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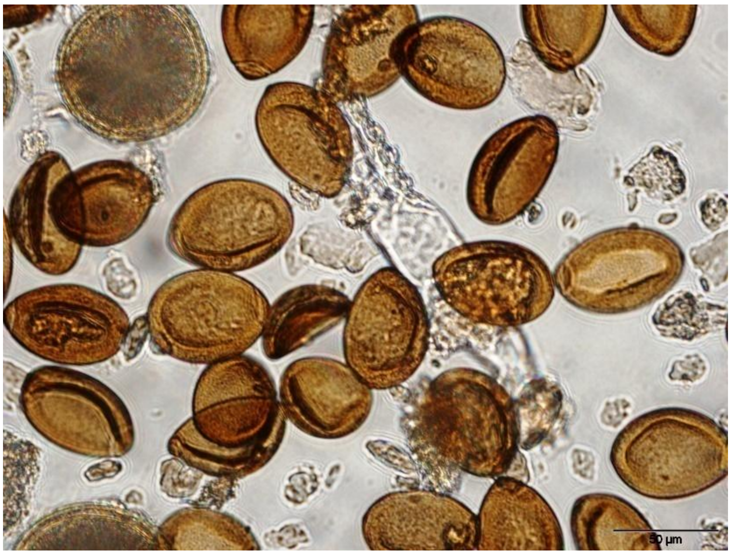

Abstract book

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PLENARY PRESENTATIONS

TOXOPLASMA GONDII IS A LATINO IMMIGRANT

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Toxoplasma gondii is an obligate intracellular protozoan parasite capable of infecting all warm-blooded animals worldwide, including humans. It is estimated that a quarter of the world's population is currently infected with this successful parasite. While infection is common in humans, clinical disease or toxoplasmosis is uncommon because the majority of *T. gondii* infections are asymptomatic. The burden of the disease is difficult to estimate but there is evidence of strong geographic variation. In France, where this parasite has been extensively studied and where prenatal screening programs have been implemented to prevent congenital toxoplasmosis, the disease is not a big problem because clinical sequelae are very unusual. Conversely, in some areas of South America, toxoplasmosis can be a big but neglected problem for the general population. We discuss the reasons for these geographical differences and bring new and important data about the origin of *T. gondii* strains now in circulation.

ECOLOGY AND EVOLUTION OF SPECIFICITY OF AVIAN MALARIA PARASITES: FROM THE TREE TO THE (RAIN) FOREST

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Most parasites are specialists, meaning that they exploit one or only a few host species, although in theory parasites would always benefit from the ability to exploit any potential host. This later strategy is in fact rare, but still it is shown by a number of parasites in the same habitat and host communities where most parasites behave as specialist. Why generalist parasites are so rare then? Research on avian haemosporidians (the parasites that cause malaria and other similar diseases of birds) has shown that the generalist strategy has evolved recurrently during the evolutionary history of these diverse parasites. But apparently it has always been an evolutionarily labile trait, with generalist parasites going extinct or reverting to the specialist state. What favours the evolution of generalist parasites? Ongoing research shows that host diversity may play a prominent role in driving the evolution of diverse exploitation strategies of parasites. In turn parasites that acquire the generalist strategy improve their dispersal capabilities favoured by their ability to colonize diverse host species, and pose a serious threat to immunologically naive hosts when moved outside their native areas. Unveiling the links between host diversity and parasite specialization improves our knowledge of disease ecology, and provides us with better tools to understand and counteract disease threats to biodiversity.

PARASITES AS BIOINDICATORS OF CHEMICALS IN THE ENVIRONMENT

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In recent years, research on environmental implications of parasites has seen a strong increase, leading to the establishment of ‘Environmental Parasitology’ as an accepted discipline. The focus of this interdisciplinary research is the question how parasitism and pollution can interact with each other. Apart from the parasitological perspective, these interactions are also important in terms of environmental research. Their sensitive reaction to pollutants and environmental disturbances makes many parasite taxa useful indicators of environmental health. Main research directions concentrate on parasites as accumulation indicators for selected pollutants and the application of parasites as effect indicators. The use of parasites as accumulation indicators specifically addresses the questions if and how parasites can be used to indicate the biological availability of certain substances, which are commonly accepted to be harmful to the environment. Based on the fact that certain groups of endoparasites are excellent accumulators of toxic metals and selected organic pollutants, one can suggest adding parasites to the list of already existing (free-living) accumulation indicators. Effect indication with parasites is a much more intricate field as it usually concentrates on complex biotic responses. If parasites are considered as effect indicators, applicable approaches mainly focus on changes in population and community structures. In the sense of parasites as integrative parts within ecosystems, environmental changes can be displayed by parasites if one of their developmental stages or one of their hosts is negatively affected. In either way such adverse effects result in numerical changes of parasites, i.e. in changes of biodiversity patterns and associated indices such as measures of diversity or the relation between monoxenic and heteroxenic species. By reviewing some of the most recent findings in the field of environmental parasitology, the talk summarizes the current state of the art and tries to identify promising ideas for future research directions.

ELIMINATION OF NEGLECTED ZOONOTIC DISEASES: IS IT A REALISTIC GOAL?

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Among the World Health Organisation’s 17 prioritized Neglected Tropical Diseases, four Neglected Zoonotic Diseases have been included in the list of diseases to be eliminated by 2030. The four disease groups are Rabies, *Taenia solium* taeniosis/cysticercosis (TSTC), echinococcosis (caused by both *Echinococcus granulosus* and *E. multilocularis*), and foodborne zoonotic trematode infections (caused by *Fasciola spp, Opisthorchis viverrini, Clonorchis sinensis,* and *Paragonimus spp*). Common for all the selected diseases is that effective drugs and/or vaccines exist. Whereas dog-associated rabies has been very well prepared including a very simple and measurable goal, strong international commitment, advocacy and strategic planning, the case is very different for the three parasitic groups.

Disease elimination requires reduction of the incidence of infection to zero within a defined geographical area as a result of deliberate efforts. For rabies the goal has been set as ‘no new dog-mediated human rabies cases for a consecutive 12-month period in country X’. In addition awareness, commitment, country strategies, road-maps and control tools are available and elimination programmes are up and running. For the parasitic zoonoses, the situation is quite different. Our current knowledge is fragmented regarding the epidemiology and burden of diseases and algorithms and step-wise approaches to control have not been thoroughly scientifically validated. So while claiming to be tool-ready to roll out elimination programmes, the lack of knowledge combined with lack of specific elimination goals, political commitment, roadmaps, and country strategies, makes it a major challenge to reach the 2030 goal.

ORAL PRESENTATIONS

IS IT POSSIBLE TO TREAT HUMAN NEMATODE INFECTIONS WITHOUT ANTIPARASITIC DRUGS?

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Benzimidazoles have proven to be insufficiently effective even in regions highly endemic in human ascariasis and trichuriasis, with the second wave of those nematodoses reaching 45-55% of the initial rate soon after de-worming. On the other hand, many nematode species, both adult and larvae forms, have been reported to be killed *in vitro* by chemical donors of nitric oxide (NO). The latter can be rapidly converted into peroxynitrite, a natural endogenous anti-parasitic compound intensely secreted by the host macrophages. Moreover, excretory/secretory soluble antigens of nematodes can stimulate the production of nitric oxide by phagocytic cells. We have assessed the efficacy of a novel treatment for ascariasis and trichuriasis *in vivo* aimed at the substrate and cofactor induction of NO synthases in the host to stimulate production of peroxynitrite, especially when benzimidazoles are found ineffective.

Significant clinical improvement and negative faecal microscopy registered on four consecutive days after finishing mebendazole therapy have been observed in 56 male and 47 female patients with ascariasis and trichuriasis. Non-responders were then given oral L-arginine combined with magnesium and zinc to stimulate production of NO/peroxynitrite in the host, after which they manifested clinical improvement accompanied by negative faecal microscopy on four consecutive days of sampling.

Conclusions: 1. Oral L-arginine together with magnesium and zinc can be used as a targeted metabolic therapy for mebendazole-resistant ascariasis and trichuriasis. 2. Sensitivity of a single microscopic stool examination is not sufficient for diagnosing ascariasis and trichuriasis in patients with chronic gastroenterological conditions. Examining the faecal samples collected during four consecutive days is much more sensitive for diagnosis and assessment of treatment efficiency. Biochemistry of beneficial effects of the aforementioned metabolic stimulation, as well as its indications, contra-indications and limitations, as well as NO synthase-independent NO production in the human body is being discussed.

DEVELOPMENT OF *HAEMOPROTEUS* SPECIES IN THE LABORATORY REARED BITING MIDGE *CULICOIDES NUBECULOSUS*

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Species of *Haemoproteus* (*Haemosporida, Haemoproteidae*) are widespread, and cause diseases both in birds and blood-sucking insects. These parasites are transmitted by *Ceratopogonidae* and *Hippoboscidae* insects, but vectors species remain unknown for many of these parasites and their genetic lineages. The aim of this study was to investigate sporogonic development of 3 widespread *Haemoproteus* species in experimentally infected biting midge *Culicoides nubeculosus* (*Ceratopogonidae*). Laboratory reared *C. nubeculosus* flies were infected experimentally by allowing them to take blood meals on passeriform birds (*Luscinia luscinia, Motacilla alba* and *Sturnus vulgaris*) naturally infected with *Haemoproteus balmorali* (cytochrome b lineage hROBIN1), *H. motacillae* (hYWT2) and *H. pastoris* (hLAMPUR01), respectively. The engorged insects were maintained in the laboratory at 22-24 ˚C, and dissected at intervals in order to follow the development of sporogonic stages. The presence of corresponding lineages of parasite in infected insects was confirmed by polymerase chain reaction (PCR). All three parasite species completed sporogony in the laboratory reared biting midge *C. nubeculosus*, and ookinetes, oocysts and sporozoites developed synchronically. Morphology of sporozoites is different in these parasite species. Sporozoites of *H. pastoris* were significantly shorter than those of *H. motacillae* and *H. balmorali*. *C. nubeculosus* is highly susceptible to *Haemoproteus* (*Parahaemoproteus*) parasites, with numerous sporozoites reported in salivary glands. This biting midge likely is the natural vector. Importantly, *C. nubeculosus* is easy to maintain in laboratory conditions, and we recommend using this insect in experimental research with avian haemoproteids.

LIFE-TIME VARIATION IN HOST FECUNDITY IN RESPONSE TO LARVAL TAPEWORM INFECTION

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Parasite infections, by definition, incur a cost to host fitness. However, the cost of infection to the host may go undetected, as it depends on the energy requirements of the parasite, and infected hosts may alter key life-history traits to compensate for losses caused by infection. In addition, the cost may be temporary and associated with periods of parasite invasion, development and growth. Parasite-associated reduction of host fecundity is a commonly exhibited effect on life history, but most studies have been based entirely on short term offspring production, without considering the life-time fecundity and quality of offspring. In this study, we measured the life-time production of larvae (quantity) and their body mass (quality) produced by infected hosts using the tapeworm *Hymenolepis diminuta* – flour beetle *Tenebrio molitor* model. Our results showed that *H. diminuta* infected *T. molitor* produce fewer offspring. However, this decrease in larval production was evident only within the first three weeks after infection, corresponding to the development of larval tapeworms into mature cysticercoids. After cysticercoid maturation, there is no difference in offspring production between infected and uninfected hosts. We did not see any evidence of infected hosts compensating for reduced offspring number by greater investment in offspring quality (i.e. increased body mass). Thus, our results indicate that the host is unable to compensate for the energy demands required by parasite development.

TOXOPLASMOSIS – AWARENESS AND KNOWLEDGE AMONG MEDICAL PRACTITIONERS IN NIGERIA

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Toxoplasmosis caused by *Toxoplasma gondii* remains associated with poverty and high disease burden. We surveyed the knowledge on toxoplasmosis among medical doctors in Nigeria. We designed a questionnaire that the participants answered without consulting literature or colleagues. Altogether 522 medical doctors, from four tertiary hospitals, completed the questionnaire. The mean number of correct answers in the knowledge questions that covered epidemiological and clinical aspects of *T. gondii* infections was 7.5, while 8.4% of the participants selected at least 12 of the 17 correct answers. The proportion of participants scoring the high score was significantly higher among participants who reported having seen a case of clinical toxoplasmosis than in those who had not. While 62% of participants knew that cats can shed *T. gondii* in their feces, 36% incorrectly suggested that humans could do that too. That *T. gondii* infection can be meat borne was known by 69%, but that it can be also waterborne only by 28% of the participants. Most of the participants, 78%, knew that clinical toxoplasmosis may involve the central nervous system, but only 37% answered it can involve the eyes. Our study revealed some knowledge gaps that could be addressed by Continuous Medical Education (CME) of medical practitioners. As there was an association between having seen a case of clinical toxoplasmosis and scoring a high knowledge score, case-based CME could be emphasized.

