Table of Contents

1. Oral Presentations (order according to the programme) .......................................................... 1
   1.1. Plenary Session I .................................................................................................................. 1
   1.2. Plenary Session II ................................................................................................................. 1
   1.3. Session I. Human parasitology and zoonoses ................................................................. 4
   1.4. Session II. Parasitology in genomic and biochemistry era ........................................... 11
   1.5. Plenary Session III ............................................................................................................. 14
   1.6. Session III. Veterinary and wildlife parasitology ......................................................... 16
   1.7. Plenary Session IV ............................................................................................................. 19
   1.8. Session IV. Pharmacology and treatment ..................................................................... 19
   1.9. Session V. Helminths – diversity, taxonomy and ultrastructure .................................. 21
   1.10. Plenary Session V ............................................................................................................. 28
   1.11. Session VI. Vectors and vector-borne diseases .......................................................... 29

2. Student Competition (order according to the programme) .................................................. 38
   2.1. Session Protists and bacteria – tiny creatures from different aspects of research ........ 38
   2.2. Session Tapeworms and flukes – does DNA, proteins or hosts rule their world? .......... 43
   2.3. Sessions Monogeneans and nematodes – from molecules to biodiversity
       Ecological and veterinary parasitology .............................................................................. 49
   2.4. Session Ticks, mites and mosquitoes – hidden beauty of little vampires .................... 56

3. Poster Session (in alphabetical order) .................................................................................. 61

The editors hold no responsibility for any content, inaccuracy or language errors in the abstracts.

Editors
Mikuláš Oros, Zuzana Vasišková

Graphic Design
Zuzana Vasišková

ISBN 978 - 80 - 968473 - 7 - 2

©Slovak Society for Parasitology at SAS
Košice, May 2014
ORAL PRESENTATIONS

PLENARY SESSION I

(SELF)-INFECTIONS WITH HELMINTHS

R. Kuchta, T. Scholz
Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic,
370 05 Ceske Budejovice, Czech Republic

The experimental (self-) infections with helminthes can be re-interpreted from a perspective that was usually not on the mind of scientists, who, mostly long time ago, subjected themselves and other individuals to these experiments. In most cases, (self-) infections were used to elucidate life cycles of parasites, their transmission, host specificity and/or pathogenicity, data which were difficult or even impossible to obtain by other means. Interestingly, many of these experiments remain poorly known as they were often published in obscure or non-English journals or have been forgotten. Moreover, some of them are incompatible with current ethical rules.

Several helminth species have been used by researchers for self-infections, including tapeworms (as Diphyllobothrium, Spirometra, Taenia), digeneans (as Fasciolopsis, Schistosoma, Plagiorchis) or nematodes (as Ancylostoma, Burgia, Ascaris, Necator, Strongyloides, Tricuris).

However, with recent research addressing the possible beneficial impact of intestinal helminths on a range of autoimmune diseases in humans, these studies, although often not complying with current ethical rules, provide valuable information. Here, we critically review cases, in which humans were infected with parasites.

PLENARY SESSION II

THE BLOOD-SUCKING NEMATODE ASHWORTHIUS SIDEMI: TRANSMISSION FROM WILDLIFE ANIMALS TO COWS

B. Moskwa, J. Bien, A. Cybulska, A. Kornacka, K. Goździk, W. Cabaj
Witold Stefański Institute of Parasitology, Polish Academy of Sciences, Twarda 51/55,
00-818 Warsaw, Poland

Ashworthioides sidemi, a blood-sucking nematode of the Trichostrongylidae family, is a primary parasite of Asiatic cervides, particularly sika deer (Cervus nippon). The introduction of this host species in Ukraine, Slovakia, the Czech Republic and France has also allowed the parasite to spread throughout the area and the neighboring countries. Sika deer were first introduced to Poland in 1895 near Pszczyna, Silesia, and then again in 1910, near the Vistula lagoon in the vicinity of Kadyń. The first evidence of A. sidemi in Poland was reported in 1997 in European bison from the Bieszczady Mountain. Further studies revealed that A. sidemi infections are common in bison, red, roe and fallow deer. Additionally, the possibility has been suggested that infections may be transmitted between wildlife animals and livestock, particularly sheep and cows, which may be grazed on the same pastures. The main interest of the study was to confirm this hypothesis. Until now, the
presence of *A. sidemi* in the abomasum and duodena in wildlife animals was confirmed during post mortem microscopic examination only. To facilitate an easier and more reliable diagnostic tool, a simple PCR technique was developed to differentiate *A. sidemi*.

The study was performed on faecal samples collected from European bison immobilized in the summer of 2012 in the Bieszczady Mountains (Poland) and from cattle fed on pastures exposed to infected wildlife in the Białowieża Primeval Forest buffer zone and Strzelowo Forest District (Mazurian Lake area). Faecal samples were cultured on Petri dishes and were incubated at 25°C for 7-14 days. DNA of L3 larvae was identified to the *A. sidemi* level according to Ljunggren and Goździk (2008) and Moskwa (in press). The amplified genomic DNA samples were analysed on 1.5% agarose gels and visualized using the Kodak 1D™ Electrophoretic Documentation and Analysis System.

The presence of DNA of L3 *A. sidemi* larvae was confirmed in both bison and cattle faecal samples. On the basis of our knowledge this is the first report on *A. sidemi* in cattle. A DNA segment of approximately 406 bp was excised from agarose gel and purified. The DNA was sequenced in both directions by Genomed (Warsaw, Poland). The sequences were edited using Vector NTI Advance™, version 10 (Invitrogen, USA). BLAST searches were performed in order to compare the sequence with those in GenBank. The obtained sequences matched sequences already published in GenBank, showing very high similarities with the isolate from European bison (accession number EF467325).

Histopathological changes observed in the walls of the abomasum and duodena of infected wildlife caused by a strong parasite presence may also become an important health problem for farm animals. Therefore further studies are needed to monitor the current spread of *A. sidemi* in wildlife and domestic animals.

*This research was supported by National Science Centre. Grant No. N308 585740*

### PROTEOMIC STUDIES OF THE MODEL CESTODE SPECIES *HYMENOLEPIS DIMINUTA*

#### CURRENT STATE OF KNOWLEDGE AND RESEARCH PERSPECTIVES

D. Mlocicki¹, J. Bien¹, R. Salamatin², A. Przybek²

W. Stefanski Institute of Parasitology PAS, Twarda 51/55 Street, 00-818 Warsaw, Poland¹;
Department of General Biology and Parasitology, Medical University of Warsaw, Chalubinskiego 5 Street, 02-004 Warsaw, Poland²

Proteomic research along the cestode life-cycle has never been performed before, and nothing is known about the protein profiles of the oncosphere, the cysticercoid and the adult *Hymenolepis diminuta*, which is one of the most common model species used in experimental cestodiasis. We have greater understanding of *H. diminuta* biology than of practically any other cestode. There are, however, fields in its biology that remain unknown; one of the serious blanks in our knowledge is the complete lack of results concerning molecular changes in protein expression during cestode ontogenesis, especially those based on modern proteomic techniques. In the current project we applied sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), two-dimensional electrophoresis (2-DE) and Western Blot to study and compare protein profiles of three developmental stages of *H. diminuta* (oncosphere, cysticercoid, adult). Comparative analysis of protein profiles were performed using QanityOne and PDQuest (Biorad) software. Our preliminary results indicate significant differences in the protein profiles and signal intensity distribution in all of the studied stages. LC-MS/MS analysis are, however, needed for more detailed comparative analysis.
Simultaneously we cultivated adult parasites in vitro to collect the excretory-secretory (E-S) products. Western Blot was used to verify if any of the somatic and/or E-S proteins may exhibit immunogenic properties. In both crude extract and E-S proteins we indicated the presence of immunogenic proteins. Their masses ranged between >30 and > 200 kDa. The preliminary results of LC-MS/MS analysis show that identified proteins are most probably involved in cestode body movement, metabolism and in general mechanisms engaged in parasite survival in the hostile environment of the host intestine. Some of these proteins are known as vaccine candidates in other flatworm species (eg. Schistosoma, Echinococcus). We expect that further detailed studies and obtained results will reveal the differences and similarities in the protein profiles of all analyzed stages, specifically in relation to their role in the cestode life cycle. These comparative proteomic analyses of the consecutive developmental stages should uncover mechanisms contributing to adaptation to environmental constraints and host-parasite interaction. Data about similarities and differences in the protein profiles between all of the developmental stages could be crucial for understanding tapeworm biology, development, adaptations to the parasitic way of life and host-parasite physiological interrelationships in very different hosts (invertebrate and vertebrate). Characterization of protein profiles along the life-cycle of H. diminuta should help to define what are the molecular mechanisms involved in the evolutionary success of tapeworms. Moreover identification and characterization of parasite proteins might help us to find new candidates for immunodiagnostics and may represent useful targets for novel molecules with therapeutic potential.

This study was funded by the National Science Centre Poland (grant number: 2012/05/B/NZ6/00769)

THE MASSIVELY PARALLEL SEQUENCING – METHODOLOGICAL PRINCIPLES AND APPLICATIONS

G. Minárik¹,², I. Králová-Hromadová³

Department of Molecular Biology, Faculty of Natural Sciences, and Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia¹; Geneton s.r.o., Bratislava, Slovakia²; Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovakia³

The last ten years in biological research have been heavily influenced by possibility of looking on genomes instead of genes. This was enabled by introduction of next generation sequencing (massively parallel sequencing - MPS) technology with unprecedented throughput in genome data production. Hand in hand with ultrahigh throughput the dramatic lowering of the cost per base sequenced made this technology widely used and now there are many applications in research of different kind of samples. The most prominent projects have been focused on human samples, when normal variation and mutations in diseases have been studied most frequently. Of these, the most prominent were projects as 1000 Genomes Project, ENCODE Project and Cancer Genome Project.

To understand how this technology can be used in particular biological research the basic knowledge of the technology is needed. Currently there are four MPS technologies used worldwide – Illumina, IonTorrent, Solid and PacBio. All are different from technological point of view with their pros and cons. Despite of their differences they all share the need for bioinformatics in the analysis of the large amount of data generated and sometimes the sequencing alone is not the crucial step in the sample analysis.
In parasitic organisms, the MPS technology will be applied in characterization of complete genomes/transcriptomes/proteomes of medically and socio-economically important species of parasitic protists (e.g. *Trypanosoma, Leishmania, Plasmodium*) and worms (e.g. *Echinococcus, Schistosoma, Trichinella*). The leading institution in this field is the Welcome Trust Sanger Institute (Hinxton, UK). The large-scale data sets on complete genomes of parasitic organisms provide an useful initial information that can further be applied in: (i) development of new diagnostic markers, potential drug targets and vaccines, (ii) better understanding of the biology of parasites and diseases they cause, (iii) evolutionary insights, (iv) functional genomics, changes in the transcriptome, proteome and epigenome in a course of life cycle of the parasite, (v) studies on energetic (mitochondrial) metabolisms and biological pathways, (vi) mapping antihelmintic drugs resistance, (vii) determination of functional proteins associated with parasite invasion and immuno-modulation.

The MPS technology provides also an effective methodology in mining particular genes/DNA regions of interest. In particular, it has provided a significant value in establishing a practical platform for the rapid sequencing, annotation and analysis of mitochondrial genomes. Besides, MPS technique enables fast and reliable mining of candidate STR (short tandem repeats) loci.

To summarize, the MPS is currently widely adopted in the field of basic and applied biological research. As it is usable also in hypothesis-free settings and for qualitative as well as quantitative analysis it is universally applicable for different kind of projects, including parasitological ones.

*This presentation is the result of the project implementation: Revogene – Research Center of Molecular Genetics (grant No - ITMS 26240220067) supported by the R&D Operational Programme funded by the ERDF. Parasitological applications were performed thanks to APVV-0653-11 and VEGA 2/0133/13 projects.*

**SESSION I**

**Human parasitology and zoonoses**

**PARASITIC ZOOHOSES IN SVALBARD**

O. Ditrich¹, E. Myšková¹2, L. Honsova¹, M. Kváč², B. Sak², T. Tym¹²

Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic¹; Biology Centre AS CR v.v.i, Institute of Parasitology, České Budějovice, Czech Republic²

The occurrence of etiological agents of parasitic zoonoses in high Arctic was detected. Droppings of terrestrial mammals and birds, collected in central part of Svalbard, were examined for the presence of intestinal parasites. Till now, 432 from 16 species have been inspected. Classical coprological methods and much more sensitive molecular analyses (PCR and consequencial sequencing) were used for the detection and the determination of parasites. Several species belonging to cryptosporidia (Apicomplexa) and Microsporidia (Fungi) and one tapeworm species (Cestoda) were recorded: all of them can produce human infections as well.

*Cryptosporidium parvum* genotype IIa was revealed in Pink-Footed Goose (*Anser brachyrhynchos*), *Cryptosporidium* goose genotype II in Barnacle Goose (*Branta leucopsis*) and *Cryptosporidium muris* TS 03 in Svalbard Reindeer (*Rangifer tarandus platyrhynchus*). *Encephalitozoon cuniculi* genotype II was found in reindeer (*R.tarandus platyrhynchus*), Polar Fox
Alopex lagopus), Polar Bear (Ursus maritimus) domestic dog (Canis lupus familiaris) and Barnacle Goose (Branta leucopsis). Two new genotypes of Enterocytozoon bieneusi were identified, the first one in Svalbard Reindeer and and the second in Pink-Footed Goose. Other genotypes of E. bieneusi were recorded in Polar Fox (cf. genotype EbPC), and Black-legged Kittiwake (Rissa tridactyla) – probably genotype RO7. These findings represent the first records of cryptosporidia and microsporidia in Svalbard and the proof that the extreme conditions of high Arctic can enable their surviving and circulation. However, some of parasites common in temperate zone (e. g. Giardia) were absent in our material.

Fox tapeworm (Echinococcus multilocularis) was found in droppings and cadaver of one Polar Fox in Nybyen near Longyearbyen. The life cycle of this highly dangerous parasite in Svalbard has been enabled by anthropogenic unintentional introduction of Sibling Vole (Microtus levis). However, two Sibling Voles caught in dog sled base in Longyearbyen were negative for the hydatid cysts. Human settlement in Svalbard represents the cause of propagation of some etiological agents of parasitic zoonoses in Svalbard.

WILDLIFE HELMINTH ZOONOSES IN THE TATRA NATIONAL PARK ECOSYSTEMS

Z. Hurníková1, M. Mitepáková1, B. Chovancová2
Institute of Parasitology SAS, Košice, Slovakia1; TANAP Research Station, Tatranská Lomnica, Slovakia2

The Tatra National Park situated in northern part of Slovakia and covering an area of 738 km² represents the most important recreational area of the country. A strong windstorm in 2004 and the others climatic and anthropogenic changes eventuated in migration and immigration of new wildlife population, an increase of game numbers followed by growth of carnivorous predators and an increase of biodiversity and population density of small mammals. All these changes may affect the parasite fauna composition and represent enhanced risk of transmission and spread of parasitic diseases. Thus, from a public health perspective, the monitoring of serious helminth zoonoses – alveolar echinococcosis, trichinellosis, and dirofilariosis is of great importance.

The aim of the study has been to perform a long-lasting and detailed survey on the occurrence of above mentioned parasitic diseases in specific ecosystems of the Tatra National Park. Between 2007 and 2013 more than 200 wild carnivores, the final hosts of Echinococcus multilocularis, Trichinella spp. and Dirofilaria spp., were examined using standard parasitological methods.

E. multilocularis tapeworm was detected in small intestines of 40.3 % red foxes (Vulpes vulpes) originated from the High Tatra recreational centres, in 3 out of 9 investigated raccoon dogs, one wild cat and one domestic cat. The high prevalence of E. multilocularis represents a serious risk in terms of human health, concerning in particular tourists, hunters and field workers. Infection threat is superimposed by the fact that hitherto no effective treatment is known for alveolar echinococcosis, which implies permanent damage to the health of the patient, often with lethal consequences.

Trichinella larvae were found in muscles of 18.8 % examined foxes, in rock martens and pine martens, polecats, wolf, otter, brown bear, lynx and domestic cat. The high number of infected animals from family Mustelidae (33 %) points out that these carnivores represent an important reservoir of the parasite in the territory of the Tatra Mountain. Since these synanthropic predators
often live in close proximity to human settlements, they pose a real risk of transmission of sylvatic trichinellosis into domestic cycle, which was confirmed by the finding of infected domestic cat.

*Dirofilaria repens*, zoonotic agent of subcutaneous dirofilariosis was diagnosed in 3 out of 19 examined red foxes and 1 from 3 rock martens (*Martes foina*). This is the first finding of D. *repens* in red foxes in northern mountainous part of Slovakia and the first discovery of the parasite in rock marten in the country. Although alpine area of the Tatra Mountains is the coldest part of Slovakia, some effects of climate changes that affected the diversity of fauna and flora become already evident here. The long-term research revealed an increasing tendency of air temperature and slight increase in number of precipitation days in the Tatra Mountains.

The high number of infected carnivorous animals inhabiting recreational areas of the Tatra National Park represents permanent transmission risk of important parasitic zoonoses to human and their pets.

The study was supported by the Science Grant Agency VEGA No. 2/0011/12.

LUNG HELMINTHES AND HELMINTHOSES OF ANIMALS AND HUMANS

S. Movsesyan1,2, G. Boyakhchian2, F. Chubarian, M. Nikogosyan2, R. Petrosyan2,
L. Arutyunova2, A. Gevorkyan2, M. Panayotova-Pencheva3, A. Demiaszkiewicz4,
M. Voronin1

Center of Parasitology, A.N. Severtsov Institute of Ecology and Evolution, RAS, 33 Leninskii Prospect, 119071, Moscow, Russia1; Scientific Center of Zoology and Hydroecology, NAS RA, 0014, Yerevan, P. Sevak str. 7, Armenia2; Institute of experimental morphology, pathology and anthropology with museum of BAS, 1113, Sofia, acad. G. Bonchev str. 25, Bulgaria3;
W. Stefansky Institute of Parasitology, PAS, 00-818, Warsaw, Twarda str. 51/55, Poland4

Helminth fauna material had been obtained and studied from 18 host species, including Cervidae — 7, Bovidae — 8, Canidae, Hyaenidae, Lagomorpha — 1 species each (located 15 species in Russia, 7 in Bulgaria, 6 in Poland and 4 in Armenia). Also studied were agricultural animals: cattle, sheep, goats. Another group of objects were many intermediate host species, including land and freshwater mollusks. To assess situation in respect to echinococcosis, one of our authors (Gevorkyan A.) performed original research on data kindly provided by medical institutions of Republic of Armenia. Also in Armenia we studied therapy and prophylaxis of sheep and goats against protostrongyliosis. Species variety of lung helminthes of animals and humans in the regions studied includes three taxonomic groups: Protostrongylidae — 28 species, including 26 in Russia, 11 in Bulgaria, 9 in Poland and 6 in Armenia; Dictiocaulidae — 2 species; Taeniidae — 2 species. Species common for all the regions were *Muellerius capillaris, Dyciotocallus filaria, D. viviparus, Echinococcus granulosus*. Goats and sheep in Armenia are infected with lung nematodes at levels of 20,4-44,1% and 16,5-37,0 %, respectively; in Bulgaria these levels are 88,09% for goats and 62,35% for sheep, with 39% of mixed infections; in Poland it is 10-72% for sheep, 46,1-67,6% for *Dama dama*, 37% for *Alces alces*, 7,4-18,5% for *Mouflon musimon*. Life cycles of Protostrongylidae lung nematodes include 77 land mollusk species, including 50 in Russia, 39 in Armenia, 20 in Bulgaria, 12 in Poland. The species found to be the most common and often infected in these regions were *Helicigona*
arbustorum, Cepaea nemoralis, Pyramidula selecta, Zanitoides nitidus, Helicella derbentina, Napaeopsis hohenackeri.

Echinococcosis situation in Armenia was as follows: infection level for cattle was 49%, for goat and sheep — 22%, for pigs — 25%. Level of human infection during 10 latter years can be characterized with the following: there were 420 cases of infection in Yerevan, and 118-199 in each countryside region during this period. Age distribution was as follows: greatest number of cases — 314 — was among people between 30 and 40 years old; among those between 10 and 30 y.o. there were 269 cases; for 40 to 50 y.o. there were 267 ones; for those older than 50 y.o. - 164. Larval echinococcosis have been found not only in lungs, but also in other organs: liver, spleen, brain, kidneys, abdominal cavity. There were also cases of combined infection of several organs. The organs most commonly attacked were lungs and liver (mean 43% or more each) while other organs (brain, kidneys, abdominal cavity, etc.) were infected in 1% of cases each. Methods for fighting echinococcosis are well developed and known in medical and veterinary practice. As for protostrongyloidoses, measures against them are developed quite insufficiently. As a result of our (Boyakhchian G.) experiments on sheep we propose the following highly effective anthelmintics: albenazol as bolus of 300 mg to be administered per os for animals weighting 30-40 kg and as bolus of 600 mg for those of 60-80 kg, alben as pills of 360 mg to use per os for animals of 40-45 kg and atazol forte (300 mg) as pills for animals weighting 30-40 kg.

EPIDEMIOLOGY AND CLINICAL EXPRESSION OF IMPORTED MALARIA IN PATIENTS HOSPITALIZED IN THE TROPICAL CENTRE IN POZNAN IN 2001-2012

K. Mrówka, M. Paul, J. Stefaniak
Department and Clinic of Tropical and Parasitic Diseases, University of Medical Sciences, Poznan, Poland

Background: Recently, there has been a constant increase in a number of people travelling to tropical countries and hence a significant risk of importing malaria to Poland and Central Europe. Malaria constitutes a direct risk of severe complications and death, so it is of a great importance to diagnose the infection as urgency in the initial phase of the disease and thus have no delay in administration of causative treatment. We presented the largest group of travellers with confirmed Plasmodium spp. infections imported to Poland that has been so far described in the literature.

Materials and methods: Epidemiological, clinical and laboratory studies were carried out on 54 patients hospitalized or consulted in the University Centre of Tropical Medicine in Poznan (Poland) from January 2001 to October 2012. Epidemiological interview was based on a questionnaire, including information on destination and type of a travel, duration of a stay, compliance with protection measures against mosquitoes’ bites and antimalarial chemoprophylaxis. A severity of the clinical course of the disease was evaluated using standardized criteria of the World Health Organization. The acute episode of malaria was confirmed in all of the hospitalized patients on the basis of positive thick and thin blood films examinations.

Results: In the analyzed group, 83% of the patients were infected in sub-Saharan Africa, mainly in Cameroon and Ghana. The most predominant species of the diagnosed parasite was Plasmodium falciparum (65%). Majority of the cases suited the WHO definition of uncomplicated malaria (68%), but 1/3 of the patients were diagnosed of severe complications as cerebral malaria, respiratory distress, renal failure, circulatory collapse or DIC syndrome. Anti-parasitic treatment with
quinine, mefloquine, artemisinin derivatives and doxycycline has been administrated with no fatal outcome; one patient required exchange blood transfusion.

Conclusion: Malaria is a significant cause of febrile illnesses in travelers returning from the tropics and must be diagnosed and treated in specialized centers which have practical clinical experience in tropical medicine and good laboratory practice.

ELISA TEST FOR DETECTION OF BLASTOCYSTIS SPP. IN HUMAN FAECES: COMPARISON OF THREE METHODS

I. Kucsera, M. Molnár, E. Gályász, J. Danka, E. Orosz
National Center for Epidemiology, Department of Parasitology, Budapest, Hungary

Objectives: Blastocystis is an enteric protozoan parasite highly prevalent in humans and animals. It is associated worldwide with aspecific symptoms, like diarrhoea, abdominal pain, anal itching, excess gas, and irritable bowel disease. Therefore the Blastocystis infected patients often remained non-diagnosed. Detection of Blastocystis is routinely performed by microscopy, culture, and sedimentation concentration technique. These methods are time consuming, laborious and require special skilled personnel. Microscopy is difficult since Blastocystis has several morphological forms (vacuolar, cyst, amoeboid, granular, multivacuolar, and avacuolar). Concentration technics destroy some of the forms during stool processing, therefore are unreliable. Culture requires 2-4 days for diagnosis and may allow preferential growth of specific strains while eliminating others. ELISA-based test for detection of Blastocystis antigens in stool could be a proper alternative to currently used methods, especially the microscopy. This work compares results of stool examination by microscopy, cultivation and antigen detection test (CoproELISA Blastocystis, Savyon, Israel).

Material and Methods: 74 stool samples routinely sent to the laboratory have been tested microscopically and by CoproELISA Blastocystis. 63 samples were tested with cultivation. 6 human Blastocystis strains isolated and maintained in the laboratory have been initiated to testing. For microscopic examination direct wet mount and Modified Merthiolate-Iodine -Formalin (MIF) preparation, for cultivation modified Boeck and Drbohlav’s diphasic medium and for Blastocystis antigen detection CoproELISA Blastocystis (Savyon, Israel) have been used.

Results: Microscopically in 16,2% (12/74) of samples Blastocystis was detected, by cultivation in 40,6% (28/69) and by ELISA 38,7% (31/80). Comparing cultivation and ELISA: 28 by cultivation positive samples (strains included) were positive in 96.4% (27/28) by ELISA. Testing 41 culture-negative samples by ELISA further 4 positives have been detected (9.7%, 4/41). Based on this comparison the calculated parameters of the ELISA test are: Sensitivity: 96.4% and Specificity: 90.2%.

Conclusions: The ELISA results showed high correlation with results obtained by cultivation, as a standard method. ELISA is expeditious in providing reliable results. Considering this, the ELISA is expected to be the method of choice for diagnosis of Blastocystis in the common laboratory.
VERMAMOeba ASSOCIATED KERATITIS – FIRST CASE IN SLOVAKIA
M. Garajová¹, M. Mrva¹, F. Ondriska², P. Žiak³

Department of Zoology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia¹; HPL (Ltd) Department of Parasitology, Microbiological Laboratory, Bratislava, Slovakia²; Faculty Hospital and Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia³

Amoebic keratitis is a painful progressive sight threatening eye disease of immunocompetent individuals. The rising number of cases is connected with the increasing use of contact lenses worldwide. Various species of ubiquitous free-living genus Acanthamoeba were confirmed as causative agents of this disease. However, other amoebae such as Vahlkampfia spp. and Vermamoeba vermiformis (formerly classified as Hartmannella vermiformis) have been occasionally reported to be associated with keratitis. Although V. vermiformis was included in the list of human pathogens, its pathogenicity remains controversial.

In March 2013 a 22-years-old woman wearing soft contact lenses was treated on the eye clinic due to bilateral keratoconjunctivitis with several small stromal infiltrates detected in the affected cornea of the right eye. From both contact lenses, their cases and cleaning solution Pseudomonas aeruginosa, Klebsiella oxytoca and non-fermenting Gram-negative bacteria were isolated and antibacterial therapy started (levofloxacin) with partial effect. Nevertheless, cultivation of the contact lens from the right eye revealed amoebae and a subsequent therapy with propamidine isethionate (Brolene drops) led to improvement of the eye condition. Trophozoites of amoebae were elongated, 25 – 45 μm, L:B ratio of 7.5 – 9.5, with a large and permanent hyaline frontal cap. The posterior end was narrowed, occasionally with a uroid. Amoebae were mostly monopodial, only during changes of locomotion they were temporarily Y-shaped or polypodial. The single vesicular nucleus possessed a central nucleolus. A single contractile vacuole was situated near the posterior end. Cysts were spherical, 6 – 8 μm, with a single nucleus situated in the centre and with double cyst wall. On the basis of morphological features the strain was identified as Vermamoeba vermiformis (Page, 1967) Smirnov & Cavalier-Smith, 2011.

In the previous works, Vermamoeba was detected from keratitis cases rarely and mainly in coinfections with Acanthamoeba. To the best of our knowledge, this is the third report of corneal infection associated exclusively with Vermamoeba without any other amoeba species involved.

The research was supported by grants VEGA 1/0796/12 and UK/263/2014. This publication is the result of the project implementation: Comenius University in Bratislava Science Park supported by the Research and Development Operational Programme funded by the ERDF Grant number: ITMS 26240220086.
SEROPREVALENCE OF TOXOCAROSIS IN ROMA POPULATION FROM SEGREGATED SETTLEMENTS AND IN MAJORITY POPULATION OF EASTERN SLOVAKIA

D. Antolová¹, P. Jarčuška², M. Janičko², M. Halánová², L. Čechová², L. Čisláková², M. Škutová¹, K. Reiterová¹, HepaMeta team
Institute of Parasitology SAS, Košice, Slovakia¹; P. J. Šafárik University in Košice, Faculty of Medicine, Košice, Slovakia²

Larval toxocarosis is a serious parasitozoanosis caused by larval stages of Toxocara canis and Toxocara cati, frequent parasites of domestic and wild carnivores. Humans can acquire infection by ingestion of embryonated eggs or encapsulated Toxocara larvae from tissues of infected paratenic hosts. Roma people belong to one of the oldest and largest minorities in Europe. In Slovakia, estimated real number of Roma citizens exceeds 400,000 with approximately one sixth of them living in segregated settlements. The aim of the study was to map the seroprevalence of toxocariosis in the population living in segregated Roma settlements in Eastern Slovakia and to compare it with the majority population.

Antibodies to Toxocara were detected in 95 (22.1 %) out of 429 Roma people examined, while only 3 (1.9 %) people out of majority population living in catchment area close to target Roma population and one (0.4 %) person from majority population living in area without Roma population where found to be positive. The prevalence of antibodies in majority population decreased with age, whereas in Roma it increased from 15.8 % in persons between 18 and 30; to 23.3 % in those between 30 and 40; 24.8 % in people between 40 and 50 and to 35.7 % in people older than 50 years. The gender did not influence the prevalence of antibodies significantly but both, Roma (22.8%) and non-Roma (1.9 %) women were positive more often than men (20.9 % and 0.0 %). Analysis of clinical symptoms revealed significantly more frequent occurrence of headache, influenza like symptoms, muscle pain and diarrhoea in persons with Toxocara antibodies than in negative individuals, but the frequency of symptoms did not correlate with the antibody titers. Analyses showed strong correlation between sanitary equipment of houses and positivity of their inhabitants, missing of canalisation, running water, functioning bathroom and electricity was associated with significantly higher prevalence of antibodies.

We can conclude that living conditions in segregated settlements, with large number of people and domestic animals concentrated on small areas and limited access to water resources, electricity and sewage system, significantly contribute to the spread not only of toxocariosis, but also other parasitic and infectious diseases. This issue is even more important in the light of migration of socially deprived minority population observed in last years, increasing the possibility of the spread of infectious diseases over large distances.

The research was supported by the Research and Development Support Agency, Contract No. APVV-00-032-11; the Agency of the Slovak Ministry of Education for the Structural Funds of EU, project CEMIO-ITMS: 26220120058 (0.3), by the project “Social determinants of health in socially and physically disadvantaged and other groups of population” (CZ.1.07/2.3.00/20.0063), by Roche Slovensko, s. r. o. and also within the framework of the VEGA project, grant 2/0127/13.
SESSION II
Parasitology in genomic and biochemistry era

TRANSCRIPTOME AND GENOME ANALYSIS OF *EUDIPLOZOOON NIPPONICUM*
H. Dvořáková¹, L. Jedlíčková¹, J. Vorel¹, P. Roudnický², E. Dzika³, B. Koubková², M. Gelnar², M. Kašny¹,², L. Mikeš¹

Department of Parasitology, Faculty of Science, Charles University in Prague, Viničná 7, 12844 Prague 2, Czech Republic¹; Department of Botany and Zoology, Faculty of Science, Masaryk University Brno, Kamenice 753/5, 62500 Brno Bohunice, Czech Republic²; Faculty of Medical Science, University of Warmia and Mazury in Olsztyn, Żołnierska 14c, 10-561 Olsztyn, Poland³

General knowledge of the biological processes taking place in all organisms, including helminths, is based also on our understanding of their detailed molecular biological and biochemical principles. For whole group Monogenea this kind of information is still extremely limited. Recently the “50 Helminth Genomes Project” was started, but only one monogenean species was involved - *Protopolystoma xenopodis*. We would like to change this insufficient status and therefore we adopted *Eudiplozoon nipponicum* (Monogenea: Diplozoidae) as a model organism and started some preliminary genome, transcriptome and proteome analyses in respect to reveal the important parasitic molecules, such as the proteolytic enzymes - peptidases and their inhibitors. By using the 454 sequencing technologies (GS FLX System, Roche), a total of 324 941 reads were generated (average length 423 bp) and assembled into 6910 contigs. The homology searches were performed and particular peptidases/inhibitors were identified; 29 contigs of cysteine peptidases (e.g. cathepsin L) and 7 contigs of their inhibitors (e.g. cystatins); 12 contigs of serine peptidases (e.g. cathepsin A) and 7 contigs of their inhibitors (e.g. serpin). Their further characterization is in process.

Acknowledgements: Czech Science Foundation grant no. P506/12/1258, GBP505/12/G112 and grants of the Charles University in Prague (UNCE 204017, PRVOUK P41, SVV 267210/2013, GAUK 502313).

KUNITZ DOMAIN PROTEINS AS POSSIBLE ANTICOAGULATION FACTORS IN MONOGENEANS
K. Skipalová¹, L. Jedlíčková¹, H. Dvořáková¹, M. Kašny¹,², M. Gelnar², L. Mikeš¹

Department of Parasitology, Faculty of Science, Charles University in Prague, Viničná 7, 12844 Prague 2, Czech Republic¹; Department of Botany and Zoology, Faculty of Science, Masaryk University Brno, Kamenice 753/5, 62500 Brno Bohunice, Czech Republic²

Among monogeneans, there are groups of blood-feeding parasites occupying the gills of fishes (fam. Diplozoidae) or the urinary bladder of amphibians (fam. Polystomatidae). All hematophagous parasites need to avoid host’s hemostasis (blood coagulation) to facilitate blood intake. For this purpose, various anticoagulant factors and anti-platelet aggregation agents are employed. Although antihemostatic molecules have not been reported yet from monogeneans, their presence is highly probable in sanguinivorous species. As hemocoagulation systems of animals are
based on the action of a cascade of serine proteases, antihemostatic molecules often act as serine protease inhibitors that target one or more enzymes (coagulation factors).

Our study is focused on molecular, biochemical and functional characterization of potential anticoagulants involved in blocking of factors in fish coagulation cascade. We measured inhibitory activity of excretory-secretory products and homogenates from adult diplozoids *Eudiplozoon nipponicum*, *Paradiplozoon homoin* and *Paradiplozoon bliccae* towards coagulation factors IIa and Xa with negative results. Employing transcriptomic data of *E. nipponicum* and bioinformatics, we found three groups of potential anticoagulants - annexins, serpins and Kunitz-domain proteins. For further analyses we focused on the Kunitz protein family. These Kunitz proteins contain one or more structurally related inhibitory domains. We described similarities between Kunitz sequences from *Eudiplozoon nipponicum* and their homologs from the polystomatid *Protopolystoma xenopodis* and other less related hematophagous parasites, which exhibit anticoagulant activity (e.g. the ticks *Ixodes scapularis*, *Rhipicephalus microplus* or *Haemaphysalis longicornis*).

To obtain the full length Kunitz DNA sequences, we used Rapid Amplification of cDNA Ends (3’ and 5’ RACE PCR). Separate Kunitz domains will be prepared in the recombinant form for further biochemical and functional tests.

Acknowledgements: Czech Science Foundation grant no. P506/12/1258, grants of the Charles University in Prague GAUK 502313, UNCE 204017, PRVOUK P41 and SVV 260074/2014.

**EVOLUTIONARY AND POPULATION CHARACTERIZATION OF ANISAKIS SIMPLEX S.S. USING MITOCHONDRIAL DNA**

A. Kijewska\(^1\), J. Dzido\(^2\), J. Rokicki\(^2\)

Institute of Oceanology PAS, Department of Genetics and Marine Biotechnology, Sopot, Poland\(^1\); University of Gdańsk, Department of Invertebrate Zoology, Gdańsk, Poland\(^2\)

*Anisakis simplex* s.s. is parasite of marine mammals. Previous studies demonstrated a noticeable genetic distance between of the parasite. The number of samples and their geographical origin was sufficient to compare Atlantic and Pacific populations. The Bayesian analysis provided a strong support for population split into 2 groups: one representing *A. simplex* s.s. samples from the Pacific, and the other representing all the Atlantic samples. It is highly probable that observed subpopulations were constituted after the connection between the Atlantic and Pacific had been closed and the communication between these 2 oceans became aggravated. We confirmed the division of *A. simplex* population into two separate, genetically distant subpopulations (**F**\(_{ST}\) = 0.46371, \(p<0.00001\); **S**\(_{ST}\) = 1, \(p=0.0027\)). Moreover, these subpopulations seem to be completely separated with minimal chance for exchanging migrants between each other. Characteristics of both subpopulations suggested also the Pacific one to be older because of higher number of mitochondrial lines than in the Atlantic population. The Atlantic Ocean had been infiltrated by a fairly low number of individuals what probably was a reason of a bottleneck effect which affects the present structure of the Atlantic subpopulation.
TRANSPORT OF PYRUVATE INTO THE MITOCHONDRION OF *TRYPANOSOMA BRUCEI*

J. Šťáfková¹, J. Mach¹, F. Bringaud², J. Tachezy¹

Department of Parasitology, Faculty of Science, Charles University in Prague, Czech Republic¹; Centre de Résonance Magnétique des Systèmes Biologiques, UMR5536, Université de Bordeaux, CNRS, France²

*Trypanosoma brucei* is a pathogen of livestock and humans transmitted by tse-tse flies in sub-Saharan Africa. Different life-cycle stages of trypanosomes present adaptations to their specific environment. In bloodstream and procyclic *T. brucei*, these include changes to mitochondrial morphology and function, and overall metabolic rearrangements reflected by different spectra of metabolic end products. In bloodstream *T. brucei*, ATP is generated primarily by glycolysis, thus pyruvate produced by glycolysis is the predominant excreted product of metabolism. The mitochondrion of the bloodstream stage is not considered to play a role in ATP generation. In contrast, procyclic-stage *T. brucei*, found in the midgut of the insect vector where glucose is scarce, depend on mitochondrial catabolic pathways for ATP production. Proline and threonine are candidate carbon sources for these stages. *In vitro*, these are eventually metabolized to succinate, acetate and glycine.

Regulating the availability of pyruvate in the mitochondrion is one of the modes of balancing oxidative phosphorylation and glycolysis; in bloodstream *T. brucei* this balance is shifted heavily towards glycolysis. We seek to determine whether *T. brucei* transports pyruvate into the mitochondrion using a mitochondrial pyruvate carrier homologous to the one recently identified in fruit fly, human and yeast cells (MPC). In addition, we address the relative importance of the pyruvate transporter in procyclic and bloodstream trypanosomes.

To this end, we identified two MPC homologs in the genome *T. brucei* and confirmed the mitochondrial localization of the epitope-tagged proteins in both procyclic and bloodstream stages. We generated MPC1 knock-out cell lines in both these stages, showing that the putative pyruvate transporter is dispensable for *T. brucei* under standard culture conditions. The end product analysis using HPLC and NMR strongly supports the involvement of MPC1 in transport of pyruvate. In addition, adaptations of mitochondrial metabolism observed in the knock-out cell lines let us comment on mitochondrial metabolism in *T. brucei* in general.

A NOVEL APPROACH TO PROTEIN LOCALIZATION IN PARASITIC WORMS

J. Bulantová¹, M. Kašný¹,², D. Potěšíl³, Z. Zdráhal³, L. Staněk⁴, P. Horák¹

Department of Parasitology, Faculty of Science, Charles University in Prague, Vinicna 7, 128 00 Prague, Czech Republic¹; Department of Botany and Zoology, Faculty of Science, Masaryk University Brno, Kamenice 753/5 625 00 Brno Bohunice, Czech Republic²; National Centre for Biomolecular Research, Masaryk University Brno, Kamenice 753/5, 625 00 Brno Bohunice, Czech Republic³; Institute of Pathology of 1st Faculty of Medicine and General Teaching Hospital, Charles University Prague, Studničkova 2, 128 00 Prague, Czech Republic⁴

Currently, in the era of "Next Generation Sequencing", the genome/proteome databases are still faster and faster filled with comprehensive sequential data. There are many possibilities how to
effectively use these robust data sets, e.g. for interpretation of mass spectra of analyzed proteins leading to their identification, prediction of protein functions, or better understanding of protein specific roles in biological systems. In the context of protein functions it is useful to complete this computational information by localization of protein specific expression in cells/tissues and protein mode of action.

Localization of the selected protein in particular tissues by using common immunotechniques is a time consuming process that usually includes cloning of gene, preparation of the recombinant form, its purification, immunization of experimental animals, purification of antibodies, preparation of histological slides and immuno-histochemical staining by use of sera from immunized animals.

Several years ago (Helminthological days 2008, Ředkovec, Czech Republic), the potential of mass spectrometry in modern proteomic studies focused on parasites, namely trematodes, was presented as a possible “shortcut” in the process of protein immuno-localization mentioned above. MALDI-MSI technique (Matrix-Assisted Laser Desorption/Ionization Mass Spectrometric Imaging) was highlighted as a perspective method for direct determination and monitoring of biomolecules in the organic tissue.

Recently we optimized the method also for identification and localization of particular proteins; we adopted microdissection technique combined with mass spectrometry analysis. Predefined organs (e.g. intestine, penetration glands, vitellaria) or specific tissue parts (tegument, musculature) were thoroughly microdissected from cryosections of two model species - *Trichobilharzia regenti* (cercariae) and *Fascioloides magna* (adults). Microdissection ensures subsequent precise analysis of protein distribution directly in organs of interest, without possible contamination. By application of this approach cathepsin L from *F. magna* intestine was identified.

Acknowledgement: Czech Science Foundation (Grant No. 13-29577S) and Charles University in Prague (UNCE 204017, PRVOUK P41, SVV 260074/2014, GAUK 502313).

---

**PLENIARY SESSION III**

**TRICHINELLA INFECTIONS IN WILDLIFE IN THE CZECH REPUBLIC**

B. Koudela¹, J. Harna²,³, M. Pijáček²,³

University of Veterinary and Pharmaceutical Sciences, Palackého 1-3, 612 42 Brno, Czech Republic¹; State Veterinary Institute, Jakoubka ze Stříbra č. 1, 779 00 Olomouc, Czech Republic²; Czech National Reference Laboratory for Parasites, Jakoubka ze Stříbra č. 1, 779 00 Olomouc, Czech Republic³

Trichinellosis is a foodborne zoonotic disease caused by the consumption of raw meat and raw meat-derived products from animals infected with nematode worms of the genus *Trichinella*. In Europe, the parasite was more prevalent in wildlife than in farmed animals and wildlife animals serve as the major reservoir hosts. Between years 2001 and 2012, almost 1.33 million wild boar were hunted and of them 0.887 million wild boars (*Sus scrofa*) (66.7 %) were tested for *Trichinella* sp. in the Czech Republic. *Trichinella* infection was demonstrated in 16 wild boars (0.0018 %). Although the prevalence of *Trichinella* sp. infection in wild boars is very low, the spatial analysis reveals that the
level of risk differs by region in the Czech Republic. Larvae from wild boars were identified as *T. britovi* (75%), *T. spiralis* (6.25%) and *T. pseudospiralis* (18.75%); mixed infection was not found.

In December 2010 and January 2011, *Trichinella* sp. larvae were detected in three wild boars hunted in the eastern part of the Czech Republic. The larvae were identified as *T. pseudospiralis* by multiplex PCR. All *T. pseudospiralis*-positive wild boars had similar weight (around 35 kg) and were shot at the same baited site by the same hunter. A common origin of the infection in the three wild boars has been hypothesized: 1) the meat used as bait for attracting the wild boars was the source of *T. pseudospiralis* infections; or 2) the three wild boars belonged to the same wild boar herd which had fed on the same carcass of an infected wild animal. These findings support the tendency of a more frequent detection of the non-encapsulated species *T. pseudospiralis* in Europe, which probably is related to the increased number of tested wild boars and to the use of the artificial digestion instead of the less sensible trichinoscopy to detect *Trichinella* larvae in meat samples.

In the last decades, since the administration of an oral vaccination against rabies, the red fox population in Czech Republic has increased exponentially. Monitoring the prevalence of *Trichinella* spp. in the main reservoir animal, the red fox, was also performed using artificial digestion method. The results of investigation performed in 1164 (2001-2003) and 1019 (2006-2007) red foxes showed that *Trichinella* infection is present in low prevalence (2001-2003: 0.6%; 2006-2007: 0.49%) across the Czech Republic but the prevalence increased significantly (3 positive red foxes since January to April 2014).

*Trichinella* larvae were also detected in two hunted badger (*Meles meles*) near the town Frýdek-Místek where *Trichinella* infection was earlier confirmed in wild boars and red foxes. The parasites were identified as *T. britovi*. This is the first report of the identification of *T. britovi* larvae from badgers in the Czech Republic.

**Acknowledgements:** the authors thank Drs. Michaela Dokulilová, Ivan Pavlásek, Karol Račka, Antonín Prouza for their help in sample collection. Furthermore, the authors are very grateful to members of the European Union Reference Laboratory for Parasites in Rome who tested *Trichinella* larvae by multiplex PCR.

---

**NEGLECTED LARVAL CESTODE INFECTIONS – CURRENT SITUATION AND CONTROL**

G. Hrčková, S. Velebný

Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovak Republic

Millions of humans and animals are simultaneously infected with different helminth species. However, an important group of disease-causing organisms has often been excluded from such surveys, namely the zoonotic larval cestodoses. In 2007, the World Health Organization (WHO) included echinococcosis (*Echinococcus multilocularis* and *E. granulosus*) and cysticercosis (*Taenia solium* and *T. saginata*) as a part of a neglected zoonosis subgroup for its strategic plan for the control of neglected tropical diseases (NTDs). Introducing part of the lecture deals with geographical prevalence of these diseases, as well as with pathological consequences of the infection with related cestodes. WHO also identified five drugs (mebendazole, albendazole, diethylcarbamazine citrate, ivermectin and praziquantel) in the NTDs area because they have the greatest and most immediate impact on public health. Moreover, praziquantel (PZQ) is drug of choice for the treatment of all *Schistosoma* species. The therapeutical potential of PZQ for the above mentioned human and animal
Cestode infections is documented by the results obtained in many experimental as well as clinical studies – as a single dose regime or in combination with other drugs (e.g. with albendazole and cimetidine).

PZQ has limited solubility in biofluids, the result of which is that only a low concentration of the active drug can reach parasites localized in the parenchymal tissues. In this respect, modified drug formulations, which could overcome this problem, can lead to the improvement of efficacy. Liposomes, spherical vesicles made from natural lipids, offer many advantages as drug carriers. Immunosuppression of host immune response, triggered by parasite-derived molecules, is a phenomenon characteristic of helminth infections which complicates the cure of the illness. It can be manipulated with some drugs (e.g. PZQ) and especially with external immunomodulatory substances. Until a new compound with antiparasitic effect and simultaneous stimulatory activity towards the host’s immunity is available, safe and cheap alternative approaches need to be investigated. There are numerous compounds isolated from natural sources without having toxic side-effects, which are currently evaluated in antiparasitic therapy, either as single drugs or in combination with current drugs. _Mesocestoides vogae_ (syn. _Mesocestoides corti_, Etges, 1991) is considered as a good experimental model to study cestode biology and the effects of drugs, because it can be easily manipulated both _in vivo_ and _in vitro_ and due to its relatively close relationship with cestodes of medical relevance, such as species of _Echinococcus_ or _Taenia_.

The findings summarized in this lecture demonstrate that the efficacy of praziquantel towards larval cestodes can be markedly improved after its incorporation into suitable liposomal drug carriers. Moreover, co-administration of the immunomodulatory substances glucan, silymarin and transfer factor offered a very effective tool to activate cells of the host immune defence system, which is immunosuppressed. Combined therapy proved to have multiple advantages over the classical therapy, regarding the efficacy and host pathophysiology.

_Support was given by project Grant Agency VEGA no. 2/150/13._

**SESSION III**

**Veterinary and wild-life parasitology**

**IMPACT OF MANAGEMENT PRACTICES ON THE COURSE OF THE BLOOD-SUCKING NEMATODE _ASHWORTHIUS SIDEMI_ INVASION IN WILD-LIVING EUROPEAN BISON (_BISON BONASUS_) IN BIAŁOWIEŻA PRIMEVAL FOREST, POLAND**

M. Kołodziej-Sobocińska¹, A. Demiaszkiewicz², A. M. Pyziel², J. Lachowicz², R. Kowalczyk¹

Mammal Research Institute, Polish Academy of Sciences, Waszkiewicza 1, 17-230 Białowieża, Poland¹; W. Stefański Institute of Parasitology, Polish Academy of Sciences, Twarda 51/55, 00-818 Warsaw, Poland²

The largest terrestrial mammal in Europe, the European bison (_Bison bonasus_ L., 1758) – has been reintroduced to 30 locations in Eastern Europe after becoming extinct in the wild. It is managed as a forest specialist, however evolutionary history and morphological features indicate adaptation to open or mixed habitats. In most locations bison are regularly provided with supplementary feed in winter, in an effort to reduce damage to forest stands and agricultural crops, while also limiting bison...
dispersal. This leads to unnatural bison aggregations and may lead to an increase in parasitic load, which is one of the major threats to the species. In the last few decades bison have assimilated several new species of parasite, including the blood-sucking nematode found in the abomasum - Ashworthius sidemi (Trichostrongylidae) – specific to Asian deer species. This parasite was first found in Bialowieża Forest in 2000 in one of the culled bison. After 4 years A. sidemi was found in all investigated individuals and average infection intensity rose to 12 000 (max. 77 600) nematodes in 2011. Since 2012, a decline of prevalence and intensity of infection has been observed, which indicates that a balance between host and parasite is occurring. We investigated the influence of management practices and intensity of supplementary feeding on parasitic load of A. sidemi. Our results confirm that A. sidemi infection intensity is significantly higher (up to 7 times) in intensively fed bison, which spend up to five months at feeding sites, in comparison to less intensively or non-fed bison, which utilize much larger ranges during winter. The rate of infection is also influenced by age but not by the sex of bison. The highest infection rate was recorded in sub-adult bison in comparison to calves and adults. Our results show that the intensity of the infection significantly influenced blood parameters such as red blood count and hemoglobin. When the intensity of A. sidemi infection was high, the blood parameter values were lower. We assumed that the sudden appearance of a new and strong invasion of the parasite in bison population in Bialowieża Forest had an impact on the overall condition of the animals, which is expressed in changes in blood parameters. Additionally, the results suggest that it is necessary to change the general approach to management practices of the bison population both in Bialowieża Primeval Forest and other locations, where similar management is practiced. A split of large winter aggregations may help to manage parasitic infections and also increase bison fitness.

SPATIAL DISTRIBUTION OF FASCIOLA HEPATICA IN SWEDEN AND IMPACT OF METEOROLOGICAL AND ENVIRONMENTAL FACTORS

A. Novobilský1, J. Novák2, J. Höglund3

Swedish University of Agricultural Sciences, Department of Biomedical Sciences and Veterinary Public Health, Section for Parasitology, 750 07, Uppsala, Sweden1; Tartu University, Department of Geography, Vanemuise 46, 51 014, Tartu, Estonia2

The liver fluke Fasciola hepatica is the causative agent of a production limiting parasitic disease affecting livestock worldwide. In the European Union, financial losses due to F. hepatica in ruminant production were recently estimated to € 1.1-2.0 billion per annum. According to data from meat inspections, an increasing prevalence of fasciolosis has been documented in Sweden in the last decade.

Spatial models based on Geographic Information Systems (GIS) can be used to develop risk maps and to identify predictors for fasciolosis. In the present study, a serological survey of F. hepatica in Swedish beef cattle was performed to determine F. hepatica seroprevalence and high-risk areas in Sweden and to characterize potential risk factors for fasciolosis in beef cattle. A total of 2767 serum samples (1-5 per herd) were collected from first season grazing beef cattle on 2135 herds between November 2006 and May 2007. The F. hepatica antibody levels in serum samples were determined using an in-house F. hepatica excretory/secretory antigen specific ELISA. All examined herds were identified based on 5-digit (80%) and 3-digit (20%) postal codes. Post code area
(PSA) layer was constructed using ArcGIS software as combination of five-digit and three-digit postal codes area polygons. To localize hot spots with fasciolosis, spatial clustering of positive and negative herds was tested using a spatial scan statistic. Identification of clusters was performed by Bernoulli model using the SaTScan software (www.satscan.org). A logistic regression models using climate, environmental data and GIS were developed to evaluate risk factors.

