

Proceedings of the Thirteenth Helminthological Days held at Ředkovec (Czech Republic) May 9 – 13, 2005

The conference 13th Helminthological Days organized by the Helminthological Section of the Czech Parasitological Society, was held at Ředkovec in the lovely countryside of the Bohemian-Moravian Highlands (CzR), May 9 – 13, 2005. A total of 65 Czech, Slovak, Austrian, French and Mexican helminthologists, including many students, participated in this meeting.

Most papers were devoted to fish helminths, their ecology, morphology and biology, several presentations reported new data from human and veterinary helminthology.

Mystery of cercarial surface structures of *Trichobilharzia* spp.

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Cercariae of *Trichobilharzia* spp. infect a wide range of birds and play an important role as the etiological agent of human cercarial dermatitis, which is now considered as an emerging disease. Unfortunately, the discrimination of species within the genus *Trichobilharzia* based on cercarial morphology is problematic. The dimensions, morphology of the excretory system, position and structure of the penetration glands and distribution of surface papillae (chaetotaxy) are used for species determination but the taxonomic value of these characteristics is often unclear. Another species of trematodes have also been studied and chaetotaxy was often used as a taxonomic criterion.

In our study, the cercariae of *T. szidati* and *T. regenti* were obtained from experimentally infected *Lymnaea stagnalis* and *Radix peregra*, respectively. Their surface structures (including chaetotaxy) were characterised and compared using scanning electron microscopy. In addition the sensory papillae were stained with 0.5 % silver nitrate and observed under a light microscope.

In both species, the tegumental surface bore posteriorly directed spines. The length of spines on the body was about 1 µm, on the tail stem and furca about 3 µm; they were densely distributed. The anterior part of the body was comprised of a head organ with gland duct openings, multiciliated, uniciliated and aciliated sensory receptors. A pore-like mouth was situated on the mid-ventral surface of the

head organ. The surface of the ventral sucker (acetabulum) was often covered with undetermined secretions. In our opinion the sucker probably contains some unknown gland duct openings. Two types of uniciliate sensory endings were distinguished: sensory endings with and without tegumental collar and with cilia of different length. Furthermore, pit-like sensory receptors on the body of cercariae were observed.

In addition to numerous studies on the genus *Trichobilharzia*, we found an extra sensory papillae on the ventral surface of cercariae of *T. regenti*. We demonstrated that chaetotaxy is a useful tool to distinguish cercariae of *T. szidati* and *T. regenti*.

Labelled lectins as a tool for differentiation of bird schistosome cercariae and for detection of changes in glycosylation during penetration of cercariae into their hosts

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The glycocalyx (saccharide coat) of cercariae is a structure that provides osmotic protection and physiological adaptation to changing environment during trematode life cycles. For cercariae in the water, this structure is absolutely essential, but after penetration into a host it could be a source of possible activation of host immune system. In order to avoid this activation, cercariae shed their saccharide coat after penetration.

Glycosylation of living cercariae was described for two species of the family Schistosomatidae: *Schistosoma mansoni* (Linder, 1984) and *Trichobilharzia szidati* (Horák & Mikeš, 1995; Horák *et al.*, 1997). On cross-sections it was characterized also for cercariae of *Trichobilharzia regenti* (Frýzková, 2004). Comparison of these four studies has shown significant differences in the glycosylation of these species. It was supposed that the type of glycosylation could be used for determination of cercariae of morphologically undistinguishable schistosome species.

During a control experiment employing a set of fluorescein-labelled lectins and two bird schistosomes (*Trichobilharzia szidati* and *Trichobilharzia regenti*) parallelly, no

clear differences were observed between these cercariae. The results of this experiment led to a conclusion that the glycosylation of cercarial tegument might not be a reliable discriminative character for determination of related schistosome species.

However, changes in glycosylation of cercariae and following post-penetration stages seem to be a good marker of transformation not only for schistosomes but also for other host-penetrating species. For example, binding of lectins specific for N-acetyl-D-galactosamine, D-galactose lactose, D-mannose/D-glucose and D-glucosamine has been detected to cercariae of the fish eye fluke, *Diplostomum pseudospathaceum*. After penetration into the host, the reactions of lectins specific for lactose and N-acetyl-D-galactosamine appeared weaker, while the reactions of lectins specific for D-mannose/D-glucose have even not been detected. A similar tendency to reduce external glycosylation of cercariae after penetration has also been observed in cercariae of the fish blood fluke, *Sanguinicola inermis*. Whether the penetration gland proteases are involved in removal of surface glycoproteins is a subject for discussion. The project was supported by grant of the Grant Agency of the Czech Republic no. 524/04/P082.

Ultrastructure of male reproductive systems in trematodes (Digenea)

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The ultrastructural characters of spermiogenesis and spermatozoon are useful tools in the interpretation of relationships in Platyhelminthes. The lack of data in the Digenea does not allow us to use these morphological characters for elucidation of interrelationships, as in Cestoda. Four species of Digenea parasitizing Mediterranean fish have been studied using transmission electron microscopy: *Diptherostomum brusinae* (Zoogonidae), *Helicometra fasciata*, *Poracanthium furcatum* (Opecoelidae) and *Monorchis parvus* (Monorchidae). During spermiogenesis, various processes have been highlighted. In *H. fasciata* and *M. parvus*, the flagellar rotation presents a particular 120° angle. A new element, called centriolar extension, was described in *M. parvus*. *P. furcatum* has a centriolar inclusion at the level of the posterior extremity of its centriole. The spermatozoon of the four species follows the general model described in the Trepaxonemata: two axonemes of the 9 + "1" pattern, a nucleus, mitochondria and cortical microtubules. Some ultrastructural elements are useful tools to compare the male gametes: spine-like bodies, lateral expansion, external ornamentations of the plasma membrane and the number of mitochondria. A particular structure of the anterior extremity of the spermatozoon of *M. parvus* is

described for the first time.

This study was supported by the Grant Agency of the Czech Republic (project no. 206/03/1317).

European bird schistosomes

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Overview of bird schistosomes occurring in Europe was given. Close attention paid to bird schistosomes and use of molecular biology techniques in their taxonomy conduces to discovery of new species. Three new species of *Bilharziella* were described from anatid birds in France. In the Czech Republic, *Trichobilharzia* sp. was found in *Motacilla alba*. It represents the first report of *Trichobilharzia* in a passeriform bird in Europe. Several unknown *Trichobilharzia* species were found also in anatid birds in France and Iceland. Unknown species of *Gigantobilharzia* and *Dendritobilharzia* were reported from the Czech Republic and France, respectively. Several findings of bird schistosomes remain still undetermined. The new findings show that the revision of bird schistosome system is necessary. Morphological approach has to be combine with molecular biology (sequencing) and ecology (life cycles) approaches.

Terminal phase of infection with bird schistosome of *Trichobilharzia regenti*

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Trichobilharzia regenti uses in its life cycle the snail *Radix peregra* and the birds (waterfowl) as intermediate and definitive hosts, respectively. Definitive hosts are infected with larvae (cercariae) penetrating their skin. Invading cercariae transform themselves to migrating schistosomula. Within the definitive host body schistosomula migrate via the nervous tissue to the target location – the nasal cavity, where the matured worms produce eggs.

In order to characterize the terminal phase of *T. regenti* infection in the birds' nasal cavity 27 ducks were infected with freshly shed cercariae. Ducks (2 – 3 specimens) were sacrificed on days 12, 14, 15, 16, 17, 19, 21, 22, 24, 25 and 27 days p.i. The soft tissues of the nasal cavity were processed for classical histology (fixed with Bouin fixative, embedded in paraffin, stained with H/E). The exact intra- and extra-vascular location of worms, their intestinal con-

tent, the periods of egg production, the egg distribution and the developmental stage of eggs were observed. The pathological impact of the parasite presence at this location was evaluated.

The first worms appeared in the nasal mucosa 14 days p.i. The highest abundance was recorded 15 days p.i. Since 24 days p.i. no worms were detected. The worms were localized extravascularly, in the connective tissue between cartilage of the turbinate and the glandular epithelium of the nasal mucosa. The intestine of adults contained dark pigmented granules indicating their feeding on red blood cells. This is in contrary to the migrating juvenile worms feeding on the particles of the nervous tissue.