THE STATUS OF WHIPWORMS OF THE GENUS *TRICHURIS* IN ROE DEER (*CAPREOLUS CAPREOLUS*) IN FRANCE

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According to the literature, the genus *Trichuris* Roederer 1761 includes five species parasitizing Roe deer (*Capreolus capreolus*). Three of them have also been identified in domestic ruminants hitherto. Since 1986, we have investigated the helminthofauna of cervids in France with a special emphasis on Roe deer. Identification has been performed by morphological examination after mounting in lactophenol d'Amann and by molecular analysis targeting both ribosomal (D2, ITS1 and ITS2) and mitochondrial (CoI) domains. In wild French roe deer populations sampled without sympatry with domestic ruminants, we have found only one *Trichuris* species, *T. capreoli* (Artjuch, 1948) represented by females worms only. However, we have observed few *Trichuris* males belonging to various species but only when sympatry between Roe deer and domestic ruminants (especially sheep) has occurred. Based on these previous observations, the relationship between the number of females and faecal egg counts was investigated. Data were collected on 244 hunted roe deer of both sexes and all age classes coming from two sampling areas. Total worm burden was assessed by complete examination of the digestive tract. Faecal egg counts (included gastro-intestinal strongyles, GIS) were determined by the modified Mc Master procedure. Various species of GIS worms were identified. GIS eggs were also found but their specific morphological identification remains confusing. *Trichuris* species diversity was dramatically lowest with preeminence of *T. capreoli*. Then, observed *Trichuris* eggs were mainly laid by this alone species. This noteworthy *Trichuris* feature in French Roe deer populations suggests that *Trichuris* egg prevalence or abundance could be a potential indicator of ecological change in roe deer populations.

THE RISK OF HUMAN TRICHINELLOSIS FROM CONTROLLED AND NON-CONTROLLED HOUSING SYSTEMS

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We developed a Quantitative Microbiological Risk Assessment (QMRA) model for *Trichinella* throughout the food chain that allows quantification of trichinellosis incidence under various scenarios. At the meat inspection, the EU reference method (EU-RM) for the detection of *Trichinella*, utilizing pepsin digestion, is regarded the golden standard having a detection sensitivity of 1 – 3 Trichinella muscle larvae (ML) per gram of meat. However, at the same time, the EU-RM is prone to larval loss. Nevertheless, well-trained laboratories are able to correctly identify *Trichinella* spiked and negative control samples, even at very low spike levels. In view of future pepsin shortage, alternative tests have been developed to allow continuation of individual carcass control. The aim of our model study was 1) to compare the risk of human trichinellosis from pigs kept under different housing systems, 2) to explore the effect of test sensitivity and 3) to explore the trichinellosis risk attribution of raw meat products. We tested a hypothetical scenario in which all 120 million pigs from controlled housing that are tested annually in the EU, would origin from non-controlled housing. Using this scenario, we estimate 470 (95% CI 79 – 1060) annual cases of human trichinellosis for the whole EU. In comparison, we estimate at most 0.09 ± 0.04 trichinellosis cases/year for the whole EU from controlled housing, which quantitatively illustrates the efficacy of this housing system. A test sensitivity of 85% relative to EU-RM in these scenarios resulted in twelve to fifteen times more trichinellosis cases/year respectively in the EU. The *Trichinella* QMRA was used to clearly demonstrate the quantitative effect of different housing systems and test sensitivities and may be of assistance in evaluating alternative methods, kits or chemicals for the detection of *Trichinella* ML in meat.

TICK INFESTATION, HAEMATOLOGICAL AND CLINICAL PARAMETERS IN ROE DEER FAWNS

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In the summer of 2016 a total of 52 neonate roe deer fawns captured in two different areas in Sweden, Grimsö (n = 24) in South central Sweden and Bogesund (n = 28) in the inner archipelago just north of Stockholm have been examined at least once for presence of attached ticks. Clinical parameters (n = 32) have been recorded and a blood sample (n = 49) has been taken and analysed for haematology. The residual number of ticks after correcting for fawn age has been calculated and finally fawn mortality has been calculated, until the end of February, 2017. Death has been recorded for 57.7% of fawns, 41.7% from Grimsö and 71.4% from Bogesund. The main death cause has been predation in both sites. Statistical analysis of recorded parameters has been performed using capture site (Grimsö and Bogesund) and status (dead or alive February 2017) as independent variables. Preliminary results do not indicate different tick infestation levels between survivors or dead animals, while it is confirmed that roe deer fawns from Bogesund had higher tick burdens. Haematology seems to reveal that fawns from Bogesund were slightly more anaemic than those from Grimsö. This is indirectly confirmed by clinical findings (i.e. increased respiratory and heart rate), perhaps related to infections by various pathogens. At current, we have analysed samples from neonates from 2013 and 2014 for tick-borne pathogens (i.e. *Babesia, Borrelia, Anaplasma)* and possible clinical findings will be discussed in the light of these results. These findings can provide baseline parameters useful for further investigations in roe deer, both as hosts involved in tick life cycle as well as in other research fields. The marking and handling of roe deer in this study were approved by the Ethical Committee on Animal Experiments, Uppsala, Sweden (Approval Dnr: C149/2015).

BEHAVIORAL AND TOXICOLOGICAL EFFECT OF MOLLUSCICIDES ON MIRACIDIA OF *SCHISTOSOMA MANSONI* TESTED BY A VIDEO RECORDING PROCEDURE DEVELOPED ON THE MODEL ORGANISM *ARTEMIA SALINA*

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The model organism the brine shrimp *Artemia salina* is comparable to the larvae of schistosoma based on size, longevity and velocity and found suitable for testing the toxidity of the selected substances based on the assay using video recordings. The test conditions are constant temperature and uniform illumination. Four groups of substances are tested in each 4 different concentrations. The substances are potassium dichromate, which is standard in general toxicological tests. Copper sulfate and NPK fertilizer, which were the first used molluscicides used in the attempts to eradicate intermediate snail host and liquid soap used everywhere in localities of the intermediate snail. The viscosity of all concentrations of the substances is analyzed to determine the extent to which the viscosity has an influence on the velocity of the miracidia. The miracidia shows a high degree of resistance to the potential environmental toxic substances used in this study. One of the substance shows extreme high acute toxicity on the miracids with an LC 100 in about 30 seconds. This substance or group of substances, soap containing 10 % detergent, is suggested as a potential mean in breaking the infection cyclus of Schistosomiasis by killing and controlling the miracidia.

DIENTAMOEBA FRAGILIS IN CHILDREN ATTENDING DAY CARE CENTERS IN COPENHAGEN, DENMARK: PREVALENCE, RISK FACTORS, AND OBSERVATIONS OVER TIME

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Dientamoeba fragilis is an intestinal protozoan of debated clinical significance. Here, we present cross-sectional and longitudinal observations on *D. fragilis* in children attending day care facilities in Copenhagen, Denmark. We used questionnaire data of the first observational point and real-time PCR results for *D. fragilis* from all the stool samples collected during a dynamic 1-year multi-day-care-center cohort study. A total of 142 children aged 0.9–6.6 (median 2.8) years were included in this study. *D. fragilis* was detected in the first stool sample of 97 (68.3%) of the 142 children. Older age and a history of traveling abroad were risk factors for testing positive. Moreover, univariable analyses indicated having siblings as a risk factor. There was no statistical association between a recent history of gastrointestinal symptoms and testing positive for *D. fragilis*. The longitudinal analysis included data on the 108 children who were represented by at least two stool samples. Of them, 32 tested first negative and later positive, and the last sample from all of the 108 children was positive. The results of this study support *D. fragilis* as a common commensal in this population. (This work was also presented at ECCMID 2017, Vienna, Austria, 22.04.-25.04.2017; the abstract text is the text of the e-poster. Publication: Pikka Jokelainen, Betina Hebbelstrup Jensen, Bente Utoft Andreassen, Andreas Munk Petersen, Dennis Röser, Karen A. Krogfelt, Henrik V. Nielsen, Christen R. Stensvold. 2017. *Dientamoeba fragilis*, a Commensal in Children in Danish Day Care Centers. J. Clin. Microbiol. doi:10.1128/JCM.00037-17.)

GENETIC DIVERSITY OF *ECHINOCOCCUS MULTILOCULARIS* IN RED FOXES IN POLAND - FIRST REPORT OF A HAPLOTYPE OF PROBABLE ASIAN ORIGIN

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In the last several years, increasing research efforts have focused on the genetic diversity of *E. multilocularis*. On the one hand, the genetic diversity in Europe is explained by the mainland-island migration from historical centre to peripheral areas. Other studies suggest spread of Asian genotypes towards Europe. Poland with its geographical location (central-east Europe), is an interesting area for such investigations. The aim of the present study was to estimate the genetic diversity of the cestode *E. multilocularis* in Poland based on sequence analysis of the mitochondrial genes of worms isolated from red foxes. Overall, 83 adults of *E. multilocularis* from the same number of foxes in different parts of Poland were used for analysis. Sequences of the three mitochondrial genes, cytochrome b (cob), NADH dehydrogenase subunit 2 (nad2) and cytochrome c oxidase subunit 1 (cox1), were analysed. Seventy-four individual biological samples were successfully sequenced. Combined sequence analysis of these three genes exhibited fifteen Polish haplotypes (EmPL1–EmPL15). Most isolates were classified to the EmPL1 haplotype, which occurred mainly in the east, north and centre of Poland. Haplotype EmPL4 and other haplotypes appeared predominantly in the south and west area. Fourteen haplotypes were grouped in the European clade. One Polish haplotype (EmPL9) was assigned to the Asian clade with haplotypes from Japan and Kazakhstan. This haplotype was found only in northeast Poland and this is the westernmost location of *E. multilocularis* haplotype belonging to the Asian clade in Europe. It could be suggested that in Poland there is a western border of Asian-derived haplotype distribution. The investigation demonstrated that populations of *E. multilocularis* in Poland (and probably also in Eastern Europe) included not only different European haplotypes but also those of the Asian origin. Full description of investigation was published in Folia Parasitologica, 2017,64:007.

CURRENT STATUS OF DISTRIBUTION AND FUTURE RESEARCH PERSPECTIVES OF *DIPHYLLOBOTHRIUM LATUM* AND *DIPHYLLOBOTHRIUM DENDRITICUM* IN EUROPE

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Tapeworms of the genus *Diphyllobothrium* cause an important fish-borne zoonosis responsible for about 20 million human infections worldwide. The life cycle of *Diphyllobothrium* requires 3 hosts for completion: 1) copepods (first intermediate hosts), 2) freshwater, anadromous or marine fish (second intermediate hosts), and 3) fish-eating mammals, including humans, or birds (definitive hosts). Out of about 50 currently recognized species of *Diphyllobothrium,* at least 14 of them have been reported from humans. In Europe, *Diphyllobothrium latum* and *Diphyllobothrium dendriticum* are the most frequent species of the genus. While infections in humans are very often not native and are linked with global tourism and local eating habits, detection of plerocercoids in fish reflects the occurrence of the parasite in a natural environment. Based on the ichtyoparasitological examinations, *D. latum* has been reported mainly in Alpine lakes region in northern Italy (Iseo, Como and Maggiore lakes), Switzerland (Geneva, Biel and Morat lakes), but even in Lake Peipsi in Estonia and in several localities in Russia and France. On the other hand, *D. dendriticum* occurs frequently in circumpolar areas, especially in Scandinavian countries such as Norway and Finland, but also in Loch Doyne and Loch Lomond in Scotland and in northern Russia. The aim of our research is to complete the data on *Diphyllobothrium* occurrence in middle and south-eastern Europe, in particular in the Danube River basin, where the ichtyoparasitological research has not been aimed at diphyllobothriosis for long time (e.g. the sporadic occurrence of *D. latum* in Danube River in Slovakia was detected more than 50 years ago). We are also interested in results of ichtyoparasitological examinations in regions, where *Diphyllobothrium* identifications were based only on detections of eggs during coprological examinations (Białowieża, Poland). This work was supported by the projects APVV-15-004 and VEGA 2/0134/17.

THE JOURNEY BETWEEN TWO CONTINENTS: A CASE OF INVASIVE TREMATODE *FASCIOLOIDES MAGNA*

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Fascioloides magna (Trematoda: *Fasciolidae)* is a liver parasite of a wide range of free-living and domestic ruminants, known due to its large spatial distribution, invasive character, and potential to colonize new territories. The aim of our study was to assess the population genetic structure in *F. magna* across five enzootic regions in North America and three natural foci in Europe. Short variable regions of two mitochondrial genes (cox1, nad1) and 11 microsatellite loci were applied as a methodological tool. The analysis of dispersal routes of the parasite in North America revealed west-east and south-north lineages that partially overlapped in the central part of the continent, where different host populations historically met. The exact origin of European populations of *F. magna* and their potential translocation routes were determined. Flukes from the first European focus, Italy, were related to *F. magna* from northern Pacific coast, while parasites from the Czech focus originated from south-eastern USA, particularly South Carolina. The Danube floodplain forests (third focus) did not display relationship with any North American population; instead the Czech origin of the Danube population was indicated. The results of microsatellite analyses were compared to outputs from mitochondrial haplotype data and correlated with natural migration of final cervid hosts of *F. magna*. Moreover, the newly emerging European populations of giant liver fluke in Poland and Germany were genotyped using cox1 and nad1 mitochondrial markers. The study revealed genetic uniformity of specimens from Germany and Poland with individuals from the Czech focus, what indicated that fascioloidosis has been spreading beyond rather strict and well established Czech focus into neighbouring countries. This work was supported by the Slovak Grand Agency VEGA (project VEGA no. 2/0134/17) and Bilateral Mobility Project SAS-ASCR 16-20.

RISK FACTORS OF ECHINOCOCCOSIS IN LATVIA

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Echinococcosis is a chronic zoonotic disease causing serious global socio-economic losses in human and animal hosts. Scientific literature on echinococcosis reporting data on risk factors is limited and study like that was never conducted in Latvia.

In this study, structured questionnaire was made containing inquiries about different risk factors based on available literature data. This was hospital-based study where we questioned echinococcosis patients group and control group that were not affected by echinococcosis and had similar demographic characteristics as those of the echinococcosis patients. For immunological risk factors, we fully genotyped for HLA- DRB1; DQB1; DQA1- using RT-PCR with sequence-specific primers. Associations of DRB1; DQB1; DQA1 alleles in patients were examined individually using the χ^2 test.

