

PARASITES OF A 'SUPERMODEL': PHYSIOLOGY, GROWTH AND BEHAVIOUR OF  
SCHISTOCEPHALUS-INFECTED STICKLEBACKS

By I. BARBER

*University of Leicester, UK*

The three spined stickleback *Gasterosteus aculeatus* – which has been described as a ‘supermodel’ in evolutionary ecology – is often infected with plerocercoids of the avian tapeworm *Schistocephalus solidus*. In this talk I will focus on the utility of the stickleback-*Schistocephalus* model for examining the fitness consequences of parasite infection for host fish. I will introduce the host system and the various tools that are available for undertaking such studies and then outline three lines of research designed to examine the impacts of infection on (i) the antipredator behaviour, (ii) the growth and energetics and (iii) the sexual development and reproductive behaviour of host fish. I will end by outlining potential avenues for future research utilising the stickleback-*Schistocephalus* model system.

## NAVIGATING A COMPLEX LIFE CYCLE: WHEN SHOULD PARASITES MOVE BETWEEN HOSTS?

By D. BENESH

*Max Planck Institute for Evolutionary Biology, Germany*

The size at and timing of ontogenetic transitions (e.g. maturation, metamorphosis) are important life history characteristics with clear effects on fitness, but these traits have not received much attention in complex life cycle parasites. We know little about how and why parasites split their growth and ontogeny among multiple obligate hosts. Using the tapeworm *Schistocephalus solidus* as a model, I explored the parasite's strategy for moving from its first intermediate host, copepods, to its second intermediate host, sticklebacks. Parasites seem to benefit from reaching a large size in copepods, but what are the costs associated with this? Parasites that grew fast also matured earlier, indicating that prolonged development is not necessary to achieve a large transitional size. Rapid parasite growth did not reduce host survival, even in small, starved hosts. Moreover, in both behavioural assays and predation trials, rapid parasite growth did not result in an increased risk of host predation. Thus, rapid larval growth is obviously beneficial, but it does not seem to directly or indirectly affect host survival. Detecting what costs constrain larval parasite growth is necessary to understand complex life cycles as adaptive strategies.

## INFECTION BY BIRD SCHISTOSOMES: AN OVERLOOKED PROBLEM?

By L.KOLÁŘOVÁ

*National Reference Laboratory for Tissue Helminthoses, Institute for Postgradual Medical Education and 1<sup>st</sup> Faculty of Medicine, Charles University, Prague, Czech Republic*

Larval stages (cercariae) of bird schistosomes are known causative agent of common non-communicable water born allergic skin disease known under the names swimmer's itch or cercarial dermatitis. Human disease is characterised by the development of maculopapular eruptions which develops after repeated contacts with the cercariae in fresh- as well as salt-water bodies. Cercarial dermatitis occurs globally, however, human infections by the cercariae are not monitored till present. Nevertheless, increasing number of new outbreaks indicates the re-emergence of the disease in many European recreational areas. Recent findings of new genera and species of bird schistosomes indicate a broad spectrum of causative agents with different life-cycles, host specificity and pathogenicity. The ability of the cercariae to penetrate into the skin of non-compatible hosts (mammals) has been confirmed for schistosomes of the genera *Trichobilharzia*, *Gigantobilharzia*, and *Ornithobilharzia*. Moreover, the studies on the *Trichobilharzia szidati* and *T. regenti* parasite behaviour in mammals showed that the infections by the cercariae can be linked to more than skin symptoms. Despite the failure to develop fully, bird schistosomes can escape from the skin and migrate in the mammalian hosts under certain circumstances. The studies on mice experimentally infected by *T. regenti* showed that parasite escape from the skin and following migration depends primarily on the host immune status. It has been observed that initial infection characterised by a mixed Th1/Th2 immune response is so weak to trap the schistosomula in the skin and, therefore, facilitates the development of internal organ disorders. On the other hand re-infections lead to strongly Th2 polarised immune response, which manifests by the development of maculopapular skin eruption which is able to trap the parasites in the host skin. From human point of view, the fate of bird schistosomes after penetration into human body remains to be clarified. Nevertheless, the recent findings brought novel insights on potential pathogenic effect of bird schistosomes to non-compatible hosts, including man.

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## RECENT ACHIEVEMENTS IN THE MOLECULAR IDENTIFICATION AND CHARACTERISATION OF NEURONAL ANTHELMINTIC DRUG TARGETS

By G. VON SAMSON-HIMMELSTJERNA

*Institute for Parasitology, University of Veterinary Medicine Hannover, Germany*

During the past decades the intensive use of anthelmintics has unfortunately led to the widespread development of resistant parasite isolates, namely concerning nematode parasites. Therefore, it is of great significance that most recently two new antinematocidal drug classes were identified. These are the cyclooctadepsipeptides with the drug emodepside available for cats and dogs and more recently the amino-acetonitrile derivatives of which the compound monepantel is currently launched for use in sheep in New Zealand. In the model nematode *Caenorhabditis elegans* forward genetic screens revealed that the later drug class appears to act via binding to the ACR-23 protein which belongs to the nematode specific DEG-3 clade of nicotinic receptor subunits. Using *in vitro* selected monepantel-resistant isolates of *Haemonchus contortus* it was found that the homologue HCACR-23H was associated with resistance. The focus of this presentation will be on the cyclooctadepsipeptides, where at least two different putative neuronal receptors were identified. The first was the latrophilin-like G-protein coupled receptor LAT-1 in *C. elegans* and its homologue HC110-R in *H. contortus*. Emodepside binding studies, modulation of latrotoxin induced calcium influx in recombinantly HC110-R expressing eukaryotic cells exposed to the drug as well as functional studies in mutant *C. elegans* have demonstrated that LAT-1 is involved with inhibition of pharyngeal muscle activity in nematodes. More recently by using mutant *C. elegans* lines the calcium-gated potassium channel SLO-1 was found to be associated with emodepsides inhibitory activity on nematode body muscles. By functional rescue of the emodepside susceptible phenotype in these resistant *C. elegans* lines through transformation with the respective orthologue genes of different parasitic nematodes like *C. oncophora* and *Ancylostoma caninum* we could further substantiate the relevance of this receptor for the emodepside activity.

# ECOLOGICAL, GENETIC, AND BEHAVIORAL ASPECTS OF HOST-PARASITE INTERACTIONS IN FISH EMBRYOS

By C. WEDEKIND

*University of Lausanne, Switzerland*

Parasites are ubiquitous, even if no primary infection can be observed: animals and plants are associated with symbiotic microbial communities whose role ranges from mutualism through commensalism to parasitism. We study the interaction between fish embryos and symbiotic microbes that have the potential of becoming virulent pathogens. We experimentally disentangle genetic from maternal effects by producing half-sib families in full-factorial breeding designs (“North Carolina II design”), and we concentrate on salmonids like brown trout and whitefish because they have external fertilization and no parental care, i.e. differential parental investment is not possible and sire effects on embryo performance therefore directly reveal genetic effects. We raise the embryos in multiple batches or singly in wells of cell-culture plates and expose them experimentally to various stressors and/or microbial pathogens. We found that the micro-ecological environment crucially affects the virulence of microbial pathogens. On the hosts’ side, maternal effects are very important in determining the embryos’ resistance to pathogens. At later stages of embryogenesis, the embryos’ own genetics also plays a role, especially their MHC genotype (major histocompatibility complex, a group of genes that are important in immune recognition). Moreover, exposure to high concentrations of pathogens can induce early hatching that allows the larvae to quickly leave the contaminated place. Hence, ecology, genetics, and behavior determine fish embryos’ resistance to microbial pathogens. Because fish embryos can be produced and raised under very controlled conditions and in very high numbers, they are excellent vertebrate models for studying host-pathogen interactions.

## TICK HETEROGENEITY PECULIARITIES UTILIZATION AS A FORECASTING TOOL OF VARIOUS PATHOGEN DISTRIBUTIONS

By A.N. ALEKSEEV & H.V. DUBININA

*Zoological Institute, RAS, St. Petersburg, Russia*

Preferential frequency of occurrence of some tick parasites, agents of tick-borne diseases on various territories is known but the causes of this phenomenon as a rule are connected by authors mainly with specificity of abiotic factors, such as climate, highness above the sea level and conditioned with these peculiarities plant associations. Peculiarities and specificity of genetic types of vector' populations were not taken into account either for interpretation or for forecasting of pathogen distribution. An attempt to use for this purpose malatdehydrogenase (MDH) enzyme electrophoretotypes was made. It permits to marker genovariation of *Ixodes persulcatus* distributed on the different territories of Russia, where various disease agents are circulating. Six identified genotypes prevalence was compared with the prevalence of tick-borne encephalitis virus (TBEV) and of babesiosis morbidity. Obtained correlations between these quotients permitted to suppose that the estimation of MDH genotype differences and the prevalence of intracellular tick parasites may serve as a tool for forecasting of distribution of populations of *Ixodes* ticks predominately infected by various intracellular tick parasites, agents of tick-borne diseases. Number of genotyped ticks was 1877, number of ticks in which TBEV was identified 15186, number of patients suspected for babesiosis 100. Pearson correlation between two ranges was used to estimate the correctness of conclusions.

INFECTION WITH *TRICHURIS SUIIS* AND *OESOPHAGOSTOMUM DENTATUM* IN PIGS:  
EFFECTS ON PARASITE POPULATION DYNAMICS IN EXPERIMENTAL CO-  
INFECTIONS

By A. ANDREASEN, H.H. PETERSEN, H. KRINGEL, S.M THAMSBORG & A.  
ROEPSTORFF

*Department of Veterinary Disease Biology, Faculty of Life Sciences, University of Copenhagen,  
Denmark*

The aim of this study was to examine possible interactions between *Trichuris suis* and *Oesophagostomum dentatum* infections in pigs with regard to the population dynamics of the worms such as faecal egg excretion, worm burden, location and length. Forty-eight helminth naïve pigs were allocated into 4 groups in a 2x2 factorial design. Two groups were trickle inoculated with either 10 *T. suis* eggs/kg/day (Group T) or 20 *O. dentatum* L3/kg/day (Group O). One group (OT) was trickle infected with both 10 *T. suis* eggs/kg/day and 20 *O. dentatum* L3/kg/day. The remaining group C served as an uninfected control. All trickle infections continued until necropsy. In each group, six pigs were necropsied 5 weeks post first inoculation (wpi) and the remaining 6 pigs were necropsied 10 wpi. A significantly higher faecal *O. dentatum* egg excretion was seen in Group O compared with Group OT ( $p < 0.05$ ). Faecal egg counts remained high in Group O while a marked decrease was seen in Group OT from 4 wpi. The faecal *T. suis* egg excretion was generally higher in Group OT compared to Group T. The *Oesophagostomum* worm burden was significantly higher in Group O compared to Group OT both at 5 weeks ( $p < 0.01$ ) and 10 weeks ( $p < 0.05$ ). At both necropsies the mean *T. suis* worm burden was higher in Group OT compared to Group T, although not significantly so. The weighted mean location for *O. dentatum* was more posteriorly in the large intestine in Group O compared to Group OT, while *T. suis* was located in the proximal part of the large intestine in both Groups T and OT. The length of adult *O. dentatum* females seemed to be slightly shorter in group OT. The results clearly indicate that *Trichuris* may negatively influence *Oesophagostomum* populations in co-infected individuals – we observed an almost ten fold reduction in *Oesophagostomum* worm burdens in these animals. In contrast the presence of *Oesophagostomum* may potentially enhance populations of *Trichuris*. We conclude that co-infections may result in altered parasite population dynamics compared to mono-species infections.

MICROEVOLUTIONARY ASPECTS OF MORPHOLOGICAL VARIABILITY AND SPECIFICITY OF CESTODES BY THE EXAMPLE OF *PROTEOCEPHALUS LONGICOLLIS* (ZEDER, 1800) (PROTEOCEPHALIDAE), A PARASITE OF SALMONIDS

By L.V. ANIKIEVA & E.P. IESCHKO

*Institute of Biology Karelian Research Centre of RAS, Russia*

There has been studied morphological variability to salmonids cestode *Proteocephalus longicollis*, a parasite of smelt *Osmerus eperlanus* L., which was spontaneously introduced into Lake Syamozero. It was ascertained that for last 20 years *P. longicollis* has become a mass representative of established parasitic fauna of smelt, thereby showing the absence of its strict specificity. It was shown that morphological polymorphism of *P. longicollis* from smelt combines features of ecoforms characteristic for local hosts, those are whitefish (*Coregonus lavaretus* L.) and vendace (*Coregonus albula* L.). By the nature of variability cestodes from smelt are more similar to ecoforms from whitefish. It is concluded that when a parasite colonise a host that is new for the water body, a new hostal group is forming, which makes for the parasite number maintenance and its population stability under changing environment of freshwater ecosystems

## ECHINOCOCCOSIS IN THE WILD CANIDS IN LATVIA

G. BAGRADE<sup>1,2</sup> & J. OZOLIŅŠ<sup>2</sup>

<sup>1</sup> *Natural History Museum of Latvia*

<sup>2</sup> *State Forest Research Institute "SILAVA", Latvia*

The objective of this research was to provide first data of *Echinococcus spp.* in canids in Latvia; their intensity and extensity of invasion. 34 wolves, 45 foxes and 19 raccoon dogs were examined according to conventional helminthological methods.

Both *Echinococcus* species known in Europe – *E.multilocularis* and *E.granulosus* – have been found in Latvia. *E. multilocularis* has been found in fox, wolf and raccoon dog populations and *E.granulosus* in the wolf population.

*E.multilocularis* in Latvia is most common in foxes with a parasite extensity of 35.6% and an intensity of 1-1438 parasites per animal. In wolves the parasites are found in 5.9% of cases with an intensity of 62 -380 parasites per animal and in raccoon dogs they are found only in one of 19 animals with an intensity of 114 parasites per animal. In the Ventspils district *E.multilocularis* has been found in one fox, one wolf and one raccoon dog. The wolf and raccoon dog were hunted even in the same location – Ugāle. *E.granulosus* has been found only once – in an adult male wolf hunted in the Saulkrasti forestry. Parasite intensity was very high in the animal – 989 parasites.

Since the *Echinococcus* genus, specifically the *E.granulosus* species, is very genetically diverse, the *E.granulosus* and some *E.multilocularis* samples were submitted for genotype determination. Genetic analyses were performed in Parasitological Institute of the Slovak Academy of Sciences in Košice (Slovakia) for some of *Echinococcus* from foxes and in Department of Zoology, Institute of Ecology and Earth Sciences, University of Tartu (Estonia) for *E.multilocularis* and *E.granulosus* from wolves.

The results of our study supplement the information available about the *Echinococcus* parasites in the Baltics and in Europe.

