae. cinereus,* predominated. In years with no major floods, larvae of the snow-melt mosquito species, such as *Ae. cantans or Ae. punctor*, prevailed. On the edges of fish ponds, females of *Culex modestus,* a potential vector of the West Nile virus, collected by EVS CO_2 traps were very abundant, along with omnipresent *Cx. pipiens/Cx. torrentium, Ae. vexans, Ae. sticticus,* and *Ae. cinereus.* No WNV was isolated from *Cx. modestus* in 2016-2017.

Using EVS CO_2 traps, two females of *Uranotaenia unguiculata* were trapped in the reed zones of the Černičný pond (49°4´41´´N, 14°45´20´´E) and Malý Tisý pond (49°3´15´´N, 14°44´57´´E) on 2 and 3 August 2017. It was the first record of *Ur. unguiculata* in Bohemia and in one of the northernmost points of distribution of this thermophilic species in Europe. Another new species for the area under study was *Anopheles daciae*. In August 2017, approx. 50 km northward of the study area border, on a parking lot of the gas station at km 64 of the D3 highway, eight introduced specimens of invasive *Ae. albopictus* were recorded. No mosquitoes of the exotic species *Ae. japonicus* has been found in the Třeboň basin.

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Human *Dirofilaria repens* infection in Ukraine (2013–2017)

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Dirifilaria repens seems to be one of the human and animal parasites that have been recently quite extensively spreading all over Europe.

In Ukraine, more than 1.5 thousand cases of human dirofilariasis were recorded until the end of 2012 (Sałamatin *et al*. 2013; Acta Parasitologica 58(4): 592–598). The aim of this study was to assess current epidemiological situation of human dirofilariasis and analyse the changes over the period of 5 consecutive years following 2012.

The epidemiological data covering the period between 2013–2017 were analysed. Data originates from the reports of Laboratory Centers owned by the Ministry of Health of Ukraine.

A total number of 706 new cases have been recorded within the period of 2013–2017. Nearly half of them occurred in only 5 administrative units: Kyiv city (77 cases), Dnipropetrovsk region (80), Zaporizhzhya region (59), Donets'k region (56) and Mykolaiv region (46).

The analysis of the records from 1997–2017 shows that after the period of exponential growth within 1997–2012 (*op. cit.*) the number of new cases reached some plateau (257 cases in 2012 and 252 in 2013) after which a decrease in the number of newly registered cases was recorded: 2014 – 156 cases, 2015 – 108 cases. This decrease can only partially be explained by the insufficient epidemiological data from the regions that are occupied by Russia (Crimean Peninsula as well as parts of the Luhans'k and Donets'k regions). The same phenomenon of decreasing number of new records is seen in majority of Ukrainian regions.

High number of human dirofilariasis recorded in Ukraine as compared with other countries is a proof of relatively good functioning of the registration system of *D. repens* infections as well as good diagnostic procedures. Well-functioning human dirofilariasis reporting and monitoring system existing in Ukraine may provide good reference point for other European countries and allow monitoring dynamics of human dirofilariasis spread in the coming decades.

We would like to thank Aleksander Masny (National Institute of Public Health – National Institute of Hygiene, Warsaw) for his support.

Thelazia callipaeda is spreading across Europe

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In last decade, the occurrence of new parasitic species *Thelazia callipaeda* has been observed in several parts of Europe. *T. callipaeda* also called as "oriental eyeworm" is responsible for ophthalmological infection of animals as well as human. Symptoms of thelaziosis ranging from mild to severe and in some case, untreated disease can lead to blindness. Previously, *T. callipaeda* occurred in area of Far East and in Mediterranean, but nowadays increasing number of clinical cases of dogs and human are described in central Europe and thelaziosis becoming one of the emerging diseases in this area. Curiously, molecular analyses of worms extracted from different definitive hosts, as well as human revealed that only one haplotype (h1) circulated in European countries. Only recently, the first autochthonous cases of thelaziosis were diagnosed in dogs in Slovakia. In present study, autochthonous cases of canine thelaziosis are described and the results of the first epidemiological study of *T. callipaeda* in domestic and wild carnivores are reported.

Between years 2016 and 2017 eight clinical cases of canine thelaziosis were diagnosed in Slovakia. Various clinical signs were observed in infested dogs including unilateral or bilateral conjunctivitis, mucopurulent secretion, hyperlacrimation, epiphora and others. After consecutive ophthalmological examinations, filiform worms were extracted from one or both eyes. The worms were identified as adult *T. callipaeda* by morphological and molecular determination. Five infected animals never travel abroad and travel history of other three dogs is unknown, but autochthonous origin of the infections is assumed in the all cases. Subsequently, an extensive epidemiology research in wild and domestic animals was performed. Total of 790 carcases (744 red foxes, 31 dogs, 10 cats, 3 racoon dogs, 1 grey wolf and 1 brown bear) were examined for presence of *T. callipeada* in animal's ocular tissues during the control of the effect of the antirabic vaccination in whole area of Slovakia. The "oriental eyeworm" were detected in eyes of nine red foxes. None of other animal hosts have been found infected. BLAST analyses of the *cox*1 gene showed that all specimens isolated from Slovak red foxes and dogs were identified as haplotype 1.Infected animals were diagnosed in different parts of the country, but the majority of them originated in the Košice region in eastern Slovakia. According these results, we can expect that this area might become endemic in a short period of time. Also, our results confirmed the presence of only one haplotype, as same as in other European countries. Thelaziosis outbreak in Slovakia creates serious risk for animal, but also for human health.

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Session IV – Veterinary and Plant Parasitology

Plenary lecture

Marián Várady, Alžbeta Konigová, Martin Babják, Michaela Urda Dolinská (SK): Diagnostic Challenges and Forgotten Parasites of Domestic Animals

Oral presentations

Michal Babják, Alžbeta Königová, Michaela Urda Dolinská, Štefánia Megyesi, Ladislav Molnár, Edina Sesztáková, Marián Várady (SK): Transmission of Gastrointestinal Nematode *Haemonchus contortus* between Sheep and Roe Deer under Field Conditions

Štefánia Megyesi, Alžbeta Königová, Ladislav Molnár, Martin Babják, Edina Szestáková, Peter Major, Michaela Urda Dolinská, Marián Várady (SK): Wild Ruminants as Potential Risk Factor for Transmission of Resistant Abomasal Nematodes

Michaela Urda Dolinská, Alžbeta Konigová, Tomas Kupčinskas, Michal Babják, Marián Várady (SK): Experimentally Infected Small Ruminants with Composite Infection of *Haemonchus contortus*

Dominika Mravčáková, Michal Babják, Magdalena Bryszak, Ľubomíra Grešáková, Klaudia Čobanová, Svetlana Kišidayová, Alžbeta Königová, Adam Cieslak, Zora Váradyová, Marián Várady (SK): The Use of Nutraceuticals in Lambs Experimentally Infected with *Haemonchus contortus*

Jana Jurová, Marek Renčo (SK): Can Invasive Common Milkweed Threat the Communities of Soil Nematodes?

Marie Varloud, Noemi Szilagyi, Julian Liebenberg, Josephus Fourie (CEVA): Confirmation of the Fast Transmission of *Babesia canis* to Dogs by Infected and Pre-Fed Male *Dermacentor reticulatus* Ticks within 8h of Infestation

Marie Varloud, Noemi Szilagyi, Julian Liebenberg, Josephus Fourie (CEVA): Anti-Attachment Efficacy of a Topical Administration of Dinotefuran-Permethrin -Pyriproxyfen against *Dermacentor reticulatus* Adult Ticks in Dogs over 1 Month

Poster presentations

Kristína Mravcová, Gabriela, Štrkolcová, Mária, Goldová (SK): Genotyping of *Giardia duodenalis* Isolates in Dogs from Shelters

Júlia Jarošová, Daniela Antolová, Martina Miterpáková, Viktória Čabanová (SK): *Echinococcus multilocularis* in Dogs and Red Foxes in Slovakia – the Risk of Infection

Daniela Antolová, Bronislava Víchová (SK): Canine Alveolar Echinococcosis – Diagnostic Principles and Strategies

Nikola Holubová, Martin Kváč, Lenka Hlásková, Dana Květoňová, Bohumil Sak (CZ): Susceptibility of Chicken Embryos to *Cryptosporidium* spp. Infection

Bohumil Sak, Dana Květoňová, Tereza Vecková,, Lenka Hlásková, Martin Kváč (CZ): Risk Assessment of *Encephalitozoon cuniculi* Infection Acquired from Fermented Meat Products

Jacek Sroka, Jacek Karamon, Weronika Piotrowska, Ewa Bilska-Zając, Paweł Kusyk, Tomasz Cencek (PL): *Toxoplasma gondii* in Retail Raw Meat Products and Goat Milk in Poland

Nina Samoylovskaya (RU): Materials on Prophylaxis of Parasitic Diseases in Elk on Natural Sites of Russia

Nina Samoylovskaya, V.V. Belimenko, A.M. Gulyukin, N.S. Malisheva, E.V. Novosad (RU): Diagnosing Strongylatoses in Elks on Natural Sites of Russia

Dawid Jańczak, Adam Kaczmarek, Elżbieta Gołąb (PL): Molecular Evaluation of *Toxoplasma gondii* Infection in Game Animals

František Tóth, Júlia Bystrianska, Ingrid Papajová, Jindřich Šoltys, Peter Šalamún, Naďa Sasáková, Gabriela Gregová, Ján Venglovský (SK): Parasite Contamination of Sewage Sludge from Municipal Wastewater Treatment Plants in Slovakia

Jolanta Zdybel, Jacek Karamon, Jacek Sroka, Mirosław Różycki, Tomasz Cencek (PL): Parasitological Contamination of Dehydrated Municipal Sewage Sludge in Poland

Petra Vanková, Milo Bystrický, Marica Kuzmiak Theiszová, Zuzana Bírošová (SK): Knowledge Junction - Open Access to the Scientific Information and the Risk Assessment`s Tools

Diagnostic challenges and forgotten parasites of domestic animals

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Diagnosis in parasitology has a profound impact on animal health and welfare and, in some cases, public health. Although, over the last decades, there has been progress in the diagnosis of parasitic diseases, gaps in the diagnosis of parasites in domestic animals still exist. For instance, difficulties in obtaining samples for diagnostic purposes, inappropriate sample storage and poor sensitivity of the commonly used techniques, may represent major obstacles in diagnosing parasitic diseases. In Slovakia, veterinarians and livestock farmers depend on the specialized workplaces that perform this diagnosis. To a large extent, it is the regional Institutes of the State Veterinary and Food Administration of the Slovak Republic. These shortcomings could lead to the conclusion that some domestic animal parasitoses that we thought were almost completely eradicated are emerging again. According to our knowledges in recent years, parasites such as *Fasciola hepatica*, *Dicrocoelium dendriticum, Paramphystomum cervi, Parascaris equorum, Anoplocephala spp*. have been re-emerged, but these specialized Institutes have no records about this. This lecture will try to answer some of the questions why this is so.

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Transmission of gastrointestinal nematode *Haemonchus contortus* between sheep and roe deer under field conditions

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Wild animals may be carriers of infectious pathogens, which can be transmitted to domestic ruminants. The rapid spread of anthelmintic resistance (AR) raised the question of the role of wildlife ruminants in transmission of resistant parasites to domestic sheep and goats. Less information is available about the status of AR and course of parasitic infection in wild ruminants. Main goal in our study was confirm the transmission of gastrointestinal blood-sucking nematode *Haemonchus contortus* between sheep and roe deer, which shared the same pastures. Two lambs were infected with 2500 third stage larvae (L_3) of resistant strain (MHco4) of *Haemonchus contortus* and imported to clean pasture, where any animals never been grazed before. 28 days after infection counts of eggs per gram (EPG) for each lamb were determined by modified McMaster technique. A value of EPG varies between 8000-10000 EPG. Subsequently, lambs were moved to clean pasture, where any animals never been grazed before. After 6 weeks 2 roe deer were introduced to the same pasture. Faecal samples of roe deer were collected and EPG were determined on day 21, 28, 35, 42 and 49. *In vitro* egg hatch test (EHT) and larval development test were used for determine ED_{50} and LD_{50} for thiabendazole (TBZ) and ivermectin aglycone (IVM) in roe deer samples. Part of samples had been used to prepare coprocultures and L_3 were harvested by Baermann technique. Selected L_3 larvae were identified according morphological features. Maximum intensity of infection in roe deer was documented on day 35, where was observed 800 EPG for roe deer no.1 and 4400 EPG for roe deer no.2. Values of ED_{50} in EHT were 0,111±0,004 - 0,115 ±0,008 µg/ml TBZ. In LDT LD_{50} values for TBZ were 0,021±0,002 - 0,031±0,001 µg/ml and for IVM 46, 67 ± 5,508 ng.ml^{-1}. All L_3 larvae isolated from coprocultures were classified as *Haemonchus contortus*. Values obtained from *in vitro* tests and isolated larvae from coprocultures confirm transmission of multi resistant *Haemonchus contortus* between sheep and roe deer under field conditions.

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Wild ruminants as a potential risk factor for transmission of resistant abomasal nematodes

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Wild ruminants pose a potential threat for the transmission of resistant parasites in domestic small ruminants. Considerable research efforts have been directed towards defining *in vitro* tests that may diagnose resistance in pre-parasitic stages of nematodes. However, little is known about how *in vitro* data obtained in experimental *Haemonchus contortus* infection differ in domestic and wild ruminants. The present study was designed to compare two *in vitro* methods - egg hatch test (EHT) and larval development test (LDT) as tools for detection of the level of benzimidazole resistance in *H. contortus* in mouflons (*Ovis musimon*) and sheep. In this experimental study 6 lambs and 6 mouflons, were each infected with 8 000 L3 larvae of the MHco4 resistant and the MHco3 susceptible isolate of *H. contortus*. In mouflons and sheep the ED_{50} values in EHT of susceptible *H. contortus* strain varied from 0.043±0.005 to 0.064±0.008 mg/ml of thiabendazole (TBZ), which correlated with a low level of resistance obtained by LDT (LD_{50} 0.007±0.002 - 0.009±0.001 mg/ml TBZ). In contrast, higher ED_{50} (0.091±0.001 to 0.111±0.004 mg/ml TBZ) and LD_{50} values (0.021±0.002 to 0.031±0.001 mg/ml TBZ) were determined both in mouflons and sheep in the *H. contortus* resistant strain. Both *in vitro* tests confirmed the same level of benzimidazole resistance for both species. In conclusion, *in vitro* test results showed that mouflons can potentially serve as reservoir hosts of benzimidazole resistant *H. contortus* nematodes.

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Experimentally infected small ruminants with composite infection of *Haemonchus contortus.*

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Anthelmintic resistance is permanent problem of sheep and goats not only in Slovakia but also worldwide. This status is reflected in economy characterized by low production, the death and subsequently financial loss. Both species of hosts share similar genera of nematodes, but in goats they usually develop anthelmintic resistance more rapidly. Sheep and goats differ in many ways; for example, goats have a higher metabolic rate and require higher dose rates for drugs. The main goal of this study was to compare response of the benzimidazole treatment between these animal species. Additionally, the data obtained from *in vitro* method and genotyping in sheep and goats have been compared. The experiment included 54 goats and 54 sheep. Animals were divided into 6 groups. Each group consisted of 9 animals. Sheep and goats in each group were infected with 2500 *Haemonchus contortus* L3 larvae from each of the composite isolate with different resistant alleles composition. Composite infection doses consisted of 10, 20, 30, 40, 60 and 80% of resistant alleles. *In vivo* (Faecal egg count reduction test) and *in vitro* (Egg hatch test) assays were performed. Additionally, molecular determination of the beta-tubulin isotype 1 codon 200 BZ-resistance associated allele frequencies has been performed. Although both animal species share certain differences in drug pharmacokinetics, our results confirmed a comparable response to benzimidazole treatment. Similarly, both genotyping and in vitro test results were similar.

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The use of nutraceuticals in lambs experimentally infected with *Haemonchus contortus*

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Nutraceutical is a food containing health-giving additives and having medicinal benefit. Secondary metabolites of medicinal plants (PSMs), which have been used for centuries in traditional medicine and veterinary practice to treat various digestive or parasitic disorders, have important biological activities. On the other hand the trace element zinc is essential for the proper functioning of both unspecific and specific immune defensive mechanisms and reduces the impact of many diseases by preventing disassembly of the immune system. Both PSMs and zinc are compounds with applications in nutraceuticals and functional food. The aim of this study was to determine the effect of dietary supplementation with a mixture of medicinal plants and organic zinc (Zn-glycinate) on hematological and parasitological parameters of lambs infected experimentally with the gastrointestinal parasitic nematode *Haemonchus contortus*. Mixture of medicinal plants contained plants common in central Europe (11.8% each of *Althaea officinalis*, *Petasites hybridus*, *Inula helenium*, *Plantago lanceolata*, *Rosmarinus officinalis*, *Solidago virgaurea*, *Fumaria officinalis*, *Hyssopus officinalis* and 5.6% *Foeniculum vulgare).* All lambs were infected orally with approximately 5000 third-stage larvae of a strain of *H. contortus* susceptible to anthelmintics (MHco1). Twenty-four female lambs 3-4 months of age were divided into four groups: unsupplemented animals (control), animals supplemented with mixture of medicinal plants (mix), animals supplemented with organic zinc (Zn) and animals supplemented with plants and organic zinc (Znmix). Eggs per gram (EPG) of feces were quantified 20, 28, 35, 42, 49, 56, 62 and 70 d post-infection and mean abomasal worm counts were assessed 70 d post-infection. Samples of blood were collected from each animal 7, 35, 49 and 70 d post-infection. Quantitative analyses of the bioactive compounds in mixture of medicinal plants identified three the most abundant groups of total identified bioactive components: flavonoids (54%), diterpenes (27%) and phenolic acids (19%). Egg counts in the lambs treated with mix, Zn and Znmix decreased after 49 d. The EPGs in the Zn and Znmix groups were significantly lower on day 56 ($P < 0.05$ and $P < 0.01$, respectively), and the EPGs and mean worm counts were significantly lower on day 70 in all supplemented groups ($P < 0.05$ and $P < 0.01$, respectively). The evaluation of red blood cells identified clinical signs of haemonchosis, such as anemia, in each animal after day 35. White blood cell and differential counts did not differ significantly between the infected lambs ($P > 0.05$). Direct anthelmintic impact on the viability of nematodes was not fully demonstrated, but the treatments with herbal nutraceuticals and zinc likely indirectly contributed to the increase in the resistance of the lambs to nematode infection. The use of mixture of medicinal plants with bioactive compounds together with some essential trace elements represents a promising nutraceuticals for controlling nematode infections on principles of organic farming.

This study was supported by funds from the Slovak Research and Development Agency (APVV 14-0169).

Can invasive Common milkweed threat the communities of soil nematodes?