In total, we investigated 47 echinococcosis patients and control group was 50 healthy individuals. Exposure associated with increased odds of infection was “living in rural areas”, “being female”, “dog ownership” and “number of dogs owned”. Also, possible significance had factors involving ownership of kitchen gardens, for example, “eating raw/unwashed vegetables”. Checking for immunogenetic markers for susceptibility we found following haplotypes HLA-DRB1*17:01/DQB1*03:01/DQA1*01:02, gene frequency (gf) 0.10 , OR=11,42 in comparison with control group gf 0,01; HLA-DRB1*11:01/ DQB1*03:01/DQA1*01:03, gf 0.17, OR=20,63,gf 0,01 in control group and HLA- DRB1*11:01/DQB1*03:01/DQA1*03:01, gf 0.31 , OR=22.05,but gf 0,02 in control group.

Living in endemic rural areas, in which free roaming dogs have access to offal and food sources and being a dog owner and growing vegetables in small gardens seem to be significant risk factors for acquiring this parasitic infection. As well it was possible to distinguish several genetic haplotypes in patients possibly contributing to acquiring infection. Results of data analyzed here may potentially be useful in planning community interventions aimed at controlling echinococcosis.

THE ROLE OF PARASITES FOR THE HEALTH OF WILLOW PTARMIGAN (*LAGOPUS LAGOPUS*) IN NORWAY

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As willow ptarmigan (*Lagopus lagopus*) are an important game species in Norway, the identification of potential health threats is important. Although there has been some investigation of the role of parasites for Norwegian ptarmigan populations, these studies are few and are concentrated in only small parts of the country. Still, the results of these and studies in other countries have indicated that the prevalence of endoparasites (e.g. *Trichostrongylus tenuis, Hymenolepis microps, Ascaridia* spp., and *Syngamus trachea*) can affect factors such as individual body mass and breeding success of ptarmigan. In addition, Louping ill virus (LIV), a flavivirus transmitted by ticks (*Ixodes ricinius*), is known to be lethal in red grouse (*Lagopus lagopus scoticus*) in Scotland. Although this virus is present in Norway, it has not been investigated in ptarmigan. The purpose of this presentation is to review the results of these and other major studies performed in Fennoscandia, the UK, and Iceland investigating the effect of parasites on the health of ptarmigan and other related grouse species (e.g. rock ptarmigan, *Lagopus muta*; black grouse, *Lyrurus tetrix*; capercallie, *Tetrao urogallus*; hazel grouse, *Tetrastes bonasia*). This will provide background for a project planned to map the presence/absence of endoparasites and LIV antibodies found in over 150 willow ptarmigan collected from approximately 40 localities distributed from southern to northern Norway, 2014-2017. Investigations for this project are underway; however, results are pending.

IS THE FINNISH NATIONAL PARASITE STATUS OF *DIPHYLLOBOTHRIUM LATUM* OUTDATED?

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The broad or fish tapeworm *Diphyllobothrium latum* was declared the National Parasite of Finland during the 1950s. Then, at locations in eastern part of the country, the majority of people were infected by “the Worm”. Infection was so natural that some people considered the absence of the parasite to be a sign of bad health. Even though sporadic reports exist on the presence of the parasite in Finland already in the 19th century, no actual prevalence figures are available from the time. Therefore, the hypothesis has emerged that the tapeworm life cycle was so seldom fulfilled that the parasite could not possibly reach the high prevalence and status it got later. In 1917, Finland declared independence from Russia and became a republican governance. Both economic and political reasons led the society to urbanize and “sub-urbanize” creating the need and wish to build water pipes and sewers. While in the rural community, human faeces was a valuable resource as a fertilizer in agriculture, in the borough it became a disgusting waste. Water closets were introduced before proper wastewater treatment. First, the wealthy people acquired this new invention and thus supplied the water system with of *D. latum* eggs. It was then a common habit to eat raw, salted pike and also roe of pike, perch and burbot. According to the hypothesis, poor people got their share of infection pressure from the well-off “upper crust” via these predatory fish species. In 1953, partly because of the cost of infection in the form of wasting expensive food, the Finnish Red Cross initiated an eradication campaign. The highly successful campaign warned against eating raw fish. Now, *D. latum* infection in humans is rare. However, even if humans are the best hosts, the parasite can also live in other fish-eating mammals, and the National Parasite is not eradicated from Finland. Because of current wastewater treatment methods, it can probably never fully regain its former glory, even if the trend of enjoying raw fish would increase further.

Perhaps due to climate change, zoonotic opistorchid liver flukes *Metorchis bilis* and *Pseudamphistomum truncatum* have recently been found established in the southeasternmost part of Finland, where they circulate between cyprinid fish species (roach) and piscivorous seals, foxes and white-tailed eagles. The roach is a very common, although not a highly valued fish in Finland. Because it is a neglected food source, and because fishing is regarded as a good means of removing nutrients from eutrophicated water systems, authorities wish to increase the consumption of both roach and related fish species. Salting and drying of roach to be used as snacks is perhaps gaining popularity, but the effect of the treatment on the viability of trematode metacercariae is not properly known and should be studied.

FROM NATURE INTO THE LAB: COMPARISON OF EXPERIMENTAL STUDIES ON AVIAN MALARIA PARASITES

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Recent experimental studies show that birds get sick from avian haemosporidian parasites. Until now there are described more than 50 species causing avian malaria, however, the information about the virulence of different species of *Plasmodium* spp. is fragmented and scarce. The lack of information about virulence of many avian *Plasmodium* spp. is because these parasites as well as other haemosporidians are transmitted by blood sucking dipterans and studies combining vertebrate host, vector and parasite are complex and difficult to conduct. Such experiments are impossible if vectors of certain species are unknown, what is rather common issue. To gain information about the virulence of malaria from mistnetting studies is incorrect as birds especially at primary stage of infections decrease activity and thus rarely are presented in such datasets. One way of testing parasite virulence is by conductiong of experimental infections using subinoculation of blood stages of avian malarial parasites into vertebrate host in controlled lab conditions. Using this method, mosquito bite stage is omitted and instead of sporozoites (sporogonic transmission stage) erythrocytic meronts (blood stage) are inoculated. These differences in a way how animal was infected may lead to significant delay in a peak of parasitemia. Moreover, this delay may give a vertebrate host some leg-up to be better immune prepared what may lead to lower number of parasitized erythrocytes comparing to direct invasion of meronts into blood circulation. Here we presented our results to evaluate the development of infection caused by *Plasmodium relictum* (genetic lineage pSGS1) and its virulence to vertebrate host (Siskins *Carduelis spinus*) after conducting experimental infections using three different methods in parallel: a) by inoculation of infected blood; b) by inoculation of sporozoites; c) by mosquito (*Culex pipiens pipiens* form molestus) bite.

CRYPTIC SPECIATION: FIRST CASE IN AVIAN PLASMODIUM PARASITES

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Avian malaria and related haemosporidian parasites are important agents of diseases in domestic and wild birds. Over 50 avian *Plasmodium* species have been described based on similarity (morphology) species concept, with insufficient information about sporogonic development in vector. Studies based on the polymerase chain reaction (PCR) show huge diversity of haemosporidians indicating possible existence of diverse group of cryptic species. *Plasmodium* sp. was isolated from Red-backed shrike *Lanius collurio* for morphological and molecular characterization. Domestic canaries *Serinus canaria domestica* were used for experimental infection with the aims to obtain information about 1) patterns of development of this parasite in vertebrate host, 2) to determine specificity and virulence in experimental birds and 3) to determine sporogonic development in three common European mosquito species. New cryptic species of *Plasmodium* (lineage pCOLL4) was detected. Its blood stages are indistinguishable from those of *Plasmodium circumflexum*. Parasitemia developed in all infected canaries. High parasitemia (up to 75%) and phanerozoites causes death of approximately 36% of infected birds. The parasite develops gametes, ookinetes and oocysts in *Culex pipiens pipiens*, *Culex pipiens pipiens* form molestus and *Aedes vexans*, however, sporogony is abortive on the oocyst stage. Using both similarity and genetic species concepts we report the first case of cryptic speciation in avian *Plasmodium* parasites. This infection is pathogenic to avian hosts and causes death of birds due to anaemia and cerebral paralysis. This information is of epidemiological importance and worth more attention in veterinary medicine and wildlife conservation. This study was partly funded by the Research Council of Lithuania (MIP-038/2015).

THE PREVALENCE OF SEROLOGIC MARKERS OF TICK-BORNE DISEASES IN PATIENTS INFECTED WITH HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1)

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Tick-borne diseases (TBD) are a great concern for public health. When untreated, they may lead to severe complications or even death. An increased number and activity of ticks observed in natural and urban areas increases the risk of tick-borne infections, including HIV-infected subjects. For routine detection of tick-borne diseases mainly serologic methods are used. In group of HIV-infected patients one can consider negative results associated with immune system disturbances or false positive results due to coinfection with another pathogens or unspecific activation of the immune system. The aim of the study is to analyse the incidence of tick borne-diseases among HIV-1-infected patients. Study was performed using blood serum samples from 150 HIV-1-infected patients and 200 non infected patients (controls). There were no subjects or controls with clinical symptoms indicating the need for routine diagnostic of tick-borne pathogens. The prevalence of specific IgM and IgG antibodies in HIV - infected patients was as follows: *B. burgdorferi* (IgM - 29%; IgG-3.3%), *Babesia microti* (IgM-9%; IgG-0%), *Anaplasma phagocytophilum* (IgM -3.3%; IgG- 1.3%), and *Ehrlichia* sp.(IgM- 2.6%; IgG- 2%) and *Bartonella* sp. (IgM -6.6%; IgG-1,3%). The prevalence of antibodies in control group was as follows: *B. burgdorferi* (IgM-20%; IgG-5%), *B. microti* (IgM-2%, IgG-1%) *A. phagocytophilum* (IgM -2%; IgG-1%), and *Ehrlichia* sp.(IgM- 2% ; IgG- 2%). and *Bartonella* sp (IgM -3% IgG-4%). There were no statistically significant differences between both groups. False positive or indefinable results were observed in 30% HIV- infected patients with IgM antibodies against *B. burgdorferi*, out of whom 35% showed coinfection with *T. pallidum*. The coinfection may explain observed nonspecific results. The results obtained in the study suggest high prevalence of serological markers of tick- borne pathogens; unspecific reactivity against *B. burgdorferi* s.l. and the dominant role of clinical symptoms rather than serological tests results. The study was funded by the Ministry of Science and Higher Education (MNISW) Iuventus Plus grant nr IP2014050373.

SADURIA ENTOMON (LINNAEUS, 1758) AS A TRANSMITTER OF *HYSTEROTHYLACIUM ADUNCUM* TO COD (*GADUS MORHUA*) FROM BALTIC SEA

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Invertebrates are important components of the diet of many fish species. They may also be a source of fish infection with parasites as invertebrates are intermediate hosts in the life cycles of nematodes or acanthocephalans. Cod changes the diet preferences during the life time. Diet of young and older fish is dependent on their ability to prey. Young fish occur mostly near the coast and feed on invertebrates, particularly *Crustacea*, like *Saduria entomon*. Older and larger cod prefer deeper offshore waters and feed mainly on fish. Parasite fauna of cod from the Baltic Sea is well known and have been studied by many authors; however, the life cycles of Baltic cod parasites are described only in general. The aim of the study was to examine *S. entomon* (Linnaeus, 1758) obtained from the stomach of Baltic cod, for the presence of the parasites. The research material consists of the stomachs of Baltic cod, collected during two commercial cruises in the Polish Exclusive Economic Zone in February and May 2015. The total number of 98 cods' stomachs have been sampled and analyzed. All invertebrates found in stomachs have been collected. Parasites have been found only in *S. entomon*. This is the first evidence in situ of the presence of *S. entomon* (6 individuals) infected with larvae (L3) *H. aduncum* found in the stomachs of Baltic Sea cod. It may indicate that *S. entomon* play a role as intermediate hosts in the life cycles of *H. aduncum* in the southern Baltic Sea.

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CHANGES IN WHITE BLOOD CELLS IN EXPERIMENTALLY INFECTED EUROPEAN SISKINS (*CARDUELIS SPINUS*) BY MALARIA PARASITE PLASMODIUM RELICTUM (LINEAGE SGS1)

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Like in mammals, bird's white blood cells belong to the innate immunity. These cells are the body's first line of defence against different infectious agents. Peripheral blood leukocytes can change rapidly in numbers and their relative proportion may be affected by parasite infection. Leukocyte profile is often used in parasitological studies to describe an immune status of birds. However, little is known about immune response to avian malaria parasites. In our project we studied changes in absolute value of leukocytes and their percentage ratio (limphocytes, heterophils, eosinophils, basophils, monocytes) in European siskins (*Carduelis spinus*) in response to infection by *Plasmodium relictum* (lineage SGS1) at the stage of high primary parasitemia. 22 siskins were treated with subinoculation of infected blood mixture infected of donor blood and 22 birds were used as control. Blood samples for leukocyte counting were taken every fourth day. Besides, we measured hematocrit value and body mass of all experimental birds. In our study, we did not find significant changes in the numbers of basophils and eosinophils. However, we observed a gradual increase of monocytes within group of infected siskins, reaching their maximum with decreasing of erythrocytic stage of parasitemia. The interpretation of leukocyte formula changes during primary infection is not easy job as many different factors can cause those changes and circulating white blood cells show significant individual fluctuations.