The numerous eggs appeared 15 days p.i. and they were grouped in clusters of 5 – 12 specimens. The highest number of eggs was recorded 22 days p.i. The eggs were localized in the connective tissue tight to the cartilage. The eggs recorded 15 days p.i. were immature. The first fully developed miracidia within the eggs appeared 17 days p.i. Since 19 days p.i. either eggs with fully developed miracidia, or miracidia already shed and located freely in the tissue were recorded.

The worms and the eggs present in the nasal cavity caused serious pathological changes of the soft nasal tissues. Focal hemorrhages sized up to 700 µm were dispersed all over the nasal mucosa. This injury persisted from 17 days p.i. The infiltrates of lymphocytes were present around the accumulated eggs. The granuloma appeared around the eggs since 22 days p.i.

The work has been supported by Charles University Grant Agency (215/2004/B-BIO/PrF) and Czech Ministry of Education (FRVS 690/2004).

***Trichobilharzia regenti* - a causative agent of histopathologic changes of non-specific host's nervous tissue**

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Similarly as for definitive hosts (ducks), cercariae of the avian schistosome - *Trichobilharzia regenti* - are able to find and penetrate into mammalian skin where a transformation to schistosomula takes place; few hours later, the parasites invade the peripheral nerves and migrate further to the hosts CNS. Contrary to final host, however, the parasites never mature in mammals and die in various intervals post infection.

In both ducks and mammals, the pathogenesis of the CNS infections is similar and accompanied by temporary neuromotor symptoms (a complete or partial leg paralysis and/or balance disorders).

In order to characterize pathological changes in various parts of the mouse CNS (BALB/c and SCID strains, both 6 weeks old) during different phases of *T. regenti* infection, the nervous tissue was histologically and immunohistologically investigated. The interactions between various nervous cells (neurons, astrocytes) and the parasites were observed as well.

Our results confirmed the ability of schistosomes to migrate in white and grey matter as well as between meninges of the mouse spinal cord and brain. The histopathological studies showed oedema and spongiosis in various parts of the host CNS where neuronal injuries as well as proliferation of astrocytes were detected. Higher amount of damaged neurons and increase of astrocytes proliferation with progression of the infection were observed. These changes might be a source of some neuromotor symptoms developing in the infected mice.

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Cellular immune reactions of mice with alveolar echinococcosis after glucan therapy

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New alternative therapeutic strategies have been developed in experimental models due to the extreme severity of the alveolar echinococcosis (AE) (*Echinococcus multilocularis*) and its unusual resistance to surgical and chemotherapeutic strategies. One of the possibilities considered is immunotherapy.

The effect of nonspecific immunomodulator β-1,3-D glucan (GI) (*Pleurotus ostreatus*) alone or in combination with anthelmintic albendazole (ABZ) on selected cellular immunological parameters in mice with AE was observed. Glucan was administered at a dose of 5 mg/kg body weight (b.w.) intramuscularly twice a week from week 5 to 6 post infection (p.i.) and ABZ at a dose of 10 mg/kg b.w. orally twice a week from week 5 to 10 p.i.

GI administration stimulated the proliferative response of T and B lymphocytes to concanavalin A and lipopolysaccharid, which was suppressed by the parasite, only on week 8 and 6 p.i. The enhanced T and B lymphocyte proliferative activity during ABZ therapy was noticed on week 6 – 12.p.i.

In contrast to the group of infected non-treated mice, we recorded the short-time increase of CD4+T lymphocyte subpopulation on week 8 p.i. after the GI application. The CD8+T lymphocyte subpopulation was not influenced by the immunotherapy.

GI alone and also GI+ABZ significantly inhibited the Th2

response during the whole experiment (low IL-5 cytokine level in the serum of treated mice). Th1 response was stimulated by GI+ABZ therapy (IFN- γ concentration in serum risen two times from the week 10 p.i. till the end of the experiment in comparison with the other groups).

The long-time increase of O₂⁻ production after GI application was observed on the week 6 – 14 p.i. The GI+ABZ combination elongated the stimulative effect of immunomodulator till the week 18 p.i.

The activation of the immune system after the immunotherapy restricted the metacystode growth in the host until the week 8 p.i. We have found the most significant larval growth reduction in group of mice treated with ABZ. In comparison with ABZ therapy, the GI administration didn't have such reducing effect on parasitic biomass. GI+ABZ even partly inhibited the parasitostatic influence of ABZ and the amount of cysts in this group of mice increased.

The results suggest induction of Th1 immune response in mice with AE and treated by glucan. Th1 reaction is connected with resistance to *Echinococcus* infection. GI also stimulated the metabolic macrophage activity and caused the long-time increase of O₂⁻ production.

Despite the positive influence of immunotherapy on the host nonspecific immunity glucan didn't restrict the metacystode growth and in combination with ABZ partially suppressed the efficiency of anthelmintic. These results indicate that β -1,3-D glucan is not suitable drug for the AE immunotherapy.

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Parasitic helminths of stranded manatees and cetaceans along the coast of the Yucatan Peninsula, Mexico

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Marine mammal strandings along the coast of Mexico are very common and poorly documented. Between 1991 and 2004, around 20 cetacean and sirenid stranding events (130 dead individuals) were reported in the coasts of the Yucatan Peninsula. This survey shows the results of necropsies applied to 13 recently dead specimens of the most commonly stranded species: the Caribbean manatee, *Trichechus manatus manatus*, pygmy sperm whale, *Kogia breviceps* and short-finned pilot whale, *Globicephala macrorhynchus*, from 6 localities (Guerrero lagoon, Raudales lagoon, Cozumel, Chetumal Bay, Progreso, El Cuyo).

Three helminth species were recovered from 6 manatees: 1 nematode (*Heterocheilus tunicatus*) and 2 trematodes (*Cochleotrema cochleotrema*, *Chiorchis groschaffii*). Pygmy sperm whale (n = 4) harbored 3 nematodes (*Anisakis brevispiculata*, *A. simplex*, *Pseudoterranova ceticola*). Short-finned pilot whale (n = 3) had 2 nematodes (*Stenurus globicephala*, *S. minor*) and 1 trematode (*Nasitrema globicephala*). All helminths showed host specificity since each host species had its own parasite fauna. Manatees and pygmy sperm whales recovered from different localities carried a similar nematode fauna, it could be related with their broad distribution in the region. In spite of histopathological studies were not achieved, it is important to remark that *Stenurus globicephala* might be a significant factor on the natural mortality, organic weakening or disorientation of cetaceans because of its presence into the cranial sinuses of the hosts.

Cysteine and serine peptidases of *Trichobilharzia regenti* and *Schistosoma mansoni* cercariae

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Cercariae of the family Schistosomatidae actively penetrate the host skin. The mechanisms of penetration are not still sufficiently understood. However, due to the pathogenicity of human schistosomes (e.g. *Schistosoma mansoni*) and difficulties in maintenance of some species, non-human species can serve as an alternative laboratory model. The *Trichobilharzia regenti* species is a bird schistosome with a unique kind of migration among schistosomes. In contrast to *S. mansoni*, *T. regenti* migrate through the peripheral and central nervous system (spinal cord, brain and olfactory nerves) to the nasal cavity of a water bird definitive host of the family Anatidae (Hrádková and Horák, 2002).

Proteolytic enzymes (peptidases) of *T. regenti* and *S. mansoni* cercariae are probably essential for the penetration process. Among these enzymes, cysteine and serine peptidases of cercariae of *T. regenti* were biochemically characterized and compared to peptidases of *S. mansoni* cercariae. Cercarial extracts were fractionated and activities of both peptidase types were detected.

Two protein bands, 17 kDa and 28 kDa, from a *T. regenti* fraction with the highest cysteine peptidase activity were characterized by mass spectrometry methods and de novo sequenced.