## PROBLEMS OF SALMON RIVER: PARASITOLOGICAL VIEW

By J. BARSKAYA<sup>1</sup>, D. LEBEDEVA<sup>1</sup>, J. LUMME<sup>2</sup> & A. VESELOV<sup>1</sup>,

<sup>1</sup> *Institute of Biology, Karelian Research Center RAS, Russia*

<sup>2</sup> *University of Oulu, Finland*

The river Lizhma draining to the Lake Onega is a salmon river, challenged by increasing eutrophication caused by fish farming. The cases of accidental release of farm fish to the river are potentially dangerous for natural ecosystem. For example, *Gyrodactylus salaris* is endemic in the Lake Onega, and the salmon population has developed a complete resistance against the local parasite strains. However, increased fish farming may threaten the evolutionary status quo, by introducing new parasite clones and species. We investigated all parasites on and in juveniles (ages 2+ to 3+) of the native Atlantic salmon (*Salmo salar*) and a rainbow trout escaped from fish farms (*Onchorynhus mykiss*), caught in the river. Most of parasite species were common for both hosts. It revealed cross of habitats and probably food competition. Unusually high invasion of salmon fry by *Proteacephalus* larvae demonstrates changes in the eutrophication level. *Onchorynhus mykiss* was heavily infected by the dangerous monogenean *Gyrodactylus salaris*. Molecular genetic investigation revealed that the worms belonged to the fish farm specific clone (RBT clone). The salmon population in Lizhma was occasionally infected by endemic *G. salaris* strain, but it is only present in some years, not permanently. Hybridization of RBT clone and salmon specific parasite has been observed in the Lake Kuito (river Pista), and in the River Laerdalselva in Norway, leading to a more pathogenic form. The situation in the salmon rivers in the Lake Onega area should be monitored continuously. Supported by RFBR: 08-04-91771, 08-04-91864.

# *IXODES (EXOPALPIGER) TRIANGULICEPS* IN THE FOCUS OF TICK-BORNE ENCEPHALITIS IN THE NORTHERN PERIPHERY OF THE SPECIES RANGE

By L. BESPYATOVA, S. BUGMYRIN & E. IESHKO

*Institute of Biology, Karelian Research Centre, Russian Academy of Science, Russia*

The aim of the study was to research the host range and abundance of the ixodid tick in long-term dynamics in the natural focus of tick-borne encephalitis (TBE) in the middle taiga of Republic of Karelia, Russia. The material was gathered during nine years from a permanent sample plot (62°04' N, 33°55' E) in an active TBE focus area in secondary forests made up of small-leaved species: birch, aspen and speckled alder mixed with spruce and pine. *I. trianguliceps* was found on 7 species of small mammals out of the 11 species captured. Ticks of the *I. persulcatus* - *I. trianguliceps* complex in the natural focus area fed on the same species of small mammals, which is a sufficient prerequisite for exchange of agents among these species (Bespyatova et. al. 2006). At all stages of its development *I. trianguliceps* parasitizes on small mammals. The main feeding hosts for the tick were the species dominant in the area – bank vole and common shrew. They hosted 61.7% and 27.8 % of all ticks, respectively. The leading host at all active development stages was bank vole, which sustained 54.3% of larvae, 76.6% of nymphs and 75.5% of imago ticks from all hosts. *Sorex araneus* hosted only larvae and nymphs – 35.7% and 13.7%, respectively. Infection parameters in bank vole (prevalence – 53.3%; intensity – 2.6) were the highest among small mammals. Differences in infection of mature and juvenile bank vole individuals with *I. trianguliceps* were insignificant (46%, 1.0 specimen and 38%, 1.3, respectively). Analysis of among-year variations in abundance (feeding pressure at the larval and nymph phases) revealed no direct correlation with the abundance of the main host – bank vole. *Ix. trianguliceps* abundance peaks lagged one year behind rises in bank vole abundance. Round-the-year activity of the tick, abundances rises in spring and autumn, wide host range promote the spread of the infection agents and maintenance of the natural focus area of the infection.

## THE FIRST REPORT ON IXODES PERSULCATUS FOUNDED IN THE EAST FINLAND

By S. BUGMYRIN<sup>1</sup>, L. BESPATOVA<sup>1</sup>, T. HOKKANEN<sup>2</sup>, E. IESHKO<sup>1</sup>, F. FEDOROV<sup>1</sup>, A. YAKIMOVA<sup>1</sup>, L. ROMANOVA<sup>3</sup>, Y.U. KOROTKOV<sup>3</sup>, L. BURENKOVA<sup>3</sup> & G. KARGANOVA<sup>3</sup>

<sup>1</sup> *Institute of Biology, Karelian Research Centre RAS, Russia*

<sup>2</sup> *North Karelia biosphere reserve, Finland*

<sup>3</sup> *M.P. Chumakov's Institute of Poliomyelitis and Viral Encephalitides RAMS, Russia*

The occurrence of the ticks in the east Finland was studied. The material was collected on May 20-30, 2008 near the Mekrijärvi Research Station, University of Joensuu. Imago and nymphs of ticks were collected by flagging. In total 160 flag–km are gone. Larvae and also nymphs were collected from small mammals caught by snap-traps. The host sampling was consisted of 12 specimens of 2 species of small mammals (*Sorex araneus* – 2, *Clethrionomys glareolus* – 10 spm). The total number of ticks was equal 2 larvae, 6 nymphs and 26 imagoes. The ticks founded concern to species *Ixodes persulcatus* (31 spm.), *I. ricinus* (1 spm.) and *I. trianguliceps* (2 spm.). Prevalence and abundance of *I. persulcatus* and *I. trianguliceps* on small mammals were 17 %; 0.2 spm. and 8 %; 0.2, respectively. Number of taiga tick imagoes has made 0.17 ind./flag–km. *Borrelia burgdorferi* sensu lato and tick-borne encephalitis virus were not revealed at the investigated ticks. This is the first record of the taiga tick in the region which allows speaking about essential change of this species area in the European Northwest. The research was supported by FTP (№ 02.512.11.2171) and RFBR (№ 08-04-90720-mob\_st).

## MULTIPLEX PCR ON SINGLE UNEMBRYONATED *ASCARIS SUUM* EGGS

By J. CARLSGART, A. ROEPSTORFF & P. NEJSUM

*Danish Centre for Experimental Parasitology, Department of Veterinary Disease Biology,  
Faculty of Life Sciences, University of Copenhagen, Denmark*

A sensitive and inexpensive method for DNA isolation and amplification by PCR from single unembryonated *Ascaris* sp. eggs is described. The resistant shell of single eggs was crushed mechanically and PCR applied to the crude egg contents without any further purification steps. The ITS1 region of the rDNA and three regions of the mtDNA could be successfully amplified. Using two primer sets, it was possible to amplify the rDNA and mtDNA simultaneously in one single reaction. The ability to perform PCR on single unembryonated eggs may result in better and more precise species identification of eggs recovered from faecal material, environmental samples, and possibly archaeological samples and in this way e.g. explore transmissions routes of human and pig *Ascaris* and identify zoonotic infections. In addition, single egg PCR makes it possible to perform population genetic studies without having to recover adult worms by deworming or autopsy.

ABOUT DISTRIBUTION OF *HYDROMERMIS CONTORTA* LINSTOW, 1889 (NEMATODA: MERMITHIDAE) IN THE WATER BODIES OF KALININGRAD REGION (RUSSIA)

By N. CHUKALOVA & A.A. GUSEV

*Atlantic Research Institute of Marine Fisheries and Oceanography, Russia*

The life cycle of Mermithidae family include parasitic and free-living stages. *Hydromermis contorta* is the typical species for fresh waters of Europe and North America. In Kaliningrad region this species was recorded firstly in 2008 (Chukalova, Gusev, 2008). The purpose for present work is to study the distribution of *H. contorta* in the main water bodies of Kaliningrad region. The investigations were carried out in the Sheshupe, Angrapa, Pissa, Deima, Neman, Primorskaya rivers, in the Pravdinsk pound, in the Vishtynetskoe Lake and in the Vistula and the Curonian lagoons in 2008. The benthos was sampled in the littoral zone (0.5-1.0 m depths) using the Petersen dredge with 0.0225 m<sup>2</sup> sampling area. The processing of benthos and mermithide identification was carried out by standard methods (Salazkin et al., 1984; Rubtsov, 1972). *Hydromermis contorta* was found in all researched water bodies except the Sheshupe river and the Vistula Lagoon. The parasitic larvae were revealed in hemocoel of *Chironomus plumosus* larvae, postparasitic larvae and adult helminthes-in macrozoobenthos. The parasitic larvae were found out in summer (in 10-15 mm chironomids) and in winter (in chironomids under 5 mm). In autumn the parasitic forms were absent. The infestation of *Ch. plumosus* larvae was the highest in Neman river (P=0.52%). Free-living stages of *H. contorta* were revealed in all seasons. The adult helminthes dominated in autumn (52-64% of total), postparasitic larvae-in summer (65-82%). The highest abundance and biomass the free-living mermithids reached in the Angrapa river (513 sp./m<sup>2</sup> and 0.2 g/m<sup>2</sup> accordingly), the lowest- in the Deima and the Primorskaya rivers (27 sp./m<sup>2</sup> and 0.04 g/m<sup>2</sup>). Thus, the nematodes *Hydromermis contorta* were revealed in 8 from 10 main water bodies of Kaliningrad region. The mermithids were the most spread in the Angrapa and the Neman rivers. The free-living and parasitic stages of *H. contorta* reached the highest abundance and biomass in summer.

THE STUDY ON *TOXOCARA CANIS* MIGRATION ROUTES IN MICE USING AN  
EPIFLUORESCENCE METHOD

By P. KOLBEKOVÁ<sup>1,2</sup>, D. VĚTVIČKA<sup>3</sup> & L. KOLÁŘOVÁ<sup>1,4</sup>

<sup>1</sup>National Reference Laboratory for Tissue Helminthoses Institute for Postgradual Medical Education,  
Prague, Czech Republic

<sup>2</sup>Department of Microbiology, 3<sup>rd</sup> Faculty of Medicine, Charles University, Prague, Czech Republic

<sup>3</sup>Department of Immunology and Gnotobiology, Institute of Microbiology, Academy of Science, Prague,  
Czech Republic

<sup>4</sup>1<sup>st</sup> Faculty of Medicine, Charles University, Prague, Czech Republic

Larvae of *Toxocara canis* and *T. cati* are the causative agent of common human disease known as larval toxocariasis. The infection is acquired after ingestion of the embryonated parasite eggs, from which the larvae hatch in the small intestine, penetrate into the wall and migrate to various organs. Traditionally, the pathway is characterized by migration of the larvae via the livers and lungs to various organs. The human disease is most commonly associated with location of the larvae in host visceral organs, however, ocular and even CNS infections are also reported. The involvement of visceral organs during the infection can be explained by traditional view on the parasite pathways but migration of the larvae from the lungs to the eye and CNS is under discussion. It is difficult explain how can relatively large parasites (approx. 400 x 15-20 µm) pass by pulmonary capillaries the diameter of which is 8 µm. The aim of the study was, therefore, to explain whether the parasites affecting the eye and CNS can utilize other migration routes.

Female Balb/c mice were infected by oral intubation with 2500 and 5000 *T. canis* L3 larvae. Prior to the infection, the larvae were stained by fluorescent [carboxyfluorescein succinimidyl ester](#) (CFSE, Sigma), enabling the observation of the parasites *in vivo*. Distribution of the larvae and progress of pathological changes in the mouse gut, liver, lungs, brain and the other organs was observed by Small Animal Imaging System - Olympus OV 100 (SAIS) and on histological sections, respectively, at 6, 12, 24, 36, 48, 60 h p.i. and 5, 10, 13, 17 days p.i.

Preliminary results suggest that the larvae escape from the gut to liver during the first 12 hours p.i. After 24 h p.i., most of the larvae were found in the liver and lungs. The CNS involvement by *Toxocara* larvae started at 60 h p.i.; with progress of infection the number of parasites in brain increased reaching maximum on day 17 p.i. Histology confirmed the findings by SAIS and showed, that the parasites reaching CNS did not migrated through the mouse lungs. The presented results suggest that similar pathway might be used by the parasites also in humans and highlights the need to intensify the study on migration routes of nematodes in mammals.

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AN ATTEMPT TO ANALYSIS OF LIFE CYCLES OF RENICOLID TREMATODES  
(DIGENEA, RENICOLIDAE) WITH ITS1 REGION OF THE rDNA SEQUENCES

By N.K. GALAKTIONOV<sup>1</sup>, K.V. GALAKTIONOV<sup>2</sup> & K. SKIRNISSON<sup>3</sup>

<sup>1</sup> *Institute of Cytology, St. Petersburg, Russia*

<sup>2</sup> *White Sea Biological Station, Zoological Institute, St. Petersburg, Russia*

<sup>3</sup> *Institute for Experimental Pathology, Keldur, Iceland*

The life cycles of trematodes of marine and coastal birds are studied poorly even for widespread and common species. It is the case for representatives of genus *Renicola* which are pathogenic parasites of birds' kidneys and ureters. Previously (Skirnisson et al., 2003) basing on comparison of sequences of ITS1 region of the rDNA the renicolid cercariae from Icelandic intertidal gastropod *Nucella lapillus* were identified as *Renicola somateria* parasitic in common eider. In this study we analyzed renicolid cercariae *Cercaria parvicaudata* from periwinkles *Littorina* spp. (sampled in Iceland and in the White Sea) and *Cercaria pacifica* from *Littorina squalida* (sampled in the Sea Okhotsk), and adults *Renicola cf. lari* from Icelandic herring gull (*Larus argentatus*) and *Renicola* sp. from the Sea of Okhotsk common eider (*Somateria mollissima*). Genomic DNA was isolated and purified with GlassMilk Kit (Sileks). The ITS1 region of the rDNA amplified by using of trematodes specific primers Tits1F 5'-GGTAAGTGCAAGTCATAAGC-3' and Tits1R 5'-GCTGCGCTCTTCATCGACA-3'. *Agarose electrophoresis* confirm the presence of specifically amplified ~500bp products in each of analyzed samples. Products extracted from the gel were cloned by InsTA cloning Kit (Fermentas) and sequenced. Bootstrap test of aligned by ClustalW sequences performed by Maximum Parsimony analysis indicates that the European and the Sea of Okhotsk renicolids form two clusters with 100 bootstrap values for ITS1 sequences. The ITS1 of *Cercaria pacifica* demonstrate 99.6% of identity with *Renicola* sp. adults from the Sea of Okhotsk common eider that infer they are two developmental stages of the same species, while *Cercaria parvicaudata* and *Renicola cf. lari* most probably represent two different species with ITS1 intraspecific variation about 2%.