DIROFILARIA SPP. IN MOSQUITO VECTORS AND HOSTS IN EASTERN AND NORTH-EASTERN EUROPE

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The filarioid nematodes *Dirofilaria repens* and *D. immitis* are transmitted by mosquitoes, primarily causing subcutaneous or cardiopulmonary dirofilariasis in dogs, respectively. A spread of canine and human *Dirofilaria* infections was observed in eastern, north-eastern and central Europe. Autochthonous human cases were also reported from Moldova, Romania, Belarus and Latvia. Therefore 4,481 specimens of 22 mosquito taxa were sampled in Moldova between 2010 and 2015. In Belarus 467 mosquitoes of five taxa were collected in 2015. Overall, 1,083 mosquitoes of six taxa (*An. maculipennis* s.l., *An. hyrcanus*, *Ae. vexans*, *Cx. pipiens* s.l., *Cq. richiardii* and *Ur. unguiculata*) were collected in Danube Delta, Romania in 2014 and 2015. Mosquitoes were analyzed using real-time PCR targeting COI (*D. repens*) and 16S rRNA (*D. immitis*) gene fragments. In total 1,395 blood samples collected from dogs in Latvia between 2013 and 2016 were analyzed for the presence of microfilaria using blood smear microscopy and KNOTT test. Canine antigen Heartworm test (Idexx Labs) was used to detect *D. immitis* antigen. PCR targeting COI gene fragment was used to detect *D. repens* DNA in canine blood. Positive mosquito pools for *D. repens* were found in Moldova at 13 trapping sites and in 17 of the 22 screened mosquito taxa, while *D. immitis* was detected in four taxa. In Romania positive mosquito pools for *D. repens* were composed of *An. maculipennis* s.l. and *Ae. vexans*, while *An. hyrcanus* and *Cq. richiardii* were positive for *D. immitis*. In Belarus, one pool of *An. claviger* s.l. was positive for *D. repens* and another pool of *Cx. pipiens* s.l./*Cx. torrentium* for *D. immitis*. The mean prevalence of *D. repens* in dogs from Latvia between 2013 and 2016 was 23.5%, whereas between 2008 and 2012 was 3.8% with regularly reported canine autochthonous cases since 2010. *D. immitis* antigen was not detected in canine blood samples from Latvia.

PATHOLOGICAL CHANGES IN THE COURSE OF VERTICAL TRANSMISSION OF *BABESIA MICROTI*

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Babesia microti is an obligate, intraerythrocytic parasite of humans and rodents causing a malaria-like infection – babesiosis. There are few described cases of vertical transmission of *B. microti* in humans and animals but little is known about impact of *B. microti* infection on the pregnancy. The aim of our study was to trace pathological changes in pregnant females infected with *B. microti* and in their pups with congenital infection. We determined the occurrence and the success of vertical transmission in females with acute and chronic infections of *B. microti*. In experimental groups, females were mated on the 1st day of *Babesia* infection (Group G0); in the post- acute phase of the parasite infection (G28); and in the chronic phase of the parasite infection (G90 and G150 group). One group of females was infected on the 12th day of pregnancy (G12). Pups were obtained from 5% of females infected in mid-pregnancy (G12), 58% of females mated in the post-acute phase (G28), from 33% of females in groups G90 and G150. Congenital *B. microti* infections were detected by PCR amplification of Babesia 18S rDNA in almost all pups (96%) from the experimental groups G28, G90 and G150 and from 100% pups from group G12. Pathomorphological and physiological changes were recorded in different organs collected from female mice infected with *B. microti* during the pregnancy. In females with acute babesiosis we observed pathological changes in embryos development, resulting in malformation of limbs, growth inhibition and death. Acute phase of babesiosis causes pathological changes in the mother tissues and inhibits development of the offspring, which may result in pregnancy termination, inhibition of growth or the death of the offspring. Chronic babesiosis in mothers results in congenital babesiosis in their offspring.
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POSTER PRESENTATIONS

POPULATION STUDY OF *CARYOPHYLLAEUS LATICEPS* (CESTODA: *CARYOPHYLLIDEA),* AN INTESTINAL MONOZOIC TAPEWORM OF FISH, USING MICROSATELLITES MARKERS

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Caryophyllidean tapeworms (Platyhelminthes: *Eucestoda)* represent the unique group of tapeworms having monozoic body plan with only a single set of reproductive organs. They are widely distributed intestinal parasites of cypriniform and siluriform fish occurring in all zoogeographical regions except the Neotropics. Caryophyllideans apparently played an important role in evolutionary processes of Cestoda since they represent a basal group of tapeworms. *Caryophyllaeus laticeps* is a common caryophyllidean tapeworm parasitizing a wide spectrum of cyprinid fish in the Palearctic region. The morphological studies of different host and geographical populations of *C. laticeps* revealed very high morphological plasticity, indicating that *C. laticeps* may consist of several species complexes. Molecular study based on the partial mitochondrial cytochrome c oxidase subunit I and partial large subunit of the ribosomal DNA revealed several phylogenetic sub-lineages of *C. laticeps*, which corresponded with different morphotype groups. Finally, recent morphological revision of genus *Caryophyllaeus* resulted in redescription of *Caryophyllaeus* sp. from common nase (*Chondrostoma nasus*) from Austria as the new species *Caryophyllaeus chondrostomi* sp. n. Barčák, Oros, Hanzelová & Scholz, 2017. In order to assess genetic interrelationships among *C. laticeps* population from different European localities and fish hosts, six microsatellite loci were designed by microsatellite library screening using NGS approach. Application of microsatellite markers revealed that *C. chondrostomi* displayed distinct and specific allelic pattern, different from all other *C. laticeps* populations. Thus, the results on microsatellite analyses supported the description of *C. chondrostomi* as a new species. This work was supported by the Slovak Grand Agency VEGA (project VEGA no. 2/0134/17) and APVV-0653-11.

TECHNICAL DESIGN OF ASSAYS FOR THE INVESTIGATION OF CHEMOTAXIS IN TREMATODE MIRACIDIA

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Development of the device for analysis of the chemotaxic effects on motile microorganisms are reviewed. The technical design is shown for the devices used at the institute and the current setup is presented for video recording of the effect of chemoattracants and chemorepellants on the nauplius larvae of *Artemia salina*, as the model for miracids of *Schistosoma mansoni*.

COPRO-ELISA FOR DIAGNOSIS OF CANINE ECHINOCOCCOSIS

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Echinococcosis became highly endemic zoonotic disease in the former Soviet Central Asian Republics, including Kazakhstan, due to increasing practice of home slaughtering and non-compliance with the mandatory periodical deworming of dogs. In this context, timely identification of dogs infected with the causative agent of the disease – *Echinococcus granulosus*, as well as environmental monitoring for the presence of the parasite acquire particular relevance. Known methods of *in vivo* diagnosis of canine echinococcosis are ineffective. The results of studies on the development of the ELISA test to identify dogs infested with echinococcosis, based on the detection of tapeworm antigen in fecal samples are presented in this paper. Rabbit polyclonal and mouse monoclonal antibodies specific to excretory-secretory antigen of larval and adult forms of *E. granulosus* are the main reagents of test system. Testing the diagnosticum on fecal samples of experimentally infected dogs showed that it allows differencing coproantigens of two closely related cestodes: *E. granulosus* and *Taenia hydatigena*. Coproantigen of *E. granulosus* was detected in the dogs' feces on the 5th day after infection and it was detected before the 30th day of the experiment (observation time). The research results indicate the possibility of using ELISA-test for the lifetime diagnostics of canine echinococcosis. The authors conclude that for the implementation of ELISA test in the diagnosis of echinococcosis it is necessary to set the duration of the elimination coproantigen after deworming, which will determine the terms of dogs' re-examination in order to confirm the release of the body from the parasite.

PREVALENCE OF DIROFILARIASIS INFECTIONS IN DOGS IN LATVIA

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The nematodes *Dirofilaria* spp. are a zoonotic mosquito-borne haematophagous filariid parasite of carnivores worldwide.

Due to climate changes dirofilariasis is spreading to new areas around the world. These changes could be partially attributed to the growing interest of the scientists in dirofilariasis, especially with respect to human infections. Climate change has increased the range of mosquito vectors of *Dirofilaria* spp. in Latvia as well.

The aim of our study was to expand the current knowledge on the prevalence of *Dirofilaria* infections in dogs in Latvia, to assess the risk factors of heartworm disease or subcutaneous dermal swelling and nodules in dogs. In addition, there is a need to finally fill white areas in the epizootological map of Baltic region in relation to the prevalence of canine dirofilariasis.

We have tested hematological blood samples of domestic dogs collected in 2008-2016 years mainly from the central part of Latvia. We have analyzed samples to the presence of *Dirofilaria* spp. larvae using blood film microscopy and modified Knott's technique. In total, 559 (4.4 %) out of 12686 examined animals were found positive for *Dirofilaria* spp. by any of these tests.

Canine antigen Heartworm test (Idexx Lab Snap test) was used to detect *D. immitis* antigen in 74 positive blood samples. *D. immitis* antigen could not be found in any sample. Positive blood samples of years 2008 – 2013 with microfilaria were examined by PCR technique. *D. immitis* was not detected in canine blood samples.

Other positive samples of years 2014 – 2016 we are currently analyzing by molecular techniques and continue our epidemiological study of *Dirofilaria* spp. infection in dogs in Latvia.

We should note that none of the dogs infected with *Dirofilaria* spp. have left our country, all being autohtone cases.

D. immitis could not be confirmed yet, but data from border countries suggests that the successful establishment of this nematode species in Latvia is a credible scenario for the near future.

TOXOPLASMA GONDII SEROPREVALENCE IN SHEEP IN LATVIA

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Toxoplasma gondii is a protozoan parasite with both public health and veterinary importance. We conducted a cross-sectional study to estimate *T. gondii* seroprevalence in sheep in Latvia. We tested sera from 1039 sheep from 84 farms, collected between 2012 and 2013, for antibodies against *T. gondii*, using an in-house enzyme-linked immunosorbent assay. Antibodies against *T. gondii* were detected in 179 (17.2%, 95% confidence interval 15.0-19.6) of the sheep. At least one seropositive sheep was found on 37 (44.0%, 95% confidence interval 33.7-54.8) of the farms. The seroprevalence was 10.8% (95% confidence interval 6.8-16.3) in juveniles (≤1 year) and 18.4% (95% confidence interval 16.0-21.1) in adults (>1 year) ($P < 0.05$). The results show that a substantial proportion of sheep are exposed to *T. gondii* in Latvia. (Publication: Deksne, G., Ligere, B., Šneidere, A., Jokelainen, P. 2017. Seroprevalence and factors associated with *Toxoplasma gondii* infections in sheep in Latvia: Latvian Dark Headed sheep breed associated with higher seroprevalence. Vector Borne Zoonotic Dis. [epub ahead of print])

EFFECT OF IGG CONCENTRATION IN BOVINE COLOSTRUM AND CALF'S BLOOD SERUM ON *CRYPTOSPORIDIUM* SPP. INVASION IN CALVES

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The present work aimed to investigate interrelation between level of IgG in bovine colostrum and calf's blood serum and its influence on the *Cryptosporidium* spp. invasion in calves. Research was conducted in a dairy cattle farm in Zemgale region during the winter and spring 2016. In the present work, colostrum samples (n=58) and blood samples (n=58) were measured using Bio-X ELISA kit for the quantitative determination of Bovine immunoglobulin G in blood serum and colostrum. First milking colostrum samples were obtained. Blood samples were taken from calves at the age of 2 days. Coprological samples were taken from calves at the age of 0, 5 and 10 days. For the presence of *Cryptosporidium* spp. standardized ovoscopic method was used. Statistically significant positive correlation was established between the colostrum IgG concentration in cows and blood serum IgG concentration in their calf's ($r = 0.4$, $P = 0.001$). But there was no significant correlation between level of IgG in calf's blood serum and *Cryptosporidium* spp. invasion. Positive correlation between IgG in colostrum and the calf blood serum indicate that calves that got colostrum with higher level of IgG will have higher IgG concentration in serum at first days of age. But high concentration of IgG in calf's serum does not affect *Cryptosporidium* spp. invasion. Studies will be continued in dairy cattle farms, were there is a higher *Cryptosporidium* spp. invasion.

QUESTIONNAIRE STUDY ON OCCURRENCE OF DIARRHOEA DURING VETERINARY STUDIES IN ESTONIA

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In Estonia, rumours of cryptosporidiosis among veterinary students have been circulating for years. In particular, veterinary students with Finnish background have described severe symptoms resembling cryptosporidiosis during their university studies in Estonia. At the same time, only few cases of cryptosporidiosis have been reported in the national statistics. Using an online questionnaire, we surveyed the occurrence of diarrhoea during veterinary studies in Estonia, and whether there was evidence supporting Finnish veterinary students being more commonly affected than Estonian veterinary students. Altogether 106 current and former veterinary students completed the questionnaire in September 2016, and more than half of them reported having had diarrhoea during their studies. The Finnish veterinary students had three times higher odds to reportedly have had diarrhoea during their studies than the Estonian veterinary students. Individuals in both groups reported symptoms resembling cryptosporidiosis, and the results suggested that there had been outbreaks of diarrhoea and diarrhoea resembling cryptosporidiosis. Most of the participants knew that humans can shed *Cryptosporidium* and that cattle can shed *Cryptosporidium*, while almost a third did not know what measures can kill or inactivate *Cryptosporidium* oocysts.