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Invasive plants can threaten biodiversity and alter the environment in which they are introduced. They can change ecosystem services by altering community composition, structure and abundance of native species (plants, belowground fauna) in invaded area. Common milkweed known as *Asclepias syriaca* belongs to the important invasive plant species in Slovakia. The main aim of our study was to investigate how soil nematodes have answered to *A. syriaca* (AS) invasion. Permanent grassland habitat for this study have been chosen near to the Ipeľ River at a locality of Selešťany. We selected 10 m × 50 m area invaded by *As*. An area of the same size which was not yet colonized by invasive plant was chosen in proximity to the corresponding invaded one as control (CO). In the invaded area, we randomly marked five 1 m × 1 m squares which had a similar cover of *A. syriaca.* Similarly, five squares with an equal spatial distribution were marked in the related uninvaded area. Soil samples were collected from each square three times within the vegetation period (May, July, September) in 2017. The results showed that obligate plant parasites and bacterivores have best reflected the changes in soil nematode communities under the influence of AS invasion during whole sampling period. Their relative abundance was significantly higher in CO and AS respectively ($P<0.05$, 0.01) at two sampling dates. On the contrary, miscellaneous (variable) results were yielded for the average total nematode abundance, species numbers, and relative abundance of fungivores, predators and omnivores between invaded and uninvaded as well as three sampling dates. Our short-term study provides some evidence that *A. syriaca* can affect nematode abundance, species numbers and relative abundance of particular nematode trophic groups, however differently among three sampling dates. Therefore we can´t confirm negative or positive impact of this invasive plant on nematode trophic structure in general. So, multiannual investigations are needed to reach more significant conclusions.

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Confirmation of the fast transmission of *Babesia canis* to dogs by infected and pre-fed male *Dermacentor reticulatus* ticks within 8h of infestation

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In an exploratory study, the transmission of *Babesia canis* was demonstrated within 8h of infestation by pre-fed ticks (Varloud et al., 2018). The present study allowed to investigate further this fast transmission of *Babesia canis* to dogs by infected and pre-fed male *Dermacentor reticulatus*.

The protocol was approved by an ethics committee. Donor sheep were infested with *Babesia canis* infected male and uninfected female ticks for 88h. Ticks were subsequently removed, infested on seven new host dogs on day 0 and were allowed to attach and feed before removal at 8h after infestation. Each dog was infested with approximately 50 infected pre-fed male and 25 uninfected unfed female ticks. The dogs were maintained under clinical surveillance that include body temperature measurement and blood samples were collected for blood smear, IFA and PCR analysis.

The tick infection rate was 64% (30 infected / 47 tested). Pre-fed and infected male ticks re-attached easily on dogs (46-72% attachment) and only showed subtle signs of blood-feeding. Four out of seven dogs were infected after being exposed to pre-fed male ticks for 8h. All the infected dogs had elevated body temperature (>39.4°C) starting from day 5 to 7. Three of the infected dogs had blood visible in their urine 7 days after the challenge which resolved within 24-48h of treatment. All the infected dogs were positive to blood smear, IFA and PCR. The infected dogs were immediatly treated and recovered.

These results confirmed the risk of fast *Babesia* transmission within the first 8 hours of tick infestation in dogs. Since no minimal transmission time can be established for all possible natural situations, a strategy of prevention based on tick anti-attachment is recommended.

Reference:

Varloud M, Liebenberg J, Fourie J. 2018. Early Babesia canis transmission in dogs within 24 h and 8 h of infestation with infected pre-activated male Dermacentor reticulatus ticks. Parasites & Vectors. 11:41.

This study was sponsored by Ceva Santé Animale.

Anti-attachment efficacy of a topical administration of dinotefuran-permethrin-pyriproxyfen against *Dermacentor reticulatus* adult ticks in dogs over 1 month

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This study allowed to assess the anti-attachment efficacy of a topical (dinotefuran-permethrin-pyriproxyfen, DPP, Vectra 3D) on dogs challenged with *Dermacentor reticulatus* ticks 2 or 4 weeks after administration. *Dermacentor reticulatus* is the vector of *Babesia canis* and transmission of this pathogen was recently demonstrated within 8h of infestation by pre-fed ticks (Varloud et al., 2018).

The experiment was blinded. The dogs were allocated to 3 groups based on gender and body weight. The dogs were untreated (n=7; 14.8±2.7kg BW) or administered 3.6mL of DPP as a line-on on day 0 (n=7; 13.7±2.1kg BW) or day 14 (n=7; 13.8±2.7kg BW). The dogs were exposed to 75 adult *Dermacentor reticulatus* ticks on day 28 and were maintained in an individual crate for 4h after the release of ticks on the crate floor, close to the dogs. The ticks were counted in the crate 4h after infestation and on the dogs 8h after infestation before being categorized as live, moribund or dead (from the crate and dogs) and attached or free (from the dogs).

In the control group, there were in average 4.1 live, 1.6 moribund and 0.7 dead ticks per dog in the crate 4h after infestation and 43.1 ticks attached on dogs 8h after infestation. In the DPP-D0 group, there were in average 2.7 live, 25.4 moribund and 10.3 dead ticks per dog in the crate 4h after infestation and 3.7 ticks attached on dogs 8h after infestation. The anti-attachment efficacy was 94.1%. In the DPP-D14 group, there were in average 0.4 live, 29.9 moribund and 22.9 dead ticks per dog in the crate 4h after infestation and 3.4 ticks attached on dogs 8h after infestation. The anti-attachment efficacy was 95.9%. The product was well tolerated.

The results demonstrate the successful and rapid preventive actions of DPP against ticks in dogs. The anti-attachment efficacy above 94% is expected to provide protection against the fast transmission of tick-borne diseases such as babesiosis.

Reference:

Varloud M, Liebenberg J, Fourie J. 2018. Early Babesia canis transmission in dogs within 24 h and 8 h of infestation with infected pre-activated male Dermacentor reticulatus ticks. Parasites & Vectors. 11:41.

This study was sponsored by Ceva Santé Animale.

Genotyping of *Giardia duodenalis* isolates in dogs from shelters

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Giardiasis is protozoal worldwide expanded parasitosis and one of the most common intestinal infection in humans, domestic and wilds animals. We include this infection among opportunistic infection, which are transmitted by contaminated water and food - *waterborne and foodborne diseases.* The World Health Organization recognized *Giardia* as zoonotic parasite. In the present, *Giardia duodenalis* is divided into eight genetically different assemblages (A – H) from which A and B have the most important zoonotic potential. Assemblages C – H are strictly host-specific: C and D are found largely in canids, E in domestic mammals, F in cats, G in rodents and H in seals. *Giardia duodenalis* is located in the distal part of duodenum, proximal and central part of jejunum, where they infect the cells of the small intestine and block nutrient absorption. If the infection is clinically manifested, gastrointestinal symptoms frequently include diarrhoea, spastic pain in the epigastric region and steatorrhea.

Giardia duodenalis is one of the most common pathogen causing infections diarrhoea in people around the world. This infection occurs in all age categories, but the most vulnerable are children under 2 years old. In dogs and cats, giardiasis affects young individuals and individuals with reduce immunity, but the disease is also diagnosed in adult animals. Potential mechanism of transmission *Giardia duodenalis* can be from human to animal, from animal to animal, or zoonotic transmission (from animal to human). The aim of this study was to identify assemblage *G. duodenalis* in shelters dogs.

Fecal samples were examined microscopically by flotation method with zinc sulphate (specific gravity 1.18 g.cm^{-3}) for the presence of cyst *Giardia duodenalis*, prior to molecular testing. DNA was extracted from the fecal samples using the ZR Fecal DNA MiniPrep™ kit (Zymo Research, USA) according to manufacturer`s instructions and then subjected to a nested PCR. The nested PCR protocol was used to amplify a partial sequence of the triose phosphate isomerase (*tpi*) gene, as previously described by Sulaiman *et al.* (2003). Obtained *tpi* sequence of our tested samples, were submitted to the GenBank and accepted for publishing in GenBank databases under the accession numbers. From totally 8 examined samples, we had 4 positive. After performing BLAST analysis and then comparing sequences of our positive samples with GenBank sequences already existing, we found a similarity from 99% to 100% with assemblage C in all 4 samples. This samples were coincided with accession numbers JN587492 obtained in a dog from Croatia and AY228641 obtained in a dog from USA.

*This study was funded by the projects of the Scientific Grant Agency of the Ministry of Education SR and Slovak Academy of Sciences,*VEGA 1/0536/18.

Echinococcus multilocularis in dogs and red foxes in Slovakia – the risk of infection

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Echinococcus multilocularis is a tapeworm that may cause a zoonotic infection resulting in a highly pathogenic and potentially fatal chronic liver infestation called human alveolar echinococcosis. Red foxes (*Vulpes vulpes*) provide a natural reservoir of infection but the urbanisation of the red fox and it's increase in numbers across Europe have brought *E. multilocularis* into increased contact with domestic dogs that may also harbour infection. The study aimed at monitoring of the occurrence of *E. multilocularis* in dogs and in red foxes in Slovakia. Fecal samples of dogs (n= 22) from dog shelters, segregated settlements and dogs kept outdoors were examined coprologically and small intestines of red foxes (n= 12) were examined by sedimentation and counting technique. DNA from samples positive for taeniid eggs was isolated, examined by PCR and specific products were sequenced. Preliminary results showed the presence of *E. multilocularis* in 50% of red foxes and in 13.6% dogs. All three positive dogs were from Roma settlements from eastern Slovakia. Our results confirmed the persistence of the circulation of *E. multilocularis* in wildlife in Slovakia, but also the risk of infection connected with the contact with dogs.
The research was supported by the Slovak Research and Development Agency, project No. APVV-15-0114.

Canine alveolar echinococcosis – diagnostic principles and strategies

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Canine alveolar echinococcosis is serious parasitic disease of dogs caused by larval stages of *Echinococcus multilocularis.* Cases of the diseases have been have rarely reported in dogs from Europe (Switzerland, Germany, Belgium) and Canada, and in 2016 the infection in 11-years old female of Siberian Husky was recorded also in Slovakia.

In general, all diagnostic procedures used for the diagnosis of alveolar echinococcosis in humans can be used also in veterinary medicine (serology, imaging techniques, and molecular analyses). Nevertheless, in many countries the possibilities to diagnose the disease in dogs are limited, because commercial laboratories usually do not perform *E. multilocularis* serological tests and practical veterinarians have no access to histological or PCR examination of samples obtained during surgery. Different serological approaches using commercial human diagnostic sets, as well as in-house ELISA and Western blot methods with native and recombinant antigens have been evaluated for their suitability in veterinary diagnostic practice. In presented work, results of such studies were compared to define the methods suitable for the use in veterinary practice. Moreover, the summary of clinical symptoms and outcomes of the different therapeutic approaches presented by different authors are summarised.
Research was supported by the Slovak Research and Development Agency, project No. APVV-15-0114.

Susceptibility of chicken embryos to *Cryptosporidium* spp. infection

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The genus *Cryptosporidium* comprises species of protist parasites that infect epithelial cells in the microvillus border of the gastrointestinal tract, lungs and the bursa of Fabricius of vertebrate hosts. Most *Cryptosporidium* species and genotypes have a narrow host specificity, and, with the exception of *C. parvum*, those that infect a broader host range do not infect different classes of vertebrates. Cryptosporidium parvum has been detected in more than 200 species of mammals and birds. Reports of *C. parvum* in birds have been from field studies and have not been verified experimentally. This study examined the infectivity of *C. parvum* (mammalian species) and *C. baileyi* (bird species) for one-day-old chickens and chicken embryos. *Cryptosporidium baileyi* was infectious for both one-day-old chickens and chicken embryos. Following embryo infection, hatched chickens shed oocysts of *C. baileyi* from the first day after hatching with an infection intensity up to 45,000,000 oocysts per gram of faeces (OPG). In comparison, chickens infected at one day old shed a maximum of 150,000 OPG beginning at four days post infection (DPI). *Cryptosporidium baileyi* infection in one-day-old chickens was localised to the small intestine and trachea, and the infection resolved within 50 days. In chickens that hatched from infected embryos, all organs were infected with *C. baileyi* and the chicken died within 14 days post hatching. *Cryptosporidium parvum* was not infectious for one-day old chickens. Chickens that hatched from embryos infected with *C. parvum* shed oocysts from day 0 to at least 30 day post hatching, with an infection intensity of 2,000–50,000 OPG, and C. parvum was found in the small and large intestine and trachea.

This study was funded by Grant Agency of the University of South Bohemia (002/2016/Z; 017/2018/Z) and supported by MEYS CR (LM2015062 Czech-BioImaging).

Risk assessment of *Encephalitozoon cuniculi* infection acquired from fermented meat products

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Food safety is together with safe water availability a growing concern mainly in developed countries. Many pathogens which can result in the illness or death of the person or other animals such as bacteria, viruses, mold, and fungi could be transmitted via poorly treated food. Microsporidia, worldwide distributed intracellular spore-forming unicellular parasites infecting a wide range of vertebrate and invertebrate hosts, have been originally recognized as the causative agents of opportunistic infections in individuals with deficient immunity, but the introduction of a method for molecular detection of microsporidia showed that microsporidia, mainly Encephalitozoon cuniculi, successfully survive in immunocompetent human and animals. In the present experimental study performed on murine-derived sausages we reported the risk of E. cuniculi infection associated with fermented salami consumption as the non-heat treated food. Moreover, we recorded the presence of E. cuniculi in fresh pork meat used in sausage production.

This study was funded from the Czech Science Foundation (17-12871S).

Toxoplasma gondii in retail raw meat products and goat milk in Poland

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Human toxoplasmosis still poses a public health problem. People usually become *T. gondii* infection by consumption of raw or undercooked meat containing parasite cysts, or by food and water contaminated with parasite. Since *T. gondii* has already been identified in the milk of goats, the possibility of acquiring infection by ingesting unpasteurised goat milk should be also taken into consideration.

In the present study, samples of raw meat products (cured bacon, raw or smoked sausages, hams and minced meat) sold in Polish stores were examined on the presence of *T. gondii* DNA. Samples were digested by pepsin solution, followed by the DNA isolation. PCR was performed based on the amplification of 35-fold-repetitive B1 fragment gene of *T. gondii*.

Among 1514 examined samples, in 122 samples (8.1%) *T. gondii* DNA was detected. The highest percentage of positive results was found in samples of ham (13.9%), lower in samples of smoked bacons (8.9%) and sausages (8.5%) and lowest in minced meat samples (4.7%). The highest percentages of positive results were obtained for samples from Podkarpackie province (17.9%), lower from Małopolskie (12.6%), Lubelskie (10.8%) and Pomorskie (5.5%) provinces. The lowest percentages of positive results were found in provinces: Podlaskie (3.7%) and Warmińsko-Mazurskie (1.4%).

The study on the presence the *T. gondii* DNA in goat milk also was performed. First, 73 goats (females) from 36 farms located in Poland were examined serologically by Direct Agglutination Test (DAT) to estimate the *T. gondii* serological status. Milk samples from 60 selected lactating females were examined for the presence of *T. gondii* DNA by Real time PCR and nested PCR (B1 gene). To estimate the clonal type of detected T. gondii, multilocus PCR was performed using 6 markers. In DAT, positive results were found in 70% of 73 goats. Among examined 60 milk samples, 65% were positive in Real time PCR and 43% in nested PCR. It is noteworthy that 11 samples positive in PCR were collected from seronegative goats. The multilocus PCR analysis mostly revealed the occurrence of genotype III, which is relatively rare in Europe.

The detection of *T. gondii* DNA in raw meat products and goat milk can indicate a potential risk threat to people health. However, to complete the risk assessment for consumers, the additional studies confirming the presence of a live parasite are needed.

Materials on prophylaxis of parasitic diseases in elk on natural sites of Russia

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This guidebook is based on the research carried out on the territory of Losiny Ostrov national park (Moscow) and Kostromskaya Elk Farm (Kostroma region).

The present guidance materials can be used as aids for heads of scientific departments, workers of national parks, nature reserves, wildlife sanctuaries and other NAPPs (natural areas of preferential protection), for protection inspectors, workers of biological stations and elk farms, veterinary specialists, parasitologists and biologists.

Wild animals of different species living on natural sites as well as domestic animals are susceptible to many infectious diseases. There were contagious, fungous and parasitic diseases registered in game animals and birds. Parasitic diseases in wild animals can manifest themselves as single cases or affect large groups of animals on large territories that is to become epizootic. This significantly affects wild animals population in because in many cases leads to their mass infection and death. Invasion diseases of game animals and birds can be differentiated by nature of causative agent as well as by nature of run of the disease and damage caused by them to national parks, nature reserves, hunting farms, etc.

Of the wild hoofed of the Central part of Russia the absolute honour is Cervidae representative – elk.

Consolidating the research results it can be concluded that elks of Losiny Ostrov national park and of Kostromskaya Elk Farm are infected with various helminths species simultaneously and the species common to them are: *Dicrocoelium lanceatum*; *Moniezia benedeni*; *Dictyocaulus filaria*; *Strongyloides papillosus*; *Eimeria bovis* and *E. ellipsoidalis*.

The helminths fauna of elks is dominated by nematodes. IE by helminths came up to 100%, of which the larger part was represented by intestinal Strongylata.

Prophylaxis has as its main goal to prevent distribution of invasive diseases and not to allow the healthy animals get infected. Prophylaxis methods vary but the wide use is made of biological and chemoprophylactic ones which are aimed at destruction of invasive source – eggs, larvae, mature adults, intermediate hosts or carriers.

The whole complexity and difficulty of organization veterinary- prophylactic measures concerning preventing the occurrence of invasive diseases in wild animals is in the fact that the main source of causative agents is not the live-stock but wild animals.

Veterinary- prophylactic measures are aimed at not letting causative agents of invasive diseases into the environment of wild animals and at meeting veterinary-zoological and technical requirements on keeping, extra nutrition and regular feeding of animals, at getting healthy calves and reducing waste among the wild hoofed.

For achieving the above listed goals in parks and other NAPPs it is necessary to plan and hold the following general economic and veterinary-prophylactic measures.

Diagnosing strongylatoses in elks on natural sites of Russia

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Collection of excrement samples to diagnose strongylatoses in elks should be carried out with due account for the biology of the animal in question, namely to determine the favorite habitats (stations), traces of their presence, feeding places, food supply etc.

The best lands for elks are young forests, healing fire-sites and cutting areas, located within solid old wood. Elks also like lowland forest with dense osiery, close to wood swamps and water basins, rich in aquatic and swamp vegetation.

In summer the elks of Losiny Ostrov concentrate near the saline soil present in Losino-pogonny and Mytishchinsky forest parks. Especially willingly they stick to the wood of the northern part of the Losiny Ostrov and close to the swampy areas of the Verchnye-Yauzsky Water and Swamp Complex, located between the Mytishchinsky and Losino-pogonny forest parks of the central part of the Park. It should be mentioned that the elks can migrate over the whole territory throughout the year.

The terms of examining elks should be determined with the account for the local climate conditions, the biology of causative agents, epizootology of diseases.

