

**Bulletin
of the
SCANDINAVIAN-BALTIC
SOCIETY FOR PARASITOLOGY**



**PROCEEDINGS OF THE 1st SYMPOSIUM OF THE SCANDINAVIAN-
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26-29 May, 2005**

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BULLETIN OF THE SCANDINAVIAN-BALTIC SOCIETY FOR PARASITOLOGY

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Cover: In Norse mythology, the giant ash tree - Yggdrasil - spreads its limbs over the entire mankind. The ash has three roots, each of them sucking water from its own spring. The first spring - Hvergelmir - is found in the ice cold North; next to the spring, the serpent Níðhoggr is ceaselessly gnawing at the roots of the ash. The second spring - Mímisbrunnr - is the source of wisdom and is guarded by Mímir. The third spring - Urðarbrunnr - is guarded by three women, the Norns, which mete out man's thread of life.

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Editors: Romualda Petkevičiūtė and Gediminas Valkiūnas

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PREFACE

WELCOME BY THE PRESIDENT OF THE SCANDINAVIAN-BALTIC SOCIETY FOR PARASITOLOGY

KARL SKIRNISSON

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Dear colleagues,

I extend to you a warm welcome to the first Symposium of the Scandinavian-Baltic Society for Parasitology (SBSP). As probably all of you know, the unification of the Scandinavian and the Baltic Societies took place at the last General Assembly of the Scandinavian Society for Parasitology in June 2003 in Bergen, Norway. We made this step after extensive discussions and careful preparations, which already started in 1999. New statutes were accepted (see: <http://www.hi.is/pub/sbsp/>) and one of the first tasks of the SBSP Board was to decide to arrange this first Symposium of the Society in Vilnius in Lithuania.

It is well known that parasitologists in the countries which surround the Baltic Sea more or less deal with the same parasitic fauna. Consequently, colleagues in this geographical area frequently face similar problems and share scientific interest in many fields. This fact was one of the important arguments why Baltic and Scandinavian parasitologists wished to join in one strong Society. I sincerely hope that the activities of the previous Boards of the Scandinavian and Baltic Societies offer the SBSP members in future years a better opportunity to exchange information and strengthen scientific communication and cooperation in our region. However, I stress that the success of the new Society mainly depends on the activities and interest of its members. If I consider the very good attendance of this first SBSP Symposium, and the high scientific value of the contributions, which is clearly reflected in our first SBSP Bulletin, I sincerely believe that our decision to join the two Societies was a correct step.

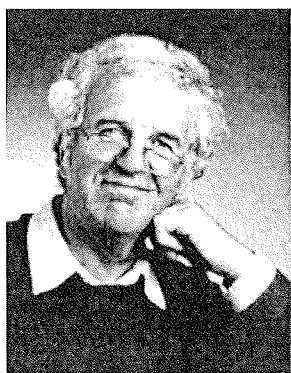
We all know that the members of the Scandinavian-Baltic Society for Parasitology are a heterogeneous group of scientists with different background, education and interests. Some of us studied veterinary or human medicine; others have finished their degree in biology or even other fields of science. Most of the members are active in research, either working at universities or at other governmental institutions, and some work for private companies. However, the diverse background and different activities should not be considered as a weakness of our Society. On the contrary it should be regarded as an excellent educational opportunity when parasitologists from a certain geographical region are brought together for a few days for the exchange of

information and discussions of parasitological subjects. In past decades these arrangements definitely inspired and improved the skills of Nordic parasitologists and I hope that the same will happen in future years in our new Society.

Unfortunately, we were forced to stop the ordinary publishing of the SSP /SBSP Bulletin. We had to face the facts that our members decided to publish their results elsewhere, and also the financial burden was too high. But please bear in mind that we decided to keep the Bulletin to print our Symposium issues and that Jorun Tharaldsen is still our experienced Editor. As we managed to create a home page we got a new forum to spread information to our members in a cheap and effective way. The Webmaster of SBSP is Arni Kristmundsson. The SBSP Board is indebted both to the editor of the Bulletin, Jorun Tharaldsen, and to the SBSP Webmaster, Arni Kristmundsson for their skilled work.

It is also an honour and privilege to thank the local organizing committee (Tatjana Iezhova, Vytautas Kontrimavičius, Jadvyga Olechnovičienė, Romualda Petkevičiūtė, Gražina Stanevičiūtė) which is headed by Gediminas Valkiūnas for arranging the first SBSP Symposium. They have set up a very interesting scientific programme, which covers broad issues highly relevant to medical, veterinary as well as general parasitology. And they have embraced the participation of two neighbouring groups, The European Veterinary Parasitology College (EVPC) and the EchinoRisk EU-project group, into satellite meetings along with the Symposium. In addition, they have arranged a lovely social programme, which enables us to meet, discuss and enjoy the stay in Vilnius. A special welcome is directed to the invited speakers. The SBSP Board is also indebted to various agencies and sponsors who contribute to the success of our meeting. Last but not least, we thank all the participants who are attending the Symposium. We hope that you bring back home new knowledge and some fresh ideas, and also the intention to attend the next SBSP meeting, which according to the scheme should be arranged in Finland in 2007.

Dear colleagues, now, I have been on the Board of SBSP for two years. Before that, I spent six years on the Board of the SSP and now my time for work on behalf of Nordic and Baltic parasitologists is running out. I will use this opportunity to express my gratitude to all the colleagues, which I met and worked with during all these years. Thanks to all of you for a fantastic time.



LATE PROFESSOR PETER NANSEN AND THE BALTICS

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The 1st Symposium of the Scandinavian-Baltic Society for Parasitology is dedicated to the memory of prominent veterinary parasitologist Professor Peter Nansen.

Peter Nansen was a strong supporter and spokesman for a close Nordic collaboration, and he also had a special affection for the Baltic countries and their natural association with the Nordic neighbours. In 1990 he participated in a Baltic Environmental Conference, and the same year he became the Baltic News section editor of the Bulletin of the Scandinavian Society for Parasitology. In 1997-1998, he served as member of the steering committee of the Nordic Veterinary and Agricultural Universities Baltic Section, NOVABA. Peter Nansen was the instigator and co-organiser of various symposia and research projects in Lithuania 1991-1997, and during 1993-1997 he went on numerous visits to veterinary and academic institutions in all three countries. He actively pursued collaborative efforts and thus supervised or co-supervised several Ph.D. and M.Sc. students from Lithuania: Prof. Saulius Petkevičius, Drs. Mindaugas Šarkūnas, Alvydas Malakauskas, and from Estonia: Dr.Heli Talvik. Many parasitologists from the Baltic States were invited by Peter Nansen to visit the Danish Centre for Experimental Parasitology and to give talks there.

As a symbol of appreciation, Peter Nansen became *doc.hon.causa* at the Estonian University of Agriculture in 1993, foreign member of the Lithuanian Academy of Sciences in 1994, and *doc.hon.causa* at the Lithuanian Veterinary Academy in 1997.

Peter Nansen served as President of the Scandinavian Society of Parasitology during 1985-1989, and became honorary member of the SSP in 1997. He was very much in favour of the joining of the Nordic and Baltic Societies for Parasitology and, e.g., was a major instigator in ensuring, in 1991, the first participation by representatives from all three Baltic countries in the SSP Conference in Uppsala.

During the Closing Session of the 12th Baltic Republics Scientific Conference on Parasitology, which was held in Vilnius in 1993, Peter Nansen said that parasitologists of Nordic and Baltic countries would be members of one joint Society for Parasitology in the future. Today this is reality, which is in many respects due to Peter Nansen's friendly and professional activities.

INVITED SPEAKERS

PATTERNS OF CYTOCHROME *b* SEQUENCE DIVERSITY IN AVIAN HAEMOSPORIDIANS: DIVERSITY AMONG HOST SPECIES AND POPULATIONS

S. BENSCH

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Avian blood parasites of the genera *Plasmodium* and *Haemoproteus* are sister taxa and more closely related to each other than to *Plasmodium* parasites infecting mammals. For convenience, I will call members of these genera “avian malaria parasites”, although from a disease point of view only *Plasmodium* parasite are considered malaria parasites (Pérez-Tris *et al.*, 2005).

A traditional view based on an assumption of strict host specificity of malaria parasites, held that each bird species had its own parasite species and hence, the number of avian malaria species should then roughly correspond to the number of bird species (in the order of 10,000). Much detailed morphological data have then accumulated and this enormous material was recently compiled in a book titled “Avian malaria parasites and other haemosporidia” (Valkiunas, 2005). These morphological based analyses instead suggested that the number of avian malaria species were much more limited than suggested from the one host – one parasite scenario. Rather than 10,000 species, the morphologically based estimated was closer to a few hundred. Some of the identified species showed large distributions over host species belonging to different families and sometimes orders.

Recently developed PCR-based methods are generating sequence data that can provide much more detailed information of malaria parasite lineages than possible to retrieve from morphology alone (Bensch *et al.*, 2000; Ricklefs & Fallon, 2002). That these mtDNA-lineages to a large extent are reflecting species diversity has been confirmed by sequence analyses of nuclear genes (Bensch *et al.* 2004) and by their often distinct host-distributions. Studies of cytochrome *b* gene diversity of avian malaria parasites indicate that there might be as many species of parasites as there are species of bird hosts, and the distribution of lineages shows rich examples of both remarkable host specificity and wide host distribution. Most of this diversity is however cryptic and it remains to be demonstrated what this means in terms of host fitness effects. This diversity of parasite species is indicating that there must be situations with severe competition over hosts and vectors, which might be responsible for observed temporal and spatial patterns in the composition of avian malarial communities (Bensch & Ekesson, 2003, Fallon *et al.*, 2004).

I will exemplify the potential for detailed molecular based population studies of avian malaria parasites, with data from a long term study of great reed warblers *Acrocephalus arundinaceus* carried out in southern Sweden. Between 1987 and 2003, we typed 782 blood samples by PCR and found 18 different parasite lineages of *Plasmodium* (11) and *Haemoproteus* (7). The more common lineages showed evidence of temporal dynamics over the study period, and one lineage was associated with MHC [major histocompatibility complex, (Westerdahl *et al.* 2004)] variation in the host, however prevalence was not associated with correlates to fitness (reproductive success and survival). We found interesting changes in parasite lineages between years and within individuals and I will present some preliminary data on how quantitative PCR (Q-PCR) can be used to decipher these complex patterns.

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EPIDEMIOLOGY AND TRANSMISSION BIOLOGY OF *ECHINOCOCCUS MULTILOCULARIS* IN EUROPE

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Human alveolar echinococcosis (AE), caused by the metacestode stage of *Echinococcus multilocularis*, is a serious zoonosis with mortality rates of up to 100% in untreated patients. AE is occurring in large areas of Central and Eastern Europe at low prevalence rates. Approximately 100 new cases are diagnosed every year in Europe but exact data are not available for all countries. Considerable advances in diagnoses and surgery since the 1970s have improved the prognosis of this predominantly hepatic disease. In inoperable cases, long term chemotherapy is essential as treatment is parasitostatic rather than parasitocidal.