FLATWORMS OF THE FAMILY *MICROPHALLIDAE,* WARD, 1901 (TREMATODA: *PLAGIORCHIIDA)* – RARE PARASITES OF MALLARD *ANAS PLATYRHYNCHOS L.* IN CENTRAL EUROPE

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Trematodes of the family *Microphallidae* are extremely rare in the mallard *Anas platyrhynchos L.*, 1758 in Central Europe. A few species of these trematodes have been recorded in Poland in other ducks wintering on the Baltic, but never in the mallard. Parasitological necropsies were performed of 24 mallards acquired in the county of Olsztyn (north-eastern Poland) from 20 August to 9 December 2016. About 4,000 *Levinseniella propinqua* Jägerskiöld, 1907 trematodes were found in the intestine of one mallard (male, immature) in December 2016. Parasites may be transported by birds during seasonal migration. The seasonal migration routes of the mallard are not known in detail. The mallard population from the north of the Eurasian continent is known to migrate in autumn to areas located in eastern Poland. They stay there for several days, feeding mainly on stubble fields, and later continue southward and westward. Part of the population spends the winter in cities, such as Olsztyn, where mallards form the largest population among all wintering birds, numbering about 3,000 individuals. It is very likely that the trematodes were transported during the mallards' migration from their feeding grounds, located in northern Europe, i.e. from Estonia, Latvia, Finland or Russia. The mallard is an aquatic phytophage, and the presence of *L. propinqua* in the mallard is a consequence of the contribution of crustaceans in its diet, containing infective larvae of these trematodes. The intestines of the mallards also contained trematodes that are typical of these hosts throughout the continent: *Australapatemon minor, Cotylurus cornutus, Bilharziella polonica, Echinostoma revolutum, E. paraulum, Echinoparyphium recurvatum, Hypoderaeum conoideum* and *Notocotylus attenuatus*.

HELMINTHS OF RODENTS AND SHREWS IN LATVIA

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Rodents’ are the largest class of mammals, with over 40% of mammalian species belonging to the order *Rodentia.* Types of biotopes where rodents live are: bogs and meadows, pastures, woodland (mixed forest, deciduous and coniferous trees, spruce forest, and swampy forests), shrubs and outdoors places in agriculture and horticulture farms. Small mammals have significant role as host of many parasites species and its study in every geographical area has veterinary and medical importance to prevent transmission of diseases to domestic animals and human. During the study period of 2015–2017, more than 200 small mammals (*Apodemus flavicollis, A. agrarius, A. sylvaticus, Apodemus* sp., *Rattus rattus, Mus musculus, Myodes glareolus, Micromys minutus, Microtus arvalis, Microtus* sp., *Arvicola amphibious, Sicista betulina, Sorex araneus, S. minutus, Sorex* sp. and *Neomys fodiens*) from three regions of Latvia – Latgale, Kurzeme, Vidzeme were examined for the presence of parasites. The method of full parasitological examination by Anikanova (2007) was used. The helminth fauna composition was represented by 12 species from the following taxonomic groups: Trematoda – 2 species, Cestoda – 4 species, and Nematoda – 6 species. Similar investigations were reported by Grikieniene (2005) in Lithuania. The overall infection prevalence was 59.1%, but in our investigation prevalence was lower – 34.5%.

MOLECULAR DETECTION OF SUBCLINICAL INFECTIONS WITH KUDOA THYRSITES (*MYXOSPORA*) IN ATLANTIC MACKEREL (*SCOMBER SCOMBRUS*)

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Several species of the myxozoan genus *Kudoa* Meglitsch, 1947 are of concern as they may induce post-mortem myoliquefaction of the fish hosts' somatic musculature, commonly known as "soft flesh". The species *K. thyrsites* represents a serious problem for the Atlantic salmon (*Salmo salar*) aquaculture industry in Pacific North America, as well as for the NE Atlantic mackerel fisheries. Its life cycle is still unknown, e.g. if there exists an alternate host for the parasites' actinospore stage. This study aimed to develop a method for molecular detection of subclinical infection with *K. thyrsites* in Atlantic mackerel from the North Sea. Parasite DNA was extracted from muscle, heart, foregut and spleen, using genomic DNA isolation kit (Beckman Coulter, USA). Presence of any *K. thyrsites* stages was examined by PCR conducted in 50 µl solution containing 50 ng template DNA mixed with 10x high-fidelity buffer, 10 mM dNTPs, 50 mM MgSO4, 10 mM of each primer, Platinum Taq DNA polymerase, and MilliQ dH2O. The primer pair K.thyr18Sfor_3 (5'-GGTCATATGCTCGTCTCAAAG-3') and K.thyr18Srev_3 (5'-TCGGTCAAGACAATTTAACCG-3') was applied for amplification of parasite nuclear SSU rDNA. The resulting PCR-products were visualized on a 1.5 % agarose gel with positive *K. thyrsites* samples showing bands at 1250 – 1300 bp. Microscopically confirmed positive samples were identified as *K. thyrsites* by DNA sequencing for control. The current method is able to detect subclinical *K. thyrsites* infections in Atlantic mackerel with high accuracy, i.e. before any clinical manifestation of "soft flesh". Considering the ongoing climate change and vast extension of the mackerel's northerly distribution range, the risk of spread of the parasite into Norwegian salmon culture facilities clearly exists. Thus, a method for reliable detection of subclinical *K. thyrsites* infections may be useful in future surveys of Norwegian farmed salmon in order to prevent economic losses due to the parasite.

CONFOCAL LASER SCANNING MICROSCOPY FOR *SARCOCYSTIS* SPP. PARASITE DIAGNOSTIC

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Nowadays, genus *Sarcocystis* is estimated to include more than 200 species of protist parasites which are identified relied on morphological features using mostly light microscopy, or by using molecular biology methods. However, the morphological features of different species of sarcocyst within one type of the host might be quite similar under light microscope. In this occasion, other microscopy methods are applied, such as TEM and SEM which allows examining the ultrastructure of the parasite. Still, there is a lack of information about application of confocal laser scanning microscopy on *Sarcocystis* morphological investigations and only one report is known where structure of bradyzoites were described. The aims of the investigation are: sarcocyst staining protocol optimization for confocal laser scanning microscopy; and evaluation of confocal laser scanning microscopy efficiency in *Sarcocystis* parasites investigations. Investigation was carried out in Daugavpils University in the Laboratory of Parasitology and Histology. From non-stained muscles of wild boars 30 *Sarcocystis* cysts were isolated and placed in five different fixators (10% formalin neutral buffered solution, Bouin's solution, alcohol-formalin-acetic acid AFA solution, 96° ethanol and Carnoy's solution), as well as cysts without fixation were dyed. After fixation cysts were stained with fluorescent dye Benz[de]antracen-7-ona, synthesized in Laboratory of Organic Chemistry and Synthesis of Daugavpils University. Dyed sarcocysts were examined under laser scanning confocal microscope Nikon Eclipse Ti-E. As a result, all 30 *Sarcocystis* cysts emitted fluorescence; however clarity of the structure depended from a fixator utilized. Thus, cysts fixed in 10% formalin neutral buffered solution had more clear structure, where cyst wall and internal saturation were distinguishable, whereas non-fixed sarcocysts were dim and unclear. However, obtained results do not allow identifying *Sarcocystis* species. Thus, investigation on sarcocyst staining protocol optimization will carry on.

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HIGH NUMBER OF *TRICHINELLA BRITOVI* DOUBLE INFECTIONS OCCURS IN WILD ANIMALS OF LATVIA

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Nematode worms of genus *Trichinella* are zoonotic parasites with a broad geographical distribution. *Trichinella* spp. in Latvia are frequently transmitted by a sylvatic cycle and seldom by a domestic cycle. These zoonotic parasites are found in many host species including unusual hosts such as beavers. According to the Centre for Disease Prevention and Control of Latvia, overall from 1955 to 2000 and from 2001 and 2016, 302 and 303 trichinellosis cases were documented, respectively. *Trichinella britovi* is the prevalent species in Latvian wildlife. The aim of the present study was to analyze the polymorphism of *T. britovi* from wildlife. Six microsatellite markers were investigated for an average of nine *T. britovi* single larvae from 45 wild animals belonging to eight carnivore species. A polymorphism was detected at two loci with five and seven alleles. The Fst index shows an average value of 0.102, suggesting a reproductive isolation among individuals belonging to different isolates. According to the Baysian analysis implemented in STRUCTURE ver 2.3.3, the genetic structure is characterized by a very high admisture level. The genetic differentiation (Fst = 0.102) and the Baysian analysis suggest that a high number of *T. britovi* double infections occurs in wild animals of Latvia. This event is supported by the high prevalence of infection among wild carnivores of Latvia.

GASTROINTESTINAL PARASITES IN REINDEER (*RANGIFER TARANDUS TARANDUS*) CALVES

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We conducted a cross-sectional study to estimate the prevalence of gastrointestinal parasites in reindeer (*Rangifer tarandus tarandus*) calves. The 480 reindeer calves included in the study were aged 6-7 months, originated from nine geographically defined reindeer herding cooperatives in Finland and one in Norway, and were slaughtered in September-November 2015 in ten reindeer slaughterhouses. The individual fecal samples were examined using a modified McMaster method. *Strongylida* eggs were detected in 75.6%, *Eimeria* sp. oocysts in 50.6%, *Moniezia* sp. eggs in 28.1%, *Nematodirus* sp. eggs in 22.1%, *Capillaria* sp. eggs in 9.4%, and *Trichuris* sp. eggs in 0.6% of the samples. The prevalence varied by geographical region.

DIRECT GENETIC CHARACTERIZATION OF *TOXOPLASMA GONDII* FROM CLINICAL SAMPLES FROM DENMARK: NOT ONLY GENOTYPE II

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The *Toxoplasma gondii* genotypes circulating in the Nordic countries, including Denmark, are largely unknown. We genetically characterized *T. gondii* strains that had been detected in human clinical samples at the national reference laboratory in Denmark during the years 2011-2016. Samples that had tested positive for *T. gondii* DNA using diagnostic real-time PCR and had a cycle threshold value <33 were included in this study and subjected to direct genetic characterization of *T. gondii* based on length-polymorphism of 15 microsatellite markers. A total of 23 DNA samples from 22 individual patients were analyzed. The results were consistent with genotype II with 15/15 markers amplified from six samples from central nervous system (CNS) including two samples from one patient, four ocular samples and one unspecified sample, with 14/15 markers from one CNS-sample, and with 3/15 markers from two ocular samples; with genotype III with 15/15 markers from two ocular samples; with genotype Africa 1 with 15/15 markers from one amniotic fluid sample and from one CNS-sample, and with 7/15 markers from one CNS-sample; with atypical genotype with 15/15 markers from one CNS-sample; and with a HG12-like genotype with 8/15 markers from one CNS-sample. The results were consistent with predominance of genotype II in Europe – however, a substantial proportion of the strains were non-type-II strains.

(This work was also presented at the 14th *Toxoplasma gondii* research community biennial meeting, Portugal, 31.05.-04.06.2017.)

A COPROLOGICAL SURVEY OF PARASITES OF MUSK DEER *MOSCHUS MOSCHIFERUS* IN RUSSIA

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148 samples of feces were examined from two populations of Musk Deer *Moschus moschiferus moschiferus* and *M. m. turovi* from Altai and Primorye Regions of Russia. The most prevalent endoparasites were revealed lung protostrongylid nematodes. Their larvae were found in 92.6% of total samples. Of three distinguished protostrongylid species *Pneumocaulus kadenizii* had the dominant rate, 91.2%. The larvae of *Protostrongylus* sp. were detected in samples from the Altaic population, their prevalence reached 20.5%. The not identified protostrongylid larvae having specific morphology were found in 7.3% of animals from Far Eastern population. The lung nematodes obviously have the greatest epizootic significance among parasitoses in the surveyed populations of musk deer. The eggs of gastrointestinal nematodes were also revealed in approximately similar rates in both populations. The eggs of *Trichuris* sp. were found in 15.5 %, *Nematodirus* sp. in 6.8%, and *Pygarginema skrjabini* in 2.7% of total samples. In 22% of the samples from Primorye and 41% from Altai there were eggs of hepatic trematodes *Dicrocoelium* sp. Three samples (2.8%) from Primorye contained trematode eggs, morphologically identical to *Hasstilesia ovis*. This is the first report of this intestinal trematode species in musk deer. In addition to helminths, the oocysts of three species of *Eimeria* were discovered. *E. moschus* was found in 10.1%, *E. jinfengshanenisis* in 22.3% and *Eimeria* sp. in 14.9% of total samples from both populations. The coccidian infection has been reported for the first time in musk deer in Russia. The study was supported by RSF project No 14-16-00026.