On the basis of the results of monthly examining the elks of the Park during the period of 2006-2010 the seasonal periodicity of digestive tract strongylatoses has been determined, characterized by invasion peaks in late August, September and early October.

The infestation of elks in the Losiny Ostrov National Park with strongylata larvae vary considerably throughout the year. In January the extensity of invasion made 34.7 per cent, in February and March it reduced to the corresponding 31.6 and 23 per cent, and in June it rose again to 42 per cent. The invasion reached its peak in August, September and October, making 68.8, 65.2 and 58.3 per cent, correspondingly. Then the invasion level reduced gradually, making 53.3 per cent in November. By strongylatoses of the digestive tract elks (older than 1 year of age) living on the territory of the biological station show seasonal periodicity of infestation with peaks in August, September and October being 71.3, 69.4 and 73 per cent, respectively. Youngsters of 5-6 months of age the figures of invasion extensity began growing in late September – early October and reached 36.7 per cent from those in June.

Starting from June, the invasion level by strongylatoses of the digestive tract of elks was growing (28.7 ± 1.4 per cent) and reached its maximum in September/October (34.7 ± 1.4 per cent). In winter and spring the figures of invasion extensity decline.

Systematic diagnostic examination of elks is better to be carried out in late May or early June and in September of early October.

Molecular evaluation of *Toxoplasma gondii* infection in game animals

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Toxoplasmosis caused by unicellular protozoa *Toxoplasma gondii* is the most common zoonotic infection, worldwide. Human infection is usually caused by ingestion of oocyst or tissue cysts from thermally unprocessed animal products. *T. gondii* strains exhibit various levels of genetic diversity and virulence. Highly pathogenic strains can break immune response and cause superinvasions with severe clinical course. Toxoplasmosis in most animal species is asymptomatic, and sanitary tests do not include this disease entity. The aim of the study was the evaluation of *T. gondii* prevalence in game meat and genetic characterisation of the isolated strains.

Game meat samples: wild boar (52), roe deer (40), red deer (23), fallow deer (12), mouflon (12), pheasant (13), brown hare (5) and mallard duck (5) were tested for the presence of *T. gondii* DNA by nested PCR according to the methodology described by Grigg i Boothroyd (2001). The unilocus PCR-RFLP typing for the B1 gene coding fragment of *T. gondii* was performed using Eco72I restriction enzyme.

T. gondii DNA was detected in 17,5% (7/40) roe deer and 5,8% (3/52) wild boar meat smaples. The unilocus PCR-RFLP typing for the B1 gene coding fragment showed the presence of clonal lineage I (8) and lineage II / III (2) of *T. gondii*.

The results show that *T. gondii* infection is common among game animals. In the absence of proper thermal processing of game meat, it can be an important source of the infection for humans.

Parasite contamination of sewage sludge from municipal wastewater treatment plants in Slovakia

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Sewage sludge represent potential reservoirs for the pathogenic microorganisms. Out of them the agents of parasitic infections epidemiologically should be considered very hazardous. Therefore, the objective of this study was to monitor the parasite contamination of sewage sludge from municipal wastewater treatment plants (WWTPs). The samples of sewage sludge were collected from WWTPs in Košice, Snina, Sabinov, Lipany, Nitra and Bratislava. A rich parasitological fauna was found: eggs of *Toxocara* sp., *Toxascaris leonina*, *Ascaris* sp., *Trichuris* sp., *Capillaria* sp., *Hymenolepis diminuta*, *Heterakis* sp., strongyle-type eggs and oo(cysts) of Eimeria sp. In conclusion, the presence of helminth eggs and protozoan oo(cysts) in sewage sludge does imply a risk of transmission of parasites that are of health risk to humans.

This work was supported by the scientific Grant Agency of the Ministry of Education of the Slovak Republic, and by support from the Slovak Academy of Sciences, VEGA no. 2/0125/17 (1.0).

Parasitological contamination of dehydrated municipal sewage sludge in Poland

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The objective of the study was evaluation of the real contamination with the live eggs of intestinal parasites (*Ascaris, Toxocara* and *Trichuris*, ATT) of sewage sludge produced by municipal waste treatment plants in Poland. Ninety two municipal, mechanical-biological sewage treatment plants located in the territory of the whole Poland were selected. They belonged to various types of agglomerations": group 0 (large), group 1 (medium), group 2 (smaller) and group 3 (small). Samples were collected at the final stage of the process of sewage treatment, after the addition of flocculent into the sludge, and its dehydration. The samples were examined by own method adjusted to examination of sewage sludge dehydrated using polyelectrolytes. The viability of the isolated eggs was evaluated based on the incubation in a moist chamber. Live eggs of intestinal nematodes were found in 98,9% of samples. The largest number of samples was contaminated by the eggs of *Ascaris* spp. (95%) and *Toxocara* spp. (96%). However, Trichuris spp eggs were detected in 60% samples. The mean number of eggs in 1 kg of dry mass was: *Ascaris* – 5605, *Toxocara* - 3670 and *Trichuris* – 1075 eggs/kg d.m. The highest number of ATT eggs was detected in samples from sewage treatment plants located in south-eastern and central Poland. The highest number of ATT eggs were found in sewage sludge produced in large sewage treatment plants with (agglomerations Groups 0 and 1) – mean 15,206 and 8,911 eggs/kg, and a considerably lower number in small sewage treatment plants (Groups 2 and 3) - mean 3,603 and 3,145 eggs/kg d.m. The presented study is the first such comprehensive parasitological investigation conducted after the common introduction of polyelectrolytes into the process of dehydration of sewage sludge. The results indicate that sludge produced in municipal sewage treatment plants, are highly contaminated with parasite eggs.

This study was performed within the project N N305 601339 funded by the Polish Ministry of Science and Higher Education.

Knowledge Junction - open access to the scientific information and the Risk Assessment`s tools

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The Knowledge Junction is a curated community in the Zenodo (https://zenodo.org/communities/efsa-kj?page=1&size=20) repository for research sharing. It is used by the OpenAIRE project, and was commissioned by the EC to support their nascent Open Data Policy by providing a catch-all repository for EC funded research. Presented document informs national scientific experts about the possibility to obtain and exchange evidence and supporting materials used in food and feed safety risk assessments. Member State organizations, EU countries and Pre-Accession countries are encouraged to upload, through national representatives, all information relevant to risk assessment activities including risk assessment mandates, outputs (opinions, reports, statements, guidance documents), national work plans related to risk assessment, relevant technical reports, any other relevant document, data and software.

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Scientists and experts interested in publishing their scientific papers at the Knoledge Junction may contact us via e-mail: efsa.focalpoint@land.gov.sk.

More detailed information on the procedures for inserting documents as well as searching for relevant publications is available on the EFSA web site (https://www.efsa.europa.eu/en/events/event/170925-0).

Session V – Wild-Life Parasitology and Zoonoses

Oral presentations

Jana Bulantová, Zuzana Pokrupová, Tomáš Macháček, Libuše Turjanicová, Petr Horák (CZ): Increasing Risk of Cercarial Dermatitis in Urban Areas of Czech Republic

Eliza Kondzior, Małgorzata Tokarska, Rafał Kowalczyk, Iwona Ruczyńska, Wojciech Sobociński, Marta Kołodziej-Sobocińska (PL): The First Case of Genetically Confirmed Sparganosis (*Spirometra erinacei uropaei*) in Reptiles in Europe

Aleksandra Jurewicz, Radomir Józef Jasiak (PL): Prevalence of Endoparasites in Alpacas (*Vicugna pacos*) and Llamas (*Lama glama*) in Poland

Anna M. Pyziel, Izabella Dolka, Żaneta Steiner-Bogdaszewska, Jan Wiśniewski (PL): Pathological Lesions in Lungs of Free-Roaming Red Deer *Cervus elaphus* (L.) Associated with *Dictyocaulus cervi* Infection in North-East Poland

Jiřina Marková, Pavlína Pittermannová, Eva Bártová, Jana Sedláčková, Hana Banďouchová (CZ): SDirect Detection of Protozoal and Microsporidian Parasites in Vespertilionid Bats from Central Europe

Zuzana Čadková, Michaela Svrčková, Kateřina Široká, Jiřina Száková, Jaroslav Vadlejch (CZ): Gastrointestinal Nematodes of Small Terrestrial Mammals from two Post-Mining Area in the Czech Republic – Potential Effect of Risk Element Contamination

Remigiusz Gałęcki (PL): *Cryptosporidium* spp. in Cervids as a Potential Source of Hazard for Farm Animals and Humans - Preliminary Research

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Júlia Jarošová, Daniela Antolová, Zuzana Hurníková, Miloš Halán, Gabriela Chovancová (SK): Intestinal Parasites in Rodents from Pet Shops and in Free Living Small Mammals, with Particular Attention on *Hymenolepis nana* Infection

Poster presentations

Pavlína Pittermannová, Jiřina Marková, Veronika Trávníčková, Alena Žákovská, Eva Bártová (CZ): *Encephalitozoon* spp. in Wild Rodents in ZOO Brno

Božena Kočíková, Igor Majláth, Viktória Majláthová (SK): Atypical Protozoan Parasites Infecting Lacertid Lizards in Central Europe

Emília Dvorožňáková, Marta Kołodziej-Sobocińska, Bronislava Víchová, Zuzana Hurníková, Karol Zub (SK): Circulation of Zoonotic Pathogens in Wild Rodents Living in Different Ecosystems of the National Parks in Poland and Slovakia

Gabriela Chovancová, Milan Kornajčík, Zuzana Hurníková, Martina Miterpáková (SK): *Calodium hepaticum* (syn. *Capillaria hepatica*) in Muroidea and Insectivora from Tatra National Park (Slovakia) – Preliminary Study

Anna Mácová, Jana Kvíčerová, Jasna Kraljik, Aneta Maršíková, Ladislav Mošanský, Ivana Heglasová, Michal Stanko (CZ): Dominant Rodents in Big Cities and their Potential Risk as Reservoirs of Infectious Agents

Jasna Kraljik, Ladislav Mošanský, Jana Kvičerová, Dana Miklisová, Markéta Derdáková, Michal Stanko (SK): *Bartonella* Infection in Communities of Small Mammals in Slovakia and Czech Republic

Grzegorz Karbowiak, Kateryna Slivinska, Tomasz Szewczyk, Joanna Werszko (PL): *Bartonella* Parasitizing Rodents in Chernobyl Exclusion Zone, Ukraine

Dovilė Nugaraitė, Vytautas Mažeika, Algimantas Paulauskas (LT): First Data on the Parasite Fauna of the Eurasian Otter *Lutra lutra* (Linnaeus, 1758) in Lithuania

Jacek Karamon, Joanna Dąbrowska, Maciej Kochanowski, Jolanta Zdybel, Ewa Bilska-Zając, Mirosław Różycki, Jacek Sroka, Tomasz Cencek (PL): Intestinal Parasites of Red Foxes (*Vulpes vulpes*) in Different Parts of Poland

Marcin Popiołek, Alicja Bajon, Agnieszka Piróg, Natalia Kuśmierek (PL): Soil Contamination with Eggs of *Baylisascaris procyonis* (Ascarididae, Nematoda) in Urban Areas Invaded by the Raccoon (*Procyon lotor* L.)

Yuliya M Didyk, Igor Akimov (SK): *Trichinella* (Nematoda, Trichinellidae) amongst Wild and Domestic Mammals in Ukraine

Zuzana Hurníková, Petronela Komorová, Gabriela Chovancová (SK): *Trichinella pseudospiralis* in Slovakia

Marta Basiaga (PL): Occurrence of Parasites in the Great Cormorant (*Phalacrocorax carbo*) from Southern Poland (Kraków District)

Petronela Komorová, Zuzana Hurníková (SK): Prevalence of *Toxoplasma gondii* (Coccidia, Sarcocystidae) in Birds of Prey from Slovakia

Lucyna Holec-Gąsior, Bartłomiej Ferra, Karolina Kujawska, Rafał Kowalczyk, Marta Kołodziej-Sobocińska (PL): Epidemiological Study of *Toxoplasma gondii* Infection in European Bison (*Bison bonasus*) in NE Poland

Miroslaw Rozycki, Ewa Bilska–Zając, Jacek Karamon, Jolanta Zdybel, Katarzyna Gradziel Krukowska, Ewa

Chmurzyńska, Tomasz Cencek (PL): Presence of Anisakids in Baltic Cod and Herring
Dalibor Uhrovič, Mikuláš Oros (SK): Helminth Fauna of Fishes of Liptovská Mara Water Reservoir with Emphasis on Invasive Species
Karolina Majerová, David Modrý, Lada Hofmannová, Jan Votýpka (CZ): Importance of Road-Killed Vertebrates as Sentinel Hosts within the Active Surveillance of Selected Pathogens

Increasing risk of cercarial dermatitis in urban areas of the Czech Republic

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Water in the environment is a big issue of the last years in the Czech Republic. Huge financial resources from the state government or European Union are spent for renewals of old ponds used previously for intensive production of freshwater fishes, revitalizations of areas with mined sources of brown coal, or planning of ponds for water retention in landscape or creation of suitable living space for protected species of animals, mainly amphibians.

Popularity of water bodies increased also in densely inhabited areas of big cities; numerous small decorative water bodies are built on private gardens, or in city parks. Requirements for appearance of official recreational areas connected with water environment are also changing in big cities including Prague. Thus, artificial water bodies made of concrete are gradually changing in more natural water reservoirs. New water bodies serving primarily as areas enabling swimming and other water activities for residents are built *de novo* as well to satisfy contemporary request of citizens for living in close contact with “nature” in city centres.

All mentioned projects usually result in increase of biodiversity on locality, which is connected also with higher probability of simultaneous occurrence of avian schistosomes causing cercarial dermatitis, their definitive hosts (mainly anatid birds), intermediate fresh water snail hosts and swimming humans as accidental hosts. Consequently, contact of people with cercariae of avian schistosomes is more frequent than in the past, which seems to be one of the main reasons why cercarial dermatitis is classified as emerging disease.

Thanks to this fact, we have also more possibilities to observe and analyse various manifestations of cercarial dermatitis in humans. We are often not able to confirm and determine the infection agents, or infected people are unwilling to cooperate in long term studies. Unexpectedly, we got a chance to make a systematic follow-up study of symptoms and immune response in ten human cases of cercarial dermatitis originating in small decorative waterbodies from inner city centre of Prague, which will be presented as a case report.

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The first case of genetically confirmed sparganosis (*Spirometra erinaceieuropaei*) in reptiles in Europe

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Sparganosis is a food- and water-borne disease caused by spargana (plerocercoids), the second larval stadium of tapeworm *Spirometra* sp. (Diphyllobothridae). Adult *Spirometra* sp. reproduces mainly in the intestines of cat-like carnivores and canids such as Eurasian lynx (*Lynx lynx*) and wolf (*Canis lupus*). The first intermediate hosts are copepods (*Cyclops* sp.), in which procercoids develop. The second intermediate or paratenic hosts for the tapeworm can be vertebrates, such as amphibians, reptiles, mammals (including man) and birds.

Amphibians and reptiles are important vectors for this parasite transmission in Asia. However, little is known about transmission routes of *Spirometra* sp. in European wildlife. In Białowieża Primeval Forest (BPF), this parasite was found in many mammalian species such as Eurasian lynx and wolf being definitive hosts and red fox (*Vulpes vulpes*), raccoon dog (*Nyctereutes procyonoides*), wild boar (*Sus scrofa*), Eurasian badger (*Meles meles*), Eurasian otter (*Lutra lutra*), common shrew (*Sorex araneus*) and American mink (*Neovison vison*), being paratenic or both hosts. The aim of our study was to investigate whether reptiles can be a vector in *Spirometra* sp. transmission in wildlife. Morphological identification of *Spirometra* sp. plerocercoids is very unreliable. Thus, the genetical methods are required for undoubt confirmation.

Material for the study was collected between March 2016 and October 2017. A total of 43 found dead reptiles from BPF (13 ind.) and Biebrza National Park (BNP) (30 ind.) were necropsied. The study material included 40 grass snakes (*Natrix natrix*), 2 sand lizards (*Lacerta agilis*) and 1 common European viper (*Viperaberus*). The *Spirometra* sp. was found only in one grass snake from BPF. One complete sparganum located subcutaneously was isolated. To identify a species over 240 bp length sequence of evolutionary conserved nuclear 18S rRNA gene was used. We compared obtained sequences with these acquired from wildboar and badger from BPF and GenBank deposits, using ClustalW Multiple alingment test. It was genetically confirmed that the grass snake was infected with *S. erinaceieuropaei*. The obtained sequence was identical to the sequences of spargana obtained from other analyzed hosts from BPF.

Our finding is a first genetically confirmed record of *Spirometra* sp. in reptile outside the Asian continent. Because of feeding habits of some mammal hosts, reptiles can be a source of *Spirometra* tapeworm infection in the wild. However, further studies are required to investigate prevalence of intermediate hosts infection, including reptiles.

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Prevalence of endoparasites in alpacas (*Vicugna pacos*) and llamas (*Lama glama*) in Poland

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Alpacas and llamas in Poland belong to exoteric animals in home and agritourism farms. They occur most often in small populations (1-2 individuals), and in larger concentrations in zoological gardens or pedigree breeding farms. The aim of the research was to show the developmental forms of endoparasites in alpacas and llamas from different farms of these animals depending on the sex and size of the herd.

From December 2017 until March 2018, samples of fresh faeces - 20 g each, were collected from 164 alpacas (76♀i 85 ♂) and 3 llamas (♀) from different farms in Greater Poland Voivodeship (140 animal specimens), Warmian-Masurian Voivodeship (6 specimens), Kuyavian-Pomeranian Voivodeship (2 specimens) and Pomerania region (16 animals) for parasitological examination.

From the samples obtained after mixing, 1 randomly chosen gram of stool was weighed and tested by Fülleborn's flotation technique with the use of Darling liquid (NaCl / glycerol 1:1) and centrifuged at 2500 rpm for 5 minutes. Microscopic slides were viewed under a light microscope at 400x magnification. The number of eggs in 1 g of feces (EPG and OPG) was calculated according to the McMaster method. Identification of developmental forms of parasites was made on the basis of the "Georgis' Parasitology for Veterinarians" by Georgis Dwight D. Bowman

In the samples collected from 164 (100%) animals, the forms of development of internal parasites were found in 20 specimens (12.19%). In alpacas most often found parasites were eggs of: *Haemonchus* sp. - in 32 (19.87%) animals and *Nematodirus* sp. in 15 specimens (9.31%). In 6 samples (3.66%), oocysts of *Eimeria* sp. were found, and in 1 sample (0.6%) an egg of *Skrjabinema* sp. was detected. Parasites were more frequently found in females - 65 (85.52%) specimens and these were eggs of *Haemonchus* sp. as well as *Nematodirus* sp. Also, more often endoparasites occurred in large clusters of alpacas. In the samples taken from llamas (♀) the presence of individual eggs of *Trichostrongylus* sp. was found. In all EPG and OPG samples tested, it was at a very low level (80 and 90). It should be noted that in 2 farms the animals were dewormed regularly.