Molecular techniques were used for screening the cDNA *T. regenti* expression library employing PCR with primers

based on the sequences of cathepsin L (*Boophilus microplus*; Renard *et al.*, 2000) and B (*T. regenti*-schistosomula; Dvořák *et al.*, 2005). Two fragments corresponding to putative cathepsin L (500 bp) and B (618 bp) were amplified. The results imply that similar strategies are probably employed for skin penetration by the two schistosomatids, although some significant differences have been found in enzymatic equipment of the cercariae.

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Cathepsin L in cercariae of *Diplostomum pseudospathaceum*

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A 24 kDa lectin with specificity to saccharide chains containing glucose and galactose or their derivatives linked in beta-1,3 and beta-1,4 positions was isolated from cercarial extracts of the fish eye fluke *D. pseudospathaceum*. It was shown to possess also cysteine peptidase activity. Fluorometric substrate assays showed a strong peptidolytic activity with Z-Phe-Arg-AMC with a maximum under slightly basic conditions; this could be inhibited by irreversible cysteine protease inhibitor E-64. Heparin, a saccharide ligand inhibiting hemagglutination activity of the protein, strongly reduced the peptidolytic activity.

Parasite mRNA was isolated from cercarial germ balls within sporocysts and transcribed by RT-PCR using a forward primer based on a tryptic peptide sequence obtained by mass spectrometry and a reverse oligo-dT primer. The whole sequence of the molecule was obtained employing 3' and 5' RACE methods. The protein showed similarities to clan CA cysteine proteases of different origin, being highly similar to cathepsin Ls of adult *Fasciola gigantica* and *F. hepatica* liver flukes. Immunohistochemistry as well as ligand histochemistry confirmed localization of the peptidase in penetration glands of cercariae. Involvement of the protein in cercarial penetration is a subject of further research.

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Cathepsin B2 from cercariae of bird schistosome *Trichobilharzia regenti*

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Bird schistosome *Trichobilharzia regenti* is recently an intensively studied parasite not only for the ability of its lar-

vae (cercariae) to cause cercarial dermatitis (swimmer's itch) in humans, but also for its unusual way of migration, which involves peripheral nerves, spinal cord, medulla oblongata, brain and final place of localization – nasal cavity of their definitive bird host (Hrádková & Horák, 2002).

Recent findings in human schistosomes show that proteolytic enzymes play a crucial role in the process of penetration and migration of the parasite; these have also been recognized as potential targets for chemotherapy and sero-diagnostics (Caffrey *et al.*, 2002). Due to the limitation by insufficient amount of material for biochemical assays, molecular methods were used to gain some information about these enzymes in *T. regenti* cercariae.

We performed isolation of poly (A) RNA from sporocysts with developing cercariae (present in the hepatopancreas of infected snails), RT-PCR and subsequent semi-nested PCR with degenerate primers designed according to conserved domains of known cathepsin B from related *Schistosoma* species. We identified a 1032 bp sequence which corresponds to a 344 aminoacid chain. The protein contains all conserved domains characteristic for a cysteine protease, namely for cathepsin B. Due to its 77 % similarity to SmCB2 (a tegumental cathepsin B2 from adult *S. mansoni* – Caffrey *et al.*, 2002) and 78 % similarity to cathepsin B endopeptidase from *S. japonicum*, we termed this sequence TrCB2.

Our finding of the cathepsin B2 in sporocysts with developing cercariae is quite interesting because all attempts to find his orthologue (SmCB2) in cercariae of human *S. mansoni* were unsuccessful (Caffrey, personal communication). This shows that bird schistosome *T. regenti* is a good comparative model to human schistosomes *S. mansoni* and *S. japonicum*.

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Metazoan parasites of *Neogobius kessleri*, recently introduced fish species in the Middle Danube basin

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The expansion of gobiid fish was registered in waters of Middle Danube basin during few recent years. In present, bighead goby (*Neogobius kessleri*) represents a dominant fish species in several localities of the Danube River basin in Slovakia. Specimens of *N. kessleri* were collected from six localities in the Slovakian part of Danube River (former and current main channel), from two adjacent backwaters,

and from lower Hron and Váh Rivers during 2002 to 2004. All fish were parasitized. Thirty three species of metazoan parasites were recovered from bighead goby, when most of the parasites were common species in the central Danube River region. In the native area of bighead goby distribution, lower Danube River, 23 parasite species have been reported. Seven widely distributed parasite species (*Bucephalus polymorphus*, *Diplostomum spathaceum*, *Tylodelphys clavata*, *Pomphorhynchus laevis*, *Camallanus lacustris*, *Ergasilus sieboldi* and glochidia of Unionidae) have occurred in both native and non-native area. In total, 88 % of all parasites found composed by glochidia of Unionidae and acanthocephalan. Glochidia of *Anodonta anatina* and *Pomphorhynchus laevis* were the dominant parasite species. Larval and immature parasites dominated in all fish examined, where bighead goby served as the intermediate or paratenic host.

Metazoan parasites of deep-sea fish, with a focus on pseudophyllidean cestodes

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Records of parasites from deep-sea fish indicate that they are as heavily infected as are most fish in shallow-water habits. The deep-sea parasite fauna is mainly composed of digeneans and crustaceans, but also cestodes, monogeneans, myxozoans, nematodes and rarely acanthocephalans. Among tapeworms (Cestoda), the Tetracyllidae and Trypanorhyncha are the most common in elasmobranchs (sharks and rays), Gyrocotylidae in chimaerids and Pseudophyllidae in teleosts. As many as seven genera of pseudophyllidean cestodes are known only from deep-sea fish, namely *Australicola*, *Bathycestus*, *Philobythos*, *Philobythoides*, *Pistana*, *Probothriocephalus* and *Tetrapapillocephalus*. During a field trip to the North Atlantic, Outer Hebrides, the following tapeworms were found in 2004: *Amphicotyle heteropleura*, *Bothriocotyle solinosomum* and *Echinophallus wageneri* in the blackfish, *Centrolophus niger* (prevalences 100 %, 50 % and 50 %, respectively), *Bathycestus brayi* in *Notacanthus bonaparte* (29 %) and *N. chemnitzii* (18 %; new host), and *Philobythos atlanticus* in *Coryphaenoides rupestris* (11 %; all cestodes immature). Gyrocotylideans (*Gyrocotyle* spp.) were found in *Hydrolagus pallidus* and *Harriotta raleighana* for the first time and may represent a new species. This study was supported by the Grant Agency of the Czech Republic (No. 524/04/0342), European Union Access to Research Infrastructures IHP (contract HRRI-CT-2003-00180), and research projects of the Institute of Parasitology, AS CR (Z60220518) and Faculty of Biological Sciences, University of South Bohemia (MSM 6007665801).

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Paraphyly of pseudophyllidean tape-worms (Cestoda): testing a phylogenetic hypothesis using sequence data (18S rDNA)

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Phylogenetic relationships of cestodes of the order Pseudophyllidea (Platyhelminthes: Cestoda) were examined using complete sequences of 18S rDNA. The results support the idea that the Pseudophyllidea are paraphyletic as indicated by previous analyses that were based on much lower number of species sequenced (Mariaux, 1998; Kodedová *et al.*, 2000; Olson *et al.*, 2001). The order is formed by two unrelated clades, "Bothriocephalidea", with close relationship to tetrafoosate orders, and "Diphyllobothriidea" forming a distinct clade with unclear relationship to other difosate orders.