THE STRATEGY OF MICROSATELLITE DESIGN OF *DIPHYLLOBOTHRIUM LATUM*

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Diphyllobothrium latum (Cestoda: *Diphyllobothriidea)* represents medically important food-borne parasite belonging to so called “broad tapeworms” or “fish tapeworms”. The complex life cycle of *D. latum* includes two intermediate hosts (crustaceans and fish) and final host (fish-eating birds or mammals, including human). In Europe, different fish species (e.g. *Perca fluviatilis*, *Esox Lucius*, *Lota lota*) were found to be infected with *D. latum* mainly in the Alpine lakes region in Italy, Switzerland, France, but even in Estonia and Russia. However, the distribution pattern and genetic interrelationships of European populations, their exact origin and transmission routes are not known. Microsatellites, or short tandem repeats (STR), are genetically informative and suitable tools for population genetics studies. Therefore, we aimed to develop and characterize polymorphic microsatellite loci in *D. latum* using next-generation sequencing (NGS) approach. For NGS analysis and selection of suitable STR loci, DNA pool was prepared from *D. latum* plerocercoids from perch (Iseo Lake, Italy). In total, 518 amplicon candidates generated after NGS provided the best resolution, 122 of them were tested by PCR amplification. In total, 110 candidate loci yielded PCR products of expected size and in 78 of them the declared repetitive motif was identified by Sanger sequencing. The next steps of the microsatellite design will be: i) determination of STR allele polymorphism by fragment analysis with fluorescently labelled primers; ii) statistical analyses (determination of observed (Ho) and expected (He) heterozygosity, deviations from Hardy-Weinberg equilibrium, etc.). The statistically informative microsatellite loci will be applied in future population genetics studies of *D. latum*. This work was supported by the projects APVV-15-004 and VEGA 2/0134/17.

SEARCH FOR EVIDENCE OF *TRICHINELLA* EXPOSURE AND INFECTION IN MOOSE (*ALCES ALCES*) HUNTED IN ESTONIA

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Trichinella spp. are zoonotic helminth parasites endemic to Estonia (sylvatic cycle). Like other herbivores, the moose (*Alces alces*) might be an accidental host for *Trichinella*. We investigated moose hunted for human consumption in Estonia for evidence of *Trichinella* exposure and infection using serological, digestion, and molecular methods. Samples were collected by hunters during the hunting season of 2015.

Using an artificial digestion method, muscle samples from 460 moose were analyzed for the presence of larvae. Sera or plasma from 463 moose were screened for the presence of anti-*Trichinella* immunoglobulin G antibodies with a commercial ELISA test. Samples yielding a positive or doubtful ELISA result and samples from moose that had larvae or were included in a pool that had larvae were further tested using Western blot analysis.

Nematode larvae were found in six digested samples, five of which were pooled samples. Five of the samples were subjected to molecular analyses, using a conventional targeted PCR and a 16S/18S microbiome platform. *Trichinella* DNA was not detected. Twelve moose tested positive using ELISA. None of the 32 samples tested using Western blot were positive.

The ELISA results indicated presence of anti-*Trichinella* antibodies in 2.6% of the moose, but the Western blot and molecular analyses did not confirm exposure or presence of *Trichinella* spp. in these animals.

ANTHELMINTIC RESISTANCE AGAINST GASTROINTESTINAL NEMATODES OF SHEEP IN LITHUANIA

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The intensive and incorrect use of anthelmintics, such as under-dosing and treatments with the same anthelmintics led to the development of anthelmintic resistance (AR), especially in gastrointestinal nematodes of sheep worldwide. The aim of the present study was therefore to examine the prevalence of anthelmintic resistance in gastrointestinal nematodes to ivermectin (on 21 farm), benzimidazoles (on 25 farms) and levamisole (on 6 farms) in Lithuanian sheep flocks, with previous history of the use of benzimidazoles, levamisole and/or ivermectin for at least of 3-5 years. The prevalence of AR was examined using the *in vitro* egg hatch (EHT) and larval development tests (LDT). Data were analysed using a threshold discriminating concentration of 21.6 ng/ml^{-1} for ivermectin-aglycone, 2 μg/ml^{-1} for levamisole in LDT and 0.1 μg/ml^{-1} for thiabendazole in EHT. Based on farm questionnaire in Lithuania, the most commonly used anthelmintics were ivermectin (68.6%), and benzimidazoles (27.5%). Levamisole was used very sporadically (3.9%). The *in vitro* survey showed the anthelmintic resistance to ivermectin on 13 farms (61.9 %; 95% CI 38.4-81.9), to levamisole on 2 farms (33.4%; 95% CI 4.3-77.7) and to benzimidazoles on all sheep farms ivestigated (100%; 95% CI 88.7-100). *Teladorsagia* and *Trichostrongylus* were the most resistant ($P<0.05$) gastrointestinal nematodes identified on sheep farms. The AR of gastrointestinal nematodes to most classes of anthelmintics is widespread on Lithuanian sheep farms.

THE STUDY OF THE POSSIBILITY OF CROSS-INFECTION OF LABORATORY RATS WITH *GIARDIA* ISOLATES FROM HUMANS AND DOMESTIC ANIMALS

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Giardiasis is one of the most common protozoan diseases in many countries of the world. People and many species of animals are susceptible to infection. But experiments to confirm the possibility of cross-infection with different genotypes are not sufficiently considered in scientific literature. *Giardia duodenalis* assemblages A and B isolated from different people, cats and dogs were used in experiments on cross-infection of laboratory rats. Rats, free from parasitic infections, were orally infected with cysts of giardia in doses ranging from 3 to 45 thousand per animal. A daily study of the feces of rats for the presence of cysts was performed after infection. Laboratory rats were successfully infected in 4 out of 5 experiments with *Giardia* isolates from humans. The prepatent period lasted from 2 to 24 days; the patent period was from 90 to 464 days. The infection of rats with *Giardia* isolates from dogs was successful in 3 out of 8 experiments. The prepatent period lasted from 17 to 25 days; the patent period was from 30 to 520 days. Only in 1 case of 2 experiments the rats were successfully infected with isolates from cats. The prepatent period lasted 30 days; the patent period was 150 days. In all the experiments, animals showed an intermittent character of cysts excretion with feces into the environment, the intensity of infection was from 10 to 5500 cysts per gram of feces. It should be noted that not all animals in the experimental groups were successfully infected. This may depend not only on the genotype of giardia, but also on the viability and infectiousness of cysts, and on the individual susceptibility of laboratory animals. The study was supported by RSF project No 14-16-00026.

HELMINTH FAUNA OF THE GASTROINTESTINAL TRACT OF THE POLAR BEARS

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Polar bear *Ursus maritimus* is a circumpolar species included in IUCN Red List having conservation status as vulnerable with high risk of endangerment in the wild. This bear considered to be practically free of parasites. The only helminth species reported from polar bear in the wild is *Trichinella spiralis* s.l. During 2013-2015 samples of feces have been taken in different regions of the Arctic in different seasons of year. One pool of samples was taken in the snow-free period (August-September) on the territory of the Chukotka coast and Wrangel Island. Another one was collected on the ice floes in the Kara, Laptev and Chukchi seas in winter. Among 27 specimens collected in the snow-free period only in one sample (3.7%) the eggs of nematodes morphologically identical to the eggs of *Toxascaris leonina* were found. In 3 (33%) of 9 samples taken in winter eggs and larvae of helminths were found. There were revealed the cestode eggs *Diphyllobothrium* sp., small eggs (21-23 µm) of intestinal trematodes, most likely *Heterophyidae;* nematode eggs of *Toxascaris leonina* and strongylid eggs morphologically identical to *Uncinaria stenocephala*. Larvae of the first stage of lung nematodes *Crenosoma* sp. were found as well. The significant difference observed in animals invasion in snowless and winter periods, can be explained by a sharp seasonal differences in the structure of bears nutrition. Lack of food and presence of crude vegetable fibres in the diet in the snow-free period may probably cause the elimination of intestinal helminths. Supported by RSF 14-16-00026.

PHYLOGENETIC RELATIONSHIPS OF EIMERIAN PARASITES INFECTING SHREWS: ANOTHER PIECE TO THE PUZZLE

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Examination of faecal samples from 111 insectivores (genera *Crocidura, Neomys* and *Sorex)* from various localities in Europe revealed that around one third of the samples were infected with coccidian, however, often in a very low infection rate. Obtained 11 sequences of cytochrome c oxidase I gene of *Eimeria* spp. used for phylogenetic analyses clustered into two distinct groups. One was host-specific for *Crocidura* species, the other was mixed, with the sequence of *Eimeria* from *Neomys* on a separate branch. A single sequence of *Isospora*, from *Sorex araneus*, grouped with isosporans from moles. No obvious geographic pattern was observed. The first cluster resembled *E. ropotomae* by inner structures and by the wall characteristics, but not precisely in size. The rest of the samples was not fully sporulated, thus we could not reliably assign them to the species. The only better resolvable samples were *Isospora* species (most likely *I. araneae*) and *Eimeria* from *Neomys* (most likely *E. neomyi*).

DETECTION OF TAENIID EGGS FROM BERRY SAMPLES: A PILOT STUDY

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We studied berries as a vehicle for the taeniid eggs by using non-zoonotic *Taenia laticollis* eggs obtained from lynx carcasses as a model. All family *Taeniidae* tapeworm eggs appear nearly similar in their structure and physical characteristics. Consequently, information gained in this experiment can be applied also for understanding the transmission of echinococcus eggs. We experimentally contaminated European blueberry shrubs in cultivation boxes in a controlled environment. We sprayed shrubs in four cultivation boxes with different amounts of *T. laticollis* eggs (0 eggs, 15 500 eggs, 31 000 eggs and 155000 eggs in 100 ml of water) and collected berries manually after 24 hours. The mass of collected berries varied between 19 and 28 g (mean 22.9 g). We washed the samples in water, strained the water through a 60 µm sieve to remove the coarse rubbish and through 20 µm sieve to collect eggs, extracted DNA from the collected fine debris, and used PCR with NAD-1 taenia-specific and T. laticollis-specific primers designed for this purpose. We detected taeniid DNA in all three of the samples by using *T. laticollis*-spesific primers and in two samples by using NAD-1 primers. In recovery tests, with the *T. laticollis*-specific primer we detected taeniid DNA in 50 g berry samples spiked with a minimum of five eggs and with the taenia-specific primer with a minimum of ten eggs. Five eggs was the minimum number of eggs used in recovery tests. The results indicate that berries may serve as a vehicle in the transmission of taeniid eggs when DNA is used as an indicator. Further, a field trial experiment is planned to confirm these results in natural conditions.

EVALUATION OF SURGICAL CASES OF CYSTIC ECHINOCOCCOSIS: EXPERIENCE OF HERACLES PROJECT IN ROMANIA, PRELIMINARY RESULTS

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Echinococcus granulosus cestode is hosted as adult stage in dogs' intestine and, as larval stage (metacestode) causes cystic echinococcosis (CE) in various species of mammals and accidentally in humans, an important zoonotic disease responsible for a lot of important damages in both human and animal populations, with a lot of social and economic loss. Heracles FP7 Project is focused to contribute to a better understanding of the disease and to improve the diagnosis of CE. Between October 2013-February 2016, in Colentina Clinical Hospital, 309 CE patients were admitted. They all agreed and signed the consent forms. Out of these, 150 where addressed to surgery: 108 cases open surgery, six laparoscopies, 36 percutaneous treatments. Biological materials (membranes/fluids, blood samples) were collected and analyzed from 63 patients (89 cysts) out of 150. The age was between 19 and 76 (higher number between 31 and 50 y.o.). The average size of the cysts: <5cm (19), 5-10cm (54), >10cm (16). Location of the cysts: liver (72), lung (4), spleen (2), peritoneal (10), kidney (1). The WHO classification of the liver cysts (72) is: CE1 (35), CE2 (10), CE3a (6), CE3b (14), CE4 (6), CE5 (1). Laboratory tests performed: serology ELISA (48/63 positive – 76.19%). Serum samples from all the patients were referred to Biobank and will be retested with the new kits developed within the Project. Viability test for protoscolices: 77/89 samples were positive, recording the presence of protoscolices and/or hooks. Genotyping is under process. All the surgical cases are enrolling in ERCE (European Registry of Echinococcosis).

Conclusion: CE is a serious health problem in Romania. The benefit of serological diagnosis is limited. Heracles FP7 Project opens new possibilities for further studies of CE diagnosis and treatment, offering a new perspective of this complex disease.

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MOLECULAR DATA CONFIRM THE TAXONOMIC POSITION OF *HYMENOLEPIS ERINACEI* (CYCLOPHYLLIDEA: HYMENOLEPIDIDAE), WITH NOTES ON HOST SWITCHING IN THE FAMILY HYMENOLEPIDIDAE

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Hymenolepis erinacei is a cestode which parasite in hedgehogs of the genus *Erinaceus.* The taxonomic position of this hymenolepidid has been debated for a considerable period of time. Consequently, the aim of this study was to explore the systematic position of *H. erinacei* based on molecular sequence data and phylogenetic analysis. For the study 26 hedgehogs representing *Erinaceus roumanicus*, killed by motor vehicles on roads, were collected in south-eastern Lithuania and examined during the period from March to September, 2013–2016. Specimens of *H. erinacei* were detected in intestines of 12 hedgehogs (46 %). This cestode species was recorded for the first time in Lithuania. Four cestodes were used for detailed morphological study; *H. erinacei* is characterised by unarmed scolex, oval embryophore without filaments and "v" form guard of antero-lateral embryonic hooks. Also, partial mitochondrial 16S rDNA gene (855 bp) and nuclear 28S rDNA gene (up to 1517 bp) were amplified and sequenced; phylogenetic trees were obtained and analysed. The phylogenetic analyses highlight that the genus *Hymenolepis* is best supported group among cestodes of mammals in the family *Hymenolepididae* and unequvocally establishes taxonomic position of *H. erinacei*. The genus *Hymenolepis* forms a monophyletic group of cestodes with one hedgehog and other rodents as hosts. We suppose, after host switching from insectivorous animals to rodents only *H. erinacei* remains with the ancestral host in the genus *Hymenolepis.* The knowledge about helminths of hedgehogs in different regions and effective methods for parasite monitoring are important because hedgehogs have become exotic pets leading to an increase for their importance in small animal veterinary practice. *Hymenolepis erinacei* is the only species parasitizing hedgehogs of the genus *Erinaceus.* This research was funded by a grant (No. MIP-43/2015) from the Research Council of Lithuania.