THE STUDY OF CYST-FORMING COCCIDIA (*SARCOCYSTIS*, *FRENKELIA*) OF  
SYNANTHROPIC RODENTS IN LITHUANIA

By J. GRIKIENIENĖ

*Institute of Ecology of Vilnius University, Lithuania*

House mouse (*Mus musculus*), brown rat (*Rattus norvegicus*) and black rat (*R. rattus*) harbour many disease agents. In Lithuania, house mouse and brown rat are very common, whereas black rat is quite rare. Information on parasites of synanthropic rodent's species in Baltic countries is rather scarce. This paper aims to fill in this gap to a certain extent and to present own results of the investigations of muscle and brain parasites in these rodents of Lithuania. We focussed on two parasite genera: *Sarcocystis* Lankester, 1882 and *Frenkelia* Biocca, 1968 (Coccidia: Sarcocystidae). Attempts to transmit these heteroxenous coccidians from natural definitive hosts to lab. rodents will also be discussed.

Most of mice and brown rats were caught by snap traps in various localities, whereas all black rats in one (Varėna) district. To find *Sarcocystis* or *Frenkelia* cysts, we examined muscle and brain samples by compressor microscopy method as well as histologically.

In all, 205 house mice, 126 lab. mice, 73 brown and 62 black rats were examined for sarcocysts and part of them also for *Frenkelia* cysts. Only cysts of *Sarcocystis* were determined. Prevalence of infection among house mice was low, 2.3%, while among brown and black rats 21.9% and 35.4%, respectively. Infection intensity varied from a few sarcocysts up to mass infection in some cases. The size of sarcocysts ranged widely and in some cases they were seen with the naked eye. Morphology of sarcocyst wall, sizes of cystozoites, intermediate host-specificity and literature data indicate presence of two *Sarcocystis* species: *S. muris* in house mice and *S. rodentifelis* in both species of rats. Our attempts to transmit *Sarcocystis/Frenkelia* spp. to lab. mice and rats by sporocysts isolated from the intestines of some predatory birds were unsuccessful.

These results add to the findings in the investigation into cyst-forming coccidians fauna and parasites' prevalence and distribution among rodents in the Baltic region.

## IN VITRO INVASION OF *EIMERIA TENELLA* INTO MDBK CELLS

By S. HENIKL, K. TEICHMANN, A. KÖSTELBAUER & G. SCHATZMAYR

*BIOMIN Research Center, Austria*

*E. tenella* is one causative agent of coccidiosis, which is the major parasitic disease of poultry. Coccidiosis is mainly treated preventively by anticoccidial drugs and live vaccines. Regarding the problem of drug resistance, a lot of effort is made to find new strategies to fight the disease. In this connection, an in vitro screening assay was established to study anticoccidial effects using Madin-Darby bovine kidney (MDBK) cells as host cells for parasite invasion (PI). In preliminary trials, the influence of the state of confluence of the MDBK cells, the pH value and the incubation time on the PI were studied. MDBK cells were cultivated in 96-well plates and were infected with fluorescence-labelled *E. tenella* sporozoites (SZ). After incubation, PI was studied by epifluorescence microscopy. Based on these results, parameters for the assay were defined and the resulting method was used to test the anticoccidial drug monensin [0.13 µM]. PI was influenced by all three tested parameters. A direct correlation between the seeding density of the MDBK cells and the PI was determined. Slightly acidic pH values increased the PI, whereas pH 8 decreased the number of invaded SZ. After 2 h, ca. 40 % of the SZ invaded the MDBK cells, taking the number of invaded SZ after 20 h as 100 %. Regarding the results of the preliminary trials, the MDBK seeding density was defined to be 4E+04 cells/well in 200 µL and an incubation time of 2 h after infection was chosen. Using these parameters, monensin inhibited the PI almost completely. Summing up, MDBK cells were shown to be suitable to study the invasion of *E. tenella* SZ. In terms of testing potential anticoccidial substances, the influence of the pH on the PI has to be regarded. To avoid negative effects of the test substances on the MDBK cells, the incubation time after infection was defined to be 2 h. The established method enables a rapid screening of a high number of substances that could help to fight coccidiosis.

## HERD HEALTH MONITORING OF PARASITES IN ORGANIC DAIRY FARMS IN SWEDEN

J. HÖGLUND<sup>1</sup>, M. ARESKOG<sup>1</sup>, F. DAHLSTRÖM<sup>2</sup>, A. ENGSTRÖM<sup>2</sup> & S. SOLLENBERG<sup>1</sup>

<sup>1</sup>*Department of Biomedicine and Veterinary Public Health, Section for Parasitology and*

<sup>2</sup>*Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Switzerland*

The diagnostic value of tools used for the detection of major pasture-borne helminths in cattle, such as *Dictyocaulus viviparus*, *Fasciola hepatica* and *Ostertagia ostertagi*, is evaluated in organic dairy herds in Sweden. First, the parasite status of 8 herds that were investigated in 1997 and 1998, is re-examined. Nematode faecal egg counts (FEC) from ~10 first-season grazers per herd, and from pooled samples collected from fresh dung pats, were conducted on several occasions during the 2008 grazing season with a modified McMaster technique and FECPAC, respectively. In addition, serum pepsinogen concentrations (SPC) and lungworm antibody levels were determined with the Ceditest<sup>®</sup>-ELISA in blood collected around housing. Secondly, antibody levels against *Ostertagia* and *Fasciola* were determined from 105 additional organic farms and 105 neighbouring conventional farms in September 2008. Both parasites were diagnosed by detection of antibodies in bulk tank milk using the Svanovir<sup>®</sup> and Institute Pourquier<sup>®</sup> ELISAs, respectively.

There was a positive correlation ( $r=0.81$ ) between the mean FEC and FECPAC results. This shows that an accurate FEC reflecting the situation in first-season grazing animals can be determined in fresh faecal samples collected directly from pasture. In agreement with previous results, the magnitude of gastrointestinal nematode infections varied, with 3 herds that had SPC indicating subclinical levels of ostertagiosis. Elevated lungworm antibodies were detected in sera from 4 herds. The results from the bulk tank milk survey showed that the mean optical density ratio of *Ostertagia* was significantly higher in the milk from organic than conventional herds, i.e. 0.82 (95% CL=0.78–0.86) versus 0.66 (0.61–0.71). In contrast, there was no difference in the seroprevalence of *Fasciola hepatica*, which was diagnosed only in 8 (7.6%) organic and 7 (6.7%) conventional herds, respectively.

INFESTATION CHARACTERISTICS AND ABUNDANCE DYNAMICS OF THE  
JUVENILE ATLANTIC SALMON PARASITE *GYRODACTYLUS SALARIS*

By E. IESHKO<sup>1</sup>, J. BARSKAYA<sup>1</sup>, D. LEBEDEVA<sup>1</sup>, I. SHCHUROV<sup>2</sup> & V. SHIROKOV<sup>2</sup>

<sup>1</sup>*Institute of Biology, Karelian Research Centre, Russian Academy of Science, Russia*  
<sup>2</sup>*Northern Fisheries Research Institute, Russia*

Latest data on the occurrence of the dangerous parasite of juvenile Atlantic salmon *Gyrodactylus salaris* in rivers of East Fennoscandia are presented. The monogenean distribution and infestation characteristics are demonstrated for rivers of the White, Barents, and Baltic Sea watersheds. The highest infection levels are observed in autumn months. The occurrence of *G. salaris* RBT clones in wild salmon juveniles from rivers supporting trout farms is discussed. Epizootic events in juvenile salmon caused by *G. salaris* interventions beyond its natural range are described with the examples of the River Keret' (Russia) and River Vefsna (Norway). Supported by RFBR: 08-04-91771, 08-04-91864.

## PIG PARASITES IN DIFFERENT REARING SYSTEMS

By T. JÄRVIS, E. MÄGI & B. LASSEN

*Estonian University of Life Sciences, Institute of Veterinary Medicine and Animal Sciences,  
Estonia*

In order to determine the level and distribution of pig parasites in Estonian pig herds and in purpose to study whether management practices in the farms allow pig intestinal parasite infection, the data of our recent parasitological survey in 11 different districts are presented and analysed.

Current study includes 26 industrial large farms with pig number of 250 -10500, 16 smaller conventional farms (13 - 41 pigs), 23 small farms (1-7 pigs), 2 ecological outdoor farms and one wild boar farm. In total 3080 faecal samples were collected in 2006-2008 and investigated for parasite eggs and oocysts.

Parasite eggs or oocysts were found in 22% faecal samples. Helminth intestinal infections on industrial large farms were limited to *Ascaris suum* and *Oesophagostomum* spp. with low (20-100) epg-s in some sow and fattener groups. Infection prevalence of pigs in smaller farm fattener groups was 48% with *Ascaris suum*, 78% with *Oesophagostomum* spp. and 61 % *Eimeria* spp. with high opg-s in all age groups (up to 10,000). On small farms 44% infection with *Ascaris suum*, 66% with *Oesophagostomum* spp. and 38% *Eimeria* spp. has been observed. Pigs infected on the ecological farms: 89% *Oesophagostomum* spp., 32% *Ascaris suum*, 9% *Strongyloides ransomi*, 63% *Eimeria* spp. and 31% *Cryptosporidium* sp. The wild boar farm results were: *Oesophagostomum* spp. 64%, *Trichuris suis* 21%, *Metastrongylus* sp. 7% and *Eimeria* spp. 100% with low epg/opg-s. The identified *Eimeria* species in domestic pigs were: *E. porci*, *E. polita*, *E. suis*, *E. neodebliecki*.

Industrial management on large industrial farms with regular antiparasitic treatments seems to be successfully reducing the parasite infection level and species variety. On smaller conventional farms where antiparasitic treatments are carried out irregularly, high infection level with *Oesophagostomum* spp. and *Ascaris suum* was detected. Pigs on ecological farms with any pig treatments occurred to be more intensively infected than on other farms.

## SEROPREVALENCE OF *TOXOPLASMA GONDII* IN SHEEP IN FINLAND

By P. JOKELAINEN<sup>1</sup>, S. KNAAPI<sup>1</sup>, A. NÄREAHO<sup>1</sup>, A. OKSANEN<sup>2</sup> &  
A. SUKURA<sup>1</sup>

<sup>1</sup> *Department of Basic Veterinary Sciences, Faculty of Veterinary Medicine, University of Helsinki, (FINPAR), Finland*

<sup>2</sup> *Finnish Food Safety Authority Evira, Fish and Wildlife Health Research Unit, (FINPAR), Finland*

Although sheep is regarded as an important host for *Toxoplasma gondii* worldwide, data on the prevalence of this zoonotic parasite in sheep in Finland have been lacking. There are about 140 000 sheep in Finland and this serological study demonstrates the infection is prevalent. In addition, it reveals an intriguing north-south gradient in the seroprevalence.

Serum samples of 1940 sheep (20 animals over 1 year of age from 97 separate flocks) were analyzed in 2008. The sera included in this study originated from a larger disease surveillance program. *T. gondii*-specific IgG antibodies were detected in 477 (24.6%) of the samples with a commercial direct agglutination test (Toxo-Screen DA, bioMérieux) at a serum dilution of 1:40. Of the 97 flocks examined, 74 (76.3%) had seropositive sheep.

Seroprevalences in the six provinces of Finland suggested a tendency of lower prevalences in the north to higher in the south. In the northernmost province of Lapland the prevalence was 8.6% and in the province of Oulu (south from Lapland) 21.3%. South from the province of Oulu the seroprevalences were: in Eastern Finland 31.2%, in Western Finland 25.8%, in Southern Finland 21.5% and in Åland (the most south-western archipelago) 36.4%. Statistical analysis confirmed the gradient: 31 seropositive sheep of 360 examined from Lapland were compared with 64 of 300 from the province of Oulu ( $P < 0.001$ ) and with 160 of 440 from the Åland Islands ( $P < 0.001$ ). Seropositive animals were found in 9 of 18 flocks in Lapland, and 19 of 22 in the Åland Islands ( $P < 0.05$ ).

As a conclusion, *Toxoplasma gondii* is prevalent in sheep in Finland, and this should be considered a potential source of human infections.

## TRICHINELLA SPECIES IN WILDLIFE OF LATVIA

By M. KIRJUSINA<sup>1,2</sup>, G. JAUNBAUERE<sup>2,1</sup> & E. POZIO<sup>3</sup>

<sup>1</sup> *Laboratory of Parasitology, Institute of Systematic Biology, Daugavpils University, Latvia*

<sup>2</sup> *Division of Parasitology and Ichtiopathology, National Diagnostic Centre, Latvia*

<sup>3</sup> *Community Reference Laboratory for Parasites, Istituto Superiore di Sanita, Rome, Italy*

Trichinellosis is a zoonotic disease caused by different species of nematoda of the genus *Trichinella*. The natural cycle of *Trichinella* spp. involves many wild carnivorous and omnivorous species. In Latvia in the last years, pork from wild boars (*Sus scrofa*) has been an important source of trichinellosis for humans. Among wild carnivores, the raccoon dog (*Nyctereutes procyonoides*) and the red fox (*Vulpes vulpes*) play an important role as reservoir of *Trichinella* spp. infection. The aim of the present work was to evaluate the prevalence of *Trichinella* spp. infection among wildlife of Latvia and to identify the etiological agents at the species level.

Raccoon dogs, red foxes and wild boars were collected to be tested for *Trichinella* spp. Red foxes and raccoons were getting from Rabies vaccination program and only rabies negative animals were tested for *Trichinella* infection.

During the hunting season 2007-2008, 3,586 wild boars, 45 red foxes and 56 raccoon dogs were collected from four different geographical regions of Latvia. Muscle samples (25 g) from each animal were tested by the magnetic stirrer artificial digestion to detect *Trichinella* spp. larvae and to evaluate the worm burden. *Trichinella* larvae were detected in 107 animals from all regions of Latvia. The overall prevalence of infection in the red foxes, raccoon dogs and in the wild boars was 77.7 %, 71.4 % and 0.08%, respectively, with regional differences, being the Zemgale region that with the highest prevalence, but also the region from which most of the investigated animals originated.

*Trichinella* larvae collected from 55 positive animals have been identified at the species level by multiplex PCR. Two *Trichinella* species have been identified: *Trichinella britovi* and *T. nativa*. All *Trichinella* positive animals harboured *T. britovi* and one red fox and one raccoon dog were infected with both *T. britovi* and *T. nativa*. The average count of *Trichinella* larvae was 21.4 and 27.1 lpg in raccoon dogs and in red foxes, respectively. After artificial digestion of muscle samples from 275 wild boars, mesocercariae of *Alaria alata* have been detected in 17 (6.2%) wild boars with a worm burden of 1-12 mesocercariae per animal.

THE *STRONGYLIDAE* SPECIES COMPOSITION IN HORSES FROM A RIDING CLUB –  
PRELIMINARY STUDY

By S. KORNAŚ, M. SKALSKA, M. BASIAGA, B. NOWOSAD &  
P. NOSAL

*University of Agriculture in Krakow, Faculty of Animal Sciences, Department of Zoology and  
Ecology, Poland*

The aim of the study was to determine the species composition of nematodes (*Strongylidae*) in regularly dewormed horses from a riding club.