In recent years, AE has also been diagnosed in animal species which do not play a role in the transmission cycle. We have diagnosed 22 cases of AE in dogs and around 20 in captive monkeys. In two dogs, concurrent infections of the intestine and of the liver were observed. Furthermore, small liver lesions caused by *E. multilocularis* have been diagnosed in slaughter pigs. However, in these animals as well as in experimentally infected pigs, the lesions did not develop to metacestode tissue containing protoscoleces (Deplazes *et al*, 2005).

The life-cycle of *E. multilocularis* in Europe predominantly involves foxes *Vulpes vulpes* as definitive hosts and many species of rodents as intermediate hosts. Although infections have also been found in other wild or domestic carnivores (Eckert and Deplazes, 2004), foxes are thought to be the main sources for the environmental contamination with eggs of *E. multilocularis* in most endemic areas. It is not known whether the *E. multilocularis* cycle can be maintained independently from fox reservoir by any of the other definitive host species. Therefore, diagnoses of intestinal infections with *E. multilocularis* in domestic cats or dogs in Europe do not prove the existence of a synanthropic cycle.

In naturally infected foxes, a highly over-dispersed distribution of *E. multilocularis* is observed which may be due to different mechanisms. The dynamics of infection with high initial numbers of worms in foxes is characterised by a rapid loss of the worm burden within the first month of patency. However, residual worms can survive a few months longer. Furthermore, protoscolex numbers in naturally infected voles *Arvicola terrestris* are also over-dispersed leading to an aggregated infection pressure for foxes (Stieger *et al*, 2002). Field studies have shown that young foxes harboured significantly more *E. multilocularis* than older animals. This might be an indication for age dependant susceptibility or for the acquisition of a partial intestinal immunity. Additionally, ecological aspects such as diverse predation habits of the different age groups of foxes could also contribute to this phenomenon.

Although foxes are the major reservoirs of *E. multilocularis*, the significance of other potential hosts in transmission is unclear. In a recent study at the Danish Centre for Experimental Parasitology, experimental infections of foxes, dogs, racoon dogs and cats with 20,000 *E. multilocularis* protoscoleces have provided important information regarding the biotic potential, the establishment of infection, the dynamics of egg excretion and the life-span of infection (Kapel *et al.*, 2005). In this study, infections in foxes had the highest biotic potential in terms of parasite establishment (average of 16,800 worms per fox, 35 dpi), with a total estimated eggs excretion of more than 400,000 eggs per fox during patency. The high biotic potential of *E. multilocularis* in foxes is not surprising as they appear to be the key definitive host. However, in dogs and racoon dogs, parasite establishment and egg production (over 250,000) were in a comparative range. Infections in cats resulted in low worm burdens (average of 642 worms per cat, 35 dpi) and very few eggs being excreted, suggesting an insignificant biotic potential of cats.

A possible role of dogs as sources of human AE in Europe has been substantiated by other studies. In some regions, high prevalence rates of *E. multilocularis* of up to 7% have been recorded in dogs. However, even in the general dog population of Switzerland where there is a low prevalence of 0.3%, the lifetime incidence of infection in dogs reaches around 10% (Deplazes *et al.*, 2004). Taken together, the high dog population, the high reproductive potential of *E. multilocularis* in this species, the fact that dogs can be contaminated with eggs on their fur and the close proximity to humans underlie the zoonotic potential of this host. Recent epidemiological studies in Europe indeed have suggested that dog ownership is a statistically significant risk factor for acquiring human AE (Kern *et al.*, 2004).

Recent studies have shown that *E. multilocularis* has a wider geographic range than previously anticipated (Romig, 2002, EchinoRisk data presented during this meeting). There is evidence for growing populations of red foxes in most European areas, for increasing invasions of cities by foxes and for establishment of the parasite cycle in urban areas (Deplazes *et al.*, 2004). In high endemic areas of southern Germany, a tenfold increase of the parasite density was estimated to have occurred within the previous 10-15 years (Romig, 2002). In view of this changing epidemiological situation, the veterinary and health authorities should initiate all possible measures for monitoring or preventing *E. multilocularis* infections. Significant progress has been made in the development of sensitive and specific new techniques for the *intra vitam* and *post mortem* diagnosis of the intestinal *E. multilocularis* infection in definitive hosts, notably the detection of coproantigen by ELISA and of copro-DNA by PCR (Deplazes *et al.* 2003).

Regarding prevention, research on possible control strategies is of major interest, apart from information campaigns about this zoonosis and its potential risks. A reduction in the abundance of intermediate rodent hosts is very difficult to achieve and is controversial from the ecological point of view. Similarly, a general reduction in fox numbers is difficult to attain with conventional methods.

Several attempts have been made to control *E. multilocularis* by anthelmintic treatment of wild foxes. Studies undertaken in Germany have demonstrated the general feasibility of such an approach by distributing baits containing praziquantel in densities of 20 per km² using small aircraft. Hence, the prevalence in foxes decreased significantly but eradication was not achieved.

Fox baiting on a small scale in urban areas was evaluated in the city of Zurich. A camera trap study revealed that domestic cats and foxes were most frequently photographed at baiting places, but neither the cats nor the few observed stone martens and badgers fed on the baits. Most of the removed baits were taken by foxes (48%), but also hedgehogs (19%), dogs (9%), snails (10%) and mice (4%) consumed the baits (Hegglin *et al*, 2004).

The cycle of *E. multilocularis* in urban settings seems to be determined by the small home-ranges of foxes and the focal distribution of suitable intermediate hosts. Hence, local interventions in the cycle should be feasible aiming at reducing the infection pressure in defined areas which are intensively used by humans (e.g. public parks, swimming pool areas, private gardens). This has been supported by field experimental studies in Zürich. Manual distribution of praziquantel-containing baits (50 baits km⁻² per month) has reduced the infection pressure on defined small urban patches with high *E. multilocularis* egg contaminations resulting in a significant lower prevalence in the rodent intermediate hosts (Hegglin *et al*, 2003).

AE contributes to high levels of human morbidity, some mortality and causes high costs for treatment. However, in most endemic areas, lack of accurate estimates of disease burden hamper to set priorities for control of infectious diseases given finite resources (Carabin *et al*, 2005).

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NEOSPORA AND NEOSPOROSIS: AN UPDATE ON EPIDEMIOLOGY AND DIAGNOSIS

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Neospora caninum ranges within the most important causative agent of bovine abortion worldwide, and neosporosis has been recognized as an economically important disease with considerable impact on livestock industry. Today it is clear that endogenous transplacental infection (= vertical transmission) through maternal recrudescence of tachyzoites is the most frequent mode of passing *N. caninum* to the fetus (responsible for approximately 90% of abortion cases). This process can occur repeatedly in the same animal, the likelihood of abortion is higher in heifers and slightly decreases by age. Oral uptake of sporulated oocysts derived from definitive hosts represents an exogenous mode of transmission (approximately 1-5%). Other exogenous (= horizontal) means of infection are still under discussion.

In cattle, asymptomatic infection with no signs of disease is the norm. Disease occurs when the parasite multiplies in the developing fetus and its placenta and causes sufficient damage to trigger either abortion or stillbirth, or - rarely - a postnatally manifesting neuromuscular syndrome. Infection of the fetus early in gestation is more likely to be fatal to the conceptus than infection later in gestation, on the other hand infection is more likely to be transmitted in late rather than early pregnancy. The final question why fetal or placental damage – resulting in abortion – occurs only in certain individual cases and not in most other cows hasn't yet been resolved, but various factors, including immunosuppressive events e.g. food toxins, stress or concomitant infections are assumed to play a role. The issue if a persistent *N. caninum*-infection may affect the health or the production potential of bovines is still under controversial discussion, few data are indicative so far. In a Swiss study performed by Haessig and Gottstein (2002), a higher incidence of mastitis was revealed in neosporosis case

farms. Furthermore, Thurmond and Hietala (1997) documented a lower milk yield in *N. caninum* seropositive cows, others could not confirm these observations.

Diagnosis. Fetal diagnosis includes methodically primarily histopathological or immunohistochemical investigations - always complemented by PCR - of the fetal brain and optionally of other organs. Post mortem analyses demonstrate the presence of multifocal necrosis in fetal brain, heart, lung, liver, placenta and lymph nodes. Histopathological examination may reveal minimal histological lesions such as focal gliosis in the CNS as well as severe neural lesions, such as nonsuppurative encephalomyelitis characterized by multifocal nonsuppurative infiltration, with or without multifocal necrosis and multifocal to diffuse nonsuppurative leukocytic infiltration of the meninges, and occasionally calcifications. The presence of multiple small foci of necrosis in a placenta or brain may be suggestive of a protozoan infection in a fetus, but since this lesion is not pathognostic, additional assays need to be performed for determining the etiology, such as immunohistochemistry. The high correlation between PCR and detection of necrotic lesions in histopathology provides a solid evidence for *N. caninum* being the cause of abortion (Sager *et al.*, 2001). PCR appears less affected by autolysis than histopathology. Thus, autolytic and mummified fetuses can be investigated, from whose brains a histological examination is not feasible anymore. Placentas may be examined together with aborted fetuses for *N. caninum* by PCR, but in our hands *N. caninum*-DNA could only be detected in the placenta if it was also detectable in the fetus as well (Gottstein *et al.*, 1998).

Serological methods (IFAT, ELISA and Immunoblotting) are useful for the indirect diagnosis of *N. caninum* infection in adult cattle, newborn calves and up to a certain level in aborted fetuses. The detection of specific antibodies is usually done in serum and, more recently, in colostral milk samples and milk as well. Cows aborting due to an infection with *N. caninum* often have high levels of antibodies shortly after the abortion. Therefore post-abortion serology is a useful tool to aid in a diagnosis of neosporosis. However, the mere presence of antibodies to *N. caninum* does not prove that the infection caused the abortion, as many chronically infected cows are serologically positive. This means that antibodies to *N. caninum* may be present in a dam, which aborted due to another cause. If knowledge of a cow infection status, not related to an abortion, is needed e.g. for reasons of purchase or sale, one has to consider that antibodies in infected cattle may fluctuate substantially and may even drop below the cut-off value of the serological test used. Thus, Conrad and coworkers showed fluctuation of *N. caninum* antibody concentrations during pregnancy, in some cases concentrations even dropped below the detection limits (Conrad *et al.*, 1993). Similar findings of seronegative but nevertheless *Neospora*-aborting cows have been found by Sager *et al.* (2001).

The IgG avidity ELISA (Björkman *et al.*, 1999) can be used to discriminate between recent and chronic *N. caninum* infections. This allows e.g. to determine if abortion storms have been related to primary exogenous infections or if they were

triggered of in a persistently infected population by e.g. immunosuppressive events leading to recrudescence (Sager *et al*, 2005).