The national seroprevalence of *F. hepatica* in Swedish beef cattle was 9.8%. The spatial scan statistic revealed two significant clusters (p=0.01) with high risk of *F. hepatica* infection: Skåne (prevalence 35.2%) and Västra Götaland (prevalence 65.3%). In regression models, temperature in spring month, rainfall in first quarter of year (both 10-year average), magnesium in soil and cattle density were positive predictors. Negative predictors were rainfall in the third and fourth quarter of year, coniferous forest, copper and phosphorus in soil. Our data suggests that risk for *F. hepatica* infection on spatial level is affected rather by environmental factors than climate.

**NEOSPORA CANINUM IN FARMING AND WILDLIFE ANIMALS IN POLAND**

B. Moskwa, J. Bień, A. Cybulsk, A. Kornacka, W. Cabaj

Witold Stefański Institute of Parasitology, Polish Academy of Sciences, Twarda 51/55, 00-818 Warsaw, Poland

*Neospora caninum* is a protozoan parasite originally reported in dogs and commonly diagnosed in bovine abortions worldwide. In the life cycle of *N. caninum*, both horizontal and vertical transmission of the parasite has been reported. Consequences of vertical transmission during pregnancy in cattle may result an abortion of foetus, birth of a weak calf which may show some neurological signs, or birth of a clinically normal but persistently infected calf. It is documented that the parasite is widely spread among non-carnivorous wildlife. However, the incidence and effects of *N. caninum* vertical transmission in wildlife animals remain unexplained.

In Europe, the presence of *N. caninum* has been confirmed serologically in red deer (*Cervus elaphus*), roe deer (*Cervus capreolus*), fallow deer (*Dama dama*) and sika deer (*Cervus nippon*). Enzyme-linked immunoassay (ELISA) is one of the most commonly used tests for the *N. caninum* diagnosis. In our studies ELISA (IDEXX Laboratories Inc., Westbrook, ME, USA) was performed according to manufacturer’s instruction and with some modifications (Bien et al., 2012). Additionally, the antibody titer was also confirmed by NAT, cELISA and WB.

Until now approximately 5500 cows serum samples were examined. The herds consisted of 10 to 450 cows and the seroprevalence varied from 1.5% to 80%.

Additionally, during veterinary handling the blood was taken from jugular vein of 606 fallow deer, 160 red deer, 120 sika deer in Kosewo Górne Field Station belongs to the Institute of Parasitology. The overall seroprevalence was found to be 3.3% (for fallow deer) and 11% (for red deer), however it decreased in consecutive years. It is worth noting that antibodies against *N. caninum* were not detected in none of sika deer examined in 2012-2014. Among 47 free living red deer near Kosewo Górne 6 were seropositive (13%).

The majority of 664 serum samples from European bison were obtained in the Bialowieża National Park. Antibodies against *N. caninum* have been found in 72 serum samples. Overall seroprevalence was found to be 10.9%, however it varied in consecutive years.

Blood samples were collected from seven moose hunted in the north-east region of Poland. Antibodies to *N. caninum* were detected in one of seven examined animals.
Our results strongly confirmed the presence of antibodies against *N. caninum* in farming and wildlife animals in Poland.

**PLENARY SESSION IV**

**CAN ANTHelmINTICS WIN THE FIGHT AGAINST PARASITES?**  
M. Várady, A. Königová, M. Dolinská  
Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovakia

Parasitoses, particularly those caused by gastrointestinal nematodes, are certainly one of the most important diseases that constrain productivity due to the large scale effects that they have on the nutrient economy of domestic animals. Unfortunately, the extensive use of these chemicals has led to the emergence of resistant parasite populations. Globally there are over 50 countries with reported cases of anthelmintic resistance (AR); many cases involve multiple drug groups and/or multiple species. Although the incidence of resistance is difficult to ascertain, the trend is for higher levels of resistance on more farms in more countries. Research into AR has not progressed far beyond the stage of “descriptive research”. Some progress has been made in developing control strategies and in diagnosing resistance. However, the lack of reliable biological and molecular tests means that we are not able to follow the emergence and spread of resistance alleles.

The following research priorities during 25 years study of AR in the Department of Experimental Parasitology of SAS have been identified: 1) To monitor the prevalence of AR in parasites of domestic animals in Slovakia; 2) To develop and improve the in vitro techniques for monitoring of AR; 3) To develop the strategies to delay the onset of resistance. The results obtained are discussed and possible benefits will be highlighted.

*The study was supported by Grant Agency VEGA, Grant No. 2/0151/13 of the Scientific Agency of the Slovak Academy of Sciences and Slovak Research and Development Agency Project No. 0539-10.*

**SESSION IV**  
**Pharmacology and treatment**

**EFFECTS OF PROBIOTIC BACTERIA ON PHAGOCYTOSIS AND RESPIRATORY BURST ACTIVITY OF BLOOD POLYMORPHONUCLEAR LEUKOCYTES IN MICE INFECTED WITH TRICHINELLA SPIRALIS**  
E. Dvorožňaková¹, Z. Hurníková¹², V. Revajová², A. Lauková³  
Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovakia¹; University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia²; Institute of Animal Physiology, Slovak Academy of Sciences, Košice, Slovakia³

Probiotic strains confer a beneficial property to the host as immune stimulation, protection against pathogens; have the capacity to control intestinal parasite infection but also some nongut infection. However, molecular mechanisms mediating the beneficial effects are as yet poorly
understood. Phagocytosis and respiratory burst are two of the most important functions of leukocytes and essential for the elimination of invading pathogens. Phagocytosis is a complex process mediated through various cell surface receptors and enzymes activated by reactive oxygen species produced in the process of respiratory burst.

Bacteriocinogenic and probiotic strains of different origin (Enterococcus faecium AL41, Enterococcus durans ED26E/7, Lactobacillus fermentum AD1-CCM7421, Lactobacillus plantarum 17L/1) were administered daily in dose of 10^6 cfu/ml in 100 µl and mice were infected with 400 larvae of T. spiralis on 7th day of treatment. T. spiralis infection increased phagocytic activity of leukocytes at 1st week post infection (p.i.), but a decrease in the percentage of phagocytes was recorded at weeks 3 and 4 p.i. The ingestion capability of phagocytes was significantly increased during the first two weeks of the infection. Administration of bacterial strains to mice with T. spiralis infection stimulated phagocytic activity of leukocytes for a longer time, from 1 to 3 weeks of the infection and the phagocytosis was inhibited only at week 4 p.i. Similarly, the ingestion capability of phagocytes in mice treated with bacterial strains was stimulated first three weeks of the infection. Only strain L. fermentum AD1-CCM7421 increased the ingestion capability before the infection, i.e. after one week of its administration. The highest stimulative effect on phagocytosis was induced by strains E. durans ED26E/7 and L. plantarum 17L/1. The percentage of cells with respiratory burst was increased after T. spiralis infection with the exception of week 3 p.i., but the high percentage of metabolic active cells persisted for all time after the infection in mice treated with bacterial strains. Enzymatic activity was already increased at 1st week of treatment with E. durans ED26E/7 and L. plantarum 17L/1. T. spiralis infection induced an increased enzymatic activity at weeks 2 p.i. and then 4 and 5 p.i. But in mice treated with bacterial strains the enzymatic stimulation was observed for all time after the infection, with the highest intensity caused by strains E. durans ED26E/7, L. fermentum AD1-CCM7421 and L. plantarum 17L/1.

The results indicate phagocytic and metabolic activity of blood leukocytes is inhibited at weeks 3 and 4 post T. spiralis infection, i.e. in the time of massive blood migration of newborn larvae into the host muscles. The administration of bacterial strains with a probiotic effect prevented the inhibition at week 3 p.i. and stimulated phagocytosis and respiratory burst of blood leukocytes that could contribute to a decreased larval migration and a destruction of newborn larvae and then reduced parasite burden in the host. The highest protective effect against T. spiralis infection was induced by strains E. durans ED26E/7 and L. plantarum 17L/1.

The work was supported by the Project “Competence centre for biomodulators and nutritional supplements - Probiotech (ITMS-26220220152)”.

ANTHELMINTIC RESISTANCE: IN VITRO VERSUS IN VIVO DETECTION IN GOATS
A. Königová¹, M. Dolinská¹, T. Kupčinskas³, G.von Samson-Himmelstjerna², M. Várady¹
Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovakia¹; Freie Universität Berlin, Institute for Parasitology and Tropical Veterinary Medicine, Robert-von Ostertag Str. 7-13, 14163 Berlin, Germany²; Lithuanian University of Health Sciences, Tilžes 18, LT-47181 Kaunas, Lithuania³

A variety of in vivo and in vitro methods are available for monitoring anthelmintic resistance. Considerable research efforts have been directed towards defining in vitro tests that may diagnose
resistance in pre-parasitic stages of goat parasites. The objective of the study was to compare in vivo FECR test, two in vitro methods (EHA and LDT) and resistance/susceptible allele frequency determination in *H. contortus* infected goats. In this study 54 goat kids were each infected with mixture of 2500 L3 larvae of resistant (benzimidazole, ivermectin) and susceptible isolate of *H. contortus*. Composite infection doses consisted of 10, 20, 30, 40, 60 and 80 % of known resistant isolate. Faecal samples for *in vitro* tests were collected on separate days. The results of *in vivo* FECR test showed that albendazole treatment reduced infections by 91.3, 78.0, 63.3, 48.4, 36.5 and 41.4 %.

The results of ED<sub>50</sub> values in the *in vitro* EHA varied from 0.09±0.01 to 15.63±12.10 µg/ml TBZ. The LD<sub>50</sub> values from 0.014±0.002 to 12.31±2.41 µg/ml TBZ were defined by *in vitro* LDT in composite *H. contortus* isolates. Results of Pyrosequencing<sup>TM</sup> method revealed that frequency of susceptible and resistant alleles of *H. contortus* in larvae derived from different groups of goats was closely comparable with composite larvae which we have used during infection. The results of *in vitro* tests show solid base for an estimation of clinical resistance *in vivo*. By using of hatching percentage *in vitro* EHA (0.3 and 0.5 µg/ml TBZ) we can precisely estimate the efficacy of drug *in vivo*.

*The study was supported by Grant Agency VEGA, Grant No. 2/0151/13 of the Scientific Agency of the Slovak Academy of Sciences and Slovak Research and Development Agency Project No. 0539-10.*

---

**SESSION V**

**Helminths – diversity, taxonomy and ultrastructure**

**DIVERSITY OF FISH NEMATODES IN BRAZIL**

F.M. Vieira<sup>1</sup>, T. Scholz<sup>2</sup>, J.L. Luque<sup>1</sup>

Departamento de Parasitologia Animal, Universidade Federal Rural do Rio de Janeiro, Seropédica, BR 465 Km 7, Rio de Janeiro, Brazil<sup>1</sup>; Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic<sup>2</sup>

Diversity of fresh-, brackish water and marine fish in Brazil is the highest on the planet, with approximately 4300 species recognized. Parasitic roundworms (Nematoda) form an integral component of communities of helminths parasitizing Brazilian teleosts. The first report on nematodes parasitic in marine fish from Brazil represents the description of *Trichocephalus gibbosus* (now *Onchophora melanopechala*) by Rudolphi in 1819 based on specimens collected from *Thunnus thynnus* (Linnaeus) off the coast of Brazil by Pohl in 1817. The first nematodes reported from freshwater fish in Brazil were *Lecanocephalus spinulosus* (now *Goexia spinulosa*) and *Cheiracanthus gracilis* (now *Gnathostoma gracile*), both described by Diesing in 1839 from *Arapaima gigas* (Schinz). However, the first comprehensive study on fish nematodes represented an account by Travassos et al. (1928) who listed several nematodes from freshwater fish in Brazil. Subsequently, a large number of taxonomic papers with descriptions of new species and genera of fish nematodes have been published, especially in recent years. At present, 142 nominal species of adult and larval nematodes (plus 75 unidentified taxa) in 74 genera have been found in more than 300 species of marine, brackish and freshwater fish. Despite a considerable progress in mapping diversity of fish nematodes in Brazil, the current knowledge of their species composition, spectrum of fish hosts, distribution and
life-cycles is rather limited. In fact, parasitic nematodes have been reported from as few as 7.6% of fish species occurring in Brazil. As a result, our current knowledge of nematodes parasitizing fish in Brazil is not sufficient to answer some basic questions concerning their biodiversity, biology, ecology and phylogenetic relationships. However, some general patterns regarding the distribution of nematodes in Brazilian fishes can be recognized. For example, the number of nematode species parasitizing freshwater fishes is higher than that of marine and brackish fish species, with the highest species richness in perciform and siluriform teleosts. The most frequent, widely distributed group of fish nematodes represent ascaridoids, with about one half of all published records. Common occurrence of anisakid nematodes in a number of marine fish may be of public health concern and may have negative effect on fish marketing. Since only a small portion of the potential fish hosts of anisakids and other potential causative agents of zoonotic, fish-borne human diseases have been examined, further studies are needed to evaluate the impact of these fish parasites on human health, aquaculture, fisheries and ecosystems in Brazil.

F.M.V. was supported by a Postdoctoral fellowship from FAPERJ/CAPES (Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro/Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil). J.L.L. was supported by a Research fellowship from CNPq (Conselho Nacional de Pesquisa e Desenvolvimento Tecnologico, Brazil). Stay of T. S. in Brazil as “CAPES/BRASIL Grantee” at the Universidade Federal Rural de Rio de Janeiro, Seropédica (laboratory of José Luque) was supported by the Ministério da Educação, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

DETECTION OF HEXAMERMIS ALBICANS NEMATODE IN SUCCINEA PUTRIS AMBERSNAIL
IN HUNGARY
A. Juhasz

Department. of Parasitology and Zoology, Faculty of Veterinary Sciences, Szent István University, István u. 2, Budapest 1078, Hungary

Parasites of snails are unique organisms that develop only in them and do not infect other hosts. Mermithid nematodes are a special group of the so-called parasitoid organisms, which kill their hosts during their development. These parasites have not been investigated in Hungary so far. The research of these aquatic and terrestrial worms is extremely difficult because the adult specimens can only be found in soil, where they lay their eggs as well. Arthropods, snails and lumbricid worms take larvae of mermithids emerged from eggs and the parasitic larvae grow to adult stage inside them.

We detected the worm Hexamermis albicans that parasitizes Succinea putris land snails in the swampy area of Gemenc forestry in Hungary. With external inspection the infection of snails cannot be recognized in the affected snails. Specimens of snails were collected after a heavy summer rain, we preserved them in alcohol, and dissected their mantle cavity for the worms.

The prevalence and intensity of parasites were detected. The identification of the worms was performed by morphological analysis of preadult stages using identification keys. Microscopic slide preparations were prepared suitable for morphological inspection and subsequent molecular analysis of worms which was removed from the bodies of hosts. Detection of the adult forms and eggs of H. albicans in soil has not been successful so far.
Sequence of 18S rDNS region of the worm showed low similarity with the DNA sequences of other Mermithid species available in GenBank. This proves the unique position of *H. albicans* among other Mermithid worms which develop in arthropods.

Remarkable feature of *H. albicans* is that unlike other parasites as trematodes, this worm does not damage the reproductive system of snails during its development. Thus, eggs can be formed by the host snail, despite of the fact that the parasite grows in it.

**SURVEYS ON THE PARASITE FAUNA OF WILD VERTEBRATES IN SOUTHERN QUINTANA ROO, MEXICO**

D. González-Solís

El Colegio de la Frontera Sur, unidad Chetumal. Av. Centenario km. 5.5, Chetumal, Quintana Roo 77014, México

Mexico is considered one of the most megadiverse countries around the world. The richness and diversity of flora and fauna is spectacular, but many of these organisms are considered as threatened or endangered and should be protected. During the implementation of conservation programs of charismatic species in the southern region of Quintana Roo, samples (fecal and intestines) of different vertebrates were collected. Coproparasitoscopic and helminthological examinations were applied to six stranded manatees (*Trichechus manatus manatus*), 79 feces of wild jaguar (*Panthera onca*) and puma (*Puma concolor*), 26 cocodriles (*C. acutus*), 14 boas (*Boa constrictor*), and one intestine and 19 feces of the Central American river turtle (*Dermatemys mawii*). Results showed the presence of three parasite species in *T. m. manatus*, (*Chiorichis groschafti*, *Heterocheilus tunicatus*, *Pulmonicola cochleotrema*), 10 in the two felids (*Alaria marcionae*, *Ancylostoma sp.*), *Capillaria sp.*, *Dermacenter sp.*, *Isospora felis*, *Tenaia taeniformis*, *Toxocara cati*, *Toxascaris leonina*, *Trichurus sp.*, *Uncinaria sp.*), seven in *C. acutus* (*Contraecum sp.* type 1, *Contraecum sp.* type 2, *Dujardinascaris helicina*, *Glossocercus sp.*, *Gorgorhynchoides sp.*, *Pseudoterranova sp.*, *Terranova crocodili*), five in *B. constrictor* (*Contraecum sp.* type 2, *Cruzia sp.*, *Kalicephalus subulatus*, *Physocyphalus sp.*, *Staphylodora horridum*), and 11 in *D. mawii* (*Amphibiocapillaria serpentina*, *Caballerdiscus tabascensis*, *C. resupinatus*, *Choanophorus rovirosai*, *Dermatemytrema trifoliatum*, *Octangioides tlcatalpensis*, *Parachiorchis parvicaecabulatus*, *Placobdella ringueleti*, *Pseudocleptodiscus margaritae*, *Siroxyx contortus*, *Telorchis sp.*). Parasite fauna of the aforementioned vertebrates are relatively poor in the specific richness, but abundant in the number of individuals. The parasitological surveys of most of these species represent the first one in the region.

**NEW CIRCUMSCRIPTION OF MONOBOTHRIUM NYBELIN, 1922 AND PROMONOBOTHRIUM MACKIEWICZ, 1968 (CESTODA: CARYOPHYLLIDEA), PARASITES OF FRESHWATER FISHES IN THE Holarctic Region**

M. Oros1,2, T. Scholz1, J. Brabec4, A. Choudhury5, A. Waeschenbach4

Institute of Parasitology, Biology Centre of the Academy of Science of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic1; Institute of Parasitology, Slovak Academy of Sciences, Hlinkova 3, 04001 Košice, Slovakia5; Division of Natural Sciences, St.
Species composition and delimitation of two genera of caryophyllidean cestodes, *Monobothrium* Diesing, 1863 and *Promonobothrium* Mackiewicz, 1968, parasites of cypriniform fishes (cyprinids and suckers) in the Holarctic Region, are revised on the basis of molecular phylogenetic studies and morphological characterization. Species of the former genus, which originally included morphologically distinct parasites of cyprinids (Cyprinidae) in Europe and suckers (Catostomidae) in North America, are split into 3 different genera. *Monobothrium* becomes monotypic, with *M. wageneri* Nybelin, 1922 from tench, *Tinca tinca*, in Europe representing its type and only species. Another European species, *M. auriculatum* Kulakovskaya, 1961, a parasite of *Leuciscus danilevskii* (Cyprinidae) in Ukraine, is transferred to *Caryophyllaeus* Gmelin, 1790 as *C. auriculatus* (Kulakovskaya, 1961) n. comb. Five remaining species parasitizing suckers in North America, which were formerly placed in *Monobothrium*, are transferred to *Promonobothrium* based on their close morphological resemblance with the type- and previously the only species of *Promonobothrium*, *P. minytremi* Mackiewicz, 1968, and molecular data. Multilocus phylogenetic analysis of nuclear ribosomal genes (18S and 28S rDNA) and mitochondrial genes *rrnL* and *cox1* placed *P. minytremi* within a clade composed of 4 Nearctic species, previous members of *Monobothrium*. The species of *Promonobothrium* differ from *M. wageneri* in the possession of an external seminal vesicle (absent in the latter species), the absence of postvariant vitelline follicles in North American species (present in *M. wageneri*), and a different scolex morphology, which is monobothriate, loculomonobothriate or loculotrunicate, i.e., equipped with loculi and an terminal introvert (*versus* claviform, bluntly ended, with 6 weak, shallow longitudinal grooves in *M. wageneri*).

*This study was supported by the Czech Science Foundation (project No. P505/12/G112), Institute of Parasitology (RVO: 60077344), Slovak Research and Development Agency (project no. APVV-0653-11), and Grant Agency VEGA (No. 2/0129/12).*

---

**EVERYTHING IS NEW: HOST, PARASITE AND INSIGHT INTO TAXONOMIC DIVISION OF DIPLOZOIDAE**

K. Civáňová¹, B. Koubková¹, M. Koyun²

Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic¹; Department of Biology, Science and Art Faculty, Bingöl University, 12000 Bingöl, Turkey²

Monogeneans belonging to the Diplozoidae are common parasites on the gills of cyprinid fish but host specificity of diplozoids differs and relates to the geographical origin of the host. The size and shape of sclerotized parts of attachment apparatus of adults (four pairs of clamps and a pair of small central hooks) are commonly used for the species identification of diplozoids. The length of the central hook sickle and the shape of the anterior end of median plate and anterior joining sclerites of the clamps were confirmed to be the most significant features for species discrimination.

In Turkey, only four diplozoid species have been recorded up to date mostly on cyprinid hosts genera. In our study, morphological approach in combination with recent molecular
analyses and made a new parasite species description (*Paradiplazoon bingolensis* sp. n.) in new cyprinid host species (*Garra rufa*, Heckel 1843).

Samples of fish were collected from the Göynük Stream, a tributary of the Murat River, Turkey. Adult parasites of the new species were found on the gill apparatus of garra during each particular fishing-out. Parasites were fixed and stored in absolute ethanol at 4 °C. For morphometric analysis, one opisthaptor of each specimen was cut off and soaked in 10 % sodium dodecyl sulphate, washed in distilled water and mounted on a microscope slide and fixed with a mixture of ammonium picrate and glycerin. A light microscope equipped with differential interference contrast, a digital image analysis system (AnalySIS Five Auto) and drawing attachment were used. Genomic DNA was extracted, the second internal transcribed spacer (ITS2) rDNA, a meaningful genetic marker for species identification and analysis of parasite population variation, was amplified and sequenced (ABI 3130 Genetic Analyzer). The evolutionary distances between relative diplozoid species (previously and newly described) were computed, phylogeny reconstruction was performed and evolutionary history was inferred by neighbor joining, maximum parsimony and maximum likelihood statistical methods.

To date, no species of diplozoid has been identified in *G. rufa*. *P. bingolensis* is distinguished from the other valid species in the genus by the size of the central hook sickle, morphology of the clamp sclerites, especially by the shape (form) of the connection of the anterior jaws to the median plate. Important distinguishing feature is that the posterior jaw in this species is not divided on medial and lateral sclerites as in the other genus representatives. Even molecular characterization based on variability of ITS2 of the ribosomal DNA region provided additional support of separation of this new species from the valid one and demonstrated the uniqueness of this new parasite species. However, phylogenetic comparison of recently described diplozoids revealed uncertainties in the current taxonomic division of the Diplozoidae family, confirmed vague description of some (Chinese) species and suggest the reclassification of this parasite group.

*The research was supported by Czech Science Foundation project no. P506/12/1258. The results presentation supported Czech Science Foundation by project no. P505/12/G112.*

**THE SYSTEMATIC POSITION OF CORTREMA MAGNICAUDATA (PLATYHELMINTHES, DIGENEA, CORTREMATIDAE) WITH NOTES ON MOLECULAR PHYLOGENY**

G. Zaleśny1, G. Kanarek2, J. Sitko3, V.V. Tkach4

Institute of Biology, Department of Invertebrate Systematics and Ecology, Wrocław University of Environmental and Life Sciences, Kożuchowska 5b, 51 631 Wrocław, Poland1; Ornithological Station, Museum and Institute of Zoology Polish Academy of Sciences, Nadwiślańska 108, 80 680 Gdaňsk, Poland2; Moravian Ornithological Station, Comenius Museum, Horní náměstí 1, 751 52 Přerov, Czech Republic3; Department of Biology, University of North Dakota, 1 Campus Drive and Cornell Street, Grand Forks, ND 58202, USA4

In present work, we evaluate the phylogenetic relationships of the family Cortrematidae using newly obtained sequences of nuclear large ribosomal subunit DNA sequence data of *Cortrema magnicaudata*, collected from Barn Swallow, *Hirundo rustica* in Czech Republic. The phylogenetic position of *Cortrema* is discussed, and some modifications to the classification of the Microphalloidea
and some of its constituent genera and families are proposed. The systematic and taxonomic history of *Cortrema* is extremely complex. Different authors included it in the Troglotrematidae, Lecithodendriidae or its own family within subfamilies Troglotrematoidea, Lecithodendrioida or Gorgoderoida. Our study has revealed the phylogenetic affinities and allowed to define the correct systematic position of *Cortrema*. The phylogenetic analysis has clearly demonstrated that this genus belongs to the family Pleurogenidæ within the superfamily Microphalloidea. The results are partly in agreement with opinion of other authors who supported establishing of the Cortrematidae as an independent family based on adult morphology, but stated that the presence of non-virgulate xiphidiocercarie in life-cycle of *C. corti* along with the use of pulmonate snails (*Lymnea* (*Fossaria*) *ollula*, *Succinea chinensis*) as intermediate hosts indicated a lack of close phylogenetic relationships with the Lecithodendriidae.

**GERMINATIVE STEM CELL ULTRASTRUCTURE IN CESTODE HEXACANTH LARVAE**

D.B. Conn¹, Z. Świderski²

Department of Invertebrate Zoology, Museum of Comparative Zoology, Harvard University, Cambridge, MA 02138, USA¹; Department of Biology and One Health Center, Berry College, Mount Berry, GA, 30149, USA¹; 3W. Stefański Institute of Parasitology, Polish Academy of Sciences, 00-818 Warsaw, Poland²; Department of General Biology and Parasitology, Medical University of Warsaw, Chalubińskiego Street, Warsaw, Poland²

Understanding the biology of pluripotent stem cells is among the most important quests in modern developmental biology. Stem cells maintain developmental plasticity in various stages of an organism’s ontogeny. Depending on the species, maintenance of stem cells may give that organism the capacity to perform such critical functions as replacing defective cells, undergoing differentiation of successive stages of histogenesis and organogenesis, arresting development of a cell pool for metamorphosis into sequential life stages, or maintaining the capacity for asexual reproduction. Among cestodes, stem cells exist in four primary situations: 1) germinative stem cells of all pre-metamorphic hexacanth larvae; 2) germinative stem cells of all post-metamorphic juveniles (metacestodes); 3) progenitor stem cells responsible for asexual proliferation in post-metamorphic metacestodes such as hydatid cysts; 4) proglottid‐forming germinative stem cells that reside in the neck region, ultimately differentiating into the sexual organs of the adult strobilat stage of polyzoic eucestodes.

To establish baseline data for understanding cestode stem cells, we examined the ultrastructure of germinative stem cells in the hexacanth larvae of several cestode species representing diverse taxa: *Echinococcus granulosus* and *Echinococcus multilocularis* (Cyclophyllidea, Taeniidae); *Catenotaenia pusilla* (Cyclophyllidea, Catenotaeniidae); *Anoplocephaloides dentata* and *Inermicapsifer madagascariensis* (Cyclophyllidea, Ano-plocephalidae); and *Bothriocephalus clavibothrium* (Bothriocephalidae, Bothriocephalidae). Stem cells of all of these species are grouped together at the posterior poles of the hexacanth. All other hexacanth cells and structures serve only as adaptations for aiding the transmission of the germinative stem cell pool to the intermediate host, after which they rapidly degenerate. The number of hexacanth stem cells varies with species, ranging from two in *C. pusilla* to 12 in *B. clavibothrium*. These cells play an important role in cestode ontogenesis, as through metamorphosis they are involved in the growth and differentiation of the...
cercoid, the next stage of the life cycle. These large stem cells are approximately 7-8 µm in diameter and frequently lobate in shape. They are characterised by a large, lobed nucleus, measuring approximately 4-5 µm in diameter, with a prominent, electron-dense nucleolus and numerous heterochromatin islands distributed peripherally in the karyoplasm, adjacent to the nuclear membrane. They possess a thin layer of granular cytoplasm that is densely supplied with free ribosomes, numerous small mitochondria, and a few short profiles of granular endoplasmic reticulum (GER). These characteristics of the nuclear and cytoplasmic ultrastructure of the stem cells of all the hexacanths we examined clearly indicate their developmental potential for rapid growth and the multiplication during ontogenesis of the cercoid stages, up to and including the establishment of the fully formed metacestode, and incorporating both metacestode somatic cells as well as the next generation of stem cells, i.e., the germinative stem cell pool reserved for adult reproductive system development and the proliferative pool reserved for asexual reproduction in those taxa (e.g., *Echinococcus*) that maintain that reproductive strategy.

### COMPARATIVE ULTRASTRUCTURE OF THE INTRAUTERINE EGGS OF FOUR EUROPEAN TREMATODES

Z. Świderski¹, D.B. Conn²

1 W. Stefánski Institute of Parasitology, Polish Academy of Sciences, 00-818 Warsaw, Poland; 2 Department of General Biology and Parasitology, Medical University of Warsaw, Chałubińskiego Street, Warsaw, Poland; Department of Biology and One Health Center, Berry College, Mount Berry, GA, 30149, USA; Department of Invertebrate Zoology, Museum of Comparative Zoology, Harvard University, Cambridge, MA 02138, USA

Descriptions have been reported on the eggs of several individual trematode species examined ultrastructurally. Herein, we synthesize our previously published descriptions, supplemented by new previously unpublished data, to present a comparative analysis of the intrauterine eggs of four digenean species, representing four genera and three families of the superfamilly Microphalloidea, all native parasites of wildlife in Europe. The trematodes were collected from four different host species in four European countries.

Trematodes examined and analysed here included: 1) *Mediogonimus jourdanei* (Prosthogonimidae) from voles, *Myodes glareolus* (Mammalia: Rodentia), collected in France; 2) *Maritrema felii* (Microphallidae) from shrews, *Crocidura russula* (Mammalia: Soricimorpha), collected in Spain; 3) *Bradesia turgida* (Pleurogenidae) from frogs, *Pelophylax ridibundus* (Amphibia: Anura: Ranidae), collected in Russia; and 4) *Prostococcus confusus* (Pleurogenidae), from frogs, *Rana lessonae* (Amphibia: Anura: Ranidae), collected in Belarus. All were studied by preparing whole worms by standard techniques for transmission electron microscopy (TEM), so that eggs could be studied in situ within the uterus of the parent worm.

The eggs of all four species followed general aspects of the pattern that we and others have described previously for all trematodes and cestodes; however, there are some variations among species in details as well as in some more generalized features described here. The early embryo (*M. felii* and *P. confusus*) or the fully formed miracidium (*M. jourdanei* and *B. turgida*), is surrounded by three envelopes: 1) an inner embryonic envelope formed from mesomeres; 2) an outer embryonic envelope formed from macromeres and vitelloidocyte remnants; 3) an eggshell formed from secreted
components of vitellocytes and Mehlis’ gland. In addition, the eggs of *B. turgida* and *P. confusus* were enclosed by a thick layer external to the eggshell, and consisting of electron-lucent material proximally and electron-dense islands distally, attached to a bounding membrane. This unique layer, which we designate as a “cocoon”, is of undetermined function, though we suggest that it is protective. It has not been described in any other trematode. The macromeres forming the outer envelope deteriorate very quickly in *M. jordanei*, *B. turgida*, and *P. confusus*, but persist for longer in *M. feliui*, in which they undergo migration to the poles of the egg prior to forming a syncytium. In all species, the embryo proper and ultimately the miracidium forms from micromeres. Some of these undergo apoptosis during early embryogenesis, but others persist through embryonic development, and ultimately their derivative cells form the completed miracidium larva.

Our data demonstrate complete development of miracidia larvae within the intrauterine eggs of both *M. jordanei* and *B. turgida*, thus constituting late-stage ovoviviparity in these two species, but not attaining true viviparity; in contrast, *M. feliui* and *P. confusus* eggs contain only early embryos, thus constituting very early-stage ovoviparity. Further studies are needed to explore egg ultrastructure in other digenean taxa, and to correlate structure with function in the life cycle.

**PLENARY SESSION V**

**ECOLOGICAL CORRELATES OF LOUSE DIVERSITY: A REVIEW**

L. Rózsa

MTA-ELTE-MTM Ecology Research Group, Budapest; Dept. of Evolutionary Zoology and Human Biology, University of Debrecen, Hungary

This research was supported by the EU and Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0001 ‘National Excellence’ Program

Identifying environmental factors that influence the taxonomic diversity of parasite communities is a major challenge of ecological parasitology. Accordingly, several attempts were made to find environmental correlates (host characters) that can explain louse diversity. Early efforts were totally fruitless for several reasons. First, louse richness of host species (or higher taxa) is partly an inherited character that has a phylogenetic inertia, thus we need to control for host phylogenetic effects statistically. Second, species richness is a poor measure of louse diversity because of the uncertainty of species concepts. Third, the two major clades of lice (Amblyceridae and Ischnocera) often exhibits quite different responses to similar environmental effects. Finally, known values of louse taxonomic richness are strongly biased by differential sampling intensity.

After applying statistical controls for host phylogenies and differential study intensity, switching from species richness to the usage of genera richness, and analysing the major louse clades separately, the following host characters emerged as major determinants of louse taxonomic richness.

1. Host diving behaviour (obtaining food from under the water surface) significantly reduces the richness of louse assemblages as compared to terrestrial sister-clades;
2. Host clades’ evolutionary switch to a brood-parasitic way of life (like cuckoos) reduces louse richness probably due to the loss of the vertical way of transmission.
3. Within cuckoo lice, cuckoo species with more foster parent species tend to harbour more diverse Ischnoceran lice assemblages than more foster-specialist cuckoos. Cuckoo body size also had a positive effect in their Ischnoceran richness.

4. The intensity of host defence capabilities increase Amblyceran richness (but not Ischnoceran richness) in birds. This was true for 3 different measures, the time allocated for grooming, the intensity of nestling T-cell immune responses and also for the uropygial gland size, all characters presumed to be related to antiparasitic defences. Thus stronger antiparasitic defences appear to enhance Amblyceran diversity.

5. An analysis of lice harboired by the global seabird fauna indicates that host geographic area size and population size co-varies positively with both Amblyceran and Ischnoceran species richness, while host body size appeared to have a negative effect.

6. An analysis of the louse genera diversity of avian families showed that avian clades exhibiting higher cognitive capabilities also harbour more diverse Amblyceran faunas.

7. Finally, a recent study indicates that Eichler’s ‘rule’ formulates the most powerful determinant of louse richness. As predicted by this ‘rule’, more diverse host taxa tend to harbour more diverse parasite taxa.

The present view of the ecological factors shaping louse diversity is rather crude, however. The first problem is that statistical controls for sampling biases are rather ineffective and often doubtful. Another major problem is that we cannot unite all these host characters into a single statistical model due to limitations in data availability.

**SESSION VI**

**Vectors and vector-borne diseases**

**IS THE HOUSE MOSQUITO (CULEX PIPIENS L.) THE MOST ABUNDANT CULEX SPECIES IN THE CZECH REPUBLIC?**

F. Rettich

National Reference Laboratory for Disinfection and Vector Control, National Institute of Public Health (NIPH), Prague, Czech Republic

In the Czech Republic, five species of the genus Culex (if Cx. molestus is accepted as a “good” species) occur regularly. Mostly synanthropic Culex pipiens sensu stricto (Culex pipiens pipiens) and fully synanthropic Culex molestus (Culex pipiens molestus) are often considered as two bioforms of one species, Culex pipiens. Cx. molestus is anthropophilic (and autogenous). Its larvae breed underground, in flooded cellars or septic tanks. Culex pipiens, in Central Europe, in latitudes above the 48th parallel, is mostly ornithophilic and feeds on humans quite rarely at the end of the mosquito season (August-October) inside houses and only at altitudes up to 250 m a.s.l. Cx. pipiens used to be considered the most prevalent Culex mosquito. Unlike Culex pipiens, Culex torrentium was described as a rare and more or less mountain species. (Kramář, 1958). To identify and differentiate the two species, Cx.pipiens and Cx. torrentium, using larval or female morphological features is extremely difficult and not reliable. The key identification clue is the male hypopygia mounted on microscope slides.
From 2007 till 2013, we collected Culex pipiens/torrentium larvae or pupae in the field, from various localities and biotops and reared them to the adult stage. To establish the Culex pipiens/torrentium ratio, we used only males. Of a total of 2,395 identified specimens, 68.3% were assigned to Cx. torrentium and 31.7% only to Cx. pipiens. Cx. pipiens larvae prevailed in breeding places with polluted water e.g. flooded meadows with decaying hay. Cx. torrentium larvae preferred cleaner water, e.g. in rainwater drums or clean forest and meadow pools. Finding of Cx. torrentium prevalence may have important consequences for the interpretation of results of the studies on the major enzootic and link-vectors of mosquito-borne bird-associated viruses (West Nile and Usutu viruses). Larvae of two more species of the genus Culex, Cx. territans and Cx. modestus, often breed on the edges of fish ponds. Cx. modestus is quite abundant in southern Moravia and southern Bohemia (Votýpka et al., 2008, Šebesta, 2012). Cx. modestus bites only people in reed zones. Cx. territans feeds exclusively on amphibian blood. Two females of the sixth Culex species, Culex martini, have been collected using CO2 light traps (Šebesta, 2012).

*Part of this study has been published in J.C. Hensson, F.Rettich, J. Lundstrom et al., 2013. The arborvirus vector Culex torrentium is more prevalent than Culex pipiens in northern and central Europe. Medical and Vet. Entomol. doi: 10.111/mve.12024)*

**THE VECTOR COMPETENCE OF PHLEBOTOMUS ORIENTALIS FOR LEISHMANIA DONOVANI**

J. Votýpka¹, V. Seblova¹, M. Vlkova¹, J. Valenzuela², R. Jochim², A. Hailu³, A. Warburg⁴, P. Volt⁴

Charles University, Faculty of Science, Dpt. Parasitology, Vinicna 7, Prague, Czech Republic¹; Vector Molecular Biology Section, NIAID/NIH, Rockville, MD, USA²; Department of Microbiology, Immunology & Parasitology; School of Medicine, Addis Ababa University, Ethiopia³; Department of Microbiology and Molecular Genetics, The Kuvin Centre for the Study of Infectious and Tropical Diseases, Hadassah Medical School, The Hebrew University of Jerusalem, Israel⁴

*Phlebotomus orientalis* is the main vector of *Leishmania donovani* in eastern Africa and thus a sand fly species of high importance. We studied various properties of *P. orientalis* populations from both endemic (Addis Zemen) a non-endemic (Melka Werer) areas in Ethiopia with respect to *L. donovani* transmission. We successfully demonstrated the ability of laboratory colonies arising from these populations to crossbreed by obtaining F1 and F2 progeny with hybrids similar or even higher fecundity than parental colonies. Comparison of the populations by sequencing of two genes (cytB and COI) and by RAPD (a multilocus method) revealed no genetic differences. We demonstrated that both populations are highly susceptible to experimental infections by *L. donovani* and even a small number of ingested parasites results in heavy infections in *P. orientalis* females. In addition, we characterized the transcriptomes, proteomes and the main salivary antigens in both colonies. We compared the enzymatic activities of important salivary enzymes, namely apyrase and hyaluronidase, and also tested the cross-reactivity of salivary antigens using sera of repeatedly bitten mice. In both *P. orientalis* colonies we found transcripts belonging to 13 main protein families; based on the phylogenetic analysis we showed that *P. orientalis* is closely related to *P. tobbi* and *P. perniciosus*. Similar enzyme activity levels and high cross-reactivity of salivary antigens were found in both
colonies. Overall, the composition of salivary proteins was almost identical, regardless of whether the colonies originate from the focus of visceral leishmaniasis or from a non-endemic area. These findings, together with the identical developmental pattern of *L. donovani* in both *P. orientalis* colonies, suggest that the absence of visceral leishmaniasis in the non-endemic area of Melka Werer cannot be attributed to reduced vectorial competence of local *P. orientalis* populations to *L. donovani*.

**PREVALENCE OF TICK-BORNE PATHOGENS IN *DERMACENTOR RETICULATUS* TICKS IN LITHUANIA AND IN CENTRAL EUROPE**

A. Paulauskas¹, J.Radzijevskaja¹, A. Banaityte¹, M. Stanko², G. Karbowiak³, B. Petko²

Vytatás Magnus University, Lithuania¹; Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovak Republic²; W. Stefanski Institute of Parasitology, Polish Academy of Sciences, Poland³

The meadow tick *Dermacentor reticulatus* is recognized as significant tick-borne disease vector in Europe. However, information on prevalence of tick-borne pathogens is scarce, especially in Lithuania, where the large-scale investigation of tick-borne pathogen in *D. reticulatus* tick never has been done before. To determine whether *D. reticulatus* ticks in Lithuania are infected by *Babesia* spp., *Rickettsia* spp., *Anaplasma phagocytophilum*, and *Borrelia burgdorferi* s.l., we collected questing ticks in 32 locations during April – May of 2013, and examined them for the presence of DNA of these pathogens. A total of 1218 unfed *D. reticulatus* ticks were collected, and a representative number of samples were investigated for prevalence of DNA of *Babesia* spp. (n=1057), *Rickettsia* spp. (n=1062), *Borrelia burgdorferi* s.l. (n=875), *Anaplasma phagocytophilum* (n=571). Standard PCR, nested PCR and Real-Time PCR analyses and pathogen-specific primers were used to estimate the prevalence of infection. The 18S rRNA gene of *Babesia* spp., the *gltA* and *ompA* genes of *Rickettsia* spp., the *ospA* gene of *B. burgdorferi* s.l., and the *msp2* gene of *A. phagocytophilum* were used as targets in PCR detection of pathogens. Two pathogens, *Babesia canis* and *Rickettsia* spp. were detected in present study. The overall prevalence of *B. canis* in ticks was 0.95%, and ranged from 0 to 6.7% in different locations. Sequencing of PCR products led to identify *B. canis canis* subspecies. DNA of *Rickettsia* spp. was detected in 5.9 % (n=63) of tested ticks. The prevalence of *Rickettsia* pathogens ranged from 0% to 36.9 % in different locations. Twenty *Rickettsia* spp. positive samples were sequenced and *Rickettsia raoultii* was identified in all samples. *A. phagocytophilum* and *B. burgdorferi* s.l. were not detected in this study. However, in our previous investigations, *A. phagocytophilum* was found in *D. reticulatus* in two locations of Lithuania (not included in this study) with overall prevalence 8% (7/87). *B. burgdorferi* s.l. also was previously detected in *D. reticulatus* ticks engorged on badger.

We investigated the prevalence of *Babesia* spp. and *Rickettsia* spp. in *D. reticulatus* ticks collected during 2012-2013 in 2 locations of Slovakia (n=53), and 7 locations of Poland (n=194). In Slovakia prevalence of *Rickettsia* spp. ranged from 0% to 16.7% in investigated localities. In Poland, *Babesia canis* and *Rickettsia* spp. were detected with prevalence range in different locations from 0% to 2.7% and from 0% to 59.6%, respectively.

*The study was partially supported by the Research Council of Lithuania (grant no. MIP-053/2013), and by the projects of Slovak Research and Development Agency APVV 0267-10 and VEGA 1/0390/12.*
BORRELIOSIS IN PRAGUE: PREVALENCE IN TICKS, BLOOD DONORS AND PATIENTS OF THE NATIONAL REFERENCE LABORATORY AS COMPARED WITH REPORTED INCIDENCE DATA

P. Kodym¹, J. Hořejší², Z. Kurzová³, P. Balátová¹, D. Berenová¹, J. Kracíková², Z. Jágrová³, M. Malý⁴

National Reference Laboratory for Lyme Borreliosis, National Institute of Public Health (NIPH), Prague, Czech Republic¹; Blood Transfusion Centre, Royal Vinohrady Teaching Hospital, Prague, Czech Republic²; Regional Public Health Authority of Prague³; Department of Biostatistics and Informatics, NIPH, Prague, Czech Republic⁴

According to data collected by the EPIDAT information system of infectious diseases, the Prague region has the lowest incidence of borreliosis in the Czech Republic, fluctuating between 9.3 - 16.7 cases per 100 000 population per year in 2005-2013, while yearly means for the Czech Republic ranged between 31.4 - 46.1 cases per 100 000 population in the same period. In order to further characterise the epidemiological situation in Prague, the authors make use of additional data sources. Important parameters such as the prevalence of spirochetes in ticks and the difference in seroprevalence in the healthy population and in patients with suspected Borrelia infection are summarized and discussed.

Ixodes ricinus ticks were collected by Prague Regional Public Health Authority personnel at 8 localities on the city periphery during 2005-2013 and examined with dark field microscopy. Plasma and sera samples of 100 blood donors and of 100 patients sent to the National Reference Laboratory for Lyme Borreliosis for examination (mostly under diagnosis “A69.2”), all collected on the cusp of of February/March 2013 before the start of the tick activity period, were assayed for anti-Borrelia IgM and IgG with ELISA test (Vidia, Prague, Czech Republic).

Spirochetes were detected in 10% of a total 4184 ticks examined, specifically in 7.8% of nymphs, 11.8% of males, and in 13.6% of females. Prevalence differed significantly in individual Prague localities; the lowest continued to be in Točná (mean: 6.5 %), the highest in Klánovice forest (15.9%). Mean prevalence fluctuated year by year between 8.3% (2007) and 20.7% (2011). Surprisingly, these fluctuations correlated with reported yearly incidence rates of human Lyme borreliosis in Prague. Serological examination revealed 71% seronegativity in blood donors. Anti-Borrelia IgM antibodies alone (equivocal or positive values) were detected in 7%, IgG in 17%, and both IgG and IgM in 5% of subjects. In contrast, the patients with suspected borreliosis showed 60% seronegativity with IgM only present in 13%, IgG in 16% and IgG + IgM in 11%.

From these preliminary results we can conclude that spirochetes are relatively prevalent in ticks from some localities in Prague. This is probably the reason why 29% of samples from absolutely healthy blood donors from the region of the lowest incidence of borreliosis, which were collected at least 4 months after the end of the autumn tick activity peak, contained some anti-Borrelia antibodies. Asymptomatic borreliosis thus seems to be very common in the Czech population. An unimpressive difference from seroprevalence in patients whose indication for testing should be based on symptoms or anamnesis shows that unsatisfactory clinical selection and unjustified diagnosis of “borreliosis” is relatively common in the Czech Republic.

This study was supported by the Grant Agency of the Ministry of Health of the Czech Republic (IGA MZ ČR – No. NT/13467 – 4), and by institutional funds of the National Institute of Public Health (č.1 RVO-SZÚ/2014).
OCCURRENCE OF ANAPLASMA PHAGOCYTOPHILUM AND BORRELLIA BURGDORFERI S.L. IN WILD RODENT POPULATIONS – ECOLOGICAL APPROACH

J. Hildebrand, E. Gajda, K. Buňkowska-Gawlik, A. Perek-Matysiak
Department of Parasitology, University of Wroclaw, Przybyszewskiego 63, 51-148 Wroclaw, Poland

Anaplasma phagocytophilum and Borrelia burgdorferi s.l. are agents of vector-borne diseases transmitted by Ixodes ricinus mainly. The role of rodents in epidemiology of Lyme borreliosis (LB) is known and confirmed while human granulocytic anaplasmosis (HGA) is still discussed. Borrelia afzelii, strongly associated with small mammals, is one of the major human pathogenic species and causes specific clinical symptoms in humans, e.g. erythema migrans and neuroborreliosis. According to literature data mice and voles are reported as reservoir hosts for Anaplasma phagocytophilum however some molecular research not assign to them significant role in HGA (Bown et al. 2006, 2009; Loewenich et al. 2014).

Aim/hypothesis: Are there any ecological differentiating factors involved in the pattern of occurrence of both pathogens in rodent populations?

Rodents (n=314) represented by Apodemus agrarius, A. flavicollis and Myodes glareolus, were captured in live traps in four localities of south-western Poland (2011-2012). For the analysis of pathogens, Borrelia burgdorferi s.l. and Anaplasma phagocytophilum, spleen samples were obtained from rodents. The choice of genetic markers (flaB and msp2) and primers was based on the literature data and our preliminary results. Conventional PCR was used for the detection of DNA of examined pathogens. Selected PCR positive products were purified and sequenced. BLAST searches were conducted in order to elucidate any homologies with previously deposited sequences in GenBank. During our research we were able to detect varied species and genotypes of B. burgdorferi s.l. and A. phagocytophilum, including those with zoonotic potential.

In examined rodent populations the prevalence of B. burgdorferi s.l. and A. phagocytophilum ranged from 7 to 22 % and from 14 to 17 % respectively. Ecological analysis between infection level with regard species of host, season, study sites and additionally co-occurrence of pathogens were performed.

MOLECULAR EVIDENCE AND THE SEASONAL DYNAMICS OF TICK-BORNE PATHOGENS IN QUESTING TICKS FROM THE EASTERN SLOVAK KARST REGION, CENTRAL EUROPE

B. Vichová¹, L. Blaňárová¹, M. Bona⁴, M. Stanko¹,², L. Mošanský³, J. Kraljik¹,³, B. Peňko¹
Institute of Parasitology SAS, Hlinkova 3, 040 01 Košice, SR¹; Institute of Zoology SAS, Löfflerova 10, 040 01 Košice, SR²; Department of Zoology, Faculty of Natural Sciences, Comenius University, Mlynská dolina B-1, 842 15 Bratislava, SR³; Department of Anatomy, Faculty of Medicine, UPJŠ, Košice, SR⁴

The aim of this study was to investigate the prevalence of infection and the seasonal dynamics of Borrelia spp., Babesia spp. and Anaplasma phagocytophilum in questing ticks (Ixodes spp., Dermacentor spp., Haemaphysalis spp.) collected in the Slovak Karst National Park (48°34.899 N, 20° 46.743 E) in eastern Slovakia. This area is characterized by the presence of several endemic plant and animal species, and also by the co-occurrence of at least 5 tick species.
Ticks were collected in two-week intervals, during the one year period (2011 - 2012). Altogether 802 ticks were collected and identified into the species. *Ixodes ricinus* was the most prevalent tick species, followed by *Haemaphysalis inermis*, *Dermacentor marginatus* and *Dermacentor reticulatus*. *Haemaphysalis concina* and *Ixodes frontalis* were collected in single exemplar each. Temporal analysis has shown differences in the species diversity during the seasons. As the winter (December - February) was characterized by the occurrence of *H. inermis* only, for the summer (June - August) the occurrence of *I. ricinus* was typical. In spring (March - May), all four tick species were recorded, while in the autumn (September - November) only 3 species were collected at the site. *D. marginatus* was not present. The questing tick abundance curve had a uni-modal character with a peak in spring, while the curve of the tick species diversity was bi-modal with the peaks in the spring and autumn. In total, 660 ticks of four species were examined for the presence of tick-borne pathogens by the molecular methods. *I. ricinus* ticks carried the widest spectrum of pathogens with the highest prevalence of spirochetes from *Borrelia burgdorferi* s.l. complex (±12.0%). The predominant genospecies were *B. garinii* (±40.0%) and *B. afzelii* (±37.0%), followed by *B. lusitaniae*, *B. valaisiana* and *B. burgdorferi* sensu stricto. In addition, one *D. marginatus* tick tested positive for *B. afzelii* and one *H. inermis* carried *B. valaisiana*. The second, most prevailing pathogen of *I. ricinus* ticks was *Babesia* spp. (±8.0%). Sequencing allowed distinguishing between *B. microti* "Jena strain" (±65.0%) and *B. venatorum* (22.0%). *A. phagocytophilum* was confirmed in almost ±3.0% of tested *I. ricinus* ticks.

This study confirmed that the karst area in eastern Slovakia represents a significant biodiversity and tick-borne diseases hotspot.