It was characteristic that no symptoms of parasitic diseases were observed in animals from which samples were taken for examination. Although there has been a low level of endoparasites prevalence, it is advisable to continuously monitor the animals in this type of culture.

Pathological lesions in the lungs of free-roaming red deer *Cervus elaphus* (L.) associated with *Dictyocaulus cervi* infection in north-east Poland

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The lungworms of the genus *Dictyocaulus* are causative agents of parasitic bronchitis characterised by coughing and severe lung pathology in various ungulate hosts. Recently, *D. cervi* a new lungworm of red deer was discovered in northeast Poland. Examinations performed during the 2017/18 hunting season found large lungworms in the lungs of 15 of 19 red deer in Piska Forest: the area where the species was described. Molecular biology analysis revealed exclusively *D. cervi* infection. Samples of large lungworms and the infected lungs were preserved individually in buffered 10% formalin and submitted to the Department of Pathology and Veterinary Diagnostics for histopathological study. The lung samples were dehydrated and embedded in paraffin, and 4-μm-thick sections were stained with hematoxylin and eosin (H-E). Parasite-positive specimens were also stained with Periodic acid according to the Schiff method (PAS). Additionally, several samples were stained with Masson and Ziehl-Nielsen (Z-N). The occurrence of *Dictyocaulus* was associated with various degrees of histopathological lesions in the lungs, regardless of intensity of parasitic infection. Bronchial and bronchiolar tissue were involved by inflammatory cells, particularly eosinophils, lymphocytes, plasma cells and macrophages with additional bronchiolar hyperplasia of the lymphoid tissue. Moreover, hyperplasia of bronchiolar epithelium and sloughing of epithelial cells into the bronchiolar lumen were observed. In one case, a cross section of an adult *Dictyocaulus* lungworm was visible in the lumen of the bronchus characterised by a histopathological picture of lymphocytic and eosinophilic bronchitis. In 12 out of 15 cases, the infection was associated with interstitial pneumonia, which 11 cases, was accompanied by the presence of lungworm larvae in the interalveolar space. Additionally, focal haemorrhages, emphysema, atelectasis, thickening of alveolar septa and subpleural fibrosis were observed. Interestingly, in lung of one deer some pulmonary arteries were obstructed by a foreign body with honeycomb structures (suggesting the nematode cuticle).

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Direct detection of protozoal and microsporidian parasites in vespertilionid bats from Central Europe

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Vespertilionid bats belong to cosmopolitan family with many different species and comprise almost third of all bats in the world. There is not so many information about endoparasites in this animal species from Central Europe. Because bats live in colony, infected individuals could spread parasites inside their colony or be a source of infection for predators. The aim of this study was to examine bats from the Czech Republic and Slovakia for the presence of *Toxoplasma gondii*, *Neospora caninum* and *Encephalitozoon* spp. in their tissues. In total, one hundred bats from family Vespertilionidae (52 *Myotis myotis*, 43 *Nyctalus noctula* and 5 *Vespertilio murinus*) collected during last seven years were used. Animals were found dead in wild or close to human residences. After dissection, DNA was isolated from brain (*T. gondii* and *N. caninum*) or small intestine (*Encephalitozoon* spp.). Regarding protozoal parasites, *T. gondii* was found in one brain of *M. myotis* male by single PCR (TGR1E sequence), while all bats were negative for *N. caninum* (Nc-5 region). Microsporidian DNA of *Encephalitozoon* spp. was proved in 43 % bats (31 *M. myotis*, 11 *N. noctula* and one *V. murinus*) using nested PCR (ITS gene region). This is the first screening of endoparasites in bats from Central Europe showing the high prevalence of microsporidia in species of family Vespertilionidae.

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Gastrointestinal nematodes of small terrestrial mammals from two post-mining area in the Czech Republic – potential effect of risk element contamination

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Small terrestrial mammals were trapped at 20 sites in two post-mining areas which differ in the source and level of soil contamination. Concerning north-western Bohemia (Most region), open-coast brown coal mining and

petrochemical industry can be considered as the main sources of environmental pollution whereas the ore mining and metallurgic industry have caused enormous contamination in Příbram region. The host data set comprised of 442 individuals from 11 species: *Microtus arvalis, Microtus agrestis, Myodes glareolus, Apodemus flavicollis, Apodemus sylvaticus. Apodemus microps, Micromys minutus, Mus musculus musculus, Sorex araneus, Crocidura leucodon* and *Crocidura suaveolens*. Based on parasitological dissection, following 17 nematod species were found in gastrointestinal tracts: *Syphacia frederici, S. nigeriana, S. obvelata, S. stroma, S. vanderbureli, Trichuris muris, T. arvicolae, Heligmosomoides polygyrus, H. laevis, Heligmosomum costellatum, H. pseudocostellatum, H. mixtum, Longistriata (Carolinensis) minutus, Capillaria muris-sylvatici, Capillaria* sp.(1-from *Apodemus*), *Capillaria* sp.(2-from *Sorex*) and *Ganguleterakis* sp.. Significantly higher prevalence of gastrointestinal nematodes was detected in Příbram region (45 %) when compared to that of Most area (34 %), (χ^2 test, p< 0,01). The infection intensity varied from 1 – 427 worms/host. Concerning the average intensity, the opposite trend compared to prevalence was detected when hosts from Příbram region harboured significantly less nematodes (8 ind.) than these from Most area (42 ind.); (t-test, p< 0,01). In both regions, the majority of the nematode positive hosts was infected with just one species. However, the spectrum of GI nematode partially differed between both areas of interest; *S. frederici, T. muris* together with *Ganguleterakis* sp. were found only in the Most region whereas *H. leavis*, *S. obvelata, S. vanderbruli*, *L. minutus, C. muris-sylvatici*, and *Capillaria* from *Sorex* sp. were specific for Příbram area. Though the Příbram area is 10–45 times more contaminated by risk elements (As, Cd, Pb, and Zn) than north-western Bohemia, no significant correlations were proved between either pollutant concentrations and prevalence nor the intensity of nematode infection at particular sampling sites.

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Cryptosporidium spp. in cervids as a potential source of hazard to farm animals and humans - preliminary research

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The presence of endoparasites in free living deers is quite common. Due to constant movement in the environment, it is difficult to collect samples belonging to specific individuals. In addition, these animals can be a source of parasites threatening directly to livestock and indirectly to humans. In Poland, according to the data of the Polish Hunting Association, the cervids population in 2016 was 1019501 individuals. In our region the most common species of cervids are: european roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*) and red deer (*Cervus elaphus*). The common food base contribute to the occurrence of zoonotic and zooterionotic parasites, including *Cryptosporidium* spp. These animals can be a reservoir of this protozoa directly to farm animals and indirectly to humans.

Therefore, the aim of the study was to assess the occurrence of *Cryptosporidium* spp. in cervids associated with the choice of pastures used by farm animals with the determination of predilection groups depending on age and sex. The research was carried out during winter 2017/2018 in 7 voivodships with the largest number of forest areas. One hundred and sixty samples of fresh faeces weighing 20 g each were collected randomly for parasitological tests. Fecal samples were collected in grazing places of farm animals located close to housing estates with documented penetration by cervids. Samples were tested by formalin-ether concentration method and Ziehl-Nielsen staining method, then segregated by age of animals, which was established on the basis of the shape and size of droppings: group I (animals <1 year), group II (> 1 year) and sex (male droppings, have one side with the spike and on the other concavity, and the females droppings on both sides are the same, rounded). Predilection groups were demonstrated by logistic regression, where the dependentem variable was occurrence of protozoa and independent variables were age and sex, with a significance level of p <0.05.

The presence of *Cryptosporidium* spp. oocysts was found in 92 (57.5%) samples. In group I oocysts were found in 28 (46.67%) out of 60 samples; in group II in 64 (64%) out of 100 samples. In males oocysts were found in 51 (63.75%) out of 80 samples and in females in 41 (51.25%) out of 80 samples. The logistic regression model was statistically significant. It has been shown that adults were 2.06 times more likely to have *Cryptosporidium* spp. than juveniles ($\chi2$ = 4.67; d.f.= 1; 95%CI= 1.07–3.97; P=0.03). There was no sex dependency. When testing only young specimens, it was shown that males were 3.33 times more likely to have *Cryptosporidium* spp. ($\chi2$ = 4.34; d.f.= 1; 95%CI= 0.12–0.96; P=0.04).

Research carried out suggests that cervids are an important vector of *Cryptosporidium* spp. in the environment. In order to protect the welfare of livestock and public health, monitoring studies should be carried out at specific places where different animal species intermingle.

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Increased parasitic load in captive-released European bison (*Bison bonasus*) has important implications for reintroduction programs

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Diseases play an important role in wild populations, strongly influencing their condition and demography. They are often a real threat to rare and endangered species, whose survival is one of the main issues in conservation programmes. Captive bred animals, widely used in reintroduction programmes, are regularly dewormed to prevent spread of parasitic diseases and in most cases they have no prior contact with pathogens occurring in the wild. Thus, they are very likely to be immunologically naïve and more susceptible to pathogens present in the environment. The European bison (*Bison bonasus*) was extirpated in the wild at the beginning of 20th century and later restored with captive survivors. Bison are one of the main species in rewilding programmes in Europe, and there have been discussions on the need to establish new herds and create a more continuous distribution range. Apart from small herd size, isolation and low genetic variation due to a severe genetic bottleneck after extinction in the wild, diseases and parasites are the main threats to bison. Among the 88 parasite species discovered in European bison, the blood-sucking nematode *Ashworthius sidemi* is probably the most pathogenic. The parasite introduction and spread in bison populations has been well documented from the initial phase of its progression.

We analysed infection of invasive blood-sucking nematode *Ashworthius sidemi* in captive bred bison released to the wild in the Białowieża Primeval Forest (BPF), NE Poland in comparison to wild and captive individuals. Mean *A. sidemi* infection intensity of released bison (29 137 nematodes) was over three-fold higher than in wild bison (8 756 nematodes) and over 2 000 times higher than in captive bison (14 nematodes). It indicates a rapid acquisition and increase in the infection intensity in previously dewormed bison released from captivity.

Our study indicates that it is not solely biological factors that determine the spread of parasites in wildlife, but that management practices can also have a strong influence. The impacts of disease have been neglected in mammal reintroduction programmes. Thus, the management protocols should be established and standardized for endangered species reintroductions. These would help to avoid hazards and prevent failure of the programmes caused by exposure to new diseases and weakening of naïve individuals released from captivity. In recent years, European bison have been introduced to new places in Europe; by understanding the factors that can cause failure of bison reintroductions we can design more optimal conservation management approaches and reduce the conservation risk though exposure of naïve captive animals to pathogens present in the wild.

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Seroepidemiology of *Toxocara* spp. in selected population groups in Slovakia

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Roundworms of genus *Toxocara*, common in dogs and cats throughout the world, are the primary cause of visceral larva migrans (VLM) in humans. Soil contaminated with *Toxocara* spp. embryonated eggs is the main source of infection for men; or alternatively infection can be transmitted via dirty hands, raw vegetables, handling of infected definitive host, and by encapsulated larvae in the raw or undercooked tissues or viscera of infected paratenic hosts.

The aim of our study was to determine the seroprevalence of human toxocarosis in the selected population groups in Slovakia. Totally 377 serum samples, divided into four groups, hunters (88 sera), students (126 sera), veterinarians and veterinary assistants (50) and control group of healthy population (113 samples) were included to the study. The presence of specific antibodies in samples was detected serologically by ELISA method using *T. canis* excretory/secretory antigen.

Out of 377 serum samples, anti-*Toxocara* antibodies were detected in 9 (2.4 %) cases. In detail, six (6.8 %) hunters; one (0.8 %) student, one (2.0 %) veterinarian and one (0.9 %) of control samples were serologically positive. The occurrence of antibodies to *Toxocara* was significantly higher ($p < 0.05$) in the group of hunters than in students and control group. There was higher incidence of antibodies in men than in women, but the difference was not statistically significant ($p > 0.05$). Our results suggest that the profession and activity can significantly influence the occurrence of larval toxocarosis in human population.

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Intestinal parasites in rodents from pet shops and in free living small mammals, with particular attention on *Hymenolepis nana* infection

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Small mammals are potential reservoir hosts of many diseases with zoonotic potential, some of them also of parasitic origin. The study aimed at monitoring of the occurrence of intestinal parasites in small rodents from pet shops and breed clubs, as well as in small mammals from rural and urban areas of Slovakia.

Coprological analysis revealed 55.4% overall prevalence of intestinal parasites in fecal samples from 112 cages holding group-housed small animals from pet shops and breed clubs, namely rats, hamsters, mice and Mongolian gerbils. Due to the coprophagia and the direct life cycle of parasites recorded in this survey we suppose that most animals living in the same cage were infected with the same parasitic species. Based on this fact, 378 (72.1%) animals suffered from parasitic infection. Most commonly infected species were laboratory mice (99.1%) in which eggs of *Hymenolepis nana* (27.2%), *Aspiculuris* spp., *Syphacia* spp. and coccidia oocysts were detected. Laboratory rats were infected less frequently (67.6%); with *H. nana* (16.0%), *Syphacia* spp., *Aspiculuris* spp., *Capillaria* spp. and oocysts of coccidia recorded. Intestinal parasites were observed in 54.4% of examined hamsters, in which eggs of *H. nana* (35.5%), *Aspiculuris* spp., *Syphacia* spp. and *Capillaria* spp. were detected. In total, 14.8% of Guinea pigs were infected, with *Aspiculuris* spp., *Syphacia* spp., *Capillaria* spp., *Paraspidodera uncinata* and coccidia oocyst detected. Only one parasite species, *Syphacia* spp. was found in the Mongolian gerbils, but as many as 85.7% of the animals were positive. The presence of *Hymenolepis nana* was confirmed also by molecular analyses in all positive rodent species, mice, rats and hamsters.

In total, 10 rodents from Košice city and 220 free living small mammals from National Park High Tatras were examined. *Hymenolepis nana* was recorded only in one brown rat (*Rattus norvegicus*) from urban area of Košice. Preliminary results showed the presence of tapeworms of *Cladotaenia* spp. in *Apodemus agrarius* and *Staphylocystis furcata* in *Sorex araneus* from National Park High Tatras.

Our results revealed that zoonotic species *H. nana* occurs commonly in small rodents from pet shops and breed clubs, while it is rare in free living animals. Therefore the attention should be payed to the management and hygiene conditions of breeding, as well as to regular parasitological investigation and subsequent deworming of pet rodents.

Encephalitozoon spp. in wild rodents in ZOO Brno

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Encephalitozoon spp. belongs to obligatory intracellular microsporidia, which is causing systematic disease in animals. Encephalitozoonosis is zoonosis, dangerous especially for immunocompromised individuals. Infection is often asymptomatic but sometimes it develop neurologic abnormalities and progressive renal failure. Parasite is spreading especially by urine and faeces. Since we know little of its occurrence in wild rodents, the aim of this study was to detect *Encephalitozoon* spp. in wild rodents in ZOO Brno. Trapping of rodents was done by using spring traps and life-hunt traps in years 2016 – 2017. In total, 54 rodents were trapped including 27 Yellow-necked Field Mouse (*Apodemus flavicollis*), 19 Long-tailed Field Mouse (*A. sylvaticus*), 5 Eurasian Harvest Mouse (*Micromys minutus*) and 3 Bank Vole (*Clethrionomys glareolus*). *Encephalitozoon* spp. was detected in liver of 13 (24 %) rodents by nested PCR. Positive animals included 9 Yellow-necked Field Mouse, 3 Long-tailed Field Mouse and 1 Bank Vole. Our results demonstrate that wild rodents in zoo Brno are coming into contact with *Encephalitozoon* spp., and they can serve as source of infection for zoo animals, but also for humans (zoo personnel and visitors).

We would like to thank Mgr. Vendula Sychrová from ZOO Brno for organizing of trapping of wild rodents in zoo.

Atypical protozoan parasites infecting lacertid lizards in Central Europe

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Reptiles often serve as hosts for various endo- and ectoparasites including protozoan blood and intestinal parasites. In this study, we report the occurrence of protozoans belonging to three families: Tritrichomonadidae, Proteromonadidae, and Lankesterellidae, in lizard blood samples. A total of 738 individuals of 4 lizard species (*Lacerta agilis*, *Lacerta viridis*, *Podarcis muralis*, and *Zootoca vivipara*) captured from 4 European countries (Hungary, Poland, Romania, and Slovakia) were examined. One blood smear obtained in 2008 from *L. agilis* (Odolanów, Poland) was found to be infected with trophozoites representing three genera: *Karyolysus lacazei*, *Tritrichomonas* sp., and *Proteromonas* sp. Moreover, *Proteromonas* sp. were detected by molecular methods in another individual of *Lacerta agilis exigua* (Romania, Vadu), which revealed 100% similarity with the isolate of *Proteromonas lacertae* isolated from the American Type Culture Collection (U37108). Sporozoites of *Schellackia* sp. were detected in blood smears from 2 individuals of *L. viridis* from Slovakia. Molecular detection revealed the occurrence of *Schellackia* sp. in 2 individuals, *L. viridis* (Slovakia) and *Lacerta agilis erythronota* (Romania) and showed 99% and 90% similarity, respectively, with isolates of *Schellackia*-like parasites obtained from *Lacerta schreiberi* (JX984675, JX984674) and *Podarcis hispanica* (JX984676, JQ762306, KJ189384) from Portugal and Spain. This study represents documentation of multiple infections (trophozoites of *Karyolysus* sp., *Tritrichomonas* sp., and *Proteromonas* sp.) detected in a sand lizard from Poland, and the first report of *Schellackia* in populations of a green lizards (Slovakia) and sand lizards (Romania), as well as the first evidence of *Proteromonas* sp. from the Romania population of sand lizards.