Another question that can be addressed serologically concerns the origin of antibodies found in seropositive newborn calf, i.e. by self-production or via the maternal colostrum. As only bovine IgG1 is transmitted colostrally from the dam to the off-spring, the isotypic discrimination between IgG2 and IgG2 anti-*Neospora* serum antibodies allows to determine the origin of the respective immunoglobulins and thus to estimate more precisely the time point of infection. Precolostral positive calf serology – if positive – proves a prenatal infection of the fetus, a negative serology – however, does not allow to completely exclude prenatal infection, as presumably not all infected fetuses have - or have had enough time - to produce their own antibodies.

Immunoblotting has revealed its high diagnostic potential with regard to operating characteristics (e.g. sensitivity, specificity and predictive values) and is usually carried out as a complementary test when the conventional serology (IFAT and ELISA) provided ambiguous results (Sondgen *et al.*, 2001). Immunoblotting may be of crucial help to elucidate potential cross-reactivities due to other apicomplexan infections such as bovine toxoplasmosis or sarcocystosis.

A more definitive diagnosis can be achieved when the abortion problem is examined on a herd level. Firstly, an initial estimation of the serological status of the whole herd is essential. A classical approach is to accomplish an individual serological analysis by means of IFAT or ELISA and employing a cut-off value for maximal sensitivity to diagnose infection according to previous works on validation of serological tests on bovine neosporosis. Once *Neospora* infection has been evidenced as a reason for an abortion problem in a herd, a seroepidemiological approach can be proposed. The rationale is to determine if the proportion of seropositivity in aborting cows is higher than in non-aborting cows. If *Neospora* infection contributed to the abortion problem, then the proportion of seropositive aborted cows should be significantly higher than the proportion of seropositive non-aborting cows.

- Studies e.g. in Switzerland (Gottstein *et al*, 1998, Sager *et al*, 2001) demonstrated that *N. caninum* and respective cerebral lesions can be revealed in 21-29% of aborted fetuses, and at the farm level, *N. caninum* is causatively linked to an abortion problem in 33% of farms. *Neospora*-positive heifers exhibit a slightly higher abortion risk than older cows (4x elevated when compared to a seronegative cow versus 3x). The distribution of abortions in dependence of the gestational age focuses between the 5th and the 7th month after insemination. Other infectious agents such as BVD virus (detected in 7% of aborted fetuses) and bacteria (4%) are not causatively associated to *N. caninum*-infection. As mentioned above, serological means alone appear not to be very reliable for predicting on-going or forthcoming *Neospora*-abortion-problems.

Since the dog has been defined as a definitive host for *N. caninum* (McAllister *et al*, 1998), several reports have putatively linked abortion storms in farms to a horizontal transmission of this parasite by oocyst-contaminated cattle food. It has been shown that the presence of dogs on the farm increases the risk of *N. caninum*-abortions in cattle. The isolation of *N. caninum* from the feces of naturally infected dogs has – so far - been reported a few times only. Diagnostically, *N. caninum* oocysts need to be discriminated from *Hammondia heydorni* and other similar oocysts by PCR (Hill *et al*, 2001). First studies tackling the epidemiological role canine definitive hosts play in the cattle abortion story have recently been or are presently undertaken. Preliminary data indicate a very low prevalence of oocyst-excretion in central European dogs. Experimental infection of pregnant cows with defined numbers of sporulated oocysts (1'500 – 115'000) demonstrated that such infection can cause transplacental transmission and abortion in cattle (Gondim *et al*, 2004).

Present possibilities to reduce the potential risk of horizontal transmission or *Neospora*-related abortion on a farm are as follows:

- Reliably diagnose *N. caninum*-associated abortion problems by using appropriate tools, serology alone is inadequate. There is need to demonstrate the presence of the parasite and (histo)-pathological damage in the fetus.

- Identify *N. caninum*-positive breeding lines, and envisage their removal from breeding upon appropriate economic assessment of the measure. Alternatively, embryo-transfer may help to save the breeding line.

- In view of potential exogenous (horizontal) transmission, we recommend to properly discard any infectious sources for both definitive and intermediate hosts as best as possible. This includes primarily appropriate disposal of any aborted fetal materials and placentas, and an appropriate food and defecation hygiene for farm dogs and other foreign dogs having access to the farm and its pastures.

- If a farm has a low-prevalence level, it may be appropriate to avoid the introduction of *N. caninum*-seropositive breeding animals into the farm.

Efforts have been made to develop vaccines for the prevention of abortion in cattle (Andrianarivo *et al*, 1999, Romero *et al*, 2004). However, no study so far has demonstrated that a vaccine can reliably prevent fetal infection in pregnant cattle (Andrianarivo *et al*, 2000).

Strategically, it may be interesting to assess efficacy of treating newborn calves originating from seropositive mothers with an appropriate drug. Such a metaphylactic chemotherapy is anticipated to eliminate postnatally developing tachyzoites and thus prevent the establishment of chronic infection in the offspring, and thus to produce *Neospora*-free breeding lines independent of the initial infection status. Corresponding studies e.g. using toltrazuril (Kritzner *et al*, 2002) are presently under investigation.

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COMPLEMENT EVASION IN THE HOST-PARASITE RELATIONSHIP

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The complement system is a first-line defense mechanism against parasites in promoting opsonophagocytosis, eosinophil-mediated killing and direct lysis. All parasites causing deep infections and getting into contact with human plasma must, in one way or another, avoid the destructive effects of complement. For this, parasites utilize a spectrum of evasion mechanisms to hinder different steps of complement activation. The build-up of physical barriers on the outer layers can protect many parasite forms from complement attack. Pathogenic protozoa can synthesize specific complement inhibitors or enzymes that cleave complement factors. Examples include trypanosomal C2 binding protein (CRIT), decay-accelerating factor-like molecule (T-DAF) and a C3-cleaving neutral cysteine protease of *Entamoeba histolytica*. Another common mechanism is to acquire complement inhibitors from the host. *Onchocerca volvulus* microfilariae and *Echinococcus granulosus* bind the soluble alternative pathway inhibitor factor H to their surfaces, and *Schistosoma mansoni* worms bind the classical pathway inhibitor C4bp. Upon prolonged growth the parasites can also acquire a protective shield of GPI-anchored membrane regulators, like CD59, from the cells of their hosts. Most of the evasion mechanisms, e.g. those of the malarial parasites, and the respective underlying molecules, however, still remain to be elucidated. Their identification and analysis could help in the development of new therapeutic and preventive approaches to control the different parasitic diseases.

STRESS IN THE LIFE OF A PARASITIC NEMATODE

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Parasitic nematodes, as all animals, are subject to stresses throughout their lives that constrain them in various ways. A significant feature of the environment of endoparasitic nematodes is the immune response of their hosts. Here, I will consider how the host immune response acts as a stress on the parasitic nematode *Strongyloides ratti* in three ways: (i) by affecting the development of its free-level generation; (ii) by reducing the fitness of individual parasites and (iii) by bringing about density-dependent effects.

Strongyloides is a genus of nematodes that parasitise many vertebrate groups. Two species infect humans (*S. stercoralis* and *S. fuelleborni*); some 50 – 100 million people are infected, principally with *S. stercoralis*. In the life-cycle of *Strongyloides* spp., the parasitic generation are female only worms, that lie embedded in the mucosa of the small intestine and reproduce by parthenogenesis (Viney, 1994). Eggs that pass out of the host can develop either into infective third stage larvae (iL3s) ('direct' development) or into free-living adult males and females ('indirect' development). These latter stages mate by sexual reproduction (Viney *et al*, 1993) and their progeny, in turn, develop into iL3s.

For *S. ratti* (a parasite of rats), many factors affect the choice between direct and indirect development of the free-living generation: these include, the temperature external to the host and the immune status of the host (with respect to *S. ratti*) from which the eggs and larvae are passed. The consequence of this is that eggs and larvae passed from a host that is mounting an anti-*S. ratti* immune response, preferentially develop indirectly into free-living adults, compared with stages passed from a host not mounting such an immune response (Gemmill *et al*, 1997; Harvey *et al*, 2000). Thus, here, the host immune response affects the development of the free-living generation of *S. ratti* and, in so doing, affects the fitness of this generation.

These findings raise intriguing questions about the sensory biology of *S. ratti*: how does *S. ratti* sense the host immune response; how does the host immune response affect the development of larvae external to the host? Our understanding of these factors (and their interactions) that affect the development of the free-living generation, suggest that *S. ratti* larvae external to the host have some 'memory' of the host immune environment from which they came.

The host immune response also has direct effects on the parasitic stages of *S. ratti*. Laboratory rats infected with *S. ratti* achieve a patent infection five days post infection (PI). Following infection, an anti-*S. ratti* immune response develops and worms are gradually lost from hosts, such that by *c.* day 30 PI, the infection has, essentially, been cleared from the rats. As an anti-*S. ratti* immune response develops,

parasitic females become progressively shorter (approximately halving in length), their *per capita* fecundity is reduced and they become more posteriorly distributed along the small intestine. These negative effects of the host immune response are enhanced in rats previously immunised with *S. ratti* and do not occur (or are reversed) in immunodeficient (or immunosuppressed) hosts (Wilkes *et al*, 2004). The combined effect of this is that the survival and reproduction of parasitic females is severely curtailed by the host immune response.

Density-dependent processes (*i.e.* when aspects of an organisms biology are negatively affected by the density of con-specifics) are a common feature of free-living and parasitic organisms, including parasitic nematodes. For, *S. ratti*, such density-dependent processes act on the survival and *per capita* fecundity of parasitic females. However, these density-dependent processes only occur late in a primary infection, *i.e.* when an anti-*S. ratti* immune response develops. A comparison of infections in immunologically normal and immunologically deficient (nude) rats, showed that density-dependent effects only occurred in immunocompetent rats, thereby proving that these density-dependent process are immune-dependent (Paterson & Viney, 2002).

In summary, the host immune response has individual and population-level effects on the parasitic stages of *S. ratti*; it also has profound effects on the development of the free-living generation. Therefore, in one sense, the immune response is clearly acting as a stress; however, it also appears to be used by *S. ratti* as an important developmental cue in its free-living life.

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EARLY EVOLUTION OF THE CELLULAR DOMAINS: FROM PROKARYOTIC SEX, TO SYMBIOSIS, TO EUKARYA?

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The cellularity of life is firmly established, but cells may not yet be generated *de novo*. From theoretical and empirical analysis, steps in cellular evolution from an ancestral cell to the two types of prokaryotic monera and the more complex eukarya are now emerging. The principle of cellularity is partly violated in certain parasitic and symbiotic cases, but how such events relate to basic cellularity is today not fully understood.

Formation of eukarya involved symbiosis of distinct bacteria that form the mitochondrial and chloroplastic organelles of modern eukarya. It remains unclear whether such events represent the mechanism by which eukarya arose, or whether they were specific steps in this evolutionary process.