*The study was supported by the project of Research & Development Operational Programme funded by the ERDF (code ITMS: 26220220116) (0,1); by the projects of Slovak Research and Development Agency APVV 0267-10, VEGA 2/0113/12, VEGA 1/0390/12 and VEGA 2/0055/11.*

**ECO-EPIDEMIOLGY OF LYME BORRELIOSIS IN DIVERSE HABITATS OF SLOVAKIA**

M. Derdákova1,2, V. Tarageľová1, R. Václav1, M. Chvostáč1, S. Kolenčík4, K. Cabadajová4, E. Ondrísková3, T. Vaculová1, D. Selyemová1, B. Peňko2

Institute of Zoology, SAS, Dúbravská cesta 9, 845 06 Bratislava, Slovakia1; Institute of Parasitology, SAS, Hlinkova 3, 040 01 Košice, Slovakia2; Institute of Virology, Dúbravská cesta 9, 845 06 Bratislava, Slovakia3; Faculty of Natural Sciences Comenius University, Mlynská dolina, 842 15 Bratislava, Slovakia4

Over the past decades, *Borrelia burgdorferi* sensu lato, the causative agent of Lyme borreliosis, has attracted a lot of scientific attention. At the time of its discovery, it was thought to be an uniform organism. Currently 19 different genospecies belong to this complex out of which at least 9 are present in Europe. Prevalence in ticks in Slovakia varies between 10 to 40%. The specific associations of different genospecies with the reservoir hosts as well as clinical symptoms have been assigned. This association is not strict and difference have been observed. The incidence of LB in Slovakia is up to 20 cases per 100 000 inhabitants.

We have analyzed prevalence, genetic variability and ecological associations of *B. burgdorferi* s.l. in different habitats of Slovakia. More than 5500 questing *Ixodes ricinus* ticks were sampled from 10 diverse habitats including mountain spruce forest, lowland deciduous forest, xerothermic steppe,
suburban forest, urban park, game reserve and woodland-farmland ecotone. Moreover, at two sites (urban and sylvatic) host feeding ticks collected from birds, lizards and rodents were analyzed as well.

18.7% ticks were positive for *B. burgdorferi* s.l. There was significant difference in prevalences between the habitats. The lowest prevalence (6% ) was in urban park in 2012, the highest prevalence - 46% was detected in 2010 at site in foothill area of Central Slovakia. Moreover, we observed significant differences in prevalences between studied years. We have detected 7 genospecies occurring in Europe. *B. afzelii, B. garinii* (including *B. bavariensis*) and *B. valaisiana* were detected at each studied site as the most prevalent with the few exceptions. RFLP method revealed the presence of two different genotypes, that was confirmed by phylogenetic analyses. Using the SSCP method we were able to distinguish *B. bavariensis* from other *B. garinii* genotypes. In habitats with higher biodiversity and abundance of hosts – natural lowland forests - the most commonly detected genospecies were *B. afzelii, B. garinii and B. valaisiana*.

In habitats with lower biodiversity and abundance of hosts –the genospecies that are not so common for this region dominated or were present (*B. lusitaniae* –mountains; *B. spielmanii, B. burgdorferi* s.s.-urban park). This might indicate that introduction of new genospecies might be more easily established in the areas without presence or with low occurrence of original-native genospecies represented by the lower abundance and diversity of reservoir hosts.

*The study was supported by the project APVV-0267-10.*

**URBAN HEDGEHOGS AS POTENTIAL RISK FACTORS FOR TICK-BORNE ZOONOTIC BACTERIA**

**IN A CITY PARK, BUDAPEST**

G. Földvári¹, S. Jahfari², K. Rigó¹, M. Jablonszky¹, S. Szekeres¹, G. Majoros¹, M. Tóth³, V. Molnár⁴, E.C. Coipan², H. Sprong²

Department of Parasitology and Zoology, Faculty of Veterinary Science, Szent István University, Budapest, Hungary¹; Laboratory for Zoonoses and Environmental Microbiology, National Institute for Public Health and Environment, Bilthoven, Netherlands²; Hungarian Natural History Museum, Budapest, Hungary³; Budapest Zoo and Botanical Garden, Budapest, Hungary⁴

We carried out an eco-epidemiological study of an urban population of the Northern white-breasted hedgehog (*Erinaceus roumanicus*) in a park of Budapest for three years. Our preliminary results showed that large number of ticks including imported tick species can survive in close proximity to humans if hedgehogs are present. Here we examined the role of hedgehogs in the urban ecology of Lyme borreliosis and two other emerging tick-borne bacteria.

Hedgehogs were live-captured and anesthetized; DNA was extracted from ear tissue samples and PCR, qPCR and sequencing was performed. Surprisingly 216/230 (94%) ear tissue samples were positive for the LB spirochete. Prevalence of *B. burgdorferi* s.l. in ticks flagged in the park was 33.15% (177/534). Sequencing showed the presence of *Borrelia afzelii, Borrelia spielmanii* and *Borrelia bavariensis* in hedgehogs and in questing ticks. *Candidatus* Neoehrlichia mikurensis was detected in 2.3% (2/88) and *Anaplasma phagocytophilum* in 76% (67/88) of tested hedgehogs. Prevalence of *A. phagocytophilum* and *Candidatus* *N. mikurensis* in questing ticks was 22.1% and 19.29%, respectively.
The recently emerged Candidatus N. mikurensis was detected for the first time in non-rodent hosts and this is the first report of A. phagocytophilum in E. rounanicus. Our data draw attention to the possible risk of human infection with these emerging bacteria in urban habitats. We hypothesise, however that a significant difference exists in the tick density of open grassy areas (where visitors tend to spend their time) versus bushes with dense vegetation causing a lower real risk of human infections than expected.

24H AND 48H ACARICIDAL EFFICACY OF A TOPICAL ADMINISTRATION OF DINOTEFURAN-PERMETHRIN-PYRIPROXYFEN OR PERMETHRIN-PYRIPROXYFEN ON DOGS AGAINST WEEKLY INFESTATIONS OF IXODES RICINUS ADULT TICKS

M. Varlou1, S. Warin1, M. Murphy3, C. Moran3, S. McGrath3, N. Szilagy1
Ceva, 10 avenue de la ballastière, 33500 Libourne, France1; Ceva-Phylaxia Ltd. Szállás u. 5. Budapest, Hungary2; Charles River Laboratories Preclinical Services Ireland Ltd, Ballina, Co. Mayo, Ireland3

Introduction. Ticks are hematophagous parasites that must perform a blood-meal before oviposition. Ixodes ricinus, also known as the castor-bean tick, is a common parasite of dogs. It can transmit pathogens such as Anaplasma phagocytophilum, through the blood meal. This study was conducted to assess the acarical efficacy of a topical acaricide, with (dinotefuran-permethrin-pyriproxyfen, DPP, Vectra® 3D) or without dinotefuran (permethrin-pyriproxyfen, PP) on dogs against viable unfed adult Ixodes ricinus.

Material and methods. The protocol for this study was approved by an independent ethics committee. Twenty-four dogs were allocated based on pre-treatment tick attachment rate (day -4) to one of three groups: an untreated control group (n=8, 10.0 ± 1.2 kg BW), a DPP treated group or a PP treated group. Dogs in the treated groups were administered a volume close to the minimal recommended dose: 1.6 mL of DPP (n=8, 10.4 ± 0.5 kg BW) or 1.53 mL of PP (n=8, 9.4 ± 0.9 kg BW) on day 0. Following inclusion, all dogs were infested with 50 (±4) viable unfed adult ticks (Ixodes ricinus) on days -2, 7, 14, 21, 28 and 35. Tick numbers were assessed in situ on dogs on day 1 (24 ± 2 hour post treatment) and also 24 ± 2 hour after each infestation. Ticks were combed and removed 2 days after each infestation before being classified as live or dead, attached or free, engorged or unengorged. Live ticks, as well as killed attached engorged ticks were considered as a failure. Comparisons between treatments were performed on each group at each time point on the arithmetic mean number of ticks by ANOVA after log transformation on the arithmetic tick (count + 1) data. When the analysis was not suitable because of non-normality of the ANOVA residuals or unequal variances of the outcomes, tick counts were analysed using non-parametric tests. Veterinary examinations, bodyweight, general health observations and clinical assessments were evaluated clinically throughout the study.

Results and conclusion. Even at a dose close to the minimal recommended one, the DPP treated group had ≥ 90% acarical efficacy at 24 h and at 48 h timepoints for one month after administration. The PP treated group had ≥ 90% acarical efficacy at 24 h and at 48 h timepoints from one week post dosing up to one month after administration. This study demonstrated that dinotefuran had no negative impact on the acarical efficacy of permethrin. The results underlined the sustained high immediate (24h post-treatment) and residual (24h post-infestation) effect of DPP
for one month against *Ixodes* ticks infesting dogs. Re-treatment is required monthly for optimal protection against these parasites. Topical application of DPP and PP was well tolerated.

**ANTI-FEEDING EFFICACY OF A TOPICAL ADMINISTRATION OF DINOTEFURAN-PERMETHRIN-PYRIPROXYFEN OR PERMETHRIN-PYRIPROXYFEN ON DOGS AGAINST WEEKLY INFESTATIONS OF *IXODES RICINUS* ADULT TICKS**

M. Varloud¹, S. Warin¹, M. Murphy³, C. Moran³, S. McGrath³, N. Szilagyi²

Ceva, 10 avenue de la ballastière, 33500 Libourne, France¹; Ceva-Phylaxia Ltd. Szállás u. 5. Budapest, Hungary²; Charles River Laboratories Preclinical Services Ireland Ltd, Ballina, Co. Mayo, Ireland³

Introduction. Ticks are hematophageous parasites that must perform a blood-meal before molting to their next developmental stage and for oviposition. *Ixodes ricinus*, also known as the castor-bean tick, is a common parasite of dogs. It can transmit pathogens while blood feeding. This study was conducted to assess the anti-feeding efficacy of a topical ectoparasiticide, with (dinotefuran-permethrin-pyriproxyfen, DPP, Vectra® 3D) or without dinotefuran (permethrin-pyriproxyfen, PP) on dogs against viable and unfed adult *Ixodes ricinus*.

Material and methods. This protocol was approved by an independent ethics committee. Twenty-four dogs were allocated based on pre-treatment tick retention rate (day -4) to one of three groups: an untreated control group (n=8, 10.0 ± 1.2 kg BW), a DPP treated group, a PP treated group. Dogs in the treated groups were administered a volume close to the minimal recommended dose: 1.6 mL of DPP (n=8, 10.4 ± 0.5 kg BW) or 1.53 mL of PP (n=8, 9.4 ± 0.9 kg BW) on day 0. Following inclusion, all dogs were infested with 50 (±4) viable unfed adult ticks (*Ixodes ricinus*) on days -2, 7, 14, 21, 28 and 35. Ticks were combed and removed 2 days after infestation before being classified as engorged or unengorged by squashing ticks on a white background. Engorged ticks were considered as a failure. Comparisons between treatments were performed on each group at each time point on the arithmetic mean number of ticks by ANOVA after log transformation on the arithmetic tick (count + 1) data. When the analysis was not suitable because of non-normality of the ANOVA residuals or unequal variances of the outcomes, tick counts were analysed using non-parametric tests.

Results and conclusion. Even at a dose close to the minimal recommended one, the DPP treatment exhibited an immediate and month-lasting anti-feeding efficacy >90%. This study demonstrated that dinotefuran had no negative impact on the anti-feeding efficacy of permethrin. The results underlined the sustained high immediate (48h post-treatment) and residual (48h post-infestation) efficacy effect of DPP for one month against *Ixodes* ticks infesting dogs. Re-treatment is required monthly for optimal protection against these parasites.
UROSPORA OVALIS – COELOMIC GREGARINE FROM THE WHITE SEA POLYCHAETE TRAVISIA FORBESII

A. Diakin¹, G.G. Paskerova², T.G. Simdyanov³, A. Valigurová¹

Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic¹; Department of Invertebrate Zoology, Faculty of Biology, Saint – Petersburg State University, Saint – Petersburg, Russia²; Department of Invertebrate Zoology, Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia³

Urosporids (Apicomplexa: Urosporidae) parasitize a wide range of animals such as echinoderms, nemertean3, annelids and ascidians3, inhabiting their coelom, intestine and its derivatives. As also known from gregarines of family Monocystidae3, translocation within the host causes changes in their morphology and motility mode together with a structural modification of the cortical zone (epicyte and ectocyte). Particularly, gregarines of the family Urosporidae exhibit considerable morphological plasticity, which correlates with different modes of movement and variations in structure of their cortical zone, according to their localizations within the host. This family comprises representatives inhabiting various host biotopes and showing different types of motility: a typical gliding movement (Urospora travisiae), metaboly (Urospora ovalis) or peristalsis of large gamonts (Pterospora floridiensis), unusual movements such as regular pulsing of cytoplasm within the central part of the cell and its posterior trunks (Pterospora floridiensis and P. schizosoma), as well as completely immotile species (Urospora chiridota, Lithocystis spp. and Gonospora spp.).

Coelomic trophozoites of Urospora ovalis are spindle-shaped and lack any signs of cell polarity. They show low metabolic activity during which the wave of constriction goes along the cell from one end towards the opposite end. Transmission electron microscopy confirms that trophozoites are covered by a trimembrane pellicle, typical for gregarines. It forms numerous longitudinal epicytic folds that extend from one to another cell pole and have a finger-like shape in cross-section. In the apical part of these folds, two or three apical arcs can be poorly observed between the plasma membrane and the external cytmembrane. Just beneath the internal cytmembrane, there is a single rod of electron-dense material situated. The subjacent fibrillar layer (the so-called internal lamina), forming bridges at the bases of the epicytic folds, can be found under the pellicle. Micropores were found in between the epicytic folds. In the region of cell constriction, the pellicle and cortical zone form the so-called superfolds, which can be seen under light microscope as distinct striations. The superfolds are also covered by epicytic folds. The cytoplasm of gregarines is obviously subdivided into two regions: cortical zone with various filamentous structures, and endoplasm containing nucleus, majority of cellular organelles, amylopectin granules and other inclusions.
New SSU rDNA sequences of *U. ovalis*, together with *Urospora travisiae* (also found in the body cavity of the same host), belong to the Lecudinoidea clade, however they affiliate not with other coelomic urosporids (*Pterospora* spp. and *Lithocystis* spp.), but with intestinal lecudinids (*Difficilina* spp.).

Our new obtained data confirm the theory on independent and parallel origin of coelomic gregarines. We also assumed on parallel evolution of cell cortex morphological adaptations as a response to parasitism in different host biotopes.

*Financial support provided by Czech Science Foundation No. P505/12/G112.*

**CYTOMORPHOLOGICAL CHANGES IN PATHOGENIC ACANTHAMOEBA STRAINS AFTER THE APPLICATION OF HETEROCYCLIC ALKYLPHOSPHOCHOLINES**

M. Garajová⁴, M. Mrva⁴, L. Timko², M. Lukáč², F. Ondriska³

Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic¹; Faculty of Pharmacy, Comenius University, Bratislava, Slovak Republic²; HPL (Ltd), Microbiological Laboratory, Bratislava, Slovak Republic³

Amphizoic amoebae of the genus *Acanthamoeba* Volkonsky, 1931 are causative agents of painful and difficultly treatable human diseases: *Acanthamoeba* keratitis (AK) in immunocompetent individuals, granulomatous amoebic encephalitis (GAE) and disseminated infections in immunodeficient individuals. To date, any standard and effective therapy was not developed and presently used therapeutic agents are frequently ineffective and with many side effects. The development of new drugs continues intensively due to the increasing number of cases. Currently considerable attention is focused on alkylphosphocholines (APCs) – phospholipid analogues with lytical effect on cell membranes of diverse parasitic protists. However, their mechanism of their amoebicidal activity has not been completely comprehended yet.

The aim of our study was to detect cytomorphological changes of *Acanthamoeba* spp. clinical isolates from AK cases after application of four new synthesised APCs with ammonium cation bounded in heterocycle: IF16-P-4-Pip and IF16-P-2-MetPip with piperidine, IF16-P-Azep with azepan and IF16-P-Morf with morpholine, which were tested *in vitro*.

The cytotoxic effect of APCs against trophozoites was visible within 1 hour particularly at the highest tested concentrations (250–500 μM). The signs of cytotoxicity included ceasing of locomotion, rounding up of cells, resorption of acanthopodia and detachment of trophozoites from the bottom of the well. After lysis dead cells were stained densely by trypan blue. The sole identifiable cell organelle was nucleus situated in the centre or excentrically with distinct nucleolus. After the application of three APCs, IF16-P-4-Pip, IF16-P-2-MetPip, and IF16-P-Azep, at low concentrations of 62.5–125 μM, rounded pseudocysts were formed as a response to an acute stress after 1 hour of exposure. After 24 hours of incubation, pseudocysts were present after the treatment with IF16-P-4-Pip and IF16-P-Azep. After 48 hours of incubation, the presence of pseudocysts was noted only in wells treated with IF16-P-Azep.

The signs of cytotoxic activity of APCs against cysts were visible within 1 hour at the highest tested concentrations (250–1000 μM). Destroyed cells densely stained by trypan blue were characteristic with shrinkage of the cytoplasm and cytoplasmic membrane separated from the
endocyst. The double-layered cyst wall remained undamaged. After the application of IF16-P-2-MetPip at the concentration of 1 mM, large self-assembled spherical vesicles of this APC of 20–30 μm were frequently observed enclosing one or two inactivated cysts.

The present results of the amoebicidal activity of heterocyclic APCs suggest their possible therapeutic potential and detected cytomorphological changes contribute to the comprehension of their action.

The research was supported by grants VEGA 1/0796/12, UK/263/2014 and UK/123/2014. This work was supported by the Slovak Research and Development Agency under the contract No. APVV-0516-12. This publication is the result of the project implementation: Comenius University in Bratislava Science Park supported by the Research and Development Operational Programme funded by the ERDF Grant number: ITMS 26240220086.

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF BLOOD APICOMPLEXAN PARASITES (KARYOLYSUS, SCHELLACKIA) INFECTING SOME EUROPEAN LIZARDS

B. Haklová¹, A. Hiňanová², I. Majláth¹,², K. Račka³, D.J. Harris⁴, G. Földvári⁵, P. Tryjanowski⁶, N. Kokošová², V. Majlátová¹

Institute of Parasitology SAS, Hlinkova 3, 040 01 Košice, Slovak Republic¹; Institute of Biology and Ecology, University of P. J. Šafárik in Košice, Moyzesova 11, 040 01 Košice, Slovak Republic²; Department of Parasitology, State Veterinary Institute Jihlava, Rantišovská 93, 586 05 Jihlava, Czech Republic³; CI-BIO-UP, Centro de Investigación em Biodiversidad e Recursos Genéticos da Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal⁶; Department of Parasitology and Zoology, Faculty of Veterinary Science, Szent István University, 2 István Street, Budapest H-1078, Hungary⁵; Department of Behavioural Ecology, Adam Mickiewicz University, Poznań, Poland⁶

The blood parasites of genus Karyolysus Labbé, 1894 and Schellackia Reichenow, 1919 represent haemogregarines parasites found in various lizards families (e.g. Polychrotidae, Agamidae, Gekkonidae, Lacertidae, Scincidae and Chamaeleonidae). We examined 608 individuals of reptiles from six different localities in Europe (Greece, Hungary, Poland, Romania, Slovakia and Sweden) using both, microscopic and molecular methods. In this study we examined and assessed phylogenetic relationships of all isolates of these blood parasites for the first time. A total of 460 blood smears were examined, from which protozoan parasites were found in 63 samples (12.2%). Based on morphology, we observed two species of Karyolysus: K. latus, characteristic by lentiform or bean-shaped trophozoites with large reticulated centrally placed nucleus and vacuolated cytoplasm, gametocytes with rounded ends and non-vacuolated cytoplasm; and K. lacazei, with thin and elongated trophozoites, vacuolated cytoplasm and slender, thin gametocytes with one end bent. In total, 538 samples from 15 reptile species and 4 subspecies tested by polymerase chain reaction (PCR), the presence of parasites were observed in 63 individuals (11.7%). We also obtained sequences of blood parasites from ectoparasites (Ixodes ricinus ticks and Ophionyssus saurorum) collected from reptiles. Contrary, all 133 snakes from Europe belonging to six genera (Natrix, Zamenis, Coronella, Coluber, Dolichophis and Viper) were infection-free. Smears of Ophionyssus mites collected from lizards contained several free gametocytes liberated from erythrocytes after
bloodsucking, and moreover sporokinetes were also found in the smear preparations from the mite eggs. Comparison of the eight isolates revealed the existence of four haplotypes, all part of the same lineage within sequences of parasites identified as belonging to the genus Hepatozoon sp. Schellackia sp. was the only blood parasite observed in Lacerta viridis from Slovakia (Zádiel). Phylogenetic analysis revealed relatedness of isolate obtained in this study with Schellackia –like parasites derived from lizard species captured in Spain as well as with Eimeria arnyi and E. ranae.

The study was supported by the project of Research & Development Operational Programme funded by the ERDF (code ITMS: 26220220116) (0,5) and by the Scientific Grant Agency of the Ministry of Education of Slovak Republic and the Slovak Academy of Sciences VEGA 1/0417/14. This study was conducted under the frame of the EurNegVec COST Action TD1303.

PREVALENCE OF BARTONELLA SPP. AMONG SMALL MAMMALS IN EASTERN SLOVAKIA

J. Kraljík1,4, M. Stanko1,2, L. Mošanský1, L. Blaňarová1, D. Miklisová1, M. Bona3
Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovakia1; Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovakia2; Department of Anatomy, Faculty of Medicine, UPJŠ, Šrobárova 2, 041 80 Košice, Slovakia3; Department of Zoology, Faculty of Natural Sciences, Comenius University, Mlynská dolina, 842 15 Bratislava, Slovakia4

Bartonella species are fastidious hemotropic gram negative bacteria which are transmitted by blood-sucking arthropods to a wide variety of vertebrate animals including rodents. The aim of our study was to investigate whether these bacteria are prevalent in small mammals in different studying sites of Slovakia. For this aim, a total of 854 individuals belonging to six rodent species and four insectivore species were collected by means of live traps during the years 2011 and 2012 at four sampling sites of Eastern Slovakia (Čermel, Hýľov, Rozhanovce and Botanical garden Košice). Dominant species of small mammals were Apodemus agrarius (37.6 %), followed by Clethrionomys glareolus (28.3 %) and A. flavidus (28.1 %). 827 spleen samples were further screened for the presence of bartonellae using genus-specific primers targeting the 16S-23S intergenic spacer region. The overall prevalence of infection with Bartonella spp. was 18.9 % (157 samples). Among dominant species of small mammals the lowest prevalence of Bartonella spp. infection was recorded in A. agrarius (9.8 % in 2011 and 8.5 % in 2012) while the highest prevalence was found in A. flavidus (37.5 % in 2011 and 27.6 % in 2012). Nucleotide sequences were manually edited in MEGA 5 and compared with GenBank entries by BLAST. Preliminary results indicate the presence of Bartonella grahamii (3.8 %) and Bartonella taylorii (6.4 %) and that bartonellae are widely distributed among dominant species of small mammals in Eastern Slovakia. However, continued long-term surveillance of associated parasites as potential vectors is needed to confirm the circulation of these pathogens in natural foci of Slovakia and to establish the potential zoonotic risks for humans.

The research was supported by Slovak Research and Development Agency APVV 0267-10, VEGA 1/0390/12, VEGA 2/0060/14 and EU project FP7-261504 EDENext.
The aim of the present study was to diagnose the presence of waterborne protozoan parasites *Giardia intestinalis* and *Cryptosporidium* spp. and to detect helminth status in selected groups of children of majority population and Roma children from Medzov in Kosice region. We examined 239 faecal samples of children, divided into 2 groups: majority (66), minority (173). Micro epidemiological studies in children from orphanage (19) and children from 2 classes in the third years of school (21) was also performed.

In majority group were healthy children who passed out regular preventive examinations and they noted none signs, pointing to possible infection. Children from minority group were from area with low hygienic standard. These both groups of children were divided to 4 age groups: 1-5 years old, 6-9 years old, 10-14 years old, and >14 years old. In orphanages were children from minority group. In the third years of school were children from majority and minority groups together in the same classes. The faecal samples were analysed by flotation concentration method and perianal strips for eggs of *Enterobius vermicularis*. Direct ELISA method commercial test for faecal *Cryptosporidium* antigen was used.

In positive samples we detected cysts *Giardia intestinalis* and eggs of 4 species of helminths (*Ascaris lumbricoides, Trichuris trichiura, Enterobius vermicularis, Taenia* spp.). *Cryptosporidium* spp. in 9.30 % (4/43) of majority group of children and 9.26 % (10/108) of minority group. Helminth eggs were detected in 19.70 % (13/66) of majority group and 70.52 % (122/173) of minority group of children. In majority group of *Enterobius vermicularis* in 18.18 % (12/66) and *Taenia* spp. tapeworm eggs in the stool of one child 1.51 % (1/66) was detected.

In minority group of children *Ascaris lumbricoides* was the most frequently observed species in 63.00 % (109/173) of population, followed by *Trichuris trichiura* in 17.34 % (30/173) of the examined samples, *Giardia intestinalis* in 13.29 % (23/173), *Cryptosporidium* spp. in 9.26 % (10/108), *Enterobius vermicularis* was detected in 7.51 % (13/173) and *Taenia* spp. in 0.58 % of examined specimens. The highest occurrence of positive samples was found in the group of 6 to 9 years old children.

Micro epidemiological study in children from orphanage have shown the presence of *Giardia intestinalis* in 47.37 % (9/19), *Ascaris lumbricoides* in 10.52 % (2/19) and *Enterobius vermicularis* in 5.26 % (1/19). Micro epidemiological study in children from 2 classes confirmed the present of *Enterobius vermicularis* in 38.46 % (5/13) of majority group of children and in 25.00 % (2/8) of minority group. *Cryptosporidium* spp. was not found.

*The study was supported by the by grants of State Agency VEGA No. 1/0831/12, 1/0063/13.*
SESSION
Tapeworms and flukes – does DNA, proteins or hosts rule their world?

HOST-DRIVEN POLYMORPHISM IN CARYOPHYLLAEUS GMELIN, 1790 (CESTODA: CARYOPHYLLIDEA), PARASITES OF CYPRINID FISHES
D. Barčák1, M. Oros1, V. Hanelová1, T. Scholz2
Institute of Parasitology, Slovak Academy of Sciences, Hlinkova 3, 04001 Košice, Slovakia1; Institute of Parasitology, Biology Centre of the Academy of Science of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic2

In the present study, Caryophyllaeus brachycolis Janiszewska, 1953 and C. laticeps (Pallas, 1781), are redescribed on the basis of newly collected specimens. Several morphotypes from different fish hosts within both species were recognized, which indicates host-driven morphological plasticity of these euryxenous parasites. Within C. brachycolis, morphotype 1 from barbels (Barbinae) and chubs (Leuciscinae) is characterised by a more robust body with the spatulate scolex, which is only slightly wider than a very short neck region, and the anterior position of the testes and vitelline follicles, which begin immediately posterior to the scolex. Specimens of morphotype 2 from breams (Abramis, Ballerus and Blicca; Abraminae), which were previously misidentified as Caryophyllaeus laticeps, possess a more slender body with a flabellate scolex, which is much wider than a long neck, and the first testes begin at a considerable distance posterior to the first vitelline follicles. In C. laticeps five morphotypes from different hosts (Abramis, Ballerus, Chondrostoma, Cyprinus and Vimba) and geographical regions were recognized. Despite conspicuous differences in scolex morphology and the anterior extent of the testes and vitelline follicles, the different morphotypes within both species are identical in the morphology of the posterior end of the body. Specimens of C. brachycolis are typical by its large cirrus-sac, which is thick-walled, elongate pyriform, and the distribution of the vitelline follicles, which surround medially vas deferens near the cirrus-sac. In contrast, C. laticeps possesses a small, subspherical, thin-walled cirrus-sac containing a well-developed internal seminal vesicle, and a well developed and strongly coiled vas deferens not surrounded by vitelline follicles medially. If host-related morphological polymorphism in taxonomically important characteristics is confirmed in other groups of fish cestodes, it may complicate identification of these parasites.

This study was supported by the Slovak Research and Development Agency (project no. APVV-0653-11), Grant Agency VEGA (No. 2/0129/12), Czech Science Foundation (project No. PS05/12/G112) and Institute of Parasitology (RVO: 60077344).
DIFFERENTIAL EXPRESSION AND GLYCOSYLATION OF PROTEINS IN THE RAT ILEAL EPITELIUM IN RESPONSE TO *ECHINOSTOMA CAPRONI* INFECTION

A. Cortés¹, C. Muñoz-Antolí³, J. Sotillo¹,², J.G. Esteban¹, R. Toledo¹

Departament de Biologia Cel·lular i Parasitologia, Facultat de Farmàcia, Universitat de València. Avda. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain¹; Center for Biodiscovery and Molecular Development of Therapeutics, James Cook University, Cairns, Queensland, Australia²

*Echinostoma caproni* (Trematoda: Echinostomatidae) is an intestinal trematode with no tissue phases in the definitive host. Although *E. caproni* is able to parasitize a wide range of laboratory rodent hosts its compatibility differs considerably among rodent species. In high compatible hosts, such as mice, the infection becomes chronic, while in hosts of low compatibility, such as rats, the worms are expelled from 2 weeks post-infection. Because of these characteristics, the *E. caproni*-rodent systems are highly suitable for elucidating the host-specific components that determine the course of infections with intestinal helminths.

In order to further investigate the factors determining the expulsion of intestinal helminths, we analized changes in the protein expression and glycosilation induced by *E. caproni* in Wistar rat, a host of low compatibility in which the parasites are rapidly rejected. To determine the changes in protein expression, two-dimensional difference gel electrophoresis (2D-DIGE) was employed using protein extracts from the intestine of naive and infected rats. The patterns of glycosylation were analyzed by lectin blotting. Those spots showing differential expression or glycosylation were analyzed by mass spectrometry. A total of 33 protein spots differentially expressed were identified (26 were found to be over-expressed and 7 down-regulated). Moreover, *E. caproni* induced changes in the glycosylation status of 8 proteins that were successfully identified.

Most of these proteins were related to the cytoskeleton, such as type I and type II keratins, actin and T-plastin; and the maintenance of the functional integrity of the intestinal epithelium, for example annexin 4A. The elevated number of proteins related with the preservation and healing of the intestinal tissue that were found to be over-expressed after *E. caproni* infection appears to be of great importance to explain the low level of local tissue damage. Furthermore, this suggests that the regeneration of the intestinal tissue is a major effector mechanism, responsible for the early rejection of adult worms that occurs in this host. Several proteins involved in the energy metabolism were also altered in the ileum of rats as a consequence of the *E. caproni* infection. These changes suggest that the infection shifts the cellular metabolism to a more aerobic-oxidative profile than that observed in non-infected animals. Although the precise mechanisms operating in this modification and their implications on the course of the infection are difficult to predict according to our current knowledge, this finding could be of relevance for a better understanding of the mechanisms involved in the control of infections at mucosal surfaces.
DEVELOPMENT OF POLYMORPHIC MICROSATELLITE LOCI IN GIANT LIVER FLUKE

FASCIOLOIDES MAGNA (TREMATODA: FASCIOLIDAE)

L. Zvijáková1, G. Minárik2,3,4, E. Bazsalovicsová1, J. Štefka5, L. Pálková3, I. Kráľová-Hromadová1

Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovakia1; Department of Molecular Biology, Faculty of Natural Science, and Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia2; Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Sasinkova 4, Bratislava, Slovakia3; Geneton Ltd., Ilkovičova 3, Bratislava, Slovakia4; Biology Centre ASCR, Institute of Parasitology, České Budějovice, Czech Republic4

The giant liver fluke, Fascioloides magna, is an important liver parasite of wide range of free-living and domestic ruminants. Its pathological effect in definitive host depends on the type of host; while in specific hosts (white-tailed deer Odocoileus virginianus, wapiti Cervus elaphus canadensis, caribou Rangifer tarandus, red deer Cervus elaphus elaphus, and fallow deer Dama dama) is infection very often subclinical, fascioloidosis in domestic ruminants (cattle Bos taurus, goat Capra hircus and sheep Ovis aries) may be lethal. Fascioloides magna is not only a parasite of veterinary importance; the giant liver fluke is due to its invasive character also very interesting model species for population genetics. The original continent of F. magna is North America, where parasite occurs in five enzootic regions throughout Canada and USA. The giant liver fluke was introduced to Europe along with its deer host in the 19th century and has established three permanent natural foci. While genetic interrelationships among European population of parasite are known, the population genetics of North American populations of F. magna and exact origin of middle European fluke populations have not been resolved yet. For this purpose, the so far most suitable molecular markers seem to be the microsatellite markers, which characteristics (multilocus genes, codominance, Hardy-Weinberg equilibrium) predestine them for population genetics studies.

The next-generation sequencing approach was applied for initial selection of candidate microsatellite loci and primer design. Out of 667 microsatellite loci candidates (length 90-309 bp), 118 provided the best resolution and were applied for further PCR amplification analysis. In total, 56 assays provided PCR products of expected size and were sequenced from both sides; the declared repetitive motif was confirmed in 36 of them. After fragment analysis, 24 microsatellite loci were excluded due to either failure to amplify PCR product in several individuals (potential of homozygotes for null alleles), or owing to the fact that some loci were detected to be monomorphic. The remaining 12 loci were tested on the representatives of both, North American and European populations in order to ensure their global applicability in future studies of F. magna. The 12 loci tested in multiplex panels were determined to be polymorphic (2-8 alleles per locus) and provided statistically significant tests for genotypic equilibrium and deviations from Hardy-Weinberg proportions.

This work was financially supported by the project Revogene – Research Center of Molecular Genetics (grant No - ITMS 26240220067) supported by the R&D Operational Programme funded by the ERDF, and by VEGA 2/0133/13 project.
SUBSTRATE SPECIFICITY OF RECOMBINANT ISOFORMS OF CATHEPSIN B1 FROM THE BIRD SCHISTOSOME TRICHOBILHARZIA REGENTI

H. Dvořáková¹, C.R. Caffrey², A.J. O’Donoghue³, L. Mikeš¹

Department of Parasitology, Faculty of Science, Charles University of Prague, Viničná 7, 128 44 Prague 2, Czech Republic¹; Center for Discovery and Innovation in Parasitic Diseases, Dept. of Pathology, University of California San Francisco, 1700 4th Street, San Francisco, CA 94158, USA²; Department of Pharmaceutical Chemistry, University of California San Francisco, 16th Street, San Francisco, CA 94158-2330, USA³

Peptidases are a very important group of enzymes essential in biological processes of all organisms including parasites. One of the keys to understanding the biological role of peptidases in any organism is the knowledge of preferred cleavage sites within their substrates, i.e. substrate specificity of peptidases. Characterization of the substrate specificity can provide a clue to natural protein substrates of a particular peptidase and may serve as a basis for design of selective substrates or inhibitors to study peptidase activity. Therefore, two recombinant isoforms of cathepsin B1 of the bird parasite Trichobilharzia regenti (TrcB1.1 and TrCB1.4, GenBank AY648119 and 22) expressed in Pichia pastoris have been analyzed for substrate specificity using a positional scanning synthetic combinatorial library. This library allows determination of amino acid preferences of peptidases in a defined position within tetrapeptide substrates (P1-P4). Recombinant TrCB1.1 exhibited preference for Lys and Arg in P1 position, a broader specificity in P2 (Met>Val>Ala>Ser>nLeu>Ile>Leu>Asp), Leu and other hydrophobic amino acids in P3 and Arg, Lys in P4 position of the tetrapeptide. Recombinant TrCB1.4 has a broader specificity in P1 position (Arg>Met>nLeu>Lys), but has a narrow specificity in P2 (mainly small aliphatic hydrophobic amino acids). The P2 specificity of both recombinant enzymes was distinct from most cathepsins B, which generally prefer large hydrophobic amino acids in this position. It is noteworthy that recombinant TrCB1.4 was unable to hydrolyze tetrapeptides with Arg in P2, contrary to other cathepsins B of most organisms (including TrCB1.1). There are no significant differences in preferences between both recombinant isoforms in P3 and P4 positions of the peptide substrate. Although the amino acid sequence identity of TrCB1 isoforms is high (98-99% between the putatively active isoforms), even small changes in amino acid sequences may cause variations in substrate specificities. The consequences of different amino acid preferences of the two recombinant isoforms will be discussed in the context of their putative biological functions.

Acknowledgements: Czech Science Foundation (Grant No. 13-29577S)
SEARCHING FOR SUITABLE ANTIGENS FOR SERODIAGNOSTICS OF BIRD SCHISTOSOME INFECTIONS CAUSED BY TRICHOBILHARZIA REGENTI

L. Turjičková¹, M. Kašný¹,², L. Mikeš³

Department of Parasitology, Faculty of Science, Charles University in Prague, Viničná 7, 12844 Prague 2, Czech Republic¹; Institute of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic²

Trichobilharzia regenti is a schistosome parasitizing anatid birds. After cercariae penetrate the skin of a host, schistosomula migrate via the nerve tissue to the place of final localization, nasal mucosa. T. regenti can also penetrate into mammalian hosts including humans where they cause an itchy hypersensitive skin response, known as cercarial dermatitis. The aim of our recent research was to describe the antibody response of experimentally infected specific definitive hosts Anas platyrhynchos to various antigens of T. regenti in order to find antigens which could be used for immunodiagnostics.

Sera from experimentally infected ducks have been collected in predefined intervals. They were examined using ELISA and western blot methods. Homogenates of cercariae and schistosomula were used as antigens. Results of ELISA demonstrated that the level of specific IgY antibodies had been gradually increasing from 12th day post infection and peaked around the 30th day post infection. This trend was observed with antibodies from sera of infected ducks regardless of age or infectious dose applied to experimental ducks.

The level of IgM antibodies specific to the early stage of infection sequentially grew and culminated around the 15th day post infection, then declined. Using Western blotting, we identified several antigens of cercariae and schistosomula recognized by IgY from infected ducks. Their identification by mass spectrometry is under way.

Previous studies focused on the immune response of nonspecific mammalian hosts revealed two antigens strongly recognized by IgG1 and IgE antibodies characterized as trios phosphate isomerase (TPI, MW 25 kDa) and glyceraldehyde 3-P dehydrogenase (MW 34 kDa).

TPI was produced in a recombinant form using Escherichia coli bacterial expression system. Its immunodiagnostic potential was tested in ELISA and Western blots with sera of ducks experimentally infected by trichobilharziae.

The work was supported by the Czech Science Foundation grant No. 13-29577S and by the Charles University in Prague (GAUK 243-259305)

MODULATION OF DENDRITIC CELL FUNCTION BY EXCRETORY-SECRETORY PRODUCTS OF THE MODEL TAPEWORM MESOSTEOIDES VOGAE

E. Vendeľová¹, J.K. Nono², G. Hrčková¹, M.B. Lutz³, K. Brehm²

Institute of Parasitology of the Slovak Academy of Sciences, Košice, Slovakia¹; University of Würzburg, Institute of Hygiene and Microbiology, Würzburg, Germany²; University of Würzburg, Institute of Virology and Immunobiology, Würzburg, Germany³

Despite the fact that cestodes cause debilitating chronic diseases with long asymptomatic state, their immunomodulatory potential is highly understudied as compared to other helminths
expression to biochemical we amounts (Trema Eudiplozoon and reveal were primary cystatins and helminths. Although considerably recruited to the site of infection, host DC failed to produce elevated amounts of IL-12p70 in M. vogae injected mice when compared to mock-injected controls. In vitro, we found that neither excreted/secreted (E/S) products nor somatic extracts of the parasite were able to induce conventional maturation of DC (as judged by MHC II class and CD86 surface marker expression and cytokine production). Moreover, whereas the parasite secretion significantly suppressed LPS-induced production of IL12p70, somatic extracts failed to do so. A bio-assay guided biochemical analysis of the parasite secreted product(s) revealed that the observed effect was most likely driven by a glycoprotein. Finally, an analysis of the protein profiles of parasite secreted (active) and somatic (inactive) antigens provided us with several differentially expressed proteins that could constitute efficient anti-inflammatory factors. The identification strategy of such factor(s) and the possible implication of our findings for cestode immunomodulation will be discussed.

EXPLORING THE CYSTATINS OF HELMINTHS
J. Ilgová¹, B. Koudela³, M. Gelnar¹, M. Kašny¹,²

Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic¹; Department of Parasitology, Faculty of Science, Charles University in Prague, Czech Republic²; Department of Pathological Morphology and Parasitology, Faculty of Veterinary Medicine, Brno, Czech Republic³; University of Veterinary and Pharmaceutical Science Brno, Czech Republic⁴

Parasitic organisms release numerous types of molecules essential for their survival. Cystatins - cysteine peptidase inhibitors (CPI) were determined as important protein molecules which participate in the processes in the host-parasite interface. Their effect can influence the host immune response, particularly the processing and presentation of antigens, production of nitric oxide, etc. The aim of our study is to expand the information about cystatins produced by helminths and to reveal their functions in four selected parasitic model species representing the major groups of helminths. We used bioinformatic tools to analyze the transcriptomic and/or genomic data of Eudiplozaon nipponicum (Monogenea), Hymenolepis diminuta (Cestoda), Fascioloides magna (Trematoda) and Trichinella spiralis (Nematoda) and we searched for the presence of conserved cystatin domains. The selected genes of CPI of diverse families - stefins, cystatins and kininogens were amplified, cloned and sequenced and significant differences in their nucleotide/amino acid primary structure were revealed. The production of recombinant forms of CPI is in process in order to characterize their structures and functions.

This research was supported by the Masaryk University (Grant n. MUNI/A/0888/2013), by the Czech Science Foundation (Grant n. GBP505/12/G112; GAP506/12/1258), by the Ministry of Education, Youth and Sport Czech Republic (Grant CONTACT II n. LH12096) and by the Charles University in Prague (UNCE 204017, PRVOUK P41, SVV 267210/2013).
SESSIONS

- Monogeneans and nematodes – from molecules to biodiversity
- Ecological and veterinary parasitology

EFFECTS OF HYBRIDIZATION BETWEEN COMMON AND MEDITERRANEAN BARBEL ON THE COMPOSITION OF MONOGENEAN PARASITE COMMUNITIES IN SOUTHERN FRANCE

L. Gettová¹, A. Gilles², A. Šimková¹
Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, Brno, 611 37, Czech Republic¹; Aix-Marseille Université, IMBE, UMR CNRS 7263, Evolution Génome Environnement, Case 36, 3 Place Victor Hugo, 13331 Marseille Cedex 3, France²

The Monogenea represent a heterogeneous group of mainly fish ectoparasites, which generally display a high level of host specificity and high morphological variability of attachment organs. In our project, we aimed to investigate the composition of monogenean parasite communities in the hybrid zone between common barbel *Barbus barbus* and mediterranean barbel *B. meridionalis* which was documented in southern France at the end of the 20th century. Hybrid zones provide direct insight into the formation of interactions between parasites and their hosts, however, host-parasite interactions have been so far studied in only a few hybridizing systems. In our study, we hypothesized lower parasite load in *B. meridionalis* with restricted population sizes comparing to the populations of *B. barbus* characteristic by wide area of distribution. In addition, lower level of monogenean infection in hybrids in contrast to parental barbel species as a result of heterosis effect was expected.

In 2007-2013, together 327 fish individuals were collected from both sympatric and allopatric populations, and subsequently dissected for the presence of metazoan parasites. Based on molecular identification using 30 microsatellite markers, the occurrence of pure *B. barbus* (191) and *B. meridionalis* (71) individuals and their respective hybrids (65) were detected.

Of all parasitic groups, Monogenea were the most dominant almost in all *Barbus* populations. Within the family Dactylogyridae, *Dactylogyrus carpathicus* parasitized both *Barbus* species from the investigated sites, while *D. malleus* was specific for *B. barbus* which is in concordance with previously published data. On the other hand, in our study *D. dyki* was present solely in allopatric populations of *B. barbus*, while its occurrence was previously documented also in *B. meridionalis* populations. Our study reported for the first time the presence of *D. extensus* in *B. meridionalis*. This parasite has been considered as a specific parasite of common carp (*Cyprinus carpio*). The family Gyrodactyliidae was represented by *Gyrodactylus katharineri* and *G. markewitschi* parasitizing both *B. barbus* and *B. meridionalis*. Furthermore, at least three *Gyrodactylus* species were detected in investigated populations, one of them host-specific for *B. barbus* individuals. However, molecular identification is further required to determine these parasites to a species level. *Paradiplozoon homoion*, the only representative of the family Diplozoidae, occurred rarely and in low abundances in populations of both *Barbus* species. Overall, significantly higher abundance of monogenean parasites was found in *B. barbus* comparing to *B. meridionalis*. Significantly lower monogenean abundance was revealed in
hybrids when compared to both parental Barbus species. Nevertheless, obvious differences in the intensity of infection of monogeneans were observed between particular Barbus populations. The effect of locality on similarity of parasite communities cannot be therefore excluded. Geographic as well as genetic distances between investigated populations will be tested as possible factors influencing the composition of monogenean parasite communities along the Barbus hybrid zone.

This study was founded by the GACR project No P505/12/0375.

BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF PEPTIDASES FROM MONOGENEAN PARASITES OF THE FAMILY DIPLOZOIDAE (HETERONCHOINEA)

L. Jedličková1, H. Dvořáková1, M. Kašný1,2, L. Mikes1

Department of Parasitology, Faculty of Science, Charles University in Prague, Viničná 7, 128 44 Prague 2, Czech Republic; Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

Members of the family Diplozoidae are blood-feeding parasites occuring mainly on the gills of cyprinid fishes. They may have significant effects on their hosts, e.g. mechanical damage of gill filaments accompanied by a risk of secondary bacterial or mycotic infections, or by induction of hypochromic microcytic anemia.

Blood processing mechanisms in monogeneans of the subclass Heteronchoinea are unknown. So far, only ultrastructural and histochemical analyses have been performed and it is supposed that digestion is intracellular and takes place in a specialized type of gut cells. Only very limited information is available about the biochemistry of digestion. In our study, we focused on molecular and biochemical characterization of peptidases which can be involved in degradation of blood proteins for nutrition.

Cysteine class peptidases were confirmed in samples of excretory/secretory products and in soluble protein extracts from adult Paradiplozoon blliccae and Eudiplozoon nipponicum by using fluorogenic oligopeptide substrates, a set of specific inhibitors and a labelled cysteine peptidase-specific probe DCG-04. Soluble protein extracts of adult worms labelled with biotinylated or fluorescent DCG-04 were separated by one/two dimensional electrophoresis. In the gels after electrophoresis or on blots, bands/spots around 35 kDa were detected for P. blliccae and around 35-25 kDa for E. nipponicum. Labelled spots from 2D electrophoresis were identified by mass spectrometry as cathepsin(s) L.

From the results of transcriptome analysis we have predicted 30 peptidase genes in E. nipponicum; 9 of them may be included in the process of blood digestion. Cathepsin L and cathepsin B of E. nipponicum have been expressed in Pichia pastoris yeast and are being biochemically characterized. We suppose that cathepsins L are major hemoglobinolytic peptidases in freshwater monogeneans of the family Diplozoidae.

Acknowledgements: Czech Science Foundation grant no. P506/12/1258 and grant of the Charles University in Prague GAUK 502313
MONOGENEANS (DACTYLOGYRIDAE) FROM THE GILLS OF AFRICAN TETRAS (CHARACIFORMES: ALESTIDAE) IN KENYA AND ZIMBABWE

M.L. Kičinžaová, E. Řehulková
Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

The Alestidae, often called African characids (or tetras), is the most specious (with about 120 species) family of characiform fishes in Africa. These fish hosts are known to harbor monogeneans representing 3 genera of the Dactylogyridae, namely *Afrocleidodiscus Annulotrema* and *Characidotrema*. Current evidence based on morphological data suggests that species of the two last genera have evolutionary relationships with species parasitizing Neotropical characiform fishes, i.e. those of *Annulotrematoides* and *Jainus*, respectively. During several field campaigns (2005 – 2012) in Kenya, Senegal, Sudan and Zimbabwe, a total of 15 species of the Alestidae have been examined for monogeneans and more than 30 species (3 genera) of the Dactylogyridae were found.

Our current research deals with the taxonomic evaluation of monogeneans collected in Turkana Lake (Kenya) and Kariba Lake and Zambezi River (Zimbabwe). Examination of the material from Kenya revealed the presence of 8 species of *Annulotrema* on the gills of 4 species (3 genera, i.e. *Alestes, Brycinus* and *Hydrocynus*) of the Alestidae. Two species, namely *Annulotrema besalis* and *A. nili*, represent previously described taxa and other 6 species are probably new for science. Investigation of monogeneans found on the gills of 2 alestid species (2 genera, i.e. *Brycinus* and *Hydrocynus*) from Zimbabwe showed the presence of 6 species of *Annulotrema* and 1 species of *Afrocleidodiscus*. Morphological analysis of sclerotized structures of the *Annulotrema* species found in the two regions suggests that there are at least 3 groups within the dactylogyrids parasitizing these fishes, with regard to the basic structure of the male copulatory organ (MCO). The first group includes 5 species with MCO composed of an arcuate copulatory tube articulated to an accessory piece with lightly sclerotized proximal part; the second group (2 species) is characterized by MCO with sock-like base and non-articulated accessory piece with richly intertwined distal part; the third group consists of 3 species with an oval base of the copulatory tube and articulated accessory piece with sickle shaped distal part. The shared characters mentioned above may indicate a close relationship among the respective *Annulotrema* spp. Nonetheless, phylogenetic analyses on species of *Annulotrema*, including those 10 found by us, will be necessary to confirm their apparent close relatedness.

*This study was supported by the Czech Science Foundation (project no. LC 522 and P505/12/G112).*

---

STUDY OF SPECIES DIVERSITY OF VIVIPAROUS MONOGENEANS PARASITIZING AFRICAN CICHLIDS: LOOKING FOR A NEEDLE IN A HAYSTACK

P. Zahradničková¹, M. Barson², W.J. Luus-Powell³, M. Gelnar¹, I. Přikrylová¹,³
Department of Botany and Zoology, Faculty of Science, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic¹; Department of Biological Sciences, University of Zimbabwe, PO Box MP167, Mt. Pleasant Harare, Zimbabwe²; Department of Biodiversity, University of Limpopo, Turfloop Campus, Private Bag X1106, Sovenga, 0727 South Africa³

...
The diversity of monogenean ectoparasites of Gyrodactylidae Cobbold, 1864 is well known with more than 440 described species worldwide. However, to date, gyroactylids from the African continent has not been well-researched. In order to better understand the diversity of viviparous monogeneans parasitizing African cichlids, various species of cichlids were collected and examined during 2011-2013 from Zimbabwe, South Africa, Burundi and Democratic Republic of Congo. In addition viviparous parasites of northern African cichlids, originating from Sudan and Egypt were also included in the study. Thus material from four African riverine basins were examined, i.e. Limpopo River basin (Nwanedzi River, South Africa), Zambezi River basin (Zambezi River, Kariba and Chivero Lakes, Zimbabwe), Congo River basin (Lake Tanganyika, Burundi and Democratic Republic of Congo) and Nile River basin (Nile River, Sudan and Egypt). Viviparous monogeneans of the genus *Gyrodactylus* Nordmann, 1832 were recorded from a wide range of cichlid hosts, representing five different tribes. The tribes and infected host were as follows: Ectodini - *Ophalmotilapia nasuta* (Poll & Matthes, 1962); Eretmodini - *Eretmodomus markingsmithi* Burgess, 2012; Haplochromini - *Astatotilapia burtoni* (Günther, 1894), *Ctenochromis horei* (Günther, 1894), *Pharyngochromis acuticeps* (Steindachner, 1866), *Pseudocrenilabrus philander* (Weber, 1987); Tropheini - *Simochromis diaphragma* (Günther, 1894), and Tilapiini - *Oreochromis niloticus* (L.), *Tilapia rendalli* (Boulenger, 1897), *Tilapia sparrmanii* (Smith, 1840), *Tilapia zillii* (Gervais, 1844) and *Tilapia* sp. Identification of parasite species based on haptoral sclerite morphometry and nuclear ribosomal DNA internal transcribed spacer (ITS) sequences identified 15 *Gyrodactylus* species. Only three of them are currently known to science i.e. *Gyrodactylus nyanzae* Paperna, 1973, *Gyrodactylus sturmbaueri* Vanhove, Snoeks, Volckaert & Huyse, 2011, and *Gyrodactylus yacatli* García-Vásquez, Hansen, Christison, Bron & Shinn, 2011. Identification of 12 undescribed *Gyrodactylus* species insinuates high diversity of these parasites in Africa. Based on the shape of the haptoral complex, species were divided into groups within each of them the species can be distinguished based on measurements of the haptoral sclerites and/or the shape of marginal hook sickles. The use of principal component analyses (PCA) helped to distinguish between the different *Gyrodactylus* species and their relative positions were plotted. Phylogenetic analyses, the maximum likelihood (ML) and Bayesian interference (BI), revealed interspecific relationship and evolutionary lineages indicating different origin of *Gyrodactylus* species parasitizing African cichlids.