The financial support was provided by the Scientific Grant Agency of the Ministry of Education, Science, Research, and Sport of the Slovak Republic and Slovak Academy of Sciences (VEGA), Grant No. 1/0417/14 and by the European Network for Neglected Vectors and Vector-Borne Infections, European Cooperation in Science and Technology (COST) Action TD1303

Circulation of zoonotic pathogens in wild rodents living in different ecosystems of the national parks in Poland and Slovakia

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Small mammals serve as reservoir hosts for zoonotic protozoa, helminths, and tick-borne bacteria and the prevalence of these pathogens in wild rodents may provide information on their circulation in nature. Białowieża Primeval Forest (BPF) in Poland is one of the best preserved lowland forests in Europe inhabited by rich communities of animals. The Tatra National Park (TANAP) in Slovakia protects the Slovak areas of the High Tatra mountain and also serves as a favourite destination for tourism, sport and health resorts, where free-living animals may come into contact with humans. We focused on detection of protozoa – *Babesia* spp., helminths – *Trichinella* spp., *Toxocara* spp., *Echinococcus* spp., and tick-borne bacteria – *Anaplasma* spp., *Candidatus* Neoehrlichia mikurensis, *Borrelia* spp. A total of 106 wild rodents: *Microtus oeconomus* (73), *Apodemus flavicollis* (18), *Clethrionomys glareolus* (13), *Glis glis* (2) were collected in BPF in Poland between 2010 and 2014. No *Trichinella* spp. larvae were found in muscles; prevalence of *Trichinella*-antibody was 3.8 % - *A. flavicollis* (2), *C. glareolus* (2), *Toxocara*-antibody 2.8% - *M. oeconomus* (1), *A. flavicollis* (1), *C. glareolus* (1), and no *Echinococcus*-antibody was detected. Occurrence of parasite-specific antibodies indicates the contact of rodents with the parasites and their presence in BPF. A total of 131 wild rodents were obtained from TANAP in Slovakia during 2016-2017. No parasite larvae were found in muscles; prevalence of *Trichinella*-antibody was 6.10 % – *Mus musculus* (4), *A. flavicollis* (2), *Rattus norvegicus* (2); *Toxocara*-antibody 9.2 % – *M. musculus* (6), *A. flavicollis* (2), *R. norvegicus* (2), *Sorex araneus* (2); and *Echinococcus*-antibody 4.6% – *M. musculus* (3), *C. glareolus* (3). The higher occurrence of synanthropic rodents (*M. musculus* and *R. norvegicus*) resulted in a higher prevalence of anti-parasitic antibodies to *Trichinella* spp., *Toxocara* spp., and *Echinococcus* spp.

No *Anaplasma* spp., *Candidatus* N. mikurensis, *Borrelia* spp. bacteria were confirmed in rodents from BPF. In total, 6.6 % of spleen samples tested positive for *Babesia* spp. Sequence analysis confirmed the presence of *Babesia microti* in *M. oeconomus* (7). Diversity of vector-borne pathogens in rodents from TANAP area was higher: the highest prevalence was recorded for *Candidatus* N. mikurensis (7.1 %), followed by 4.3 % rate of *Anaplasma phagocytophilum* and *Babesia* infection (*B. microti*). One out of 70 DNA samples tested positive for spirochaetes from *Borrelia burgdorferi* sensu lato complex.

The results of this study showed the zoonotic parasites circulation in forest habitats of BPF with a low prevalence; no tick-borne bacterial infestation was found in small rodents from BPF. On the contrary, this study demonstrated higher diversity of tick-borne agents in rodents from TANAP and also the contact of zoonotic parasites with wild rodents. Monitoring of pathogens occurrence in rodents should be continued, as they represent an important reservoir of zoonotic diseases.

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Calodium hepaticum (syn. *Capillaria hepatica*) in Muroidea and Insectivora from Tatra National Park (Slovakia) – preliminary study

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Callodium hepaticum (syn. *Capillaria hepatica*) is a worldwide-distributed species of zoonotic nematode causing hepatic capillariosis. The main hosts of the parasite are rodents belonged to Murinae and Arvicolinae but *C. hepaticum* indicates very low host specificity and was recorded in a wide spectrum of Mammalia. In humans, an infestation is documented in the form of spurious infection or real hepatic capillariosis mainly diagnosed coincidentally or at autopsy. Individuals in close contact with rodent habitats are at higher risk of exposure to the parasite eggs.

In an attempt to obtain information of *C. hepaticum* occurrence in the territory of the Tatra National Park, livers of small mammals from Muroidea and Insectivora group were investigated in the preliminary study. The livers were homogenized and processed by artificial digestion technique for the parasite eggs recovering. Eggs found were identified by morphology using light microscopy and stored for further morphological analyses.

Up to now 231 individuals, belonged to genera *Apodemus*, *Arvicola*, *Clethrionomys*, *Microtus*, *Mus*, *Neomys*, *Rattus*, *Sorex*, and *Talpa* were examined. *C. hepaticum* eggs were detected in one individual of *Arvicola terrestris* and *Apodemus flavicolis*, one *Rattus norvegicus* and six subjects of *Sorex araneus*. The mean prevalence reached 3.9 %, and more than 11 % of examined *S. araneus* were tested positive.

The fact that infected animals were trapped or found dead near the settlements and recreational centres of the Tatra National Park, confirms the existence of an infection pressure for public health. Further, more detailed monitoring should be performed to reveal the real distribution of the parasite in nature.

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Dominant rodents in big cities and their potential risk as reservoirs of infectious agents

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During the spring and autumn periods of 2016 and 2017, we performed several field studies on rodents inhabiting urban, suburban, and rural areas in the eastern Slovakia and southern Bohemia. Altogether 207 rodent individuals representing 7 species (*Apodemus agrarius*, *A. flavicollis*, *A. microps*, *A. sylvaticus*, *Micromys minutus*, *Microtus arvalis*, and *Myodes glareolus*) were trapped and examined for reservoirs of infectious agents and their potential risk. Rodents were inspected for the presence of ectoparasites. Two species of ticks (*Ixodes ricinus*, *I. trianguliceps*), 4 species of mites (Gamasida: *Eulaelaps stabularis*, *Haemogamasus nidi*, *Hirstionyssus sunci*, *Laelaps agilis*), 10 species of fleas (*Amalaraeus penicilliger*, *Ctenopthalmus agyrtes*, *C. assimilis*, *C. solutus*, *C. uncinatus*, *Daratopsylla* *dasycnema*, *Hystrichopsylla talpae*, *Megabothris turbidus*, *Palaeopsylla soricis*, *Peromyscopsylla bidentata*), and 2 genera of lice (*Hoplopleura* and *Polyplax*) were detected.

Coprological examination of rodent faeces revealed the presence of helminth eggs (*Capillaria*, *Trichuris*, and undetermined genera of the families Strongylidae and Oxyuridae), but surprisingly no tapeworms were detected. We also recorded coccidian oocysts (*Eimeria alorani*, *E. apionodes*, *E. jerfinica*, and *E. uptoni*).

Part of the samples analyzed by PCR amplifying specific bacterial and viral markers revealed the presence of the Tula hantavirus in *M. arvalis* in Borek (Czech Republic), *Bartonella* sp. in *A. flavicollis*, *M. glareolus* and *M. minutus* in Čermel´ (Slovakia), and in *A. sylvaticus* and *M. arvalis* in Borek (Czech Republic). Several species of *Rickettsia* were found in various hosts in Čermel´ and Hrhov (Slovakia). *Borrelia miyamotoi* was detected only in a single individual of *M. arvalis*, whereas *Anaplasma* sp. was not confirmed in any of the studied samples.

The spectrum of the ectoparasite species, particularly fleas, corresponded with the host structure as well as with the seasonality of the field studies. Despite the extensiveness of the trappings we confirmed a broad spectrum of ectoparasites as potential vectors of pathogens even in the modified environments of urban and suburban character. Examination of zoonotic pathogens revealed the existence of foci close to human settlements and activities.

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Bartonella infection in communities of small mammals in Slovakia and Czech Republic

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The aim of this study was to identify and compare the spectrum of *Bartonella* species among small mammals captured in different habitat types in Slovakia and Czech Republic. This was achieved by analyzing partial sequences of citrate synthase gene (*gltA*), RNA polymerase beta-subunit gene (*rpoB*) and *groEL* gene, which encodes the 60 kDa heat-shock protein. The results showed similar infection prevalence between localities, ranging from 15,8 % in Borek, Czech Republic to 18,3 % in Čermeľ and 21,3 % in Hrhov (Slovakia). Phylogeny of analyzed sequences revealed high diversity of Bartonella strains including the most prevalent *Bartonella taylorii*, *Bartonella grahamii*, *Bartonella doshiae* and also unidentified genotypes. The differences in *Bartonella* composition were observed among different rodent species as well as between localities.

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Bartonella parasitizing rodents in Chernobyl Exclusion Zone, Ukraine

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The Chernobyl Exclusion Zone (CEZ) is established around the point of the 1986 disaster at the Chernobyl nuclear power plant. The parasite fauna of small rodents in Chernobyl Exclusion Zone is weakly known. The preliminary study was conducted in to the south of Chernobyl city, in September 2017. The mammals were caught in live-traps; the traps were checked two times per day. Captured mammals were individually identified, the spleen samples were collected. In total 36 rodents were caught – yellow-necked mice (*Apodemus flavicollis*), striped field mice (*Apodemus agrarius*), wood mice (*Apodemus sylvaticus*), bank vole (*Myodes glareolus*), common vole (*Microtus arvalis*). The spleen samples were collected to DNA isolation, using the NucleoSpinTissue Kit. PCR reaction was performed using primers 1400F (CGCATTGGCTTACTTCGTATG) and 2300R (GTAGACTGATTAGAACGCTG) (Renesto et al. 2001), four of the the positive products were sequenced.

The infections were detected in Apodemus flavicollis, Microtus arvalis, Apodemus agrarius, Myodes glareolus, the prevalence of infections were 75.0%, 33.3%, 28.0%, 15.4%, respectively. Two *Bartonella* sequences obtained from A. flavicollis showed 99% homology to sequence CP001562, origin from the

Sweden, one sequence to AF165995 from France. The isolate from A. agrarius showed 100% homology to sequence AB779535 from Russia.

First data on the parasite fauna of the Eurasian otter *Lutra lutra* (Linnaeus, 1758) in Lithuania

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The Eurasian otter *Lutra lutra* (Linnaeus, 1758) is a semi-aquatic mustelid belonging to subfamily Lutrinae. Its range covers parts of three continents: Europe, Asia and North Africa. The Eurasian Otter is protected species according to international legislation and conventions. Since 2004 otter is listed as "Near Threatened" by the IUCN Red List. The otter is becoming an increasingly frequent object of parasitological studies. Parasites act as a factor which could have an impact of otter population dynamic, therefore the knowledge's of parasites could play an important role in species protection. Today Eurasian otter population covered about 95% territory in Lithuania, however, no parasites were previously reported from the country. The aim of this study was to explore the parasite fauna of otters in Lithuania for the first time.

Six road-killed Eurasian otters collected between 2016–2017 were examined for helminths using the total helminthological examination of individual organs (Ivashkin et al., 1971). Spleen samples of otters were tested by PCR for the presence of *Filaria*, *Borrelia*, *Babesia*, *Anaplasma*, and Hemotropic *Mycoplasma*.

A total four species or higher taxa of helminths were recorded: flukes *Pseudamphistomum truncatum* (Rudolphi, 1819), *Isthmiophora melis* (Schrank, 1788) Lühe, 1909, and *Phyllodistomum folium* (Olfers, 1816), and nematodes unidentified to the species level.

Mycoplasma sp. was detected in four otter spleen samples. Comparison of the partial 16S rRNA gene with all sequences in GenBank showed 100% similarity with Mycoplasma sp. from red fox (*Vulpes vulpes*) (Accession number KY175160.1) collected from Slovakia.

Intestinal parasites of red foxes (*Vulpes vulpes*) in different parts of Poland

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The aim of the study was to determine the prevalence of intestinal helminths in red foxes in different parts of Poland with particular regard to zoonotic parasites. Four hundred and seventy three red foxes (*Vulpes vulpes*) shot in different part of Poland were used in the study The investigation area were divided on 4 following regions: North (N) (n=114) (Pomorskie and Kujawsko-Pomorskie Province), North-East (NoE) (n=143) (Warmińsko-Mazurskie and Podlaskie Province), South-Est (SoE) (n=150) (Małopolskie and Podkarpackie Province) and South-West (SoW) (n=66) (Opolskie Province). Intestines were examined with the use of the sedimentation and counting technique (SCT). Additionaly, samples of faeces from 344 red foxes were examined with the use of flotation. Overal, parasites were detected (by SCT) in 98,9% of red foxes. The average prevalence of detected parasites were following: *Mesocestoides* spp. (84.1%), hookworms (67.9%), *Alaria alata* (61,5%), *Toxocara/Toxascaris* (49.5%), *Taenia* spp. (42.5%), *Echinococcus multilocularis* (25.6%) and *Trichuris vulpis* (2%). Significant differences between regions were observed especially in case of *E. multilocularis* (no statistically significant differences were noted only between NoE and SoE and between N and SoW). Moreover, the differences were found between northern and southern regions in case of *Alaria alata* and between SoE and other regions in case of *Mesocestoides* spp. infection. Prevalence of other parasites was on a similar level in each region. The percentage of positive samples obtained with coproscopic examination (with exception of *Trichuris*) were sigificantly lower than SCT. Analysis of prevalence estimated in individual regions with the use of both method (flotation and SCT) showed high correlation in case of all parasite species (with exception of Mesocestoides spp.). Additionaly, flotation method allowed to detect eggs of lung nematode (*Capillaria aerophila*, syn. *Eucoleus aerophilus*), which was the most prevalent (76.2%), and coccidia oocysts (7.6%). The study showed a very high percentage of red foxes infected with intestinal helminths in different parts of Poland. Additionally, dangerous zoonotic parasites were found, what should be taken into consideration in assessment of infection risk for humans.

Investigation was conducted in the frame of the grant of Polish Ministry of Science and Higher Education No. NN 308 631 938.

Soil contamination with eggs of *Baylisascaris procyonis* (Ascarididae, Nematoda) in urban areas invaded by the raccoon (*Procyon lotor* L.)

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One of the fastest spreading wild mammals at present in Europe is the raccoon (*Procyon lotor*), introduced from North America. Besides the damage done by the raccoon in native zoocoenoses, an important problem is the possibility of transmission of many zoonotic pathogens, including parasites, into new areas. From the medical point of view, the geohelminth *Baylisascaris procyonis* is one of the most important pathogens carried by raccoons. Studies on the level of soil contamination with eggs of *B. procyonis* were carried out in Kostrzyn on the Odra, a town with a population of around 20,000 located in north-western Poland on the German border, where the raccoon density was estimated at 7 to 25 individuals per 10 km^2. Soil samples were taken from April 2014 to May 2015 in recreation areas of regularly documented raccoon occurrence, considering phenology. The sampling sites were divided into two groups based on raccoon habitat preferences. Some sites were located near watercourses or water bodies; the remaining sites were located in the town's built-up area. A total of 190 samples were subject to parasitological analysis. Eggs of *B. procyonis* were found in 10 samples (5.26%). The mean number of eggs per sample was 2.6. Considering the location of the sites, eggs of *B. procyonis* were only found in places situated along the river, canals or at small water bodies. Our seasonal analysis showed that the frequency of occurrence of eggs of *B. procyonis* in the examined soil was similar in the spring, summer and winter (mild winter) (from 5.71 to 6,25), while no eggs were found in the autumn. The highest mean number of eggs of *B. procyonis* per sample was recorded in the winter; it was smaller in the summer and the smallest in the spring. The results seem to be significant from both epi- and zoonotic points of view, and the presence of eggs of *B. procyonis* in the soil of urban areas poses a real threat to human and animal health.

Trichinella (Nematoda, Trichinellidae) amongst wild and domestic mammals in Ukraine

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Trichinellosis is one of the most dangerous helminthic diseases common to humans and animals. It is caused by the nematodes from the genus *Trichinella* Railliet, 1895. *Trichinella* infections were found in almost all mammal species, as well as reptiles and birds (Pozio & Murrell, 2006). In Ukraine *Trichinella* infection was detected in humans, domestic pigs and games (Shelemba, 1999; Derbal, 1997; Veterinary Reports 1991-2016). More than 1 200 cases of human trichinellosis were found in Ukraine over the past 30 years (Sanitary-Epidemiological Service Reports 1986-2016). Infected pork was the main source of human *Trichinella* infection in past, but the consumption of infected game caused most recent human trichinellosis cases (Artemenko, 1997, Shelemba, 1999, Didyk, 2006). The main purpose of our study was to evaluate the prevalence of *Trichinella* among wild animals in Ukraine and to identify the species composition.

Materials were collected from 2002 to 2016 during the hunting seasons in all regions of Ukraine. The muscle tissue samples from ungulates (wild boar, roe) and carnivores (bear, lynx, wolf, fox, marten, badger) were studied. The samples were examined following the standard protocol of ITRC. The identification of parasites was carried out by multiplex PCR analyses (Borsuk et al., 2003; Pozio & La Rosa, 2003).

Trichinella infection was detected in all regions of Ukraine. Larvae were found in 3% of investigated wild boars, 15.5% wolves, 16% red foxes, 12% martens and 10% badgers. Wolves and foxes were found to be the main reservoir of *Trichinella* in a sylvatic cycle. Our studies demonstrate the presence of tree species of *Trichinella* in Ukraine: *T. britovi* (ITRC codes: ISS1590, 1591, 1592, 1593) was found in wild boars, wolves, foxes and martens in all Ukraine; *T. spiralis* (ISS1594) was found in the domestic pigs from the Kyiv region; *T. nativa* (ISS1595) was found in wolves and foxes from Chernihiv, Sumy, Poltava and Kherson regions. Mixed infections (*T. britovi-T. spiralis)* were found in southern regions*. T. britovi* found to be a dominant species in the sylvatic cycle in Ukraine. High prevalence of *Trichinella* in a sylvatic cycle is caused by high densities of predators' populations that correspondingly lead to increased levels of scavenging and cannibalism. Humans also have big influence on *Trichinella* prevalence, because hunters leave animal carcasses in forests like "baits" or throw it away as garbage near villages.

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Trichinella pseudospiralis in Slovakia

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Trichinella pseudospiralis is a tissue dwelling zoonotic parasite with cospomopitan distribution. The species is the only of the genus capable of infecting birds as well as mammals, including humans. The presence of the parasite in the Central Europe was for the first time recorded in 2003 in Eastern Slovakia. The first focus of *T. pseudospiralis* was documented in pigs, rats, and a cat and from a pig breeding farm in Eastern Slovakia. In following years the parasite was found in co-infection with *T. britovi* in wild boars and red foxes from the same region. Recently, the infection was diagnosed in two Common kestrels, one Peregrine falcon and one Golden eagle. All infected birds originated from Eastern Slovakia. These results indicate that the parasite is already present in the environment of this part of Slovakia, as no of the infected birds belongs to regular migrants and most likely contracted the infection in their residence area. The most current finding of the parasite was from a wild boar in western part of the country, where the species has never been detected to date.

As regards the increasing number of reports of *Trichinella pseudospiralis* in Slovakia, we can conclude that most probable „culprit“ is the introduction of artificial digestion method followed by larva identification according to the Commission Regulation 2075/2005 that allows reliable detection of *T. pseudospiralis* larvae, together with numerous sample group investigated. Most studies on trichinellosis are primarily focused on animals intended for human consumption or hunted for sport and due to protected status of majority of birds of prey species it is difficult to acquire an adequate number of samples for extensive epidemiological studies. This is the reason why the natural cycle of this zoonotic parasite is still not fully known. Further research is needed to understand the host-parasite relationship in birds of prey as a flight-mobile reservoir of *Trichinella pseudospiralis* and routes of further its spreading and circulation in wildlife.