Comparative study on the fauna of parasitic oxyuroids (Nematoda: Oxyuroidea) of reptiles from Azerbaijan and selected areas of the Near East

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Parasitic nematodes (Nematoda) represent the largest group of helminths, and are of mayor medical and veterinary importance. They also represent the most frequent parasites occurring in reptiles. Oxyuroid nematodes (Oxyuroidea) of amphibians and reptiles (family Pharyngodonidae) are one of the evolutionary oldest groups of nematodes of vertebrates. Thus they may serve as a suitable model to study various aspects of relationships between parasites and their hosts such as host specificity, coevolution with their hosts or the phylogeny of individual taxa of the nematodes. Despite of this fact, the data on oxyuroid nematodes of reptiles are rather scarce in literature and so far no synoptic survey dealing with this problem has been carried out. In this study, 180 lizards from the families Agamidae, Gekkonidae, Lacertidae and Scincidae were collected in Azerbaijan and selected areas of the Near East (Egypt, Iraq, Jordan, Lebanon and Syria) and examined for oxyuroid nematodes with emphasis given to the following genera: *Parapharyngodon*, *Pharyngodon*, *Skrjabinodon*, *Spauligodon* and *Thelandros* (Pharyngodonidae). A total of 8 species were found: *Parapharyngodon brevicaudatus*, *Pharyngodon mamillatus*, *Pharyngodon* cf. *termezensis*, *Spauligodon* cf. *eremiasi*, *Spauligodon saxicolae* and *The-*

landros popovi in Azerbaijan and *Parapharyngodon tyche* and *Thelandros* sp. in Syria, from which nematodes marked as *Thelandros* sp. possibly represent a new species. This study represents the first record of *Pharyngodon* cf. *termezensis* in Azerbaijan and the very first record of pharyngodonid nematodes from reptiles in Syria. *Eremias velox caucasica* represents a new host to *Spauligodon* cf. *eremiasi*, *Darevskia caucasica* is a new host to *Spauligodon saxicolae* and *Laudakia stellio picea* and *Trapelus ruderatus* represent new hosts to *Parapharyngodon tyche*. The nematodes were taxonomically evaluated, including scanning electron microscope (SEM) observations, and several aspects of their ecology and parasite-host relationships were discussed. A preliminary survey on the prevalence, intensity, host specificity and geographical distribution of oxyuroid nematodes of reptiles from the selected areas of the Near East was carried out. It was found that oxyuroid nematodes of reptiles can serve as a suitable model for the studies on coevolution of parasites and their hosts and that the Near East represents a good model locality because in this area overlap the areas of geographical distribution of the families of lizards studied.

Molecular phylogeny of dracunculoid nematodes based on SSU rRNA genes

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Phylogenetic relationships of the superfamily Dracunculoida (Nematoda: Spirurida) were studied using the SSU rDNA sequences. In phylogenetic trees constructed by maximum likelihood and parsimony methods dracunculoid nematodes constituted a monophyletic group to the exclusion of Anguillicolidae (*Anguillicola crassus*). First molecular data obtained for *Dracunculus medinensis* enabled assessment of the relationships of this important human parasite with other dracunculoids. SSU rDNA based trees are only in partial agreement with trees constructed on the basis of morphology and life cycles.

Helminthofauna of *Chondropython (Morelia) viridis* and its treatment with anthelmintics

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The Green Tree Python (*Chondropython viridis*) is a rare

species of pythons in Czech terraria, famous by his bad adaptation in captivity. One of the factors breaking the adaptation are parasites in wild captured animals. Some of these parasites can be rather pathogenic.

For 2 years we have monitored the health status of imported snakes. We examined coprological samples from living animals and made dissections of dead snakes. Unfortunately there was no possibility to make an examination for blood parasites.

In coprological samples (n=79) of 57 Green Tree Pythons we identified eggs of *Kapsulotaenia* sp., *Capillaria* sp., *Physaloptera* sp., *Kalicephalus* sp., eggs of the nematodes from the Heterakoidea group and pentastome eggs.

From necropsies (n=45), the same groups of parasites were recorded as from coprological samples plus one specimen of an unidentified spirurid larva.

Infections in snakes were treated with several sorts of anthelmintics (Panacur, Paraquistel, Fenbion) with different effect. The most sensitive to treatment were *Kalicephalus* sp. and heterakoid worms. In *Capillaria* sp. and *Physaloptera* sp., significant reduction of egg number was noted following treatment. Effects on *Kapsulotaenia* sp. and pentastomes were not observed.

A link between variability of MHC IIB genes and metazoan parasites in the selected populations of the chub (*Leuciscus cephalus* Linnaeus, 1958)

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The genes of the MHC (major histocompatibility complex) play a central role in mediating a vertebrate's immune response to diseases and parasites. The MHC family is divided into two major subfamilies, class I and class II. In the teleost fishes, the class I and II genes are not localized on the same linkage group. The MHC loci are characterized by high levels of polymorphism, and the products of these genes are the sign of individuality. The allelic lineages of MHC are often shared by related species (this feature is called as trans-species polymorphism). Variability of MHC loci is maintained by two main types of mechanisms: the pathogen-driven balancing selection and reproductive mechanisms. There are two basic types of the hypothesis of the pathogen-driven balancing selection: (1) overdominance hypothesis based on the advantage being heterozygotes at MHC alleles, which permits to recognize wider range of antigens derived from the pathogens than in the case of homozygotes, (2) negative frequency dependent selection hypothesis (or rare-allele advantage) proposing that the individuals with a rare MHC allele respond better to new pa-

thogens. The hypothesis of MHC dependent mating preferences proposes that selection of MHC alleles contributing to the immune recognition of pathogens or parasites could be connected with sexual selection which may provide a choice of "good genes" in order to increase the offspring resistance. Considering the class II B the polymorphism is concentrated in exon 2 corresponding to B1 domain of this class which includes the peptide binding regions (PBR). The PBR should interact with the parasitic antigens or parasites, the source of the antigen, are likely the selective agents. The goal of this study was to investigate the variability in exon 2 of class II beta-encoding genes (DAB genes) in four populations of the chub (*Leuciscus cephalus* L.) collected from the different water basins not historically connected, and evaluate the potential role of the metazoan parasites on the MHC genes differentiation. The results indicate the relationship between MHC allelic diversity and parasites.

Inter- and intraspecific genetic variability of diphyllbothriid tapeworms

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We examined inter- and intraspecific genetic structure of diphyllbothriid tapeworms using ITS-2 rDNA sequences. At the interspecific level, our data show: (1) that the inclusion of ligulids (previously family Ligulidae) to the Diphyllbothriidae is correct; (2) *Schistocephalus* is the most basal taxon of the Diphyllbothriidae; (3) *Digramma* appears within samples of *Ligula*, thus suggesting its invalidity as a genus. At the intraspecific level, the analysis was focused on resolving the genetic structure of *Ligula intestinalis* population in respect to its fish-host spectrum and geographical distribution. Another two species, *Digramma interrupta* and *Ligula colymbi*, whose monophyly in respect to *L. intestinalis* has been questioned, were included into the analysis. Our results show that (1) several ITS-2 haplotype groups of *L. intestinalis* can be identified within the population; (2) the haplotypes obtained from *Gobio gobio* isolates (N. Ireland) and from *Giardinichtys multiradiatus* isolates (Mexico) were found to be more closely related to *Digramma interrupta* than to *L. intestinalis* samples; (3) *Ligula colymbi* was found to be an assemblage of various "L. intestinalis-like" haplotypes.

A link between immunocompetence of chub (*Leuciscus cephalus* L.) and parasitism

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The role of immune function is recently widely investigated in the context of trade-off between the different life-history traits. Each potential host is exposed to variety of parasites that may negatively influence its viability, and then the host must develop a performing immune system in order to generate the resistance towards the parasites and to reduce the fitness losses caused by parasitism. Therefore, we hypothesized and tested a link between fish immunocompetence and parasitism. We performed the investigation of chub parasitofauna from one locality of Svitava River in four seasonal samples (spring, early summer, late summer and autumn) during 2004. Several measures connected fish immunocompetence and physiological status were selected and measured: blood cell counts, hematocrit, leucocrit, phagocyte activity and spleen size. We tested the seasonal changes in those parameters. Parasite communities of all seasonal fish samples were analysed. The total parasite species richness varied from 17 to 20 per sample. The highest parasite diversity was recorded in the first sample (Shannon index diversity). Monogenea were the group with the highest prevalence and mean abundance in all samples. Digenea were the second abundant group in all samples. The high values of prevalence but low mean abundance were recorded for Acanthocephala in the first and third samples (spring and later summer). The positive correlation between spontaneous and activated phagocyte activity was found in the majority of fish in all samples. The statistical significant differences were found among seasonal samples for the groups Monogenea and Mollusca being more abundant in spring, Cestoda being more abundant in early summer and Acanthocephala being more abundant in autumn. Infracommunity metazoan parasite species richness was significantly higher in spring sample. Moreover, we found significantly higher numbers of protozoan *Myxobolus* spp. in autumn sample. The analyses of immunocompetence revealed that spleen size is higher in autumn sample. Measures connected with phagocyte activity were higher in late summer or autumn samples. Special types of neutrophils were found in significantly higher numbers in the spring samples. The results indicated the potential connection between fish immunocompetence and parasitism.