*Study was financially supported by the projects P505/11/P470 and GBP505/12/G112 from the Grant Agency of the Czech Republic.*

---

**THE TAXONOMIC STATUS OF SPECIES OF PHYSALOPTERA RUDOLPHI, 1819 (NEMATODA: PHYSALOPTERIDAE) PARASITIZING REPTILES IN BRAZIL**

F.B. Pereira¹, T. Scholz², J.L. Luque¹

Departamento de Parasitologia Animal, Universidade Federal Rural do Rio de Janeiro, Seropédica, BR 465 Km 7, Rio de Janeiro, Brazil¹; Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic²

*Physaloptera* Rudolphi, 1819 is a species-rich nematode genus which includes 105 nominal species found in the stomach of all groups of tetrapods. These parasites have global distribution and most of the species have been described from mammals. To date, ten *Physaloptera* species have been described in reptilian hosts, seven of which in reptiles from Brazil. To date, ten *Physaloptera*
species have been described in reptilian hosts, seven of which from Brazil. Of these seven species, two have been described recently namely, *P. tupinambae* Pereira, Alves, Rocha & Souza Lima, 2012 and *P. bainae* Pereira, Alves, Rocha, Souza Lima & Luque, 2014, both parasitic in the large “tegu” lizard *Salvator merianae* (Duméril & Biron). Additionally, *P. monodens* Molin, 1860 originally described from *Boa constrictor* (Linnaeus), is considered a junior synonym of *P. obtusissima* Molin, 1860 described from a “colubri”, in fact an unidentified snake. The remaining four species, as well as *P. obtusissima*, require taxonomic revision. For example, *P. bonnei* Ortlepp, 1922 was described from a type host defined only as “Sapakara”, which in Suriname is the word to designate large teiid (Teiidae) lizards; this lizard probably belongs to *Tupinambis teguixin* (Linnaeus). *Physaloptera liophis* parasitic in the snake *Erytrolamprus miliaris* (Linnaeus), *P. bonnei* and *P. obtusissima* were never reported since their original description. It is a strange fact, because several reptile species have been analyzed with frequency for parasites. On the other hand, *P. retusa* Rudolphi, 1860 originally described from *T. teguixin* is the most reported physalopterid among the Brazilian reptilian fauna, occurring on several lizard species. Other problem is that the majority of *P. retusa* taxonomic reports contains several morphological and morphometric divergences from each other and from the redescription of type material. *Physaloptera lutzi* Cristófaro, Guimarães & Rodrigues, 1976 a parasite originally described from *Ameiva ameiva* (Linnaeus), is also often reported from other lizards, but different from *P. retusa* not has divergences on morphology and morphometry. Another problem is that most of the reports of *Physaloptera* parasitizing reptiles in Brazil are not from experts on nematode taxonomy, which can cause misidentifications of the parasite. Among all the *Physaloptera* spp. from reptiles in Brazil, only *P. bainae* has studies of ultra-structure revealed by scanning electron microscopy (SEM). Analyses by SEM are very important on this group of parasites, because they can reveal important interspecific features as have been occurred for *Physaloptera* of mammals and reptiles from other localities. Additionally, except for *P. bainae* and *P. tupinambae* all the other species require a redescription, because they lack important details such as good drawings, accurate description of structures present in the apical region of anterior end and more morphometric data. Therefore, a detailed evaluation of *Physaloptera* specimens deposited in helminthological collections from Brazil and mainly from Vienna is required to resolve these problems.

**COMPARISON OF GASTROINTESTINAL HELMINTH INFECTION INTENSITY WITH HEMATOLOGIC PARAMETERS AND ALBUMIN IN SHEEP AND GOATS**

Z. Kazatelová¹, V. Svobodová¹, J. Illek², E. Vernerová³

Department of Pathological Morphology and Parasitology, Faculty of Veterinary Medicine VFU, Brno¹; Large animal clinical laboratory, Faculty of Veterinary Medicine VFU, Brno, Czech Republic²; Institute for State Control of Veterinary Biological and Medicines, Brno, Czech Republic³

Parasite occurrence in sheep and goat farms is one of the main problems which the breeders and keepers have to deal with. When the parasitic infection raises more than the organism is able to accept it manifests by general health alterations; mostly diarrhea, weight loss, swellings and anaemia. The intensity of clinical signs often depends on nutrition quality.

This study focuses on the severity of parasitical gastroenteritis (PGE) in comparison with haematological and biochemical parameters.

We overall visited 14 farms in the Czech Republic and we took 207 blood and faeces samples from 53 goats and 154 sheep. The tested animals were of different breeds (Romney, Suffolk, Clun forest, Romanov sheep, Friesian milk sheep etc.) They were also in different age groups. We focused
mainly on one and two-year-old animals and we also preferred more those in worse health state with clinical signs (diarrhoea, low body condition).

We took the blood and faeces samples from goats and sheep from April 2013 to March 2014. Faeces sampling was performed individually from rectum and blood was taken from the jugular vein to sample tubes. Faeces was tested by flotation, sedimentation and valued semi-quantitatively (+,++,+++). EPG assessment was done with the Flotac method but only if the parasitic occurrence was higher than one +. In blood samples we analyzed albumin, numbers of red and white blood cells, hematocrit, haemoglobin, mean cell volume (MCV), mean corpuscular haemoglobin MCH), mean cell haemoglobin cobcreation (MCHC) and in indicated cases also other parameters as zinc, beta-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), calcium, phosphorus, protein, bilirubin, aspartate aminotransferase (AST), iron, creatine kinase (CK), magnesium.

The overall prevalence of gastrointestinal nematodes was 77, 27% in sheep and 86, 79% in goats. The prevalence of tapeworms was 10, 90% in sheep and 0% in goats. Albumin values were in helminth infested animals under the physiological level in 91,30% in goats and 45,95% in sheep. Hematological parameters were measured bellow the physiological level in hematocrit in 71,74% goats and 58,33% sheep; haemoglobin in 52,17% goats and 35,0% sheep. Less influenced components of the blood were erythrocytes – erythrocytopenia was found only in 8,70% of goats and 4,20% of sheep. The animals positive with endo-parasites had leucocytosis in 45,65% in goats and 47,06% in sheep. Goats older than 3 years bear the situation considerably worse in contrast to sheep; the worst health alterations were in one-year-old and younger sheep.

Alterations of analyzed parameters were clearly worse in farms were we noticed inadequate nutrition. Goats are more vulnerable and if we want to keep their good health status and high quality milk production we have to provide sufficient anti-parasitic programmes, nutrition and overall welfare. If we secure adequate breeding and keeping conditions, the animals are able to live with mild parasitic infection without any clinical signs or production loss.

ASCARIDOIDEA FROM BARENTS SEA

K. Najda1, A. Kijewska2, J. Dzido1, J. Rokicki1

University of Gdańsk, Wita Stwosza 59, 80-308 Gdańsk, Poland1; Institute of Oceanology PAS, Powstańców Warszawy 55, 81-712 Sopot, Poland2

The Barents Sea contains around 150 different fish species. Among these are some of the world’s biggest fish stocks, including the cod, haddock, saithe and Greenland halibut. Those economically important fish species are hosts for many parasites, including nematodes from Ascaridoidea superfamily.

Members of the Ascaridoidea (Nematoda: Secernentea) are widespread around the world. Adults of most of these parasites live in the alimentary tract of the vertebrate host (fish, birds, mammals), and are present in both marine and freshwater environments. The life cycles of marine ascarooids are heterogenous. The study shows that the parasitic fauna of fishes being the paratenic, intermediate or final hosts reflects the feeding behavior of the hosts linking the parasites' life cycles. The degree of accumulation of parasites and the proportions between individual parasite species may probably be associated with diet composition and its alteration as the fish grow.

The survey was developed in October and November in 2006 and 2011 year, on the Barents Sea. The parasitic fauna of 14 fish species belonging to families: Pleuronectidae, Gadidae, Sebastidae,
Macrouridae, Rajidae, Cyclopteridae, Anarhichadidae, Zoarcidae, Lotidae and Argentinidae was studied. Greenland halibut was dominating in all hauls. Six nematode species were recorded, including first record of *Contracecum osculatum* C reported from the Greenland halibut.

The fish species supporting the highest nematode species richness are known to occur in deeper water, as well as in the epipelagic zone. As opposed to other fish species examined, the Greenland halibut is not exclusively bathydemersal or pelagic, but its depth range covers almost the entire depth range of all the fish species examined in this study. This fact lends support to the hypothesis that some Ascaridoidea species are decidedly epipelagic and pelagic, whereas others complete their life cycles at specific depths. The scrutiny of parasite phylogeny from the perspective of the intermediate and paratenic host ranges could be a good supplement to the analysis of anisakid distribution. The occurrence and abundance of anisakids reflects the distribution of hosts and their diet preferences. An examination of all these factors altogether could result in a more detailed understanding of the interactions between fish stocks and the quality of their environment.

This study was supported: by the National Science Centre grant No. DEC-2011/01/B/NZ8/04194 and from European Social Fund in as a part of the project "Educators for the elite - integrated training program for PhD students, post-docs and professors as academic teachers at University of Gdansk" within the framework of Human Capital Operational Programme, Action IV.

**COMPARISON OF URBAN AND RURAL ECOSYSTEMS FROM PARASITOLOGICAL POINT OF VIEW**

**J. Pipiková, I. Papajová**

Institute of Parasitology SAS, Hlinkova 3, 040 01 Košice, Slovak Republic

The aim of this epidemiological study was to observe the parasitic contamination of abiotic environmental components in different types of ecosystems and to detect possible source of their contamination. As model ecosystems were selected villages Rudňany and Petrová (rural ecosystem) and towns Snina and Zvolen (urban ecosystem).

Total 109 samples of dog’s droppings, 51 samples of stool and 10 samples of soil were collected in model rural localities. 90.32% of dog’s excrements from Rudňany and in 97.87% of samples from Petrová contain at least one species of endoparasite. 11 different species of intestinal parasites were detected in faecal samples from rural ecosystem as follows: eggs of family *Ancylostomatidae* (83.49%), *Ascaris* spp. (55.96%), *Toxocara* spp. (41.28%), *Trichuris* spp. (24.77), oocysts of *Isospora* spp. (21.10%), eggs of *Toxascaris leonina* (18.35%), sporocysts of *Sarcocystis* spp. (16.51%), cysts of *Giardia duodenalis* (11.92), eggs of *Capillaria* spp. (5.50%), *Taenia* spp. (5.50%) and *Dipylidium caninum* (0.92%). In children population was in the stool detected eggs of *Ascaris lumbricoides* (58.82), *Trichuris trichiura* (11.76%), *Hymenolepis nana* (3.9%) and *Giardia duodenalis* (1.9%). The most frequent eggs in soil from rural areas were *Ascaris* spp. (60.00%).

From model urban localities, total 24 samples of dog’s droppings, 53 samples of stool, 33 samples of soil and sand from children playgrounds and 10 samples of surface and waste water were collected. Dog’s excrements from urban areas contain 4 species of endoparasites as follows: *Toxocara* spp. (20.83 %), eggs of family *Ancylostomatidae* (20.83%), *Capillaria* spp. (4.19%) and sporocysts of *Sarcocystis* spp. (4.19%). Of all 53 stool samples only one was positive to *A. lumbricoides*. Soil and sand contain mostly propagative germs of *Toxocara* spp. (13.33%).
Based on the results we can conclude, that rural ecosystem is more polluted with endoparasitic germs than urban. Increased risk of transmission of parasitoses in rural ecosystem is predominantly in the areas with low hygienic standards, low level of health awareness, and poor level of technical infrastructure. In Slovak Republic is such type of environment represented mostly by villages with Roma settlements.

This study has been realized thanks to the financial support of the project VEGA No. 2/0140/13

SESSIONS

Ticks, mites and mosquitoes – hidden beauty of little vampires

DISTINCT ANAPLASMA PHAGOCYTOPHILUM GENOTYPES ASSOCIATED WITH IXODES TRIANGULICEP S TICKS AND RODENTS IN CENTRAL EUROPE

L. Blaňarová1, M. Stanko1,2, G. Carpi3,4, D. Miklisová1, B. Víchová1, L. Mošanský1, M. Bona5, J. Kraljik1,6, B. Petko1, M. Derdákova1,2

Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovakia1; Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovakia2; Fondazione Edmund Mach, Trento, Italy3 The Pennsylvania State University, USA4; Department of Anatomy, Faculty of Medicine, UPJŠ, Šrobárova 2, 041 80 Košice, Slovakia5; Department of Zoology, Faculty of Natural Sciences, Comenius University, Mlynská dolina, 842 15 Bratislava, Slovakia6

Rodents are important reservoir hosts of tick-borne pathogens. Anaplasma phagocytophilum is the causative agent of granulocytic anaplasmosis of both medical and veterinary importance. In Europe, this pathogen is primarily transmitted by the generalist Ixodes ricinus tick among a wide range of vertebrate hosts. However, to what degree A. phagocytophilum exhibits host specificity and vector association is poorly understood. To assess the extent of vector association of this pathogen and to clarify its ecology in Central Europe we have analyzed and compared the genetic variability of A. phagocytophilum strains from questing I. ricinus and feeding I. ricinus and Ixodes trianguliceps ticks, as well as from rodents tissue samples at four sites in Slovakia.

Tick collections and trapping of rodents were performed in years 2011 – 2013 at four sites in Eastern Slovakia which were characterized by contrasting occurrence of I. ricinus and I. trianguliceps ticks. Genetic variability of this pathogen was studied by DNA amplification and sequencing of four loci followed by Bayesian phylogenetic analyses. A. phagocytophilum was detected in questing I. ricinus ticks (0.7%) from all studied sites and in host feeding I. trianguliceps ticks (15.2%), as well as in rodent biopsies (ear - 1.6%, spleen - 2.2%), whereas A. phagocytophilum was not detected in rodents in those sites where I. trianguliceps ticks were absent. Moreover, Bayesian phylogenetic analyses have shown the presence of two distinct clades and tree topologies were concordant for all four investigated loci. Importantly, the first clade contained A. phagocytophilum genotypes from questing I. ricinus and feeding I. ricinus from a broad array of hosts (i.e.: humans, ungulates, birds and dogs). The second clade comprised solely genotypes found in rodents and feeding I. trianguliceps.
In this study we have confirmed that *A. phagocytophilum* strains display specific host and vector associations also in Central Europe similarly to the situation in United Kingdom and that *A. phagocytophilum* genotypes associated with rodents are probably transmitted solely by *I. trianguliceps* ticks, thus implying that rodent-associated strains may be not of risk for humans.

*The study was supported by the projects of Research & Development Operational Programme funded by the ERDF (code ITMS: 26220220116) (0.2), VEGA 2/0055/11, by the Slovak Research and Development Agency under contract No. APVV – 0267 - 10, EU project FP7 EDENext.*

**TICKS AND RODENTS WITH ANAPLASMA PHAGOCYTOPHILUM AND CANDIDATUS NEOEHRlichIA MIKURENSIS INFECTION IN SOUTHERN HUNGARY**

S. Szekeres\(^1\), K. Rigó\(^1\), G. Majoros\(^1\), E. C. Coipan\(^2\), S. Jahafari\(^2\), H. Sprong\(^2\), G. Földvári\(^1\)

Department of Parasitology and Zoology, Faculty of Veterinary Science, Szent Istvan University, Budapest, Hungary\(^1\); Laboratory for Zoonoses and Environmental Microbiology, National Institute for Public Health and Environment (RIVM), Antonie van Leeuwenhoeklaan 9, P.O. Box 1, Bilthoven, Netherlands\(^2\)

*A. phagocytophilum* has been long known as the pathogen causing “tick borne fever”. Wild ruminants and rodents have the biggest role in pathogen life cycle and ticks can infect domestic species and also humans. Ca. *N. mikurensis* is a new human pathogenic bacterium belonging also to Anaplasmataceae first detected in the late 1990s. The key role of rodents as reservoir host has recently been proven. In order to investigate these tick-borne bacteria in South-Hungary we collected small mammals with live-traps (2010-2013) and questing ticks with flagging in 2012. We euthanized the small mammals and collected tissue samples and removed all the ectoparasites and stored in 70% alcohol. We found low tick infestation (8%). Samples were analysed for *A. phagocytophilum* and Ca. *N. mikurensis* with multiplex quantitative real-time PCR.

A part of msp2 gene from *A. phagocytophilum* and the groEL heat shock protein gene from Ca. *N. mikurensis* were amplified to examine the infection. We found in the tissue samples 7% (skin) and 5.27 % (spleen) *A. phagocytophilum* and 2.25% (skin) and 2.87% (spleen) Ca. *N. mikurensis* infection. Among questing ticks we found three *I. ricinus* (female, male, nymph) with Ca. *N. mikurensis* (1.85%) and five ticks with *A. phagocytophilum* (two *D. reticulatus* female, two *H. concinna* female, one *I. ricinus* male) (3.1%) infection. We found one Ixodes ricinus nymph removed from male *Apodemus flavicollis* with *A. phagocytophilum* infection. This study showed the presence of these pathogens with relatively low prevalence in questing ticks, engorged ticks and rodent tissue samples.
FOSTER SPECIES OR PHYLOGENY DOES MATTER? QUILL MITE FAUNA OF AVIAN BROOD PARASITES
M. Klimovičová1, M. Skoracki2, M. Hromada1

Laboratory and Museum of Evolutionary Ecology, Department of Ecology, Faculty of Humanities and Natural Sciences, University of Prešov, 17. novembra 1, 081 16 Prešov, Slovakia1; Department of Animal Morphology, Faculty of Biology, Adam Mickiewicz University, Umultowska 89, 61–614 Poznań, Poland2

Avian brood parasites lay their eggs in nests of other species (hosts) which incubate and rear their offspring. This uncommon reproduction strategy refers only to 1% of all avian species. Biology and ecology of brood parasites and their hosts, including ectoparasites of both, has been intensively studied. Ectoparasites (e.g. lice and various groups of parasitic mites) are primarily transmitted vertically, from parents to their offsprings, but this is, of course, not possible in brood parasites. Ectoparasites typical for hosts have been only rarely found on fledglings of cuckoos and never on adults. Therefore, it seems that there exist some substantial difference in ectoparasites’ ecological niches on foster species and their brood parasites. In our study, we primarily focused on question, whether the similar pattern occurs also in quill mites. Quill mites (Syringophilidae) are the permanent, highly specialised mono- or oligoxenous ectoparasites of birds. We investigated thoroughly 23 species of avian brood parasites in four families and we found out quill mites in five species from family Cuculidae, four Viduidae, one Picidae and one from Icteridae; we have studied also their sister, non-parasitic lineages. We have found out that whole lineage of parasitic cuckoos plus their most closely related non-parasitic species are infected byclade-specific quill mite. Similarly, all parasitic birds of clade Viduidae plus one weaver finch harbour one common quill mite species. Brood parasitic honeyguides parasitized by the same quill mite like three its hosts, however, identical species harbour also whole woodpecker clade (17 species). On the other side, parasitic cowbird does not share quill mite with its host, but another five icterid birds harbour a different common quill mite. The pattern we have found seems to support the hypothesis that, in avian brood parasites, there is substantially lesser species richness of ectoparasites. In different brood parasitic lineages, both phylogeny and transmission from closely related avian hosts seem to form their quill mite fauna.

SPECIES DIVERSITY AND SEASONAL DYNAMICS OF CULICOIDES IN EASTERN SLOVAKIA
A. Sarvašová1, A. Kočišová1, I. Sopoliga2, H. Hlavatá3

University of Veterinary Medicine and Pharmacy in Košice, Department of Parasitology, Komenského 73, 04181 Košice1; University of Veterinary Medicine and Pharmacy in Košice, Special Centre for study of Breeding and Diseases of Wild Animals, Fish and Bees, Rozhanovce2; Slovak Hydrometeorological Institute, Žumbierska 20, Košice3

Since 2008, Europe faced with outbreaks of diseases caused by Bluetongue and Schmallenberg viruses which were transmitted by the biting midges of the genus Culicoides (Diptera: Ceratopogonidae). The comprehensive of epidemiology is limited by the lack of knowledge regarding bionomics of the Culicoides in central Europe. We studied diversity, distribution and population dynamic of Culicoides in Eastern Slovakia. We implemented an entomological survey in three sites: two farms with cattle (Michaľany, Tulčík) and a game park in Rozhanovce. On each site, midges were weekly collected using UV light
trap between 2011 and 2013. Meteorological data were obtained from Slovak Hydrometeorological Institute.

A total of 157,785 Culicoides were collected, belonging to at least 41 species. During this survey 5 new species for Slovak fauna have been recorded, namely C. picturatus, C. clastrieri, C. gejgelensis, C. griseidorsum and C. odiatus. The highest species diversity (35 species) and abundance (Σ=97,945) was in south eastern Slovakia (Michaľany) compared to Tulčik (27 species, Σ=41,670) and Rozhanovce (31 species, Σ=18,167). The potential vectors of pathogens, C. obsoletus/C. scoticus, were found to be highly abundant with 79% of the total specimen trapped in Tulčik and 68% in Rozhanovce. The second most abundant species were the species of the Pulicaris complex with 14% and 12% respectively. In Michaľany, most of the Culicoides captured belonged to the Pulicaris complex (49%) and C. obsoletus/C. scoticus (35%). The species of the Nubeculosus complex were present in high abundance (8%) only in this lowland area of south eastern Slovakia. The most abundant species C. obsoletus/C. scoticus completed 3 generations throughout the season and 2-3 generations was determined for C. pulicaris/C. punctatus.

The population dynamics of Culicoides depend on environmental factors and primarily the weather. In general, only few Culicoides were collected in temperatures below 8 °C. Statistical analyses showed a positive correlation between Culicoides abundance and the average of temperature. The dependence on minimal daily temperature was significant in Michaľany and Rozhanovce. Midge abundance was negatively correlated by wind speed in Tulčik and by relative humidity in Rozhanovce.

Adults’ activity of the biting midges was pointed out from April to November meaning a vector free period from December to March. The eastern Slovakia provides suitable biotopes for developments of Culicoides especially potential vectors of pathogens.

This research was supported by grant VEGA No. 1/0236/12, basic research of National Reference Laboratory for Pesticides of the University of Veterinary Medicine in Košice and execution of the Project “Centre of Excellence for Parasitology” (ITMS code: 26220120022) upon the support of the operation program Research and Development, financed by the European Regional Development Fund (part 0.5).

GENETIC DIVERSITY OF ANAPLASMA PHAGOCYTOPHILUM AND RESERVOIR COMPETENCE OF WILD LIFE ANIMALS FOR TICK-BORNE PATHOGENS IN NORTHERN ITALY

I. Baráková¹, G. Carpi¹, F. Rosso¹, M. Chvostač², V. Tagliapietra², H.C. Hauffe², A. Rizzoli³, M. Derdáková¹

Institute of Zoology, SAS, Dúbravská cesta 9, 845 06 Bratislava, Slovakia¹; Fondazione Edmund Mach, Via Edmund Mach, 1, 38010 San Michele All’adige Trento, Italy²; Yale School of Public Health, 60 College Street, New Haven, CT 06520, USA³

Tick borne diseases are zoonoses causing infection in humans and domestic animals. The most spread zoonoses in Europe are the one caused by Borrelia burgdorferi sensu lato. Anaplasma phagocytophilum, Babesia spp., and Rickettsia spp. Impact of global, climatic and social changes occurring in recent years has increased incidence of ticks and number of infected people and animals. Movement of ticks into higher altitudes also causes the emergence of new foci.
Our study was carried out in the Valle dei Laghi (northeastern Italian Alps). We have studied the prevalence and genetic variability of *A. phagocytophilum* and its ecological associations with hosts and vectors in the area. A total of 821 questing *I. ricinus* ticks were collected by dragging and 284 engorged ixodid ticks were collected from humans and from hunted or live-trapped wild-life animals. Further they were analyzed for the prevalence of *A. phagocytophilum*. All positive samples were used for amplification of *groEL* and *msp4* genes for further phylogenetic analysis. In addition 448 larve ticks from wild ungulates, birds and rodents were analyzed for reservoir competence for *A. phagocytophilum, Babesia spp.*, *B. burgdorferi* s.l. and *Rickettsia spp.*

Overall *A. phagocytophilum* prevalence in questing *Ixodes ricinus* ticks was 1.8%, in ticks from various host was: 4.3% in ticks from humans, 9.1% in ticks from dogs, 14.3% in ticks from wild ungulates, 7.7% in ticks from sheep, 10.7% in ticks from birds and 6.1% in ticks from rodents. Prevalence in rodent blood samples (*A. flavicolis, M. avellanarius, M. glareolus*) was only 0.3%. Phylogenetic analyses of *msp4* and *groEL* showed two distinct enzootic cycles of *A. phagocytophilum* associated with different reservoirs as well as vectors. Strains that infect humans belong to the clade formed out of strains from engorged ticks collected from dogs, wild ungulates, sheep and birds. Strains obtained from rodents are most likely transmitted by other tick vector and may not represent an immediate threat to humans in northern Italy. Our results shows different ecology of *A. phagocytophilum* as it is in United States.

Moreover, from total of 339 engorged larvae *I. ricinus* ticks from rodents 9.1% were positive for *B. burgdorferi* sensu lato, 5.3% positive for *Rickettsia spp.* and 1.8% positive for *Babesia spp.* From 99 engorged larvae *I. ricinus* ticks from wild ungulates 6% were positive for *A. phagocytophilum*, 6% positive for *Rickettsia spp.* and 1% positive for *Babesia spp.* From 10 engorged larvae *I. ricinus* ticks from birds 80% were positive for *B. burgdorferi* s.l. High degree of genetic variability was observed.

Our study revealed that in mountainous habitats of Norther Italy foci of tick-borne diseases are emerging. Small mammals and wild ungulates are the most important hosts for ticks as well as reservoirs for pathogens, however they participate differently in their ecology and circulation of tick borne agents in natural foci.

*The study was supported by the European Union grant FP7-261504 EDENext*
Common diagnostic practice in parasitology includes also distinguishing of parasitic organisms from various artefacts. It involves differentiation between parasites and pseudoparasites, spurious parasites and parasite delusions. Pseudoparasites are usually characterised as ingested objects that resemble parasite forms, e.g. pollen grains, plant hairs, grain mites, air bubbles, spores and different animal or plant debris. Spurious or false parasites are commensal or temporary parasites being accidentally ingested and passed through the animal, sometimes briefly surviving in the intestine. Typical feature of parasite delusion also called Morgellons disease is impossibility to find any signs of parasitic organisms in the sample, while the patient describes the imaginary parasite and clinical signs of the disease in detail. The sufferers firmly believe that they have the pruritus due to an infestation with insects or parasites. Patients usually present with clothing fibres, pieces of skin or other debris contained in plastic wrap, on adhesive tape, or in matchboxes. Incorrect diagnosis and identification of objects as parasites results to economic losses due to expenses for further diagnostic tests and treatment. Moreover, unnecessary treatment with antiparasitic drugs can affect the health and welfare of animals or humans. The work presents some of pseudoparasitic findings diagnosed during our praxis.

*The work was supported by the Science Grant Agency VEGA 2/0127/13 (0.5) and project “Application Centre for Protection of Humans, Animals and Plants against Parasites” (code ITMS: 26220220018), supported by the Research & Development Operational Programme funded by the ERDF (0.5).*

**THE INDIVIDUAL SUSCEPTIBILITY FOR GASTROINTESTINAL NEMATODES (STRONGYLIDAE) INFECTION IN HORSES**

M. Basiaga¹, S. Kornaś¹, Z. Podstawski², K. Szawica¹, J. Kowal¹, P. Nosal¹  
Department of Zoology and Ecology, University of Agriculture in Krakow, al. Mickiewicza 24/28, 30-059 Krakow, Poland¹; Department of Horse Breeding, University of Agriculture in Krakow, al. Mickiewicza 24/28, 30-059 Krakow, Poland²

Parasites infection in livestock depends on many factors. Their occurrence is attached with low management conditions of animal welfare. Deworming is a procedure that reduces the level of the infection, but does not remove parasites from environment. In horses, the most common parasites are nematodes from Strongylidae family which inhabits cecum and colon of the host. These parasites are reported in Poland in more than 90% of horses. The purpose of the observation was to
demonstrate that the presence of parasites does not depend only on the welfare, but host individual’s susceptibility.

The study was conducted from Autumn 2012 to Spring 2014, including 18 horses from Experimental Station of the Department of Horse Breeding UR in Krakow. The horses were kept under housed-pasture management system and dewormed twice a year: before and after the pasture season. The coproscopical study was performed using McMaster method. Based on the obtained results prevalence of infection as well as the mean number of eggs of parasites in 1g of feces (EPG) were determinate. On the basis of the study, horses were divided into three groups (group I - 50-350 EPG, group II - 700-1350 EPG, group III - 1600-2400 EPG) depending on the level of infection and within them the parasites reinfection was observed.

In majority of the horses, infections of strongylids (Strongylidae) were found. The study showed, what is most interesting, that during the two years of survey 5 horses among 18 checked, were constantly free from Strongylidae infection (despite the lack of deworming). Among infected horses reinfection of strongylids occurred after 60 days post-deworming. In horses from groups I and III reinfection (number of EPG) was higher in Spring, while in group II differences were slightly reversed and were as follows: Spring - 925 EPG and Autumn - 1000 EPG.

Conclusion: Effective parasite control, despite management system, should take into account individual susceptibility of animals to infections. Moreover regular use of parasitic drugs from one class, especially without knowledge about the level and type of parasitic infections, in combination with the occurrence of many subpopulation of those parasites like adult forms in the intestinal lumen, hypobiotic larvae present in the intestinal mucosa as well as invasive larvae in environment favors the emergence of drug-resistant populations of strongylids.

DNA-BASED EVIDENCE OF HOST RELATED PLASTICITY OF MORPHOLOGICAL CHARACTERS IN CARYOPHYLLAEUS LATICEPS AND CARYOPHYLLAEUS BRACHYCOLLIS (CESTODA: CARYOPHYLLIDEA)

E. Bazsalovicsová¹, I. Králová-Hromadová¹, J. Brabec², V. Hanzelová¹, M. Oros¹, L. Zvijáková¹, T. Scholz²

Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovakia¹; Institute of Parasitology, Biology Centre ASCR, České Budějovice, Czech Republic²

An extensive collection of morphologically polymorphic cestodes of the genus Caryophyllaeus (Cestoda: Caryophyllidea), parasites of cyprinid fish in the Palaearctic Region, were analysed using partial sequences of mitochondrial cytochrome c oxidase subunit I (cox1) and nuclear ribosomal large subunit RNA (lsrDNA) genes. Results of the molecular phylogenetic analysis raised the question about the validity of the traditional morphological criteria employed in caryophyllidean systematics including the scolex morphology currently used for species circumscription. Several clades within both of the Caryophyllaeus laticeps (Pallas, 1781) and Caryophyllaeus brachycollis (Janiszewska, 1951) species lineages were recognized, largely corresponding to specific morphotypes from different hosts and geographical regions. This suggested host- and probably geography-related intraspecific morphological variability and confirmed euryxenous host specificity of both taxa, especially C. laticeps, but did not seem to support the existence of cryptic species within this cestode genus. Results of the present study cast doubts on the traditionally used morphological concept of
species of the order Caryophyllidea, especially due to high morphological plasticity of the scolex and the anterior end of the body - the characteristics widely used for caryophyllidean species delimitation. The current data pointed out the fact that outcomes of traditional morphological descriptions and molecular analyses may be in conflict. In genus Caryophyllaeus, morphological polymorphisms were not supported by molecular data and morphological plasticity is apparently a result of host effect and geographical adaptation. Since this phenomenon has already been observed in other groups of the order Caryophyllidea, it should be taken into consideration when envisaging their taxonomy.

This study was supported by the Slovak Research and Development Agency (project no. APVV-0653-11), and Grant Agency VEGA (No. 2/0129/12).

SHORT-TERM STABILITY OF BORRELLIA GARINII IN THE CEREBROSPINAL FLUID
D. Berenová, L. Šípková, A. Lukavská, M. Malý, J. Hořejší, P. Kodym
National Institute of Public Health, Šrobárova 48, Prague 10, Czech Republic

The aim of our study was to find out the optimal conditions for short-term storage of cerebrospinal fluid (CSF) samples for direct diagnosis of Lyme disease. A mixture of Borrelia negative CSFs spiked with a defined amount of cultured Borrelia garinii was used. Borrelia stability was investigated over seven days at four different temperatures (RT, +4°C, -20°C, and -70°C). Quantitative changes in CSF Borrelia were measured by RT-PCR and morphological changes in the spirochetes were observed with an electron microscope. RT-PCR results were statistically evaluated. We found +4°C to be an optimal temperature for short-term storage of CSF to be observed under an electron microscope. There was no significant difference between the temperatures tested in the average quantity of Borrelia measured by RT-PCR. On the contrary, electron optical diagnosis of frozen samples and samples stored at RT showed destructive morphological changes and decrease in spirochete counts.

Our results show that the optimum conditions for the preanalytical phase of investigation of one type of material can differ depending on the diagnostic method employed.

This study was supported by grant IGA MZ ČR – No. NT/13467 – 4 from the Internal Grant Agency of the Ministry of Health of the Czech Republic and institutional funds of the National Institute of Public Health (No.1 RVO-SZÚ/2014).

DETECTION OF RICKETTSIA SPP. IN TICKS AND HOSTS FROM CENTRAL PART OF SLOVAKIA
L. Berthová, J. Valariková, M. Quevedo Diaz, E. Špitalská
Institute of Virology, SAS, Dubravska cesta 9, 845 05 Bratislava, Slovakia

Rickettsiae are obligate Gram negative aerobic intracellular bacteria, causing serious diseases in humans. Transmission is provided by arthropods, mainly ticks which serve as vectors and reservoir of infection, respectively. Occurrence of rickettsiae in Slovakia has been continuously monitored and the presence of 5 species (6 strains) has been confirmed: R. slovaca, R. helvetica, R. raoultii, R. monacensis strains IRS3 and IRS4 and R. conorii conorii. From all the species (except of R. conorii
conorii) were obtained isolates. In addition one case of R. africae was reported. This bacterium was identified in Ceratophyllus garei flea collected from Reed warbler (Acrocephalus scirpaceus) crossing Slovakia during spring migration. The aim of this study was to investigate presence of rickettsiae in ticks, rodents and birds in forest habitat in Bojnice.

Together 167 questing ticks (147 Dermacentor marginatus, 6 Haemaphysalis concinna, 5 H. inermis and 4 Ixodes ricinus) were collected by dragging white blanket over the vegetation during 2012. Seventy-four birds of 20 species were caught in mist nets. Each individual was ringed and blood sample was taken by puncture of vena branchialis. Fifty-two (40 Apodemus flavicollis and 12 Clethrionomys glareolus) rodents were live trapped. The total number of trap nights was 400. Trapped rodents were sacrificed humanely, blood samples were obtained from sinus orbitalis and spleens were collected. Seventy three and 21 Ixodes ricinus ticks were removed from rodents and birds, respectively. All tissues and ticks were stored in 70 % ethanol in 4°C. Total genomic DNA was extracted using the Machrey-Nagel NucleoSpin tissue kit and phenol-chlorophorm from tissues and ticks, respectively. Rickettsiae were detected by PCR - amplification of gltA gene. Seroprevalence of rodent sera was evaluated by ELISA.

Rickettsia spp. was found in 17 questing ticks, 16 - D.m. and one H.i., and in 14 I. ricinus ticks collected from rodents. From 52 rodents trapped none of spleens was positive. In rodents we have not detected anti-Spotted fever group (SFG) rickettsiae antibodies. Analyses of blood samples from birds and ticks (N = 21) removed from birds, is in progress.

The results of our study confirm the occurrence of spotted fever group Rickettsia sp. in ixodid ticks from vegetation and hosts, respectively. People visiting forests should be warned of potential risk of tick-bite and the potential transmission of rickettsiae or other tick-borne pathogens.

This study was financially supported by the project VEGA No. 2/0061/13, VEGA No. 2/0193/12 and SRDA-0280-12.

ARE RODENTS RESERVOIRS OF SPOTTED FEVER GROUP RICKETTSIAE?

L. Berthová1, E. Špitalská1, L. Mydlová2, Z. Svitálková2, M. Kazimírová2

Institute of Zoology, SAS, Dúbravská cesta 9, 845 06 Bratislava, Slovakia1; Institute of Virology, SAS, Dúbravská cesta 9, 845 05 Bratislava, Slovakia2

Rickettsiae are gram-negative intracellular bacteria associated with eukaryotic cells within which they live, divide by binary fission and may cause diseases (generally specified as rickettsioses). Their life cycle involves invertebrate hosts (which act as vectors and reservoirs, too) and vertebrate hosts. Plenty of tick-bites are reported by people visiting forests and forest parks in Europe every year. Rodents commonly found in forests are important hosts for larvae and nymphs of ixodid ticks, mainly Ixodes ricinus. The role of rodents as reservoirs of Rickettsia still needs exploration, whereas the wide-spread I. ricinus tick may serve as vector and also long-term reservoir of rickettsiae because of its ability to transmit the pathogen transstadially as well as transovarially. To date, 28 Rickettsia species with validated and published names have been reported. Seventeen of them are recognized as human pathogens. All Rickettsia species found in Slovakia (R. slovaca, R. helvetica, R. raoultii, R. monacensis, R. conorii conorii) belong to the spotted fever group (SFG) and are transmitted by ixodid ticks. The aim of this study was to compare prevalence of SFG rickettsiae in rodents caught in a forest habitat (Prievidza) and a suburban habitat (Bratislava - Železná studnička), located in W and SW Slovakia, respectively.
Small rodents trapping was carried out by using live traps in both habitats in 2012 and 2013. Captured rodents were anaesthetized and sacrificed humanely, identified and examined for ectoparasites. Blood samples and sera were prepared and spleens dissected. Antibodies against spotted fever group rickettsiae in sera were screened by ELISA. Rickettsia was detected in DNA isolated from spleen and blood by PCR targeting the gltA gene.

Rodent trapping during 1600 trap-nights yielded 109 Apodemus flavicollis, 70 Clethrionomys glareolus and one Apodemus sp., which were infested with a total of 122 ixodid ticks (Ixodes ricinus and Haemaphysalis concinna) and 282 ticks (the same tick species) in Prievidza and Bratislava, respectively. We have detected rickettsial DNA in 14.05 % and 17.89 % of ticks collected from rodents. We haven’t detected anti- SFG rickettsiae antibodies. All spleens and blood samples of rodents from Bratislava were negative for rickettsial DNA. Analysis of rodent blood samples from Prievidza is in progress.

The results of the study suggest that rodents are probably not reservoir hosts of SFG rickettsiae in Slovakia.

This study was financially supported by the FP7 project EDENext (No. 261504), grant APVV DO7RP–0014–11, APVV-0280-12 and grant VEGA No. 2/0061/13.

ASSOCIATION OF CANDIDATUS NEOEHRlichIA MIKURENSIS AND BABESIA MICROTI WITH RODENTS AS RESERVOIR HOSTS IN NATURAL FOI OF SLOVAKIA (CENTRAL EUROPE)

L. Blaňarová1, M. Stanko1,2, D. Miklisová1, B. Víchová1, L. Mošanský1, M. Bona3, J. Kraljik1,2, B. Peťko3, M. Derdákova1,2

Institute of Parasitology of the SAS, Hlinkova 3, 040 01 Košice, Slovakia1; Institute of Zoology of the SAS, Löfflerova 10, 040 01 Košice, Slovakia2; Department of Anatomy, Faculty of Medicine, UPJŠ, Šrobárova 2, 041 80 Košice, Slovakia3; Department of Zoology, Faculty of Natural Sciences, Comenius University, Mlynská dolina, 842 15 Bratislava, Slovakia4

Rodents are important reservoir hosts of many tick-borne pathogens. Their importance in the circulation of human pathogens Neoehrlichia mikurensis and intraerythrocytic protozoan parasite, Babesia microti has been recently proposed. The aim was to identify the presence and genetic diversity of N. mikurensis and B. microti circulating in the natural foci between the rodents, Ixodes ricinus and Ixodes trianguliceps ticks and to study their ecological association.

In 2011–2013, rodents were captured at four sampling sites in Eastern Slovakia (Čermeľ, Hýľov, Botanická záhrada, Rozhanovce). Ear and spleen biopsies, feeding I. ricinus and I. trianguliceps ticks from rodents and questing I. ricinus ticks were investigated for the presence of pathogens by molecular methods followed by DNA sequencing.

N. mikurensis was detected in questing I. ricinus ticks (2.5%), spleen biopsies of rodents (1.6%), as well as in feeding I. ricinus (0.3%) and I. trianguliceps (2.7%) ticks from rodents. The 16S rRNA and gltA sequences of N. mikurensis obtained in this study confirmed high degree of homology. DNA of B. microti was found in biopsies of rodents (ears-0.6%, spleen-1.9%), feeding (0.4%) and questing I. ricinus ticks (5.5%). None of the 112 I. trianguliceps ticks were infected with B. microti. BLAST analysis of B. microti nucleotide sequences confirmed the presence of two genotypes, „Jena strain” (92%) and „Munich strain” (8%).
Results of our study confirmed the importance of rodents in the circulation of both emerging pathogens in the natural foci. This might pose a potential threat for humans.

The study was supported by the projects of Research & Development Operational Programme funded by the ERDF [code ITMS: 26220220116] (0.1), VEGA 2/0055/11, VEGA 2/0113/12, the Slovak Research and Development Agency under contract No. APVV–0267-10, EU project FP7 EDENext.

MOSQUITO FAUNA IN MEANDERS OF THE HORNAD RIVER IN THE KOŠICE BASIN

E. Bocková, A. Kočišová

University of Veterinary Medicine and Pharmacy in Košice; Department of Parasitology,
Komenského 73, 041 81 Košice, Slovak Republic

Meanders of the Hornad River that stretch from Trstené pri Hornáde village to the Milhost village represent the last remnants of the riparian forest preserved throughout the Košice Basin and at the same time they constitute the richest ecosystem in the Central European conditions. These ecosystems have been presented as an active and dynamic entity with characteristic habitats. High soil moisture, presence of water resources and wetlands create ideal conditions for the survival of a wide range of animals, including mosquitoes, which are an essential part of floodplain forests.

The aim of our study was to investigate the fauna of mosquito larvae and adult mosquitoes in the condition of floodplain forest and meanders in the alluvium of the Hornad River in the Košice area. The mosquito larvae were collected using a sieve with a small-diameter mesh in periodic waters, wetlands and blind river branches. Adult mosquitoes were sampled using CO2 - baited CDC light traps supplemented with dry ice. Mosquitoes were identified using available identification keys (Becker et al., 2010; Kramar, 1958).

During the three-year research (2011-2013) 3,281 larvae and 10,866 adult mosquitoes of 20 species were captured. They were: Aedes cinereus, Ae. rossicus, Ae. vexans, Anopheles claviger, An. maculipennis, An. plumbeus, Coquillettidia richiardii, Culiseta annulata, Culex modestus, Cx. pipiens, Cx. territans (larvae only), Ochlerotatus annulipes, Oc. cantans, Oc. communis (larvae only), Oc. caspius, Oc. cataphylla, Oc. flavescens, Oc. geniculatus, Oc. pector (only larvae) and Oc. sticticus.

The dominant species of adult mosquitoes were (D > 5 %) Ae. vexans (47.0 %), Oc. sticticus (15.4 %) and Cx. pipiens (26.7 %), in the case of larvae were dominant Cs. annulata (36.8 %), Ae. vexans (31.6 %) and Cx. pipiens (20.6 %).

Floodplain forests represent a very diverse and valuable group of forest communities and they currently rank among the habitats of the European importance. Due to specific environmental conditions of floodplain forests, water regime and frequent flooding, the negative side of these biocenosis are mosquitoes and the seasonal calamity associated with them.

The epidemiological role of mosquitoes as (potential) vectors of pathogens in this area, when considering the geographical location of the studied area, i.e. close proximity to neighboring villages (Gyňov, Trstené pri Hornáde, Milhost and others) should not be underestimated.

This research was supported by the grant VEGA No. 1/0236/12, basic research of the National Reference Laboratory for Pesticides of the University of Veterinary Medicine in Košice and execution of the Project “Centre of Excellence for Parasitology” (ITMS code: 26220120022) upon the support of
**EVIDENCE OF DIOFILARIA REPENS IN MOSQUITOES USING PCR**

E. Bocková, A. Kočišová
University of Veterinary Medicine and Pharmacy in Košice

Slovakia is one of the endemic areas in terms of the incidence of carnivores’ heartworm, especially dogs. Three areas of risk have been identified in the country, namely the Borská, Danubian and Eastern Lowlands.

Our previous surveys have been focused on dogs as definitive hosts of heartworm. We started the initial monitoring aimed to identifying the (potential) vectors of microfilariae of *Dirofilaria* spp. in 2013. The detection of microfilariae in mosquitoes was done by PCR using species-specific primers for *D. repens* and *D. immitis*. PCR assays were performed on 192 pools, each pool containing 50 or 100 mosquitoes of the same species, collected on the same location.

We examined 12,000 mosquitoes belonging to eight species - *Ae. vexans*, *Ae. cinereus*, *Ae. rossicus*, *Culex pipiens*, *Culicesta annulata*, *Ochlerotatus sticticus*, *Oc. cantans* and *Oc. caspius* from six monitored sites (Košice and Trebišov district).

We confirmed *Ae. vexans* as the vector of *D. repens*, which were captured in Košice district – Košické Olšany (48°44’0.96"S, 21°20’44.52"W) and subsequently we detected the microfilariae in mosquitoes from Paňovce (48°38’45.96"N, 21°3’47.52"V). Vector of *D. immitis* have not been yet detected.

This studies represents the first molecular evidence of *D. repens* microfilariae in mosquitoes in Slovakia.

Slovakia is characterized by typical climate with extremely suitable conditions for life cycle and survival of mosquitoes and the spread of diseases. In view of the increasing trend of the spread of dog’s heartworm in this area, from the epidemiological point of view is needed in addition to the definitive hosts due attention to the research vectors.

From the epidemiological point of view of the increasing trend of the spread dirofilariosis in Slovakia is indispensable to continue in research of the potential mosquito vectors.

*This research was supported by the grant VEGA No. 1/0236/12, basic research of the National Reference Laboratory for Pesticides of the University of Veterinary Medicine in Košice and execution of the Project “Centre of Excellence for Parasitology” (ITMS code: 26220120022) upon the support of the operation program Research and Development, financed by the European Regional Development Fund (part 0.5).*

**IMMUNODIAGNOSTIC APPROACHES FOR THE DETECTION OF HUMAN TOXOCARIASIS**

V. Boldiš, F. Ondriska
HPL (Ltd) Medical laboratories, Istrijská 20, 84107, Bratislava, Slovakia

Human toxocariasis is an important zoonosis caused by infection with the larvae of *Toxocara canis* (dog roundworm) and *T. cati* (feline roundworm). Humans become infected by the ingestion of
eggs, which originate from the feces of the definite host (dog or cat) and embryonate in the environment. Depending on the organs affected and the specificity of the symptoms, the predominant clinical syndromes are classified as visceral larva migrans, ocular larva migrans and neurotoxocariasis. *Toxocara* infections in humans are relatively frequent, as indicated by a seroprevalence of 8.4 % in healthy population coming from Bratislava.

Our objective was to evaluate if detection of IgA, IgG anti-*Toxocara* antibodies and determination of the avidity of the specific IgG in human sera could improve the diagnosis of toxocariasis.

Over 3 years, we investigated the presence of IgA, low- and high-avidity IgG antibodies against *Toxocara* spp. in 179 individual patients referred for suspected human toxocariasis (based on seropositivity for IgG antibodies to *Toxocara* spp.), in respect of some determinants such as sex, age, IgG titers, eosinophilia, increased total-IgE, domicile, geophagia, dog/cat ownership, anamnesis. These mentioned characteristics of patients were obtained from physicians involved in the current treatment of the person. Patient information has been provided in 49.2 % of examined cases. *Toxocara* spp. specific IgA and IgG were analyzed using excretory-secretory (ES)-enzyme-linked immunosorbent assay (ELISA) method. The IgG avidity test was performed by ELISA in duplicate rows of 96-well microtiter plates. One row was washed with urea and the other with PBS, then the avidity index (AI) was calculated. The anti-*Toxocara* IgA antibodies were detected in 28.6 % cases. The IgA seropositivity was 63.6 % in examined adults (aged ≥41 years), and 25.6 % in examined children (aged 1 – 10 years). The difference was statistically significant (p = 0.0169). In all patients who were IgA seropositive, high, middle and low IgG titres were detected in 40.9 %, 21.7 % and 5.9 % cases, respectively (p = 0.0482). Low-avidity IgG antibodies against *Toxocara* spp. was found in 30.7 % patients. The seroprevalence of low-avidity IgG antibodies in eosinophilic group (42.1 %) was significantly higher than in non-eosinophilic group (22.0 %; p = 0.0428). Substantially higher prevalence of eosinophilia was detected in children between 1 and 10 years of age (55.6 %) than was that in adults between 41 and more years of age (17.6 %; p = 0.0093). Significant difference in seroprevalence of total IgE in patients coming from towns (48.8 %) and patients coming from villages (21.3 %) was confirmed (p = 0.0066). Similarly, significantly higher prevalence of increased total-IgE antibodies in patients with negative anamnesis for toxocariasis (43.9 %) was recorded in comparison to their presence in patients with positive anamnesis (16.1 %; p = 0.0088). The sensitivity of ELISA IgA and IgG avidity assay were 57.1 % and 43.8 %, respectively. The ELISA for IgA showed the highest specificity (100 %), followed by and IgG avidity assay (83.3 %).

The disadvantage of serology for diagnosis of toxocariasis is that these methods cannot be used to accurately distinguish between recent and distant infection. However, our results suggest that measurement of *Toxocara* spp. specific IgA and IgG avidity may be useful for determination of acute toxocariasis but these tests have to be accompanied by other immunological markers and determinants of examined patients such as *Toxocara* spp. specific IgG, eosinophilia, increased total-IgE, age etc.
MOLECULAR CYTOGENETICS OF ARCHAIC FISH FLUKE ASPIDOASTER LIMACOIDES (TREMATODA: ASPIDOASTREA)

M. Bombarová¹, M. Špakulová¹, M. Skurka¹,2
Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovakia¹; University of Prešov, Faculty of Humanities and Natural Sciences2

Aspidogastrean fluke Aspidogaster limacoides (Aspidogastriidae) was studied using molecular and cytogenetic approaches, resulting in a detailed analysis of karyotype and chromosomal localization of ribosomal genes. The karyotype of A. limacoides comprised 6 medium-sized pairs of chromosomes (the first and the last pair being two-armed while four remaining pairs are one-armed; 2n = 12, n = 1m + 1m-sm + 4t) and differed significantly from the complement of congeneric A. conchicola composed of only 5 short pairs of one armed subtelo – or telocentrics (2n = 10, n = 1st + 4t, according to Petkevičiute, 2001). Chromosomes of A. limacoides possessed small amount of heterochromatin distributed near the centromeres. C-banding with combination of fluorescent DAPI staining revealed weak pericentromeric heterochromatin bands on the pairs Nos. 1, 3, and 6. Silver staining highlighted a single nuclear organizer located in pericentromeric region of the third chromosome pair and thereafter fluorescent in situ hybridization with rDNA probe revealed the ribosomal genes cluster located at the apical end of the third chromosome pair. The secondary constriction was apparent in meiotic prophase, specifically in early diplotene. The present results provide a pilot study on molecular cytogenetics within a group of aspidogastrean flukes and only fifth description of the karyotypes within the group. Therefore, the observed cytogenetic characters can hardly be interpreted in terms of karyotype evolution without further cytogenetic information.