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Occurrence of parasites in the great cormorant (*Phalacrocorax carbo*) from southern Poland (Kraków district)

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The great cormorant (*Phalacrocorax carbo*) is a migratory fish-eating bird. In the southern Poland (Małopolska, Kraków district), these birds stay only over the winter and no nesting colonies have been so far found. Nevertheless, these cormorants can cause a decline of fish populations and be a potential source of parasitic infections for fish. Therefore, the aim of the study was to evaluate the infection parameters and the species composition of the great cormorant‘s internal parasites.

A total of 33 cormorants, obtained in January 2017 in the area of Krakow, were subjected to full parasitological examination. Studies were carried out with the permission of the Regional Director for Environmental Protection in Kraków (decision no. OP-I.6401.235.2017.PKw).

The conducted research revealed the presence of 36 046 helminths specimen belonging to 14 taxa, i.e. six from the Digenea: *Hysteromorpha triloba, Holostephanus dubinini, Metorchis bilis, Paryphostomum radiatum*, *Petasiger phalacrocoracis* and *P. exaeretus;* one Cestoda: *Paradilepis scolecina;* six belonging to the Nematoda: *Eustrongylides excisus, Desmidocercella incognita, Syncuaria squamata, Contracaecum rudolphii, Cyathostoma* sp. and Capillariidae; in addition one Acanthocephala was found. In one host, from 5 to 11 parasitic taxa were found (average: 8.6 ± 1.5). The overall mean intensity of infection was 1092.1 (range 320-2583). The most prevalent species was *Contracaecum rudolphii,* stated in all dissected birds.

Observed, high level of infection may indicate the accumulation of parasites developmental stages in the aquatic ecosystem of the Krakow district. For the majority of the observed parasite taxa, fish are the intermediate and/or paratenic hosts, and therefore the cormorant may pose a potential threat to its health condition.

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Prevalence of *Toxoplasma gondii* (Coccidia, Sarcocystidae) in birds of prey from Slovakia

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Toxoplasma gondii infects practically all warm-blood animals, including birds of prey. Carnivorous birds represent intermediate hosts of this protozoan parasite and may be infected in two ways – by ingestion of oocysts from the contaminated environment and more often by feeding on their prey in which the tissue-inhabiting stages of *T. gondii* may be present. The feeding ecology of predatory birds helps to indicate infections of *T. gondii* in their prey, expacially in small mammals which are at the same time the natural prey of felids that are definitive hosts of the parasite.

In this study 80 individuals of birds of prey from the order Falconiformes, Accipitriformes and Strigiformes were examined for the presence of *T. gondii* using PCR analysis acording to Homan et al. (2000). DNA was extracted from the samples of pectoral mucsles of following species: *Falco peregrinus* (1), *Falco subbuteo* (3), *Falco tinnunculus* (26), *Accipiter gentilis* (3), *Accipiter nisus* (2), *Aquila heliaca* (4), *Buteo buteo* (12), *Buteo lagopus* (2), *Buteo rufinus* (4), *Circus aeruginosus* (1), *Asio otus* (7), *Athene noctua* (1), *Bubo bubo* (2), *Glaucidium passerinum* (1), *Strix aluco* (2), *Strix uralensis* (8) and *Tyto alba* (1). The carcasses of free living birds of prey were colected in the territory of Slovakia and subjected to post-mortem examination using parasitological autopsy.

In the order Falconiformes one individual of *F. peregrinus* (P=100%) and two individuals of *F. tinnunculus* (P=7,7%) were positive for *T. gondii.* Regarding order Accipitriformes no infected specimens were found in our samples. *T. gondii* was also detected in one representative of the species *A. otus* (P=14,3%) and *S. uralensis* (P=12,5%) both belonging to the order Strigiformes. When considering overall prevalence it represents 6,3% (5/80) of all examined birds of prey.

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Epidemiological study of *Toxoplasma gondii* infection in European bison (*Bison bonasus*) in NE Poland

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Toxoplasmosis is a zoonotic disease caused by the protozoan *Toxoplasma gondii*, which infects humans and most warm-blooded animals throughout the world. This disease is of great medical and veterinary importance because *T. gondii* infection may cause abortion or congenital disease in its intermediate hosts, including man. Serological studies on the frequency of anti-*T. gondii* antibodies have proved the infection dissemination in many different farm and wild animals. The European bison (*Bison bonasus*), the largest herbivorous animal in Europe, is a species that has been saved from the extinction and restored in the wild from captive survivors. Diseases and parasites are among the main threats to the species. Thus, the aim of this study was to estimate anti-*T. gondii* IgG antibodies in wild living European bison inhabiting Białowieża Forest (BF) and Knyszyn Forest (KF) in NE Poland and in captivity. Serology for *T. gondii* was undertaken using the direct agglutination test (Toxo-Screen DA, bioMérieux) and the in-house enzyme-linked immunosorbent assay (ELISA) based on native *Toxoplasma* lysate antigen (TLA). Of 367 examined individuals (322 in BF and 45 in KF), fifteen (4.1%) were found to be positive for the presence of *T. gondii*-specific IgG antibodies. All seropositive animals were found in the Białowieża Forest (both in wild living population and in captivity), mainly among animals intensively fed with hay in winter or utilising meadows on the edge of the forest. These results indicate that *T. gondii* infection in European bison living in the natural environment is relatively low. Lack of animals infected with *T. gondii* in Knyszyn Forest is probably related to lower sample size or different risk of parasite transmission in both areas. Therefore, this study is an important contribution to better understanding the epidemiology of toxoplasmosis in two bison populations.

In order to obtain serum samples of an intermediate host of the parasite for further testing, this study was supported by Polish National Science Centre grant UMO-2015/17/B/NZ6/03480. Bison serum samples were collected in the frame of the project no. 2012/07/B/NZ8/00066 financed by Polish National Science Centre.

Presence of anisakids in baltic cod and herring

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Human fish-borne parasitic diseases are mainly caused by nematodes. Infections are caused by consumption of viable parasites. Allergic reactions may occur while consumed fish contains live larvae (sensitisation). Once sensitised, response to allergens can be highly destructive. Prevention of sensitisation is most effective tool to control of *A. simplex* alergy. Marinating and cold smoking methods are not sufficient to kill *A. simplex* thus freezing remain the most effective processes to prevent anisakiosis. All wild caught fish are considered at risk of containing viable parasites. For wild-catch fish, no sea fishing grounds can be considered free of *A. simplex* but the data are limited. As there is a lack of sufficient data on the geographical distribution, prevalence and intensity of parasites of public health importance in fishery products, all wild-catch products are considered at risk of containing parasites. Parasites can affect the commercial value of fish and thus result in significant economic losses. Fish caught from fishing grounds in the Baltic Sea, may present possible health risks from: *Anisakis simplex*, *Contracaecum osculatum*, *Pseudoterranova decipiens*. The *A. simplex* and *Pseudoterranova decipiens* have been found in fishery products in International Council for the Exploration of the Sea (ICES) subdivisions 22, 23, 24, 25, 26, thus the public health risks due to the presence of these parasites cannot be excluded. Part of the SeaQual project is devoted to assess the presence of Anisakids in fish from Baltic Sea. The aim of the carried study is to collect the data on the presence of parasites in wild caught fish from the Baltic Sea for further risk assessment. For the study herring (*Clupea herrengus*) and cod (*Gadus morhua*) caught in the Baltic Sea were examined. Samples were collected from the sea catch provided in April 2016 i 2017 by the research vessel "Baltica". Totally 246 herring and 228 cod samples were collected. Fish samples were examined by candling method, candling with UV and digestion of muscle tissue. All the methods were validated; candling and digestion were accredited by the Polish Centre for Accreditation. Overall, 14.23% of herring and 9.65% of cod were found to be positive with the use of digestion method. The prevalence in herrings varied from 02.35% (ICES 25) up to, 23% (ICES 26). For cod varied from 0% Gdańsk Gulf through 6% (ICES 24) up to 15% (ICES 25). From cod muscle as *Anisakis simplex* s.s. 20 larvae were identified and 2 larvae were classified as *P. decipiens*. Larvae originated from herring were identified as *Anisakis simplex* s.s. in 30 cases, and 5 larvae were classified as *P. decipiens*. Species of anisakids were identified by PCR-RFLP according to EURLP protocol. As the result of the study guideline for parasitological examination of fish for Veterinary Service was elaborated.

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Helminth fauna of fishes of Liptovská Mara Water Reservoir with emphasis on invasive species

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The water reservoir Liptovská Mara is one of the largest (2160 ha) and most important water structure belonging to the Váh cascade system in northern Slovakia. Ichtyoparasitological survey was carried out between the years 2013 and 2015 in six localities of the water reservoir. In total, 160 fish belonging to ten species of the family Anguillidae (1 species), Cyprinidae (6), Percidae (2) and Salmonidae (1) were examined. Parasites were isolated from the intestine of fish hosts, washed in saline, fixed in hot 4% formaldehyde solution, stained with acid iron hydrochloric carmine, dehydrated and mounted in Canada balsam. Analysis of the parasite community structure was carried out and was characterized by quantitative ecological parameters including prevalence and intensity of infection and was calculated for each parasite and its host. A total of 13 species of gastrointestinal helminths belonging to the groups Acanthocephala (3 species), Cestoda (6), Nematoda (3) and Trematoda (1) were identified in the examined fish. The overall prevalence was 49.4% with an average infection rate of 37.7 (1–517) and as many as 24 host–parasite combinations were detected.

Within the study of biodiversity the invasive species *Atractolytocestus huronensis* Anthony, 1958 and *Khawia* *sinensis* Hsü, 1935, which are specific parasites of common carp, *Cyprinus carpio* L., have been found. The detailed taxonomical, morphological and morphometrical characteristics by using light and scanning electron microscopy of these non-indigenous parasites were also provided. These epizootiologically important parasites of carp appear to be a successful colonizer of new habitats and a skilful competitor, which may significantly interfere in the structure of native communities of fish parasites and influence their biodiversity.

The data on biodiversity of organisms and original faunistic data are of major value in terms of prognosis of ecosystem changes, which are expected in respect of accrued environmental problems of global and local characters.

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Importance of road-killed vertebrates as sentinel hosts within the active surveillance of selected pathogens

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In the last decades, several novel diseases emerged and the distribution of many pathogens and their vectors changed. Within the One health concept, zoonoses are one of the most important groups of the emerging diseases. Vectors, reservoir hosts and distribution of the diseases need to be studied in all over the world. However, the vast majority of the available surveillances are focused basicaly on the geographic distribution of arthropod vectors, while information about the reservoir hosts species, their prevalence of infections and related potentional risk for humans and domestic animals are limited.

Our research is focused on using selected road-killed vertebrates for detecting mainly tick-borne pathogens (TBPs) in the Czech Republic. The main idea of our project is focused on effort to test the capacity of urban wildlife for surveillance of the pathogen presence in cities. Hedgehogs (*Erinaceus europaeus* and *E. roumanicus*), squirrels (*Sciurus vulgaris*) and blackbirds (*Turdus merula*) were chosen as representatives of wild animals that thrive in urban areas, that are ordinarily infested by ticks and seems to be suitable hosts for many important zoonotic pathogens.

In the first year of our project, we have already proved that this method can be very usefull for TBPs surveillance. From 50 road kill carcasses of selected host species, 49 specimens were found to be positive at least for one TBP (above all *Anaplasma phagocytophilum* and *Borrelia burgdorferi* s.l.) by using the real-time PCR method.

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Session VI – Human and Clinical Parasitology

Plenary lecture

Michal Giboda (CZ): Migrants and Parasites – Facts and Fictions [Migranti a parazity – fakty a mýty]

Oral presentations

Martin Novotný, Zuzana Paraličová, Milota Kopčová (SK): Rare Case of Visceral Leishmaniosis Imported from Croatia [Zriedkavý prípad viscerálnej leišmaniózy importovanej z Chorvátska]

Lenka Richterová, Ivana Zicklerová (CZ): Malaria in the Czech Republic in 2015-2017 [Malárie v České republice v letech 2015 – 2017]

Zuzana Paraličová, Martin Novotný (SK): Echinococcosis - Overlooked Diagnosis [Echinokokóza – často prehliadaná diagnóza]

František Ondriska, Daniel Pinďák, Vojtech Boldiš, Daniela Antolová, Pavol Poliačik, Natália Farraová, Pavel Vahala, Ľubica Piesecká, Katarína Tináková (SK): Case Report of Alveococcosis with Repeated Cyst Resection [Prípad alveolárnej echinokokózy s opakovanou resekciou cysty]

Róbert Rosoľanka, Katarína Šimeková, Mária Szilágyiová (SK): Alveolar Echinococcosis from the Perspective of an Infectologist [Alveolárna echinokokóza z pohľadu infektológa]

Iva Kolářová (CZ): The Effectiveness of Bioresonance Methods Used To Diagnose or To Treat Parasitic Infections [Efektívnosť biorezonančných metód používaných na diagnostiku alebo liečbu parazitárnych infekcií]

Slávka Dubinská, Pavol Dubinský (SK): Neuropsychiatric disorders in diseases related to parasitic infection [Neuropsychiatricke poruchy pri ochoreniach viazaných na parazitárne infekcie]

Poster presentations

Zuzana Hůzová, Lenka Richterová (CZ): Intestinal parasitoses in the Czech Republic not only in 2017 – Report of National Reference Laboratory for Diagnostics of Intestinal Parasitoses [Střevní parazitózy v ČR nejen v roce 2017 - Zpráva NRL pro diagnostiku střevních parazitóz]

Barbora Voxová, Zuzana Čermáková (CZ): Malaria Diagnosis in the University Hospital Hradec Králové [Vyšetření na malárii ve fakultní nemocnici Hradec Králové]

Lenka Lecová, Pavla Tůmová, Libor Mazánek, Jana Dluhošová, Hana Typovská, Veronika Kotrašová, Věra Tichácková, Eva Nohýnková (CZ): A Case of a True Zoonotic Giardiasis: Infection of a Child via Giardia Cysts in a Pet Chinchilla Droppings [Zoonózny prípad giardiózy: Infekcia dieťaťa cystami z výkalov činčily]

Jana Pipiková, Ingrid Papajová, Jindřich Šoltys, Júlia Bystrianska, Viktória Majláthová (SK): Molecular Characterization of *Giardia duodenalis* in the Children from the Minority Population with Low Hygienic Standards in Eastern Slovakia [Molekulárna charakterizácia *Giardia duodenalis* u detí z minoritnej populácie s nízkym hygienickým štandardom na východnom Slovensku]

Lucia Chomová, Hana Némová, Emília Pavleová, Viera Nagyová (SK): Amoebae Presence Monitoring in Waters of Health Care and Recreational Institutions [Monitorovanie prítomnosti améb vo vodách zo zdravotníckych a rekreačných zariadení]

Veronika Bednárová, Katarína Sitková, Peter Juriš, Stela Majlingová, Kinga Dedinská, Juliana Gabzdilová, Monika Halánová (SK): Cryptosporidiosis in Immunocompromised Patients Kryptosporidióza u imunokompromitovaných pacientov]

Júlia Bystrianska, Ingrid Papajová, Jindřich Šoltys, Jana Pipiková, Ingrid Schusterová, Oleksandr Dobrovanov, Karol Králinský (SK): Helminth Infections among the Hospitalized Children in Košice and Lučenec [Helmintózy detí hospitalizovaných v Košiciach a Lučenci]

Zuzana Vasilková, Adriána Dudlová, Peter Juriš (SK): *Enterobius vermicularis* in Children in the Eastern Slovakia [*Enterobius vermicularis* u detí z východného Slovenska]

Kamila Bírová, František Ondriska, Jana Špajdelová, Ondrej Lachký, Pavol Ščasný, Vojtech Boldiš (SK): Influence of Latent Toxoplasmosis on Reproductive Functions. Preliminary Results of the Study [Vplyv latentnej toxoplazmózy na reprodukčné funkcie. Predbežné výsledky výskumu]

Petr Kodym, Zuzana Kurzová, Blanka Širocká, Marek Malý, Markéta Geleneky (CZ): Advantages and Limitations of IgG Avidity Test in the Diagnostics of Acute Toxoplasmosis [Výhody a obmedzenia IgG Avidity Testu v diagnostike akútnej toxoplazmózy]

Katarína Reiterová, Daniela Antolová (SK): Prognostic Value of *Echinococcus granulosus* Hydatid Fluid and Antigen B in Diagnosis of Human Cystic Echinococcosis [Prognostická hodnota antigénu z hydatickej tekutiny a antigénu B *Echinococcus granulosus* v diagnostike cystickej echinokokózy u ľudí]

Adam Kaczmarek, Elzbieta Golab, Rusłan Sałamatin (PL): Comparative Study of Different Techniques for Detection of *Blastocystis* spp. in Human Faecal Samples Porovnávacia štúdia rôznych techník na diagnostiku *Blastocystis* spp. vo vzorkách ľudskej stolice]

Daniel Krsek, Jan Suchan, Pavla Vacková, Petr Kodym (CZ): Role of Electron Microscopy in Rapid Detection of Infectious Agens [Úloha elektrónovej mikroskopie v rýchlej detekcii infekčných agens]

Iva Kolářová, Oleg Ditrich (CZ): Alternative Medicine Devices Used To Diagnose or To Treat Parasitic Infections (A Lesson from the Czech Republic) [Prostriedky alternatívnej medicíny využívané na diagnostiku alebo liečbu parazitárnych infekcií (Skúsenosti z Čiech]

Martina Miterpáková, Daniela Antolová, Viktória Čabanová (SK): Bioresonance and Delusional Parasitic Infestations - a Great Challenge for Parasitologists [Biorezonancia a bludy o napadnutí parazitmi – veľká výzva pre parazitológov]

Migrants and parasites – facts and fictions

Michal Giboda

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In order to get real data on prevalence of human parasites in 5 468 foreigners from 87 countries entering to Czechoslovakia, Active Case Detection method (ACD) was used. Such method applied in populations screening reveals any parasite irrespective of the presence or absence of symptoms and sign of infection. An opposite method is Passive Case Detection (PCD), which reveals only those who visit any medical facilities due to their health disorders. Majority of the foreigners were 18 to 35 years old university students, another group consists from factory workers. The most numerous group came from Southeast Asia – 1713 students and workers, the second one from Tropical and South Africa – 1510, mostly students. According to the international law, everyone who crosses the Czechoslovak border supposed to be healthy.

The most prevalent intestinal parasites were Soil Transmitted Helminth (STH) in order *Trichuris trichiura, Ascaris lumbricoides* and *Hookworms*. Intensity of infection, measured as Eggs per Gram of faeces (EPG) in all three STH belonged approximately in 90 % to the light infections. There were no remarkable differences in number of eosinophils, level of haemoglobin, and haematocrit between infected and non-infected individuals. Imported malaria consists from 50 infections *with Plasmodium falciparum* and 16 with *Plasmodium vivax*. 74.0 % of those infected with *P. falciparum* were asymptomatic carriers who harboured less than 10 000 asexual parasites/μl of periferic blood. In malaria-endemic areas, as western Cambodia, nearly 75% of asymptomatic P. falciparum infections are diagnosed. Onset of clinical symptoms was between second and nineteenth day after arrival to Czechoslovakia. Onset of symptoms in 16 persons infected with *P. vivax* was 2 days until 10 months after arrival. A radical cure for *P. vivax* infections, targeting liver stages of infection with Primaquine, prevented any relapse, but recrudescence of *P. falciparum* in peripheric blood was seen after treatment of resistant strain of *P. falciparum* from Rwanda.