Host specificity of congeneric monogeneans (*Dactylogyrus*) in cyprinid fish

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Processes connected with host specificity were investigated in congeneric monogenean parasites including 51 *Dactylogyrus* species parasitizing 19 fish species belonging to Cyprinidae and one fish species belonging to Percidae. Phylogenetic information obtained from molecular analysis of 18S rDNA and ITS1 of *Dactylogyrus* and cytochrome *b* of cyprinid fish were included in the analyses. We tested whether host specificity is constrained by parasite and host phylogeny. Morphometric correlates of host specificity i.e. parasite body size and variables connected with attachment organs were studied. Determinants of host specificity were investigated following the hypothesis of specialisation on more predictable resources such maximal host body size, maximal longevity and abundance. Host range was calculated for each parasite species. Moreover, index of host specificity was defined for this study including host relatedness.

Host specificity was constrained by parasite phylogeny, i.e. it was related to intra-host speciation of *Dactylogyrus* species. No relationship between host specificity and fish host phylogeny was found. Specialist trait was showed as an ancestral state for *Dactylogyrus* species. We confirmed the specialisation on more predictable resource, i.e. the specialist parasites with larger body size colonized the fish with higher maximal longevity. Specialists with larger anchors (a part of attachment organs) tend to live on longer lived and/or larger body sized fish species. No morphometric association between the traits of preferred host and generalist parasites was found (*Dactylogyrus* generalists parasitize one preferred and several supplementary hosts). Evolution of morphology of attachment organ is connected with host specificity and fish relatedness. However, it seems that even generalists can generate morphological adaptation to their hosts.

The analysis of the occurrence and seasonal dynamics of the Monogenean parasites infesting gudgeon (*Gobio gobio*, L.) in the River Haná

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During the two year study a total of 230 specimens of gudgeon (*Gobio gobio*, L.) were investigated for occurrence of monogenean parasites. Fish were examined monthly. 3685

and 2260 specimens of seven species from three genera were found in 2001 and 2002, respectively. The most abundant monogenean species were *Dactylogyrus cryptomeris*, *Gyrodactylus gobiensis* and *G. gobii* in both studied years. Other parasites observed were specialist species for gudgeon *G. markakulensis* and generalists *G. vimbi*, *G. gasterostei* and with the lowest prevalence *Paradiplozoon homoion*. Average abundance of *D. cryptomeris* was about 8 specimens per fish in both years. In the case of gyrodactylids the average abundance was 25 and 11 in 2001 and 2002, respectively. The presence of monogeneans is closely connected to water temperature. The peak of gyrodactylids was in both years two months earlier than the peak of dactylogyrids. The difference was the gyrodactylids reached maximum numbers in March in 2001 and May in 2002 following the curve of water temperature. The maximum intensity of infection was 92 specimens of *D. cryptomeris* (July 2002), 148 of *Gyrodactylus* sp. (May 2001) and 2 *P. homoion* (July 2002) on single fish. All the specimens of *D. cryptomeris* and *P. homoion* were unreservedly attached on the gills. Almost 90 % of Gyrodactylids were situated on the fins, than body surface and the gills. In spite of the same parasite species in both years were found, in 2001 the highest number of fish was attacked by five, in 2002 by three monogenean species.

Microhabitat specificity of *Gyrodactylus* species Nordmann, 1832 on stone loach (*Barbatula barbatula* L., 1758) in the Haná River

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Microhabitat specificity of 8 *Gyrodactylus* species parasitising gills, fins and body surface of stone loach (*Barbatula barbatula*) originating from River Haná, Czech Republic, were investigated during the period from January 2001 to December 2002. A total of 10484 gyrodactylids were collected from 300 specimens of the host fish infected with range of 1 – 202 parasites. At low intensities (1 – 10 parasites per fish), the ventral fins were preferred site of attachment (in 41 %), followed by the pectoral and dorsal fins. With increasing intensity (11 – 50 parasites and more), parasites mainly on the ventral and pectoral fins were located. However, the distribution of parasites on the fish varied during the year with infection intensity. Also preferred sites of each species parasitising stone loach were analysed. Specimens of *G. nemachili* and *G. menschikowi* occur on the gills of the host, very rarely on the external sites. In 51 %, the specimens of *G. barbatuli*, as the species with the biggest and most massive central hooks of attachment apparatus from all species parasitising *B. barbatula*, were present on the body surface. The presence of *G. jiroveci*, *G. papernai*, *G. pseudonemachili*, *G. sedelnikowi*

and *G. pavlovskyi* was restricted on the external sites with slight differences in the preferred location of each species, the moving on the gills was found exceptionally. In relation to the size and shape of parasite opisthaptor hard parts microhabitat specificity of particular parasite species were analysed.

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Comparison of the effect of quality dust lime and dust rejects from lime production on the survival of *Ascaris suum* eggs

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The effect of two types of lime on the survival of parasitic germs in organic wastes was studied under laboratory conditions. Quality dust lime and dust rejects from lime production caught on the electrostatic precipitator was used in the experiment. A 24 hour after application of both types of lime at concentration 20 g.kg⁻¹ manure more than 80 % of model unembryonated *Ascaris suum* was devitalised. *A. suum* eggs were totally devitalised as early as till 36 days after application of lime in manure. For sanitation of organic wastes, the use of dust rejects from lime production is thus appropriate due to their cost availability.

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Occurrence of gastrointestinal parasites and bacteria in a combined small-scale farm of sheep and goats

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The aim of our study was to estimate a parasitostatus in a combined small-scale farm of sheep (Tsigaya and cross-breed) and goats (Slovak White Short-haired) with an ecological breeding, (i.e. without any treatment on the basis of chemical components), where animals were kept on deep bedding. Totally 120 faecal samples (30 from each category – lambs, adult sheep, kids, adult goats) were coprologically examined and the total counts of selected bacterial genera (staphylococci, streptococci, enterococci and Gram-negative bacteria from the family Enterobacteriaceae) were

evaluated as well. Results were compared with results from examinations of both animal species from several separated large-scale farms in Slovakia (445 and 530 faecal samples from sheep and goats, respectively). In the observed combined small-scale farm the lower numbers of *Eimeria* oocysts (687.5 – 4610 OPG) were recorded in comparison with large-scale farms (4916 – 11941 OPG). On the contrary, the numbers of gastrointestinal and lung parasites eggs and/or larvae determined in the small-scale farm were higher than those in the large-scale farms. The exception to the results described above was the number of *Trichuris* spp. and *Muellerius* spp. eggs and/or larvae determined in sheep. In the small-scale farm the occurrence of eggs and/or larvae of both parasite species was only sporadic, however in large-scale farms the numbers of *Trichuris* spp. eggs ranged from 45 EPG (adult sheep) to 91 EPG (lambs) and numbers of *Muellerius* spp. larvae ranged from 21 LPG (adult sheep) to 50 LPG (lambs). There were not significant differences between animals of both species from both kinds of farms in the total counts of selected bacterial genera. The total counts of Gram-positive bacteria ranged from 10⁵ to 10⁷ CFU.g⁻¹ and the total counts of Gram-negative bacteria were 10⁷ – 10⁸ CFU.g⁻¹. Although the higher numbers of helminth eggs were found out in the small-scale farm, clinical symptoms of helminthoses were not observed in examined animals in contrast to large-scale farms. Despite animals from the small-scale farm are kept on deep bedding without anticoccidial and anthelmintic treatment, their health condition is relatively satisfying. It is probably due to low concentration of animals and breeding of breeds resistant to infective, parasitic and/or dietary diseases, which is confirmed by our results.