This work was supported by the project of the Grant Agency of the Slovak Republic VEGA (2/0168/13).

ZOONOTIC ROLE OF ASCARIS SUUM IN HUMAN INFECTION IN ITALY

S. Cavallero¹, B. Gambetta¹, V. Perrone², S. Gabrielli¹, M. Dutto², S. D’Amelio¹
Department of Public Health and Infectious Diseases, Parasitology Section, Sapienza University of Rome, Italy¹; Department of Prevention Local Health Unit ASL CN1, 3. USL Rome B, Italy²

Humans and pigs can be infected with Ascaris lumbricoides Linneaus, 1758 and Ascaris suum Goeze, 1782 and both parasites are two of the most important soil-transmitted helminthes of public health and socio-economic issue. Ascariasis is considered a Neglected Tropical Desease with a global prevalence estimated to be about 1.2 billion (WHO, 2006).The absence of morphological distinct keys and genetic similarities between the two species determine difficulties to solve the question on their taxonomic rank. Interestingly, evidences about hybridization, gene flow and cross-infection have been demonstrated (Anderson , 2001; Peng and Criscione, 2012; Galvin et al.,1968). The uncertainty on the taxonomic status complicate the picture of zoonotic potential of Ascaris as the development of control programmes (Leles et al., 2012).

The main source of human infection appear mostly due to A. lumbricoides in endemic regions, while in Italy is likely more relevant the zoonotic potential of Ascaris suum, as in other non-
endemic regions (Europe). Moreover, the presence of A. suum and putative hybrids in human Italian infections has been reported (Cavallero et al., 2013).

The aim of the present study is to characterize specimens of Ascaris spp. collected in humans and pigs from Italy in order to identify the etiological agent of human cases and to compare the results with genotypes circulating in pigs, to update knowledge on molecular epidemiology of Ascariasis in non-endemic regions.

A total of 8 human and 122 pig specimens were characterized to species level using PCR-RFLP approach on nuclear rDNA ITS (Zhu X et al., 1999) to differentiate A. suum, A. lumbricoides and hybrid genotype. Sequence analysis of mtDNA cox1 were performed on a subsample to define the cluster affiliation (Anderson TJC, Jaenike J 1997 Parasitology 115:325; Snabel V et al 2012 Jpn J Infect Dis 65:179) using parsimony network and Bayesian phylogenetic analyses.

A total of 120/130 positive amplicons were obtained (7 from humans and 113 from pigs).

PCR- RFLP analysis revealed that 57% of the human nematodes displayed A. suum pattern and 43% showed A. lumbricoides pattern. Most of the pig samples showed the typical A. suum pattern (86,7%), about 2% showed A. lumbricoides pattern and 11,5% of specimens were hybrids.

Phylogenetic analyses revealed that both human and pig samples belong to clade A and C. Comparing to results from ITS genotyping, no clear association between genotype and clade were observed.

The present study confirms the zoonotic role of A. suum in human infections in Italy, although the low number of samples examined doesn’t allow undisputable conclusions: all Italian patients showed the A. suum ITS-RFLP pattern, while no data on patients nationality were available for the few cases showing A. lumbricoides. Samples from pig host showed mostly A. suum genotype, although both A. lumbricoides and hybrid genotypes were present. The results confirm cross-infection and gene-flow between the two species. Moreover, two samples of human origin were included for the first time in clade C, as previous studies reported solely specimens of pig origin. These results confirms the need to revise taxonomic status of Ascaris spp., since hybrid genotype were observed in pigs and no fixed polymorphism was clearly attributable to A. suum or A. lumbricoides.

WILD BOARS AS A RESERVOIR OF NEMATODES FROM GENUS TRICHINELLA IN THE SYLVATIC CYCLE IN POLAND

A. Cybulska, J. Bień, A. Kornacka, W. Cabaj, B. Moskwa
Witold Stefański Institute of Parasitology, Polish Academy of Sciences, 00-818 Warsaw, Twarda 51/55, Poland

Trichinellosis is a zoonosis caused by parasitic nematodes of the genus Trichinella. The members of the genus are able to infect a broad spectrum of mammalian hosts, making them one of the world’s most widely distributed group of nematodes. A total of 12 genotypes have been identified in the genus Trichinella. The majority of Trichinella spp. cause disease in humans. Four species of Trichinella: T. spiralis, T. nativa, T. britovi and T. pseudospiralis are represented in Poland. The aim of the study was to determine the current prevalence and spread of these parasites in wild boars in Poland.

The study was performed on 793 wild boars from different regions of Poland collected in 2011-2014.
Muscle samples were taken from diaphragm pillars and tongue. Each sample was examined individually by artificial pepsin-HCl digestion method by Gamble et al. (2000). Muscle larvae were identified as Trichinella based on gross morphology. Genomic DNA was extracted from single larvae. Trichinella larvae were identified at species level by multiplex polymerase chain reaction (multiplex PCR).

The overall prevalence in examined samples was found to be 1.8% (14/793). The weight of examined muscle samples varied from 4.2 to 23.3 g. The intensity of infection was not high, and infected wild boars harbored from 0.04 to 9.7 larvae per gram (LPG). Recovered muscle larvae were identified as two Trichinella species: T. spiralis and T. britovi (65% and 33%, respectively).

Until now, in Poland three Trichinella species: T. spiralis, T. britovi and T. pseudospiralis have been detected in wild boars. However, our studies have confirmed only two of them: T. spiralis and T. britovi.

Based on data published by the National Institute of Public Health – National Institute of Hygiene, the source of most Trichinella cases in Poland has been wild boar meat.

While it is worth noting, that wild boar infections are not common and the intensity of infection is low, our results confirmed that wild boars can be infected with nematodes belonging to the genus Trichinella.

This fact strongly confirms the absolute necessity of the study of wild boar meat intended for human consumption. The importance of meat inspection towards determining the presence of Trichinella should not be underestimated. It is worth being reminded that two Trichinella species: T. spiralis and T. britovi were found in sausages prepared from wild boar hunted in 1999 in the vicinity of Sępólno Krajeńskie. The product was a source of infection for more than 200 people. Further, in 2005, 40 people, including a 16-month-old baby, were infected with Trichinella after ingestion of wild boar sausages, from animals hunted around Kurów, near Poznań.

This research was partially supported by the National Centre for Research and Development. Grant No. 12 0126 10.

EFFECT OF INSECTICIDES ON NEMATODE COMMUNITIES IN MAIZE CROP

A. Čerevková1, M. Renčo1, L. Cagáň2

Institute of Parasitology, SAS, Hlinkova 3, 040 01 Košice, Slovak Republic1; Slovak Agricultural University, A. Hlinku 2, 94976 Nitra, Slovak Republic2

The influence of Western corn rootworm seed coating and granular insecticides on the seasonal fluctuations of soil nematode communities was studied during period of May to September, 2011 at maize field in Komoča (coordinates 47°58’N; 18°02’E, Slovak Republic). Four soil treatments were used in this experiment: variant 1 – a granular application with tefluthrin (199.5 g a.i./ha); variant 2 – a granular application with clothianidin (110 g a.i./ha); variant 3 – a seed treatment with clothianidin (1.25 µg a.i./seed); and control – a maize field without insecticides.

During the investigated period, 19 117 soil nematode individuals were captured and 9 orders, 33 genera and 37 species were identified. Acrobeloides nanus, Cephalobus persegnis, Eucephalobus striatus and Basiria gracilis were the dominant species, accounting for 48 % of the total number of individuals. The mean abundance and species diversity index were significantly lower for variant 2. Bacterial feeders were the dominant trophic group for all 4 variants. The numbers of nematodes in
particular trophic groups (i.e., bacterial feeders, fungal feeders and omnivores) were significantly different between variant 2 and the control. The Σ Maturity index, Maturity index and Plant parasitic index did not show significant differences among the variants. The higher values of the Enrichment and Structure indices were observed in the first month of the investigation in all 4 variants. A cluster analysis showed that nematode species population densities were strongly affected by the date of soil sample collection and by the variants used in the experiment.

This research was supported by grants of Slovak Scientific Agency VEGA Grant No. 2/0079/13 and Grant No. 1/0894/11

TREHALASE mRNA EXPRESSION IN THE BODY OF ASCARIS SUUM
M. Dmitryjuk, E. Zaobidna, E. Łopieńska-Biernat
University of Warmia and Mazury, Faculty of Biology and Biotechnology, Department of Biochemistry, Oczapowski 1A Str., 10-957 Olsztyn

Trehalase (EC 3.2.1.28) hydrolyzes the α-1,1- glycoside binding of trehalose (1-α-α-glucopyranosyl-α-α-glucopyranoside), releasing two molecules of glucose. Metabolism of α,α-trehalose is of particular interest in case of parasites. This disaccharide plays in nematodes crucial roles in protecting cellular structures against most environmental stresses, it provides energy, is the major circulating blood sugar and it is also important in the proces of hatching. Trehalase activity was previously determined in nematodes. Therefore, the aim of this study was to determine of the trehalase mRNA (tre gene) expression in the tissues of adults Ascaris suum female and male.

The total RNA was isolated from tissues (muscle head portion- MH, muscle central portion - MC and muscle tail portion - MT, intestine –I, reproductive system -RS and haemolymph - H) of female and male roundworms with kit total miniRNA (AABiotechnology). cDNA synthesis and amplification were conducted with using TranScriba Kit and StartWarm 2xPCR Master Mix (A&A Biotechnology Poland). Quantitative real-time PCR was performed using a SYBRGreen PCR-MIX Taq™ (A&ABiotechnology) according to the manufacturer’s instructions. Mean values and standard deviations were used for the analysis of relative transcript levels for each tissue using the 2−ΔΔCT method. The data were presented as the fold change in gene expression normalized to an endogenous reference gene gapdh and relative to the control (RQ=1; MH or appropriate tissue of females). Transcript levels were shown by AB analysis software (7500 v2.0). All samples were tested in triplicate on Applied Biosystems™7500 Fast RealTime PCR Systems (Life Technologies, USA). Melting curves were constructed after amplification.

PCR and Real-time reverse transcriptase qPCR analysis showed tre expression in all tested tissues in male and female of parasite except haemolymph. Relative to the MH were noted the increase of tre expression in MC and MT muscles (RQ=4.4±0.96 and RQ= 19.9±4.8, respectively), in I (RQ=8.4±0.95) and in RS (RQ=11.3±0.5) of females. In the case of males were noted the increase of tre expression in MC (RQ=3.73±0.15), MT (RQ=1,2±0.1), the highest in intestine (RQ=22±0.95) and the decrease of tre expression in RS (RQ=0.2±0.05). The analysis of tre expression in males relative to females showed the increase of tre gene expression in MH and MM (RQ= 3.8±0.1 and RQ=3.2±0.1, respectively) and in intestine (RQ=9.9±0.43). In MT and RS we observed the decrease of tre expression in males relative to females (RQ=0.23±0.05 and RQ=0.066±0.05, respectively).
In conclusion, we can say that in the female the highest expression of tre gene occurs in the muscles of tail portion (MT) and in the reproductive system (RS). The high expression of trehalase mRNA perhaps is related here from the production and submission of a large number of eggs by the female. In turn, in males is observed the significant increase of tre expression in the intestine (I).

**INVASION OF THE TAPEWORM PROTEOCEPHALUS LONGICOLLIS (ZEDER, 1800) IN SMELT (OSMERUS EPERLANUS) FROM LAKE HAŃCZA (NORTHERN POLAND)**

J. Dziekońska-Rynko¹, K. Mierzejewska², P. Hliwa³

Department of Zoology, Faculty of Biology, University of Warmia and Mazury in Olsztyn, Oczapowskiego 5, 10-957 Olsztyn, Poland¹; Department of Fish Biology and Pisciculture, Faculty of Environmental Sciences, University of Warmia and Mazury in Olsztyn, Oczapowskiego 2, 10-719 Olsztyn, Poland²; Department of Ichthyology, Faculty of Environmental Sciences, University of Warmia and Mazury in Olsztyn, Oczapowskiego 5, 10-957 Olsztyn, Poland³

Smelt in Polish lakes shoaling in pelagic zones, from July to November taking up the diurnal vertical migrations following the zooplankton, the favorite food items in the diet of this fish. Older and larger individuals (mostly at age 2) become predators. In spring the smelt is grouping together near the shallow areas with sandy bottom for spawning. Since the end of the XX century, the rapid decrease of the smelt populations is observed in many polish lakes. This species, known as sensitive to eutrophication and water pollution is, at least locally threatened.

The aim of this work was to determine the species composition and quantitative structure of the community of gastrointestinal parasites in the smelt from Lake Hańcza (Easternsuwskie Lakeland). This is the deepest lake in Poland and in the whole central part of North European Plain (depending on data 108-111 m. in depth). It belongs to the group of post-glacial, oligo-mesotrophic lakes. Parasitological examinations of fish (n = 15) with an average body length of 9.52 ± 1.28 cm and an average body weight of 6.24 ± 2.2 g. were carried out in March 2014. Fish were captured by seine net for smelt during commercial catches. The digestive tract was longitudinally dissected, than decanted with saline solution (0.85% NaCl). The isolated parasites were identified in fresh preparations, than photographed and measured under the microscope Olympus CX41 equipped with a Multiscan v. 4.2 computer software for image analysis. Cestoda were classified according to Pojmańska (1991).

The tapeworm *Proteocephalus longicollis* was recorded in the digestive tract of the all examined fish (the prevalence 100%) at the mean intensity of infection 42 ± 20.3 specimens per fish. The mean length of strobila was 10.12 ± 2.7 mm, but the majority of detected specimens, were the juveniles with the length of strobila ranging between 3 to 5 mm. The parasite is commonly distributed in polish fish species of two families Osmeriformes and Salmoniformes, which are the final host of the cestode. Parasite is transmitted to the fish with copepods including *Cyclops strenuus*, *Eucyclops* sp. and *Diaptomus castor* which are the first intermediate host. There was no correlation between body weight and/or the body length of fish and the number of tapeworms, probably due to the small size range of the fish examined. The digestive tract of the smelt from Lake Hańcza, was inhabited solely by *P. longocollis*. 
CONSTRUCTION OF A BACTERIAL EXPRESSION SYSTEM FOR THE PRODUCTION OF GRA1-GRA2-GRA6 TOXOPLASMA GONDII RECOMBINANT CHIMERIC ANTIGEN WITH POTENTIAL DIAGNOSTIC UTILITY

B. Ferra, L. Holec-Gąsior, J. Kur
Gdańsk University of Technology, Chemical Faculty, Department of Microbiology
Narutowicza 11/12, 80-233 Gdańsk, Poland

Toxoplasma gondii is an obligate intracellular parasite of warm-blooded animals and humans. It is estimated that one-third of the human population is chronically infected. Human infection of T. gondii are generally asymptomatic, however, diagnosis of T. gondii infection is of great medical importance, especially for pregnant women and immunocompromised patients (e.g. AIDS or cancer patients). Primary infection of pregnant woman is often associated with fetal infection, which can lead to miscarriage, stillbirth or severe neonatal malformation. In immunosuppressed patients toxoplasmosis can result in encephalitis, pneumonia, myocarditis, conjunctivitis, and nervous system damages. The diagnosis of toxoplasmosis is usually based on the detection of specific IgG, IgM or IgA antibodies. Most commercial kits use whole Toxoplasma lysate antigen (TLA) for antibody detection, however, in some cases, performed tests give ambiguous results. For this reason, new diagnostic tools, such as recombinant antigens are still sought.

The aim of this study was to design an efficient Escherichia coli expression system for the production of GRA1-GRA2-GRA6 T. gondii recombinant chimeric antigen with potential diagnostic utility. Using the methods of genetic engineering, there was constructed a recombinant plasmid encoding immunodominant fragments of three T. gondii dense granule antigens (GRA1, GRA2 and GRA6). Efficient expression of the gene encoding the chimeric protein was obtained in the E. coli strain Rosetta(DE3)pLacI. The recombinant chimeric antigen was purified during a one-step chromatography procedure using metal affinity chromatography. The potential usefulness of the recombinant chimeric antigen GRA1-GRA2-GRA6 in serodiagnosis of human toxoplasmosis was demonstrated in the IgG ELISA test. There were used 298 sera from patients infected with T. gondii and healthy individuals (175 seropositive and 123 seronegative). All of the sera was previously tested using commercial tests (VIDAS TOXO IgM, VIDAS TOXO IgG II and VIDAS TOXO IgG AVIDITY, bioMérieux). The result obtained for chimeric antigen were compared with those of IgG ELISAs using a TLA and a mixture of three recombinant antigens (GRA1+GRA2+GRA6). The sensitivity of the IgG ELISA calculated from all of the positive serum samples was similar for chimeric antigen, combination of antigens and the TLA, and amounted to 99.4%, 98.9% and 97.1%, respectively.

Therefore, the study shows that the new diagnostic tool such as GRA1-GRA2-GRA6 chimeric antigen are very useful for the detection of anti-T. gondii IgG antibodies, and can be used in diagnostics instead TLA.

This research work was co-financed by the European Commission in the frame of the European Social Fund, by the European Social Fund, the State Budget and the Pomorskie Voivodeship Budget according to the Operational Programme Human Capital, Priority VIII, Action 8.2, Under-action 8.2.2: ‘Regional Innovative Strategy’ within the system project of the Pomorskie Voivodeship “InnoDoktorant – Scholarships for PhD students, VIth edition”.
Epidemiological study of occurrence of selected parasitic diseases in Roma children

M. Haláňová¹, Z. Kalinová¹, L. Čisláková¹, M. Halán², M. Goldová², G. Štrkolcová²,
A. Valenčáková²

P. J. Šafárik University in Košice, Faculty of Medicine, Košice, Slovakia¹; University of Veterinary Medicine and Pharmacy, Košice, Slovakia²

The number of parasites followed the rapid growing of human population worldwide, not only in developing but also in developed countries. Many of them are diagnosed in children and adolescents. Children come into contact with parasites in the sandbox, at play and in contact with domestic animals, but also the consumption of contaminated food and water. Very often is also as way of transmission the direct contact between children. Giardia intestinalis and amoebae, together with Cryptosporidium spp. belong to the most commonly detected intestinal protozoa. From a group of helminths there are Ascaris lumbricoides, Trichuris trichiura, Enterobius vermicularis, hookworms, and tapeworms Taenia spp. It is well known that all of these infections are very often endemic in places with poor sanitation and crowded living conditions and are associated with source of water, age, and socioeconomic status of community. In Slovakia, such places are largely represented by the Roma settlements and housing. According the Statistical Office of the Slovak Republic, 105,738 citizens from a total population of 5,397,036 in Slovakia are of Roma nationality, which represents only 2% of the total population. However, the actual estimated number of Roma citizens in Slovakia ranges from around 320,000 – 500,000, which represents between 8.0 to 10% of the total population. Almost half of the Roma population in Slovakia is under the age of 18 years old. Geographically, the highest number of Roma (approximately 60%) lives in eastern Slovakia.

In our study we analysed the incidence of selected parasitic infections - giardiasis, ascariasis, trichuriasis and enterobiasis in children aged 0-6 years coming from the majority and minority groups in Kosice neighborhood (village Medzev).

A total of 75 faecal samples of clinically healthy children were examined for the presence of intestinal parasites, 58 (77.3%) of whom were Roma and 17 (22.7%) of whom were from the majority population.

For separation of helminths eggs and protozoan cysts zinc sulphate flotation concentration method was used.

Ascaris lumbricoides was found to be the leading parasite (46.7%; 35 children), followed by Trichuris trichiura (13.3%; 10 children). Enterobius vermicularis was detected in 5.3% (4 children) of examined children. From protozoan parasites Giardia intestinalis was observed in 3 children (4%). In all cases except one child from the majority parasites were detected among Roma children.

Our obtained results showed that the risk of occurrence of observed parasitic infections is higher for children from minority groups compared with children from the majority group. As the highest risk factor was confirmed using individual source of drinking water in the home together with poor sanitation and crowded living conditions.

This work was supported by VEGA MŠ SR 1/0390/12, 1/0831/12 and 1/0063/12.
GASTRO-INTESTINAL PARASITES IN TATRA CHAMOIS (RUPICAPRA RUPICAPRA TATRICA)

Z. Hurníková¹, M. Miterpáková¹, B. Chovancová²
Institute of Parasitology SAS, Košice, Slovakia¹;
TANAP Research Station, Tatranská Lomnica, Slovakia²

Tatra chamois (Rupicapra rupicapra tatrica) is a significant representative of the Tatra endemic fauna species that has been classified as critically endangered. According to the annual count in 2013, the population of chamois in the territory of the High Tatra Mountains represents 872 individuals in the Slovak part and 314 individuals in the Polish Tatra National Park.

The numbers and health status of these animals is affected by plurality of different critical factors – from climatic conditions through industrial pollution of the environment to individual anthropogenic impacts. In terms of health hazard, a significant role play parasitic infections that can lead to significant depletion of the entire population. The research on parasite fauna of Tatra chamois in Slovakia took place for several decades, but it was targeted only on the incidence of lung nematodes, which were considered the greatest threat. In contrast, the most recent research focused on gastrointestinal parasites was conducted in early 80’s of the last century (Mituch et al., 1984). Thus, the aim of our work was to study the occurrence of gastrointestinal parasites of Tatra chamois in current environmental and climatic conditions.

During the pilot phase in 2013, in total 164 chamois faecal samples from the Slovak side of the High Tatra, 33 samples collected in Polish part of the mountain and for comparison also 15 samples from geographically separated area of Slovak side of the Western Tatra were examined using standard coprological methods.

The results revealed the overall prevalence of gastrointestinal parasites in chamois of the Slovak part of the High Tatras reaching 58.5 %. Most frequent were protozoa - Eimeria spp. (41.5 %), helminths were represented by Moniezia spp. tapeworms (29.3 %); eggs of family Trichostrongylidae were found in 9.1 % of examined samples. Sporadically the eggs of the genera Nematodirus and Capillaria were detected. The chamois living on the Polish side of the Tatras were infected by Eimeria spp. (21.2 %), Moniezia spp. (15.2 %) and Trichostrongylidae (9.1 %). Examination of 15 chamois faecal samples from the Western Tatra showed significantly lower occurrence of intestinal parasites with Eimeria spp. oocysts detected in one sample and strongylid eggs present in a single specimen.

The initial research on gastrointestinal parasites of the Tatra chamois introduced one disputable finding - a relatively high prevalence of the genus Moniezia. In the Slovak part of the High Tatra, nearly 30 % of samples were positive for the tapeworm eggs that is significantly more in comparison with other European studies (see Stancampiano et al., 2001; Hoby et al., 2006; Morrondo et al., 2010; Marreros et al., 2011). These differences may be related to the presence of suitable intermediate hosts (Oribatida mites) in the environment, which is evidently closely linked to the climate and microclimate conditions - oribatid mite community composition would show a strong response to changes in habitat and land-use. Research initiated in the High Tatra after the huge windstorm in 2004 revealed an increase in density of soil mites (and overpopulation of Oribatida mites), but also the decline in species diversity (Kalúž et Ferenčík, 2008).

All the above mentioned points to the need of continuing intensive research on parasite composition and distribution in Tatra chamois, in particular broader temporal, ecological and zoological contexts.

This study was supported by bilateral Slovak-Polish project APVV SK-PL-0098-12.
FOSSILIZED MITES (ACARI) IN BALTIC AMBER

W. Chmielewski
Department of Bee Products, Apiculture Division, Institute of Horticulture, Kazimierska 2, 24-100 Puławy, Poland

Paleontological studies conducted in various scientific centers on amber animal inclusions show that majority of these fossils belong to arthropods (Arthropoda), mainly insects (Insecta) and arachnids (Arachnida), including mites (Acari). However present knowledge on acarological inclusions seems to be comparatively scarce, e.g. by comparison with literature data on amber fossils of insects.

The aim of these studies is presentation of some pictures showing fossil mites and prepared mainly on the base of author’s private collection of inclusions in Baltic amber and comparison of their results with some related pieces of information in literature (Krzemińska et al. 1993, Ross 1998, Weitschat, Wichard 1998, Kosmowska-Ceranowicz 2001).

The samples of raw amber for analyses conducted and presented herein were collected from the beaches of Polish coast of Baltic Sea, mainly in Krynica Morska. Some polished pieces of material and amber products were purchased in jewelry and souvenir shops. Following the information of producers (jewelers) and sellers of these products they were made from local raw material (Baltic amber). Introductory acarological analyses of collected material, i.e. total some hundreds pieces of raw amber and polished products, were overlooked under stereoscopic microscope. After these analyses from the point of usefulness of samples for further investigations, 460 comparatively light and transparent pieces were selected. Over 10% of these samples were containing various acarological inclusions. Some of good maintained fossilized mite specimens were used for photo-documentation and more particular examination with using microscope techniques. Particular methods were described in earlier publications on amber inclusions of arthropods (Baker et al. 2003, Chmielewski 2011).

Obtained results show great biodiversity of mites found in analyzed material. Fossils of representatives of various systematical groups (suborders, families) of these arachnids were stated. Majority of examined samples were containing fossils of mite species free living in the trees and soil, i.e. moos mites, Oribatida and Astigmata. A part of specimens from these last group was remembering nowadays acaroids (Acaroidea) observed often in the similar habitats. Suborders Prostigmata and Mesostigmata were represented mainly by forms near to nowadays predatory and parasitic representatives of families Bdellidae, Cheyletidae, Erythraeidae, Tenuifilidae, Trombidiidae and Trombiculidae. Comparative morphological studies show that the general view and scheme of body structures of fossilized mites found in amber are approached to key characteristics of present days living species. Some of fossilized mite specimens are probably ancestors of nowadays evolutionary changed mites, which are animal and human parasites or predators of other small arthropods, or pests of plants and stored products, species of medical, sanitary and economic importance.
OCCURRENCE OF TRICHINOSIS IN RED FOXES (VULPES VULPES) IN POLAND IN 2010 – 2013

E. Chmurzyńska, M. Różycki, E.B. Zając, T. Cencek, J. Karamon
National Veterinary Research Institute, Puławy, 57 Partyzantów Avenue,
24-100 Puławy, Poland

The parasites of the genus Trichinella are maintained by a wild cycle involving omnivores and carnivores animals from reptiles to birds and mammals. The domestic cycle occurs where there are high risk farming practices. Trichinella still represents a concern for the public health of the European Union due to hundreds of human infections documented yearly as an effect of the consumption of raw pork from free-ranging and backyard pigs or wild boars. The fact that a most number of cases of human infections (932), reported in the years 2000 – 2010, were mainly caused by the consumption of wild boar meat, particularly emphasizes the importance of preventive measures designed to reduce the risk for the human population. Four genotypes (T. spiralis, T. britovi, T. nativa and T. pseudospiralis) are known to be circulating in Europe. The purpose of the presented study was to identify the species of Trichinella infecting the wildlife in Poland and to determine their prevalence ratio. Among other animals the red fox (Vulpes vulpes) is considered to be one of the main vectors of trichinosis. In the past 20 years the population of these animals in Poland increased several times due to introduced vaccination against rabies. According to the data obtained from Polish Hunters Association population of red fox reached more than 220 thousand. The data’s on occurrence on Trichinella in red foxes were very limited. In 2010 in PIWet PIB – NVRI monitoring of Trichinella in red foxes was launched. Samples of muscle from 1952 foxes were provided by regional veterinary laboratories (ZHW) of the 16 provinces (267 districts). Samples were taken from foxes shot for the monitoring of rabies. Samples were digested in accordance with method ZP/PB-44 in NVRL. Larvae of Trichinella spp. were detected in 57 samples (2.92 %). Identification of species was made by multiplex PCR according to protocol by Pozio and La Rosa (2010). PCR positive results were obtained in of 55 samples. The amplification product characteristic for T. britovi was found in 41 samples (74.6 %), T. spiralis in 12 (21.8%) and T. nativa in one sample (1.8%). One mixed infection with T. spiralis/T. britovi (1.8%) was found. The average degree of infection was low and amounted to 0.98 larvae / g. Analysis of geographical distribution of cases of trichinosis in the population of foxes showed a particularly severe invasion in the north - eastern part of the country. The dominant species of Trichinella in a population of red foxes in Poland was T. britovi. During the study rare species at this altitude T. nativa was found. It has to be undersignet that this genotype is extremly freez resistant and his occurrence in North-East Part of Poland (where traditionally raw sausages are prodused) can hardly implicate epidemiological situation in Poland.
INFLUENCE OF LOCAL BIODIVERSITY ON THE ECOLOGY OF BORRELIA BURGDORFERI SENSU LATO AND ANAPLASMA PHAGOCYTOPHILUM

M. Chvostáč, L. Berthová, M. Derdákova

Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovakia; Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia; Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovakia

In Slovakia, Ixodes ricinus is the most important vector of infectious agents. Among others it transmits spirochaetes from the Borrelia burgdorferi sensu lato complex and Anaplasma phagocytophilum. Both bacteria show considerable genetic diversity that is closely associated with specific reservoir hosts.

In our study we have analyzed the occurrence and seasonal dynamics of I. ricinus and its infection with B. burgdorferi s.l. and A. phagocytophilum in habitat with low abundance of rodents and permanent population of deer and miscellaneous birds species to study the influence of the biodiversity of hosts on their ecology. We also tested the prevalence of pathogens in ticks collected from birds living in our study area.

Ticks were collected in urban forest Sitina at the SAS campus in monthly intervals from March 2011 until the November 2012. Birds were caught in 2012 by net-traps and feeding ticks were removed. Questing and feeding ticks were tested for the prevalence and genetic variability of B. burgdorferi sensu lato and A. phagocytophilum by molecular methods.

Out of 543 I. ricinus ticks from vegetation, 5.8 % were infected with A. phagocytophilum. The total prevalence for B. burgdorferi s. l. was 6.8 % represented by 6 genospecies with the dominance of B. garinii. 12 % of ticks, collected from birds were borrelia-positive with eudominance of B. garinii. None of the tested feeding tick from birds was infected with A. phagocytophilum.

The total prevalence of B. burgdorferi s.l. in questing ticks was lower than reported mean prevalence for Europe. We have confirmed that in area without rodents, roe deer, that is incompetent reservoir for B. burgdorferi s.l., can hosts a large number of ticks and has a dilution effect on B. burgdorferi s.l. prevalence. On the other side, roe deer is an important reservoir for A. phagocytophilum which prevalence was higher as compared to the general prevalence in Europe. Also birds are important reservoir hosts for ticks and B. burgdorferi s.l. in the study area. Their importance on A. phagocytophilum circulation seems to be extrinsic.

The study was supported by the grant VEGA - 2/0055/ and APVV-0267-10.

THE SURVEY OF CANINE BLOOD FILARIOSES IN SLOVAKIA

A. Iglódyová, M. Miterpáková

Institute of Parasitology SAS, Košice, Slovakia

The environmental and climate conditions in Slovakia are favourable for the occurrence and spreading of mosquito transmitted diseases, include also blood filarioses. Recent 7 years show that Slovakia is a country with endemic areas of Dirofilaria spp.

Blood filarioses are natural parasites mainly in primates and carnivores. Dog is main definitive host and reservoir of two most important species of the genus Dirofilaria, D. immitis and D.
repens. These dirofilariae may occasionally infect also human. Another species with possible occurrence in Slovakia is Acanthocheilonema reconditum.

The aim of the present study was to collect data from all over Slovakia and assess the risk of spreading of blood filarioses.

Between 2007-2014, 3603 blood samples of dogs from various regions of Slovakia have been examined. Dog were divided by sex, age, utilization, housing, breed weight and type of coat. To detection of microfilariae we used blood smear and modified Knott test. In positive cases differential species diagnostic was performed using PCR.

Microfilariae were detected in 429 (11.91 %) blood samples. We recorded D. repens and D. immitis. The results of our research show that D. repens is the predominant species circulating in Slovakia. Mixed infection caused by D. immitis and D. repens was confirmed in 9 cases. One heartworm infection caused by D. immitis was recorded.

We noted significant regional differences in Dirofilaria prevalence. While in southern regions the prevalence is high (over 20.0 %), in the territory of northern Slovakia prevalence reaches only to 5.0 %.

We recorded no difference between males and females. Significant risk factor were age, utilization, housing, breed size and type of coat. Dogs older than 3 years were more often infected than dogs under 3 years. Working and shelter dogs were often positive than pet dogs. The prevalence of dirofilariosis was significantly higher in outdoor dogs, shorthaired dogs, large and giant breeds in comparison with indoor dogs, longhaired and wirehaired, small and medium breeds.

Although blood filariosis were until recently considered an exotic disease, our results confirm as dirofilariosis is current problem not only in tropical and subtropical area, but also in moderate climate zone countries including Slovakia.

The study was supported by the Science Grant Agency VEGA No. 2/0011/12.

CADMIUM IMMUNOTOXICITY AND ITS EFFECT ON TRICHINELLA SPIRALIS INFECTION

M. Jalčová¹, E. Dvorochňáková¹, Z. Hurníková¹², P. Dubinský¹
Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovakia¹; University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia²

Exposure of host organisms to heavy metals ions impairs their immunocompetence and enhances the susceptibility to infections. Heavy metals affect physiological cellular functions and modulate the immune system. For the purposes of environmental evaluations, the most suitable entities are the parasites with great geographic range and numerous hosts, such as Trichinella spiralis nematodes, who have cosmopolitan distribution in moderate and tropical climate zones. The study was focused on cadmium immunotoxicity and accumulation ability in mice infected with T. spiralis.

Mice were exposed to the intoxication with cadmium chloride dissolved in drinking water at concentrations 100 and 200 mg/l and infected with 500 larvae of T. spiralis per mouse on day 30 of the intoxication. Cadmium (Cd) intoxication stimulated the proliferative activity of T lymphocytes until day 40, with a higher intensity after the higher dose of cadmium. T. spiralis infection inhibited the proliferation first 2 weeks post infection (p.i.), but in intoxicated mice the suppression was weaker and the higher Cd dose stimulated T cell proliferation from day 40, i.e. day 10 p.i. Splenic CD4 T cell numbers started to increase from day 30 of Cd intoxication, particularly after the higher Cd
dose. This stimulation was not sufficient to restore a CD4 T cell decrease induced by *T. spiralis* infection during the first 2 weeks p.i., but the higher Cd dose increased the occurrence of CD4 T helpers from day 20 p.i. Cd intoxication stimulated splenic CD8 cytotoxic cells only the first 30 or 40 days and did not prevent from its temporary decrease during 2 weeks p.i., but it reduced this decrease. The higher Cd dose stimulated CD8 T subpopulation on day 20 p.i. The metabolic activity of peritoneal macrophages was not affected by Cd intoxication, but it stimulated the production of superoxide anion early (day 15 p.i.) and with a higher intensity in intoxicated and infected mice than in infected mice without Cd intoxication. The stimulation of cellular immunity by cadmium probably contributed to the parasite reduction in intoxicated mice. There was found a reduction of worm numbers in the intestinal phase at 47 % and 7.2 % induced by intoxicating Cd dose of 100 and 200 mg/l, respectively. Numbers of larvae in the muscles were reduced at 27.6 % and 4.96 % by the lower and higher Cd doses, respectively. Cadmium was accumulated predominantly in the liver and kidneys in proportion to size of Cd dose. *T. spiralis* infection did not influence Cd concentrations in the host tissues after intoxication with the lower Cd dose, only after the higher Cd dose, the metal accumulation was nonsignificantly higher than in intoxicated mice without the infection. In the muscles, where the muscle phase of trichinellosis is developing, the lower Cd accumulation was found. Differences in Cd concentrations on day 15 p.i. corresponded with different intoxicating doses and were balanced on day 30 p.i. (i.e. day 60 of Cd intoxication).

The obtained results confirmed the immunostimulatory effect of cadmium on functional activity of T cells and macrophages, which were of use in the host defence against *T. spiralis* infection, particularly during the muscle phase, and contributed to the significant reduction of parasite burden.

*The work was supported by the project VEGA 2/0093/11 and the Research and Development Operational Programme funded by the ERDF (code ITMS: 26220220116).*

**EFFECT OF MERCURY INTOXICATION ON IMMUNE RESPONSE OF MICE TO ECHINOCOCCUS MULTILOCULARIS INFECTION**

M. Jačová, E. Dvorožňáková, P. Dubínský
Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovakia

Under conditions of pollution both host and parasite are susceptible to the pathogenic effects of toxicants, which in turn may result in detrimental changes to their immunological and physiological processes. Mercury (Hg) represents the most toxic metal in the environment, it markedly inhibits cellular activities and initiates development of autoimmune diseases. The aim of the study was to test mercury effect on cellular immunity in mice infected with *Echinococcus multilocularis*.

Mice were exposed to the intoxication with mercuric chloride dissolved in drinking water at concentrations 0.3 and 3 mg/l and infected with 3000 protoscoleces of *E. multilocularis* per mouse on day 30 of the intoxication. Hg intoxication suppressed the proliferative activity of T lymphocytes for all days of the experiment. *E. multilocularis* infection induced a similar inhibition of T cell proliferation and Hg intoxication did not change this decrease. Splenic CD4 T cell numbers were not markedly affected by Hg intoxication. *E. multilocularis* infection induced only a short increase in CD4 T cells on day 60 post infection (p.i.), but numbers of these cells in Hg intoxicated mice did not reach
this peak. Hg intoxication did not influence splenic CD8 cytotoxic cells and a moderate increase in this subpopulation induced by *E. multilocularis* infection was significantly overwhelmed in intoxicated and subsequently infected mice in proportion to size of Hg dose. The metabolic activity of peritoneal macrophages was affected by Hg significantly, the highest production of superoxide anion was recorded on days 30 and 45 of the intoxication and macrophage’s activity was stimulated also after *E. multilocularis* infection. Parasite cysts started their progressive growth during the 3rd month of the infection, but the cyst development was slowed-down in intoxicated mice with a higher reducing effect (at 54.3%) in mice intoxicated with the high Hg dose. Hg accumulation in the host parenchymal organs correlated with a size of Hg dose. The highest Hg accumulation was recorded in the kidneys, followed by the liver and lungs, trace Hg concentrations were found in the muscles. The Hg values in the liver, lungs and muscles reached the maximum on day 60 of the intoxication with the high Hg dose. *E. multilocularis* infection did not influence Hg accumulation in mice intoxicated with the low Hg dose, but there was found a decreasing trend during the first 2 months of the infection in mice intoxicated with the high Hg dose. A significant decline of Hg concentration in all tissues was found in mice intoxicated with the high Hg dose after the infection on day 120 p.i., what could be caused by the accumulative capacity of parasite cysts.

The obtained results showed the stimulatory effect of mercury on macrophage’s activity and cytotoxic CD8 T lymphocytes, which are effector cells of the host immunity and participate in the host defence against development of *E. multilocularis* infection. We supposed the macrophage stimulation caused by mercury intoxication contributed to the reduction of the parasite cysts and decreased the parasite infection in the intoxicated host. The results suggest Hg intoxication does not weaken the host but makes it more resistant to *E. multilocularis* infection in the environment contaminated with mercury.

The work was supported by the project VEGA 2/0093/11 and the Research and Development Operational Programme funded by the ERDF (code ITMS: 26220220116).

HELMINTH FAUNA OF ARIONIDAE SLUGS IN POLAND

W. Jeżewski\(^1\), Z. Laskowski\(^1\), J. Hildebrand\(^2\), I. Rzą\d{}\(^3\), G. Zaleśny\(^4\)

W. Stefański Institute of Parasitology, Polish Academy of Sciences, Twarda 51/55, 00-818 Warszawa, Poland\(^1\); Department of Parasitology, University of Wrocław, Przybyszewskiego 63/77, 51-148 Wrocław, Poland\(^2\); Department of Ecology and Environmental Protection, University of Szczecin, Wąska 13, 75-415 Szczecin, Poland\(^3\); Department of Invertebrate Systematics and Ecology, Institute of Biology, Wrocław University of Environmental and Life Sciences, Kożuchowska 5b, 51-631 Wrocław, Poland\(^4\)

Many species of slugs serve as intermediate hosts for parasites of vertebrates and final definitive hosts for helminths. Juvenile stages of parasites develop and completed part of their life cycle inside the molluscs.

In the period from March 2010 to November 2013 we collected slugs from the genus *Arion* in several localities of Poland. The helminthfauna of seven species of *Arion*: *A. circumscriptus*, *A. hortensis*, *A. distinctus*, *A. fasciatus*, *A. lusitanicus*, *A. rufus*, *A. subfuscus* was described and analysed. Slugs were euthanized with chloroform, then foot, body cavity and internal organs were examined
for the presence of helminths. All worms were collected alive, washed in physiological salt solution, killed by heat in fresh water (60–70°C) and examined. Most of the specimens were preserved in 70% ethanol to molecular examination. Identification of parasites was carried out based on morphological and morphometric parameters and molecular data.

This study report ten taxon of parasites belonging to Digenea (4), Cestoda (1) and Nematoda (6). Six genus of helminths, in larval stage, are known to be associated with slugs: 

- Brachylaima (sporocyst, cercaria and metacercaria); 
- Choanotaenia (cysticercoids); 
- Alloionema, Aelurostrongylus, Filaroides, Skrjabingylus (from first-stage larvae to infective larvae), while for nematodes of Agfa and Angiostoma species slugs serve as definitive host.

This work was supported by the Grant no. N N303 499938 from the Ministery of Education and Science, Poland.

METASTRONGYLOIDS LARVAE IN SLUGS IN POLAND

W. Jeżewski¹, Z. Laskowski¹, J. Hildebrand²

W. Stefański Institute of Parasitology, Polish Academy of Sciences, Twarda 51/55, 00-818 Warsaw, Poland¹; Department of Parasitology, University of Wrocław, Przybyszewskiego 63/77, 51-148 Wrocław, Poland²

Wild carnivores (canids, mustelids and felids) are the definitive hosts of the Metastrongylidae nematodes from the genus: Aelurostrongylus (Railliet, 1898), Angiostrogylus Kamensky, 1905, Filaroides Van Beneden, 1858 and Skrjabingylus Petrov, 1927. Snails and slugs are the intermediate hosts, infected through penetration of the epidermis or ingestion of first stage larvae. After entering the intermediate host, L1s develops into third-stage larvae (L3s) and encyst mainly in the intestinal ligament. Definitive hosts are probably infected by eating paratenic host, e.g., rodents, birds, amphibians and reptiles or intermediate host.

Slugs were collected from March 2010 to November 2013 in several localities in Poland (e.g. Kazimierz Dolny in Central Poland, Szklarska Poreba in South-West and Pruszcz Gdański in North). Foot, body cavity and internal organs were examined for the presence of cysts containing nematode larvae. The cysts were broken to release the larvae. All nematodes were collected alive, killed by heat in fresh water and preserved in 70% ethanol.

In this study we reported the metastrongylids larvae in the naturally infected slugs Arion rufus, Limax maximus and in invasive synantropic slug Arion lusitanicus. Morphological and morphometric analyses were confirmed by molecular methods.

This work was supported by Grant no. N N303 499938 from the Ministry of Education and Science, Poland.
ENDOPARASITOSIS AS OPORTUNIC INFECTON IN PAEDIATRIC PATIENTS WITH PULMONARY DISEASES

P. Juriš1, 2, A. Dudlová2, I. Papajová3, P. Jarčuška2, J. Fáby4, J. Melter4

St. Elizabeth College of Health and Social Work, Bratislava, SR1; Faculty of Medicine, UPJŠ, SNP 1, 040 11, Košice, SR2; Institute of Parasitology SAS, Hlinkova 3, 040 01 Košice, SR3; Clinic of Paediatric Tuberculosis and Respiratory Diseases, JFM CU and Šrobár Institute of Paediatric Tuberculosis and Respiratory Diseases, Dolný Smokovec, SR4

The goal of the study was the ovoscopic diagnostics of endoparasitic infections (protozoa and helminths) in hospitalised paediatric patients of the Paediatric clinic of respiratory diseases. Stool examination of hospitalised paediatric patients with respiratory diseases revealed the total prevalence of endoparasites of 15.03 %, out of which the prevalence of helminth was 6.22 % and protozoa 8.80%. Represented are the following species: protozoa Giardia sp., Cryptosporidium sp., Entamoeba sp. and helminths Ascaris lumbricoides, Trichuris trichiura, Hymenolepis spp. The most represented species of protozoa are Cryptosporidium sp. (4.66%) of helminths A. lumbricoides (3.11 %). In paediatric atients were confirmed increased values of IgE antibodies (IgE> 160, N = 5/17 patients positive for protozoa, N = 6/12 patients positive for helminth), and increased values of Eo (Eo> 5%, N = 6/17 positive patients for protozoa, N = 7/17 positive patients for helminth).

This study has been realized thanks to the financial support of the projects VEGA No. 2/0140/13 and 1/0501/13.

THE DURATION OF WINTER INACTIVITY OF DERMACENTOR RETICULATUS TICK

G. Karbowiak, T. Szewczyk

W.Stefanski Institute of Parasitology of Polish Academy of Sciences,
Twarda 51/55, 00-818 Warsaw, Poland

The goal of the study was to establish the moment of the end of Dermacentor reticulatus tick activity in late autumn, and the moment of the start of activity beginning in spring. The study was conducted on the D. reticulatus tick population inhabiting Wal Miedzeszyński, a suburban quarter of Warsaw (52°09’07”N, 21°10’24”E), from October 2013 to the end of March 2014. The studied tick population inhabited open areas covered by grass and bushes, with single trees. Ticks were collected using the flagging method every week. The number of active ticks was established by calculating from individuals sampled by one person per 30 minutes. Males and females collected were also counted, and after this the ticks were released to avoid population destruction. Simultaneously, temperature and air humidity were noted. The observations were not conducted during snowing and/or rainfall.

The autumn activity finished after 4.12.2013, at the moment of the first snowfall and ground-frost. When the last ticks were sampled, the temperature and humidity were 3.6°C and 61.2% respectively. The number of ticks collected in autumn ranged from 16-103 (mean 45), with a female dominance. Apart from the first collection on 11.10.2013 (103 ticks) until the first ground-frost, there was no significant difference between the particular samplings. Additionally, until the first ground-
frost when the temperature descended to 0°C, there were noticed no dependences between the ticks activity and environmental factors.

The spring activity began after 11.02.2014 at the moment of snow cover disappearance. The temperature and humidity were 7.9°C and 64.3% respectively. Ticks appeared at once in great number, during the first collection on 19.02.2014. 46 individuals were collected (19 males and 27 females). Until 26.03.2014 there were collected every week 32-56 ticks (mean 50). There was observed a dominance of active females, and no correlation between tick activity and temperature or humidity values.

During winter, tick activity was observed once on 8.01.2014, during a temperature rise resulting temporary snow disappearance. The air temperature and humidity were 7.8°C and 74.9% respectively. There was one active male found.

The observations conducted show that temperature and humidity have no, or only a small influence on Dermacentor reticulatus tick activity in late autumn, Winter and Spring. It seems that only the presence of snow and ground-frost has an influence on the winter activity of this tick species.

SEROPREVALENCE OF TOXOPLASMA GONDII INFECTION IN PREGNANT WOMEN TESTED IN WARSAW (POLAND) BETWEEN 2006 AND 2013

G. Karczewski¹, R. Salamatin²,³; J. Konieczna-Salamatin⁴, W. Rożej-Bielicka², E. Golab²
Lux Med Medical Center, Warsaw, Poland¹; Department of Medical Parasitology, National Institute of Public Health – National Institute of Hygiene, Warsaw, Poland²; Department of General Biology and Parasitology, Medical University of Warsaw, Poland³
Institute of Sociology, University of Warsaw, Warsaw, Poland⁴

BACKGROUND. Toxoplasmosis is a zoonotic disease of global importance caused by the protozoan Toxoplasma gondii. In pregnant women, the primary infection can be transplacental transmitted resulting in severe and even lethal toxoplasmosis in the fetus. Serologic screening is a part of primary prevention measures of congenital infections. In Poland the screening is not mandatory, however, serological assays for pregnant women are recommended in current Ministry of Health regulations. The recommendation involves initial serology up to the 10th week of pregnancy followed by tests between 21st–26th week of pregnancy in seronegative women. Our objective was to estimate the prevalence of T. gondii infection in pregnant women diagnosed in the private medical service in Warsaw in years 2006–2013.

METHODS. The data on T. gondii infection diagnostics process in 993 pregnant women came from different parts of Poland were analyzed. The women were out – patient of private medical service LuxMed Group diagnosed for T. gondii infection between 6th July 2006 and 23rd March 2013. The IgG and IgM antibodies were determined using Architect Toxo IgG and Architect Toxo IgM tests (Abbott); >3 IU/ml IgG value was interpreted as positive and index values 0,60 and greater was interpreted as a positive IgM result. To establish IgG avidity Vidas Toxo IgG Avidity (bioMérieux) kit was used. IgG avidity indexes interpretation: < 0,200 low avidity, 0,200–0,300 borderline avidity, and > 0,300 high avidity.

RESULTS. A total of 2012 serological tests was carried out in the analyzed period. The number of IgG, IgM nad IgG avidity tests were 1012, 1087 and 71, respectively. Request for laboratory tests were authorized mainly by gynaecologists (77%). T. gondii IgG antibodies were detected in 318 (32%)
of the examined pregnant women aged 19–46 years (mean age 33.2 years). The highest percentage of IgG positive results (42.1%) was found in the age group of 31–35 years. The incidence rate of T. gondii in women aged ≥30 was similar to the rate in woman aged <30 (32.6% vs. 31.0%). Positive results of IgM test were obtained in 51 (4.7%) of studied patients. The percentage of IgM positive results in age groups ≤30 years and >30 years was 9.9% and 3.1%, respectively. Low IgG avidity was determined in 23 (32.4%) serum samples of tested women.

CONCLUSIONS. The results of our analysis showed that implementation of a congenital toxoplasmosis prevention program aimed at woman under the age of 30 years could lead to the decrease of the risk of seroconversion in pregnant women in Poland.

PREVALENCE OF BORRELIA BURGDORFERI S.L. IN IXODES RICINUS TICKS IN DIFFERENT HABITAT TYPES OF SLOVAKIA

M. Kazimírová1, L. Mydlová1, Z. Svitálková1, E. Kocianová2, M. Slovák1, L. Mošanský3, J. Kraljík3, M. Stanko1,3

1Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovakia; 2Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia; 3Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovakia

Lyme borreliosis (LB) is the most prevalent tick-borne human infection in Europe. The ecology of LB is complex and involves three main components: the pathogenic agent (the Borrelia burgdorferi s.l. complex), the vector (Ixodes ricinus) and a variety of vertebrate reservoir hosts. Increase in abundance and variation in spatial distribution of I. ricinus have been reported in recent years as a result of changes in climate, land use and wildlife management. An increased abundance of tick populations has been observed in urban and peri-urban sites across Europe, where humans and domesticated animals can be frequently exposed to bites of infected ticks. In this study, variation in prevalence and diversity of B. burgdorferi s.l. in questing I. ricinus was investigated in three habitat types in Slovakia — urban (a recreational forest park in Bratislava, SW Slovakia), natural (Fugelka, a non-fragmented forest in the Small Carpathians Mts, SW Slovakia) and agricultural (Rozhanovce, patches of oak-hornbeam forest, cultivated fields and meadows, E Slovakia). Questing nymphal and adult I. ricinus were collected by a 1 m² blanket along three 100 m transects in each habitat type monthly from April to October in 2011–2013. Borrelia infections were detected in DNA isolated from individual ticks by PCR targetting the 5S-23S rRNA intergenic spacer, followed by restriction fragment length polymorphism analysis to separate genospecies in positive samples. During the 3 years of investigation overall Borrelia infection varied between 6.5-12.5%, 10.1-18.8% and 11.5-15.8% in the urban, natural and agricultural habitat, respectively. In addition to temporal changes in prevalence of Borrelia-infected ticks, spatial differences in prevalence as well as variation in the distribution of genospecies across each habitat type were observed. Six B. burgdorferi s.l. genospecies were detected: B. burgdorferi sensu stricto, B. afzelii, B. garinii, B. valaisiana, B. lusitaniae and B. spielmanii. B. afzelii and B. garinii prevailed in each habitat type. Spatial and temporal variation in prevalence and distribution of borrelia genospecies across the research areas in association with local abiotic and biotic factors will be further analysed.