The imported schistosomiasis was from Angola and Yemen by 18 to 23 year old students. The Yemeni student excreted eggs of *Schistosoma mansoni* only, while Angolans excreted eggs either *S. haematobium* or both *S. mansoni* and *S. haematobium*. 17 (54,9%) out of the 31 *S. mansoni* infections were considered light (1 to 100 EPG in faeces) and 14 (45,1 %) moderate (101 to 800 EPG in faeces). Of the 13 *S. haematobium* infections, 12 (92.3 %) were light (less than 50 eggs/10 ml of urine) and one was heavy (208 eggs/10 ml of urine). Fiberoptic colonoscopy was done before and after the treatment in 16 person infected with *S. mansoni,* while cystoscopical and ultrasonographical examination were performed on 9 patients infected with *S. haematobium*. Biopsis for histological examination of those infected with *S. mansoni*, were taken from rectum via rectoscopy, mostly from the tunica mucosa, from those who were infected with *S. haematobium*, from the bladder by cystoskopy from tunica mucosa as well as of *tela submucosa*. The results from the long term follow up pathology will be presented. This study initiated comprehensive research on schistotomiasis resulted in 3 WHO grants, one from Medical Research Council of Canada, and the new term into epidemiology and clinic of schistomiasis „*Post transmission schistsomiasis*“ was introduced, reflecting fact that treat of parasite does not mean to heal the disease.

Rare case of visceral leishmaniasis imported from Croatia

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Leishmaniasis is typical tropical disease. Most cases of visceral leishmanias (VL) in European countries are imported, but an increased incidence of the disease has been reported during the past decade in many Mediterranean countries, where the disease is endemic. VL is usually well and quickly diagnosed in endemic countries, while most individual physicians in Middle Europe may have never seen a single patient with those disorders. It makes particularly difficult to recognize and diagnose VL. Travel history is often not sufficient for excluding VL according to evidence of *Leishmania* presence in Europe. We report a case of visceral leishmaniasis in a 57-year-old female from Slovakia, who has never travelled to tropical countries. First symptoms of a disease were developed 5 months after return from vacation in Croatia. Symptoms were variable and progressive from first picture of fever of unknown origin to generalized skin rash, splenomegaly, elevated liver enzymes and pancytopenia. The diagnosis was not determined despite of two hospitalizations and broad spectrum of investigation including bone marrow microscopy. Diagnosis of visceral leishmaniasis was done one and half year later during her 3rd hospitalization by detection of

Leishmania spp. amastigotes in the bone marrow on repeated testing. The patient didn´t respond to treatment with liposomal amphotericin B and treatment with miltefosin was needed. Imported visceral leishmaniasis is rarely seen in non-endemic regions and fever, pancytopenia and splenomegaly are diagnostic clues. The diagnosis of visceral leishmaniasis remains challenging. Diagnostic confirmation may be done by combination of serological testing, molecular testing and microscopic detection of *Leishmania* spp. amastigotes in the bone marrow.

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Malaria infections in the Czech Republic in 2015 – 2017

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A number of imported malaria infections recorded in the Czech Republic in the last years is about 30 cases per year. More than 75 % of cases are caused by *Plasmodium falciparum*. The numbers of cases recorded in the diagnostic laboratories were as follows: 34 cases in 2015, 34 cases in 2016 and 29 cases in 2017. Unfortunately, in 2016, after 13 years, we reported malaria-related deaths in three patients. We present the trend of imported malaria infections in the Czech Republic, proportion of endemic areas visited by travelers and proportions of plasmodia species. Several case reports are also presented.

Echinococcosis - overlooked diagnosis

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Echinococcosis is the most common tapeworm (cestode) infection in Slovakia. Two types of echinococcosis are endemic in Slovakia - cystic, which is caused by *Echinococcus granulosus* and alveolar caused by *Echinococcus multilocularis*. Clinical manifestation of echinococcosis is characterized with production of cysts, which occur mostly in liver, but other organs like lungs, brain, bones and spleen could be affected too. Cysts caused by *E. granulosus* are coated with rigid fibrous membrane. They produce daughter cysts inside the cyst and eventually they calcify. They usually don't cause diagnostic problems. On the other hand, alveolar cysts caused by *E. multilocularis* are made up of multiple separate vesicles and they are not bounded by fibrous membrane. They grow into the surrounding tissues and can form new metastatic cysts in distant organs. From our experience we see, they are commonly misdiagnosed as tumor, and correct diagnosis of alveolar echinococcosis was based on histological examination after surgery intervention for a "tumor", without previous serologic testing.

We describe case reports of selected patients with cystic and alveolar echinococcosis treated on the Department of Infectology and Travel Medicine, Faculty of Medicine UPJŠ and L. Pasteur University Hospital in Košice in our presentation.

Alveolar echinococcosis is commonly overlooked diagnosis. Timely and correct diagnosis is essential for the optimal treatment and to prevent unnecessary surgery. Better interdisciplinary cooperation is suitable.

The study was supported by the project VEGA 1/0084/18 (Genetická analýza vybraných nových a novo sa objavujúcich patogénov so zoonotickým potenciálom u zvierat a ľudí).

Case report of alveococcosis with repeated cyst resection

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Alveolar echinococcosis was diagnosed in Slovakia for the first time in humans in 2000. Since then, the number of cases has increased, nowadays in Slovakia, there are **67** cases of the disease **confirmed**.

Case report. 56-year old woman visited a physician because of rhinitis and inflammation of paranasal sinuses in the year 2011. After detection of decreased immunity, the patient was referred to a specialist examination on 28.11.2011. The hepatomegaly with left liver lobe hypertrophy was observed. Magnetic resonance showed a large lobular cystoid lesion of 85 x 58 x 12 mm with amorphous calcifications in the right liver lobe, and smaller deposits of a size up to 25 mm in the vicinity of the cyst. Bile ducts were dilated. The presence of IgG antibodies against *Echinococcus granulosus* and *E. multilocularis* was detected by serological examination. Because of the suspected echinococcosis, the patient was treated with albendazole. Nevertheless, the growth of the cyst progressed. In three years its size doubled with dimension of 140 x 69 x 53 mm (CT examination on 18.2.2014).

Surgical removal of cyst was performed. Species of *E. multilocularis* was confirmed by PCR in a specimen from the cyst. By CT examination on 9.10.2015, numerous calcifications were found around the resection area. Between the diaphragm and the right lobe, the progressive formation (cyst) of 50 x 33 x 74 mm in size without calcifications, and in the segment 6 the 11 mm large segment (suspected echinococcal cyst) were detected. Ductus choledochus was dilated. Based on these findings, another resection was performed on 11.1.2016, during which the cysts were removed. The serological examination of IgG antibodies against *E. multilocularis* and *E. granulosus* was negative. The control ultrasonography of the liver did not detect any cysts (after resection on 29.3.2017).

Alveolar Echinococcosis in the Eyes of an Infectologist

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Alveolar echinococcosis (AE) is a zoonotic disease, caused by the metacestode of the fox tapeworm *Echinococcus multilocularis*. Carnivores serve as a reservoir of the pathogen. In Central Europe the main definitive host is the red fox (*Vulpes vulpes*). In the life cycle of the parasite humans are so-called accidental intermediate hosts. The first human cases of AE in Slovakia were confirmed in 2000. Despite two decades of experience, the patients still encounter problems related to differential diagnosis or treatment. In humans, the infection primarily affects the liver and is considered to be a parasitic disease of this organ. Dissemination into other organs, such as the lungs, brain, or other tissues is rather rare. This so-called secondary form occurs primarily in the lungs; several authors report its occurrence in 7 to -20% of the cases. In our set of the patients, registered and treated at the Clinic of Infectology and Travel Medicine, there are two patients with the lung form. As far as we are concerned, those are the only patients with such spread into the lungs. Problems with differential diagnosis and treatment of AE are profound. It is not just a matter of inexperienced physicians and low awareness in medical community about this disease, but also a matter of problems that infectologists are facing relating to management of the patients with complicated and often inoperable forms of disease.

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The effectiveness of bioresonance methods used to diagnose or to treat parasitic infections

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The bioresonance is a pseudoscientific medical practice used in alternative medicine in many countries. The theory behind says that each species radiates electromagnetic waves of the unique frequency that can be used to diagnose each and every pathogen within the body, including parasites. To treat the illness, the patient body need to be exposed to the device-generated pathogen-specific waves that are believed to kill the pathogen. In the Czech Republic, proponents of bioresonance work on scientifically unfounded theories, primarily of Royal R. Rife and Hulda R. Clark.

Royal Raymond Rife (1888 –1971) was an American inventor who reported that a ‘beam ray’ device of his invention could weaken or destroy the pathogens by energetically exciting destructive resonances. Hulda Regehr Clark (1928– 2009) was a Canadian naturopath who claimed that all human disease was related to parasitic infection, and also claimed to be able to cure all diseases, including cancer and HIV/AIDS, by destroying these parasites by “zapping” them with electrical devices which she marketed. Rife’s and Clark’s claims could not have been

independently replicated and were dismissed by authorities as scientifically unfounded.
Nevertheless, bioresonance centers are nowadays quite common in the Czech Republic and their clients are still mystified about the effectiveness of bioresonance methods to diagnose and to treat diseases. Centers providing bioresonance methods claim that "Bioresonance is a non-invasive, painless, and quick method of testing and treatment that offers the possibility of specifically treating all kinds of diseases successfully", including parasitic infections. Confused clients (or their relatives/friends) are regularly seeking reliable information and help at Czech parasitological institutions and at the Czech parasitological society (counselling at dotazy@parazitologie.cz).
To give trustworthy counsel, we tested the effectiveness of bioresonance methods through a series of our own experiments. The diagnostic efficiency was tested using laboratory mice infected with *Plasmodium berghei* and a healthy human volunteer. The treatment efficiency was tested in vitro using a culture of *Leishmania* parasites and in vivo using laboratory mice infected with *Plasmodium berghei*. None of the experiments proved bioresonance as an efficient and reliable method to diagnose or to treat the tested pathogens.
Some of the experiments have already been published (in Czech):
Iva Kolářová, Jan Votýpka, Roman Klemeš: Biorezonance aneb Experimentální ověření léčebných frekvencí dle Dr. Clarkové, Zprávy ČPS č. 2, 2015, http://www.parazitologie.cz/doc/dotazy/Biorezonance.pdf
Roman Kuchta a kol.: Pravda o parazitech a jejich vymítačích. Vesmír, 24.9.2015., https://vesmir.cz/cz/on-line-clanky/2015/09/pravda-parazitech-jejich-vymitacich.html
Jan Votýpka, Iva Kolářová, Petr Horák a kol.: O parazitech a lidech. Triton, Praha 2018.

Neuropsychiatric disorders in diseases related to parasitic infection

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Parasitic diseases have been spread significantly among the adults. They can induce serious neurological and psychiatric disorders if they are left unrecognized. Early recognition and treatment reduce morbidity and prevent the development of CNS structural changes. It is the specific localization of *Toxoplasma gondii* and *Toxocara canis et cati* invasion. They target neural, endocrine, neuromodulatory and imunomodulatory systems. Increased dopamine activity in hippocampus and influenced impaired learning processes and memory are the main mechanisms of the neuropsychiatric complications. The relation between schizophrenia and increased *Toxoplasma*- antibodies level was found as far as the OCD symptoms. Neuropsychiatric manifestation is rarely specific. The most clinically important are: epilepsy, dementia, cognitive and affective impairment. The case presentation, as the rare example, contributes to better diagnostic process and knowledge of the psychiatrists.

Intestinal parasitic infections in the Czech Republic (not limited to 2017) – Report NRL for diagnosis of intestinal parasitoses

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In 2017, totally 2,415intestinal parasites were detected in 66 laboratories that perform diagnosis of intestinal parasites in the Czech Republic, i.e. and average of 36 findings per facility. (In 2016, it was 38 findings per laboratory). Last year, a total of 47,632 individuals were examined for the presence of intestinal parasites. Number of procedures was 97,351; out of them 95 % of the clients were the Czechs with no travel history. The detected helminth species were as follows: *Enterobius vermicularis (1964), Ascaris lumbricoides (*21), *Taenia saginata/*sp. *(*14) (8/6), *Ancylostoma/Necator (*3), *Trichuris trichiura (*3) a *Hymenolepis nana (2).* Parasitic protozoa included: *Endolimax nana* (124), *Giardia intestinalis (94), Entamoeba coli* (84), *Entamoeba hist/dispar/ nedourčeno (49) (6/26/17), Entamoeba hartmanni (28)*, *Dientamoeba fragilis (8)*, *Jodamoeba bütstchlii (8), Chilomastix mesnili (7), Cryptosporidium sp. (4), Cyclospora cayetanensis (2), Isospora belli (1).* The poster presents the trend in the number of detections of relevant intestinal parasites from 2008 to 2017.

Malaria diagnosis in the University Hospital Hradec Králové

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The surveillance of imported malaria infections in the Czech Republic has been carried out since 1990. The average numbers of imported cases between 1990 and 2010 ranged from 10 to 25 per year, but have stabilized at about 25 to 35 cases per year in the last seven years. The highest numbers (more than 30 cases) were recorded in 1995, 2011, 2014 and in 2016, with the highest recorded cases of 38 imported malaria infections.

The Laboratory of Parasitology at the Department of Clinical Microbiology, Hradec Králové has contributed its own set of data of imported malaria infections from the period between 2000 and 2007. During 18 years, 3,547 samples were tested for the presence of blood parasites using the method of thick and thin blood smears. A total of 29 positive findings were recorded as follows: *P. falciparum (20 samples), P. vivax (8)* and *P. ovalae* (1).

Despite a progressive reduction in the number of examined samples, one to three cases of malaria are being detected per year, most often imported from African countries.

A composition of detected agents has significantly changed in the last couple years. Since 2012, only *P. falciparum* has been detected. This fact is consistent with the global trend – the spread of the areas of *P. falciparum* occurrence and decrease in the occurrence of other malaria agents.

Source: *E. Nohýnková, NRL, Epidat, SZÚ (2015-2017).*

A case of a true zoonotic giardiasis: infection of a child via *Giardia* cysts in pet chinchilla droppings.

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We report a case of direct zoonotic transmission of giardiasis between a pet chinchilla and a human. Microscopic and molecular examinations of stool samples from a child and samples of chinchilla droppings revealed cysts/DNA of *Giardia intestinalis*. The transmission from chinchilla to child has been confirmed as coprophagous after the 1-year-old toddler ate pet chinchilla droppings. Molecular analysis of the *gdh* gene from both hosts classified the obtained *G. intestinalis* cysts into the assemblage B genetic group, which has been previously shown to be characteristic of both human and chinchilla giardiasis. Both *Giardia* sub-assemblages BIII and BIV were present in the chinchilla droppings, whereas only the sub-assemblage BIV was isolated from the child's stool sample. To the best of our knowledge, this is the first case report of a true zoonotic transmission of giardiasis, supporting the zoonotic potential of assemblage B.

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Molecular characterization of *Giardia duodenalis* in the children from the minority population with low hygienic standards in Eastern Slovakia

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Giardia duodenalis is a common intestinal parasite of humans and other mammals with higher prevalence rate in areas with poor sanitation, absence of safe drinking water, inadequate waste water treatment and poor hygiene practices. Presence of the selected intestinal endoparasites in children coming from areas with low hygienic and socioeconomic status was studied. This study was aimed to determine molecular characterization of *Giardia duodenalis* circulating among the children from the minority population n Eastern Slovakia. Totally 333 children stool samples for the presence of *G. duodenalis* were examined. Molecular characterization of *G. duodenalis* was performed by sequence analysis of triose phosphate isomerase gene (*tpi*) and glutamate dehydrogenase gene (*gdh*). DNA of *G. duodenalis* was detected in 21 samples (6.3%). The infection prevalence in boys was 8.3% (15/181). Meanwhile only 3.9% (6/152) girls were infected. Out of 21 positive samples 14 isolates (66.7 %) belonged to the assemblage B, 4 isolates (19.0 %) to sub-assemblage AII and 3 isolates (14.3 %) corresponded to assemblage F. Regarding the determination of sub-assemblages of assemblage B 4 isolates were characterized as sub-assemblage BIII and 6 isolates as sub-assemblage BIV. Our results provide a detailed insight about *G. duodenalis* genotypes circulating in Roma children's population living in poverty and social exclusion. Detection of assemblage F in three children raises question how do cats participate on giardiasis transmission in these locations.

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Amoebae presence monitoring in waters of health care and recreational institutions

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Amoebae are single-celled organisms most commonly living in a water-associated environment. Some of them can cause serious human illnesses related to the use of drinking water and, in particular, bathing water. Disease of the central nervous system (PAM) can cause *Naegleria fowleri* (*Heterolobosea*) and eye disease (keratitis) of some species of the genus *Acanthamoeba* (*Lobosa*). Epidemiologically significant is also the ability of amoeba to produce disinfectant-resistant cysts and their symbiotic relationship with bacteria, especially *Legionella*. Public Health Authority of the Slovak Republic monitors presence of amoeba with the possible occurrence of *Legionella* in health care and recreation institutions. Samples were taken from hot utility and drinking water at various locations in hospitals, outpatients, swimming pools and wellness in spa, hotels and resorts. Between the years 2014 and 2017, 132 samples from 18 recreational facilities and 168 samples from 27 health care institutions were collected and tested. Of the total number of samples tested, 54% of the samples were positive for amoeba in recreational establishments, 34% for samples in health care institutions. In some samples, the presence of *Acanthamoeba* sp. was confirmed by morphology and PCR methods. The presence of amoeba was examined by the culture method at several temperatures (23 ºC, 30 ºC, 37 ºC and 44 ºC) depending on the origin of the sample. To confirm the presence of amoeba in the sample, a positive finding at least at one culture temperature was sufficient. The method is accredited and performed according to procedures established at the laboratory. Now, we aimed at the genus identification of the *Acanthamoeba* strain by PCR which is based on amplification of *Acanthamoeba*-specific amplimer ASA.S1 obtained with primers JDP1 and JDP2 on the 18S rDNA. A variable part within this amplimer also identify genotype clusters, but for identification of individual genotypes are useful sequencing reactions of genotype-specific amplimer GTSA.B1, produced with primers CRN5 and 1137. Depending on the genotype, primers for ASA.S1 amplified ca. 423 to 551 bp long region, GTSA.B1 amplimer resulted in a chain from 1400 to 1700 bp long. Three internal segments of GTSA.B1 reliably distinguished all *Acanthamoeba* genotypes (Schroeder et al., 2001). Several populations of *Acanthamoeba* genus are kept in the laboratory collection. The genotype identification will be subject for our next study.The real-time PCR diagnostic method for detection of *Naegleria fowleri* has been introduced, too, based on the hybridization of specific primers and fluorescently labeled probes along with the LightCycler detection system. The *Mp2Cl5* gene sequence is the target amplification gene responsible for the production of the protein characteristic of *Naegleria fowleri* (Maďarová et al., 2010). The increase in fluorescence signal during real-time PCR indicates the presence of the gene. From the samples tested so far, none was positive for the presence of *Naegleria fowleri.*

Cryptosporidiosis in immunocompromised patients

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Cryptosporidiosis is an endemic, parasitic infection spread worldwide. It is an opportunistic infection caused by pathogenic species of cryptosporidium. Cryptosporidium contagious is highly variable. In immunocompetent individuals cryptosporidiosis results most commonly in self-limiting diarrhoea accompanied with up to 10 watery stools daily for several days. In immunocompromised individuals the infection can progress into chronic, cholera like state. Infection can cause notable deterioration of the health in patient and interrupt or stop the treatment process of the primary illness.