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Examination of immunodiagnostic in mouflons (*Ovis musimon*) infected by lancet fluke (*Dicrocoelium dendriticum*)

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Immunological methods can be used for diagnostics of dicrocoeliosis in farm and free living infected animals, too. Immunodiagnosics give us possibility to determine post infection - prepatent period, when the coprological examination is still negative because of specific antibodies appear on the second week after the infection. This is 6 – 12 weeks before the detection of the first parasitic eggs in faeces. Immunodiagnosics can be also used in case of

monitoring of parasitosis in different animal populations and for evaluation antiparasitically oriented control programmes. This study immunologically monitored lancet fluke infection in moufflon population bred in game enclosure by the detection of specific humoral immune response on two types of *Dicrocoelium dendriticum* antigens – somatic (S) and excretory-secretory (ES).

In game enclosure Vlkov p. O. (Czech Republic, 145 hectares, 120 heads of moufflons) long-term antiparasitic control programme (1996 – 2004) was realised. This programme was based on administration of albendazole. The anthelmintic therapy continually reduced parasitological values. The indirect ELISA method was selected with the aim to determine seroprevalence of specific antibodies and to use these immunological findings for wider healthy consideration of studied moufflon population. In total 82 moufflon sera obtained from previously caught animals were immunologically tested. Serological diagnostic results using both S and ES antigens were similar, only the optical density (OD) values of IgG antibodies were moderately higher (no statistically significant differences) in the test with ES antigen. The detection of specific IgG antibodies in moufflon sera by using S antigen positively evaluated 73 samples, i.e. 89.02 % seroprevalence of dicrocoeliosis. Anti-*Dicrocoelium* antibodies against ES antigen were detected in 77 samples, i.e. 93.9 % seroprevalence. The OD values of IgG antibodies exceeded the cut off point 1.5 – 2 times in both tests with S and ES antigens in majority of samples and a few sera reached higher OD values. The role of an animal age in relation to the production of specific antibodies was not found, but moufflon ewes showed higher OD values with both antigens in all age categories in comparison with moufflon rams. The specific antibody levels in infected moufflons were not markedly high. This reflects the situation in the game enclosure, where the intensity of *D. dendriticum* infection has been decreased, but the parasite still persists there and animals are suffering from chronic infection. Our results confirmed the equivalent role of somatic and excretory-secretory antigens for use in indirect ELISA test and that both antigens are suitable for serodiagnosis of dicrocoeliosis in population of moufflons, too.

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The influence of aerobic thermophilic composting for viability of model *Ascaris suum* eggs

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The survival of the model *Ascaris suum* eggs in the aerobic thermophilic composting process was studied under operating conditions in the Composting factory TOPVAR a. s. in Topolčany. The composting process proceeded at the retention time of 104-days during the winter season. Waste from the beer production, sludge from waste water treatment plant and plants substances (straw, wood chips and sawdust) were composted in the composting pile. The physical and chemical parameters: pH, dry matter, inorganic and organic matters, concentration of NH_4^+ , total nitrogen and total phosphorus and C:N ratio were observed in 3 monitoring places (2.5 m, 22.5 m and 47.5 m) of composting pile (50 m length and 1,5 m height). The pH values ranged from 6.47 to 7.71. The increase in the amount of organic matter and reduction of the concentration of dry matter, inorganic matter, NH_4^+ and C:N ratio were recorded at the end of the experiment. In contrast to middle part of the composting pile, the concentration of total nitrogen and total phosphorus in organic substance were increased in the front and rear part of the composting pile. Due to a high temperature and changes in physical-chemical properties in composted material *A. suum* eggs were totally devitalized as early as between 5 – 6th day of composting (5.66 ± 0.58 day).

Based on the results, it may be stated that that aerobic thermophilic composting process can be used for the devitalisation of helminth eggs (eggs of farm animals with zoonotic nature in their excrements as well as human helminth eggs which are potentially present in sludge from waste water treatment plant). From the hygienic and epidemiologic point of view, wastes treated by aerobic thermophilic composting process are safe and suitable for further use as fertiliser with direct application in the soil, according to the Instructions 1774/2002 European Parliament and Council, without subsequent contamination of surrounding ecosystem with endoparasite germs.

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Current distribution of the giant liver fluke *Fascioloides magna* in the Czech Republic

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The giant liver fluke *Fascioloides magna* is an important parasite of a variety of wild and domestic ruminants. The aim of the present work is to determine the current geographic distribution of *F. magna* in cervids in the Czech Republic. Faeces of red (*Cervus elaphus*), fallow (*Dama dama*) and white-tailed deer (*Odocoileus virginianus*) were

collected from 16 different areas of the Czech Republic from October 2003 to February 2005. The areas were chosen on the basis of reports from hunting associations, county veterinary offices and previous studies by Erhardová-Kotrlá in the 1960's. Samples of fresh faeces were examined by sedimentation and the eggs of *F. magna* were counted. The average number of EPG (eggs per gram) and prevalence for each area were calculated. The prevalence of *F. magna* in enzootic areas varied from 25 % to 96 %. Moreover, new foci of *F. magna* infection were discovered. The highest average number of EPG (53 eggs per gram) was detected in game fallow deer in Sedlišťe (Pilsen South county). Livers of hunted deer were dissected from each locality to confirm *F. magna* infection. Up to 40 adult flukes were found in the liver of cervids. While no clinical symptoms were observed in red and white-tailed deer, weight loss, decreased quality of the head and sporadic deaths of fallow deer were detected in one locality.

The occurrence of anthelmintic resistance in nematode parasites of sheep in the Slovak Republic

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The occurrence of anthelmintic resistance was investigated on 28 sheep flocks in Slovakia during the period from June to November 2003 and 2004. Faecal egg count reduction test (FECRT) was used to detect efficacy of albendazole (28 flocks) and ivermectin (27 flocks). Also *in vitro* egg hatch assay (EHA) and larval development assay (LDA) were used to detect benzimidazole resistance. Resistance to benzimidazoles was present on one farm (FECR = 84.9 %, C.I. = 67.9 – 92.9 %, EHA: ED50 = 0.367 µg/ml TBZ, LDA: LD50 = 0.1480 µg/ml TBZ) and suspected resistance to benzimidazoles on one farm (FECR = 98.1 %, C.I. = 84.8 – 99.8 %, EHA: ED50 = 0.072 µg/ml TBZ, LDA: LD50 = 0.137 µg/ml TBZ). On 20 farms (74.1 %) out of 27 investigated, ivermectin was less effective than 95 %, which could suggest the presence of ivermectin resistant nematodes. These results could be also explained by lower efficacy of generic ivermectin anthelmintics used in FECRT, that may not be satisfactorily effective. Thus direct comparison of the efficacy of generic product with the innovator product in selected farms needs to be determined.

Intermediate hosts of the large American liver fluke *Fascioloides magna* in the Czech Republic

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The large American liver fluke *Fascioloides magna* (Digenea: Fasciolidae) was imported to Europe in the 19th century. In North America, a number of lymnaeid snails serve as intermediate hosts of this pathogenic parasite. In Europe, only *Galba truncatula* is known to be a natural intermediate host of *F. magna* (*Lymnaea palustris* was infected experimentally). The present study surveyed the occurrence of larval stages of *F. magna* and other trematodes in *G. truncatula*, and other potential intermediate hosts in five localities in the Czech Republic with recent heavy infections of game animals (*Dama*, *Cervus*). Eleven species of aquatic molluscs were examined from May 2003 to November 2004 but larvae (rediae and cercariae) of *F. magna* were found only in *G. truncatula* (in 16 of 451 snails examined in 5 localities, i.e. prevalence 3.5 %). In 2 *Radix peregra* (0.1 %) from a small game reserve in southwestern Bohemia, rediae morphologically indistinguishable from those of *F. magna* were found. However, their species identity has to be confirmed by further studies. The snails *G. truncatula*, *R. peregra* and *Lymnaea stagnalis* were exposed in the laboratory to miracidia of *F. magna* to test their potential susceptibility to the parasite. Mature cercariae were observed only in experimentally infected *G. truncatula*, whereas mother rediae were in *L. stagnalis*.