Further deficiencies in modern cell theory include the enigma of cellular sex. Since Aug. Weismann, the consensual view of the sexual cell cycle is that it originated by evolution of meiosis from mitosis. This view was supported by the (now discredited) view that the evolution of sex was caused by the need to eliminate bad genes in haploid gametes. The evolutionary chronology of meiosis and mitosis remains to be determined. Other aspects of cellularity are also unclear: the relationship between prokaryotic fission & eukaryotic mitosis, the origin of the nucleolemma, etc. What is firmly established is that the eukarya represent an evolutionary merger of both moneric cell domains, as their genomes reflect both archaeal and bacterial monera. In contrast, eukaryotic plasmalemmas resemble the bacterial type with some new elements (i.e. their sterol contents).

Theories of cell evolution are useful if their predictions allow empirical falsifications. This normal scientific demand must apply to the field of cell evolution. Also, all cell theories must conform to the principles of darwinism.

My own work has proposed that the three domains of life account for the origins of sex, with male and female gametes descending from one of the two moneric domains. The proposal differs radically from other proposals in that evolution of cellular sex is seen as a divergent rather than a convergent phenomenon.

Among unusual predictions are that: the nearly ubiquitous extracellular envelope of the female gamete originated from the bacterial cell wall; the zygote arose from dormancy in environmental adversity; the concept that the proto-eukaryon evolved in concert with the biospheric oxygenation some 2 billion years ago; that (warm) polar biotopes supplied the first place cool enough to allow eukaryotic plasmalemmas; etc.

Hence, the *sexual theory of eukaryote evolution* suggests that the prime force in their evolution was *not* genomic, but *environmental*. Prokarya survive new environs by mutations (Darwin's 1. Law of Variation). Darwin's 2. Law of Variation ("sex") is an enigma precisely because it does not directly rely on mutations. *Only if sex already exists*, may sex work as the paramount means for adapting life to survival in new biotopes. And only eukarya have full-fledged sex.

Importantly, the first eukaryote (*proto-eukaryon*) was not nucleated. Protecting the proto-eukaryotic genome by a membrane only became essential much later in evolution in concert with their acquisition of respiration by mitochondrial organelles. This also resulted in the unique (double) nucleolemma (see below).

The sexual theory of cell evolution has the added advantage that it avoids the problem of how "half cells" (gametes) could be viable in deep time. The theory states that only haploid cells (monera) were fully viable in the first sexual cycle, while the zygote was a resting state and not a life form: it did not multiply but simply reverted to its constituent haploid cells in response to a changing environment. This recurring seasonal process is postulated to represent the origin of the first non-fission division which today constitutes the second meiotic division. The dormant nature of the proto-zygote also accounts for an essential demand in this cell theory: its *inability* to mutate.

One final aspect of cell evolution by sex concerns the gradual transition from mutualistic syntrophy of monera, to coexistence in a shared cytoplasm (proto-syngamy) with later generation of a larger eukaryotic cytoplasm. May the driving forces behind such events be postulated and chronologically timed? Life is critically dependent on a source of energy, and early life is widely postulated to have acquired energy from directly from di-hydrogen. When the global biotope slowly oxygenated after about 3 billion years ago due to photosynthesis, reliance on di-hydrogen for energy clearly was complicated: in an oxic environ di-hydrogen is not as readily accessible.

The sexual theory of cell evolution suggests that the two interacting cells types were not iso-metabolic, each relying on different energy supplies (catabolic, resp. phototrophic). In a polar biotope, each cell could survive different seasons (half the year) alone. Only by mutualism could they survive the whole year. If the (pro-female) bacterium was generating di-hydrogen during the dark season (in the clostridial manner), this would nourish the phototrophic archaeon (pro-male) until summer came. With more toxic di-oxygen generated during the summer, the dormant pro-female would be imperiled by toxic oxygen. A mutualistic archaeal pro-male could metabolize and remove di-oxygen, for instance by photo-respiration, while surviving by mutations in an oxic environ. This theory offers the first mechanistic account of syngamy. Increasing oxygenation would create a drive towards more complete syngamy as higher oxic tensions would threaten its di-hydrogen supplies, while the weakened bacterium would gain direct (non-mutational) protection by entering of oxygen-devouring pro-male. Such metabolic protection would clearly be possible only with low di-oxygen levels.

When di-oxygen levels later increased towards normoxic levels about 2.1 billion years ago, only global recombination between syngamic genomes could afford survival. This is the hallmark of the first meiotic division. Both of these events are unencumbered by nucleolemmas as they take place in prokaryotic cells. (Nucleolemma and mitochondria likely needed many hundred million years to be established as in modern symbiosis). The hallmark of eukaryotic cell evolution is thus not the nucleolemma, but the transition from viable monera to dormant *dimera*; to *dimera* with meiosis, which eventually simplified to (non-nucleated) mitosis as proto-eukaryotes spread globally after the Pre-cambrian divide in the mid-proterozoic era.

SUBMITTED PAPERS - ORAL AND POSTER PRESENTATIONS

IMPACT OF ANTHROPOGENIC PRESSURE ON *IXODES* TICK POPULATION, CADMIUM TOLERANT TICK POPULATION APPEARANCE, TICK MICROBIOCENOSIS, VECTOR CAPACITY AND IMMUNITY

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Appearance of morphologically changed *Ixodes* specimens is one of the consequences of the impact of anthropogenic pressure on the ticks population in Eurasia. It is now proved that under the selection pressure of the accumulated in the environment heavy metal ions of *Cd* the new properties of *Ixodes* ticks appeared. The exterior features of such new properties are exoskeleton anomalies. It was proved also that these ticks are widely distributed in Russia and in some European countries (England, Denmark, and Germany).

Prevalence of anomalous ticks with differently distorted exoskeleton detected stereo microscopically ranged from 12-25 to 50%. The rates of *Cd* ions detected by coulometry in the anomalous specimens to normal ones are as following (mg/kg): Denmark 6.93/5.52; Kaliningrad region (Curonian Spit) 19.44/3.6; Novgorod 5.6/2.93; St. Petersburg 5.56/3.29; Western Siberia (Irkutsk) 7.59/4.44, Far East (Vladivostok) 5.29/3.63. Thus *Cd* quantity in anomalous ticks is 1.5-2 times more than that in normal ones. Cadmium accumulation changes tick metabolism, immunity and vector capacity.

Microbiological study of microflora of two parts of *Ixodes* tick populations demonstrated that prevalence of Gram-negative and Gram-positive bacteria; bacilli, cocci and fungi were much higher in anomalous ticks (47 species) than that of normal ones (28 species). Only 14 (of 61) species of "vulgar" microflora coincided in both groups of ticks. No more than 4 species of microflora were presented in normal ticks, whereas ticks with exoskeleton anomalies contained 5-7 species of microorganisms simultaneously. Only in anomalous ticks cocci of the genus *Staphylococcus* and fungi of the genus *Penicillium* absolutely prevailed. Staphylococci are known as active stimulants of production of defensins, which increasing is a marker of tick's immune system stimulation. *Staphylococcus* injection or absorption with food stimulated gut form of lysozyme production; it is also a marker of tick's immunity stimulation. Thus, high prevalence of staphylococci as well as *Penicillium* fungi in anomalous ticks may serve an indicator of tick immune system suppression. Proved suppression of anomalous ticks immune system leads to the greater prevalence of tick-borne pathogenic for man agents such as *Borrelia*, *Ehrlichia*, *Anaplasma*, *Babesia* spp. or tick-borne encephalitis virus in this part of population. Dual and triple infections also were recorded more often in the anomalous part of tick population. More

microorganisms, which are conditional pathogenic for man (e.g. *Aspergillus* fungi), were revealed exactly among anomalous ticks.

Anthropogenic pressure and environment pollution lead to increase of prevalence of the anomalous specimens in the *Ixodes* tick populations; immunity of such ticks is suppressed and their vector capacity is increasing; dangerousness of such ticks is much greater.

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COMMUNITY STRUCTURE OF CESTODES FROM SHREWS IN SOUTHERN KARELIA

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Research of the species diversity of cestodes and their hosts, shrews, in Karelia is necessitated by the fact that the region is, first, a meeting point of two large faunal complexes – European and Siberian, and second – the northern periphery of the distribution range both for the parasites and for their hosts.

Shrews were collected from two localities in southern Karelia, between 1997 and 2002. Hosts were trapped by snap-traps arranged in a line (100 ind.). A total of 364 shrews (*Sorex araneus* - 287, *S. caecutiens* - 13, *S. minutus* - 48, *S. isodon* - 12, *S. minutissimus* - 4) were examined by the method of total helminthological dissection. Cestodes were isolated, stained and identified according to standard methods.

The cestode fauna of shrews is represented by 16 species belonging to four families: Hymenolepididae (*Ditestolepis diaphana*, *D. tripartita*, *Neoscrjabinolepis schaldybini*, *Lineolepis scutigera*, *Staphylocystis furcata*, *Spasskilepis ovaluteri*, *Vigisolepis spinulosa*, *Staphylocestoides stefanskii*, *Pseudobothrialepis matevossianae*, *Urocystis prolifer*, *Hepatocestus hepaticus*), Dilepididae (*Dilepis undula*, *Monocercus arionis*, *Polycercus lumbrici*), Taeniidae (*Taenia mustelae*) and Diphyllbothriidae (*Spirometra erinoceiropaei*).

The diversity of cestode species was the highest (16) in representatives of the European faunal complex (common shrew - 15, lesser shrew - 8). The number of cestode species parasitizing shrews of the Eastern Palearctic group was far lower (graves - 6, masked - 6, pigmy - 1). The cestode infection level was highest in the common shrew (90.6%), due to its eurytopic and eurytrophic habits. The common shrew plays a key role in maintaining the species diversity of cestodes in the biocenosis. It occupies a leading position in the community of shrews of Karelian middle taiga, and is the definitive host for the majority of known cestode species (all Hymenolepididae species and *Monocercus arionis*). The fauna of cestodes of the

common shrew comprises also larval forms of *D. undula*, *T. mustelae*, *P. lumbrici* and *S. erinaceieuropaei* which, being polyphagous species, point to its biocenotic interactions with a relatively wide range of predatory birds and mammals.

Cestode community can be classified by dominance as follows: dominants (*N. schaldybini*, *D. diaphana*), subdominants (*M. arionis*), rare (*V. spinulosa*, *St. furcata*, *D. tripartita*, *L. scutigera*, *S. ovaluteri*, *D. undula*, *P. lumbrici*) and very rare species (*P. matevossianae*, *U. prolifer*, *St. stefanskii*, *H. hepaticus*, *T. mustelae*, *S. erinoceieuropaei*). The structure of the cestode community in shrews is inconstant, varying by host species, habitats and years. Species tend to drift from one category to another. The species diversity of the cestode community in shrews of Karelia is the outcome of a wide food range of the animals and the abundance of invertebrate species they feed on.

MORPHOLOGICAL VARIABILITY OF TAPEWORMS OF THE GENUS *PROTEOCEPHALUS* (CESTODA: PROTEOCEPHALIDAE)

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The aim of the present research was to study the qualitative diversity in parasites with various host ranges. The study objects were widespread parasites of fish belonging to various evolutionary and ecological groups: *P. percae* - perch parasite, *P. torulosus* - cyprinid parasite and *P. longicollis* (syn. *P. exiguus*) - salmonid parasite.