The research was conducted in 2008 on the 7 horses in the riding club, bred in housed-pasture system. Among them, 5 were regularly dewormed with drugs based on ivermectin. The remaining 2 were brought to the riding club prior to the research starting point, and used to receive anti parasite drugs from the benzimidazole group. The level of nematodes infection was determined based on concentration Mc Master method. After that the horses were dewormed with ivermectin. Feces samples, weighing about 2kg, were gathered directly from the horses' boxes 24, 48 and 74 hours after disposing the medicine. Then, the obtained samples were analyzed i.e., found nematodes were identified to species. Based on the results, the species composition of nematodes as well as relative abundance each species of *Strongylidae* group were determined.

The presence of parasites in feces samples collected in 12th and 72nd hour was rarely noticed. In 24th hour after deworming 15 species of small strongyles (*Cyathostominae*) in horse feces has been found, which belonged to 5 genus: *Cyathostomum catinatum*, *C. pateratum*, *Coronocylus coronatus*, *C. labiatus*, *C. labratus*, *Cylicostephanus longibursatus*, *C. goldi*, *C. calicatus*, *C. minutus*, *Cylicocylus nassatus*, *C. ashworthi*, *C. leptostomum*, *C. insigne*, *C. radiatus* and *Parapoteriostomum mettami*. The number of different small strongyles species found in those horses ranged from 3 to 13 in each.

In one of the horses, which was dewormed in the previous years with benzimidazole, 13 species of *Cyathostominae*, as well as 5 species of large strongyles (*Strongylinae*) belonging to two genus: *Strongylus vulgaris*, *S. edentatus*, *S. equinus*, *Triodontophorus serratus* and *T. brevicauda* were noticed.

AN EXPERIMENTAL APPROACH FOR DIRECT INVESTIGATION OF REPRODUCTIVE  
ISOLATION OF HAEMOSPORIDIAN PARASITES

By A. KRIŽANAUSKIENĖ<sup>1</sup>, G. VALKIŪNAS<sup>1</sup>, T. IEZHOVA<sup>1</sup>, V. PALINAUSKAS<sup>1</sup> & S.  
BENSCH<sup>2</sup>

<sup>1</sup> *Institute of Ecology of Vilnius University, Lithuania*

<sup>2</sup> *Department of Animal Ecology, Ecology Building, Lund University, Sweden*

In spite of their potential as model organisms and their medical importance, parasites have been insufficiently discussed in the general literature about speciation processes. Scarcity of experimental data regarding reproductive isolation of parasites is a serious obstacle. The experimental investigations of speciation mechanisms are rare for parasitic organisms, especially protozoans, mainly because of their complicated life history strategies, the difficulties of growing and maintaining the majority of parasites and/or their hosts in the laboratory, and the problems in recognising, and quantifying mate-recognition signals. The aims of the present study were to develop a method for direct investigation of hybridization of haemosporidians in vitro and provide information about this process based on morphological analyses of avian *Haemoproteus* spp. (Haemosporida, Haemoproteidae). Five species of *Haemoproteus* spp. were sampled from naturally infected passerine birds. They were identified to species based on morphology of their gametocytes and PCR amplification of a segment of the parasite's mitochondrial cytochrome b gene. Hybridization of *Haemoproteus* spp. was initiated in vitro by mixing blood, containing mature gametocytes of different species, with 3.7% solution of sodium citrate and exposure of the mixture to air. The following hybridization experiments were performed: (i) *Haemoproteus minutus* x *Haemoproteus pallidus*, (ii) *Haemoproteus balmorali* x *Haemoproteus tartakovskiyi*, (iii) *Haemoproteus fringillae* x *H. tartakovskiyi*. The development of ookinetes of both species was blocked in the first experiment. Ookinetes of all species developed in other experiments, but presumed hybrids were distinguished only in the third experiment. The present communication indicates that controlled experiments for direct hybridization of haemosporidian parasites can be carried out in vitro. Avian haemoproteids are convenient model organisms for that.

*SARCOCYSTIS* SPP. IN THE MALLARDS (*ANAS PLATYRHYNCHOS*) AND IN THE BIRD FAMILY TURDIDAE

By L. KUTKIENĖ, P. PRAKAS, D. BUTKAUSKAS & A. SRUOGA

*Institute of Ecology of Vilnius University, Lithuania*

Birds can serve both as intermediate or definitive hosts for protists of genus *Sarcocystis*. There is an opinion that the species of *Sarcocystis* is host specific to the intermediate host. These parasites are not extensively investigated in European birds using both traditional and molecular methods. The aim of our work was to investigate prevalence of infection in the mallards (*Anas platyrhynchos*) and some species of the family Turdidae birds; to examine cyst morphology and to perform DNA analysis of some *Sarcocystis* isolates.

In the period of 1997-2008, a total of 221 mallards and 144 individuals of the family Turdidae birds were investigated. The largest part of material (all mallards and a few representatives of the family Turdidae) was collected in Lithuania, the remaining material – in the South Curonian Spit (Russia). Neck and leg muscles of the birds were examined by light microscopy. Some cyst isolates were investigated by transmission electron microscopy and DNA analysis was performed using ssu rRNA gene sequences.

Cysts of *Sarcocystis* were detected in 43 (19.5%) out of 221 investigated mallards. One type of macrocysts and 4 types (type I, II, III and IV) of microcysts were found. Morphologically macrocysts correspond to *S. rileyi*. The analysis of the cyst wall ultrastructure and DNA investigation results of type II and type IV microcysts indicate these species to be still unnamed. It is difficult to distinguish the walls of the cysts of type I and type IV by light microscopy, whereas differentiation of the cysts of type II and type III is possible. The data obtained allow us to conclude that mallards are intermediate hosts for not less than 4 species of *Sarcocystis*.

*Sarcocystis* were found in 53 (36.8%) out of 144 family Turdidae birds examined. Parasites were found in 6 species of turdids out of 8 investigated ones. Only one type of microcysts (type VI) was found in these birds. The cyst wall ultrastructure of isolates from the blackbird (*Turdus merula*) and the fieldfare (*T. pilaris*) differed only in insignificant details. Ssu rRNA gene sequences of sarcocysts from the blackbird and the fieldfare showed 100% identity. This indicates that this species of *Sarcocystis* may form muscle cysts in at least two species of turdids and above species isn't strictly host specific.

# PRESENCE OF PULMONARY PARASITES IN BANK VOLES SHOW NO ASSOCIATION WITH PUUMALA VIRUS INFECTION

By J. LAAKKONEN<sup>1</sup>, L. VOUTILAINEN<sup>2,3</sup> & H. HENTTONEN<sup>3</sup>

<sup>1</sup> *Department of Basic Veterinary Sciences, University of Helsinki, Finland*

<sup>2</sup> *Department of Virology, Haartman Institute, University of Helsinki, Finland*

<sup>3</sup> *Finnish Forest Research Institute, Vantaa, Finland*

In wild animals the increased allocation of resources to virus-specific immune response against chronic infection directs resources away from survival and reproduction, and from the immunological response against parasites. Information on possible interactions between various disease agents is important because their synergistic effects may influence host fitness, and the transmission of the disease agents involved, also to accidental hosts. Our aim was to study whether the presence of lung parasites in bank voles show any association with Puumala virus (PUUV) infection. We trapped over-wintered bank voles, *Myodes glareolus*, (N=219) in late spring from populations in the increase phase of their density cycle from Central Finland, and screened their pulmonary tissue samples for PUUV by PCR, and for pulmonary parasites *Hepatozoon* spp. and *Emmonsia* spp. by histology. The prevalence of PUUV (38%) and *Emmonsia* (11%) showed no relation to host sex but meront forms of *Hepatozoon* were significantly more common in males (24%) than in females (9%). We detected no significant associations between Puumala virus infection and the pulmonary parasites of bank voles. Although cross-sectional data does not prove interactions, information gained on concurrent infections can be used to make relevant predictions about the nature of parasite infections, and to help pinpointing the relevant disease agents for longitudinal studies on parasite interactions.

# EPIDEMIOLOGICAL FACTORS INFLUENCING OOCYST SHEDDING IN ESTONIAN DAIRY HERDS

By B. LASSEN<sup>1</sup>, A. VILTROP<sup>1</sup> & T. JÄRVIS<sup>1</sup>

<sup>1</sup> *Department of Infectious Diseases, Estonian University of Life Sciences, Estonia*

Coccidia in cattle primarily cover *Eimeria* spp. and *Cryptosporidium* spp. They are found in almost all Estonian dairy farms. Mortality due to coccidiosis is rare, and more commonly sub-clinical disease can be observed as anorexia, reduced feed uptake, and reduced weight gain. Parasite control is limited by resistance to disinfectants, limited effects of drugs, and high infectivity in small amounts of oocysts. Limiting the possibilities of parasite spread is possible through knowledge of which management factors affect the parasites. In our study we examined the amount of oocysts shed by cattle and their statistical association with collected factors covering environment, management, serology, and location. A mixed model with random effects of multivariable data was used to accomplish the analysis. The data was examined according to age categories (<3, 3-12, and >12 months), and all samples together. Removing a calf instantly from its mother after birth increase the risk of shedding more oocysts for both *E. spp.* (OR=1.47) and *C. spp.* (OR=5.00). Humidity higher than 75% and practising vacancy period before replacing animals in pens increased the risk of *E. spp.* in cows (OR=2.94 and 2.27). Calves <3 months and cows shed less oocyst (OR=0.51 and 0.41) when the herd was positive for Bovine Herpes Virus (BHV-1), while a similar effect was seen in animals between 3-12 months when housed separate from older animals (OR=0.64). Later removal of the calf from the mother statistically seems the most effective way of reducing coccidia infections according to our study. Considerations regarding cleaning procedures between pen rotation, housing of younger animals separately, and low indoor humidity, may also improve conditions regarding *Eimeria* infections.

## TREMATODS OF SOME KARELIAN BIRDS

By D. LEBEDEVA

*Institute of Biology, Karelian Research Center RAS, Russia*

There're few data on bird's parasites in Karelia (Mozgovoj et al., 1962; Martyanov, 2001). Also we have a lot of helminthes material of 1960-1962s expedition, which is still stained in ethanol in lab's museum and not use for investigation. So we tried to fill a gap in this problem, especially in trematods. As result 23 bird species were examined (78 specimens totally). They are representatives of 5 families (Anatidae, Laridae, Charadriidae, Gaviidae, Podicipitidae) from the 3 lakes: Segozero, Sjamozero, Onego. Twelve genes were revealed (*Hypoderaeum* sp., *Echinostoma* sp., *Echinoparyphium* sp., *Ignavia* sp., *Leucochloridium* sp., *Plagiorchis* sp., *Prostogonimus* sp., *Notocotylys* sp., *Diplostomum* sp., *Apatemon* sp., *Ichthyocotylurus* sp.). The Anatidae birds had the highest diversity of trematods species. Among the parasites flukes of family Strigeidae were the most frequent. Investigations were supported by the grant of President of Russian Federation for young scientists (MK-2470.2008.4).

# HOST SPECIFICITY VS HOST SWITCH.

## I. CONTEMPLATING THE PARADOX.

By J. LUMME<sup>1</sup> & M. S. ZIĘTARA<sup>2</sup>

<sup>1</sup> *Department of Biology, University of Oulu, Finland*

<sup>2</sup> *Laboratory of Comparative Biochemistry of Biological Station, University of Gdańsk, Poland*

In the life of parasites, the adaptation with host organism is crucial. To find and attach on/in, to exploit and to avoid defense, and to cross the gap between the host generations, all need specific adaptations. The most easy prediction is that parasites have to coevolve with the hosts, but there is a lot of evidence about host switching. How the host switching is possible? Evolution can not predict. We have contemplated this problem among *Gyrodactylus* ectoparasites, after learning the basic facts of the population structure. In wagneri group, the main mode of propagation is clonal. Clones are extremely host specific. The parthenogenesis maintains the genotype of the female, including heterozygosity. The reproductive capacity of *Gyrodactylus* is often considered to be large, evidenced by the rapid clone expansion. At closer look, the viviparity is extremely restrictive and costly. A female can give birth to one giant progeny at a time. The high relative unit price restricts the risk-taking in the parent-offspring transition. Parasites having millions of eggs are expected to lose most of them, due to the difficulty of targeting to the next generation host. Then some genetic variation is advantageous in the long term. But why should a mother giving birth to  $\leq 4$  full-grown offspring risk any experimental recombination, hopefully adapted to some other host? The answer, in wagneri group of *Gyrodactylus*, is that they don't risk. The females perfectly copy their own genome, without recombination, and the progeny is not testing anything new, but remains on the same, familiar-smelling host surface, for ever.

What about males. It is well known that older *Gyrodactylus* females turn hermaphrodites. What is their evolutionary interest? If a clone has propagated some time on a host, all potential mates around are identical clonemates, so sex equals selfing. Selfing is never profitable over clonal reproduction, because it leads to loss of genetic variation. So, what is the role of maleness?

The paradox is: The females are self-sufficient, well adapted, extremely host specific and not interested in sex. An all-female clone can not switch host, and will die out, after accumulating enough mutations. As males ... ?

## SPREADING OF POTATO CYST-FORMING NEMATODE IN NORTH-WEST RUSSIA

By E. MATVEEVA & L. GRUZDEVA

*Institute of Biology, Karelian Research Centre RAS, Russia*

At present potato cyst-forming nematode (PCN) *Globodera rostochiensis* Woll. is registered in most of regions of the European part of Russia. In Karelia PCN has been found in 1976 and for 30 years has spread in North (from 62°C to 67° n.l.). This spreading is stipulated with changeable climate conditions, particularly temperature. Analysis of nematode fauna and plant parasite complex in agrocenoses from 3 temperature zones showed considerable differences in the nematode community structure which depend on climatic and soil conditions and extent of soil infestation by PCN. The highest soil infestation was observed in central part, which was before considered as the northern border of PCN. Nematode community had the lowest diversity and maturity indices. This phenomenon is connected with both temperature anomalies during the last decades and insufficient control measures against potato nematode. In the northern regions of Karelia the number of infested fields is still low. Nematode communities have a high structure index (SI) of soil food web due to large share of polytrophs in the community. Human activities are the main reason of PCN distribution and climate warming is a concomitant factor.