This study was financially supported by the FP7 project EDENext (No. 261504) and grant APVV DO7RP–0014–11.
GAME AS HOSTS OF IXODID TICKS AND TICK-BORNE AGENTS IN MALÉ KARPATY MTS. (SOUTH-WESTERN SLOVAKIA)

E. Kocianová1, E. Špitalká1, L. Berthová1, Z. Svitálová2, L. Mydlová2, M. Kazimírová2
Institute of Virology, SAS, Dúbravská cesta 9, 845 05 Bratislava, SR1; Institute of Zoology, SAS, Dúbravská cesta 9, 845 06 Bratislava, SR2

Game, namely roe deer (Capreolus capreolus), red deer (Cervus elaphus), muflon (Ovis musimon), fallow deer (Dama dama) and wild boar (Sus scrofa), living in deciduous phragmented and non-phragmented forest habitats in south-western Slovakia, are obligate of hosts adult and subadult (L and N) stages of Ixodes ricinus ticks. In collaboration with local hunters, tissue samples (spleen, blood, skin) and ticks feeding on game were obtained from suburban (Bratislava forest park) and natural (Fúgelka, Harmónia) sites in the Malé Karpaty Mts (2011-2013). Ticks were obtained from all examined species, except wild boar. Majority were I. ricinus (all developmental stages): 60% larvae, 29.3% nymphs, 8.6% females and 2% males.

In DNA samples extracted from these ticks overall prevalence of rickettsiae (R. helvetica, R. monacensis and Rickettsia sp.) was 13.1% and of Coxiella burnetii 2.6% as revealed by PCR targeting the gltA and com1 genes, respectively. Positive ticks were found on all infested game species. Prevalence of Anaplasma phagocytophilum in I. ricinus collected from game was 69.5% and prevalence of Babesia sp. was 36.4% (detected by PCR targeting the msp2 and 18S rRNA genes, respectively). Presence of Neoehrlichia mikurensis and Borrelia burgdorferi s.l. was found only sporadically in nymphs and adults. Screening of spleens of game by PCR revealed the presence of A. phagocytophilum in all examined species with 100% prevalence in red deer and fallow deer. Babesia-positivity was detected only in deer, with the highest prevalence in roe deer. Spleens of game were negative for rickettsiae, Coxiella burnetii, Neoehrlichia and Borrelia.

High prevalence of tick-borne agents, namely A. phagocytophilum and Babesia sp. in the examined game and positive ticks suggest the occurrence of natural foci in studied sites in south-western Slovakia. Further studies are necessary to gain better knowledge on the epizootology and epidemiology of the studied agents.

This study is financially supported by the FP7 project EDENext (No. 261504), grants APVV DO7RP-0014-11 and APVV 0280-12 and grant VEGA No. 2/0061/13.

PRELIMINARY STUDY OF BAT ECTOPARASITES IN EASTERN SLOVAKIA

A. Kočišová1, A. Ondrejková1, D. Lőbbová2
Department of Epizootology and Parasitology, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovak Republic1; Slovak Bat Conservation Society, Andaščikova 618/1, Bardejov, Slovak Republic2

Bats are beneficial to humans and local ecosystem by reducing insect populations. They are suspected to be more vulnerable to disease and death when they carry more parasites. There are limited information available about bat ectoparasites in Slovakia. The aim of our research is to give first information on ectoparasitic fauna of bat in Eastern Slovakia.

Three hundred and twenty eight ectoparasites were collected from 58 bats of ten species (Myotis myotis, M. bechsteinii, M. nattereri, M. emarginatus, Eptesicus serotinus, Miniopterus
SCHRIEBERSII, RHINOLOPUS EURYALE, BARBASTELLA BARTASTELLUS, NYCTALUS NOCTULA AND PLEIOTUS AURITUS.

We recorded 328 ectoparasites that belonged to 5 families and 5 genera of ticks or insects. On average, 79% of bats were infested by mites, with a mean intensity of 6.65 individuals per bat. We recorded bat ectoparasites of the groups Acarina: Ixodidae (Ixodes verspertilus; 1.72%), Macronyssidae (Steatonyssus occidentalis; 5.17%) and Spinturnicidae (Spinturnix mystacina; 79.31% and S. bechsteinii; 20.68%). From the Diptera we recorded bat flies Stylidia biarticulata (Nycteribiidae) on seven bats. One species of fleas (Ischnopsyllus intermedius) we found on two bats.

This research was supported by grant APVV-0605-12.

SARCOPTIC MANGE VULNERABILITY IN CARNIVORES OF THE BIAŁOWIEŻA PRIMEVAL FOREST, POLAND

M. KOŁDZIEJ-SOBOCIŃSKA, A. ZALEWSKI, R. KOWALCZYK
Mammal Research Institute, Polish Academy of Sciences, Waszkiewicza 1, 17-230 Białowieża, Poland

Sarcoptic mange caused by Sarcoptes scabiei, is a highly contagious worldwide mite infection responsible for epizootic skin disease in populations of wild and domestic mammals. It causes skin disorders that may lead to severe generalized skin disease (mange). We investigated the spread and dynamics of sarcoptic mange in one of the best preserved carnivore communities in the Białowieża Primeval Forest, Poland over the last 20 years. Sarcoptic mange was found in 6 of 10 investigated species. The highest percentage of infected individuals was recorded in red foxes (19%), European badgers (9%), grey wolves (7%), raccoon dogs (6%), and stone martens (5%). Other species were not infected (least weasel, American mink, European polecat, pine marten) or infected sporadically (Eurasian lynx - 1 case). Rate of infection was correlated with the sum of ecological and behavioral features which may increase the spread of infection. Outbreaks of sarcoptic mange occurred simultaneously in several species of carnivores which indicates the occurrence of between species transmission. We found that the source of investigated individuals (trapped, culled and found dead) may influence results of analysis. Our study shows that in carnivore communities such as in Białowieża Forest, numerous species may serve as a reservoir of the pathogen and favor its persistence in the environment. This study indicates that sociality and use of den sites are important factors affecting sarcoptic mange spread and prevalence in wild populations of carnivores.

FIRST RECORD OF NEODIPLOSTOMUM SPATHLA (TREMATODA: DIPLOSTOMIDAE) IN AQUILA HELIACA FROM THE SLOVAK REPUBLIC

P. KOMOROVÁ¹, J. SITKO², Z. HURNÍKOVÁ¹, L. ŠIMÁK³
University of Veterinary Medicine and Pharmacy in Košice, Slovak Republic¹; Ornithological station of Comenius Museum in Přerov, Czech Republic²; Raptor Protection of Slovakia³

The Imperial Eagle (Aquila heliaca) is a highly endangered raptor species in Europe. The area of its occurrence extends from the middle of Europe across the Ukraine, Turkey and the South of Russia up to Central Siberia. In the territory of the Slovak Republic approximately 40 breeding pairs of
Imperial Eagle nest and the species is strictly protected. Main food component of these raptors comprise medium-sized mammals – ground squirrels, weasels, rats, juvenile hares and small rodents. Since 2012 we have investigated 6 individuals of these birds of prey that died naturally or as a result of human activities (car accidents, poisoning). All cadavers were examined for the presence of parasites during parasitological autopsy. Found trematodes were conserved in 70 % ethanol, stained in carmine and determined according to morphological characters.

The flukes were present in 1 of 6 examined birds. In the small intestine of a female Imperial Eagle mixed infection with trematodes was found. Two parasite species were identified, namely Strigea falconis (Szidat, 1928), and Neodiplostomum spathula (Creplin, 1829). Strigea falconis is a common fluke of Falconiformes with cosmopolitan occurrence. This is the first finding of the parasite in this bird host species in Slovakia. Except of the Slovak Republic, the parasite was detected also in other European countries – the Czech Republic, Austria, Germany and Spain (Sitko 2001; Kutzer et al., 1980; Krone, 2000; Sanmartín et al., 2004). Neodiplostomum spathula is a new parasite species for Slovak fauna. It occurs in Holarctic region and Australia. Sitko (1994) recorded this parasite in Golden Eagle (Aquila chrysaetos) from Czech Republic.

Due to the exceedingly rare opportunity for parasitological examination of these strictly protected raptors, herein reported findings of sporadic parasite species are very important for recognition of parasite fauna in the Slovak Republic.

The study was supported by the Science Grant Agency VEGA No. 1/0702/12.

FIRST RECORD OF NEODIPLOSTOMUM CANALICULATUM (TREMATODA: DIPLOSTOMIDAE) IN STRIX URALENSIS FROM THE SLOVAK REPUBLIC

P. Komorová¹, J. Sitko², Z. Hurníková³

University of Veterinary Medicine and Pharmacy in Košice, Slovak Republic¹; Ornithological station of Comenius Museum in Přerov, Czech Republic²

The Ural Owl (Strix uralensis) inhabits temperate climate zone from Europe through Asia to Japan. It is the second largest owl in Slovak Republic and occurs in deciduous and mixed forests of primeval type, especially beech forests. The Ural Owl feeds mainly small rodents, sometimes small birds, amphibians and insect. The estimated Slovak population is 700 to 1000 breeding pairs.

Since 2012, three specimens of Ural Owl were examined using the method of parasitological autopsy. After isolation of parasites from internal organs they were fixed in 70 % ethanol, stained with carmine and determined according to morphological characters.

Trematodes were detected in the small intestine of 1 of the 3 individuals. The mixed infection with Strigea strigis (Schrank, 1788) and Neodiplostomum canaliculatum (Nicoll, 1914) was diagnosed. Strigea strigis species from the family Strigeidae is typical for the birds from the order Strigiformes. This intestinal parasite is widespread in Palearctic region and Africa. In the Czech Republic it was identified in 4 host species – Short-eared Owl (Asio flammeus), Long-eared Owl (Asio otus), Eagle Owl (Bubo bubo) and Tawny Owl (Strix aluco) (Ryšavý, 1960; Tenora and Lusk, 1960; Škarda, 1964; Sitko, 1994; Sitko, 2001). The finding of Strigea strigis was also detected in owls from Netherland (Borgsteede et al., 2003). The finding of Neodiplostomum canaliculatum is the first report of this species in the territory of the Slovak Republic. This parasite is characteristic for owls and occurs in

*Strix uralensis* is, like other owls occurring in the Slovak Republic, a protected bird species. The examination of cadavers of these birds can only be done under special permit issued by the Ministry of Environment of the Slovak Republic. Moreover, the way of life of this owls living deep in forests is elusive and the findings of dead individuals are random. Hence, the descriptions of parasite fauna in the species are of high faunistic significance.

*The study was supported by the Science Grant Agency VEGA No. 1/0702/12.*

**DIPLOZOID MONOGENEANS: NEWLY IDENTIFIED DETAILS OF ULTRASTRUCTURE**

V. Konstanzová¹, B. Koubková¹, E. Dzika², M. Gelnar¹

Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic¹; Department of Medical Biology, Faculty of Medical Science, University of Warmia and Mazury in Olsztyn, 10-561 Olsztyn, Żolnierska St. 14c²

Transmission electron microscope studies of two species of blood feeding diplozoid monogeneans (*Paradiplozoon homoion* and *Diplozoon paradoxum*), gill parasites of cyprinid fish, showed some of the newly identified structures on the body surface and fine details of gastroderms.

Structure of the tegument is the same for diplozoids as that described for other monogeneans. Numerous microvillus-like tegumentary projections were observed in inturned tegument of the buccal cavity. Transversal ridges forming folds with massive tegumental spikes at the central part of body was described for the first time. Uniciliated tegumentary receptors with electron-dense collars and bulbous nerve endings were observed in the tegument near the buccal suckers.

The gastrodermis is composed of “U” shape nucleated haematin cells and a connecting syncitium, both having cytoplasmatic lamellae. In the gut lumen were observed intact erythrocytes. Small particles, presumed to be hematein, were found in the digestive cells and in the intestinal lumen. Haematin cells in different stages of blood digestion were detected.

These results contribute to the understanding of diplozoids life strategy and are helpful for the comprehension of the specific blood digestion strategy.

*The research was supported by Czech Science Foundation (project No. GAP 506/12/1258)*

**CATHAEMASIA HIANS (TREMATODA: CATHAEMASIIDAE) IN BLACK STORK (CICONIA NIGRA) IN SLOVAKIA**

A. Königová¹, L. Molnár², G. Hrčková¹, P. Major², M. Várady¹

Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovakia¹; Clinic for Birds and Exotic Animals, The University of Veterinary Medicine and Pharmacy Komenského 73, 041 81, Košice, Slovakia²

*C. hians* is a compulsory trematode of an endangered wild Black Stork in Europe, but only few reports have been published on this parasite. In the present study, *C. hians* infection was confirmed
in an infected female of Black Stork in the Slovak Republic. Trematode infestation was identified post mortem. Lesions in the gastrointestinal tract (oesophagitis) were associated with the presence of this trematode. Morphology of adult trematode *C. hians* along with inflammatory reaction in oesophagus during parasite infection was described in this study. Inflammatory connective tissue mast cells were seen in the granulomatous inflammatory foci in *submucosa* of infected oesophagus, characteristic for chronic inflammatory response. The presence of fluke *C. hians* in the Black Stork in Slovakia indicated the possibility of completing its life cycle, therefore confirming the importance to monitor the trematodosis in the wild stork in Slovakia.

*The study was supported by Grant Agency VEGA, Grant No. 2/0151/13 and VEGA, Grant No. 2/0150/13 of the Scientific Agency of the Slovak Academy of Sciences.*

**GAMETOCYST AND OOCYSTS OF *UROSPORA TRAVISIAE*, AN EXTRAORDINARY EUGREGARINE FROM MARINE POLYCHAETE**

M. Kováčiková, A. Valigurová, A. Diakin
Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

Apicomplexans represent a very successful group of unicellular parasites that consists entirely of parasitic species infecting a wide range of vertebrates and invertebrates. Most of them possess complicated life cycle, including three different types of reproduction: sporogony, merogony and gametogony. In the life cycle of gregarines, in general, gametocysts (pregametic paired association of gamonts enveloped by a thick wall) are present. The internal spaces of gregarine gametocyst represent a space where gametogony, further fusion of gamets and sporogony usually take place. The crucial step in infection spread is formation of sporozoites within a protective envelop (oocyst) during the sporogony. Despite the fact that the way of gametocyst excystation and also oocyst morphology are considered as key features in taxonomy, only few detailed studies dealing with their architecture exist.

Here we present new data on morphology of gametocysts and oocysts found in marine polychaete *Travisia forbesii*. In the host body cavity, structures named brown bodies comprising gametocysts with oocysts of gregarine *Urospora travisiae* were found. Gametocysts, whitish and round in shape, are surrounded with flattened host coelomocytes. Their envelope is thick (up to 6 µm) and consists of numerous closely adjacent layers and hyaline cover. Under this envelope, a lot of oocysts can be observed using light or electron microscopy.

The spindle-shaped oocysts are heteropolar with a transparent conical collar situated at one end and the other end is elongated in a thin curved tail. Under the scanning electron microscope, the oocyst surface appears smooth without any spines or folds, the cone and the tail are usually not preserved. Occasionally damaged oocyst walls reveal up to 8 longitudinally oriented sporozoites. The cross-sections for transmission electron microscopy confirm the presence of 4 sporozoites at both oocyst poles, with their posterior ends interdigitating in the middle part of oocyst and apical ends oriented towards the oocyst poles. Sporozoites appear banana-shaped and covered with a typical three-layered pellicle underlined by 20 longitudinal subpellicular microtubules. Sporozoites possess a large nucleus that often occupies their entire transversal section. On longitudinal sections, their anterior third is packed with several rhoptries and numerous micronemes. The nucleus is localized in
the middle or slightly posteriorly to the middle of the cell. Residual body, which is situated in between the sporozoites, usually consists of several transparent and dark inclusions. It should be also mentioned that sporogony in different oocysts from one gametocyst is asynchronous.

Our light microscopic observations agree with original description of *Urospora travisiae* oocysts published by V.A. Dogiel (Dogiel, 1910), however, this study provides completely new data on gametocyst, oocyst and sporozoite architecture in *Urospora travisiae*.

Financial support provided by Czech Science Foundation No. P505/12/G112 (ECIP).

**LIPOPTENA FORTISETOSA (DIPTERA: HIPOBOSCIDAE), ECTOPARASITIC SPECIES OBSERVED FOR A FIRST TIME ON ROE DEER IN POLAND**

J. Kowal¹, P. Nosal¹, M. Lesiak², S. Kornaś¹, M. Matysek³, M. Basiaga¹

Department of Zoology and Ecology, University of Agriculture in Krakow, al. Mickiewicza 24/28, 30-059 Krakow, Poland¹; ”Kaban” Maciej Lesiak, os. Szklane Domy 1/200, 31-972 Kraków, Poland²; Institute of Nature Conservation PAS, al. Mickiewicza 33, 31-120 Krakow, Poland³

More than 20 years have passed from the first information about presence of *Lipoptena fortisetosa* in Poland territory. Although, this parasitic dipteran was been observed later, mainly in south part of country in the field studies, the infection of cervids hasn’t been reported.

The aim of the study was to recognize *Lipoptena* spp. infection on eight roe deer from the area situated near Cracow. On all the animal carcasses, *Lipoptena cervi* (1 – 5 specimens) were found and additionally on two of them the presence *L. fortisetosa* (single specimens) were also noted.

The new observations of *L. fortisetosa* indicate continuous spread of this species on Poland territory.

**SIBLING SPECIES *GYRODACTYLUS SALARIS*/THYMALLI: THE MORPHOLOGY OF THE HARD PARTS OF HAPTOR PARASITES FROM GRAYLINGS**

M. Kulikowski¹, A. Mieszkowska², M. Rokicka², M.S. Ziętara², J. Rokicki¹

Uniwersytet Gdański, Katedra Zoologii Bezkręgowców i Parazytologii¹; Uniwersytet Gdański, Katedra Ewolucji Molekularnej²

*Gyroactylus salaris* Malmberg, 1957 is an economically important monogenean fluke, since it is a cause of high mortality of atlantic salmon *Salmo salar* (L) living in Fennoscandian Peninsula rivers belonging to the White Sea and the Atlantic basins. The aim of the study was to compare the hard parts of opisthaptor of *Gyroactylus* living on wild graylings *Thymallus thymallus* (L) from Swedish and Polish waters, with those found on salmon. Fish fins from three of nine analysed Swedish rivers tested positive for presence of *Gyroactylus* (rivers: Juktån, Sölvbacka Strömmar, Kaitum). The calculated prevalence and intensity of the infection were: 8.3% and 0.08; 23.1% and 0.35; 20.0% and 1.0 respectively. For Polish river (Radunia), the prevalence were 10% and the intensity 4.0. The results of the morphometric analyses of the haptor were as follows: for the hamuli the measurements
were similar for *G. salaris* from salmon, but the grayling parasites had shorter ventral bars and longer membranes. The analyzed specimens from grayling had slightly longer shafts of the marginal hooks than those from salmon, and the widths of the dorsal bars were identical. Taking into consideration the seasonal morphological diversity of the parasites, the observed differences were not significant to the extent enabling the distinction between the two populations. The results of the morphology of the hard parts of haptor, seem to prove that *G. thymalli* Žitnán, 1960 and *G. salaris* Malmberg, 1957 are in fact a strange of the same species.

**ARE SEROLOGICAL TESTS CAPABLE OF DISCRIMINATING BETWEEN DIFFERENT BORRELIA SPECIES?**

Z. Kurzová¹, P. Kodym¹, P. Balátová¹, D. Berenová¹, J. Hořejší¹, D. Picha²

National Reference Laboratory for Lyme Borreliosis, Centre of Epidemiology and Microbiology, National Institute of Public Health, Prague, Czech Republic¹; Bulovka Hospital, Prague, Czech Republic²

Lyme disease is caused by spirochetes of the *Borrelia burgdorferi* sensu lato complex, from which *Borrelia afzelii*, *B. garinii*, *B. burgdorferi* sensu stricto, *B. spielmani* and *B. bavariensis* are the most frequent in the Czech Republic. Some *Borrelia* species are supposed to have a specific pathogenic effect: while *B. garinii* should be related to neuroborreliosis, infection with *B. afzelii* could tend more to cutaneous symptoms. Moreover, the differences in antigenic structure could result in false negativity of serological tests if a nonspecific antigen is employed. In order to determine the *Borrelia* species infecting the patient and to avoid potential problems with test specificity, some laboratories opt to analyse the patient’s sample in parallel with ELISA and/or Western blot tests specific for antibodies to *Borrelia afzelii*, *B. garinii* and *B. burgdorferi* s.s.

The comparison was performed on serum and liquor samples collected from clinically characterized patients in Bulovka hospital, Prague. In a minority of patients the infecting Borrelia species was determined by sequencing Borrelia DNA isolated from the patient’s materials. Serum and liquor samples were tested concurrently using EIA *Borrelia afzelii* IgM, EIA *Borrelia afzelii* VlsE IgG, EIA *Borrelia burgdorferi* sensu stricto IgM EIA, *Borrelia burgdorferi* sensu stricto VlsE IgG, EIA *Borrelia garinii* IgM and EIA *Borrelia garinii* VlsE IgG (Test-Line, Brno, Czech Republic). Selected serum samples were also examined with BLOT *Borrelia afzelii* IgM, BLOT *Borrelia afzelii* VlsE IgG, BLOT *Borrelia garinii* IgM, *Borrelia garinii* VlsE IgG, BLOT *Borrelia burgdorferi*. sensu stricto IgM and BLOT *Borrelia burgdorferi*. sensu stricto VlsE IgG (Test-Line, Brno, Czech Republic). The species specific tests were also compared with methods based on recombinant *Borrelia burgdorferi* sensu lato antigens, e.g. ELISA Viditest, anti-*Borrelia* recombinant, IgM, Viditest, anti-*Borrelia* recombinant, IgG +VlsE (Vidia, Prague, Czech Republic) as well as with BLOT-LINE *Borrelia* /HGA BLOT-LINE *Borrelia* /HGA IgG (Test-Line, Brno, Czech Republic).

The comparison revealed only minor differences in results of species-specific tests. Only exceptionally the samples positive in one specific test gave negative results in another, or vice versa. Our results do not indicate any clear relationship between clinical signs and estimated *Borrelia*
species. It can be concluded that the usefulness of serological methods for determination of the species of *Borrelia* infecting the patient is limited.

*This study was supported by the Grant Agency of the Ministry of Health of the Czech Republic (IGA MZ ČR – No. NT/13467 - 4) and by institutional funds of the National Institute of Public Health (č.1 RVO-SZÚ/2014).*

**PHYLOGENY OF COCCIDIA INFECTING SMALL MAMMALS AND COEVOLUTION WITH THEIR HOSTS**

J. Kvičerová¹,², A. Mácová¹,², V. Hypša¹,²

Faculty of Science, University of South Bohemia, Branišovská 31, 370 05 České Budějovice, Czech Republic¹; Biology Centre, Institute of Parasitology, Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic²

Coccidia, the largest group of the phylum Apicomplexa, have traditionally posed many phylogenetic and evolutionary questions. However, apart from such fundamental questions, the coccidial phylogeny still contains many uncertainties regarding the origin and phylogenetic status of various taxa. Phylogenetic analyses within such taxa have revealed unexpected results conflicting with morphology and/or biological traits. There are precedents in the apicomplexan taxonomy of seemingly monophyletic groups, based on morphological and biological traits that turned out to be polyphyletic morphotypes after examination with molecular data. *Eimeria maxima*, a coccidium infecting poultry, serves as an example, and we also detected similar phenomenon when analyzing eimerians from rodents. *Eimeria*, the most speciose genus within Apicomplexa, served as a model group for addressing various questions connected to parasite speciation, host specificity and phylogeny. Here we present phylogeny of *Eimeria* species/morphotypes infecting various groups and families of commonly distributed small mammals (rodents, insectivores), and also of some rarely occurring mammals.

*This work was supported by grants P505/12/1620 and P506/14-07004S (Czech Science Foundation).*

**EFFECT OF AN ABAMECTIN FORMULATION ON THE ROOT-KNOT NEMATODE**

*MELOIDOGYNE INCognita* ON TOMATO

S. Laquale¹, N. Poiras², N. Sasanelli³, L. Poiras², S. Burteva³, A. Bivoli², I. Toderas², E. Iurcu-Straistaru², T. D’Addabbo¹

Institute for Plant Protection, CNR, Str. G. Amendola 122/D, 70126 Bari, Italy¹; Institute of Zoology, ASM, Str.Academiei 1, 2028 Chisinau, R. Moldova²; Institute of Microbiology and Biotechnology, ASM, Str.Academiei 1, 2028 Chisinau, R. Moldova³

Abamectin is a mixture of macrocyclic lactones produced by the actinomycete *Streptomyces avermitilis* especially known for its insecticidal and acaricidal activities. The effectiveness of an abamectin formulation (Vertimec® EC) for the control of the widespread and polyphagous root-knot nematode *Meloidogyne incognitawas investigated in a pot experiment on tomato (cv. Rutgers) in
glasshouse. Different concentrations (0, 0.125, 0.25, 0.5, 0.75 and 1 mL/L) of an aqueous solution of the abamectin formulation were applied as a single treatment at transplant or at transplant plus 15 days later to a soil uniformly infested by *M. incognita* (7 eggs and juveniles/mL soil). Fenamiphos (62.5 c.p. L/ha) and untreated soil were used as controls. Pots were arranged on benches in a glasshouse at 25 ± 2℃, according to a randomized block design with five replications for each treatment. After two months tomato plants were uprooted and fresh and dry top weights were recorded. Severity of the nematode attack (root gall index - RGI) was assessed on each tomato root system according to a 0 – 10 scale. Final nematode population density was calculated summing nematodes extracted from tomato roots by sodium hypochlorite’s method and those extracted from 500 mL soil with the Coolen’s method. Reproduction rate \( r = Pf/Pi \) was calculated as ratio between final and initial population density. Data from the experiment were subjected to analysis of variance and means compared by the Least Significance Difference’s test (LSD) using the PlotIT program. All abamectin solutions significantly increased fresh and dry top weight in comparison to the untreated control. Root gall index, eggs and juveniles/g root, soil nematode population density and reproduction rate were significantly reduced by all the abamectin treatments in comparison to the untreated control \( (P=0.05) \). Nematode population density and reproduction rate in the soil treated with the three highest concentrations of abamectin solution were not significantly different from those calculated in the fenamiphos treated soil.

The research was undertaken within the framework of a bilateral project between the Moldavian Academy of Science (ASM – R. Moldova) and the National Council of Research (CNR – Italy). Years 2013-14.

**THE COMPARISON OF UGTs GENE EXPRESSION BETWEEN BOTH GENDERS OF SUSCEPTIBLE AND MULTI-RESISTANT STRAIN OF HAEMONCHUS CONTORTUS**

L. Lecová, P. Matoušková, L. Skálová

Department of Biochemical Sciences, Faculty of Pharmacy, Charles University in Prague, Heyrovského 1203, 500 05, Hradec Králové, Czech Republic

Anthelmintic resistance becomes a serious problem as an inevitable consequence of the use of anthelmintics over time. Resistant parasites have several mechanisms that protect them from the effects of these drugs. Highly effective mechanisms are differences in anthelmintic metabolism and detoxification as well as differences in gene expression affecting the drug effect on the target protein without interfering in its binding affinity.

Biotransformation enzymes represent an excellent defence mechanism against the negative action of xenobiotics. An important role in the inactivation and excretion of a great variety of these compounds plays glucosidation. This task in parasites is performed by enzymes from the class of UDP-glucosyltransferases (UGTs). Taking advantage of the newly assembled genome sequences of *Haemonchus contortus*, we performed a pilot analysis of some gene candidates from UGTs family in this worm. The characterisation of the UGTs family in *H. contortus* was facilitated by the availability of genomic, mRNA and amino acid sequence for the complete UGTs family in model nematode *Caenorhabditis elegans* (WormBase). 37 findings were identified and were used for a BLASTp (protein database using a protein query) reciprocal search of the all nematodes (taxid: 6231) database to ensure the best hits were indeed UGTs. In total, 34 UGTs sequences - “tags” were identified.
In this study both genders of susceptible (ISE) and multi-resistant (WR) strain of *H. contortus* were compared. The first tested sequence was CDJ89503, containing two genes. Their mutual independence has been confirmed by Artemis (Sanger) software analysis and for their easier differentiation they have been identified as UGT2 and UGT3. The second tested sequence was CDJ96086 called as UGT10. In both strains the UGTs gene expression was higher in males compared to females. Surprisingly, higher expression was observed in the sensitive strain and not in the resistant strain as expected. Just males of WR strain showed higher expression of UGT10, but the difference was not statistically important.

*This study was supported by the operational programme ECOP, registration number CZ.1.07/2.3.00/30.0061, Increasing of the R&D capacity at Charles University through new positions for graduates of doctoral studies, Czech Republic.*

**DRUGS ON THE *IN VITRO* EFFECT OF ANTIPARASITIC EXPRESSION OF THE GLYCOGEN METABOLISM GENES OF L3 LARVAE OF ANISAKIS SIMPLEX (NEMATODA)**

E. Łopieńska – Biernat, E. Zaobidna, M. Dmitryjuk
Department of Biochemistry, Faculty of Biology and Biotechnology,
University of Warmia and Mazury, Oczapowskiego Street 1A, 10-719 Olsztyn, Poland

*Anisakis simplex* is a gastrointestinal parasitic nematode with a complex life cycle; the definitive hosts are marine mammals as well as humans. As anisakiasis is a serious condition that can be fatal to humans and may cause allergic reaction in patients sensitive to *A. simplex* allergens, it is necessary to intensify molecular biology research on the parasite’s larvae. glycogen acts as energy reserve and a as an important saccharide in the metabolism of parasites. The synthesis of glycogen in nematodes is catalyzed by glycogen synthase - GSY (EC 2.4.1.11). The degrade of glycogen by glycogen phosphorylase GLYP (EC 2.4.1.1). Genes expression of glycogen synthesis and degradation was examined basing on previously obtained partial mRNA sequences of L3 larvae of *A. simplex*, *gsy* gene with the length of 485 bp (JX 173686) and 927 bp (JX 941465) of *glyP* gene.

In the present research we determined *in vitro* (Iglesias et al., 1997) the effect of ivermectin, levamisole and combination of these drugs (10; 100; 1000; 5000 µg/ml) on the expression of mRNA *sgy* and *glyP* involved in glycogen metabolism in invasive L3 larvae of *A. simplex* after 48 and 96 hours. After 48 h nematodes were transferred to plated containing either no drugs or ivermectin, levamisole and mix of them. Isolation of total RNA was performed using total RNA mini kit according to the manufacturer’s instructions (A&A Biotechnology), RT PCR (cDNA synthesis or Reverse Transcription PCR) by TranScriba kit (A&A Biotechnology). Quantitative real-time PCR was performed using the SYBRGreen PCR-MIX ™ Taq (A&A Biotechnology). All samples were tested in triplicate on LightCycler (Applied Biosystems, FAST 7500). Melting curves were constructed after amplification. The data were analyzed and normalized relative to ribosomal gene (*ryb*) (AIC00203) (an endogenous control gene) at transcript level by an AB analysis software (7500v2.0). The mean value ± SD was used for analysis of relative transcript levels for each time point using the ΔΔct method.

A reduction in mRNA expression of *sgy* gene of L3 larvae during incubation with ivermectin was observed with increasing concentration of drug after 48 h. A similar correlation was demonstrated *glyP* expression at 96 h only in the highest concentration of the drug. Decrease the reaction of both genes was observed until the second day of incubation with levamisole. After 48 hours of incubation
of larvae in a mixing of the two drugs was found opposite effect on the tested genes. There has been a decreasing expression of glycogen phosphorylase and increase glycogen synthase.

The results showed that most effectively act on the synthesis of ivermectin, and combination drugs on glycogen breakdown. The presence 48-96 h of drugs in A. simplex resulted in a significant decrease in the level mRNA expression of glycogen metabolism genes, but drugs do not completely block these pathways. Results demonstrated outside the previously known effects on ion channels anthelmintic and the immune system of nematodes and their inhibitory effect on the expression of enzymes of carbohydrate metabolism.

**EIMERIA AND APODEMUS – PHYLOGENETIC RELATIONSHIPS AND POPULATION STRUCTURE**

A. Mácová¹², J. Kvíčerová¹², V. Hypša¹²

University of South Bohemia, Faculty of Science – České Budějovice, Czech Republic¹;

Academy of Sciences of the Czech Republic, Institute of Parasitology – České Budějovice, Czech Republic²

Study of relationships and coevolution between hosts and parasites is a remarkable field in parasitology. It is clear now, that in many cases the host phylogeny may provide a clue for deriving the parasite phylogeny and vice versa, reflecting the tight relationships between the two counterparts. This study presents research on population structure and phylogenetic relationships of eimerians parasitizing three species of field mice, Apodemus agrarius, A. flavicollis and A. sylvaticus. The phylogenetic analyses based on 101 sequences of mitochondrial gene for cytochrome c oxidase subunit I (COI) and nuclear 60 sequences of 18S rRNA gene (SSU) revealed five genetic lineages differing in their relations between phylogeny and biological traits. Most of them evinced broad host and geography distribution, except for a single lineage, restricted only to A. agrarius in eastern Slovakia. According to morphometry, the lineages corresponded to four known Eimeria species/morphotypes (E. alorani, E. apionodes, E. jerfinica and E. kaunensis).

*This study was supported by grants P505/12/1620 and 14-07004S (Czech Science Foundation).*

**DNA ISOLATION AND SEROLOGICAL DETECTION OF TOXOPLASMA GONDII IN SMALL RODENTS**

T. Machačová¹, E. Bártová¹, A. Žákovská²

University of Veterinary and Pharmaceutical Sciences Brno, Faculty of Veterinary Hygiene and Ecology, Department of Biology and Wildlife Diseases, Palackého tř. 1/3, 612 42 Brno, Czech Republic¹; Masaryk University, Faculty of Science, Department of Experimental Biology, Kamenice 753/5, Bohunice, 62500 Brno, Czech Republic²

The aim of the study was to test wild rodents in the Czech Republic for the presence of T. gondii antibodies and also to detect T. gondii DNA in livers and brain of rodents and to characterize genotypes in case of T. gondii isolation. During years 2010 – 2014, 312 wild rodents of genus Apodemus and Myodes were caught in three areas (Moravský Kras, Mohelno and Poodří) of the Czech Republic. In total, 96 blood serums were tested for presence of T. gondii antibodies by the
Indirect Fluorescent Antibody Test (IFAT); samples with titre ≥ 50 were considered positive. The blood samples were also tested by latex agglutination test (LAT). Samples of 312 livers of rodents and 21 brains of rodent embryos were used for DNA isolation and PCR detection of *T. gondii* DNA. Antibodies against *T. gondii* were found in 1 % (1/96) of rodents using IFAT and in 5.2% (5/96); *T. gondii* DNA was isolated from 4 of 312 (1%) livers and from none of 21 brains of embryos. Samples of three *T. gondii* isolates were genotyped by multiplex PCR and characterized as avirulent type II that is typical for Europe. However, these genotypes are unique in the French database of *T. gondii* isolates characterized by microsatellite markers.

**COMMON ECTOPARASITES IN EXOTIC ANIMAL PRACTICE**

P. Major¹, L. Molnár¹, A. Kočišová², K. Kuzyšinová¹, L. Kottferová¹

Clinic of Birds, Exotics and Wildlife, University of veterinary medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovak Republic¹; Department of Epizootology and Parasitology, University of veterinary medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovak Republic²

Ectoparasite infestations play an important role in exotic animal practice. The affected animals are often presented with varying symptoms, from mild to severe pruritus, dermatitis, feather loss, feather plucking, neurological disorders etc. In some cases the animals show no clinical signs, and the disease is diagnosed during the routine clinical examination. The course of the disease and the possible treatment mainly depends on the patients immunostatus, physical condition, any secondary bacterial infection, and in some cases the concurrent disease.

In this work, the authors present their own experience with ectoparasites in exotic animals. The work will focus on the most frequent cases seen during the past year, including clinical signs, diagnostic possibilities, and therapy. All the animals presented in this study were treated at the Clinic of Birds, Exotics and Wildlife in the University of veterinary medicine and Pharmacy in Košice.

**PREVALENCE OF DIROFILARIA REPENS IN MOSQUITO POPULATION IN THE VICINITY OF WARSAW**

A. Masny¹, R. Salamatin¹², E. Golab¹

Department of Medical Parasitology, National Institute of Public Health – National Institute of Hygiene, Warsaw, Poland¹; Department of General Biology and Parasitology, Medical University of Warsaw, Poland²

**BACKGROUND**

In recent years there has been growing interest in the epidemiology of *Dirofilaria repens*, a filarial parasite of carnivores, transmitted by mosquito vectors and capable of infecting humans. The presence of the parasite in the environment can be confirmed by detection of *D. repens* in the local population of mosquitoes. Apart from climate, a very important factor influencing the risk of transmission of the infection, to dogs or humans, is the prevalence of *D. repens* in mosquito population.
METHODS

The mosquito collection site was located in a residential area close to Warsaw, were presence of infected dogs was confirmed. It was previously evaluated that in this area the prevalence of canine dirofilariosis was between 10% and 20%. The mosquitoes were collected overnight, using carbon dioxide and visible light baited traps on the following dates: 19.06.2012, 03.07.2012, 08.07.2012, 15.07.2012. Collected mosquitoes were frozen in −70°C and stored for further analysis. Single insects and pools of 10 mosquitoes were analyzed. Mosquito DNA was isolated using guanidinium thiocyanate and silica suspension. Parasite DNA was detected using two sets of universal primers Replm-F0/RepIm-R0 and RepIm-Fs/RepIm-R2, for simultaneous detection of *D. repens* and *D. immitis*, by amplification of cytochrome oxidase subunit I (COI) gene fragments sized 309 bp and 436 bp, respectively. Species determination was based on PCR product sequencing.

RESULTS

*D. repens* was detected in samples collected on 03.07.2012 and 15.07.2012. The average infection rate was 1,56% (95% CI: 0,81–2,79). PCR with primers Replm-F0/RepIm-R0 detected eight infected pools and PCR with primers RepIm-Fs/RepIm-R2 detected *D. repens* DNA in four of the eight positive pools.

CONCLUSIONS

We found that *D. repens* infection rate in mosquito population from the vicinity of Warsaw was higher than the one described in other EU countries.

The twofold difference in the detection rates of both PCR assays used in the study shows that, in mosquito xenomonitoring for *D. repens*, the PCR detection rates and mosquito infection rates should not be confused.

NEW EMERGING CANINE HELMINTHOSES IN SLOVAKIA

M. Miterpáková1, Z. Hurníková1,2, A. Iglódyová1

Institute of Parasitology SAS, Košice, Slovakia1; University of Veterinary Medicine and Pharmacy, Košice, Slovakia2

Natural or human-induced modifications of ecosystems during recent years resulted into the appearance of new (emerging) parasitic diseases or have changed the epidemiology of already existing (re-emerging) infections in several European regions. The increased spread of parasitic diseases is supported mainly by climatic changes (e.g. increase of average temperature and precipitation intensity) which are crucial for the presence and distribution of various intermediate hosts (insects, snails or small mammals). On the other side, anthropogenic factors such as new farming techniques, an increase in trade and recreational activities, or intensive migration with animals also play very important role. During the past few years also strong urbanisation of wildlife animals has been seen with synanthropic animals representing significant potential reservoir source of parasitic infections transmitted from the rural environment to the urban one. From this point of view, carnivore pets are the most vulnerable group of hosts. In Central European countries, including Slovakia, several canine helminthiases have emerged during the past decades. From the medical point of view, the most important are nematodes affecting cardiopulmonary systems of dogs and cats – *Dirofilaria* spp., *Angiostrongylus* spp., *Aeulostrongylus* spp. or *Crenosoma vulpis*.

In Slovakia, research aimed at new emerging canine helmintic diseases has been initiated in 2007 with monitoring of dirofilariosis that was detected for the first time in Slovak dogs two years
before. By now, more than 3000 dogs were examined for *Dirofilaria* spp. presence. Mean prevalence rate reached about 13.0% with the highest incidence in south-western parts of the country (20.0 – 30.0%). In all infected dogs *Dirofilaria repens* was found; in 9 cases mixed infection with heartworm (*Dirofilaria immitis*) was diagnosed.

In 2013, *Angiostrongylus vasorum* was observed in Slovakia for the first time. Two dogs were found to be infected, in one of them severe clinical symptoms with almost fatal course were observed. Since 2014, the first intensive surveillance for canine angiostrongylosis has been in progress.

Additionally, we aimed our research at other metastrongyloid lungworms such as *Aeulostrongylus* spp., *Eucoleus aerophilus* or *Crenosoma vulpis*, considering the data of their occurrence in our region being very scant. However, the recent studies from Italy, Germany or Portugal revealed that this nematodes commonly found in wildlife are occurring already in companion animals.

Taking into account that geographic range of emerging canine helminthiases has expanded, intensive epidemiological research is required. Additionally, veterinary practitioners should include mentioned lungworms and heartworms into differential diagnosis and use appropriate diagnostic techniques lead to adequate treatment of infected animals. Epidemiological screening together with increased clinician awareness is also an important tool for the infections prevention and management.

This study has been realized thanks to the financial support of the Research & Development Operational Programme funded by the ERDF: Environmental protection against parasitozoanoses under the influence of global climate and social changes (code ITMS: 26220220116).

**ALBENDAZOLE RESISTANCE OF *HAEMONCHUS CONTORTUS* IN SHEEP FROM SOUTHERN POLAND**

P. Nosal¹, J. Kowal¹, M. Skalska¹, P. Sendor², M. Murawski³

Department of Zoology and Ecology, University of Agriculture in Krakow, al. Mickiewicza 24/28, 30-059 Krakow, Poland¹; Małopolska Agricultural Advisory Centre in Karniowice, os. XXXV-lecia PRL 9, 32-082 Bolechowice, Poland²; Department of Swine and Small Ruminant Breeding, University of Agriculture in Kraków, 1b Rędzina St., 30-248 Kraków, Poland³

Despite its major veterinary and economical importance, the data about the occurrence of anthelmintic resistance in gastro-intestinal nematodes of sheep in Poland are limited. During the study aimed to recognize the parasite occurrence in the flock of 30 adult Polish Mountain Sheep, formed to graze on the flood embankments in the vicinity of Cracow (southern Poland), deworming action was performed at the start of experiment, in July. The method used to assess the efficacy of the drug applied (Valbazen, albendazole), was the fecal egg count reduction test (FECRT), supported by the identification of larvae obtained from the established coproculture (Coles et al., 2006).

The obtained in coproscopic examination results indicated low level of gastro-intestinal nematode infection in grazing sheep, was similar before treatment and 10 days post-treatment and varying for the whole herd from 10 to 2100 eggs per 1g of faeces (EPG). The resistance was noted in *Haemonchus contortus*. The FECRT value evaluated for this species was low and reached in average 36.63%, ranging in individual sheep from 0 to 100%. The other gastro-intestinal nematodes
(Trichostrongylus spp., Teladorsagia spp. and Chabertia ovina), as well as pulmonary nematode species found (Dictyocaulus filaria, Muellerius capillaris), demonstrated full susceptibility to the drug.

TRANSMISSION PATTERNS OF FASCIOLOSA HEPATICA IN SWEDEN
A. Novobilský¹, K. Gustafsson², A. Engström¹, S. Sollenberg¹, D.A. Morrison¹, J. Höglund¹

Swedish University of Agricultural Sciences, Department of Biomedical Sciences and Veterinary Public Health, Section for Parasitology, 750 07, Uppsala, Sweden¹; Swedish Animal Health Service (SvDHV), PO Box 5007, 514 05 Långhem, Sweden²

Fasciolosis, caused by the common liver fluke Fasciola hepatica, is a serious and economically important disease of sheep and cattle with a worldwide distribution. Losses for livestock production consist not only of condemned livers at abattoirs, but also reduced performance (meat and milk) and sometimes even sudden deaths of animals. To improve control strategy against fasciolosis in Sweden, a deeper knowledge on epidemiology of fasciolosis is needed.

Transmission patterns of Fasciola hepatica were investigated on beef cattle (n=3) and sheep (n=3) farms in Sweden between 2011 and 2012. The dynamics of fluke infection, particularly estimated time of infection, were screened each grazing season by ELISA detection of antibodies in lambs (n=94) and first grazing season calves (n=61). Typical ‘winter infection’ was observed on two sheep farms in 2012, but the most prevalent transmission pattern was found to be ‘summer infection’, characterised by infection of animals in late summer (August-September) by F. hepatica originating from overwintered and/or spring-excreted eggs. In contrast, beef calves were infected only in September-October (‘summer infection’). Colostral transfer of F. hepatica antibodies from seropositive ewes was detected in sheep up to 11 weeks of age. Furthermore, lymnaeid and succineid snails were collected on the pastures used by these animals both in spring and in the autumn each year. In total, 1726, 588, 138, 130, 93 and 42 specimens of Galba truncatula, Lymnaea palustris, L. glabra, L. fuscus, Radix peregrina and Succinea putris, respectively, were collected and identified. These were subsequently examined for the presence of F. hepatica DNA by species-specific PCR and the findings compared against mean monthly rainfall and temperature data for each farm. The main intermediate host of the liver fluke was G. truncatula, with a prevalence range of F. hepatica infection from 0–82%. Only 1 out of 42 terrestrial S. putris tested positive for F. hepatica, casting doubt on the role of this species in transmission of F. hepatica in Sweden. The prevalence of F. hepatica in G. truncatula in general varied extensively between sites, seasons and years.

In conclusion, two main peak periods of infection were observed: May-June (from overwintered infected snails = ‘winter infection’) and August-September (from metacercariae developed and produced by snails during summer = ‘summer infection’). The occurrence and frequency of ‘winter infection’ were dependent on local environmental factors such as snail habitat availability or grazing behaviour of animals, rather than on climatic factors.
ADVANCED LIVER ALVEOCOCCOSIS MIMICKING DEGENERATING CYSTIC ECHINOCOCCOSIS: A CASE STUDY

S. Nowak
Department and Clinic of Tropical and Parasitic Diseases, Poznan University of Medical Sciences, Poland

Background: The last years, alveolar echinococcosis which is caused by a larval form of Echinococcus multilocularis has become an emerging parasitic infection in Poland. Infection occurs due to ingestion of parasite eggs shed with feces of red foxes (Vulpes vulpes), classical definitive hosts. Domestic dogs and cats may also become infected. Human is accidental host for E. multilocularis. Populations at risk include people who have contact with soil, close contact with dogs, work in domestic gardens, forest workers and farmers. Disease may have asymptomatic course for a long period of time. Liver changes caused by E. multilocularis infection, may take various forms, often mimicking neoplastic lesions – irregular, not clearly separated from normal liver tissue, with central necrosis, infiltrating surrounding tissues and organs, giving distant metastases. In some cases focal lesions described in imaging investigations may look similar to those more characteristic for E. granulosus infection – i.e. quite good separated cysts, not infiltrating healthy liver, with numerous compartments inside.

Aim of the study: To describe diagnostic and clinical difficulties in an advanced stage of E. multilocularis infection in an unusual case of a Polish patient suspected for years of classic cystic echinococcosis of the liver.

Description of the case: This study presents a case of a patient hospitalised in the Clinic of Tropical and Parasitic Diseases, Poznan University of Medical Sciences (Poland), who primarily was diagnosed as cystic echinococcosis (E. granulosus), mainly due to characteristic cystic lesions in the liver in imaging investigations and results of classical serological tests (Echinococcus spp. ELISA). Differential diagnosis by using epidemiological, clinical, imaging and immunodiagnostic examinations has been performed. Ultrasonography and computed tomography of abdomen suggested degenerating hydatid cyst of the liver of a type CE4 according to the WHO classification, with a mixed hyper- and hypodense content. In the course of further observations, progression of lesions located in liver with spreading to lungs (metastases) was revealed, despite of the implementation of basic, targeted but shortened therapy with albendazole which is typical for cystic echinococcosis, the suspicion of alveococcosis was established and lately definitely confirmed using the reference immunoblotting technique.

Conclusions: 1) In areas endemic for E. multilocularis infection in humans, irregular tumour-like lesions of the liver always require detailed differential diagnosis with alveolar echinococcosis in university centers specialised in clinical parasitology. 2) In doubtful cases, direct demonstration of parasite infection using histopathological examination of samples obtained from liver biopsy or using molecular biology tests which look for elements of parasite genome may be useful.
HELMINTHS OF MUSTELIDS (MUSTELIDAE) IN LITHUANIA

D. Nugaraitė, V. Mažeika, A. Paulauskas, J. Radžijevskaja
Vytautas Magnus University, Department of Biology, Vileikos str. 8, Kaunas LT-44404, Lithuania

Mustelids (Mustelidae) the largest family in the order Carnivora. In Lithuania pine marten (Martes martes), stone marten (Martes foina), stoat (Mustela erminea), least weasel (Mustela nivalis), European polecats (Mustela putorius), American mink (Neovison vison), Eurasian badger (Meles meles), European otter (Lutra lutra) are found. European mink (Mustela lutreola) is extinct species, not spotted in Lithuania for some decades. Parasites of mustelids have been studied in Lithuania about 40 years ago. The objective of this study was to explore the helminths of mustelids.

The material for the helminthological investigation was collected during 2011 – 2014 in different parts of Lithuania. 25 mustelids were examined: 2 pine martens, 4 stone martens, 10 European polecats and 9 American minks. Mammals were processed by method of total helminthological dissection of individual organs (Ivashkin et al., 1971). Nematodes were studied after mounting in glycerine. Before study trematodes and cestodes were stained in carmine, dehydrated, and mounted in Canada balsam. Twenty-three mustelids of 25 studied were infected with helminths. Nine taxa of the parasitic worms were found: flukes Isthmiophora melis (Schrank, 1788) and Strigea strigis (Schrank, 1788) mesocercaria, cestodes cestoda g. sp. 1, cestoda g. sp. 2 and nematodes: Eucoleus aeroophilus (Creplin, 1839), Aonchotheca putorii (Rudolphi, 1819), Crenosoma sp., Molineus patens (Rudolphi, 1845) and Nematoda g. sp. The flukes I. melis in European polecats had the highest mean abundance (A = 391.3) and prevalence (P=90%) out of all the helminthes. Flukes I. melis also were found in American mink (A= 19.4, P=66%). Second species with high parameters of infection was Strigea strigis mesocercaria: in European polecat mean abundance was 11.6 and prevalence of infection - 50%.

The study was partially supported by the Research Council of Lithuania (grant no. LEK-14/2012).

PRELIMINARY STUDIES ON ENDOPARASITES OF EXOTIC LIZARDS (LACERTILIA)

A. Okulewicz1, M. Kaźmierczak2, M. Adamczyk1
Department of Parasitology, Institute of Genetics and Microbiology, University of Wrocław, Przybyszewskiego 63/77, 51-148 Wrocław, Poland1; Department of Vertebrate Evolutionary Biology and Conservation, Institute of Environmental Biology, University of Wrocław, Sienkiewicza 21, 50-335 Wrocław, Poland2

Thirty five lizards (belonging to 27 species) housed in the City Zoological Garden in Wrocław, 13 (4 species) kept in private breeding and 9 specimens (5 species) acquired from zoological wholesales were subjected to parasitological examination (using coprological methods). Examined lizards belonged to the following families: Agamidae, Anguidae, Carphodactylidae, Chamaeleonidae, Corytophanidae, Dactyloidae, Gekkonidae, Gerrhosauridae, Iguanidae, Lacertidae, Phrynostomatidae, Scincidae and Varanidae. Endoparasites were found in 21 (60.0%) lizards from the City Zoological Garden in Wrocław, 10 (76.9%) specimens coming from private collections and 6 (66.6%) animals from zoological wholesales.
Nematodes of *Pharyngodon* sp. and *Thelandroids* sp. genera (Pharyngodoniidae, Oxyurida) were the most common intestinal parasites found in lizards from Wrocław Zoo. These parasites were detected in 40% of examined specimens belonging to the following species: *Agama agama*, Basiliscus vittatus, Celestus warreni, Chamaeleo calyptratus, Cyclura nubila, Eublepharis macularius, Eumeces schneideri, Gallotia galloti, Gerrhosaurus nigrolineatus, Lacerta lepida, Pogona vitticeps. Intensity of invasion was high (++/+++). Other parasites occurred less frequently: Strongyloides sp. in *Agama agama*, *Capillaria* sp. in *Acanthosaura capra* and *Varanus ornatus*, as well as *Nyctotherus* sp. (Ciliophora) in *Chamaeleo calyptratus* and *Eumeces schneideri*. Pseudoparasites (such as eggs of *Hymenolepis nana* and Acari eggs) were found in feces of *Varanus mertensi*, *V. ornatus* and *V. salvadori*. Following endoparasites were found in 13 lizards obtained from private breeding: eggs of Pharygonididae (Oxyurida) in *Underwoodisaurus milli*, *Chamaeleo calyptratus*, *Eublepharis macularius* and *Pogona vitticeps*, Digenea eggs in *U. milli* and protozoan (Isoptera sp.) in *P. vitticeps* and *Ch. calyptratus* as well as unidentified Ciliophora in *Ch. calyptratus*. In feces of lizards from zoological wholesale we found eggs of Pharygonididae in *Gekko gecko*, *Iguana iguana* and *Physignathus cocincinus* as well as eggs of cestodes *Oochoristica* sp. in *I. iguana* and protozoa *Choleoeimeria* sp. in *Gekko vittatus* and Ciliophora in *I. iguana* and *Varanus exanthematicus*. In feces of reptiles from Varanidae family we also detected eggs of Acari.