THE PRESENCE OF ANISAKID NEMATODES WITH ZOONOTIC POTENTIAL IN THE MUSCULATURE OF COD: BALTIC SEA CASE STUDY

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Consumption of fillets of marine fish might be the source of concern to human health due to the presence of *Anisakidae* nematodes. The parasitological analysis of muscle tissue (fillets) of cod *Gadus morhua* were performed annually from 2013 to2017 in Polish waters and gradually expanded to Danish and Swedish EEZ. Samples were collected during scientific surveys in Polish, Danish and Swedish EEZ. After standard ichtiological analysis, the fillets has been screened using white light transilluminator and then digested with artificial digestive juice to detect nematodes. Our results reveal marked increase of the prevalence of infection of cod with anisakid nematodes as compared with the 1987−1993 period. In musculature of cod third stage larvae of *Anisakis simplex* and *Pseudoterranova* sp. were found. Prevalence of cod infection differs between sampling areas. Increasing number of the grey seal *Halichoerus grypus* in the Baltic is considered to be one of the most important reasons of observed increasing prevalence of cod infection with anisakid nematodes. Sufficient monitoring of economically important fish species for the presence of anisakid nematodes is very important for food safety. This research was supported by The National Centre for Research and Development under the Strategic Program Biostrateg (grant no. 296211/4/NCBR/2016).

SPRAT (*SPRATTUS SPRATTUS*) AS TRANSMITTER OF *CONTRACAECUM OSCULATUM* TO PISCIVOROUS PREDATORS: POLISH CASE STUDY

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In the food webs of the open part of the Baltic Sea sprat (*Sprattus sprattus*) is one of the most important fish species. It was found important in the diet of piscivorous predators (salmonid and gadoid fish, marine mammals and sea birds). Sprat might be also a source of parasitic infection for these organisms. In the southern Baltic Sea (Polish EEZ) the level of sprat infection with nematodes with zoonotic potential was investigated in two time series: 1987-1989-1990 (3511 fish examined) and 2015-2016 (180 fish examined). Standard ichthyologic analyses with visual inspection of the viscera were conducted to detect nematodes. Sprats infected with *Anisakidae* parasites were caught in 2016 in central off-shore area of Polish EEZ. Anatomo-morpological identification of parasites, supported with molecular methods reveals the presence of larvae of *Contracaecum osculatum*. In the southern Baltic Sea sprat may play a role as a transmitter of that parasite to piscivorous organisms.

THE GREAT SANDEEL (*HYPEROPLUS LANCEOLATUS*) AS TRANSMITTER OF PARASITE *CONTRACAECUM OSCULATUM* TO PISCIVOROUS ORGANISMS IN THE SOUTHERN BALTIC SEA

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The great sandeel (*Hyperoplus lanceolatus*) is a common fish species that inhabits sandy substrates in the Baltic Sea. It plays an important role in the diet of marine mammals, sea birds and piscivorous fish, but in the Baltic Sea information on its parasite fauna is scarce. The great sandeels were sampled in April 2016 in two areas of the southern Baltic Sea (south of Bornholm and the Gulf of Gdansk). Standard ichthyologic analysis and visual inspection of the viscera and muscles surface were performed. Intestines and gutted fish were separately digested in artificial digestive juice to detect parasites. Anisakid nematodes were found in the great sandeel from both areas. Microscopic identification and molecular methods revealed the presence of liver worm *Contracaecum osculatum* (average prevalence of infection 8.96%). The *Anisakidae* nematodes were found for the first time in *H. lanceolatus* in the southern Baltic Sea. The great sandeel may be considered to be a transmitter of liver worm in the food web of this marine environment.

CHANGES IN THE EUROPEAN EEL INFECTION FISH *ANGUILLICOLA CRASSUS*: POLISH CASE STUDY

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The European eels (*Anguilla anguilla*) are commonly infected with the swim bladder nematode *Anguillicola crassus*. The aim of the studies was to check the level of eel infection in the Polish EEZ, southern Baltic Sea. Fish were sampled in different seasons of 2014, 2015 and 2016. Parasitological analyses of ells were focused on the presence of nematode *A. crassus*. In total 1096 eels were investigated during three years. The total number of found parasites was 6792 individuals. The correlation between infection intensity and host length, Fulton condition factor, age of the fish, area and time of sampling were taken into the account during analysis of the data. The prevalence and intensity of infection have been calculated. The mean prevalence was similar between years of sampling (more than 60%). The infection intensity fluctuated between 1 and 75 parasites per fish (in average 9.3 parasites per fish). Results were compared with last reported data from Polish waters in 2000-2002.

THE HELMINTH FAUNA OF *MERLANGIUS MERLANGUS* IN THE SOUTHERN BALTIC SEA (POLISH EEZ)

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Merlangius merlangus, known as whiting or merling, is important for the fisheries in the eastern North Atlantic Ocean and adjacent seas. The knowledge about parasite fauna of that fish species from *Gadidae* family is scarce in the southern Baltic Sea. The aim of our studies was to describe the helminth fauna of *M. merlangus* caught in Polish EEZ. Sample of fish (n = 91) was collected during survey in April 2016. Standard ichthyologic analyses were performed and viscera of fish were frozen for further inspection. The liver and digestive tract of each fish were analysed for the presence of parasites. In the digestive tract *Echinorhynchus gadi* was the dominant element of parasitofauna present in 81.32 % of examined fish (intensity 1-30 individuals per fish). Single representatives of *Hysterothylacium* sp. were found in stomach, pyloric caeca, intestines and liver. In the liver the most abundant parasite was *Contracaecum osculatum*, present in 16.48% of examined fish (intensity 1-29 individuals per fish). The Fulton’s body condition factor was calculated for infected and non infected fish. The helminth fauna of *M. merlangus* were never studied before in the Polish EEZ of southern Baltic Sea.

CHANGES IN THE HELMINTH FAUNA OF GARFISH *BELONE BELONE* IN THE SOUTHERN BALTIC SEA (POLISH EEZ)

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The garfish (or sea needle) *Belone belone* is a pelagic oceanodromous fish found in brackish and marine waters in the eastern North Atlantic Ocean and adjacent seas. In Polish waters of the southern Baltic Sea it is observed in coastal zone between April and June, during spawning migration. The aim of our studies was to describe the helminth fauna of that fish species. Sample of garfish (n = 61) was bought from the fishermen in 2015. Standard ichthyologic analysis has been performed and viscera of fish were frozen for further parasitological inspection. The parasitological analysis of liver and digestive tract was performed. The prevalence of parasitological infection was 24.59 %. In the intestines the most abundant were: nematode parasite *Hysterothylacium* sp. (prevalence 3.28 %; intensity 1-2 individuals per fish) and tapeworm *Schistocephalus solidus* (prevalence 11.48 %, intensity 1-2 individuals per fish). The prevalence of *Anisakis simplex* larvae in the liver was 13.11 %. Our results were compared with last parasitological studies conducted on garfish from Polish EEZ in 1980-ties.

THE HELMINTH FAUNA OF THE EUROPEAN PLAICE (*PLEURONECTES PLATESSA*) IN THE SOUTHERN BALTIC SEA (POLISH EEZ)

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The European plaice (*Pleuronectes platessa*) is one of the flatfish representative in the southern Baltic Sea. Parasitological studies focused on that species in the Baltic Sea are scarce. The aim of our research was to describe the helminth fauna of European plaice in that area. Samples of fish (n = 98) were collected during survey conducted in April 2016 in the southern Baltic Sea (Polish EEZ). After standard ichtyological analysis, the intestines of fish were frozen for further parasitological inspection. The most abundant parasite of digestive tract was nematode *Cucullanus heterochrous* (prevalence 73.47%; intensity of infection 1-20). In total 375 individuals of *C. heterochrous* were found (80% were females). Common were also acanthocephalans: *Echinorhynchus gadi* (prevalence 48%; intensity of infection 1-16) and *Pomphorhynchus* sp. (prevalence 12.2%; intensity of infection 1-3). The relations between presence of parasites and length of the fish as well as the area of sampling were analysed. The helminth fauna of European plaice were never investigated in mentioned area.

NEMATODE PARASITE INFECTION OF COD (*GADUS MORHUA*) LIVER FROM SWEDISH WATERS

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Parasitic infection in marine fish is a serious problem for the fishing industry in many countries. Cod, *Gadus morhua*, important for Baltic Sea fisheries, is one of the fish species that is most heavily infected with nematodes. Increasing level of cod's liver parasitic infection has been previously reported from Denmark and Poland. Conversely, there is a lack of knowledge about cod's liver infection level in Swedish waters. The aim of our study was to evaluate the prevalence, intensity of infection and species composition of nematodes in the liver of cod caught in Swedish waters. Samples of cod were collected during bottom trawl surveys in Swedish waters (February 2015 n = 165; November 2015 n = 356; February 2016 n = 409). Standard ichthyological analyses were performed on board and livers were frozen for further parasitological investigation. Thawed livers were digested in artificial digestive juice. All parasites were collected and identified on the base of anatomo-morphological features. The most abundant nematode parasite was *Contracaecum osculatum*, present in 32% of the analyzed fish in February 2015; 29% of the analyzed fish in November 2015 and 49% of the analyzed fish in February 2016. Intensity of cod infection with *C. osculatum* was between 1 and 727 parasites per fish. The total number of *C. osculatum* found in all livers was 10 971. Other nematode parasites were *Pseudoterranova* sp. (n = 10), *Anisakis* sp. (n = 88) and *Hysterothylacium* sp. (n = 12). High level of cod's liver infection with *C. osculatum* could be the result of the increasing number of the grey seal *Halichoerus grypus*, which is one of the most important final hosts in the life cycle of that parasite in the Baltic Sea.

DISEASE BURDEN OF CONGENITAL TOXOPLASMOSIS IN DENMARK

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Congenital toxoplasmosis (CT) causes substantial disease burden worldwide. We estimated the incidence, occurrence of sequelae, mortality, and burden of disease in terms of disability-adjusted life years (DALYs) of CT in Denmark in 2014. We estimated that 14 children were born with CT, of which six will have developed sequelae by the age of 12. CT resulted in a total disease burden of 123 DALYs, of which 78 and 2 were due to foetal loss and neonatal death, respectively. Based on a comparison of the estimated incidence with the number of reported cases in the period 2008–2014, for each reported CT case, at least five more CT cases went unreported. Early onset, severity, and life-long duration of sequelae have a major effect on the disease burden of CT. Our data suggest that CT is underdiagnosed or underreported in Denmark. (This work was also presented at the 14th *Toxoplasma gondii* research community biennial meeting, Portugal, 31.05.-04.06.2017.)

PRELIMINARY REPORT OF *IN VITRO* CONDITIONS ON THE HATCHING TIME OF *ALARIA ALATA* EGGS

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Alaria alata is a digenean trematode being widespread throughout all the Europe. This trematode develops in a three-host life cycle, however the life cycle can be extended by paratenic hosts. Adult *A. alata* reside in the intestines of definitive hosts. Unembryoned eggs are passed out through the faeces of the host and hatch in water. In the bright time of the day, miracidium hatches within two weeks and moves actively to find a snail host. Aim of this study is to prove the hatching of the eggs of *A. alata* on *in vitro* conditions and to find out how many days are required.

Fresh faecal samples from one red fox rectum were collected. The sedimentation technique of 1 g of faecal sample was used to isolate *A. alata* eggs. Specimens of *A. alata* were identified using the key characteristic morphological features. Approximately 100 eggs were transferred in a Petri dish and then placed under the warming lamp (+25 ^{0} C). The lamp worked diurnal and the sample in a Petri dish was examined every day.

After six days, first miracidia were observed in the eggs. However, only at 13th day of the experiment, actively moving miracidium was hatched out of the egg.

We conclude that it is possible to hatch *A. alata* eggs on *in vitro* conditions and grow viable miracidium. However, this is only a pilot study and for more detailed results a good designed experiment is required.

MORPHOMETRIC DIFFERENTIATION OF *TOXOCARA CANIS* AND *T.CATI* EGGS

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The reliable differentiation of *Toxocara canis* and *T. cati* eggs in ovoscopic assays may have great importance, for example at assessment of soil contamination. The eggs of both species have similar morphology and are usually defined as *Toxocara* sp. Thus, it is impossible to adequate evaluate the role of dogs or cats as a hazard factor in human toxocarosis. The described till now criteria for identifying eggs based on their size and shell structure should be recognized as unsatisfactory, as they have values overlapping in both species. We studied under a light microscope the morphology and carried out the morphometry of 980 eggs of *T. canis* and *T. cati*. *T. canis* eggs measures were in the range of 76-96 (m=86.5) x 65-83 (m=75.7) µm, and *T. cati*, 61 - 80 (72.3) x 50-70 (62.3) µm. Thus, while the mean sizes of the larger and smaller axes of eggs statistically differ, the intervals of values are overlapped. To increase the accuracy of the micrometric method, we propose additional metric indices: the average diameter of the egg (the arithmetic mean of measurements for the long and short axes of the egg) and the diameter of the zygote (in non-embryonated eggs). The average diameter of *T. canis* eggs varied in range 74–86 (81.1) µm, *T. cati* eggs 60–71 (67.3) µm. The diameter of a zygote due to its spherical shape does not depend on the orientation of the egg and requires only one measurement. This metrics in *T. canis* showed 56–70 (62.5) µm, in *T. cati* 45–52 (50.1) µm. The proposed parameters are less variable in comparison with the measurements on the axes and their values for different species of toxocars do not overlap. They provide the possibility for reliable species identification of *Toxocara* eggs in samples. The study was supported by RSF project No 14-16-00026.