Six qualitative features representative of the helminth major functional systems were analyzed: attachment (shape of the scolex), feeding and reproduction (shape of mature proglottids, testes, ovary lobes, vitelline glands, location of testes). The qualitative structure of *P. percae*, for which perch is the only typical host, was found to be the simplest – 13 variations of features. The qualitative structure of the species *P. torulosus* – a parasite of Leuciscinae, which we have studied in 6 fish species (*Alburnus alburnus*, *Aspius aspius*, *Leuciscus idus*, *L. leuciscus*, *Oreoleuciscus pewzowi*, *Rutilus rutilus*,) is more complex - 14 variations of features. The qualitative structure of *P. longicollis* – a parasite of coregonids and salmonids, which we have studied in representatives of the Salmonidae: *Brachymystax lenok*, *Salvelinus malma*, *Oncorhynchus mykiss*, and of the Coregonidae: *Coregonus lavaretus*, *C. albula*, *Stenodus leucichthys* is the most complex - 19 variations of features.

Thus, the research has demonstrated that the rate and characteristics of qualitative variability of a parasite species differ in time and space. Spatially they depend on the common historical fate and co-evolutionary relations between the parasite and the host. Parasite evolution has slowed down as compared with the host evolution owing to the co-existence of various phenotypes, which facilitates gene recombinations and enhances the stability and plasticity of parasite species.

RESULTS OF ECOLOGICAL AND FAUNISTIC STUDIES OF COCCIDIANS IN MAMMALS OF LITHUANIA

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The first records of the coccidians (Coccidiidae: Eimeriidae) in Lithuania date back to 1926-1928. They can be found in reports of Veterinary Department of the Lithuania Ministry of Agriculture and in the journal "Veterinary and Zootechnics", mostly on the loss of chickens because of coccidiosis; only one report concerned a case of calf coccidiosis. No other data on mammalian coccidians could be found in the period preceding our studies.

We started regular investigations of coccidians in domestic and wild mammalian in Lithuanian in 1962 and have been conducting them until 2004. The coprological flotation methods were used for the isolation of coccidia; the endogenous stages were observed in histological preparations. The cross-infection method was applied to prove the hostal specificity of some species. The following domestic animals were involved: horses, cattle, sheep, swine, rabbits, nutrias. The wild animals studies include aurochs *Bison bonasus*, elks *Alces alces*, red deers *Cervus elaphus*, roe deers *Capreolus capreolus*, wild boars *Sus scrofa*, predators (5 species), hares *Lepus europaeus*, insectivores (3 species), musc-rats and small rodents (11 species).

Coccidians were found to be widely distributed among animals of different species. About 130 species of coccidians were diagnosed parasitizing different segments of the intestine and liver. Seven new to science species of coccidians have been described.

Some of coccidian species have been found to exhibit hostal specificity. The level of the infection with coccidians has been revealed to depend on the animals species, age, population density, the character of the biope, the season of the year and only insignificantly on the sex of the animals.

AN OVERVIEW OF WILD MAMMALIAN HELMINTHOLOGY IN JAPAN

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In this paper, outlines with history of the taxonomical, faunal, and diagnostic studies on the parasitic helminths obtained from the endemic and alien mammals in Japan, and from captive mammals kept in the Japanese zoos and aquariums will be presented. Isao Iijima introduced systematic parasitology first into Japan in the early 1900s. Under his discipline, many parasitologists, including S. Goto, S. Yoshida, K. Morishita and T. Fukui appeared and launched studies on the helminth fauna of wild

mammals. Besides these Japanese researchers, some foreign parasitologists, such as H. V. Van Cleave and J. S. Wilkie, also described helminths. From 1933 to the middle 1960s, S. Yamaguti began to publish the "Studies on the helminth fauna of Japan" and "Systema Helminthum". In this era, Ozaki, Fukui, S. Ishii, T. Ogata, J. Yamashita, S. Iwata, Y. Kanda also carried out extensive studies. This era also overlapped with the period during which Japan extended its territory to neighboring countries, and many taxonomical surveys have been made on helminths in Taiwan, northeastern China, the Korean Peninsula and the southern areas. After the middle 1960s, A. G. Chabaud, who visited Japan for helminth fauna surveys, greatly stimulated Japanese helminthologists to incorporate a zoogeographical viewpoint in their surveys. Recent economic developments in Japan have enabled the researchers to extend their survey fields to Southeast Asia, the Chinese continent and Russia. It has become possible to compare helminth faunas in Japan and surrounding areas. Furthermore, the development of the wild animal and zoo medicine-part of veterinary science has made a progress of field helminthological survey on wild mammals including alien species in Japan.

We will summarize the helminth fauna studies carried out after the 1960s. These data will be outlined with zoogeographical remarks and alien invasive species issues.

BLOOD PARASITES OF GREAT SNIPE *GALLINAGO MEDIA* IN BELARUS

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The results of preliminary investigation on the fauna and distribution of haemosporidian blood parasites (Sporozoa: Haemosporida) of shorebirds (the order Charadriiformes) in southern Belarus are presented.

In all, 39 individuals of Great Snipe *Gallinago media* were investigated for blood parasites during this study. The material was collected in the floodplain meadows of the Pripyat river in the vicinity of Turov (52° 04' N, 27° 44' E) in spring 2001-2003. Males of the Great Snipe were caught on leks with mist-nets. Blood was taken from the brachial vein. All birds were then ringed and released. Blood films were air-dried, fixed in absolute ethanol, stained with Giemsa, and examined microscopically. At least 100 fields were examined using the objective $\times 20$ and then the oil objective $\times 100$.

The overall prevalence of infection was 28.2%. Haemosporidian parasites belonging to the families Leucocytozoidae and Plasmodiidae and microfilariae of filariid nematodes were seen. *Leucocytozoon legeri*, *Plasmodium* (*Giovannolaia*) sp., and *Plasmodium* (*Haemamoeba*) sp. were identified. Prevalences of *Plasmodium* spp. (23.1%), *Leucocytozoon* sp. (5.1%), and microfilariae (7.7%) were recorded. Only 2.6% of all infections were mixed infections with species of *Plasmodium* and microfilariae found simultaneously. The highest intensity of infection (3-4 parasites

per one field at the high magnification) was recorded for *Plasmodium (Giovannolaia)* sp. Species of *Haemoproteus* were not seen during this study.

According to the literature data, haemosporidian parasites usually are uncommon in shorebirds. The Great Snipe certainly is an exception. The high prevalence of *Plasmodium* spp. in the Great Snipe on leks during birds' breeding period indicates that active transmission of avian malaria probably takes place at the study site. Such high prevalence of avian *Plasmodium* spp. has been recorded for the first time in Belarus. The Great Snipe and malaria parasites are convenient model objects to test various aspects of evolutionary hypotheses about host sexual selection, immunocompetence, evolution of virulence, and the role of parasites in the maintenance of lek behaviour in the Great Snipe.

DIVERSITY AND ECOLOGY OF METAZOAN PARASITES IN BALTIC COD *ADUS MORHUA CALLARIAS* FROM THE LITHUANIAN ECONOMIC ZONE OF THE BALTIC SEA

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In the year 1999-2004 over 250 individuals of cod (0,5-8 years old, 7-108 cm body length) of both genders were investigated. The investigations were carried out all over the year. Seven species of metazoan parasites were found: Nematoda (3), Acanthocephala (3) and Cestoda (1). Shannon - Weaver infracommunity species diversity indices were $e^H = 3.54$. About 85 % of cods were infected with metazoan parasites.

Echinorhynchus gadi (Acanthocephala) is the one of cods' specific parasites species. It infects the cod of different age groups. The greatest infestation rate was in groups ranging between 25 and 56 cm. In these size groups the prevalence of infection was up to 70.58 %, intensity of infestation varied between 2-160 parasites per fish. Differences of infestation among body length groups were not significant (according to the *t*-test) and mainly depended on consecutive changes of feeding habits. The infestation of fish bigger than 85 cm length with *E. gadi* could be explained by post-cyclic transmissions from herring to cod, but not through small cod as was formerly believed. In June-August cods return after their spawning to feeding areas. During this period changes of *E. gadi* generations were detected. In the same time old and juvenile acanthocephalan were found together. Females and males were respectively of 15 and 45 (SD \pm 3 and 5) mm body length, and in 2nd-3rd maturity stage. About 72 % of females had copulatory cap "mating plug" on the posterior end. Moreover, approximately 5 % of males and some of *Hysterothylacium aduncum* also had the "mating plug" and not in all cases on the posterior end. This may be a result of

competition and sexual behavior of males. Larva of *H. aduncum* L₃ (Nematoda) reaching 10-18 mm in length were found free in intestinal lumen or encapsulated in mesentery of pyloric caecum; its' prevalence, infestation intensity in different length groups was similar (6.48 %, and 1-20 individuals per fish). This could be explained by prevalence of amphipods in diet of the cod. Matured (L₄ stage) *H. aduncum* having body length of 23–60 mm, were found in intestines. The prevalence of *Corynosoma semerme* L., *Pomphorhynchus laevis*, *Contracaecum osculatum* L., *Ascarophis* L., *Bothriocephalus* sp. was less than 3 %. The cod is a definitive host of *E. gadi*, *H. aduncum*; intermediate host of *C. semerme*, *C. osculatum*, *A. simplex*, *Ascarophis* sp.; rare host of *P. laevis*, *B. gregarius*. All these parasites are autogenic and form an ecological group of brackish and marine water.

HELMINTHS OF LYNX LYNX LYNX AND WOLF *CANIS LUPUS* IN LATVIA

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The objective of this research was to determine the species of helminths practising lynx and wolf, intensity and prevalence of invasion in order to investigate parasite – host relations of hunting fauna in Latvia.

Material for lynx helminths fauna was collected and processed from 1999 to 2005. Investigation of wolf's helminths fauna was started in 2003. Complete helminthological analysis of 71 lynx and 8 wolves was made; additional 9 lynx were partly inspected.

The complete helminthological analysis was used: stomach, intestines, liver, gall bladder, lungs, trachea, spleen, kidneys, and urinary bladder were examined. Determination of *Trichinella* spp. was carried out using digestion in artificial digestive fluid.

Seven parasite species in lynx were found belonging to the following systematical groups: Cestoda (*Taenia pisiformis*) and Nematoda (*Toxocara mystax*, *Thominx aerophilus*, *Capillaria felis-cati*, *Trichinella* sp., unidentified Nematoda sp., and *Crenosoma* sp.). Seven species were found parasiting wolf: Cestoda (*Taenia hydatigena*, *Taenia pisiformis*), Nematoda (*Uncinaria stenocephala*, *Thominx aerophilus*, *Capillaria plica*, *Trichinella* sp.) and Trematoda (*Alaria alata*). *Taenia pisiformis* was the prevalent parasite species in lynx; all examined animals were infected. Another common species was *Toxocara mystax*. Almost half of investigated animals were infected with *Trichinella* sp. Rather frequent occurrence was for *Thominx aerophilus*, considerably rarely – *Capillaria felis-cati*. There was a single

record of nematodes belonging to the genus *Crenosoma*, which is the first case documented for lynx. A larva of unidentified species of Nematoda was also found.