Our study confirmed the abundance of Oxyurida in lizards in private breeding but also in large facilities such as zoological gardens, where animals are kept under veterinary supervision. In large lizards, such as Varanidae, nematodes Oxyurida are not present. Common parasites of large lizards are often connected with their diet, which consists mostly of rodents. Therefore we found numerous pseudoparasites (such as *Hymenolepis nana* and Acari) which are typical for small rodents.

**MONITORING OF ASCARIS SUUM IN SLAUGHTER PIGS**

A. Ondrejková1, Š. Černek2, M. Prokeš1, R. Ondrejka1, Z. Hurníková1,3

Department of Epizootology and Parasitology, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovakia1; Vetservis s.r.o., Kalvária 3, 949 01 Nitra, Slovakia2; Institute of Parasitology Slovak Academy of Sciences, Dpt. Parasitic Diseases, Hlinkova 3, 040 01 Košice, Slovakia3

*Ascaris suum* is a parasitic nematode of pigs with worldwide distribution causing economic loses due do health interactions, reduced feed to gain efficiency and loses due to liver condemnation at abattoirs. During 2000 – 2009, in total 19 017 slaughtered pigs from 279 farms were in detail post-mortem inspected for pathologies by trained veterinarians at the abattoir. Monitoring encompassed whole territory of Slovakia and included pedigree breeding, livestock production and farrowing farms raising 80 – 4000 sows. Pigs from selected farms were monitored at request of farm owner, breeder or veterinarian to ascertain the health condition of slaughter pigs. For recording and evaluation of results obtained within monitoring, the integral recording form was used designed by Marek Žižlavský, DVM, PhD., Sevaron, s.r.o. All obtained data about pathological lesions were collected and evaluated.

The routine detailed post mortem examination was proceed in standard way, using adpection and palpation and can slightly differ by technological facility of the abattoir. Within the monitoring was except for inspection of skin, lungs, hearth, intestines, lymph nodes and abscesses.
performed also examination of the liver, in particular of milk spots. All found abnormalities were recorded, including their localisation, extension, and quality.

The most frequent alterations detected by post mortem examination were: lesions localised in cranio-ventral parts of the lungs (sharply defined, solid, red-blue coloured – *Mycoplasma pneumoniae*; brown to greyish brown lesions – *Pasteurella multocida*, interstitial pneumonia – mixed with SIV, PRRS, PCV-2 porcine viruses, pulmonal and costal pleuritis as the result of other etiologic agents – *Actinobacillus pleuropneumoniae*, *Mycoplasma hyorinus*, *Hemophilus parasuis*.

Investigated was also the presence of milk spots – *hepatitis interstitialis parasitaria multiplex* as a reliable indicator of infestation in organism. During the years 2000 – 2009 a significant decrease (p < 0.0001; Chi-square df 917.6.9; alpha < 0.05) in prevalence of milks spots was recorded from 39.51 % in 2000 down to 6.85 % in 2009 as a result of consistent execution of de-worming programmes and sanitation management. The intensity of infection was expressed as the number of milk spots in the liver. Most infected pigs belonged to category 1 with 1 – 4 milk spots (aver. 9.4 %); less frequent was category 2 with 5 – 15 milk spots (3.78 %). In the category 3, represented by intensity of over 15 milk spots, also decrease in numbers of positive animals was recorded over the study period with only 9 (0.96 %) pigs in 2008.

**SYNOPTIC REVIEW OF **PR**OMONOBO**THRIUM MACKIEWICZ, 1968 (CESTODA: CARYOPHYLLIDEA), PARASITES OF SUCKERS (CATOSTOMIDAE) IN NORTH AMERICA**

M. Oros³, J. Brabec³, A. Choudhury², R. Kuchta³, T. Scholz³

Institute of Parasitology, Slovak Academy of Sciences, Hlinkova 3, 04001 Košice, Slovakia¹; Division of Natural Sciences, St. Norbert College, 100 Grant Street, DePere, WI 54115 U.S.A.²; Institute of Parasitology, Biology Centre of the Academy of Science of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic³

Recently, a comprehensive study of unsegmented tapeworms (Cestoda, Caryophyllidea), possibly the most basal taxon of ‘true’ cestodes (Eucestoda) and that possess several unique biological characteristics, has started, with the main aim of critically reviewing their species composition, classification, host specificity, geographical distribution and phylogenetic relationships. In the present study, a taxonomic survey of the genus *Promonobothrium* Mackiewicz, 1968 (Cestoda: Caryophyllidea), parasites of suckers (Cypriniformes: Catostomidae) in North America, is revised on the basis of the evaluation of type specimens and newly collected material of most of the nominal species, most of them having been transferred from *Monobothrium* Diesing, 1863. Information on taxonomy, geographical distribution and host specificity, together with new data on the scolex morphology studied using scanning electron microscopy (SEM) and surface ultrastructure (i.e. microtriches) is provided. On the basis of evaluation of extensive materials, the following nominal species are considered to be valid: *Promonobothrium minytremi* Mackiewicz, 1968 (type species); *P. ingens* (Hunter, 1927); *P. hunteri* (Mackiewicz, 1963); *P. ulmeri* (Calentine and Mackiewicz, 1966) and *P. fossae* (Williams, 1974), are considered to be valid. In addition, a new species is to be described from buffalo fish (*Ictiobus bubalus* and *I. niger*) in North America. It can be distinguished from other congeners by morphology of the scolex, position of the anteriormost vitelline follicles and testes, and the presence of postovarian vitelline follicles.
This study was supported by the Slovak Research and Development Agency (project no. APVV-0653-11), Grant Agency VEGA (No. 2/0129/12), Czech Science Foundation (project No. P505/12/G112) and Institute of Parasitology (RVO: 60077344).

SURVIVAL OF SALMONELLA TYPHIMURIUM AND ASCARIS SUUM EGGS DURING LONG-TERM STORAGE OF PIG SLURRY

I. Papajová¹, N. Sasáková², J. Pipíková¹, K. Veszelits Laktičová², P. Juriš¹
Institute of Parasitology SAS, Hlinkova 3, 040 01 Košice, Slovak Republic¹; University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovak Republic²

The aim of the study was to study survival of Salmonella typhimurium and non-embryonated Ascaris suum eggs in the pig slurry stored under laboratory conditions at temperature 4°C, 20°C and 42°C. Plate counts of S. typhimurium and number of devitalized model A. suum eggs were determined on days 0, 7, 12, 22, 32, 40, 55, 90 and 115 of storage. At the same intervals level of selected physico-chemical parameters were determined. Microbiological examination showed that S. typhimurium survived in the slurry for less than 115 days at 4°C and less than 90 days at 20°C and 42°C. Devitalization of A. suum eggs increased with temperature and time of storage, but complete devitalization was not achieved even after 115 days at 42°C. The results indicate that viability of bacteria in stored pig slurry was affected first of all by the temperature during the storage. The number of devitalised A. suum eggs increased with the length of storage and the temperature. Physico-chemical parameters showed changes related to decomposition processes, but did not allow us to draw definite conclusion regarding their influence on devitalization of pathogens.

This study has been realized thanks to the financial support of the project VEGA No. 2/0140/13.

OCCURRENCE OF DERMACENTOR RETICULATUS TICKS IN THE NORTHERN BOUNDARY OF DISTRIBUTION IN CENTRAL EUROPE

A. Paulauskas, J. Radžijevskaja, A. Banaitytė, E. Galdikaitė, D. Mardosaitė, V. Mažeika, K. Šivickis
Vytautas Magnus University, Lithuania

The meadow tick Dermacentor reticulatus, is significant tick-borne disease vector in Europe. The expansion of Dermacentor reticulatus to new geographical areas has been observed in several countries in Europe.

Baltic countries are the northern boundary of the distribution of D. reticulatus in central Europe. However, the information about current distribution and habitat preference of D. reticulatus in Baltic countries still scare. A total 2571 questing D. reticulatus ticks have been collected by flagging in spring and autumn in 77 locations in Lithuania and in 5 locations in Latvia in 2013-2014 years. Tick sampling was conducted on meadows, in river valleys, in mixed and deciduous forest, and ecotones between forested and grassy areas. In Lithuania D. reticulatus ticks were found in 63 sites located in twenty two districts. In the present investigation new areas in Latvia with D. reticulatus occurrence
by at list 30 km further North from northern Lithuanian border were detected. This study provide evidence that *D. reticulatus* has extended its range in the surroundings of its former habitats in Baltic countries.

*The study was partially supported by the Research Council of Lithuania (grant no. MIP-053/2013).*

**COMBINED GASTROINTESTINAL TRACT INFESTATION WITH GIARDIA INTESTINALIS AND ISOPORA BELLi RESPONSIBLE FOR PROLONGED DIARRHOEA**

ł. Pielok

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

35-year-old patient admitted to the Tropical and Parasitic Clinic in Poznań (Poland) because of numerous loose watery stools without blood. First symptomps had begun 3 weeks before the admission to the hospital. He had been treated symptomatically without any improvement. He also lost approximately 10kg on the body weight.

On admission the patient was afebrile, the skin was dry. The examination of the abdomen cavity revealed abnormal peristaltic wave and pain in the right subcostal region. Immidietaly blood and stools samples were obtained for parasitic and bacteriological (E. coli, Salmonella, Shigella, Yersinia, Klebsiella KPC, Enterobacteriaceae NDM1) examinations.

The parasitic stool examination revealed presence of numerous cysts and trophozoits of *Giardia intestinalis*. It also detected existence of oncospheres of *Cyclospora belli*. Bacteriological cultures were negative. Combined specific therapy was initiated. The patient was given Metronidazole, Trimetoprym/Sulfametoksazole, Colistin, Rifaximine and probiotics (Enterol).

The patient completely recovered. The symptomps disapeared. The stool tests performed on dischared day and 2 weeks after were negative.

Conclusions:

1. *Giardia intestinalis/Isopora belli* coinvasion is a rare cause of gastrointestinal problems but it should be taken under consideration in differential diagnosis of diarrhoea.
2. *Coccidia* as an opportunistic microorganism may be responsible for prolonged diarrhoea mainly in immuno compromised patients but together with other pathogens could also influence the duration of symptomps in patients without immunodeficiency
3. In normal conditions *Isopora belli* invasion is self limiting disease but sometimes it has to be treated (Trimetoprym/Sulfametoksazole).

**OCCURRENCE OF ENDOPARASITIC GERMS IN PUBLIC AREAS AND BEACHES IN GREECE AND BULGARIA**

J. Pipiková, I. Papajová, I. Hovorka

Institute of Parasitology SAS, Hlinkova 3, 040 01 Košice, Slovak Republic

Dogs are important source of parasites that have potencial risk to animal and human health. Both animals and human get infected mainly by infective germs from environment. In this
epidemiological study we were focused on occurrence of endoparasitic germs in dog’s droppings and sand in selected recreational areas of Greece and Bulgaria.

Faecal and sand samples were collected in beaches and public areas in Paralia-Katerini, Olimpiaki Akti, Nei Pori, Leptokarya and Meteora in Greece. Of total 135 faecal samples, 35.55% were containing germs of intestinal parasites. Most frequently was detected the eggs from family Ancylostomatidae (58.33%), Coccidia oocysts (29.16%), eggs of Toxocara spp. (10.42%), Capillaria spp. (8.33%), Trichurus spp. (6.25%), Mesocestoides spp. (2.08%), and Ascaris spp (2.08%). All 4 samples of sand were negative. Presence of Toxocara spp. eggs was found in 20% samples of droppings from locality Meteora (20%).

From public areas of Plovdiv, Chepelare and Jasna Polana in Bulgaria were collected 36 samples of dog’s droppings and 13 samples of sand from children playgrounds. In 52.77% of faecal samples were detected eggs of intestinal parasites. Predominantly were isolated eggs of family Ancylostomatidae (94.73%) and eggs of Trichurus spp. (31.58%). One sand sample contains eggs of family Ancylostomatidae. Sand specimen from Chepelare contains eggs of Toxocara spp. with vital larvae.

High prevalence of parasites in dogs creates a risk of environmental contamination in recreational areas and subsequently forms preconditions for transmission of parasitic diseases to human.

This study has been realized thanks to the financial support of the Research and Development Operational Programme funded by the ERDF (code ITMS: 26220220116)

HELMINTH FAUNA OF THE RACCOON (PROCYON LOTOR L.) IN EUROPE – NEW SPECIES AND NEW THREATS

M. Popiolek, A. Piróg

Institute of Biology, Wroclaw University of Environmental and Life Sciences, Kożuchowska 5b, 51-631 Wroclaw, Poland

Although the impact of invasive species is one of the biggest threats to biodiversity, the attempts of complex research and solutions to that problem has started only recently. The raccoon (Procyon lotor L.), is one of the species considered invasive in Europe. Its expansion in Poland together with documented harm to indigenous fauna are a matter of serious concern. The raccoons introduce havoc in invaded zoocenos and enter the competition caused by the sympatric occurrence with the local species. Also, the transmission of parasitic species, that are new and alien for European fauna, seems to be very important problem. Such nematodes as Placoconus lotoriss, Strongyloides procynis, Baylisascaris procyonis, unidentified species of the genus Ancylostoma and family Capillariidae, but also trematodes of the genus Echinostoma, should serve as examples of taxa that have been introduced with Procyon lotor. At present, the raccoons are considered one of the fastest spreading, free roaming population in the world and more often can be encountered near the human settlements, where they search for alternative source of food. Simultaneously they become the factor of many zoonotic pathogen transmissions, including parasites. From medical point of view, one of the most important and dangerous effects of raccoon introduction in Europe is occurrence of Baylisascaris procyonis. The latter threatens not only the other, indigenous animal species, but also humans. B. procynis larve, migrating around non-specific host organism, can settle in eyes, internal organs and nervous system and cause various disabilities and dysfunctions. Parasites affect not only host populations, but also other, non-host populations as well as widely understood ecological
processes. Therefore the monitoring of raccoon parasites is essential to enrich the knowledge of this invasive carnivore biology and to assess the epizootic threats that may be caused by its further expansion.

GIANT LIVER FLUKE: COMMON ANTHELMINTIC METABOLISM
L. Prchal1, L. Rejšková1, J. Lamka2, I. Vokrál1, M. Zajíčková1, B. Szotáková1

Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic1; Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic2

Giant liver fluke (Fascioloides magna) belongs to the group of dangerous flukes, which cause severe damage to the liver of its host. The host spectrum is wide including most of the domestic and wild herbivores. F. magna is of American origin but it was introduced into Europe with imported game animals and its spread in Europe including Czech Republic is emerging in last years. Drugs from the salicylanilide group like closantel (CLO) and rafoxanide (RFX) are one of the most commonly used. Another drugs of the choice could be benzimidazoles (BZD) especially triclabendazole (TCBZ) or albendazole (ABZ). Among the others the deactivation of the drugs via metabolism of the fluke could cause the failure of the treatment.

The aim of our study is to assess the metabolism of the selected drugs (CLO, RFX, TCBZ, ABZ, and mebendazole-MBZ) in giant liver fluke ex vivo as well as in the subcellular fractions from the fluke. In our study we used the F. magna adults collected from naturally infected red deer (Cervus elaphus) and fallow deer (Dama dama). In ex vivo experiments flukes were incubated in RPMI medium with 10 μM BZD drugs or 1 μM salicylanilide drugs for 24 hours and then the metabolites were analyzed by liquid chromatography coupled with mass spectrometry. The mass weights found indicate that F. magna is able to reduce mebendazole. Despite other helminths recently studied there were no signs of ability to oxidize ABZ or TCBZ ex vivo. In vitro experiments proved the ability to metabolize MBZ and also the small amount of oxidized ABZ was found as well. No metabolites of CLO and RFX found were found so far.

The results obtained indicate that the metabolism of F. magna is in some points very different from other flukes, especially its oxidative part and it should be studied further.

The study was supported by the Czech Science Foundation (GA ČR Grant No. P502/10/0217)

INVESTIGATION OF GENETIC DIVERSITY IN DERMACENTOR RETICULATUS BASED ON MICROSATELLITE ANALYSIS
J. Radzijevskaja1, A. Paulauskas1, E. Galdikaitė1, M. Galdikas1, M. Stanko2, G. Karbowiak3, B. Petko2

Vytautas Magnus University, Lithuania1; Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovak Republic2; W. Stefanski Institute of Parasitology, Polish Academy of Sciences, Poland3

The meadow tick Dermacentor reticulatus is widespread in the temperate climate zone in Europe and Asia. According to recent observations, increasing range of geographical distribution of
D. reticulatus has been observed. However, still limited information is available about genetic diversity and population structure of this tick in Europe. Only small scale research have been done in order to investigate D. reticulatus genetic variability and most of the molecular markers available from previous studies were created to observe genetic differences between tick species. The aim of this study was to adjust relevant molecular markers for investigation of genetic diversity in D. reticulatus on local and regional scales within tick distribution area in Europe. Information on genetic variation in geographically isolated D. reticulatus populations could explain current population structure in Europe. Questing D. reticulatus ticks collected from the vegetation by flagging in Lithuania from 10 locations, and in 5 other European countries (Latvia, Poland, Ukraine, Croatia, and Slovakia) were analysed. We applied fourteen microsatellite markers previously developed and used on D. variabilis, D. albipictus and D. andersoni. All loci amplified with varying success in D. reticulatus, and five of them were chosen for further genetic analysis. The numbers of alleles per locus, allele frequencies, observed and expected heterozygosity were estimated and compared between different tick populations.

The study was partially supported by the Research Council of Lithuania (grant no. MIP-053/2013), and by the projects of Slovak Research and Development Agency APVV 0267-10 and VEGA 1/0390/12.

THE OCCURRENCE OF NEOSPORA CANINUM AND TOXOPLASMA GONDII IN WILD BOARS IN SLOVAKIA

K. Reiterová¹, S. Špilovská¹, V. Hisira²

Institute of Parasitology SAS, Hlinkova 3, 040 01 Košice, Slovak Republic¹;
Institute of Epidemiology and Preventive Veterinary Medicine, UVLF, Košice²

Neospora caninum is an apicomplexan parasite of animals, until 1988 misdiagnosed as Toxoplasma gondii. Since its first description, neosporosis has emerged as a serious disease of cows and dogs worldwide. Neosporosis is not considered zoonotic while toxoplasmosis is a serious disease of humans, sheep and many other warm-blooded animals. Domestic and wild felids are the definitive hosts of T. gondii. As it may cause abortion or congenital disease in its intermediate hosts, it is of great medical and veterinary importance. Intermediate hosts become infected mainly by ingesting food of water contaminated with oocysts excreted with feline faeces; eating raw or undercooked meat containing cysts; or congenital infection. Antibodies to N. caninum and T. gondii have been found in a variety of free range wildlife and also in captive wildlife in zoos. Recently, DNA of both parasites was reported in brains of foxes, roe deer, wild mice and wild boars. Clinical neosporosis and toxoplasmosis in wildlife is rare.

The presence of anti-Neospora and anti-Toxoplasma antibodies in sera or blood transudate of 204 wild boars (Sus scrofa) were detected by competitive and indirect ELISA. Examined animals were caught in Slovak hunting areas between the years 2009-2012. The presence of parasite DNA was determined in uncoagulated blood samples and different organs of seropositive and serologically nonexamined animals using (i) PCR with specific primers Np6 and Np21 and (ii) by PCR analyses of TGR1E gene.

Anti-Neospora antibodies were detected in 34.8% and anti-Toxoplasma antibodies in 46.1% of wild boars. Animals were coming from 8 districts, particularly from eastern Slovakia, and seropositive individuals were recorded in 6 districts. When comparing the incidence of neosporosis versus toxoplasmosis according to the age of the animals, the highest seropositivity 40.1% ver. 49.1%
was detected in the group of wild boars aged 1-2 years and the least 26.7% ver. 43.3% in group of younger than one year old animals. The females were similarly infected with both coccidian parasites more (39.4% of neosporosis, ver. 48.8% of toxoplasmosis) in comparison with males (27.6%, ver. 42.1%).

The presence of *N. caninum* DNA was confirmed in 39 blood samples (54.2%) from 71 examined, respectively in 5 samples (7.2%) from 69 wild boar kidney samples. The presence of *T. gondii* was determined by PCR analyses of TGR1E gene only in three isolates from 12 examined seropositive samples. Only genotype I. as a virulent strain of *T. gondii* was confirmed by molecular analyses.

The presence of *T. gondii* in wild boars and risk of parasite transmission to human population highlighted the need for monitoring of this parasitic zoonosis in wildlife in Slovakia. Our results suggest significant role of examined animals in the epidemiology of neosporosis and toxoplasmosis.

**Acknowledgement:** Authors are grateful to MVD. A. Čobádiová, PhD. and Dr. L. Turčeková, PhD. for their special assistance. Work was supported by the Slovak Grant VEGA, Nos. 2/0069/08; 2/0104/11 and has been realized within a frame of the project Centre of Excellence for Parasitology (Code ITMS: 26220120022) of the Operational Programme “Research & Development” funded from the European Regional Development Fund (rate 0.4).

CONTROL OF THE POTATO CYST NEMATODES *GLOBODERA ROSTOCHIENSIS* AND *GLOBODERA PALLIDA* BY THE VERMICOMPOST, VERMICOMPOST TEA AND UREA APPLICATIONS

M. Renčo¹, N. Sasanel³

Institute of Parasitology, SAS, Hlinkova 3, 040 01 Košice, Slovak Republic¹; Institute for Plant Protection, CNR, Str. G. Amendola 122/D, 70126 Bari, Italy²

Soil amendments from composted wastes may represent effective alternatives to environmentally unsafe chemical treatments in the control of plant parasitic nematodes. Three experiments were carried out to verify the efficacy of vermicompost, vermicompost tea alone or added with 45% urea in the control of the two potato cyst nematode *Globodera rostochiensis* (patotype Ro1) and *Globodera pallida* (patotypes Pa2 and Pa3). Rates of 0, 10, 20, 40 and 60 t/ha of solid vermicompost were applied to the soil at potato sowing; whereas vermicompost tea alone or in combination with urea (285 kg urea/1,000 l tea) were applied at rates of 0, 10, 20, 40, 60 l/ha to the soil, in a liquid formulation, at potato sowing and at sowing and one month later. In all trials an untreated soil was used as control. All data from the experiments were subjected to analysis of the variance (ANOVA) and means compared by the Least Significant Difference’s Test using the software PlantIT. All tested doses of vermicompost, vermicompost tea and vermicompost tea added with urea were effective to reduce number of cyst/100 g of soil, eggs and juveniles/cyst and eggs and juveniles/g of soil of both potato cyst nematode species in comparison to the untreated controls. Nevertheless, the suppressive effect of the highest dose was significantly more effective than that of the lowest at all tested materials. Several differences among the tested pathotypes of two nematode species were found. *G. rostochiensis* patotype Ro1 showed the highest sensitivity to all tested materials in comparison to *G. pallida* (both pathotypes). Moreover, aqueous vermicompost teas alone or in combination with urea were found to be more effective in both nematode species reduction in comparison to solid vermicompost. Results from these pot experiments indicate that the
use of these amendments seems to be a promising method for the control of the potato cyst nematodes in sustainable agriculture. In addition, the application of vermicompost tea in aqueous formulation considerably decreases the amount of used doses materials as well as cost of nematode management in comparison to solid vermicompost.

*This study was supported by the VEGA scientific grant agency, grant No. 2/0079/13*

**EVALUATION OF NEMATICIDAL ACTIVITY OF HYDROLYSABLE TANNINS ON THE POTATO CYST NEMATODE GLOBODERA PALLIDA**  
M. Renčo¹, N. Sasanelli²

Institute of Parasitology, SAS, Hlinkova 3, 040 01 Košice, Slovak Republic¹; Institute for Plant Protection, CNR, Str. G. Amendola 122/D, 70126 Bari, Italy²

Among the most dangerous pathogenic plant parasitic nematodes there are the cyst forming nematode *Globodera pallida*, a specific parasite of potatoes having three different pathotypes. From many years the research on low environmental impact alternatives to chemicals has received a strong impulse with a wide range of options, including the use of natural plant products. Therefore, an *in vitro* and an *in vivo* experiment were carried out to verify the potential effect of hydrolysable chestnut tannin aqueous solutions on the control of the potato cyst nematode *Globodera pallida* pathotype Pa2 and Pa3. In the *in vitro* hatching test, different tannin concentrations in a geometric scale, from 0.32 to 20.48 g/L, were used for their effect on hatching of the nematode, whereas in the *in vivo* pot experiment the soil infested by the potato cyst nematode was treated with three different doses of tannin 100 g/m²; 250 g/m² and 450 g/m² applied at potato sowing or at sowing and two weeks later to evaluate their effect on nematode control. In all trials tannin treatments were compared to untreated controls.

In the *in vitro* experiment chestnut tannin solutions significantly suppressed nematode egg hatch of both pathotypes of *G. pallida* in comparison to untreated control. Pot experiment confirmed results obtained in the hatching test, at all doses of chestnut tannin treatments the number of cyst/100 g soil, number of egg and J₁/g soil and the reproduction rate *r* (*Pf/Pi*) were significantly reduced in comparison to untreated control. Therefore, the use of chestnut tannin seems to by promising method for the control of potato cyst nematode in sustainable agriculture. Because of the tannins are present in all higher plants, the additional studies are suggested to investigate the effect of different tannins on different plant parasitic nematode species as well as on beneficial, soil free living nematodes.

*This study was supported by the VEGA scientific grant agency, grant No. 2/0079/13*

**DETECTION AND DIFFERENTIATION OF BABESIA SPP. BY REAL-TIME PCR**  
W. Rożej-Bielicka, A. Masny, E. Golab

Department of Medical Parasitology, National Institute of Public Health – National Institute of Hygiene, Warsaw, Poland

BACKGROUND. Babesia are blood-borne parasites of many mammalian species, including man. *Babesia microti*, *B. divergens* and *B. venatorum* have been shown to cause significant number
of human infections. Human are infected via the bite of ixodid ticks. The other confirmed way of transmission is transfusion of blood or blood products. The definitive diagnosis is generally made by microscopical identification of Babesia in Giemsa-stained blood smears. However, because of the low level of parasitemia, visualization and microscopical differentiation of Plasmodium and Babesia might not be possible. The development of sensitive and specific molecular assay may be an important improvement in the laboratory diagnosis of human babesiosis. The goal of the research was to develop real time PCR assay for the detection and differentiation of Babesia spp. pathogenic to people.

METHODS. Genetic markers were searched for within the small subunit 18S rRNA genomic region of Babesia spp. Based on multiple alignment of B. divergens, B. microti and B. venatorum (former EU1 strain) sequences, primers: Bhum-BDV, Bhum-BM and Bhum-rev were designed. Fragments of sizes 380 bp and 410 bp of B. divergens, B. venatorum and B. microti sequences were amplified respectively using touch-down multiplex PCR assay using high resolution melting curve analysis (HRM). HRM was carried out immediately after the PCR at the temperature increment steps of 0.1 °C from 74°C to 90°C, for 3 s at each step.

RESULTS. Using developed method HRM curves characteristic for species B. divergens and B. microti were obtained. The limit of B. microti detection of touch-down multiplex PCR with primers pair Bhum-BM, Bhum-rev was thousandfold dilution of the control DNA sample.

CONCLUSIONS. The obtained results of the research are promising however the designed method requires validation before application in laboratory diagnosis of the Babesia infections.

EVALUATION OF EFFICACY OF PT SCHEMES FOR DETECTION OF TRICHINELLA IN MEAT BY DIGESTIVE METHOD IN POLAND

M. Różyczki, E. Chmurzyńska, E.B. Zając, T. Cencek

National Veterinary Research Institute, Puławy, 57 Partyzantów Avenue, 24-100 Puławy, Poland

Examination of meat for the presence of Trichinella is a part of official post mortem examination of pig meat in Poland. Since 2009 examination is done by magnetic stirrer method, according to Annex I of Commission Decision (EC) 2075/2005 and is conducted in laboratories accredited or applying for accreditation. According to Commission Regulation 854/2004 all official laboratories should operate in a quality management system, according to EN ISO/IEC 17025. Among approaches of quality control only proficiency testing (PT) schemes can establish the bias and accuracy of the results. Participation in a PT scheme provides verification of the analytical competence of a laboratory and shows a commitment to the maintenance and improvement of performance. PTs for Trichinella were provided for all laboratories. The number of laboratories varied from 1100 in 2005 to 700 in 2013. Due to the lack of defined procedures for the preparation of samples for PT, initially (in 2005 and 2006) naturally infected material was used for the study. In 2005 PT samples were maid from cuts of muscles naturally infected. Samples showed non-uniform distribution of larvae in muscle tissue in individual muscles (varied from 15 to more than 60 per sample). Therefore, in 2006, meat sample for PT were homogenized. After homogenization samples were more uniform than in 2005, however, did not allow evaluation of PT results with the Z-score. Thus the results of early studies were rated as qualititative. In 2007, samples were prepared according to the protocol published by Isabel Vallee. Encysted larvae were recovered, than placed into pig meat samples (Trichinella free). The method provides more
uniform results, but not as much as we expect. In 2008, methodology published by Forbes and Gajadhar’a was introduced. Trichinella larvae were embedded in agarose gel and in this form inserted into the minced meat. In mid 2008, own modifications to the above method were made and agar was replaced with 8% porcine gelatin (200 Bloom). In this manner PT were continued in subsequent years. Each laboratory obtained the set of samples consisted of 4 samples, one negative and three positive (at low, medium and high levels). From year to year the number of added Trichinella larvae was reduced. The results of the analysis were evaluated in relation to the known value. The standard deviation (σ) was determined on the basis of the results reported by participants. Since 2007 results were rated with Z-score. In 2007 over 560 laboratories took part in PT, 109 obtained results inconsistent (19.3 %). The average value of Z-score was 0.72. In 2008 in PT were involved 790 laboratories. Inconsistent results obtained 115 laboratories (14.6 %). The average value of Z-score was 0.81. In next year 822 laboratories participated in PT of which 69 received inconsistent results (8.4 %). The average value of Z-score increased to 0.85. Since 2010 the inconsistent results were not higher than 5% and the mean Z-score did not exceed 1.5 while the number of larvae in the test samples was reduced to 1, 3 and 5 larvae.

After each round in laboratories that fail the test, corrective actions were undertaken and technical personnel was trained and tested again. Summarizing, after 7 years from the start of the implementation of the digestive method by introduction of quality management system field laboratories obtained better and better results in PT schemes – these results do not differ from European standards and in most cases positively exceeds them.

**MOLECULAR STUDIES OF HYDATIGERA TAENIAEFORMIS (BATSCH, 1786) (TAENIIDAE)**

R. Salamatin1,2, J. Sitko3, J. Kowal4, W. Rożej-Bielicka2, A. Masny2, V.V. Kornyushin5

Department of General Biology and Parasitology, Medical University of Warsaw, Poland1; Department of Medical Parasitology, National Institute of Public Health – National Institute of Hygiene, Warsaw, Poland2; Moravian Ornithological Station at Přerov, Comenius Museum, Přerov, Czech Republic3 Department of Zoology and Ecology, University of Agriculture in Cracow, Poland4; I. I. Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, Kyiv, Ukraine5

**BACKGROUND:** Tapeworm Hydatigera taeniaeformis (Batsch, 1786) is common intestinal parasite of domesticated and wild felines. The larval form (strobilocercus) develops in the liver of the intermediate hosts, usually rodents. According to the previous studies it is probable that single name H. taeniaeformis is used to describe distinct species (cryptic species) originating from various regions of the world (Jia et al. 2012; Acta Trop 123: 154–163). It is assumed that two or three distinct forms exist: European (lineage 1 and lineage 2) and Asian (lineage 3), according to Galimberti et al. 2012 (Mol Ecol Resour 12: 403–413). The goal of the research was to determine the genotypes of H. taeniaeformis collected in Poland and Czech.

**MATERIAL and METHODS**

*Collection of biological samples*

Strobilale stage. Two H. taeniaeformis specimens were isolated from two naturally infected cats (Felis catus), living in Warsaw.
Larval stage. Five strobilocerci were investigated. Three specimens were collected in Poland: two were isolated from the livers of muskrat (Ondatra zibethicus) and one from the liver of water vole (Arvicola sp.). Two specimens from Czech were isolated from the intestine (pseudoparasitism!) of common kestrel (Falco tinnunculus).

DNA extraction, amplification and sequencing. DNA isolation was performed manually using silica-guanidinium thiocyanate procedure (Boon et al. 1999; J Clin Microbiol 37: 615–619). Primer pair JB3 (5′-TTTTTGGGATGCCTGAGGTAT-3′), JB8.45 (5′-TAAAGAAAGACAAATGAAAAATG-3′) (Bowles et al. 1993; Acta Trop 55: 217–229) was used to amplify 444 bp of mitochondrial gene cox1, the same primers were used for both strand sequencing. The sequence data were analyzed using CLC Main Workbench 6 software (CLC bio, Aarhus, Denmark); the nucleotide sequences were compared to those present in GeneBank, all nucleotide sequences were translated and compared to the protein database to exclude presence of stop codons and indels.

RESULTS and CONCLUSIONS: All DNA sequences had the highest level of similarity to the lineage 2. The isolates from Germany (terra typical), described in literature (Jia et al. 2012), also belonged to the lineage 2. Considering that the original description of H. taeniaeformis (Batsch, 1786) was based on a German isolate, in our opinion the lineage 2 is H. taeniaeformis sensu stricto. Thus, the specimens isolated in Poland and Czech Republic represents H. taeniaeformis sensu stricto.

HUMAN DIROFILARIA REPENS INFECTION IN UKRAINE, CURRENT DATA ON EPIDEMIOLOGY

R. Salamatin1,2, T. Pavlikovska2, O. Sagach2, S. Nikolayenko2, V. Kornyushin3, V. Kharchenko3, A. Masny4, D. Cielecka1,4, J. Konieczna-Salamatin5, D.B. Conn6, E. Golab4

Department of General Biology and Parasitology, Medical University of Warsaw, Poland1; Center of Diseases Control and Monitoring of the Ministry of Health of Ukraine, Kyiv, Ukraine2; I. I. Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, Kyiv, Ukraine3; Department of Medical Parasitology, National Institute of Public Health – National Institute of Hygiene, Warsaw, Poland4; Institute of Sociology, University of Warsaw, Warsaw, Poland5; Department of Biology and One Health Center, Berry College, Mount Berry, GA, USA6

BACKGROUND: Dirofilariasis caused by the nematode Dirofilaria repens is a transmissible zoonotic disease. The number of infections in Europe has been increasing recently and D. repens has become one of the most rapidly expanding parasites of people and animals. In Ukraine the cases of dirofilariasis are a subject of epidemiological registration and are recoded in the state register that covers all Ukraine (Salamatin et al. 2013; Acta Parasitologica 58(4): 592–598).

METHODS: The analysis of literature sources covers the period since 1927 (first record) up to 1974. The epidemiological data collected from the reports of all 27 regional sanitary-epidemiological stations covering the period of 1975–2013 have been analysed. A detailed analysis of clinical data gathered during the last 5 years (2009–2013) was conducted.

RESULTS and DISCUSSION: In the period 1927—2013 in Ukraine there was registered 1787 cases of human infection. These include 1009 cases within 2009—2013. Infections were noted in all the regions of Ukraine. The majority of the cases were noted in Kyiv, followed by the oblasts of Donetsk, Zaporizhzhya, Dnipropetrovsk, Kherson, Mykolayiv and Chernihiv.
Analysing clinical data of 1009 cases, it was determined that in 653 cases (64.7%) the parasitic lesions were located in the head, including 406 cases of lesions around the eyes. Dirofilariasis of the limbs and torso constituted a lower percentage of cases – 15.0% and 11.0% respectively. *D. repens* were also detected in the sexual organs of men (40 cases), and in female mammary glands (26 cases). In 28 cases (2.8%) the location of the parasite was not specified in the data. The age of patients was from 11 months up to 90 years.

It seems interesting that despite the fact that endemic regions of *D. repens* are located in many areas in Europe, the total number of cases described in Europe without Ukraine is surprisingly low – ca. 600 (Masny et al. 2012; Parasit Vectors 6: 38) compared to Ukraine’s.

CONCLUSIONS: The Ukrainian sanitary-epidemiological services managed to achieve some measure of success, one of which is creating a system of registering *D. repens* infections in Ukraine. Therefore, it appears that in order to achieve a proper outlook on the epidemiological situation, it would be necessary to introduce mandatory registration of *D. repens* dirofilariasis cases in the EU countries.

### INTEGRATIVE TAXONOMY AND PHYLOGENETIC INSIGHT OF THE BITING MIDGE OF THE GENUS *CULICOIDES* (DIPTERA: CERATOPOGONIDAE) IN SLOVAK REPUBLIC

A. Sarvašová¹, B. Mathieu², A. Kočišová¹

University of Veterinary Medicine and Pharmacy in Košice, Department of Parasitology, Komenského 73, 04181 Košice, Slovak Republic¹; Institute of Parasitology and Tropical Pathology (IPPTS), Medicine faculty, EA7292, 3 rue Koeberlé, F-67000 Strasbourg, France²

The accurate identification of *Culicoides* to species level is tricky and a key component for epidemiological investigations. To address this issue we used the integrative taxonomy principle combining morphological and molecular data. This study went through *Culicoides* biodiversity of Slovak Republic comparing morphology and barcoding gene COI (mtDNA) from specimens. The aims of this study were to review the biodiversity of the Slovak fauna and to give a phylogenetic insight between and within the recorded species.

COI sequences were performed from *Culicoides* individuals caught in five trapping sites in Eastern Slovakia. In order to construct a phylogenetic tree, 70 original sequences of 38 species were added to 126 sequences from GenBank. The 196 total molecules were split into 3 independent analyses according a subgeneric grouping. Alignments were submitted to Maximum likelihood analysis with the HKY+I+Γ model of nucleotidic substitution.

Out of the 38 morphologically identified species, 31 species clustered to species from GenBank and barcoding data for 7 species were submitted for the first time to GenBank. Three conflicts between molecular and morphology were observed.

(i) The maximum genetic distances between *C. pallidicornis* and *C. subfasciipennis* were 3.4%, between *C. salinarius* and *C. manchuriensis* 2.4 % and between *C. festivipennis* and *C. clastrieri* 1.3%. The low interspecific genetic distances and the variations of morphological characters required further studies to evaluate the species status and to redefine the discriminant characters.

(ii) On the opposite, high intraspecific divergence within specimens morphologically identified as *C. kibunensis* was shown. Barcoding results suggested the presence of a Kibunensis complex of cryptic species. Species of this latter complex seemed to be widely distributed in Palaearctic region and a sympatric area is pointed out in Slovakia.
(iii) In addition, two specimens – referred as speA and speB - belonging to the *Culicoides* subgenus exhibited a wing pattern differing from known species of this subgenus. The barcoding data matched with 94% of identity between speA and *C. pulicaris* from Sweden and 87% of identity between speB and *C. newsteadi* from Sweden and Denmark. Further studies are necessary to determine whether these specimens referred to new species waiting to be described.

This research was supported by grant VEGA No. 1/0236/12, basic research of National Reference Laboratory for Pesticides of the University of Veterinary Medicine in Košice and execution of the Project “Centre of Excellence for Parasitology” (ITMS code: 26220120022) upon the support of the operation program Research and Development, financed by the European Regional Development Fund (part 0.5).

**Ozonated water treatments: control of the root-knot nematode**

*MELOIDOGYNE INCognITA AND HISTOLOGICAL OBSERVATIONS ON TOMATO*

N. Sasanelli¹, N. Poiras², A. Bivol², I. Toderas³, E. Iurcu-Straistaru², L. Poiras², S. Laquale¹, N. Vovlas¹, M. Renčo³, I. Papajova³

Institute for Plant Protection, CNR, Str. G. Amendola 122/D, 70126 Bari, Italy¹; Institute of Zoology, ASM, Str.Academiei 1, 2028 Chisinau, R. Moldova²; Institute of Parasitology, SAS, Hlinkova 3, 040 01 Košice, Slovak Republic³

According to the necessity to find new control strategies at low environmental impact against plants parasitic nematodes, a pot experiment on tomato (cv. Super Marmande) was carried out to verify the efficacy of soil ozonated water treatments against the root-knot nematode *Meloidogyne incognita*. Treatments were applied at transplant and for 15, 30 and 60 days and compared with: a) untreated control and b) fenamiphos treatment (synthetic nematicide) at 62.5 L/ha used as controls. Pots were arranged, according to a randomised block design, in a glasshouse (25 ± 2°C) on benches with ten replications for each treatment. Ozonated water for watering was produced *in situ* by an ozone generator dissolving ozone gas into the water until an ORP of 800 mV. At the end of the experiment, 60 days after transplant, plants were uprooted from each pot to estimate root gall index (RGI), caused by nematode attack, according to a 0-10 scale. Nematodes extracted from roots by the sodium hypochlorite’s method and those extracted from 500 mL soil with the Coolen’s method were used to calculate for each pot the final nematode population density and the reproduction rate (*r=Pf/Pi*). All data from the experiment were subjected to analysis of the variance (ANOVA) and means compared by the Least Significant Difference’s Test using the software PlotIT. Root gall index was significantly reduced in all treatments with ozonated water in comparison to the untreated control, although it was significantly higher than those observed in the chemical control. Final nematode population density and reproduction rate were significantly lower in soil ozonated water treatments for 30 and 60 days compared with the untreated control. No significant difference was observed between these two last treatments and fenamiphos.

Histological observations on *M. incognita* galled tomato roots, treated with ozonated water for different application times, were made to compare the anatomical changes induced by the nematode among the different treatments. In the roots of the untreated control and in those treated only for a limited time (15 days) were observed well developed polinucleate giant cells (feeding sites) associated with fully developed females able to deposit a large number of eggs. Undersized giant cell
feeding sites associated with immature females, with reduced ability to produce large number of eggs, were noted in tomato roots treated with ozonated water for a longer exposure time (30 and 60 days). In the chemical control was observed well developed giant cells and mature female with limited egg production.

No statistical difference was noted in the percentage of hatch from egg-masses produced in the different treatments, including the controls, subjected to an hatching test.

SYRINGOPHILID QUILL MITES: ONE FAMILY, TWO STRATEGIES OF LIFE
M. Skoracki¹, B. Sikora¹, M. Hromada², M. Klimovicova²

Department of Animal Morphology, Faculty of Biology, Adam Mickiewicz University, Umultowska 89, 61–614 Poznań, Poland¹; Laboratory and Museum of Evolutionary Ecology, Department of Ecology, Faculty of Humanities and Natural Sciences, University of Prešov, 17. novembra 1, 081 16 Prešov, Slovakia²

Quill mites of the family Syringophilidae (Acari: Prostigmata) are permanent and highly specialised parasites of birds living inside the quills of feathers. To date, this family includes over 290 species grouped in 56 genera and recorded from over 380 bird species belonging to 23 orders from both neognathous and palaeognathous birds. These mites are known from all zoogeographical realms, except Antarctica. The family has been divided into two subfamilies: Syringophilinae and Picobiinae based on their external morphology. Our recent studies on the biology and ecology of these mites support main division of the family Syringophilidae.

The members of the subfamily Syringophilinae 1) occupy large wing and tail feathers (primaries, secondaries, tertials, rectrices and coverts); 2) inside each of the quill, the intra-population including 60-120 specimens originating from single female-founder has been observed; 3) the host spectrum of individual syringophiline genera is narrow (where each genus of mites is associated with specific bird’s order, e.g. Charadriiphilus on Charadriiformes, Syringophilopsis on Passeriformes, Bubophilus on Strigiformes); 4) the phenomenon of physogastry is not observed; 5) each female of Syringophilines produces 10-12 small eggs (r-strategy); 6) the sex ratio females:males in the individual quill-intrapopulations of syringophilines is 9–11:1.

In opposite to that, the members of the subfamily Picobiinae 1) occupy small quills of body feathers exclusively and have never been observed in other types of plumage; 2) inside each quill, the intra-population including max. 5 specimens originating from single female-founder has been observed; 3) the picobiine genera are distributed on broad spectrum of their host (where each genus of mites is associated with phylogeneticaly not related host orders e.g. the genus Picobia on Passeriformes, Piciformes, Coraciformes, Rafapicobia on Passeriformes and Gruiformes); 4) the phenomenon of physogastry is common among Picobiine members and the shape and size of the physogastric forms is characteristic for particular genera; 5) each physogastric female produces only 3–4 eggs but very large in size (K-strategy); 6) the sex ratio - females:males in the individual quill-intrapopulations of picobiine mites is 2–3:1.
QUALITATIVE ANALYSIS OF LEECH FAUNA (HIRUDINEA) FROM RIVER DALJAN - LAKE OHRID (MACEDONIA)

S. Smiljkov1, D. Guseski1, L. Velkova-Jordanoska2, S. Stojanovski2
Faculty of Natural Sciences, Arhimedova Str. 3, 1000, Skopje, Macedonia1;
Hydrobiological Institute, Naum Ohridski Str. 50, 6000 Ohrid, Macedonia2

In the researching period 2012-2013, four points of the river Daljan were investigated and 6 species of leeches were found: Hirudo medicinalis, Piscicola geometra, Erpobdella octoculata, Helobdella stagnalis, Haemopis sanguisuga and Glossiphonia complanata.

Dominant species in River Daljan are Hirudo medicinalis, Glossiphonia complanata and Helobdela stagnalis.

Hirudo medicinalis is dominant species who is found in all 4 points during all 4 seasons of the researching period 2012-2013.

Piscicola geometra is found on the skin and gill on some fish from the Lake Ohrid.

HELMNTH FAUNA OF OHRID GUDGEON (GOBIO OHRIDANUS KARAMAN, 1924) FROM THE LAKE OHRID (MACEDONIA)

S. Stojanovski1, J. Rokicki2, N. Karabolovski5, L. Velkova-Jordanoska1, S. Smiljkov3, D. Blažekovič-Dimovska4
Hydrobiological Institute, Naum Ohridski Str. 50, 6000 Ohrid, Macedonia1; Department of Invertebrate Zoology, Gdansk University, Poland2; Veterinary Faculty, Prilepska bb., 7000, Bitola, Macedonia3; Faculty of Natural Sciences, Arhimedova Str. 3, 1000, Skopje, Macedonia4; Faculty of Biotechnical Sciences, Partizanska Str. bb, 7000, Bitola, Macedonia5

The parasitological examination from the Macedonian part of the Lake Ohrid showed that of 94 specimens of gudgeon (Gobio ohridanus Karaman, 1924) 61 fishes (64.89%) were infested.

In our case study the presence of 4 parasite species was established: Dactylogyrus cryptomeres f. typica, Cystidicolaoides tenuissima, Philometra ovata and Pomphorhynchus laevis.

By the parasite species, the highest prevalence and intensity of infestation was with Dactylogyrus cryptomeres f. typica. Prevalence was 59.57%, and the intensity of infestation was 6.07. The lowest one was with Cystidicolaoides tenuissima and Philometra ovata. Prevalence was 2.13%, and the intensity of infestation was 1.0.

In our case study the Monogenea Dactylogyrus cryptomeres f. typica, is recorded for the first time in the ichthyoparasitofauna of Lake Ohrid and Macedonia.
IN VITRO COMPARATIVE STUDY OF METABOLIC PATHWAYS AND EFFICACY OF MONEPANTEL AND ITS DERIVATIVES

L. Stuchlíková¹, L. Lecová¹, R. Jirásko², M. Valát¹, I. Vokřál³, J. Lamka³, B. Szotáková¹, L. Skálová¹

Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic¹; Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Studentská 573, 532 10 Pardubice, Czech Republic²; Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic³

Monepantel (MOP) and its derivatives belong to new class of amino-acetonitrile anthelmintics approved for treatment of nematodoses in small ruminants. Contrary to other anthelmintics, MOP is also effective against nematodes strains resistant to benzimidazoles, levamisole, morantel and macrocyclic lactones. High efficacy of MOP also against larval stages of nematodes was reported. However, the information about effect of MOP derivatives against the lower larval stages has not been available yet. In ovine hepatocytes, MOP is intensively biotransformed into 12 metabolites. But there is now information about metabolic pathways of MOP derivatives. Addressing this fact, the objective of this study was to compare in vitro efficacy of MOP derivatives against Haemonchus contortus larvae and metabolic pathways of MOP derivatives in ovine hepatocytes.

Eggs of Haemonchus contortus were obtained from experimentally infected lambs and micro-agar larval development test (MALDT) was used for assay of anthelmintic efficacy of MOP derivatives. Ovine hepatocytes, isolated by two-step collagenase perfusion of liver, were immobilized in Petri dishes coated with collagen and incubated with monepantel derivatives (10µM). Medium samples and homogenates of hepatocytes were extracted separately using solid-phase extraction (SPE). The samples were analysed by UHPLC/MS (QqTOF) in negative-ion mode.

The results showed that ovine enzymatic systems are able to biotransform MOP derivatives via oxidation, hydroxylation, hydrolysis, sulfation and glucuronidation. Based on obtained results, schemes of metabolic pathway of monepantel derivatives in sheep were proposed. Results of MALDT were quantified as 50 % lethal concentration (LC50), 99 % lethal concentration (LC99) and resistance factor (RF). Certain differences in metabolism and in in vitro efficacy among MOP derivatives were found.

This project was supported by Czech Science Foundation, grant No P502/10/0217 and by Grant Agency of Charles University, grant No 673612/B-CH/2012.
CIRCULATION OF BABESIA SPP. BETWEEN TICKS AND RODENTS IN SW SLOVAKIA

Z. Svitátková1, L. Mydlová1, L. Berthová2, E. Kocianová3, M. Slovák1, M. Kazimírová1

Institute of Zoology, Slovak Academy of Sciences, Dúbravská cesta 9, 845 06 Bratislava, Slovakia1; Institute of Virology, Slovak Academy of Sciences, Dúbravská cesta 9, 845 05 Bratislava, Slovakia2

Babesiosis caused by intraerythrocytic parasites of the genus Babesia is an emerging zoonotic disease of veterinary and medical importance. Babesia spp. have a natural enzootic cycle involving ixodid ticks and vertebrate hosts. Rodents (especially of the genera Apodemus and Clethrionomys) belong to important reservoir vertebrate hosts of Babesia spp. in Europe.

In this study we monitored the occurrence of Babesia spp. in questing Ixodes ricinus and Haemaphysalis concinna in two areas of Southwestern Slovakia that are differently affected by human activities and comprise different reservoir hosts. The same areas were monitored for presence of this pathogen in rodents.

Ticks were collected during 2011-2013 by blanket-dragging at two sites in the Small Carpathians Mts: the recreational area of the Bratislava forest park, and a non-fragmented woodland. The distance between the two sites is cca 40 km. By using PCR-based methods (target gene 18S rRNA) Babesia spp. was detected in 43 (1.5%) out of 2823 ticks from the forest park and in 47 (2.1%) out of 2251 ticks from the woodland site. By sequencing Babesia-positive samples B. microti, B. venatorum, B. crassa, B. canis, B. capreoli and Hepatozoon canis were identified.