## THE RICHNESS OF PATHOGEN' FAUNA OF TICKS COLLECTED FROM MIGRATORY BIRDS

By A. MOVILA<sup>1</sup>, A.N. ALEKSEEV<sup>2</sup>, H.V. DUBININA<sup>2</sup> & I. K. TODERASH<sup>1</sup>

<sup>1</sup> *Centre for General and Molecular Biology, Institute of Zoology, MAS, Chisinau, Republic of Moldova*

<sup>2</sup> *Zoological Institute, RAS, St. Petersburg, Russia*

The role of migratory birds as a source of pathogenic microorganisms relocated by them round the Globe is well known. Data concerned on bird' phoresy are more modest and limited by the knowledge of transfer of Ixodid ticks infected by borreliae and ehrlichiae. Experiments executed on the Ornithological Station "Fringilla" on Curonian Spit (Kaliningrad region, Russia) within 2000–2008 permitted to reveal in *Ixodes ricinus* preimaginal stages reattached from migratory birds a lot of microorganisms dangerous for human: tick-borne encephalitis virus –TBEV (2008, fall), *Borrelia burgdorferi* sensu stricto, *B. afzelii*, *B. garinii*, *Ehrlichia muris*, *Anaplasma phagocytophilum* (2000, spring and fall), *Rickettsia helvetica*, *R. japonica* and *Babesia* sp. EU1 (2008, spring). TBEV was never before revealed in ticks from birds in the fall, whereas neither *R. helvetica*, *R. japonica* nor *Babesia* sp EU1 were never before observed in ticks on the territory of Curonian Spit. Detection of bird influenza viruses in the pellets of migratory birds captured on the same station permitted to suppose that these agents will be detected from the parasitizing on them ticks also.

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *TRICHURIS* SPP.  
OBTAINED FROM HUMANS AND PIGS

By S. NISSEN<sup>1</sup>, P. NEJSUM<sup>1</sup>, H. CHRISTENSEN<sup>2</sup>, A. OLSEN<sup>2</sup> & S.M. THAMSBORG<sup>1</sup>

<sup>1</sup>*Danish Centre for Experimental Parasitology, Denmark*

<sup>2</sup>*Department of Veterinary Disease Biology, Faculty of Life Science, University of Copenhagen, Denmark*

The nematodes, *Trichuris trichiura* and *Trichuris suis* infecting humans and pigs, respectively are believed to be two separate but closely related species. The aim of our study was to examine the morphological and genetic diversity of *Trichuris* spp. recovered from the two hosts. Sympatric worm material isolated from 10 humans and 5 pigs in Uganda supplemented with *T. suis* from Tanzania, Denmark and USA and *T. trichiura* from England, was obtained. Based on morphology, worms from the two hosts could only be discriminated by the length of the male spicule (t-test,  $p < 0.001$ ). The second internal transcribed spacer (ITS-2) in the r-DNA was amplified by PCR and cloned. Between 1 and 6 clones from 20 worms were sequenced, which resulted in 49 human-derived and 45 pig-derived sequences that could be allocated into as many as 56 different haplotypes. A very large intra-individual variation was found within the human-derived sequences (0.2 – 45.0%) compared to the pig derived sequences (0.2 – 1.4%). This was due to the fact that the human-derived worms consisted of two main ITS-2 sequence types; one of them being unique (69% of the human-derived sequences, consensus sequence 481 nucleotides long) and the other being identical to the sequence type found in pig-derived worms (31% of the human-derived worms, consensus sequence 531 nucleotides long). The results indicated that the nematodes found in pigs belong to a genetically distinct species (*T. suis*) whereas the nematodes in humans showed considerable genetic variability either related to ancestral polymorphism or more recent cross-breeding between *T. trichiura* and *T. suis*. In addition, evidence for cross-infection of human with *T. suis* was found.

## THE OCCURRENCE OF *METASTRONGYLUS* SPP. IN WILD BOARS FROM SOUTH POLAND

By P. NOSAL, P. MORAWSKI, J. KOWAL, B. NOWOSAD & S. KORNAŚ

*University of Agriculture in Krakow, Faculty of Animal Science, Department of Zoology and Ecology, Poland*

Metastrongylosis is one of the most common and pathogenic parasitic disease affecting European wild boar (*Sus scrofa*) (Houszka 2001). The occurrence of lung nematodes of *Metastrongylus* genus was surveyed on 19 wild boars shot in a hunting area close to Cracow, Poland during three consecutive winters (2007 – 2009). Based on tooth eruption patterns, the boars were categorized into two age groups: juveniles – less than 12 months, and adults – those over 1 year of age. Their lungs were excised, separated to every lobes, and examined along the bronchial tubes (Roepstorff & Nansen 1998). The parasites found were put into 70% ethanol with 5% glycerin, and identified according to the descriptions by Tarczyński (1956), Holló (1965) and Morita *et al.* (2007).

The prevalence of the lungworms reached 78.9%, and all the infected animals harboured two (one boar) or more species (the others). The mean number of parasites in each boar was 59.2, ranging from 6 to 250 specimens. From 10 yearlings, 8 were infected with the mean intensity of 79.2 (15-250) parasites, whereas 9 adults were infected in 66.7% with 35.2 (6-75) worms per animal. Among 250 identified lungworm specimens, 48.8% belonged to *M. pudendotectus*, 32.8% to *M. elongatus* and 14.0% to *M. salmi*; 4,4% constituted *M. asymmetricus*. The male to female ratio was 1:2,4.

Till now, in Poland the occurrence of four *Metastrongylus* species were confirmed, namely *M. elongatus*, *M. pudendotectus*, *M. salmi* and *M. confusus* (Tarczyński 1956, Gadomska 1981, Drózdź & Zalewska-Schönthaler 1987). We have found the new one species for Polish fauna – *M. asymmetricus*.

# HELMINTHS OF *FELIDAE* FROM THE ZOO GARDEN AND ANIMAL SHELTER OF CRACOW, POLAND IN COPROSCOPIC EXAMINATION

By P. NOSAL, I. NOWAK, S. KORNAŚ & B. NOWOSAD

*University of Agriculture in Krakow, Faculty of Animal Science, Department of Zoology and Ecology, Poland*

During 2008, the shedding of helminth eggs was monitored in the faeces of *Felidae* kept in captivity, both in the city's zoo and animal shelter. Faecal samples were collected at bimonthly intervals (seven collections). In the zoological garden, the *Felidae* had been kept either separately or in pairs, or the females and their young together in one place. In the shelter, cats were kept in groups of 3-20 animals, or separately – sick ones or in quarantine. The zoo animals were fed exclusively on freshly killed laboratory rats (feline) or with raw beef, rabbits or frozen chickens (big cats), whereas in the shelter pelleted or canned food was given. All the animals in the zoo and shelter were regularly dewormed several times thorough the year with fenbendazole. A total of 200 faecal samples were collected randomly from 16 various species of *Felidae* housed in the zoo, and from stray cats kept in the shelter, in morning hours, with the assistance of the animals handlers, and processed by the concentration McMaster technique, with salt and sugar saturated solution as a flotation fluid.

*Toxocara cati* was the most abundant parasite, affecting from 9.1 to 17.8% of animals in the shelter and zoo, respectively, with the mean intensity of 1267 (20-2680) eggs per gram of faeces (EPG) in the shelter, and 741 (20-2540) EPG in zoo. The Pallas cats (*Felis manul*), jungle cats (*F. chaus*) and jaguarundi (*Puma yagouarundi*) harboured the highest number of these roundworms. *Toxascaris leonina* occurred only in the zoo animals, with the infection level of 23.7% and 3126 (20-40000) EPG. The sand cats (*F. margarita*), lion (*Panthera leo*) and jaguarundi were mostly affected with this species. *Capillaria* sp. appeared only in jaguarundi, thorough the whole period of study until its death, with the intensity of 160 (60-420) EPG, while *Ancylostoma tubaeformae* was noted only once in the lynx (*F. lynx*) – with 80 EPG. In the domestic cats from shelter, apart from *T. cati*, *Trichuris vulpis* was observed in few cases, with a low EPG number.

Routine monitoring of the presence of parasites in the animals kept in zoo and shelter is very important for the prevention and control of diseases affecting animals and humans, as well.

## ECHINOCOCCI IN THE BARENTS REGION

By A. OKSANEN<sup>1</sup>, A. LAVIKAINEN<sup>2</sup>, M. ISOMURSU<sup>1</sup>

<sup>1</sup>*Finnish Food Safety Authority Evira, Fish and Wildlife Health Research Unit (FINPAR),*

<sup>2</sup>*Haartman Institute – University of Helsinki (FINPAR)*

Two *Echinococcus* species/genotypes are of special interest in the Barents Region, *Echinococcus granulosus* G10 and *Echinococcus multilocularis*. The first one is currently seen sporadically in wolf-reindeer/elk -cycle in northeastern Finland, obviously also on Kola Peninsula, and uncommonly elsewhere in Fennoscandia. A reindeer herding dog-reindeer life cycle of *E. granulosus* existed in northern Fennoscandia during the first half of the 20th century. During the 1950s, infection was at least locally common; e.g. in Kautokeino in Finnmark County, Norway, about 10% of reindeer were found infected. Also several human infections were reported. Thus, Norwegian authorities initiated a campaign to fight the infection with anthelmintic treatment of herding dogs and enhanced reindeer slaughter hygiene. The measures appeared beneficial as echinococcosis became more and more uncommon. However, the same trend was also seen in Sweden and Finland, where no specific measures were taken. The obvious explanation is in the change in reindeer husbandry where herding dogs were replaced by herders riding snowmobiles. Snowmobiles cannot serve as definitive hosts for *E. granulosus*, which became virtually extinct. However, not fully, as during the early 1990s, the infection was seen emerging in reindeer of the easternmost part of the Finnish reindeer husbandry area. The definitive host proved to be wolves which apparently had found out to exploit the Finnish-Russian border where they could seek shelter from hunters and reindeer herders protecting their livestock. All parasite isolates from this origin identified so far have been *E.g.* G10. However, in Estonia two different cervid genotypes of *E. granulosus* coexist, *E.g.* G8 and *E.g.* G10. Therefore, the possibility of *E.g.* G8 as the causative agent of the dog-reindeer cycle cannot be positively excluded.

*Echinococcus multilocularis* exists in the Barents Region on Svalbard (Spitsbergen) and in Arkhangelsk Oblast. The Spitsbergen life cycle has been facilitated with the unintentional introduction of the sibling vole *Microtus rossiaemeridionalis*, probably with animal fodder transported from Leningrad Oblast to the Russian mining community of Grumant. There, the vole population has thrived, or at least survived, for decades. *Echinococcus multilocularis* was perhaps introduced later with rambling Arctic foxes infected in Siberia or Arkhangelsk Oblast. Now, the life cycle includes these two host species. This parasite has never been found in Fennoscandia, but because the infection is known, in addition to the Barents Region, from both Estonia and Denmark, the potential for introduction is rather high. In both Norway, Sweden and Finland, surveillance of infection is carried out by examination of potential definitive hosts, in Norway and Sweden, red foxes, in Finland, also raccoon dogs. In Finland, also a great number of potential intermediate hosts are tested yearly.

During, 2006-2008, for example, 823 red foxes, 581 raccoon dogs and 82 wolves supplied by hunters were sampled for echinococci in Finland. They were initially tested using coproantigen ELISA at the University of Zürich. Coproantigen positive animal intestines preserved frozen were then confirmed either with the sedimentation technique or, more recently, by demonstration of taeniid eggs by flotation technique. The identification of the taeniid eggs as *Echinococcus* is to be done by PCR. Of the 823 foxes, 73 (8.9%), 581 raccoon dogs, 15 (2.6%) and 82 wolves 16 (19.5%) were found ELISA positive. Thus far, all the red foxes and raccoon dogs where confirmation tests have been completed, have been found negative. Of the wolves, on the other

hand, a considerable proportion have been confirmed positive, demonstrating them being involved in the cervid *Echinococcus* life cycle.  
The surveillance continues.

EFFECTS OF *PLASMODIUM RELICTUM* (LINEAGE P-SGS1) ON EXPERIMENTALLY INFECTED PASSERINE BIRDS, WITH REMARKS ON THE INFECTION TREATMENT WITH MALARONE™

By V. PALINAUSKAS<sup>1</sup>, G. VALKIŪNAS<sup>1</sup>, A. KRIŽANAUSKIENĖ<sup>1</sup>, S. BENSCH<sup>2</sup>  
& C. V. BOLSHAKOV<sup>3</sup>

<sup>1</sup> *Institute of Ecology of Vilnius University, Lithuania*

<sup>2</sup> *Department of Animal Ecology, Ecology Building, Lund University, Sweden*

<sup>3</sup> *Biological Station of the Zoological Institute of RAS, Russia*

*Plasmodium relictum* (lineage P-SGS1) is widespread and cause diseases of different severity in different species of avian hosts. However, experimental studies on effects of this malaria parasite on birds are rare. The aim of this study was to evaluate patterns of development of *Plasmodium relictum* (P-SGS1) in greenfinches *Carduelis chloris*. We also used the opportunity to carry out a preliminary experimental study with the aim to treat experimentally infected greenfinches and chaffinches *Fringilla coelebs* with Malarone™, which is highly effective against *Plasmodium falciparum*. PCR-based and microscopy methods were used to determine malaria infections. To multiply the strain of *P. relictum* (P-SGS1), blood stages from cryopreserved samples were subinoculated to 4 chaffinches. When light experimental infection developed in the chaffinches, the infected blood was inoculated to 6 uninfected greenfinches. Six uninfected greenfinches were used as negative controls; they remained uninfected during this study. Light parasitemia developed in all experimental greenfinches. There was no significant effect of malaria on the body mass or haematocrit value of the experimental birds; the infection did not cause mortality or morbidity in the greenfinches. Light parasitemia (< 0.01 %) was present in all experimental greenfinches and chaffinches before treatment with Malarone™ (7mg/kg). After the treatment, parasitemia disappeared in all experimentally infected greenfinches and chaffinches. Parasites were not recorded in these birds during the remaining treatment time (19 days in total). Greenfinches were not tested after this period of time. Parasitemia relapsed in all infected chaffinches one month after end of the treatment. Hence, the cure with Malarone™ was efficient for blood stages of *P. relictum*, but exoerythrocytic stages were unaffected. This study contributes to the knowledge on pathology and treatment of avian malaria parasites.

# EFFICIENCY OF TRADITIONAL MCMASTER CHAMBER AND NEWLY DESIGNED CHAMBER FOR ENUMERATION OF GASTROINTESTINAL NEMATODE EGGS

By S. PETKEVICIUS

*Lithuanian Veterinary Academy*

The efficiency of traditional McMaster chamber and newly designed chamber for the enumeration of gastrointestinal nematode eggs was studied. Two horse, two sheep and thirteen pig farms were randomly selected, and, 264 of horse, 264 of sheep faecal and 815 of pig faecal samples were examined. Horse faeces were examined by Henriksen and Aagard (1976) and Urquhart et al. (1996) modifications, whereas pig and sheep faeces were examined by Henriksen and Aagard (1976) and Kassai (1999) modifications. All samples were evaluated in two replicates: using traditional McMaster 0.3 ml chamber and newly designed 1.5 ml chamber. In horse farms, 65.5% and 83.7% with strongyles infected horses were identified (chambers I and II,  $P < 0.05$ ). In sheep farms, the number of positive to strongyle infection animals were 81.4% and 96.2% (I and II chambers,  $P < 0.05$ ). In pig farms, 11.5% and 18.2% (chambers I and II,  $P < 0.05$ ) of pigs were infected with *Ascaris suum*. Furthermore, 14.6% and 17.8% (chambers I and II,  $P < 0.05$ ) of pigs were infected with *Oesophagostomum dentatum*, and 3.7% and 8.2% (chambers I and II,  $P < 0.05$ ) with *Trichuris suis*. There were no significant differences to both chambers in the time per samples analyzed ( $P > 0.05$ ). The darkness and transparency of traditional McMaster 0.3 ml chamber (I) and newly designed 1.5 ml chamber (II) were comparable. The new modification of chamber demonstrated statistically higher sensitivity for enumeration of gastrointestinal nematode eggs and for evaluation of farms with infected animals compared to earlier described McMaster modifications.