Alaria alata was the most common in wolf. Another prevalent parasite was *Thominx aerophilus*, as well as *Taenia hydatigena* and *Uncinaria stenocephala*. *Taenia pisiformis* and *Trichinella* sp. were of rare occurrence. *Capillaria plica* was found in a single case.

Lynx's helminths fauna does not consist of many species in Latvia and infection intensity is not so considerable to endanger health condition of animals, although several animals were infected at crucial stage threatening their health and viability. Insufficient amount of material does not provide complete information about wolf's helminths fauna; therefore it is necessary to continue this research. The analysis of animal's stomach content is being made concurrent to helminths fauna investigation, thus making a base for further research on parasites transmission possibilities in relation predator – prey. According to stomach's content analysis, the investigation of lynx and wolf prey species' helminths fauna has been started.

This study was supported by the European Social Fund.

FORMATION OF PARASITE FAUNA OF BROWN TROUT *SALMO TRUTTA* L. FROM SMALL LAKE-RIVER SYSTEMS OF NORTHERN KARELIA

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Territory of Kola Peninsula and North Karelia is one of the last areas, which freed from ice cover during degradation of Last Glacier. So, the waterbodies of this territory seems to be the youngest. Some of them, such as waterbodies of lake-river system Paanajarvi-Olanga (Northern Karelia), were not affected with anthropogenic impact. We consider them as model "waterbodies on the early stage of fish and parasite fauna development". The lake-river system Lohilampy is the part of Paanajarvi-Olanga own. Ichtyocenosis of this system includes only brown trout and perch.

The parasite fauna of brown trout parr aged 1+, 2+ and brown trout aged 5+-8+ were investigated from tributary and the lake Lohilampy.

Unique features of Lohilampy (ultraoligotrophic type, existence of isolated brown trout population, the oligocomponent composition of fish community) exhibited the peculiarities of the Lohilampy parasite fauna, which were obvious in analysis of parasite fauna formation processes.

It has been found two aspects, which pointed to brown trout parasite fauna specificity. Firstly, the core of parasite fauna is already formed in fish aged 1+. Secondly, exactly Salmonoides fish specific parasites (*Chloromyxum schurovi*,

Discocotyle sagittata, *Crepidostomum farionis* and *Cystidicola farionis*) form the core.

Too quick parasite fauna formation is possibly connected with earlier parr migration in the lake that is untypical for brown trout of large waterbodies. This migration is characterized for small lake-river systems, in which tributaries shrink in summer and are frozen in winter. The parr are down to the lake where adult fish are feeding. So in lake parr pick up new species of parasites. The core of parasite fauna is formed for fish aged 1+. The parasite fauna of brown trout 2+ consists of species, most of which were record for adult fish. It was obtained only in the Lohilampy and it wasn't received for other waterbodies of system under investigation.

The Lohilampy is closely for ultraoligotrophic status than other lakes. It is allowed to consider this lake to belong to the waterbodies which were inhabited by Salmonoidei fish after the glaciations. The composition of parasite fauna core of brown trout, including autogenic specific parasites of Salmonoidei fish, is a peculiarity of this system.

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PARASITE FAUNA OF PINK SALMON *ONCORHYNCHUS GORBUSCHA* (WALBAUM, 1792) UNDER ACCLIMATIZATION

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Pink salmon acclimatization into White Sea has been doing since 1957. Obvious evidence of naturalization of this species in waterbodies of Russian North are still absent. Thereby problem on expedience of pink salmon acclimatization had been repeatedly arisen. There are two opposite viewpoints on this problem, but none of them are confirmed by the sufficient actual data. Data obtained do not allow evaluating pink salmon influence on hydrobiocenosis states of White and Barents Sea basins. The aim of this research is to identify peculiarities of pink salmon parasite fauna formation under acclimatization.

The parasite fauna of pink salmon, which was acclimatized to the River Keret (White Sea basin) includes 13 species. Marine species are prevailed (10 species). Freshwater species are presented by parasites actively infecting hosts. Diversity of pink salmon parasite fauna of the White and Barents Sea basins was decreased as compared with parasite fauna of natural fish populations from the basin of Pacific Ocean.

Thus, because ecology of *O. gorbuscha* is similar to ecology of salmon of the genus *Salmo*, pink salmon is infected by the parasites of anadromous salmonidae fish of Kola Peninsula and Karelia in the case of introduction. The highest similarity of parasite faunae was determined for Atlantic salmon and pink salmon. This is due to marine species, represented both in core, and in sector of the overlapping parasite faunae of Atlantic salmon and pink salmon. This similarity is based on the feed composition during the feeding period in the sea. However, in this case it is difficult to suppose the degree of pink salmon and Atlantic salmon parasite fauna overlapping within large ecosystem, such as sea. This problem requires additional studies, possibly using the method of parasite marks. Furthermore, it is necessary to obtain data on parasite fauna of young pink salmon for estimation of peculiarities of pink salmon and other Salmonidae fish relations.

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PREVALENCE OF ECTOPARASITOSEs IN DOGS AND CATS AMONG OTHER SKIN DISEASES

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The purpose of this study was to evaluate the frequency of various ectoparasitoses among the dogs and cats suffering from skin diseases. All dogs and cats presented during one year with skin problems to the Small Animal Clinic were examined for infestations with fleas, mange mites and lice. The parasitic infestation was confirmed with a diagnostic methods such as examination with magnifying lens, microscopic examination of material collected on skin scrapings, in some cases serological examination and diagnostic therapy. The results of this survey showed that ectoparasitoses compose a significant part of skin diseases in pet animals. This fact must be taken into consideration when managing skin patient.

RELIABILITY OF MITOCHONDRIAL DNA IN AN ACANTHOCEPHALAN: THE PROBLEM OF PSEUDOGENES

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Mitochondrial DNA has been used extensively in phylogenetic studies particularly at the population level. However, the presence of mitochondrial-like sequences in the nuclear genome has been recognized as a potential problem for evolutionary studies utilizing mtDNA. Nuclear mitochondrial pseudogenes or numts are nuclear sequences exhibiting a high degree of homology with mitochondrial DNA sequences. Numts have been identified from a wide range of taxa, and can occur in very high copy numbers. Identification of numts is essential to avoid erroneous data and misleading conclusions. Here, we present the first evidence of the incidence of numts in an acanthocephalan. While examining the geographic distribution of mitochondrial sequence variation in *Acanthocephalus lucii*, poor PCR amplification and sequence ambiguities were common. After cloning and sequencing PCR products, it was evident that multiple sequences had been amplified, which suggested the presence of Numts. Through a combination of molecular and phylogenetic techniques, numt sequences were distinguished from genuine mitochondrial sequences. This work illustrates the possible dangers associated with the use of universal primers targeting mtDNA, and techniques to avoid numts are discussed.

PECULIARITIES OF DEVELOPMENT AND DISTRIBUTION OF BLOODSUCKING BITING MIDGES (CERATOPOGONIDAE: *CULICOIDES*) IN LITHUANIA

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Bloodsucking biting midges (*Culicoides*) are important as impertinent parasites of people and other vertebrates. They spread various bacteria, parasitic protozoa and nematodes. Biting midges had not been thoroughly investigated in Lithuania. Data published by Estonian scientist H. Remm in 1966 is the only information on the fauna of biting midges in Baltic countries. The aim of this study was to investigate peculiarities of distribution and development of biting midges in Lithuania.

Biting midges were caught by two methods: with the entomological net fortnightly in five sites, and with light traps weekly in other five sites. In addition, we investigated biting midges collected in other localities. Adult biting midges were kept in 70% alcohol. Material was collected in 2000 - 2004.

During the studies, we determined 23 species of biting midges belonging to the genus *Culicoides*. Adults of biting midges were recorded from the third week of April till the third week of November. The most abundant species were *Culicoides obsoletus* (Mg.) and *C. punctatus* (Mg.). Out of 23 *Culicoides* species found, 7 species were collected from people during the registration of bloodsucking midges.

Three species of biting midges had 3 flying periods. These species are: *C. punctatus*, *C. obsoletus* and *C. pulicaris* (L.). Nine species had two flying periods during a year. One flying period is characteristic of 11 species of biting midges. The greatest number of biting midge species was observed in Lithuania in May and July.

Some of the biting midge species were recorded only in one habitat while others were recorded in different habitats. *Culicoides obsoletus* were recorded at all study sites; it proved to be dominant at six out of ten investigated study sites. The dominant biting midge species most often varied in different habitats. Biting midges of the species *C. obsoletus*, *C. punctatus* prevailed in pinewood, *C. obsoletus*, *C. albicans* – in raised bogs; *C. obsoletus* dominated the mixed and deciduous forests. Fidelity to certain habitats may be accounted for the peculiarities of biting midge development.

THE FAUNA OF HELMINTHS OF COMMON SHREW IN THE NORTH-WESTERN PALEARCTIC

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The common shrew *Sorex araneus* is widespread Palearctic species. The fauna of helminths of this shrew has been studied to estimate distribution of the parasites in regions covered by ice during the last glaciations period.

The fauna of helminthes of 140 specimens of common shrews was studied in Lithuania (Anykščiai, Molėtai, Šakiai, Šilutė districts and Vilnius city, 1999-2003), Karelia (field station Gomselga, 2003) and in south of Belarus (Pripyatski National Park, 2004). The overall prevalence of infection was 92.1 %; 21.4 % of shrews were infected with trematodes, 88.6% with cestodes, and 86.4 % with nematodes. In total 35 species [5 species of trematodes: Brachylaemidae - *Brachylaemus fulvuus* (Belarus – B, Karelia – K, Lithuania – L), *Pseudoleucochloridium soricis* (B, L), Omphalometridae - *Rubensstrema exaspiratum* (B, K, L), *Neoglyphea sobolevi* (B, K, L), *N. locellus* (B), 16 species of cestodes: Hymenolepididae - *Vigisolepis spinulosa* (B, K, L), *Neoskrjabinolepis schaldybini* (B, K, L), *N. singularis* (B, L), *Urocystis prolifer* (K, L), *Staphylocystis furcata* (B, K, L), *Staphylocystoides stefanskii* (K, L), *Lineolepis scutigera* (B, K, L), *Ditestolepis diaphana* (B, K, L), *D. tripartita* (K, L), *Soricinia infirma* (B), *Spaskilepis ovalutari* (B, K, L), Dilepididae – *Monocercus*

arionis (B, K, L), *Dilepis undula* larvae (B, K, L), *Hepatocestus hepaticus* (B, L), *Polycercus lumbrici* larvae (B, K, L), Taeniidae - *Taenia mustelae* larvae (K), 14 species of nematodes: Heligmosomatidae - *Longistriata didas* (B, K, L), *L. depressa* (B, K, L), *L. trus* (B, K), Strongyloididae - *Parastrongyloides winchesi* (B, K, L), Capillariidae - *Capillaria incrassata* (B, K, L), *C. kutori* (B, K, L), *C. petrowi* (B), *Eucoleus oesophagicola* (B, K, L), *Hepaticola soricicola* (B, K, L), Soboliphymidae - *Soboliphyme soricis* (B, K), Anisakidae - *Porrocaecum depressum* larvae (B, K, L), *Porrocaecum* sp. larvae (B, K, L), Spiruridae - *Physocephalus sexalatus* larvae (B, L), *Agamospirura minutissima* larvae (B, L)] of helminths were found.