Rodents were trapped with live traps at the same sites in the Small Carpathian Mts. A total of 407 and 8 rodents belonging to five species were trapped in 2012 and 2013, respectively. Apodemus flavicollis and Clethrionomys glareolus prevailed. In 2012, rodents were infested with ixodid ticks (n=764) - Ixodes ricinus (94.1%) and Haemaphysalis concinna (5.9%), in 2013 (n=207) – I. ricinus (99.5%) and H. concinna (0.5%). Spleen and blood samples from rodents and ticks feeding on rodents were examined by PCR for presence of Babesia. In total, 8.8% of spleens were infected with the protozoans (dominance of B. microti and Hepatozoon sp.). We detected the protozoans also in blood samples, probably due to acute stage of parasitemia or severe infection. Babesia spp. were detected in 3.3% of host-attached ticks (dominance of B. microti).

Our results show the involvement of rodents in the natural endemic cycles of Babesia spp. in SW Slovakia and that rodents significantly contribute to the maintenance of these pathogen in nature. Results also confirm spatial and temporal differences in the prevalence of this tick-borne pathogen. Further screening of rodent tissues and molecular identification of strains of pathogenic microorganisms is in progress. Knowledge about the prevalence of this infectious agent in ticks and rodents is an important prerequisite for risk assessment of disease.

This work is supported by FP7 project EDENext (No. 261504) and grant APVV DO7RP-0014-11.
SEROPREVALENCE OF TOXOCAROSIS IN SELECTED POPULATION GROUPS IN SLOVAKIA
M. Škutová1, D. Antolová1, G. Štrkolcová2, M. NovákOVÁ1, M. GoldOVÁ2, K. Reiterová1
Institute of Parasitology SAS, Košice, Slovakia1; University of Veterinary Medicine and
Pharmacy, Košice, Slovakia2

Human toxocarosis is a cosmopolitan helmintozoonosis caused by larvae of *Toxocara canis*
and *Toxocara cati*. Humans can acquire infection by ingestion of embryonated eggs from soil
(geophagia), dirty hands or raw vegetables, handling of infected definitive host, or alternatively by
encapsulated larvae of *T. canis* 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 199 serum samples, divided into three groups, hunters (88
sera), minority population (Roma people) (36 sera) and healthy population (75 samples) were
included to the study. The presence of specific antibodies in samples was detected serologically using
*T. canis* excretory/secretory antigen.

Out of 199 serum samples, anti-*Toxocara* antibodies were detected in 20 (10.0 %) cases. In detail, six
(6.8 %) of 88 hunters; 13 (36.1 %) of 36 sera of minority population and only one serum (1.3 %) of the
healthy population were serologically positive. The seroprevalence of toxocarosis in the group
of minority population was significantly higher (p < 0.05) when compared to healthy population and
hunters. There was higher incidence of antibodies in men than in women, but the difference was not
statistically significant. Age-dependency has not been evaluated.

Our results strongly suggest the impact of living conditions, hygiene and contact with contaminated
environment on the occurrence of larval toxocarosis in human population.

The study was supported by the by the project of Research & Development Operational Programme
funded by the ERDF (code ITMS: 26220220018) (0.3); Operational Programme Education, project
Parasitological Research and Training Center of SAS (code ITMS: 26110230045) (0.3) and by the
Science Grant Agency VEGA, project No. 2/0127/13 (0.4).

WHO KILLS THE „OSCAR“?
Ľ. Šmiga1, L. Košuthová1, P. Košuth1, J. Koščo2, K. Oberhauserová1, P. Lazar1
Institute of Breeding and Diseases of Game and Fish, University of Veterinary Medicine and
Pharmacy in Košice1; Faculty of Human and Natural Sciences, University of Prešov in Prešov2

Everyday monogeneans seriously damage health or kill thousands of ornamental fish. Inadequate
controls of fish import or export facilitate spread of new and unknown monogeneans
species from original areas, farms or quarantine stations. Problematic treatment and missing
diagnosis of aquarium fish increase the importance of monogeneosis. We examined 45 specimens of
*Astronotus ocellatus* („Oscar“ cichlid) from a pet shop in Kosice. The fish were imported from a
Singapore farm. All were juveniles with two body colours - natural and albino. They had problems
with breathing (asphyxia syndrome - anaemic gills and pumping near the water suface) and nervous
paralysis which was detected during swimming. Fish were cachectic with visible depigmentation. Fish
were treated by Chloramine T and FMC (solution of formaline, methylene blue and malachite green),
but the treatment was not successful. We found monogeneans of genus *Gussevia* in 100 % prevalence in both groups and in high intensity of infection (circa 80 parasites on one gill arch). The intensity of infection would be higher, if fish were not treated. Parasites were localised on apical ending of gill filaments and caused necrosis of gill lamellae.

*The research was supported by VEGA projects No. 1/0916/14 and 1/0847/13.*

**MOLECULAR CHARACTERIZATION AND INFECTIVITY OF *HETERAKIS SPUMOSA* NEMATODES FROM BROWN RATS (*RATTUS NORVEGICUS*)**

V. Šnábel¹, K. Taira², D. Utsuki², T. Kato², F. Sunaga³, H.K. Ooi²

Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovakia¹; Laboratory of Parasitology, School of Veterinary Medicine, Azabu University, Fuchinobe, Sagamihara, Kanagawa, Japan²; Laboratory of Infectious Disease, School of Veterinary Medicine, Azabu University, Fuchinobe, Sagamihara, Kanagawa, Japan³

Intestinal parasites belonging to genus *Heterakis* (Nematoda, Heterakidae) established by Dujardin (1845) infest numerous largely ground-feeding birds and occasionally mammals. Nowadays, 10 taxa are designated as species under the genus, but classification is often ambiguous due to their resemblance, with a number of synonyms being arisen. *Heterakis spumosa* is a nematode mainly affiliated with the globally distributed invasive rodents of *Rattus* spp. of Asian origin. *H. spumosa* isolates obtained from naturally infected brown rats caught in 2007 in Sagamihara city, central Honshu (Japan) and maintained by laboratory passages, were subjected to sequencing studies of mitochondrial DNA and experimental infection in mice. In nucleotide sequences of *cox1* gene, a difference of 11.2 - 12.2% between *H. spumosa* and previously examined *Heterakis isolonche* isolates from gallinaceous birds in Japan was determined that conforms to the range expected for interspecific differences. Homogenous patterns between the two *H. spumosa* isolates were recorded, with only one 138T/C non-synonymous substitution detected in the 393 bp mitochondrial segment. In UPGMA dendrogram derived from available data in related ascarids, *H. spumosa* samples formed the subcluster with nematodes of superfamily Heterakoidea, *H. isolonche* and *Ascaridia galli*. Among the compared nematodes, the G content was highest in *H. spumosa* (26.5%), along with *B. procyonis* roundworms (26.7%). This reflects abundance of A to G transitions in *H. spumosa* and a fixation tendency toward GC via biased gene conversion. The high GC content confers to DNA higher melting point and indicates greater translational efficiency in the species given positive correlations between recombination rate and GC frequency, previously observed in *Caenorhabditis elegans* nematodes, but also in mammals, yeast, and *Drosophila* organisms.

In the study of experimental infection, ICR, AKR, B10.BR and C57BL/6 strains of mice were inoculated with 200 *H. spumosa* eggs, and the fluctuation of eggs per gram of feces (EPG) and number of worm infected were compared among mouse strains. Eggs were initially detected in feces from 32-35 DPI in ICR, AKR and B10.BR mice, and the highest mean EPG were 4800 (at 38 DPI), 2200 (at 58 DPI) and 800 (at 44 and 72 DPI) in ICR, AKR and B10.BR mice, respectively. No eggs were found in the feces of C57BL/6 strain during the experiment. At 14 DPI, similar number of juvenile worms was found from all mouse strains, whereas no worms were detected in C57BL/6 mice at 90 DPI. There may be several factors, such as sex hormones and immune responses of hosts that could affect the growth and fecundity of *H. spumosa*. The present study provided the information about the
susceptibility of different mouse strains and may serve as a unique model to study the mechanism of habitation of nematode infections in large intestine of mammals.

The study was supported by the mobility grant of the Japan Society for Promotion of Science (ID No. RC 21328101) and the Scientific Grant Agency VEGA (project No. 2/0172/13).

BAYLISASCARIS SP. INFECTION IN A PET KINKAJOU (POTOS FLAVUS): GENETIC AND MORPHOLOGICAL EVIDENCE FROM THE RECOVERED ISOLATE
V. Šnábel¹, K. Taira², Y. Une³, H. Sugiyama⁴
Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovakia¹; Laboratory of Parasitology, School of Veterinary Medicine, Azabu University, Sagamihara, Japan²; Laboratory of Pathology, School of Veterinary Medicine, Azabu University, Sagamihara, Japan³; National Institute of Infectious Disease, Shinjuku, Tokyo, Japan⁴

The nematodes of genus Baylisascaris are intestinal roundworms of carnivores such as raccoons, skunks, badgers, martens and bears, and their larvae migrate in a range of small animal paratenic hosts serving as prey. In December 2011, a routine veterinary examination of a juvenile male pet kinkajou that had been caught in nature, imported from Guyana (South America) to Japan and purchased from an exotic pet shop in the same month, revealed ascarid eggs in the feces. The collected isolate (designated as JP1) was further subjected to analysis of rDNA sequences in the second internal transcribed spacer (ITS2) and egg morphology.

The ascarid eggs detected in feces from the kinkajou were morphologically similar to those of Baylisascaris procyonis (raccoon roundworm), with eggs measuring 63-88 μm (mean: 68-76 μm) by 50 – 70 μm (mean: 55-61 μm), and to B. columnaris (skunk roundworm) with eggs measuring 72.5 ± 4.1 μm by 63.2 ± 7.5 μm. Slightly closer values with regard to the JP1 isolate were thus associated with B. columnaris, particularly in the length of transverse axis (64.4 ± 1.02 μm in JP1 isolate). Nevertheless, morphometric identification in Baylisascaris is often hampered by diversity in size and developmental stages that makes the unequivocal recognition problematic. In ITS2 sequences, the unique divergence pattern was derived for the JP1 kinkajou isolate suggesting that the nematode could not be entirely allocated to the closest recognized Baylisascaris species. The 7.8 – 8.8 % range of nucleotide difference between the JP1 isolate and the closest retrieved sequences of B. procyonis and B. columnaris therefore classified the nematode as referring to Baylisascaris sp., with its precise species categorization remaining to be determined. Eleven tandem G-A repeats identified in the polymorphic repetitive region further differentiate the roundworm from recognized congeneric species. The question emerges whether or not the present isolate pertains to any described Baylisascaris spp., for which sequencing data are not yet available in GenBank. The Baylisascaris genus currently contains eight recognized species. From these, apart from 4 species with available nuclear sequences, transmissions of B. melis linked to badgers absent in South America, and B. tasmaniensis with marsupial carnivore hosts endemic for Tasmania, exclude these taxa as plausible for infesting kinkajou in Guyana. From remaining species, B. laevis, morphologically similar to B. columnaris and B. procyonis, occurring in large rodents as marmots and ground squirrels, and B. devosi occurring in martens and fishers, are transmissible in South American wildlife. A type material from these species is needed to be subjected to further sequence analyses to better clarify the
taxonomic issue in the JP1 isolate. Given that the *Baylisascaris* sp. from the kinkajou is genetically closely affiliated with *B. procyonis* having a serious disease-producing capacity, the report appeals for precautions in informing people to avoid the transmission risk.

The study was supported by the mobility grant of the Japan Society for Promotion of Science (ID No. RC 21328101), the Scientific Grant Agency VEGA (project No. 2/0172/13), and the Grant-in-Aid from Ministry of Health, Labor and Welfare in Japan.

**NEOSPORA CANINUM ASSOCIATED ABORTION IN A DAIRY HERD IN EASTERN SLOVAKIA**

S. Špilovská, K. Reiterová

Institute of Parasitology SAS, Hlinkova 3, 040 01 Košice, Slovak Republic

*Neospora caninum* is an intracellular parasite which causes repeated abortion in cattle worldwide what has negative economic impact on their breeding. The transplacental transmission from infected dams to their offspring appears to be a major natural route of the infection. Considering the rapid spread of neosporosis in herds throughout the world, our aim was to detect the occurrence of anti-*Neospora* antibodies during the survey of 490 cows (*Bos taurus*) of the Slovak spotted breed in selected dairy herd with a high occurrence of abortions.

Sera were screened using an indirect ELISA. DNA was isolated using an isolation kit from the brain tissue of aborted foetuses. The cortical layer of brain hemispheres of one culled seropositive cow with clinical symptoms was homogenized, and, after trypsin-digestion, cultured on Vero cells.

Mean prevalence of anti-*Neospora* antibodies of cattle was 24.1%. The high seroprevalence was detected in group of heifers older than 6 months (43.3%) and in cows with a history of abortion (41%). Only 2.2% mean seroprevalence was found in cows without any history of abortion. Up to 65% seropositive heifers were born from seropositive cows (P< 0.001), what supposed the occurrence of vertical transmission of *Neospora* from mothers to their progeny. The specific 328 bp sequence of *Neospora* DNA was confirmed in three foetuses aborted by seropositive cows. Moreover, viable *Neospora* tachyzoites were *in vitro* maintained in Vero cell cultures. A total of 35% of heifers from negative cows, serologically positive for *N. caninum*, suggested that the infection is related to environmental contamination. A set of measures to control bovine neosporosis in the herd have been implemented.

This work was supported by the Slovak Grant VEGA, No. 2/0104/11 and has been realized within a frame of the project Centre of Excellence for Parasitology (Code ITMS: 26220120022) of the Operational Programme “Research & Development” funded from the European Regional Development Fund (rate 0.4).
**NEOSPORA CANINUM AND TOXOPLASMA GONDII IN POST-ABORTION SHEEP FROM THE NORTHERN REGION OF CENTRAL SLOVAKIA**

S. Špilovská, K. Reiterová

Institute of Parasitology SAS, Hlinkova 3, 040 01 Košice, Slovak Republic

*Neospora caninum* is currently well-recognized as a serious cause of abortion not only in cattle, but also in experimentally and naturally infected ewes. The role of *Toxoplasma gondii* as a serious cause of abortion and foetal mortality in sheep is also well known.

In cooperation with the State Veterinary and Food Administration in Dolný Kubín totally 154 sheep post-abortion were serologically examined for infection by *T. gondii* and *N. caninum*. Animals originated from brucellosis-free farms of 11 northern districts in central part of Slovakia. Competitive enzyme-linked immunosorbent assay (cELISA) revealed for *N. caninum* 13.6% mean seroprevalence (%I 30.5 –87.7%). Antibodies to *T. gondii* detected by an indirect enzyme-linked immunosorbent assay (IELISA) were found in 44.8% of animals (S/P% 77 – 209%). Eighteen out of all tested animals were simultaneously infected with both parasites.

Serology plays an important role in inspecting abortion outbreaks and commonly acts as a primary test undertaken to determine the cause or agent involved. The presented mean seroprevalence of neosporosis and toxoplasmosis in post-abortion sheep compared with the former results from eastern districts of Slovakia (Špilovská et al., 2009) is almost four times higher in the case of neosporosis (13.6%, versus 3.7%) and less than two times higher in the case of toxoplasmosis (44.8%, versus 24.3%). The majority of seropositive animals exhibited a humoral immune reactivity against both coccidian parasites, perhaps due to the lack of cross-protectivity between anti-*T. gondii* and anti-*N. caninum* immune responses. Adequate management might be useful and essential to control not only bacterial (*Brucella sp.*, *Chlamydia sp.*, *Coxiella burnetii*) and viral (*Pestivirus and Bluetongue virus*) abortifacent agents, but also presented coccidian parasites in the sheep flock.

*This work was supported by the Slovak Grant VEGA, No. 2/0104/11 and has been realized within a frame of the project Centre of Excellence for Parasitology (Code ITMS: 26220120022) of the Operational Programme “Research & Development” funded from the European Regional Development Fund (rate 0.4).*

**GENETIC CHARACTERIZATION OF IXODES RICINUS, DERMACENTOR RETICULATUS, HAEMAPHYSALIS INERMIS USING 12S rRNA**

E. Špitalská¹, L. Berthová¹, B. Sallay¹, J. Kraljík²

Institute of Virology, SAS, Dúbravská cesta 9, 845 05 Bratislava, SR¹; Institute of Parasitology, SAS, Hlinkova 3, 040 01 Košice, SR²

*Ixodes ricinus*, *Dermacentor marginatus*, *Dermacentor reticulatus*, *Haemaphysalis concinna*, *Haemaphysalis punctata* and *Haemaphysalis inermis* are the main exophilic tick species occurring in Slovakia and the most important ticks for humans and animals. *I. ricinus* ticks are widely distributed throughout the country. It is an important vector for pathogens of medical and veterinary importance, such as the tick-borne encephalitis virus (causative virus of tick-borne encephalitis), *Borrelia burgdorferi* sensu lato (agents of Lyme borreliosis), *Anaplasma phagocytophilum* (agent of
granulocytic anaplasmosis in humans, tick-borne fever in sheep and canine ehrlichiosis in dogs), Babesia divergens (causative agent of babesiosis in humans and redwater fever in cattle), Babesia venatorum and Babesia microti (agents of babesiosis in humans), occasionally Francisella tularensis and Rickettsia species causing rickettsial diseases. D. reticulatus and D. marginatus ticks are limited to along the rivers and Krupinská planina, respectively. They are known vectors of Rickettsia slovaca and Rickettsia raoultii distributed across Europe. They can transmit also Babesia spp., Coxiella burnetii, F. tularensis. H. inermis inhabits forest steppe sites and thermophilic oak and hornbeam-oak forests of the andesite background. It is occurring in the mountains of volcanic origin: Štiavnické Hills, Tribeč, Vtáčnik and Krupinská planina. It can transmit TBE virus and C. burnetii.

A total of 19 ticks were collected from vegetation and 17 engorged ticks were removed from dogs, cats, horses, humans in Slovakia. DNA was individually extracted using alkaline hydrolysis or DNeasy Blood and Tissue Kit (Qiagen). DNA extracts were used as templates for PCR assays using a fragment of the 12S mitochondrial rRNA. Amplification products were sequenced and the sequences were compared with those available in GenBank. Ticks were screened for the presence of Rickettsia spp., C. burnetii, Anaplasma phagocytophilum and Babesia spp. by PCR-based methods using 16S rRNA, gltA, msp2 and 18S rRNA genes.

A total of 21 sequences of I. ricinus were analysed. 18 of them showed 97.4-100% identity with sequences of I. ricinus 12S rRNA Acc. No. AF150029, tick collected from vegetation in Switzerland. Another three sequences from ticks removed from horses /one of ticks was Rickettsia-positive/ and from cat /tick was pathogen free/ differ from them, with homology 81.6-83.7% with Acc. No. AF150029. All six analysed 12S rRNA sequences of D. reticulatus collected from vegetation showed 99.4-99.7% similarity with sequences of D. reticulatus GenBank Acc. No. JQ768760 collected in Černobyl. Two analysed sequences of D. reticulatus removed from dogs showed 95.3 and 97.6% identity with sequences of D. reticulatus GenBank Acc. No. JQ768760, one of them was Rickettsia-positive. Totally, 6 analysed 12S rRNA sequences of H. inermis collected from vegetation showed 99.7% identity with sequences of H. inermis mitochondrial partial 12S rRNA gene, country Italy, Napoli Acc. No. AM410576. Sequences of 12S rRNA from one H. inermis male collected from vegetation showed only 88.8% identity with those sequences. This tick was Rickettsia-positive.

Molecular biology approaches can be valuable tools to confirm identification species, may be decisive for the classification of fragmented or damaged specimens and show differences among tick populations. Most of the 12S rRNA analysed amplicons showed >97% similarity.

This study was financially supported by the project VEGA No. 2/0061/13 and SRDA-0280-12.

**RICKETTSIA SPP. CIRCULATING IN QUESTING IXODES RICINUS TICKS IN SLOVAKIA**

E. Špitalská¹, L. Berthová¹, E. Kocianová¹, L. Mydlová², Z. Svitátková², M. Slovák², M. Kazimírová², L. Mošanský³, J. Kraljík³, M. Stanko³
Institute of Virology, SAS, Dúbravska cesta 9, 845 05 Bratislava, SR¹; Institute of Zoology, SAS, Dúbravska cesta 9, 845 06 Bratislava, SR²; Institute of Parasitology, SAS, Hlinkova 3, 040 01 Košice, SR³

Ixodes ricinus, the most common tick species in Europe, serves as an important vector for many microbial and protozoan pathogens. I. ricinus is widely distributed throughout Slovakia.
Rickettsiae are arthropod-associated gram-negative prokaryotes. Some of *Rickettsia* spp. belonging to the spotted fever group cause rickettsiosis in humans and are spread in Europe, inclusive Slovakia. The presence of different *Rickettsia* spp. was studied in three different habitats of Slovakia: urban and suburban (Bratislava), woodland (Fügelka) and agricultural (Rozhanovce).

A total of 10,055 questing *I. ricinus* ticks were collected during 2011 – 2013. Totally, 3,751 ticks were further analyzed for the presence of *Rickettsia* spp. Total genomic DNA was extracted from individual ticks using the NucleoSpin® Tissue Macherey-Nagel kit. For identification of Rickettsiae PCR amplification of *gltA* gene was used, followed by sequencing.

Overall prevalence of rickettsiae in questing ticks was 5.2%, 7.0% and 8.0% in Bratislava, Fügelka and Rozhanovce, respectively. Sequencing showed infections with *R. helvetica*, *R. monacensis* and unidentified *Rickettsia* spp. *R. helvetica* is the most often occurring Rickettsia in ticks in the studied habitats. However, *R. monacensis* occurs more often in questing ticks in Rozhanovce than in southwestern Slovakia.

Both species of *Rickettsia* genus are associated with human diseases and our study shows their circulation in all types of studied habitats - urban, suburban, woodland and agricultural.

*This study was financially supported by the FP7 project EDENext (No. 261504), grant APVV DO7RP–0014–11 and VEGA No. 2/0061/13.*

**PREVALENCE OF ENDOPARASITOSES WITH ZOONOTIC POTENTIAL IN DOGS AND CHILDREN IN COMMUNITIES WITH LOW HYGIENIC STANDARDS**

G. Štrkolcová¹, M. Goldová¹, M. Halán¹, M. Halánová³, A. Valenčáková², O. Počátková², M. Škutová⁴

Department of Epizootology and Parasitology¹; Department of Biology and Genetics, University of Veterinary Medicine and Pharmacy, Košice, Slovakia²; Department of Public Health, Pavol Jozef Šafárik University, Košice, Slovakia, ⁴ Institute of Parasitology SAS, Košice, Slovakia³

The aim of our study was to determine prevalence of protozoan parasites with zoonotic potential *Giardia intestinalis*, *Cryptosporidium* spp. and to detect helminthostatus in dogs and children in communities with low hygienic standards. *Giardia intestinalis* is a flagellated extracellular protozoan pathogen that localizes in the small intestine of a wide range of mammalian hosts including dogs and humans. Potential mechanisms of transfer include person to person, animal to animal, or zoonotic (animal to human or human to animal), usually in an indirect manner through environmental contamination. The cyst are environmentally resistant and can survive for months in soil or water basins in temperate conditions (Plutzer et al., 2010). Human and dogs, or occasionally other mammals, have been infected with genotype B, indicating this genotype clearly has zoonotic potential. Cryptosporidiosis in humans may be transmitted via anthroponotic (human-to-human) or zoonotic (animal-to-human) pathways, depending on the species of parasite (Ciao et al., 2004). The parasite causes self-limiting diarrhoea in immunocompetent individuals but the infection may be chronic and life-threatening to those that are immunocompromised (Hunter et al., 2007). The faecal samples
were analysed by flotation concentration method and direct ELISA method commercial test for faecal *Cryptosporidium* antigen was used.

In our study was examined 173 faecal samples of Roma children in settlements from East Slovakia region and 10 faecal samples of dogs. Dogs there running around freely among the people, sleeping with them together in their hut. In faecal samples of Roma children we detected *Giardia intestinalis* in 13.29 % (23/173), *Cryptosporidium* spp. in 9.26 % (10/108) and eggs of 5 species of helminths *Ascaris lumbricoides*, *Trichuris trichiura*, *Ancylostoma* spp., *Enterobius vermicularis*, *Taenia* spp. In faecal samples of dogs we detected cysts of *Giardia intestinalis* and *Cryptosporidium* spp. and eggs of helminths *Toxocara canis*, *Toxascaris leonina*, *Trichuris vulpis*, *Ancylostoma* spp.

We examined 45 faecal samples of dogs from shelter and 3 samples from shelter’s staff. Dog’s pens were overcrowded, sanitation was pure, rate of veterinary care weaker, and nutrition often unsatisfactory. In dogs we detected cysts *Giardia intestinalis* in 33.33 % (15/45) and *Cryptosporidium* spp. in 2.22 % (1/45). Eggs of helminths were also detected in 42.22 % *Toxascaris leonina* (19/45), 22.22 % *Toxocara canis* (10/45), 28.88 % *Trichuris vulpis* (13/45), 35.55 % *Ancylostoma* spp. (16/45). All examined human samples were negative.

*The study was supported by the by grants of State Agency VEGA No. 1/0831/12, No. 1/0063/13.*

---

**SEASONAL DYNAMICS OF IXODES RICINUS TICKS AND PREVALENCE OF BORRELLIA BURGDORFERI SENSU LATO AND ANAPLASMA PHAGOCYTOPHILUM IN URBAN PARKS IN BRATISLAVA**

T. Vaculová, V. Tarageľová

Institute of Zoology, SAS, Dúbravská cesta 9, 845 06 Bratislava

Ticks gradually penetrate into the urban areas and new foci of tick-borne diseases are formed. The sheep tick, *Ixodes ricinus* is geographically widespread species and it is the most common species in Europe. This tick is the main vector of tick-borne pathogens and therefore it has a major epidemiological significance. The most frequent disease transmitted by this tick is Lyme borreliosis caused by bacteria of the *Borrelia burgdorferi* sensu lato (s.l.) complex. The significant but less frequent tick-borne pathogen is *Anaplasma phagocytophilum*, agent of human and animal diseases.

In our study, two urban sites in Bratislava (Železná studienka and Horský park) were selected in order to study the seasonal dynamics of *I. ricinus* ticks and the prevalence of tick-borne pathogens in questing ticks. Both study sites are recreational parks frequently visited by people. Ticks were collected monthly by flagging from March to October 2011 and 2012. In Železná studienka the ticks were flagget in two different habitats – around the paths and playgrounds and in sylvatic area deeper in the forest. A total of 981 ticks, including 49 larvae, 515 nymphs, 227 males and 190 females were collected. Tick population density fluctuated seasonally and small differences between studied sites were recorded. The tick activity was highest in April and May.

DNA was extracted from individual ticks by alkaline hydrolysis. All samples were screened by RT-PCR or PCR for the presence of *A. phagocytophilum* (*msp2* gene of 16 rDNA) and *B. burgdorferi* s.l. (*rrfA-rrfB* intergenic spacer of 5S-23S rDNA) respectively.

Molecular screening confirmed the presence of these pathogens in both localities. *B. burgdorferi* s.l. predominated and it was detected in 13.2 % of tested ticks (n=123) (11.9 % in Železná
studienka and 15.3 % in Horský park). Six genospecies in *Borrelia*-positive samples were determined by RFLP method. The most frequent was *Borrelia afzelii* followed by *Borrelia garinii* and *Borrelia valaisiana*. The infection prevalence of *Anaplasma*-infected ticks were 5.6 % (4.4 % in Železná studienka and 7.5 % in Horský park). Our results confirmed the existence of tick-borne disease foci in the centre of Bratislava. The presence of borreliae and anaplasmae at study sites showed that competent hosts and suitable microclimatic conditions are present not only in sylvatic – natural areas but in urban habitats affected by human activity too.

*The study was supported by the grant VEGA - 0108 and APVV-0267-10.*

**BENZIMIDAZOLE AND MACROCYCLIC LACTONE DRUGS EFFICACY AND PERSISTENCE ACTIVITY AGAINST GASTROINTESTINAL NEMATODES AT SHEEP FARMS IN THE CZECH REPUBLIC**

J. Vadlejch, Z. Čadková, I. Langrová

Department of Zoology and Fisheries, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 957, 165 21 Prague 6, Suchdol, Czech Republic

The efficacies of the benzimidazole, ivermectin and moxidectin drugs against naturally acquired gastrointestinal nematodes at four sheep farms were evaluated using the faecal egg count reduction test. Panacur (2.5% oral suspension), Ecomectin (1% injectable solution) and Cydectin (2% injectable solution) were applied at the manufacturer’s recommended dosages. Using the mentioned in vivo test, anthelmintic resistance to benzimidazole (FECR 91.4 % and 92.8 %) was detected at two farms, and resistance to ivermectin (FECR 79.6 %) was detected at one farm. Moxidectin was effective at all evaluated farms. *Teladorsagia* was recognized as the only benzimidazole resistant genus in post-treatment coprocultures, whereas *Haemonchus* larvae were shown to be resistant to ivermectin.

Faecal egg counts in animals treated with evaluated anthelmintic drugs increased with time after application. Only short persistence activity was demonstrated for benzimidazoles; ivermectin suppressed gastrointestinal nematode egg production for up to 35 days, and the longest protection effect (up to 42 days post treatment) was observed in sheep drenched by moxidectin.

This study confirmed high efficacy of macrocyclic lactones against gastrointestinal nematodes of sheep. The persistent activity of these anthelmintic drugs was even longer than the manufacturer’s declaration. The results of this study indicate that it is possible to use moxidectin drugs for the control of gastrointestinal nematode infections at sheep flocks where resistance to ivermectin was already detected.

*This study has been funded by the Grant of the National Agency for Agricultural Research of the Czech Republic (No. QI111A199).*
Objective. This study was conducted with a view to determine the prevalence of selected intestinal opportunistic parasites (Microsporidia spp. and Cryptosporidium spp.) in immunocompetent groups of Roma and non-Roma children.

Introduction. Cryptosporidiosis and microsporidiosis are considered to be a widespread world zoonosis. The clinical manifestations of these infections are dependent on the type of infection, way of infection, the age of the host and the competence of the immune system. Parasites cause severe watery diarrhoea, slowly progressive weight loss and dehydration. For immunosuppressed individuals may progress to severe and fatal form.

Patients and methods. The occurrence of Cryptosporidium and Microsporidia species were investigated in 103 children (54 boys and 49 girls) between 0 – 14 years of age. The Roma children (53) come from the minority group, living in a low hygiene and health standards and 50 children represented a non-Roma control group. They permanently visited doctors for control of their health situation and did not show any of the symptoms which might indicate a possible infection. Cryptosporidium spp. - Nested PCR, Microsporidia spp. - RT-PCR. The positive PCR products were later given for sequencing and the results were compared to the sequences recorded in the Gene Bank database in NCBI by the program Blast. Basic descriptive statistics were used for the analysis of the results. The Relative Risks (RR) and their 95 % confidence intervals (95% CI) were estimated for the occurrence of Cryptosporidium and Microsporidia species. The contributions of age, gender and ethnicity on the prevalence of cryptosporidium and microsporidia infection were assessed using a logistic regression model.

Results. Examination of the samples revealed the presence of DNA Microsporidia spp. in 22 samples (33%) and the presence of DNA Cryptosporidium spp. in 6 samples (4%). RT-PCR confirmed the presence of species Encephalitozoon cuniculi in 19 samples and Enterocytozoon bieneusi in 3 samples. Co-infection of E. cuniculi and Ent. bieneusi was found in one case. The presence of Cryptosporidium parvum was confirmed by nested PCR method in sample 1 in 4 samples was confirmed species of Cryptosporidium muris. Co-infection of C. muris and E. cuniculi was found in one case. All positive samples came from a group of Roma children. In the control group of non-Roma children has not demonstrated the presence of DNA opportunistic parasites in either case. Based on the results, the Relative Risk (RR) of the cryptosporidium infection occurrence was calculated and we came to the conclusion that the risk of cryptosporidium infection was almost 7 times higher in the Roma children compared to the non-Roma children.

Conclusion. Our results confirm the actuality of occurrence of these pathogens in the population of Roma children, which can be a source of infection. Because the Microsporidia and Cryptosporidium are portable water zoonosis represent epidemiological risk. It is therefore desirable to pay attention to these diseases.

This work was GP supported by GP VEGA MŠ SR: 1/0063/13, 1/0390/12, 1/0831/12.
HISTOPATHOLOGICAL ANALYSIS OF LIVER IN FISH POPULATION OF RESERVOIR „STREZEVO” (R. MACEDONIA)
L. Velkova-Jordanoska¹, S. Smiljkov², S. Stojanovski³, G. Kostoski⁴
SI Hydrobiological Institute – Ohrid, R.Macedonia¹,³,⁴; Faculty of Natural Sciences, Arhimedova Str. 3, 1000, Skopje, Macedonia²

Some structural lesions in liver tissue have been accepted as valid biomarkers of anthropogenic stress at fish. Histopathology is the method of detecting chronic effect of exposure in the various tissues and organs to environmental stressors.

Liver pieces of 14 fish individuals collected from reservoir „Strezevo” during 2013, were excised and processed for standard histopathological analysis. The obtained results revealed pathological changes in the liver tissue on a level of hepatocellular parenchyme, and on a level of hepatic bile tract.

The granulomatous inflammatory response was proved to be a results of the presence of eggs of the parasite in the hepatocellular parenchyme of fishes liver.

MOLECULAR DETECTION AND CHARACTERIZATION OF SEVERAL BABESIA SPECIES IN SLOVAKIA
B. Víchová, L. Blaňárová, V. Majláthová, B. Haklová, B. Peťko
Institute of Parasitology SAS, Hlinkova 3, 040 01 Košice, SR

In Slovakia, babesiosis was primarily a problem of the 50’s of the last century. The local overgrowth of the ornate dog ticks (Dermacentor reticulatus) populations, transmitting Babesia divergens to the cattle, caused severe damage to livestock, particularly in the southern and southeastern Slovakia.

Further information about the spread of babesiosis disappeared for almost whole next half of the 20th century. Afterward, at the beginning of a new millennium, infections have begun to occur sporadically again, in dogs. First autochthonous report of canine babesiosis, caused by Babesia canis canis, was described in 2001, in the southeastern part of the country (Chandoga et al. 2001). Since then, the number of cases is growing exponentially every year. Currently, Slovakia is considered a country with the endemic occurrence of canine babesiosis. Agents of canine babesiosis cause severe epizootics in areas with the endemic occurrence of D. reticulatus, infection of which may locally reach up to 40% (Majláthová et al., 2011). In the past, D. reticulatus occurred primarily in western Slovakia and in some areas of eastern Slovakia (Nosek 1972). Due to global climate changes, new locations suitable for tick survival are established in areas which were not favorable in the past.

These changes support the emergence and expansion of various, also tick-borne pathogens. In Europe, human infections are caused by B. divergens, B. bovis (parasites of cattle) and B. ventorum (phylogenetically resembles to B. odocoilei), recently described in asplenic patients from Austria, Italy and Germany (Herwaldt et al., 2003; Häselbarth et al., 2007). The first case of human babesiosis in Europe was described in 1957 in Yugoslavia (Škrabalo and Deanovic, 1957). Since then, at least 40 cases have been confirmed in Europe, including 2 infections caused by B. microti in patients from Switzerland and Germany (Meer-Scherrer et al., 2004, Hildenbrand et al., 2007).

Phylogenetic analyses confirmed that B. microti is a complex species, consisting of genetically diverse isolates that constitute three clades (Goethert and Telford 2003). Within these clades, rodent isolates
are subdivided into zoonotic (e.g. Jena strain) and nonzoonotic (e.g. Munich strain). B. microti strains circulate in natural foci altogether or independently by different rodent species. There are assumptions that in the circulation of human pathogenic ecotypes, yellow-necked mouse (A. flavicollis), which is very frequent in natural foci of Slovakia, might be predominantly involved.

The main aim of our ongoing project entitled “Babesioses in Slovakia” is to determine the presence of members of the Babesiidae family in the vector ticks and hosts in natural foci of Slovakia, central Europe. To date, apart from B. canis canis, we have confirmed the circulation of both strains of B. microti in ticks and rodents, with the predominant occurrence of pathogenic “Jena strain” and sporadic evidence of “Munich strain”; the presence of B. venatorum (previously Babesia – like EU-1 strain) in ticks as well as B. odocoilei in deer.

The study was supported by the project of Research & Development Operational Programme funded by the ERDF (code ITMS: 26220220116) (0,1); by the projects of Slovak Research and Development Agency APVV 0267-10 and VEGA 2/0113/12.

**TAPEWORMS – METABOLISM AND TRANSPORT OF XENOBIOTICS**

I. Vokrál¹, Z. Seifertová¹, L. Stuchlíková², L. Prchal², B. Szotáková², L. Skálová²

Charles University in Prague, Faculty of Pharmacy in Hradec Králové, Heyrovského 1203, Hradec Králové, 500 02, Department of Pharmacology and Toxicology¹; Department of Biochemical Sciences²

Tapeworms are probably the least studied parasites in terms of metabolism and transport of xenobiots compared to flukes and roundworms. On the other hand even tapeworms can cause serious diseases of humans and animals and by that indirectly contribute to economic losses in farm animals. Knowledge about all detoxification mechanisms in tapeworms can be important for the future drug development and also can contribute to understanding of drug resistance development.

Metabolism of selected anthelmintics (albendazole, flubendazole, mebendazole and praziquantel) in two tapeworms, Hymenolepis diminuta and Moniezia expansa, was studied in vitro and ex vivo. H. diminuta is supposed to be model tapeworm while M. expansa is tapeworm with real economic impact so we can compare relevance of data obtained in laboratory to the status in field conditions. Most of found metabolites were produced during Phase I of metabolism. Only two found metabolites were from the Phase II. No metabolites of praziquantel were detected (studied only in H. diminuta). On the contrary flubendazole and mebendazole were extensively reduced in both tapeworms. Reduced flubendazole was further metabolized in H. diminuta and methylated metabolites (Phase II) were found. On the other hand only in M. expansa metabolites produced by oxidative metabolism albendazole sulfoxide and albendazole sulfone were observed.

Less information is available about drug transporters and their role in detoxification of xenobiots in tapeworms. These proteins can contribute, as observed in bacteria or cancer, to drug resistance development. There was only limited number of information about drug transporters in tapeworms till last year. Now, recently published genomes of four tapeworms clearly show presence of these transporters in tapeworms. We focused on the role of transporters in detoxification of anthelmintics from this reason. As a model tapeworm H. diminuta was used. Studied drugs were albendazole and mebendazole. For study of transport a simple method was used. Firstly, tapeworms were left in RPMI-1640 medium with tested drugs for 2 hours. Second, tapeworms were divided to two groups, one group was inactivated by deep freeze and second left alive without any influencing.
Both groups were put into the fresh drug free medium for 30 minutes. After 30 minutes of export, concentrations of drug exported to the medium were measured. Amount of mebendazole in medium of alive group was more than 40% higher and amount of albendazole more than 30% higher comparing to the medium of dead tapeworms. These results show presence of active efflux.

In conclusion, tapeworms are able, in some cases, to metabolize anthelmintics. Compared to mammals, metabolism role in detoxification processes is not so important. We assume that the export of xenobiotics from the body of tapeworms plays more important role in these processes but for final verdict further studies are needed.

TOXOCAROSIS STILL ACTUAL
B. Voxová¹, Z. Čermáková¹²

Institute of Clinical Microbiology, University Hospital, Hradec Králové, Czech Republic¹; Faculty of Medicine, Charles University, Hradec Králové, Czech Republic²

Background: Larval toxocarosis is caused by the larva of the parasite which occurs in dogs or cats - canine roundworm (Toxocara canis) and feline roundworm (Toxocara cati). Seroprevalence in dogs reaches 18%, in cats 50%. Antibodies against Toxocara spp. are present in 10-20% of the human population. The literature data report that the highest incidence of parasites occurs in children. Children are most commonly infected while playing on the ground, in contaminated sandboxes and playgrounds where roundworms contaminate the sand and soil. The eggs enter soil with feces of infected animals. Infection in humans can cause liver, lungs, muscles, eyes and brain damage, and non-specific symptoms as well.

Methods: Evaluation of laboratory diagnosis of toxocarosis based on the determination of IgG antibodies, avidity of IgG antibodies and Western blot confirmation.

Results: Our laboratory data do not correlate with the literature data, that report a high incidence of toxocarosis in childhood. Among 1083 patients examined in the last 5 years (2009-2013) in our laboratory only 3 children aged under 5 years positive in Toxocara spp IgG were detected. In a group of 106 positive patients, males slightly prevailed (52.8%). The trend of toxocarosis occurrence in the CR is steadily decreasing as well as the number of suspected patients by clinicians. The percentage of positive samples is constantly falling down - from nearly 20% to around 5%. Also detection of the infection at an early stage (with low or borderline avidity of IgG) is very rare - in our group of patients it was only in 11% of the patients.

Case report: Man (56 years, veterinarian) infected from puppies in his own praxis, clinical signs, laboratory results, anamnesis.

Conclusion: Toxocarosis is a disease whose incidence can never be completely eliminated and is still actual. In an effort to lowest prevalence of infection, it is necessary to appeal especially to breeders of dogs and cats to pay heed to the regular deworming their animals as well as to waste removal from the external environment. It is necessary to get acquainted professionals and general public with this problem at the same time.
PARASITES OF DOMESTIC FREE-RANGING CATS (FELIS SILVESTRIS CATUS) BASED ON NECROPSY

I.A. Wierzbowska¹, S. Kornaš², A. M. Piontek¹, M. Basiaga², J. Kowal², M. Lesiak³

Institute of Environmental Sciences, Jagiellonian University, 7 Gronostajowa str., 30-387 Kraków, Poland¹; Department of Zoology and Ecology, University of Agriculture in Krakow, al. Mickiewicza 24/28, 30-059 Krakow, Poland²; "Kaban" Maciej Lesiak, os. Szklane Domy 1/200, 31-972 Kraków, Poland³

The material for necropsy was taken from animals killed in road accidents in Kraków city and Kraków County between November 2011 and April 2013. Dead cats were obtained from private company "Kaban" that has an appropriate permission to collect biological material. The cats were weighed and sex determination was based on teeth development and wear. Two age categories were stated: juveniles (<2 years) and adult (> 2 years).

A total of 80 cats were examined. During the necropsy we checked the following parts of digestive tract: stomach, duodenum, small intestine and large intestine, as well as internal organs: lungs, heart, kidneys and liver. Based on the obtained results, the prevalence and intensity of infection with internal parasites was assessed. Additionally, stomach content was evaluated to provide the data on diet.

Parasitological studies revealed the presence of following parasites: tapeworms (Cestoda) - Hydatigera taeniaeformis, Dipylidium caninum, Mesocestoides lineatus and nematodes (Nematoda) - Toxocara cati, Toxascaris leonina, Ancylostoma tubaeforme. Over 40% of cats were infected with roundworm Toxocara cati (43.75%) and tapeworm Hydatigera taeniaeformis (41.25%). The intensity of infection of the above-mentioned species was respectively: 12 and 6 specimens in one host.

The analysis of stomach content from 63 animals revealed the presence of 5 species of mammals, two families of birds, at least three families of invertebrates, and various anthropogenic food.

Free-ranging domestic cats, commonly present in urban areas, may serve as an important factor in the transmission of zoonotic parasites, such as Toxocara cati which was frequently found in the present study.

SEROPREVALENCE OF LYME DISEASE AND TICK BORNE ENCEPHALITIS AMONG RISK POPULATION IN EASTERN SLOVAKIA

Ľ. Zákutná¹, E. Dorko¹, K. Rimárová¹, E. Mattová²

Institute of Public Health, Faculty of Medicine, PJ Safarik University, Kosice, Slovakia¹; Clinic of Infectology and Travel Medicine, L Pasteur University Hospital, Kosice, Slovakia²

The aim of the study was to determine the current epidemiological situation of Lyme borreliosis (LB) and tick-born encephalitis (TBE) in a selected risk groups of population in the eastern Slovakia.

The study was aimed at people working in agriculture and forestry, employees of the State Border and Customs Police and people with frequent stay in the nature. Blood with questionnaires and written informed consent were obtained by collection of samples from volunteers, especially from Košice and Prešov district. 277 samples of human sera were investigated for the presence of
specific IgG antibodies against *Borrelia (B.) burgdorferi* using serological test ELISA anti-*Borrelia plus VlsE* (EUROIMMUN, Germany). 268 sera were tested for the presence of anti-TBEV IgG antibodies by commercial test *TBE Virus IgG ELISA* (Novatec, CZ) with inactivated TBE virus antigens. Anamnestic data were obtained from questionnaires submitted.

We have detected 25.3% (n = 70) seroprevalence of LB. Seropositivity was significantly higher in men, people aged over 31 years, in farmers and foresters, in persons with positive history of rheumatological symptoms. The overall seroprevalence of anti-TBEV positive antibody was 10.4% (n = 28). However, most of them reported vaccination against TBE. Total vaccination rate for all investigated was observed in 25%. In the group of non-immunized individuals (n = 174) the occurrence of positive values was 0.6% (n = 1) and the occurrence of borderline values was 5.2% (n = 9). In the group of immunized individuals (n = 58) the post-vaccinated values of positive antibody were confirmed in 44.8% (n = 26) and the borderline values of antibody in 34.5% (n = 20). In 20.7% (n = 12) study subjects with confirmed data on vaccination against TBE, we found insufficient prophylactic status. The other 36 study subjects were not available data about vaccination, of which, however, one sample was positive.

The results confirm the frequent occurrence antibodies against *B. burgdorferi* in a risk population with a higher probability of tick bite in eastern Slovakia. In the context of TBE was reported low seroprevalence in a non-immunized individuals. On the other hand, the observed absence of booster doses of vaccine against TBE can have serious consequences just in highly endemic areas.

*The study was completed within the project VVGS – 3/GSD/2012 and VEGA 1/0198/13*

**PROPHENOLOXIDASE MRNA EXPRESSION IN APIS MELLIFERA HONEY BEE IN RESPONSE TO INFECTION WITH VARROA DESTRUCTOR**

E. Zaobidna, K. Żółtowska, E. Łopięńska – Biernat

Department of Biochemistry, Faculty of Biology and Biotechnology, University of Warmia and Mazury, 1A Oczapowskiego Street, 10-719 Olsztyn, Poland

*Varroa destructor*, ectoparasite of *Apis mellifera carnica*, is considered as one of the main reasons of colony collapse disorder phenomenon (CCD). This is a parasitic mite feeding with hemolymph of capped brood and mature individuals of *A. mellifera*. That parasite is a carrier of numerous pathogens dangerous for bees. Due to the lack in 100% effective means fighting that parasitic mite, the profound recognition of the pathogenesis of disease caused by it is an important issue. In that aspect, the analysis of its influence on honeybee resistance during infestation is very important.

Phenoloxidase (PO, EC 1.14.18.1) is a enzyme which catalyses the hydroxylation of monophenol to o- diphenol and its oxidation to o-quinone, which in a nonenzymatic reaction generates melanin (Mason, 1955, 1965). In insects, PO-mediated melanin synthesis plays a major role in immune defense. Melanin synthesis at the wound site prevents hemolymph loss, besides providing cytotoxic compounds (quinone) to encapsulate and eliminate opportunist-invading microorganisms. PO was demonstrated to exist in the hemolymph of insects as an inactive prophenoloxidase (PROPO), which can be activated via a serine protease cascade. It was found that PROPO involved in adult bees in the immune response against *V. destructor* (GLIŃSKI & JAROSZ 1988). The aim of the study was the
investigate proPO expression of honey bee A. mellifera developmental stages challenged by Varroa infestation.

The research include 5 larval stages of A. mellifera include: larvae (L8), pre-pupae (PP), pupae (P3, P4) and brood out imago infestationed and free from parasite infestation. The brood were isolated from honeycombs and separated on individual developmental stages based on morphological features according to Jay (1962, 1963). Isolated individuals were washed, and dried on liquid nitrogen. Isolation RNA from tissues with kit total miniRNA (A&A Biotechnology, Poland). RT-PCR, cDNA synthesis and amplification TranScriba Kit and StartWarm 2xPCR Master Mix (A&A Biotechnology). Quantitative real-time PCR was performed using a SYBRGreen PCR-MIX Taq™ (A&AByotechnology) according to the manufacturer’s instructions. The mean value±SD was used for analysis of relative transcript levels for each time point using the ΔΔCt method. The data were analyzed and normalized relative to rp49 (stages free from mite) transcript levels by an AB analysis software (7500v2.0). All samples were tested in triplicate on lightcycler (Applied Biosystem, FAST7500).

Time course analysis transcript level of proPO was performed by real-time qPCR to determine the regulated expression of this gene activated by Varroa.

The observed differences in response to infestation varroasis in developmental stages of A. mellifera. The increase of expression gene induced by mites in pre-pupae stage (2-times), while down-regulated (1,5-times) in larvae (Z2) and pupae (P5). The expression of mRNA proPO in adult and pupae (P3) non-detected compared with healthy stages of A. mellifera

The results provide information on the molecular response in larvae, pupae and imago of A. mellifera, confirming decrease of the activity of the immune system to Varroa infestation.
Prístroje a spotrebný materiál na analýzu, prípravu a skladovanie vzoriek

Svojím zákazníkom poskytujeme kvalifikované služby: poradenstvo, predaj laboratórneho prístrojov a spotrebného materiálu, vrátane inštalácií, školení, záručného a pozáručného servisu, preventívnych kontról a validácií.

VÝHRADNÝ ZÁSTUPCA FRIEM PRE SR A ČR:
- THERMO SCIENTIFIC - divízia laboratórnych produktov (Jouan / Heto-Holten / Heraeus / H+P / Forma / Savant / Sorvall / Barnstead / TKA / Labsystems / Finzymes)
- EVERmed - ARCTIKO - LANCER - RUSKIN
- SYNGENE - SYNBIOSIS - ERLAB
- TECNIPLAST - COMET System - TUTTNAUER

TRIGON s.r.o., Štefunkova 13, 821 03 Bratislava 2 02 635 31 241 0905 707 597 mail@trigon-plus.sk www.trigon-plus.sk
Predstavujeme Vám najrýchlejší prenosný homogenizátor pre extrakciu DNA, RNA a proteínov

**NOVINKA**

**SuperFastPrep-1**

- navrhnutý pre jednotlivé 2 ml skúmavky s lyzačnými matricami
- homogenizácia akýchkoľvek tuhých alebo zmrazených vzoriek iba za 5 sekúnd
- ľahký ručný homogenizátor
- kompaktný design, ideálny pre využitie v teréne

Je možné si ho vypožičať na vyskúšanie rezervácia tu:
www.mgp.cz/sk/veda-a-vyskum/pristroje

**Cena: 3 375 EUR + DPH**

MGP spol. s r.o.
Šustekova 2
851 04 Bratislava
Slovakia

E-mail: mgp@mgpslov.sk
Tel: +421 254 654 841
www.mgpslov.sk
www.mpbio.com
## Table of Contents

1. Oral Presentations (order according to the programme)................................................................. 1
   1.1. Plenary Session I.................................................................................................................................. 1
   1.2. Plenary Session II............................................................................................................................ 1
   1.3. Session I. Human parasitology and zoonoses..................................................................................... 4
   1.4. Session II. Parasitology in genomic and biochemistry era................................................................. 11
   1.5. Plenary Session III........................................................................................................................... 14
   1.6. Session III. Veterinary and wildlife parasitology.............................................................................. 16
   1.7. Plenary Session IV............................................................................................................................ 19
   1.8. Session IV. Pharmacology and treatment.......................................................................................... 19
   1.9. Session V. Helminths - diversity, taxonomy and ultrastructure....................................................... 21
   1.10. Plenary Session V.............................................................................................................................. 28
   1.11. Session VI. Vectors and vector-borne diseases............................................................................. 29

2. Student Competition (order according to the programme)............................................................... 38
   1.1. Session Protists and bacteria – tiny creatures from different aspects of research............................ 38
   1.2. Session Tapeworms and flukes – does DNA, proteins or hosts rule their world?......................... 43
   1.3. Sessions Monogeneans and nematodes – from molecules to biodiversity
       Ecological and veterinary parasitology................................................................................................. 49
   1.5. Session Ticks, mites and mosquitoes – hidden beauty of little vampires........................................ 56

3. Poster Session (in alphabetical order)............................................................................................... 61