CLARIFICATION OF LIFE CYCLES OF SOME EUROPEAN ALLOCREADIID  
(TREMATODA) SPECIES BASED ON ITS2 SEQUENCES

By R. PETKEVIČIŪTĖ<sup>1</sup>, V. STUNŽĖNAS<sup>1</sup>, G. STANEVIČIŪTĖ<sup>1</sup>, S. G. SOKOLOV<sup>2</sup>

<sup>1</sup> *Institute of Ecology, Vilnius University, Lithuania*

<sup>2</sup> *A. N. Severtzov Institute of Ecology & Evolution RAS, Russia*

In Europe Allocreadiidae includes the genera *Allocreadium* Looss, 1900, *Bunodera* Railliet, 1896 and *Crepidostomum* Braun, 1900. Only one species of the genus *Bunodera* was described in Europe – *B. luciopercae*. Subspecies *B. luciopercae luciopercae* (Müller, 1776) and *B. l. acerinae* Roitman et Sokolov, 1999 have been recognized based on morphology, RAPD profiles and host specificity. Different taxons revealed within *B. luciopercae* sensu lato leads to the presumption about different life cycles. In this study *B. l. luciopercae* and *B. l. acerinae* were obtained from their definitive hosts, perch and ruff. ITS2 was sequenced and compared with allocreadiid larval stages from sphaeriid clams. Adult and larval stages of *Allocreadium isoporum* (Looss, 1894), obtained from bleak and *Sphaerium rivicola*, were also included into analysis. Sequence analysis of the ITS2 clearly distinguished the adult *B. l. luciopercae* and *B. l. acerinae*; a difference as large as 3.75% at this DNA locus was determined. Identical ITS2 were revealed for adult *B. l. acerinae* and larval *B. luciopercae*, which was identified following description of Wisniewski (1958). The most surprising result was 99.16 % identity of ITS2 of larval *A. isoporum*, occurring in *S. rivicola* from Lithuanian and Ukrainian populations, and that of adult *B. luciopercae luciopercae* from perch. This strongly suggests that the parthenitae identified as *A. isoporum* following descriptions of Wisniewski (1958) and adult *B. luciopercae luciopercae* represent the same species, something unexpected. Adult *A. isoporum* obtained from bleak significantly differs from larval *A. isoporum*. The level of ITS2 sequence divergence (8.5%) between them is consistent with the level of intergeneric variation expected. So, we may have to reconsider some allocreadiids life cycles, specifically, the life-cycle of *A. isoporum* is yet to be elucidated. This study was supported by the Lithuanian Science and Studies foundation research grant C-07002.

## COMPARISON OF THREE FLOTATION METHODS FOR COUNTING EGGS IN FAECES

By A. S. RANJITKAR, A. AL-JUBERY, A. MOHAMED, P. NEJSUM & A. ROEPSTORFF

*Danish Centre for Experimental Parasitology, Department of Veterinary Disease Biology, Faculty of Life Sciences, University of Copenhagen, Denmark*

Quantitative assessment of intestinal helminth infections by flotation of eggs in faeces (faecal egg counts; FEC) is a commonly used method in veterinary parasitology. However, the precision and accuracy in the results depend of the method. We here compare three methods for FEC estimation, namely Simple McMaster, Concentration McMaster and the newly developed FLOTAC method. Faecal material was obtained from an *Ascaris suum* negative pig (as determined by the Concentration McMaster Technique) and 722,000 *A. suum* eggs were added. The faeces was mixed manually for 3 h to give a homogeneous sample with 639 eggs per gram. All three methods were evaluated by examination of 25 subsamples using the same flotation fluid (saturated NaCl with 500 g glucose per liter, specific gravity 1.27 g/ml). The FEC was found to be 184 (SD 77), 456 (SD 67) and 327 (SD 54) by Simple McMaster, Concentration McMaster, and FLOTAC method, respectively. Analysis of variance (ANOVA) showed that the FECs were all significantly different ( $P < 0.001$ ) from each other. The recovery of the eggs was highest in concentration McMaster (71.4 %), lowest in simple McMaster (28.8%) and intermediate for FLOTAC (51.2%). The Concentration McMaster therefore seems to be the method of choice for FEC estimation of *A. suum* eggs. However, in cases of low FEC the FLOTAC technique may be preferred due to a lower detection limit (1 egg per gram of faeces) where it is 20 for the Concentration McMaster method.

A NOVEL PHYLOGENY FOR THE GENUS *ECHINOCOCCUS*, BASED ON NUCLEAR DATA, CHALLENGES RELATIONSHIPS BASED ON MITOCHONDRIAL EVIDENCE

By U. SAARMA<sup>1</sup>, I. JÕGISALU<sup>1,2</sup>, E. MOKS<sup>1</sup>, A. VARCASIA<sup>3</sup>, A. LAVIKAINEN<sup>4</sup>, A. OKSANEN<sup>5</sup>, S. SIMSEK<sup>6</sup>, V. ANDRESIUK<sup>7</sup>, G. DENEGRI<sup>7</sup>, L. M. GONZÁLEZ<sup>8</sup>, E. FERRER<sup>8,9</sup>, T. GÁRATE<sup>8</sup>, L. RINALDI<sup>10</sup> & P. MARAVILLA<sup>11</sup>

<sup>1</sup>Department of Zoology, University of Tartu, Estonia

<sup>2</sup>Department of Game Monitoring, Centre of Forest Protection and Silviculture, Estonia

<sup>3</sup>Dipartimento di Biologia Animale Sezione di Parassitologia e Malattie Parassitarie, Università degli Studi di Sassari, Italy

<sup>4</sup>Department of Bacteriology and Immunology, University of Helsinki, Finland

<sup>5</sup>Finnish Food Safety Authority Evira, Oulu, Finland

<sup>6</sup>Department of Parasitology, University of Firat, Turkey

<sup>7</sup>Department of Biology, Mar del Plata National University, Argentina

<sup>8</sup>Instituto de Salud Carlos III, Centro Nacional de Microbiología, Spain

<sup>9</sup>Departamento de Parasitología and Instituto de Investigaciones Biomédicas, Universidad de Carabobo, Venezuela

<sup>10</sup>Department of Pathology and Animal Health, University of Naples Federico II, Italy

<sup>11</sup>Dirección de Investigación, Hospital General “Dr. Manuel Gea González”, Mexico

The taxonomic status of *Echinococcus*, an important zoonotic cestode genus, has remained controversial, despite numerous attempts to revise it. Although mitochondrial DNA (mtDNA) has been the source of markers of choice for reconstructing the phylogeny of the genus, results derived from mtDNA have led to significant inconsistencies with earlier species classifications based on phenotypic analysis. Here, we used nuclear DNA markers to test the phylogenetic relationships of members of the genus *Echinococcus*. The analysis of sequence data for 5 nuclear genes revealed a significantly different phylogeny for *Echinococcus* from that proposed on the basis of mitochondrial DNA sequence data, but was in agreement with earlier species classifications. The most notable results from the nuclear phylogeny were (1) *E. multilocularis* was placed as basal taxon, (2) all genotypes of *Echinococcus granulosus* grouped as a monophyletic entity, and (3) genotypes G8 and G10 clustered together. We conclude that the analysis of nuclear DNA data provides a more reliable means of inferring phylogenetic relationships within *Echinococcus* than mtDNA and suggest that mtDNA should not be used as the sole source of markers in future studies where the goal is to reconstruct a phylogeny that does not only reflect a maternal lineage, but aims to describe the evolutionary history at species level or higher.

# DO *CORYNOSOMA* SPECIES USE DIFFERENT PARATENIC FISH HOSTS IN THEIR LIFE CYCLE?

By T. H. SINISALO

*University of Jyväskylä Department of Biological and Environmental Science*

In the Bothnian Bay both ringed seals (*Phoca hispida botnica*) and grey seals (*Halichoerus grypus*) can act as the definitive host for three *Corynosoma* species (*C. strumosum*, *C. magdaleni* and *C. semerme*). *Corynosoma* species use *Monoporeia affinis* as the intermediate host in their life cycle but according to previous studies the paratenic fish host may differ between these intestinal helminth species. Stable isotope ratios of carbon and nitrogen ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) were analyzed from adult *Corynosoma* parasites from the alimentary tract of ringed seals. The  $\delta^{13}\text{C}$  values were similar between species but the  $\delta^{15}\text{N}$  ratio of *C. strumosum* was lower than that of the other species. *C. strumosum* and *C. magdaleni* were found along the full length of the small intestine but most *C. semerme* were located in the large intestine and the rectum. This spatial distribution of the worms affected the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. Combining the parasite results with the stable isotope ratios from fish and seals in the same area *C. strumosum* uses more herring as a paratenic host in its life cycle more than the other two species.

## EPIDEMIOLOGICAL STUDIES ON FLUKES IN CATTLE FROM SOUTHERN POLAND

By M. SKALSKA, S. KORNAS, B. NOWOSAD & P. NOSAL

*University of Agriculture in Krakow, Faculty of Animal Sciences, Department of Zoology and Ecology, Poland*

In the temperate climate, two species of flukes: liver fluke (*Fasciola hepatica*) and the lancet fluke (*Dicrocoelium dendriticum*) are commonly found in the liver bile ducts of the ruminants. The aim of the study was to determine the prevalence and intensity of the fluke infection in cattle slaughtered from the areas of southern Poland.

The necropsies were conducted during the years 2006 – 2007 in 5 butchereries. A total of 824 animals of both sexes and in various ages, mostly Holstein, were examined. The cattle was bred in housed or pasture system on small farms, which explains that most of the animals were defective milk cows. All slaughtered cattle on each day was examined. The 127 livers were pre-evaluated, taking into account the color of the livers' surface and their consistence, also the checking cuts has been done. Then, they were thoroughly examined in the laboratory by cutting bile ducts and the liver itself. Parasites found were washed, counted and conserved in formaldehyde solution. Based on the obtained results, the following were calculated:

- the number of livers with the anatomopathological changes characteristic for fascioliasis, i.e., thickening and focal calcification of bile ducts, parenchymatous degeneration and abscesses
- the number of livers with other types of changes, not connected with fascioliasis
- the prevalence of infection with *Fasciola hepatica* and *Dicrocoelium dendriticum*
- the mean intensity of infection with *Fasciola hepatica* and *Dicrocoelium dendriticum*

The anatomopathological changes characteristic for fascioliasis were detected in 122 animals (14.8 %), mainly in thick bile ducts, while in 5 (3.9 %) among 127 examined the observed changes did not show the consequences of fascioliasis infection. The specimens of *Fasciola hepatica* were found in 61 animals (prevalence of infection 7.4 %), with the mean intensity of infection reaching 23.5 flukes per one host. *Dicrocoelium dendriticum* was detected twice (0.2 % of animals) in smaller bile ducts with the mean intensity 18 flukes per host.

## PROTOZOAN PARASITES OF REINDEER *RANGIFER TARANDUS* IN ICELAND

By K. SKIRNISSON<sup>1</sup>, B. GUDMUNSDOTTIR<sup>1</sup> & B. GJERDE<sup>2</sup>

<sup>1</sup>*Institute for Experimental Pathology, Keldur, University of Iceland, Reykjavik, Iceland*

<sup>2</sup>*Parasitology laboratory, Norwegian School of Veterinary Science, Oslo, Norway*

So far, at least 20 protozoan parasite species belonging to 11 genera (*Babesia*, *Besnoitia* (*Fibrocystis*), *Cryptosporidium*, *Eimeria*, *Entamoeba*, *Giardia*, *Isospora*, *Maegtryphanum*, *Sarcocystis*, *Toxoplasma* and *Trypanosoma*) have been reported from reindeer throughout the world.

During 2003 to 2005 gastrointestinal and tissue protozoan parasites were studied in Iceland by examining 248 faecal samples (192 calves, 56 adults) and various tissue samples from 34 adults. Faecal samples were examined by using the McMaster and the Formalin Ethyl-Acetate concentration methods. Cysts of *Giardia* sp. and *Cryptosporidium* sp. were made detectable with the Merifluor immunofluorescent method. H&E stained sections of tissue samples were examined for cysts of *Besnoitia*, *Sarcocystis* and *Toxoplasma*. Samples from oesophagus and diaphragm of 11 animals were examined for *Sarcocystis* in Norway.

Eight protozoan parasites were found. Three *Eimeria* spp. were detected; *E. mayeri* and the previously undescribed *E. rangiferis* and *E. hreindyria*. The prevalence of the eimeriids was low. Cysts of *Giardia* were exclusively found in calves (prevalence 13.5%). *Entamoeba* sp. was commonly found both in calves (59%) and adults (65%). *Sarcocystis* spp. were found in 64% of the 11 samples examined in Norway; *S. rangi* was most commonly found (63.6%), *S. hardangeri* was found in 36.4% and *S. tarandivulpes* was present in 18.2% of the samples.

The reindeer-specific parasites in Iceland (the eimeriids and the sarcosporidians) are regarded to have been imported with the reindeer from Norway in 1787, and have since then maintained their life cycles in the population. The three eimeriids are also expected to occur in reindeer in Fennoscandia. Further three *Sarcocystis* spp. occur in reindeer in Norway, some of them could still be undetected in the Icelandic population. *Giardia* sp. and *Entamoeba* sp. infections are very common in sheep grazing with the reindeers and are probably shared by these hosts.

COMPARISON OF THE PARASITE FAUNA OF REINDEER *RANGIFER TARANDUS*  
IN ICELAND AND NORTHERN NORWAY  
AFTER MORE THAN 200 YEARS OF SEPARATION

By K. SKIRNISSON<sup>1</sup>, B. GUDMUNDSDOTTIR<sup>1</sup>, B. GJERDE<sup>2</sup> & E. HOBERG<sup>3</sup>

<sup>1</sup>*Institute for Experimental Pathology, Keldur, University of Iceland, Reykjavik, Iceland*

<sup>2</sup>*Parasitology laboratory, Norwegian School of Veterinary Science, Oslo, Norway*

<sup>3</sup>*US National Parasite Collection & Animal Parasitic Disease Laboratory  
USDA, Beltsville, Maryland, USA*

Reindeer in Iceland are descendents of 35 animals that were imported 1787 from Finnmark in Norway. Marked population fluctuations have been observed and a hunting ban prevented their extinction in the first half of the 20<sup>th</sup> century.