Digeneans of *Galba truncatula* in the „National park Donauauen“ in Austria – preliminary report

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In August 2004 we started an investigation within the area of the „Nationalpark Donauauen“, Lower Austria and Vienna, to examine the prevalence of *Fascioloides magna* in *Galba truncatula* (and *Stagnicola* spp.). This parasite of wild ruminants was originally introduced to Europe from North America and first recorded (in the wild) in Austria in 2000. *Galba truncatula* and some species of *Stagnicola* are described to act as main intermediate host of *F. magna* in Europe.

Till now 80 locations within and nearby the Nationalpark region - where infected deer has been recorded - have been investigated.

Galba truncatula was recorded at 30 locations (out of 80), mainly with high frequency at swampy shores near the water line. *Stagnicola turricola* were collected just in few cases and only in few numbers.

Till now 5626 *Galba truncatula* have been examined. 103 were infected (1.83 %) with trematodes. We found trematodes of the family Paramphistomidae and Echinostomatidae. One snail was infected with xiphidiocercariae. Only one proof record of *Fascioloides magna* occurred. This was located at Fischamend at the northern bank of the river Fischa. Some early redia stages have to be proofed with molecular biological methods. The study will be continued until September this year.

Cercariae of trematodes (Digenea) from freshwater molluscs in South-Eastern Germany: a comparison with the Czech Republic

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A total of 6421 aquatic molluscs of 28 species were examined from 16 localities in South-East Germany (near Dresden, Hof, Nürnberg, Degendorf) in 2004. Cercariae of 28 species were found in 266 (4.14 %) molluscs. The highest species diversity (10 species) was found in ponds in the vicinity of Nürnberg, where many potential definitive hosts (birds) also occurred. The species diversity of cercariae in molluscs from rivers was lower, (a maximum of 3 species). The most frequent species of cercariae were *Plagiorchis elegans*, *Echinoparyphium aconiatum*, *Opisthoglyphe ranae* and *Diplostomum pseudospathaceum*, which all occurred in *Lymnaea stagnalis*, the most frequently infected host (n=1218, prevalence 15 %). The composition of the common species was similar to that found in the Czech Republic. However, a considerable difference was noted in the occurrence of the snail *Planorbis corneus* (283 examined), which was found less frequently and was free of infection with cercariae. In the Czech Republic *P. corneus* occurs more often than *L. stagnalis* and harbours a relatively wide spectrum of cercariae (7 species).

Prosobranchia (Mollusca) as intermediate hosts of trematodes in Central Europe: an overview

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Prosobranchiate snails, mainly common in drainage basins of large rivers (Danube, Elbe, Oder), host a diverse spectrum of larval stages of trematodes (Digenea). Besides snails of the genus *Bithynia*, which can be found frequently in all types of biotopes, there are a number of

endangered species of the genera *Theodoxus*, *Lithoglyphus*, *Bythinella* and *Sadleriana* that occur only in distinct biotopes and are difficult to collect. Therefore, it is probable that many larval trematodes infecting these snails are still unknown. The trematode families found most frequently in *Bithynia tentaculata* are Lecithodendriidae, Cyathocotylidae, Prosthogonimidae, Monorchidae, Psilostomidae as well as members of the medically and veterinary important Opisthorchiidae. In Germany, Odening studied the life cycles of trematodes that use prosobranchiates as intermediate hosts in the 1960 – 70's. Recently, Jezewski (2004) reported larval stages of trematodes from *Viviparus* in Poland (Mazurian lakes). In the Czech Republic, larval trematodes were found in prosobranchiate snails by Žďárská (1963, 1964), Nezvalová (1970), Balůšek and Vojtek (1973), Ditrich and Vojtek (1977), Našincová (1992). During the present study carried out in 2003, a total of 463 *Bithynia tentaculata* snails (total prevalence 2.6 %) and 290 of *Viviparus acerosus* and *Viviparus contectus* (total prevalence 15.5 %) from South Moravia (Lednice, Dolní Věstonice, Pohořelice) were examined. In *Viviparus*, trematodes of the family Echinostomatidae (prevalence 14.5 %) were found. The trematodes found in *Bithynia* belonged to the families Lecithodendriidae (prevalence 0.2 %), Cyathocotylidae (0.4 %), Notocotylidae (0.4 %), Echinostomatidae (0.9 %) and Opecoelidae (0.2 %). Unidentified cercariae, designated as *Cercaria helvetica* XII Dubois, 1929 and *Xiphidiocercaria* sp. 5 by Našincová (1992), were also found. The affiliation of cercariae of unclear systematical status remains to be verified experimentally.

Serodiagnosis of serious parasitoses of wild boars

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The wild boar (*Sus scrofa*), important large game species in Slovakia whose number has significantly increased during the last decade, can play a significant role in circulation and maintenance of some parasites in the environment. The aim of this study was to determine the seroprevalence of trichinellosis, toxocarosis and toxoplasmosis in wild boars hunted in the Slovak Republic in 2003 and 2004. Out of 1035 wild boars investigated in both years, anti-*Trichinella* antibodies were detected in 1.3 % animals. Besides the regions with endemic occurrence of trichinellosis (Eastern and Central Slovakia), seropositive animals came also from the regions of Western Slovakia. Out of 1173 serum samples examined for the presence of antibodies to *Toxocara*, 7.2 % sera reacted positively. Totally 869 animals were examined for the presence of both, anti-*Trichinella* and anti-*Toxocara* antibodies and mixed infection was determined in 5 wild boars. Presence of anti-*Toxoplasma* antibodies was recorded in 8.1 % out of 320 individuals investigated. Concurrent occurrence of anti-*Toxo-*

plasma and anti-*Toxocara* antibodies was determined in 5 out of 292 wild boars examined. This study confirmed the role of wild boars in circulation and maintenance of trichinellosis, toxocarosis and toxoplasmosis in conditions of the Slovak Republic and underlines the importance of high hygiene standard during the handling with game.

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The survey for parasite composition in animals at Zoological Garden Košice

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Parasite infections in animals at zoological gardens represent a persisting problem. In captivity, the animal species and individuals concentration may influence the parasite resistance, as the captivity of these hosts is nothing more than ecological isolation, which includes quantitative modifications in life cycles of the resident parasites, and thus in the parasite community.

With a view to obtain more profound knowledge on parasites distribution in animals held in ZOO Košice, the pilot study was initiated in spring periods of 2004 and 2005. The faecal samples were investigated for the presence of propagative stages of parasites using standart sugar centrifugation and flotation technique. With respect to the zoological classification, the animals were arranged into the 7 groups as followed: *Ruminantia*, *Perissodactyla*, *Primates*, *Carnivora*, *Reptilia* et *Amphibia*, *Aves*, *Rodentia* et *Lagomorpha*. In 2004, a total of 378 individuals of 89 animal species were investigated, and out of 427 animals representing 80 species were examined in 2005.

In 2004, eggs and/or oocysts of parasites were found in 39 out of 89 animal species examined, which represents the prevalence of 43.8 %. The presence of parasites was most frequently detected in Equidae group, where the prevalence of Strongylidae infection reached 100 %. The most various parasite species composition was noticed in Ruminantia.

In 2005, another results in rates of parasitic infection were obtained. Out of 80 animal species under investigation, only 19 species (23.8 %) showed the presence of parasite eggs and/or oocysts in the fecal samples. In the groups of *Primates* and *Carnivora*, all positive animals arised from those recently imported and stabled in the quarantine. No presence of exotic parasite species was recorded.

The majority of animals held in the Zoological garden Košice originates from resident breeding, or was imported from other zoological gardens. Only couple individuals have been introduced from the nature. For all that, the parasitofauna of hosts in captivity is basically identical with those of domestic and sylvatic animals. Our results suggest that using targeted strategies of dehelminthisation and accu-

rate doses of antihelminthic drugs, the presence of parasites can be reduced.