In our study we analysed the cryptosporidiosis incidence in immunocompromised patients. Patients in study were aged 4 and 71. A total of 89 faecal samples were examined from which 37 samples came from adult patients and 52 samples came from child patients. For the detection of cryptosporidium we used direct ELISA that serves for qualitative detection of cryptosporidium agents in stool.

Cryptosporidium were detected in 5 samples, which represents 5.6% positivity. In child patients we detected cryptosporidium in 3 (5.8%) samples. In adult patients we detected cryptosporidium in 2 (5.4%) samples. Our obtained results showed that of occurrence of observed cryptosporidium is low. One of the reasons for low levels of capture can be permanent medical supervision and unlimited access to adequate therapy for not only the basic illness but also for the associated infections in immunocompromised patients. In case that opportunistic infection develops itself in this patient group, it is detected and eliminated in its initial infection stage. This is a reason why encountering clinically manifest cryptosporidiosis is rare at present time. Despite this, we cannot forget about the potential incidence of cryptosporidiosis in this group of patients and in case of any, including non-specific, symptoms do our best to eliminate or confirm the proper diagnosis with the goal of immediate therapy.

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Helminth infections among the hospitalized children in the Košice and Lučenec

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Nowadays intestinal helminth infections present a problem that is typical for poor communities and especially for the children. The aim of this study was to monitor the occurrence of the most important helminth infections in children hospitalized in Košice and Lučenec hospitals at whom additional parasitological examinations were performed. Totally, 144 stool samples (88 samples from Košice and 56 from Lučenec) from children aged between 0-18 years were examined. For the analysis of the helminth infections children were divided according the age, gender and minority (Roma) or majority (non-Roma) population status. The most common clinical symptoms during hospital admittance were anemia, gastroenteritis, diarrhea, abdominal pain, nausea and/or vomiting, and fever of unknown origin. The helminth eggs were detected in 22.22 % of examined children, at which *Ascaris lumbricoides* (15.60 %) and *Trichuris trichiura* (4.70 %) eggs were predominant. Helminthoses were not seen in children living in major population. Helminth ova were present in 42.31 % (22/52) of stool samples from hospitalized Roma children in Košice and 27.78% (10/36) of samples from Roma children hospitalized in Lučenec. The lowest prevalence of helminth infections was found in children older than 15 years. No significant differences in helminths prevalence among children with different gender were observed. Based on our results we can conclude that the ethnicity is one of the most important risk factor associated with the helminth infections.

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Enterobius vermicularis in children in the Eastern Slovakia

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Specimens of perianal scotch tapes were examined for the presence of *Enterobius vermicularis* eggs in children population originating from different regions of the Eastern Slovakia. 390 children – 218 girls and 172 boys were divided by age into three groups – aged from 5 months to 2 years, aged from 3 to 6 years, and aged from 7 to 15 years. The prevalence of *E. vermicularis* was P = 3.59 %. Depending on the incidence of *E. vermicularis* infection, we detected no statistically significant difference (p > 0.05). The prevalence of *E. vermicularis* in boys was P = 4.07 %, and in girls P = 3.21 %. The highest prevalence of *E. vermicularis* was recorded in the group of children aged from 3 to 6 years (P = 5.03 %). Most of the samples were positive at age 4 and 5. The lowest prevalence was in the group of children aged from 5 months to 2 years (P = 0.97 %), and the prevalence of *E. vermicularis* in the group of children aged from 7 to 15 was P = 3.91 %. The difference in the incidence of *E. vermicularis* infection among different age groups of children was not statistically significant (p > 0.05).

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Influence of latent toxoplasmosis on reproductive functions. Preliminary results of the study

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According to numerous studies of prof. Flegr and his team the presence of a parasite in latent form has an impact on human behavior. In particular, we were interested in his studies regarding the effects of latent toxoplasmosis on reproductive functions, in which they found out that: pregnancy of woman with a latent toxoplasmosis lasts longer, latent toxoplasmosis significantly favors male gender of newborns, affects the birth weight and the length of newborns. The aim of our study is to verify these findings, the preliminary results of our study are the subject of this lecture.

In the period 1.6.2017 - 31.3.2018, 1283 pregnant women were serologically examined. Of this number, 598 women gave birth at the Gynecological Clinic in Faculty Hospital Trnava and 685 women at the Hospital of Galanta, department of Gynecology. In addition to the status of toxoplasmosis in pregnant women, we determined the length of pregnancy. For newborns, we recorded gender, birth weight and length. Pregnant women were serologically tested for the presence of IgG, IgM, CFT antibodies, and possibly IgG avidity. Samples of sera were examined in the parasitological laboratory of Medirex Bratislava and the Department of Clinical Microbiology AnalytX, (Ltd). Trnava. We considered latent infection in patients with following antibody profiles: IgG positive, IgM negative, CFT positive/negative; IgG positive IgM positive, CFT positive, high avidity IgG antibodies.

In FNsP Trnava we found a latent infection in 106 (12.3%) pregnant women, in NsP Galanta in 60 pregnant women (8.8%). In FNsP Trnava, women with latent infection gave birth to 55 (51.4%) boys and 52 (48.6%) girls, 30 boys and 30 girls (both 50%) in NsP Galanta. Toxo-negative women in FNsP Trnava also gave birth to 251 boys and 251 girls (50%), in Galanta 330 (52.3%) boys and 301 (47.7%) girls. We did not detect in both institutions significant differences between sex of newborns from infected and uninfected mothers ($P \leq 0.5$). Both groups gave birth to the same number of boys and girls, and we did not confirm the outcome of Flegr et al. Likewise, we did not find any differences in the length of pregnancy, the birth weight and the physical length of boys and girls from infected and uninfected mothers.

We did not confirm the results of the prof. Flegr study in any conclusion. Our preliminary results suggest that latent toxoplasmosis does not change gender, nor does it affect the length of pregnancy or the weight and length of newborns.

Advantages and limitations of IgG avidity test in the diagnostics of acute toxoplasmosis

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The IgG avidity test is a favourite method for determination of the phase of infection, which is crucial in the diagnostics of toxoplasmosis especially in the case of pregnant women. The method is based on growing avidity ("complex affinity" = stability of binding of specific antibodies to respective antigens) during the acute phase of toxoplasmosis. Low avidity of anti-*Toxoplasma* IgG may indicate time of infection at up to 4 months previously; in this period infection during pregnancy can result in congenital toxoplasmosis, whereas prior infection will not harm the foetus.
A set of 893 serum samples collected from 642 patients was examined at the National Reference Laboratory for Toxoplasmosis using EIA Toxoplasma IgG TestLine. Avidity Index (IA) was calculated from optical densities (OD) of sera in ELISA (IA = OD of the sample treated with urea/OD in standard ELISA). Avidity was considered low if $IA \leq 0.3$. Moreover, 45 women in weeks 6-31 of pregnancy were examined at Bulovka hospital.
Low IgG avidity was found in 31.3% of samples. Among 296 samples representing probable acute toxoplasmosis (IgG+, IgM+, IgA+), 51.7% showed low IgG avidity. On the other hand, out of 372 IgM-negative samples, which can be hardly related to acute toxoplasmosis, 17.5% were low-avidity sera and the average rate of "falsely low avidity" in the whole set is 7.3%. Such cases were more frequent among samples with low levels of anti-*Toxoplasma* IgG.
Low avidity of anti-*Toxoplasma* IgG, not corresponding to other serological and clinical findings, was preserved in 46.7% of pregnant women examined.
IgG avidity testing is very useful for determining onset of infection. The period of low avidity is usually shorter than the interval of positive IgM, IgA and even IgE and the correlation of these markers is only partial. However, in some cases the low avidity can persist for several months or years after infection and is not related to the clinical status of the patient or the risk of congenital infection. "Falsely low avidity" can be related to low IgG levels, insufficient for adequate test procedure.
High avidity is a reliable indication of a prior infection.
The resulting IgG avidity index must be interpreted in context of all other diagnostic and clinical parameters.
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Prognostic value of *Echinococcus granulosus* hydatid fluid and antigen B in diagnosis of human cystic echinococcosis

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Cystic echinococcosis (CE) is a serious parasitic disease caused by larval stages of *Echinococcus granulosus* sensu lato. Humans can become infected after the accidental ingestion of parasite eggs shed with faeces of carnivores, definitive hosts of the tapeworm. Although the specificity of antigen B fraction (AgB), prepared by partial purification of fertile ovine cysts, seemed to be higher than that of crude hydatid fluid (HF) antigen, its medical relevance has been discussed. The aim of the study was to compare the sensitivity and specificity of antigen B and crude *E. granulosus* hydatid fluid antigen in the serological diagnosis of cystic echinococcosis in humans. Serum samples of 36 patients in different stage of cystic echinococcosis and 128 control sera were simultaneously tested by ELISA and Western blot techniques using AgB and HF.
HF-ELISA showed 100% sensitivity and 97.7% specificity, while the sensitivity of AgB-ELISA was slightly lower (96.4%) and specificity was higher (98.4%). Examination of eight patients after the surgery (1 – 2 years) and following albendazole treatment revealed the seropositivity in six (75%) cases by HF-ELISA, but only in two (25%) cases by AgB-ELISA. Similarly, the HF sensitivity in Western blot analyses of patients with confirmed CE was higher than the sensitivity of AgB. Lower AgB-positivity in patients after the surgical and chemotherapy treatment was also recorded.
Our results showed that crude HF is suitable for the screening of CE in suspected patients, while AgB is more appropriate confirmatory tool for specific diagnosis of the disease. Moreover, AgB-ELISA and Western blot seem to be suitable tests for estimation of the results of surgery and chemotherapy intervention. Due to the complexity

of the diagnostic procedure we would like to underline the necessity of joint cooperation between clinicians and laboratories for proper diagnosis and subsequent treatment of cystic echinococcosis in humans.

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Comparative study of different techniques for detection of *Blastocystis* spp. in human faecal samples

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Blastocystis hominis s. l. is one of the most commonly detected protozoa in the human large intestine. The aim of the study was to compare various techniques applied in laboratory diagnostics of *Blastocystis* spp. infections.

One hundred eleven human faecal samples sent for laboratory diagnostics to the Laboratory of the Department of Parasitology and Vector-Borne Diseases, National Institute of Public Health – National Institute of Hygiene (NIZP-PZH) were examined. Each sample was subjected to analysis by: classical parasitological methods (faecal smear microscopy) and *xenic in vitro culture* (XIVC) in modified Jones medium in anaerobic conditions at 37°C. All positive samples detected by microscopy and/or XIVC and 1/3 of the negative samples were subjected to PCR for *Blastocystis* detection. The DNA from the clinical and culture samples was isolated with GENOMIC MINI (A&A BIOTECHNOLOGY). A fragment of small subunit rRNA gene was amplified with primer pair BhRDr i RD5. The PCR products were sequenced and the obtained nucleotide sequences were compared to *Blastocystis* DNA sequences deposited in GenBank.

Faecal smear microscopy gave 11 positive results, 21 samples were positive in XIVC and 22 samples were positive in PCR. All positive results obtained by microscopy and XIVC were confirmed by PCR.

Based on the presented results we can conclude:

1. Faecal smear microscopy was the least efficient method of *Blastocystis* spp. detection.
2. Due to the high sensitivity and relatively low price XIVC may be applied to screening of faecal samples for *Blastocystis* spp. presence.

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The role of diagnostic electron microscopy in virus detection

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Detection of viruses by transmission electron microscopy is based on the visualization and morphological identification of virus particles in samples of tissues, cell cultures, organic fluids or environmental samples. Viruses could be classified in the family or genus level in particular cases according to size and morphology. The key methods for diagnostic electron microscopy are negative staining and rapid thin sectioning of solid tissues. Main advantages of negative staining are short processing time (1 hour) and „open view" nature of examination. Although molecular and immunological methods in many areas have replaced preferential position of TEM in routine virological diagnostics, electron microscopy, due to the speed of examination and the potential of catch all infectious agents present in the sample with one test has still irreplaceable role in some cases. In regards of emerging and re-emerging diseases (e.g. SARS, Monkeypox virus, SFTS phlebovirus), multiple infections as well as in the context of bioterrorism attack diagnostic electron microscopy remains the first choice method for detection of unsuspected infectious agens. For rapid detection of viruses in clinical material by TEM, standard negative staining method coupled with concentration methods can be used in case of a low viral load in clinical or environmental sample. In suspicion of infectious agens with high biosafety risk formaldehyde fixation of the sample is recommended because it does not alter morphology of viral particles. To increase the probability of detection of viral particles, immune clumping, immuno-electron microscopy (SPIEM), or immunogold labeling can also be used. For diagnostic of viral infections in a solid tissue samples (biopsy, autopsy), the method of regular epoxy-resin ultra-thin cuts or progressive cryoelectron microscopy techniques can be used.

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Alternative medicine devices used to diagnose or to treat parasitic infections (a lesson from the Czech Republic)

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This poster (in Czech) is dedicated to alternative medicine devices that are working on the theory of **bioresonance**. The bioresonance is a pseudoscientific medical practice used in alternative medicine in many countries. The theory behind says that each species radiates electromagnetic waves of the unique frequency that can be used to diagnose each and every pathogen within the body, including parasites. To treat the illness, the patient body needs to be exposed to the device-generated pathogen-specific waves that are believed to kill the pathogen. Claims supporting this theory could not have been independently replicated and were discredited by independent researchers.

Nevertheless, bioresonance centers are nowadays quite common in the Czech Republic and their clients are still mystified about the effectiveness of bioresonance methods to diagnose and to treat diseases. Centers providing bioresonance methods claim that "Bioresonance is a non-invasive, painless, and quick method of testing and treatment that offers the possibility of specifically treating all kinds of diseases successfully", including parasitic infections.

The main aim of this poster is to briefly introduce the most common devices available in the Czech Republic such as Zapper, Plasma Generator, Ramaloop, F-SCAN, Oberon, and Sensitiv Imago. None of those devices proved the evidence-based effectiveness and are sold solely as experimental devices. Some of them are, however, confusingly presented as medical device class II. Although there is a "medical", it only means the device is safe and performs without causing injury or harm to the user. Examples of class II devices include acupuncture needles, condoms, pregnancy testing kits, air purifiers, and powered wheelchairs. Medical devices of class II do not need a scientific review to ensure the device´s effectiveness.

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For further reading (in Czech or Slovak):
Jan Votýpka, Iva Kolářová, Petr Horák a kol.: O parazitech a lidech. Triton, Praha 2018.
Jiří Heřt: Alternativní medicína a léčitelství – kritický pohled. 2010. Volně ke stažení.
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Bioresonance and delusional parasitic infestations A great challenge for parasitologists

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Bioresonance diagnostic and therapy was "invented" in Germany in 1977 by Franz Morell, the physician and high-rank Scientologist. Nowadays, this pseudo-scientific method is experiencing its upward movement in the Central European region, especially in post-communist countries as Czech Republic and Slovakia.

According the self-assigned diagnosticians and healers, the bioresonance is able to detect a variety of diseases including parasitic infections and also to treat them by "stimulating a change of bioresonance in the cells" of human or pathogen organisms. The devices used for this bioresonance method are claimed to measure disturbances in the body´s flow and to detect "an energy imbalance".

Various parasitic species are very often diagnosed and following the healers statements the parasites are responsible for almost all diseases of modern civilisation including allergies, autism and cancers. Follow treatment referring to diagnostic represents very serious problem. It contains frequently antiparasitic drugs (praziquantel, alebendazol, ivermectin etc.), MMS solution (called Miracle Mineral Supplement) which is chlorin dioxin in fact, hydrochloric acid and "frequency drops". Very often the treatment is, fortunately, only overpriced placebo stuff.

In addition to expansive diagnostic and dangerous treatment, the tested people become frightened and emotionally

harmed. They suffer for delusional parasitosis syndrome and seek for help at parasitological laboratories.
In our work we present a detailed story of bioresonance diagnostic and therapy which our PhD. student went through voluntarily. Moreover, several examples of delusional parasitic infestation reported at Institute of Parasitology in Košice are presented.

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ECOMED

UVEDOMUJEME SI, ŽE KVALITA JE SKRYTÁ ČASTO V DETAILOCH ?

Veda podporovaná laboratórnymi analýzami sa rozrástla až do neuveriteľných rozmerov a skúma čoraz jemnejšie živé, ale aj neživé štruktúry. S tým narastajú ale aj nároky na dôslednosť a precíznosť práce, ako aj na prostriedky, ktoré sa pri takejto náročnej práci používajú.

Napriek pokročilej automatizácii laboratórnych prác je vplyv ľudského faktora a dobrej znalosti používaných prostriedkov stále veľmi významný, najmä v prípadoch mnohých analýz realizovaných v súčasných laboratóriách. Dovoľujeme si to ukázať na niekoľkých príkladoch.

V kontraste s týmito faktami je spôsob obstarávania laboratórneho vybavenia a materiálu postavený veľmi často (napríklad EKS) len na najnižšej cene! To de facto - či chceme, alebo nechceme - ohrozuje kvalitu, spoľahlivosť a dôveryhodnosť výsledkov takéhoto náročného výskumu ale napokon už aj rutinnej práce, lebo mnohé takéto náročné analýzy sa už stali súčasťou bežnej rutinnej práce, napríklad PCR.

To, že nesprávne alebo nespoľahlivé výsledky majú vždy nepríjemné následky, netreba bližšie vysvetľovať.

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„Prezentácia venovaná prehľadu portfólia spoločnosti QIAGEN, konkrétne produktom určených pre veterinárnu diagnostiku a výskum. Prehľad bude zameraný na kompletné riešenie pre molekulárnu a sérologickú veterinárnu diagnostiku, ako aj na výskum patogénov u zvierat. Bude sa týkať hlavne kitov a reagencií na efektívnu purifikáciu vírusovej DNA a RNA a bakteriálnej DNA z rôzneho typu vzoriek, taktiež prístrojovej technológie a v neposlednom rade širokého spektra patogén špecifických PCR assayí.“


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