During 2003 to 2005 faecal samples, the alimentary canal, lungs, liver, muscles, joints, nostrils, ears and skins from adult reindeer were examined for parasites. Eight protozoans (5 intestinal species, 3 sarcosporidians) and 9 helminths (a cestode, 8 nematodes) were found. Seven of them are reindeer-specific (3 eimerids, 3 sarcosporidians and *Capillaria bovis*) but the remaining species (10) also occur in other ruminants in Iceland; mainly in sheep and goats, but also in cattle (*O. ostertagi*) or even in horses (*T. axei*).

In Norway, at least 39 parasitic species have been reported. If *Giardia*, and the three eimeriids found in Iceland (must also occur in Norway) are added to this list, the number is 43. Present in Norway but apparently absent in Iceland are species-specific parasites (or parasites primarily associated with reindeer) like three additional sarcosporidians (*S. tarandi*, *S. rangiferi* and *S. grueneri*), the cestode *T. krabbei*, an abomasal nematode (*O. greuhnerii*), nematodes in the small intestine (*N. tarandi*, *N. longispiculata*) and in the large intestine (*S. tarandi*), the neurotrophic nematode *E. rangiferi*, the pentastomid sinus worm *L. arctica*, the nasal bot fly (*C. trompe*) and the warble fly (*H. tarandi*). At least two additional ectoparasites have been reported on reindeer from Fennoscandia; *C. texanus* and the reindeer louse *C. tarandi*. Many of these parasites can seriously affect reindeer health. Some of them might reduce growth rate, delay the age of first reproduction and reduce pregnancy rate. The absence of these parasites in Iceland is an important factor in contributing to the high growth rate of the healthy Icelandic reindeer population.

## HELMINTH PARASITES OF REINDEER *RANGIFER TARANDUS* IN ICELAND

By K. SKIRNISSON<sup>1</sup>, B. GUDMUNDSDOTTIR<sup>1</sup> & E. HOBERG<sup>2</sup>

<sup>1</sup>*Institute for Experimental Pathology, Keldur, University of Iceland, Reykjavik, Iceland*

<sup>2</sup>*US National Parasite Collection & Animal Parasitic Disease Laboratory  
USDA, Beltsville, Maryland, USA*

A provisional list of metazoan parasites of reindeer includes at least 65 species; seven trematodes, eight cestodes, 44 nematodes and six arthropods.

The reindeer population in Iceland has been isolated from other populations since 1787, when the ancestors (30 cows, 5 bucks) were imported from Norway.

During 2003 to 2005 carcasses of 58 reindeer were examined for cestode larvae, helminths were searched for in the large intestine of 39 and nematodes in the abomasum of 24 and in the lungs of 34 reindeer. Nostrils, ears and skins from 16 animals were examined for arthropods. The Baerman method was used to search for larvae of lung nematodes in faecal samples of 56 adults. Eggs of helminths were searched for in 248 faecal samples (192 calves, 56 adults) using the McMaster and the FEA concentration methods.

Neither trematode eggs, cestode metacestodes, pulmonary nematodes nor ectoparasites were found. However, the nematodes *O. ostertagi*, *T. circumcincta* and *T. axei* were found in the abomasum, the nematodes *C. bovis*, *N. filicollis* and *N. spathiger* and the cestode *M. expansa* occurred in the small intestine and *Ch. ovina* and *O. venulosum* were found in the large intestine. Intensity and prevalence of these parasites was in general very low. All the parasites except *C. bovis* are well known parasites of other ruminants in Iceland, mainly in sheep and goats, but also in cattle (*O. ostertagi*) or horses (*T. axei*). Therefore, these hosts can act as a source of infection for reindeer (and vice versa). In the 1950s *T. ovis* and metacestodes of *T. hydatigena* (*C. tenuicollis*) were also reported to infect reindeer in Iceland, but these species were not found in the present material.

The absence of *Ostertagia greuhneri*, *Marshallagia marshalli*, *Spiculoptera spiculoptera*, *Skrjabinema tarandi*, *Elaphostrongylus rangiferi*, *Linguatula arctica*, *Cephenomya trompe* and *Hypoderma tarandi* in reindeer from Iceland is clinically important and possible reasons are mentioned.

# THE ROLE OF INVASIVE SNAIL LITHOGLYPHUS NATICOIDES IN TRANSMITTING THE FLUKES IN AQUATIC ECOSYSTEMS

By G. STANEVIČIŪTĖ<sup>1</sup>, V. STUNŽĖNAS<sup>1</sup>, R. PETKEVIČIŪTĖ<sup>1</sup> & S. MASTITSKY<sup>2</sup>

<sup>1</sup> *Institute of Ecology, Vilnius University, Vilnius, Lithuania*  
<sup>2</sup> *Great Lakes Center, SUNY College at Buffalo, USA*

The prosobranch snail *Lithoglyphus naticoides* (C. Pfeiffer, 1928), an invasive species for Lithuania and neighbouring countries, originates from the Ponto-Caspian region. In 2008, we collected this snail in Lithuanian and Belarusian waterbodies and, for comparative examination, in the Danube River, Hungary. The diversity of intramolluscan stages of trematodes associated with *L. naticoides* and the level of infection were examined and compared in old and newly recorded populations. A total of nine species of trematodes were detected in the populations studied; *Apophallus muehlingi* was a dominant species in old populations, but not recorded in new population from Lake Lukomskoje, Belarus. A novel endosymbiont of *L. naticoides* - *Aspidogaster conchicola* was recorded in Lake Lepelskoe, Belarus. Karyological and molecular techniques were used to identify the potential variability of populations of *L. naticoides* as well as the trematode species collected in different populations of the snail. ITS2 DNA sequence analysis of *Echinochasmus* sp. revealed the relationship with Echinostomatidae species from GeneBank. The karyotypes and ITS2 DNA of *A. muehlingi* from different populations were identical. Closely related sympatric congeneric species *A. donicus* was absent in the investigated populations. The karyotypes and haplotypes of 16S mitochondrial gene of *L. naticoides* were identical in populations of Lithuania and Belarus and identical for 16S of *L. naticoides* from Poland (GeneBank information). Our data indicate that the three examined populations of *L. naticoides* were formed by a single genetic lineage. The trematode fauna diversity of different populations depends on the structure of ecosystem invaded and the presence of definitive and second intermediate hosts necessary for the completion of complex life cycles. This study was supported by the Lithuanian Science and Studies foundation research grant T-58/08.

SEARCH FOR SEASONAL DYNAMICS IN *HYSTEROETHYLACIUM ADUNCUM* AND  
*ANISAKIS SIMPLEX* LARVAE IN NORWAY POUT (*TRISOPTERUS ESMARKII*) FROM  
THE OSLOFJORD, NORWAY

By E. STRØMNES & K. ANDERSEN

*Natural History Museum, University of Oslo, Norway*

Even though Norway pout has become an economically significant species in the North-East Atlantic the knowledge of its nematode fauna is scarce. The purpose of this study was to shed more light onto this subject, in particular on the possible occurrence of seasonal variation in the nematode fauna.

The material was collected by use of nets in the outermost part of the Oslo fjord, Tjøme. Fifteen specimens of Norway pout was caught in most months from October 1995 to September 1999. A total of 539 hosts were inspected for nematodes in muscle and viscera, and the age, length and weight of the fish were recorded. Two species of anisakid nematodes were found in this survey; *Hysterothylacium aduncum* and *Anisakis simplex*. The well documented correlation between length/weight/age of host and parasite intensity was also established in this study, although hardly significant in the case of *A. simplex*. The abundance and prevalence for the total sample was considerably higher for *H. aduncum* (31 and 98 %) than for *A. simplex* (1 and 38), indicating that Norway pout is a far more important host to the former nematode than to the latter in the study area. Also, this pattern is consistent if the sample is categorized into different age groups from 0 to 4 years. The abundance fluctuated significantly from month to month during the study period, but no clear seasonal pattern appeared for any of the two parasite species involved. The finding that *H. aduncum* does not have a clear seasonal fluctuation is in accordance with the results of most other studies on this species. This parasite apparently does not have a seasonal life cycle, possibly due to its flexibility concerning intermediate- and parathenic hosts. *A. simplex* on the other hand has in some instances been found to exhibit a clear seasonality with a peak of abundance during spring. It has been speculated that this pattern is due to a high occurrence of migrating fish and/or whales into an area, the latter constituting the final host of this parasite. The density of whales in the study is low, mainly consisting of occasionally visiting porpoise (*Phocoena phocoena*). Also, the Oslofjord does not receive discernible numbers of migrating fish that could possibly introduce significant numbers of larvae to these waters. Additionally, the diet of Norway pout consist, at least for the length groups dominating the present material, to a large extent of species that is not important as intermediate hosts for the L3-larvae of this nematode. On the other hand, some of the species on the diet of this fish apparently are important as intermediate hosts for *H. aduncum*, at least to some extent explaining why the abundance of this nematode is so exceedingly high compared to *A. simplex*.

OCCURRENCE OF PAPILOMA DISEASE IN ROACH (*RUTILUS RUTILUS*): IN  
ACCORDANCE WITH ISLAND BIOGEOGRAPHY THEORY

By J. TASKINEN<sup>1</sup> & T.L. KORKEA-AHO<sup>2</sup>

<sup>1</sup> *Department of Biological and Environmental Sciences, University of Jyväskylä, Finland*

<sup>2</sup> *Department of Biosciences, University of Kuopio, Kuopio, Finland*

The theory of island biogeography predicts that the probability of a species occupying an island depends on a dynamic equilibrium between extinction and colonization. We tested how the theory could explain occurrence of epidermal papillomatosis in the cyprinid fish, roach (*Rutilus rutilus*), in 34 Finnish lakes. Lake surface area and maximum depth were regarded as variables contributing to the extinction rate of the host and the disease, so that large and deep lakes were hypothesized to have a lower probability of the extinction of both roach and papillomatosis and a higher probability of disease occurring. Altitude (elevation above sea level) and the percentage of the drainage area of the lake covered by other lakes were regarded as variables contributing to colonization, so that high altitude and a low percentage area of lakes in the neighboring area were hypothesized to increase isolation, thereby decreasing the probability of the disease occurring.

The results of discrimination analysis suggest that maximum depth, percentage of the drainage area of the lake covered by lakes in the vicinity and altitude best identified diseased lakes. Comparison of diseased and non-diseased lakes revealed that lake area could also be regarded as a variable strongly contributing positively to the occurrence of the disease. The sampling date, proportion of males and mean length of fish did not discriminate between the lakes. The probability of the disease occurring was highest in large, deep, low-altitude lakes which had a high percentage of lakes in their vicinity.

Thus, the results indicate that the colonization and extinction processes probably contribute to the occurrence of papillomatosis in roach, as predicted by the theory.

## CRYPTIC SPECIES IN HAEMOSPORIDIAN PARASITES: ARE THEY REALLY ALWAYS CRYPTIC?

By G. VALKIŪNAS<sup>1</sup>, T.A. IEZHOVA<sup>1</sup>, A. KRIŽANAUSKIENĖ<sup>1</sup>,  
V. PALINAUSKAS<sup>1</sup>,  
S. BENSCH<sup>2</sup> & R.N.M. SEHGAL<sup>3</sup>

<sup>1</sup> *Institute of Ecology, Vilnius University, Lithuania*

<sup>2</sup> *Department of Animal Ecology, Ecology Building, Lund University, Sweden*

<sup>3</sup> *Department of Biology, San Francisco State University, USA*

Recent molecular studies have revealed a remarkable genetic diversity of haemosporidians, indicating that their taxonomic diversity may be greater than in the current classifications. Numerous lineages of haemosporidians have been identified as cryptic species. The PCR-based studies suggested that avian haemosporidians with a genetic differentiation of over 5% in cytochrome b (cyt b) gene should be expected to be morphologically differentiated (Hellgren et al. 2007, *J. Parasitol.*, 93: 889-896), in other words they should be not cryptic species based on morphology of their blood stages. We addressed this issue using morphological data and phylogenetic analysis of cyt b gene of positively identified species of avian haemosporidians. Results of this study are in accord to the O. Hellgren et al.'s hypothesis. It is probable that the criterion of genetic difference of > 5% in cyt b gene reflects interspecific divergence in species of *Haemoproteus*, *Plasmodium*, *Leucocytozoon* and probably other groups of haemosporidians, so can be used for better understanding of phylogenetic trees based on this gene. It should be noted, however that genetic divergence in the cyt b gene between some readily distinguishable morphospecies of avian haemosporidian parasites is < 5%; it might be as low as 1% between some morphospecies. Thus, the molecular criterion of > 5% sequence divergence in cyt b gene for identification of haemosporidian morphospecies should be developed and applied carefully, preferably by linking molecular and phenotypic data. Accumulation of information on this subject would be helpful for the interpretation of cyt b gene phylogenetic trees of haemosporidians, and in the taxonomy of these haematozoa using molecular data. GenBank should only be used prudently for this purpose and other phylogenetic analyses because it hosts sequences from numerous misidentified parasites on the level of their species, genera and even families (see *Trends Parasitol.*, 2008, 24: 247-248).

WHAT DO PARASITES TELL US ABOUT FOOD WEBS? THE BOTHNIAN BAY,  
FINLAND

By E.T. VALTONEN<sup>1</sup>, D. J. MARCOGLIESE<sup>2</sup> & M. JULKUNEN<sup>1</sup>

<sup>1</sup>*University of Jyväskylä, Jyväskylä, Finland*

<sup>2</sup>*Environment Canada, Montreal, QC*

Parasites that are transmitted through predator-prey interactions may be used as indicators of trophic relationships between organisms. Food webs were constructed using diet alone, trophically-transmitted parasites alone, and the combination of the two based on data from 31 species of fish from the Bothnian Bay, Finland. Connectance and the mean number of links per species increased by 38% using parasites compared to diet as indicators of trophic relationships. Both measurements doubled when parasites and diet were considered together compared to diet alone. Positive correlations were found between the mean number of parasites and the number of prey taxa in the diet among the fish species. Mean total abundance and mean prevalence of parasites correlated positively with fish size among species. Trophically-transmitted parasites may be used to construct food webs and derive information about food web processes. Parasites alone provided more information than diet. However, resolution is improved by using parasites and diet together.