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A survey for parasites of red foxes (*Vulpes vulpes*) in the Slovak Republic based on fecal analysis

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Red fox (*Vulpes vulpes*) is the most abundant wild Carnivore lived in the territory of the Slovak Republic. Significant increase of its population has been observed after 1996 as a consequence of oral antirabies vaccination. The stock of red foxes increased from 6 154 in 1993 to 13 331 in 1996. Of the veterinary point of view, foxes represent major wildlife reservoirs of parasitozoonoses transmissible to humans and domestic animals, especially in rural areas. Together with increasing population of red foxes also the first finding of *Echinococcus multilocularis* in the Slovak Republic have underlined the importance of the red foxes as a source of zoonotic infectious diseases. Until recently, knowledge on the parasites of foxes in the Slovak Republic was limited to information collected during a few studies provided several decades ago or restricted on small area of one region. The aim of the present study therefore was to determine the more recent prevalence of parasites of red foxes from whole territory of the Slovak Republic in order to determine their role in the epidemiology and epizootiology of parasitoses in both humans and animals. By collecting a large group of the foxes from different regions, we have acquired the sight into the ecological factors that influence susceptibility of their populations to parasite infections. A total of 924 fecal samples of red foxes were collected and examined using standart sugar centrifugation flotation technique between September 2000 and February 2003. This study has shown that 85.6 % of red foxes in the Slovak Republic are infected with parasites. This may be important from a human health perspective because some of these parasites are zoonotic and serve as a source of infection for humans and domestic carnivores. Fifteen helminth species included two trematode species, four cestode species and nine species of nematodes were detected. *Toxascaris leonina* was the most common nematode species found in presented survey and was recorded in 47.8 % of red foxes, followed by *Trichuris vulpis* (32.8 %), *Capillaria* spp. (23.8 %) and *Ancylostoma caninum* (22.4 %). Coccidia oocysts were found in 25.6 % of red foxes.

Of the 924 foxes, 254 (27.5 %) were infected with one parasite species, 244 (26.4 %) animals had combined infection with two parasite genera, 182 (19.7 %) of faecal samples contained three species and 90 (9.7 %) contained four species. Sixteen (1.7 %) foxes were infected with five parasite species and 4 (0.4 %) of them with six species. Only

1 fox was parasitized by seven parasite species; coccidia oocysts, taeniidae eggs, *Toxocara canis*, *Toxascaris leonina*, *Ancylostoma caninum*, *Trichuris vulpis* and *Capillaria* spp. were found in its fecal sample. Twelve (57.1 %) of red foxes infected with more than 5 and more parasite species came from Banská Bystrica region. Only in 133 (14.4 %) foxes were observed no parasites.

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Eradication of pinworm infections from laboratory rat colonies using ivermectin

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Oxyurids (*Syphacia muris* and *Aspiculuris tetraptera*) are common contaminants of both conventional and specific pathogen-free rodent colonies. It is difficult to control because many anthelmintics eliminate adult worms but have no effect on ova, which can survive ex vivo for prolonged periods. The purpose of this study was to evaluate an efficiency of orally administered ivermectin diluted in water as treatment for pinworms in laboratory rat colonies.

One hundred seventy rats naturally infected with pinworms (*Syphacia muris* and *Aspiculuris tetraptera*) were randomly divided into control and treated groups. The treated group of 120 rats was divided into ten subgroups of 12 animals each. Each subgroup received one to four courses of ivermectin which alternated with drinking water every 5 days. After the last (the 4th) course of ivermectin, rats were given drinking water for 15 days.

During each treatment, ivermectin was administered for 5 days in the drinking water; based on water consumption, the ivermectin dose was 2.5 mg/kg of body weight per day. Ova production was monitored by a cellophane tape test every fifth day. The hygiene conditions of this study, such as the cage change, disinfection and cleaning of the rooms, were synchronized with the treatment cycles.

The control group of 50 rats were euthanized at the beginning of experiment (day 0) and examined for adult pinworms and larvae. Parasites were detected in all control animals.

Every fifth day all rodents of one subgroup were euthanized and their caecum and large intestinal contents were examined for adult pinworms and larvae. No worms were found in caecum and intestinal contents of all treated animals; however, the cellophane tape test was negative in all rats only after the second course of ivermectin was administered.

Hymenolepis sulcata (von Linstow, 1879): occurrence in dormice, *Glis glis* (Rodentia) in Slovak Republic

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In the course of parasitological examination of rodents in Slovak Republic, only one specimen of dormice, *Glis glis* was found and dissected. This specimen was infected with *Hymenolepis sulcata* (von Linstow, 1879). *Hymenolepis sulcata* (von Linstow, 1879)

Host and locality: *Glis glis*, Rozhanovce near Košice, Slovak republic, September, 2003; localization: small intestine; intensity: probably 2 specimens, including 1 specimen with scolex. Description. Small-sized Hymenolepidid. The exact length of the mature worms was difficult to evaluate, because the material underwent fragmentation during dissection of the entire intestine, initially fixed in 70 % ethanol. The maximum width was 1 mm. Edges of the strobila are serrate. Gravid segments were filled with eggs. Scolex rounded, with 4 suckers and a rostellum. Hooks absent. Diameter of the scolex, at the level of suckers was 130 µm, and it enlarges into 175 µm in the region below the suckers. Size of the suckers 60 – 65 × 60 – 77 µm, measurements of the rostellum was 72 × 32 µm. Genital pores localized at one side of the strobila, ventral in the border of the first third of proglottids. Cirrus sac 100 – 144 × 40 µm, passes across the excretory ducts. Both internal and external seminal vesicles are present. The vagina opens into the genital atrium behind the cirrus, forming a large seminal receptacle, extending half of the segment. Testes arranged in a form of triangle, two in aporal and one in poral position. Ovary lobular, situated in the middle of segments. Vitellaria located in the posterior half of segment in the midline. Uterus saccular, occupies the entire medullary space of the mature proglottids. Eggs measure 28 – 30 × 32 – 35 µm, embryos 21 – 22 × 25 – 26 µm and oncospherical hooks 17 µm.

H. sulcata was described for the first time by von Linstow in 1879. Until now, its occurrence in *Glis glis* was reported in different parts of Europe, namely in Slovak Republic by Tenora (1965: *Československá parazitologie*, 12: 299 – 303), in Hungary by Murai and Tenora (1977: *Parasitologia Hungarica*, 10: 63 – 66), in Switzerland by Faivre and Vaucher (1978: *Bulletin de la Société Neuchateloise*

des Sciences Naturelles, 101: 53 – 58) and in Spain by Feliu (1987: *Revista Ibérica de Parasitología*, Vol. Extraord.: 79 – 83). This study is the second report of the presence of *H. sulcata* in Slovak Republic.

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Parasite burdens and bioaccumulation of heavy metals in tissues of small mammals from sampling sites with different pollution levels

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A total of 35 small mammals (14 *Apodemus flavicollis*, 6 *Clethrionomys glareolus*, 7 *Microtus agrestis*, 3 *Mus musculus* and 5 *Sorex araneus*) from 2 sampling sites in the Czech Republic with different pollution levels were investigated with respect to their gastrointestinal parasite burdens and bioaccumulation of heavy metals in their livers and kidneys. There were 6 – 7 different parasite species

found to infect these hosts. Data from each sampling site were recorded separately. The parasites found (in descending order of prevalence) were as follows: *Heligmosomoides polygyrus*, *Paranoplocephala* sp., *Syphacia* sp., *Trichuris muris*, *Mastophorus muris*, *Capillaria* sp. Up to 3 different helminth species were found in the intestine of the individual host. Among those, *Heligmosomoides polygyrus* and cestodes (*Paranoplocephala* sp.) were the most prevalent with *Heligmosomoides polygyrus* as dominant species. The helminth communities of the small mammals from monitored areas exhibited low parasite diversity but high dominance.

Concentrations of heavy metals (Cd, Cr, Cu, Mn, Ni, Pb and Zn) in small mammal's liver and kidney were determined by ICP OES method. Our study examined the interrelation between heavy metal concentrations and parasitofauna of host (small mammals). Small mammals were used to investigate whether there is a difference in accumulation of heavy metal ions by hosts infected with helminths compared to those that are not infected. The possibility of using parasites as bio-monitoring indicators has been investigated by a number of researchers but the interaction between current bio-monitoring organisms and their parasitofauna have been left almost unexamined. The most important results from our study is the significant difference in heavy metal content between infected and uninfected host. Relatively high contents of these elements were observed in parasite-free hosts from both sampling sites. Therefore results from pollution test can be falsified if parasited test organisms are involved.