The analysis of the original data and the literary shows that the fauna of helminths of *S. araneus* in the North-Western Palearctic is considerably poorer than in the Eastern Palearctic, which was not covered by ice during the Pleistocene. The number of helminths species in the common shrew of the North-Western Palearctic is the greatest in the south and decreasing towards the north. According to *S. araneus* phylogeography, a conclusion can be made that populations from the eastern Palearctic refugium, having colonized the North Europe, distributed a rather limited number of helminths species. The core of common shrew helminths communities in the North-Western Palearctic was made up by the species of parasites, which came from the southern Pleistocene refugium.

CESTODES OF THE GENUS *APLOPARAKSIS* CLERC, 1903 (CYCLOPHYLLIDEA) FROM GULLS

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Since the genus *Aploparaksis* was established, 16 species were described or reported to infect gulls. The original descriptions of the type-specimens were brief or were lost, consequently, in the information regarding main morphological characters used for species discrimination, many misidentifications have been arisen.

The present study summarises the results of morphological re-examination of the majority of type-specimens of *Aploparaksis* described in gulls, voucher specimens from the museum collections and studies of our own material collected in North Siberia (Chukotka, the Ob' River) and specimens from North America (collection of Dr R. Rausch) and from Russia (collection of Dr A.A. Shigin, Rybinsk reservoir; Dr Z.B. Smetanina, Lake Hanka, Primorskiy Kray; Prof M.M. Belopolskaya, Yakutia; Mr S.A. Leonov, Kolyma River, and specimens from Vrangeli, Bering, Karaginskiy and Paramushir islands which were collected by our colleagues from the Far East State University, Vladivostok).

Six species have been confirmed to parasitize gulls. These are: *A. borealis* Bondarenko & Rausch, 1977, *A. brachyphallus* (Krabbe, 1869), *A. diagonalis* Spassky

& Bobova, 1961, *A. rissae* Schiller, 1951, *A. xemae* Schiller, 1951 and *Aploparaksis* n. sp. (= *A. larina* sensu Shigin, 1961.) Only the *Aploparaksis* n. sp. is supposed to be a host-specific parasite of gulls, whereas the first one and others four are recognised as being parasites of passeriform birds and sandpipers, respectively. Comparison of morphology of specimens of *A. brachyphallus* collected in Chukotka, Alaska and deposited in museum collections, with morphology of the specimens from the type-materials of *A. larina* Fuhrmann, 1921 from *L. dominicanus* in Antarctic gave good reasons to treat these species as synonyms. *A. larina* sensu Shigin (1961) is likely to be a new species of the *Aploparaksis*.

An existence of a stable infestation of *Larus argentatus* nestlings by *A. diagonalis* was described by us from the small colony in a tundra lake in Chukotka, indicating that under certain ecological conditions usual parasites of shorebirds can integrate into gull cestode communities and can be regarded as an example supporting the existence a micro-evolutionary process which is known as 'hostal radiation' or 'host switching' (in evolutionary sense).

EFFECTS OF FOREST FRAGMENTATION ON MALARIA INFECTIONS IN WILD AFRICAN RAINFOREST BIRDS

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Habitat fragmentation and deforestation are often thought to be implicated in the occurrence of epizootics and their likely transfer to humans as a result of increased contact. Understanding how habitat disturbance influences the prevalence of infectious diseases, as well as its effects on the host and geographic specificities of pathogens, therefore appears crucial to counter future outbreaks.

Blood samples from the Yellow-whiskered Greenbul *Andropadus latirostris*, the Little Greenbul *Andropadus virens* and the Olive Sunbird *Nectarinia olivacea*, three species of African rainforest birds, were collected over the last 13 years in a range of habitats, including habitats that have undergone substantial conversion over this time span. In addition, samples were collected from ecotone sites, characterized by a transitional forest-savanna mosaic. Using PCR-based detection methods and subsequent sequencing, we have screened for the presence of the blood parasites belonging to the genera *Plasmodium* and *Haemoproteus*.

Results corroborate our predictions and we show that infection rates are higher and pathogen prevalence exhibits a greater variance in forest fragments as compared to large contiguous fragments tracts. This may be explained by the clustering of hosts

in small habitats leading either to an increase in the number of infected hosts or to the extinction of parasites as a result of too few hosts. In addition, we have identified differences in parasite mitochondrial DNA haplotypes between small ecotone forest fragments and large contiguous forest sites. These results support a possible role for anthropogenic modifications of habitats in promoting the spread of infectious diseases.

INFLUENCE OF THE LIVING CONDITION ON TOXOCAROSIS RISK IN CHILDREN IN CENTRAL POLAND

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Human toxocarosis is caused by an infection with larvae of the roundworm *Toxocara* spp., a common parasite of young dogs and cats. People become infected through eating meals contaminated with infective eggs, contact with polluted soil or infected animals. The clinical manifestation of toxocarosis in man depends on the number of larvae ingested, the frequency of infection, the distribution of larvae in the body and the intensity of the host's immunological response. The most frequently infections are characteristic for children due to a lower immunological response than in adults, no admonition of hygiene rules, repeated contacts with pets and specific behaviour (geophagia).

The home surroundings (162 sites) of 205 children with diagnosed toxocarosis were examined to evaluate the correlation between the contamination of the soil with *Toxocara* spp. eggs and the incidence of clinical cases of toxocarosis. Samples of sand and soil were collected in backyards, gardens, sandboxes and playgrounds in central Poland (Mazowieckie voivodship). *Toxocara* spp. eggs were extracted using the Dada method (1979). The entire group covers 205 cases with visceral, ocular and covert toxocarosis (45.4%, 17.1% and 37.5% respectively). Patients originated from rural areas (55.6%), suburban regions (26.8%) and towns (17.6%). The highest level of soil contamination was found in suburbs (30.3% samples positive) and rural areas (24.7%), while in towns 2.8% sites were found positive. Dogs and cats as possible source of infection were kept in 41.1% and 17.1% of examined sites.

These results revealed that in suburban and rural localities there is a high risk of toxocarosis for children.

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BOVINE NATURAL KILLER CELLS PRODUCE INTERFERON GAMMA AND
KILL INFECTED AUTOLOGOUS FIBROBLASTS UPON IN VITRO INFECTION
WITH *NEOSPORA CANINUM* IN CATTLE

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Natural killer (NK) cells are known to be key players in the early innate responses to protozoan infections, and their primary role has been considered to be interferon-gamma (IFN γ) production in response to cytokines involved in the stimulation of other immune cells as well as lysis of target cells. Several studies have shown that NK cells can kill protozoa-infected cells as well as extracellular protozoan organisms, but the biological significance of NK cell cytotoxicity *in vivo* is still unclear.

We here demonstrate direct IFN γ production from *in vitro* cultivated bovine NK cells by *Neospora caninum*, a *Toxoplasma*-like protozoan known as a worldwide cause of abortions and congenital infections in cattle. Pure IL-2 activated NK cells were stimulated to produce IFN γ both directly by live and heat-inactivated tachyzoites and by infected fibroblasts, independent of IL-12 but potentiated by the addition of this cytokine. No response was seen with soluble proteins from the organism. We also show that *N. caninum*-infected autologous fibroblasts had increased susceptibility to NK cell killing than uninfected fibroblasts, in both cases dependent on perforin. In addition, we show that during these experiments, the parasite infected and proliferated inside NK cells. Together, these findings constitute the first description of an NK cell involvement in bovine neosporosis, and contribute to the field of innate immunity during protozoan infections in general.

FIRST REPORT ON PREVALENCE OF *ECHINOCOCCUS* SPP. IN NATURALLY INFECTED PIGS FROM FAMILY FARMS IN LITHUANIA

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Cystic echinococcosis (CE), caused by *Echinococcus granulosus*, is one of the most important cestode infections causing morbidity and mortality in humans and significant economic losses in livestock. It is an emerging disease in many parts of the world, in particular in Eastern Europe and the former Soviet Union. The aims of this study were to determine the prevalence of *Echinococcus* spp. in slaughter pigs from small family farms in Lithuania and to genetically identify the strain of *E. granulosus*.

Livers with putative *Echinococcus* spp. cysts were collected from 2 slaughterhouses during meat inspection. The livers were weighted, the surfaces were examined and all visible cysts or necrotic lesions were counted. After that, all lesions were excised, measured and weighted. Cysts were opened, protoscolices of each cyst were counted and frozen at -20°C for PCR. If the cyst was sterile, the germinal layer was removed and frozen. Necrotic lesions were opened and the inner wall was collected. PCR with primers specific for *E. granulosus* was performed, and parasite strains were identified by sequencing of cloned amplicons from a second PCR with primers targeting mitochondrial sequences. In addition, samples negative for *E. granulosus* were tested by a PCR specific for *E. multilocularis*.

So far 304 samples were collected from infected pigs in two slaughterhouses. This corresponds to 13.3 % of totally slaughtered pigs in the period of January-February, 2005. Ten of 21 samples were positive for *E. granulosus*. Sequence analysis of 2 samples revealed the *E. granulosus* pig strain (G7). Three cysts were identified as *E. multilocularis* by PCR, with two of them being necrotic ones.

This investigation for the first time revealed the presence of *E. granulosus* 'pig strain' as well as *E. multilocularis* in pigs in Lithuania. The high prevalence of *Echinococcus* spp. in pigs indicates that there is a high exposure to cestode eggs. The prevalence of *Echinococcus* spp. in dogs is still unknown. A high exposure of pigs to *Echinococcus* spp. eggs is feasible in small farms where unchained dogs are common. Such farms are numerous in Lithuania and conditions for *Echinococcus* live cycles are favorable in these farms. The epidemiology of *Echinococcus* spp. in Lithuania is still unclear and the presence of other strains of *E. granulosus* ('cattle strain', 'sheep strain') cannot be excluded. For this reason, further molecular epidemiological investigations are needed.