## HOST SPECIFICITY VS HOST SWITCH. II. HOW THE PARASITES SOLVED IT?

By M. ZIĘTARA<sup>1</sup> & J. LUMME<sup>2</sup>

<sup>1</sup>*University of Gdańsk, Poland*

<sup>2</sup>*University of Oulu, Finland*

Via careful theoretical considerations, it was concluded that highly host specific *Gyrodactylus* species can not switch host, because it is too risky, and selection for the new environment cannot work prior being there. However, they have switched host, even so often that we have described the speciation of wagneri group as 'adaptive radiation' to seven host fish families. What happened? Fortunately, there are some genetically tractable host-switch cases. The accumulating data shows that the stasis of parthenogenetic propagation without recombination is always radically interrupted for the switching to occur. 1. To switch from grayling (*Thymallus thymallus*) to salmon (*Salmo salar*), two strains of *G. salaris* had to hybridize, and the hybrid has to maintain heterozygosity (forever), to stay on salmon. The salmon specific superclone is monophyletic: the hybridization succeeded once, 132 000 years ago. 2. In three separate cases analyzed, the switch of *G. salaris* from rainbow trout (*Oncorhynchus mykiss*) to salmon, the RBT-specific clone has mated with salmon parasite, to 'reconstruct' the salmon specific orientation instincts. The RBT clone of *G. salaris* is also of hybrid origin, but the parents are not yet known. 3. To create a novel parasite for rainbow trout, a roach (*Rutilus rutilus*) parasite *G. pomeraniae* mated with whitefish specific *G. lavareti* (*Coregonus lavaretus*) to produce a clone thriving in fish farms. The genetic distance of the parents was about 20% in mtDNA, which is more than genus-difference in many animal groups. 4. On rainbow trout populations in Europe, all *Gyrodactylus* species seem to be of European origin. Only few of them perhaps have switched directly from European salmonids, without drastic genomic reorganization. We hypothesize that even those species are recombinant clones, when studied well enough by nuclear and mitochondrial markers. Genetic reorganization and 'impossible preadaptation' perhaps is a necessity for permanent host switch.

## LIST OF PARTICIPANTS

**Alekseev, Andrey N.**

Zoological Institute, Russian Academy  
of Sciences Universitetskaya emb., 1,  
St.-Petersburg, 199034,  
RUSSIAN FEDERATION  
E-mail: [anadev@yandex.ru](mailto:anadev@yandex.ru)

**Bagrade, Guna**

Natural History Museum of Latvia  
K. Barona 4, LV -1712, Riga,  
LATVIA  
E-mail: [guna.bagrade@ldm.gov.lv](mailto:guna.bagrade@ldm.gov.lv)

**Barskaya, Julia**

Institute of Biology, Karelian Research  
Centre, Russian Academy of Science,  
11 Pushkinskaya st., 185910  
Petrozavodsk,  
RUSSIAN FEDERATION  
E-mail: [jbarskaya@gmail.com](mailto:jbarskaya@gmail.com)

**Benesh, Daniel**

Max Planck Institute for Evolutionary  
Biology  
August-Thienemann Str. 2, 24306,  
Ploen  
GERMANY  
E-mail: [benesh@evolbio.mpg.de](mailto:benesh@evolbio.mpg.de)

**Chukalova, Natalia**

Atlantic Research Institute of Marine  
Fisheries and Oceanography  
5, Dm. Donskoy Str., 236022,  
Kaliningrad,  
RUSSIAN FEDERATION  
E-mail: [chukalova@gmail.com](mailto:chukalova@gmail.com)

**Grikienienė, Jadvyga**

Institute of Ecology, Vilnius University,  
Akademijos 2, Vilnius, LT - 08412  
LITHUANIA  
E-mail: [jagrik@ekoi.lt](mailto:jagrik@ekoi.lt)

**Henikl, Sabine**

BIOMIN Research Center,  
Technopark 1, 3430 Tulln,  
AUSTRIA  
E-mail: [sabine.henikl@biomin.net](mailto:sabine.henikl@biomin.net)

**Areskog, Marlene**

Dept of Biomedical Sciences & Veterinary  
Public Health, Section of Parasitology &  
Virology, Swedish University of  
Agricultural Sciences, 750 07 Uppsala,  
SWEDEN  
E-mail: [marlene.areskog@bvf.slu.se](mailto:marlene.areskog@bvf.slu.se)

**Barber, Iain**

Department of Biology, University of  
Leicester, LEICESTER, LE1 7RH,  
UK  
E-mail: [ib50@le.ac.uk](mailto:ib50@le.ac.uk)

**Bespyatova, Lubov**

Institute of Biology, Karelian Research  
Centre, Russian Academy of Science  
11, Pushkinskaya st, 185910 Petrozavodsk  
RUSSIAN FEDERATION  
E-mail: [annja@karelia.ru](mailto:annja@karelia.ru)

**Bugmyrin, Sergey**

Institute of Biology, Karelian Research  
Centre, Russian Academy of Science  
11 Pushkinskaya st., 185910 Petrozavodsk,  
RUSSIAN FEDERATION  
E-mail: [sbugmyr@mail.ru](mailto:sbugmyr@mail.ru)

**Galaktionov, Nikolai**

Institute of Cytology  
Tichoretsky pr.4, St. Petersburg  
RUSSIAN FEDERATION  
E-mail: [nikolai.galaktionov@gmail.com](mailto:nikolai.galaktionov@gmail.com)

**Hannes, Inger Sofie**

National Veterinary Institute, Section for  
Parasitology, P.O.Box 750 Sentrum, 0106,  
Oslo,  
NORWAY  
E-mail: [inger.hannes@vetinst.no](mailto:inger.hannes@vetinst.no)

**Höglund, Johan**

Department of Parasitology (SWEPAR)  
National Veterinary Inst. Swedish Univ.  
Agricultural Sciences  
SE-751 89 Uppsala

SWEDEN

E-mail: [johan.hoglund@bvf.slu.se](mailto:johan.hoglund@bvf.slu.se)

**Ieshko, Eugeny**

Institute of Biology, Karelian Research  
Centre, Russian Academy of Science,  
11 Pushkinskaya st., 185910  
Petrozavodsk,  
RUSSIAN FEDERATION  
E-mail: [ieshko@krc.karelia.ru](mailto:ieshko@krc.karelia.ru)

**Järvis, Toivo**

Estonian University of Life Sciences  
Kreutzwaldi 62, 51014 Tartu,  
ESTONIA  
E-mail: [toivo.jarvis@emu.ee](mailto:toivo.jarvis@emu.ee)

**Jaunbauere, Gunita**

National Diagnostic Center of Food and  
Veterinary Service , 3, Lejupes str,  
Riga, LV-1076,  
LATVIA  
E-mail: [gunita.jaunbauere@ndc.gov.lv](mailto:gunita.jaunbauere@ndc.gov.lv)

**Jokelainen, Pikka**

Department of Basic Veterinary Sciences,  
Faculty of Veterinary Medicine, P.O. Box  
66, FI-00014 University of Helsinki,  
FINLAND  
E-mail: [pikka.jokelainen@helsinki.fi](mailto:pikka.jokelainen@helsinki.fi)

**Kirjusina, Muza**

Institute of Systematic Biology,  
Daugavpils University, Vienibas 13-  
229,  
Daugavpils, LV 5401  
LATVIA  
E-mail: [muza.kirjushina@hotmail.com](mailto:muza.kirjushina@hotmail.com)

**Kolarova, Libuse**

National Reference Laboratory for Tissue  
Helminthoses, Institute for Postgradual  
Medical Education and 1<sup>st</sup> Faculty of  
Medicine, Charles University, Prague,  
CZECH REPUBLIC  
E-mail: [libuse.kolarova@ipvz.cz](mailto:libuse.kolarova@ipvz.cz)

**Kornas, Slawomir Adam**

Department of Zoology and  
Ecology, Faculty of Animal  
Sciences, University of Agriculture  
in Krakow  
POLAND  
E-mail: [skornas@ar.krakow.pl](mailto:skornas@ar.krakow.pl)

**Križanauskienė, Asta**

Institute of Ecology of Vilnius  
University,  
Akademijos 2, LT-08412, Vilnius,  
LITHUANIA  
E-mail: [asta@ekoi.lt](mailto:asta@ekoi.lt)

**Komisarovas, Jurijus**

E-mail:  
[jurijus\\_komisarovas@yahoo.com](mailto:jurijus_komisarovas@yahoo.com)

**Laakkonen, Juha**

Faculty of Veterinary Medicine,  
Anatomy, PO Box 66, FIN-00014  
University of Helsinki,  
FINLAND  
E-mail: [juha.laakkonen@helsinki.fi](mailto:juha.laakkonen@helsinki.fi)

**Lassen, Brian**

Estonian University of Life Sciences  
Kreutzwaldi 62, 51014 Tartu  
ESTONIA  
E-mail: [brian.lassen@gmail.com](mailto:brian.lassen@gmail.com)

**Lumme, Jaakko**

Department of Biology, University  
of Oulu, PL 800090014 ,  
FINLAND  
E-mail: [jaakko.lumme@oulu.fi](mailto:jaakko.lumme@oulu.fi)

**Mägi, Erika**

Estonian university of Life Sciences  
Kreutzwaldi 62, 51014 Tartu,  
ESTONIA  
E-mail: [erika.magi@emu.ee](mailto:erika.magi@emu.ee)

**Näreaho, Anu**

Veterinary Pathology,  
P.O Box 66, 00014 University of  
Helsinki  
FINLAND  
E-mail: [anu.nareaho@helsinki.fi](mailto:anu.nareaho@helsinki.fi)

**Nosal, Pawel**

Department of Zoology and Ecology,  
University of Agriculture in Krakow,  
Al. Mickiewicza 24/28, 30-059  
Kraków,  
POLAND  
E-mail: [rznosal@cyf-kr.edu.pl](mailto:rznosal@cyf-kr.edu.pl)

**Lebedeva, Daria**

Institute of Biology, Karelian Research  
Centre, Russian Academy of Science,  
11 Pushkinskaya st., 185910  
Petrozavodsk,  
RUSSIAN FEDERATION  
E-mail: [daryal78@gmail.com](mailto:daryal78@gmail.com)

**Matveeva, Elizaveta**

Institute of Biology, Karelian Research  
Centre, Russian Academy of Science  
11 Pushkinskaya st., 185910  
Petrozavodsk,  
RUSSIAN FEDERATION  
E-mail: [matveeva@krc.karelia.ru](mailto:matveeva@krc.karelia.ru)

**Moks, Epp**

Department of Zoology, Institute of Ecology  
and Earth Sciences, University of Tartu,  
Vanemuise 46, Tartu 51014,  
ESTONIA  
E-mail: [epp.moks@ut.ee](mailto:epp.moks@ut.ee)

**Nejsum, Peter**

Danish Centre for Experimental  
Parasitology, Department of Veterinary  
Disease Biology, Faculty of Life Science,  
Dyrlægevej 100, 1870 Frederiksberg C  
DENMARK  
E-mail: [pn@life.ku.dk](mailto:pn@life.ku.dk)

**Oksanen, Antti**

Finnish Food Safety Authority Evira  
Mustialankatu 3, FI-00790 Helsinki,  
FINLAND  
[Antti.Oksanen@evira.fi](mailto:Antti.Oksanen@evira.fi)

**Palinauskas, Vaidas**

Institute of Ecology, Vilnius University,  
Akademijos 2, Vilnius, LT - 08412  
LITHUANIA  
E-mail: [palinauskas@gmail.com](mailto:palinauskas@gmail.com)

**Petkevicius, Saulius**

Lithuanian Veterinary Academy  
Tilzes 18, LT-47181 Kaunas  
LITHUANIA  
E-mail: [petkevicius@lva.lt](mailto:petkevicius@lva.lt)

**Robertson, Lucy**

Norges veterinærhøgskole  
Parasitology dept., MatInf, PO Boks 8146  
dep, 0033 Oslo,  
NORWAY  
E-mail: [lucy.robertson@veths.no](mailto:lucy.robertson@veths.no)

**Skirnisson, Karl**

Institute for Experimental Pathology,  
University of Iceland, Keldur, Laboratory  
of Parasitology, IS 112 Reykjavik,  
ICELAND  
E-mail: [karlsk@hi.is](mailto:karlsk@hi.is)

**Stunžėnas, Virmantas**

Institute of Ecology, Vilnius University,  
Akademijos 2, Vilnius, LT - 08412  
LITHUANIA  
E-mail: [stunzenas@ekoi.lt](mailto:stunzenas@ekoi.lt)

**Taskinen, Jouni**

Department of Biological and  
Environmental Sciences University of  
Jyväskylä, P.O. Box 35 (YAC-315.2) FI-  
40014 University of Jyväskylä,  
FINLAND  
E-mail: [jouni.k.taskinen@jyu.fi](mailto:jouni.k.taskinen@jyu.fi)

**Osterman Lind, Eva**

National Veterinary Institute, Dept of  
Virology, Immunobiology and  
Parasitology,  
Section of Parasitology Diagnostic,  
751 89 Uppsala,  
SWEDEN  
E-mail: [gunilla.lindgren@sva.se](mailto:gunilla.lindgren@sva.se)

**Prakas, Petras**

Institute of Ecology, Vilnius University,  
Akademijos 2, Vilnius LT - 08412  
LITHUANIA  
E-mail: [pprakas@ekoi.lt](mailto:pprakas@ekoi.lt)

**Sinisalo Tuula Hannele**

University of Jyväskylä Department of  
Biological and Environmental Science  
Survontie 9 40014 Jyväskylä  
FINLAND  
E-mail: [tuula.sinisalo@jyu.fi](mailto:tuula.sinisalo@jyu.fi)

**Strømnes, Einar**

Natural History Museum, University of  
Oslo  
P.O.Box 1172 Blindern, NO-0318 Oslo  
Norway  
E-mail: [einar.stromnes@nhm.uio.no](mailto:einar.stromnes@nhm.uio.no)

**von Samson-Himmelstjerna, Georg**

Stiftung Tierärztliche Hochschule  
Hannover  
Institut für Parasitologie, Bünteweg 17  
30559 Hannover,  
GERMANY  
E-mail: [gvsamson@tiho-hannover.de](mailto:gvsamson@tiho-hannover.de)

**Thamsborg, Stig Milan**

Danish Centre for Experimental  
Parasitology,  
KU-LIFE, 100 Dyrslægevej, DK-1870  
Frederiksberg C  
DENMARK  
E-mail: [smt@life.ku.dk](mailto:smt@life.ku.dk)

**Valkiūnas, Gediminas**

Institute of Ecology, Vilnius University,  
Akademijos 2, Vilnius, LT - 08412

LITHUANIA

E-mail: [gedvalk@ekoi.lt](mailto:gedvalk@ekoi.lt)

**Valtonen, E. Tellervo**

Department of Biological and  
Environmental Science, University of  
Jyväskylä,

P.O. Box 35 (YA), Fin-40014

FINLAND

E-mail: [etvalto@bytl.jyu.fi](mailto:etvalto@bytl.jyu.fi)

**Wedekind, Claus**

University of Lausanne,

DEE, Biophore, 1015 Lausanne,

SWITZERLAND

E-mail: [claus.wedekind@unil.ch](mailto:claus.wedekind@unil.ch)

**Ziętara, Marek**

University of Gdańsk,

80-680 Gdańsk, ul Ornitologów 26,

POLAND

E-mail: [zietara@biotech.ug.gda.pl](mailto:zietara@biotech.ug.gda.pl)