SPECIFIC FEATURES OF EARLY DEVELOPMENT STAGES OF *TOXOCARA CANIS* AND *T.CATI* LARVAE IN THE EGG

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Details of the early development in *Toxocara* remain poorly understood; particularly the number of molts is still debated. In this study, we observed the development process and studied the morphology of the egg stage larvae of *Toxocara canis* and *T. cati*. In both species, two molts were definitely marked. At 25° in the eggs of *T. canis* on the fifth day observed the stage of "prelarvae" ("tadpole" stage), and on the sixth day the larvae began to move. The first molt was on the ninth day, the second molt on the 11th day. The development of *T. cati* in the egg is longer. The prelarvae stage was marked on the eighth day; the larvae began moving on the 10th day. The first molt occurred on the 13th day, the second molt on the 20th day. In both species, the first molt proceeds barely noticeable. The second molt is clearly manifested by the formation of the cuticular sheath. In *T.cati* this sheath remains on the body of larvae permanently (in our experience during the entire observation period of 6 months). In *T. canis*, larvae discard the sheath a few days after molting. The morphometry of invasive larvae of *Toxocara* revealed the statistically significant difference in species in the width (diameter) of the larvae. The width of *T. canis* larvae was 18-20 µm, and *T. cati* 14-16 µm. A characteristic morphological feature of invasive larvae of *T. canis* is a peculiar dorsal bend of the caudal end, which is absent in *T. cati*. The described differences in the morphology of L3 may be useful for species differentiation of eggs in zooscopic studies, as well as for identification of larvae localized in the tissues of paramedic hosts, including larva migrants in humans. The study was supported by RSF project No 14-16-00026.

BALTIC COD (*GADUS MORHUA*) INFECTED WITH PARASITIC NEMATODES *HYSTEROTHYLACIUM* SP. VIA *CRANGON CRANGON* (LINNAEUS, 1758)

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The Baltic cod (*Gadus morhua*) changes the diet preferences during the life time that is dependent on its ability of prey. Young fish occur mostly near the coast and feed on invertebrates, particularly *Crustacea.* The cod infection with parasitic nematodes *Hysterothylacium* sp. as well as general life cycle of these parasites is documented for the Baltic Sea. However, there is still little known which species of crustacean play the role of intermediate host in life cycle of *Hysterothylacium* sp. in the Baltic Sea. The aim of the study was to determine the intensity and prevalence of infection with parasites in invertebrate *Crangon crangon* present in the diet of cod. Cod individuals for food content analysis (n=770) were sampled in spring and autumn 2013 in the southern Baltic Sea. Parasitological analysis of invertebrates revealed the presence of *Hysterothylacium* sp. larvae in 3 of 1720 examined *C. crangon,* obtained from the stomach of cod. It suggests that *C. crangon* may play a role of intermediate host in the life cycle of *Hysterothylacium* sp. in the southern Baltic Sea. This study was financed by National Science Centrum (Poland): grant number 2015/19/N/NZ9/00173

BALTIC COD (*GADUS MORHUA*) INFECTED WITH INTESTINAL HELMINTHS VIA GAMMARUS SP.

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Invertebrates are important elements in the diet of the Baltic Sea cod (*Gadus morhua*), particularly in case of young individuals. Prey not only provides nutrients, but may be also a source of infection with parasites. The infection of cod with nematodes and acanthocephalans and the general life cycles of these parasites is well documented. Invertebrates are known as intermediate hosts in these life cycles, but the presence of parasites directly in situ in organisms that are diet components of cod have never been demonstrated before. The aim of the studies was to determine the prevalence and intensity of infection with parasites in invertebrates present in the diet of cod. In total 1075 stomachs of cod have been collected during the research and commercial cruises in the southern Baltic in 2015. Food content has been determined and invertebrates have been collected and stored for further analysis. Parasitological analysis of invertebrates *Gammarus* sp. revealed the presence of *Hysterothylacium* sp. and *Echynorhynchus gadi* (both at larval stages). The results indicate that *Gammarus* sp. may play a role of intermediate host in the life cycle of these parasites in the Baltic Sea. The presence of *Gammarus* sp. infected with *Hysterothylacium* sp. and *E. gadi* have been shown for the first time in situ in diet components of Baltic cod (*Gadus morhua*). This study was financed by National Science Centrum (Poland): grant number 2015/19/N/NZ9/00173

FASCIOLA HEPATICA SEROPREVALENCE IN CATTLE IN ESTONIA

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Trematode parasite *Fasciola hepatica* (common liver fluke) causes fasciolosis in ruminants. In cattle, the disease can affect growth, fertility, and milk production. We conducted an epidemiological study to estimate the seroprevalence and geographical distribution of *F. hepatica* in cattle in Estonia. Sera of 2461 cattle from 218 farms, collected in 2012-2013 from all 15 counties, were tested using an in-house enzyme-linked immunosorbent assay for the presence of specific anti-*F. hepatica* antibodies. The overall animal-level seroprevalence estimate was 5.9% (95% confidence interval 5.0-6.9). At least one seropositive animal was found on 28.4% (95% confidence interval 22.8-34.7) of the cattle farms; farms with at least one seropositive animal were found in all but two counties. The in-herd prevalence was 25% or higher on 14 farms, six of which were located on Saaremaa island. This is the first estimate of the seroprevalence of *F. hepatica* in cattle in Estonia. The results showed that cattle were commonly exposed to *F. hepatica*.

CHANGES IN WHITE BLOOD CELLS IN EXPERIMENTALLY INFECTED EUROPEAN SISKINS (*CARDUELIS SPINUS*) BY MALARIA PARASITE PLASMODIUM RELICTUM (LINEAGE SGS1)

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Like in mammals, bird's white blood cells belong to the innate immunity. These cells are the body's first line of defence against different infectious agents. Peripheral blood leukocytes can change rapidly in numbers and their relative proportion may be affected by parasite infection. Leukocyte profile is often used in parasitological studies to describe an immune status of birds. However little is known about immune response to avian malaria parasites. In our project we studied changes in absolute value of leukocytes and their percentage ratio (limphocytes, heterophils, eosinophils, basophils, monocytes) in European siskins (*Carduelis spinus*) in response to infection by *Plasmodium relictum* (lineage SGS1) at the stage of high primary parasitemia. 22 siskins were treated with subinoculation of infected blood mixture infected of donor blood and 22 birds were used as control. Blood samples for leukocyte counting were taken every fourth day. Besides, we measured hematocrit value and body mass of all experimental birds. In our study we did not find significant changes in the numbers of basophils and eosinophils. However we observed a gradual increase of monocytes within group of infected siskins, reaching their maximum with decreasing of erythrocytic stage of parasitemia. The interpretation of leukocyte formula changes during primary infection is not easy job as many different factors can cause those changes and circulating white blood cells show significant individual fluctuations.

ALARIA SPP. MESOCERCARIAE IN A FREE-RANGING EURASIAN BADGER (*MELES MELES*) FROM BIAŁOWIEŻA NATIONAL PARK, POLAND

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Alaria spp. are parasitic trematodes of carnivores. The parasite life's cycle comprises eight stages with two intermediate and one final host. The life cycle can be extended at the mesocercarial stage by the addition of a paratenic host such as omnivorous mammals. In Europe, *Alaria* mesocercariae have been increasingly found in game meat, particularly from wild boar and other omnivorous mammals. We present the first report of *Alaria* spp. mesocercariae infecting a free-ranging badger in the Białowieża forest, the last European primaveral forest. An adult male badger was hunted in October 2016 in the Oddizał locality, Białowieża Forest, Eastern Poland. Samples of neck muscle were tested using the well-established *Alaria* Migration Technique. In total, 28 mesocercariae were isolated from around 200 grams of neck muscle. The isolated mesocercariae were morphologically identified as *Alaria* spp.

TOXOPLASMA GONDII SEROPREVALENCE IN BREEDING PIGS IN ESTONIA

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Toxoplasma gondii is relevant from veterinary and public health point of views. While previous studies have indicated that *T. gondii* infection is common in humans, cats, and cattle in Estonia, data on the infection in the local pig population have been lacking. In this cross-sectional study, we estimated *T. gondii* seroprevalence and evaluated risk factors associated with seropositivity in breeding pigs in Estonia. Sera of 382 pigs from 14 herds were screened for anti-*T. gondii* immunoglobulin G antibodies with a commercial direct agglutination test, using 40 as the cut-off titer for seropositivity. Altogether 22 (5.8%) of the 382 pigs tested seropositive and at least one seropositive pig was found on 6 (42.9%) of the 14 herds. Gender appeared as a risk factor for seropositivity: sows had 5.6 times higher odds to test *T. gondii* seropositive than boars. Age was not a significant factor; seroprevalence did not increase with age. The results indicate that *T. gondii* was present in a substantial proportion of breeding pig herds in Estonia. The herds where no seropositive pigs were found illustrated that porcine *T. gondii* infections can be prevented. This work was also presented at the 14th *Toxoplasma gondii* research community biennial meeting in Portugal (May 31 – June 4 2017).

INFLUENCE OF FEEDING HABITS AND OTHER FACTORS ON HELMINTHOFAUNA OF MARTENS *(MARTES* SPP.) IN LATVIA AND LITHUANIA

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Pine marten and stone marten are widespread carnivorous species common in most of Europe and parts of Asia. Pine martens optimal habitats are various kinds of woodland, but stone marten typically lives in a more open areas. Materials collected in winter hunting seasons 2011 to 2014 in regions of Latvia and Lithuania. Martens (n=154) were examined for helminths using sedimentation and counting technique. Parasite species were determined microscopically using morphological characteristics. Marten feeding habits determined by examining 144 stomach contents. Food items were divided into 14 categories; mammalian taxa were identified by hair structure. Overall detected parasites belonging to four taxonomic groups: two Trematoda species, three Cestoda, 17 Nematoda and one Acanthocephala species. Helminths were localized in lungs, urinary bladder, liver, kidneys, gallbladder and intestines. Rodents (37.6%), birds (29.3%) and plants (17.7%) constituted the largest biomass and frequency of occurrence of pine marten food items. Plants (25.1%) constituted larger biomass in stone martens, but birds (12.0%) and rodents (13.7%) were consumed less. Determined food items prove to be insignificant in acquiring parasite species with highest prevalence (for example, genus *Capillaria),* as necessary intermediate hosts composed a very small percentage of biomass. No significant correlation between marten body weight and number of parasite species was found. No statistically significant differences of helminthofauna were found relative to marten species and gender, due to trophic niche overlap and little difference in behaviour of genders. Significant differences were found in mean abundance of *Capillaria plica* ($p = 0.043$), *Euparyphium melis* ($p = 0.032$) and prevalence of *C. mustelorum* ($p = 0.047$) relative to marten species. Significant differences were found in prevalence of *Capilaria putorii* ($p = 0.025$) relative to marten gender.

DO VETERINARIANS KNOW WHETHER CANINE VECTOR-BORNE PARASITES ARE ZOONOTIC?

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Canine vector-borne diseases are spreading north in Europe, and some of them are zoonotic. One of the aims of our recent questionnaire study to veterinarians in the Baltic countries (Estonia, Latvia, and Lithuania) and the Nordic countries (Denmark, Finland, Iceland, Norway, and Sweden) on selected canine parasitic vector-borne infections was to survey the knowledge on this. The questionnaire focused on canine babesiosis and infections with *Dirofilaria immitis* and *Dirofilaria repens*. The online questionnaire was open 01.01-31.01.2017, and altogether 122 veterinarians participated: 33 had their practice in the Baltic countries and 89 in the Nordic countries. Almost half (48%) of the participants knew that canine babesiosis is not a zoonosis, while a fourth (26%) knew that *D. immitis* is zoonotic and a third (34%) knew that *D. repens* is zoonotic. These results suggest a knowledge gap: the zoonotic potential of *Dirofilaria* spp. merits more awareness among veterinarians.

THE EFFICACY OF SUPRAMOLECULAR COMPLEXES OF ANTHELMINTICS AGAINST *TRICHINELLA SPIRALIS*

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The studies were made on 40 white mice which were experimentally infected with *Trichinella spiralis* at the dose of 200 larvae per head. The supramolecular complexes of albendazole with NaGA (Na_2 Glycyrrhizinic Acid) produced by mechanochemical modification of substance in activator of percussive-grating type were used in tests. Mice of different groups of 10 animals each on the third day after infection received per os supramolecular complex of albendazole and Na_2GA in different ratios (1:10; 1:5) at the dose of 2,0 mg/kg of active substance (AS) in comparison with the basic substances of albendazole which was administered orally at doses of 2.0 and 10 mg/kg. Control animals did not receive the drug. The efficacy of drugs was determined on the second day after drug administration by the results of helminthological autopsy of mice intestine and calculation the amount of larvae of *T. spiralis* in digestive material under binocular's magnification. The supramolecular complex of albendazole and Na_2GA in ratios of 1:10 and 1:5 at the dose of 2.0 mg/kg showed 92.3 and 91.8 % efficacy against larvae of *T. spiralis* respectively. Basic drug – substance of albendazole showed 90.4 % efficacy at the dose of 10 mg/kg and 23.0 % at the dose of 2.0 mg/kg. The efficacy of supramolecular complex of albendazole was approximately 4 times higher than the efficacy of the basic drug. The study was supported by RSF project No 14-16-00026.

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