SUSCEPTIBILITY OF ATLANTIC SALMON TO *GYRODACTYLUS SALARIS*
INFECTION IS ASSOCIATED WITH UN-REGULATED CYTOKINE
EXPRESSION

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Gyrodactylus salaris populations increase uncontrolled on salmon originating from rivers in Norway, Scotland and the Western part of Denmark (East Atlantic strains) whereas salmon from rivers in the Baltic area generally (with one exception) limit the parasite propagation within a few weeks. However, the basic mechanisms in these differing salmon-parasite interactions have remained unknown. *Gyrodactylus salaris* is able to attach to and propagate on Baltic salmon for a few weeks in a comparable way as seen in susceptible salmon. Thus, there seems to be no repelling substance in the skin of naive resistant salmon. Recent work has shown that both susceptible and resistant salmon reacts to infections by expulsion of mucus from their skin mucous cells but when the inflammatory reactions in these fishes are inhibited by immune suppressants, such as Dexamethasone, it does not affect Baltic salmon infections or mucous cell densities. In contrast, the East Atlantic salmon (Scottish) become even more infected and their mucous cell density increases following immuno-suppression, which indicates an inhibition of mucus-secretion by this anti-inflammatory drug. Molecular techniques for gene expression studies have confirmed that the susceptibility of the East Atlantic salmon is not caused by the lack of reactivity in the host skin. In contrast, this vulnerable fish strain seems to mount an excessive expression of IL-1 β (interleukin 1 beta), which will lead to secretion of mucus in association with an up-regulated replenishment of mucous cells. The Baltic salmon on the other hand shows no increase of the IL-1 β expression in the host skin and the fish exhibits only moderate reactions confined to depletion of mucous cells. This cell type is an important constituent of the fish epidermis and creates a microenvironment, which attracts and stimulates the parasite in naive fish. Susceptible fish are suggested to invest energy in re-establishing this microhabitat by hyper-reaction whereas the resistant fish leaves an inferior habitat due to low or no reactivity. However, it is still possible that the Baltic salmon strains also initiate production of additional repelling molecules following the first two weeks of *G. salaris* infection.

PATTERNS OF HOST-PARASITE INTERACTIONS BETWEEN THE NEMATODE *HELIGMOSOMUM MIXTUM* (SCHULZ, 1952) AND THE BANK VOLE *CLETHRIONOMYS GLAREOLUS* SCHREBER, 1780

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The aim of the investigation was to study the host-parasite interaction aspects of the nematode *Heligmosomum mixtum* and the bank vole *Clethrionomys glareolus*. The dependence of infestation on the host sex, age and weight, the seasonal and annual abundance dynamics were investigated. The material for the investigation was collected in Southern Karelia (62°04'N, 33°55'E) during 1993–2004. The host sampling consisted of 1098 specimens of the bank vole.

It has been found that the bank vole is the main host of *H. mixtum* (prevalence – 27.3%; abundance – 0.85). The frequency distribution of *H. mixtum* in the host population is described by a negative binomial distribution model (χ^2 : 21.5; df: 15; $P \geq 0.15$). The greatest infestation is characteristic for mature bank voles; males are infected more heavily than females; infestation with the nematode increases alongside with the host weight. Seasonal dynamics of the nematode abundance in a bank vole population appears as a curve with a maximum in January (75.9%; 3.0) and a minimum in August (14.9%; 0.30), however the infestation curve for 1-month-old animals captured from June through October has two peaks (in July and October), with a minimum in June.

The annual changes of *H. mixtum* abundance were not significant. The prevalence of *H. mixtum* varied within the limits of 22–38%, and the abundance account for 0.6–1.4. Under essential change of the bank vole abundance (0.3 – 5.8 per 100 trap-nights) the *H. mixtum* infection parameters were relatively stable. Therefore the number of parasites in the ecosystem over a long-term period changed synchronously with the host abundance. The synchronism in the host-parasite system dynamics is possible when the parasite has a short life span, and does not influence the host abundance. The fact that annual observed distribution of *H. mixtum* agreed with negative binomial distribution confirms stable character of the parasite-host interactions.

SWIMMERS'S ITCH IN THE RESORT ZONE OF THE NAROCH LAKE IN BELARUS

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The problem of schistosome cercariasis or swimmer's itch in the resort zone of the Narocho Lake arose in 1994, when 300 cases of the disease accompanied with

hypersensitivity reactions were recorded. In 1995, an attempt to establish the reasons of distribution of this disease among humans was undertaken by epidemiologists under the leadership of professor S. A. Be'er and scientists of the Institute of Zoology NAS of Belarus for the first time. During the last ten years, the epidemiological situation became much worse. The prevalence of the infection in humans increased almost twice. This is because of (i) the high density of waterfowl birds (common hosts of schistosomes) in coastal zone of the lake, (ii) the formation of nonmigratory populations of these birds, and (iii) the high density of pulmonary mollusks which are intermediate hosts of the trematodes *Bilharziella polonica* (Kowalewsky, 1895) and *Trichobilharzia ocellata* (La Valette, 1854).

In summer and autumn 2004, the investigations on fauna and density of the waterfowls in the northwest part of the Naroch Lake were carried out. In August and November 2004, 13 species of birds were recorded: waterfowls (9) and gulls (4). Coots *Fulica atra* are dominant in their number (more than 3,000 individuals). *Cygnis olor* (140 individuals), *Anas platyrhynchos* (150-300), *Podiceps cristatus* (260) are mass species also. During seasonal migrations, *Bucephala clangula* (240 individuals), *Larus ridibundus* (280), and *Larus canus* (670) were recorded to be common.

After dissection of 121 individuals of the waterfowls (*A. platyrhynchos*, 93; *F. atra*, 23; *C. olor*, 5), it was found that the prevalence of infection of the mallard *A. platyrhynchos* with trematodes is 64.2 %. Both males and females of *B. polonica* and *T. ocellata* were seen in the investigated birds. The prevalence of the schistosome infection in the mallards is a subject of seasonal dynamics: it is minimal in spring (22.2 %) and maximal in autumn (48.0 %). Schistosomes were not seen in swans and coots. During autumnal migration, part of the duck population of the Naroch Lake can bring the infection to other water bodies, which are located on the birds' migratory way toward their wintering grounds. Part of the mallard population, which is wintering on clearing water bodies constructions, is responsible in preservation of the infection.

Thus, the mallard, a mass species of the waterfowls, is the main avian host of the schistosomes in the resort zone of the Naroch Lake. The elucidation of a role of other species of the waterfowls in maintenance of the infection nidus is a subject of our further research.

TWO CASES OF CANINE *ECHINOCOCCUS MULTILOCULARIS* METACESTODOSIS IN FRANCE AND CONSEQUENCES

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Two cases of canine *Echinococcus multilocularis* metacestodosis were recently described in France for the first time. This occurrence draws attention to a possible extension of this zoonosis.

The first case was a 6 year old female Coton de Tuléar dog (dog no.1) which presented abdominal distention but was otherwise in apparently good general condition. Examination showed a voluminous intraabdominal mass resistant at palpation with hypothesis of tumoral disease. Endoscopy revealed a mass with a vesicular aspect which filled all abdominal cavity. Histology of biopsy samples showed typical elements of *E. multilocularis* larvae. Despite a 9 month treatment of albendazole associated with praziquantel, worsening of the condition required euthanasia. The second case concerned a 3 year old female Beauceron (dog no.2) which suffered from anorexia, emaciation, abdominal distention, vertebral pain since 3 months and hind limbs paralysis. The seriousness of symptoms justified euthanasia.

Post-mortem diagnosis was supported by histological examination and microscopic observation of the parasite from lesions in both dogs. Lesions were extensive to the liver and other various tissues and organs (epiploon, spleen, kidneys, diaphragm and lungs in dog no.1; epiploon, kidneys, muscles of the lumbar area and L4 lumbar vertebra in dog no.2). Diagnosis was also confirmed in dog no.1 by serology (ELISA positive with EmII/3-10 antigen), Sandwich-ELISA EmG11 and PCR positive from liver, and a successful experimental inoculation to merion.

One case (no.2) was originating from an enzootic eastern part of France (Massif des Vosges), but the other (no.1) had never lived outside the Champagne region which is not known at risk until now. Way of contamination is unknown but both dogs were frequently in contact with faeces (to roll in and coprophagia) and foxes were present in their living areas.

These two cases has various consequences, first for veterinary medicine as practionners must take into account *E. multilocularis* larval infestation in the differential diagnosis of tumoral diseases, other canine metacestodosis (cysticercosis

due to *Taenia crassiceps*) and various paralytic syndroms. Secondly, the potential risk of zoonotic contamination of such dogs is of importance for veterinary staff, laboratory workers and owners as adult *Echinococcus* sp. was found by some authors in other similar described cases. The description of *E. multilocularis* metacestodosis in aberrant hosts such as dog or wild boar is a signal which would justify more systematic surveys in carnivores population even outside the enzootic zones classically recognized in France. This situation may be compared with those seen in neighbouring countries such as Belgium, Germany and Switzerland with the marked increase and the urbanization in fox populations.

PARASITES AND PARASITIC DISEASES OF BREAM *ABRAMIS BRAMA* FROM THE CURONIAN LAGOON IN 2004

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Fauna of parasites of fish from the Curonian Lagoon (South Baltic) was well studied during 20th century, but complex investigations on fish's health were absent. The objective of our study was to investigate diseases and parasites of bream *Abramis brama* L. from the Curonian Lagoon.

In all, 71 specimens of the bream (body length 17.0- 60.0 cm) from different parts of the Curonian Lagoon (Russian Zone) were investigated by standard morphopathological, parasitological and hematological methods in summer 2004.

Macroscopic morphopathological analysis detected reddening of bream skin surfaces (49.3%), elevated scales (36.6%) and hemorrhages (77.5%). Pigmented (20.0%) and unpigmented epitheliomas (8.4%), myomas (1.4%) and ulcers (4.2%) were revealed on skin surfaces and in muscles of mature (upwards 6- years) fish only. Skeletal deformations (5.6%) and inflammation symptoms (1.1%) were found in immature and mature (up to 6- years) bream. Evident macroscopic changes of skin, gills, kidneys, liver, heart and intestine were found.

Thirty-seven parasite species belonging to the following systematic groups were found: Myxosporea (10 species), Coccidia (2), Microsporidia (1), Monogenea (3), Cestoda (3), Trematoda (6), Nematoda (7), Copepoda (3), Hirudinea (1), Mollusca (1). The great majority of the parasites are common species of the bream in this region. Some nematodes (5 species) and cestodes (1) were recorded for the first time. The protozoan parasites (myxosporeans and coccidians) were found in altered kidneys tissue (17.9%). *Posthodiplostomum cuticola* mtc. were detected in skin, pharynx and gill black spot (40.5%). Heart hypertrophy probably was related to the presence of *Ichthyocotylurus platicephalus* mtc. The *Paradilepis scolecina* pl. (76.7%) and nematode *Raphidascaris acus* l. (10.0%) were found in injured liver tissue. *Goezia ascaroides* l. were detected in intestinal hemorrhagic areas (33.3%). The parasitic

copepods *Tracheliastes maculatus* were detected in skin hemorrhagic areas (20.0%). Glochidia were common on skin surface with elevated scales symptom (27.2%). Relations between pathological changes of internal organs (spleen, kidney and liver) of the bream and intensity of hemopoetical processes were found. Presence of gill monogeneans caused the anemia of fish. Increase in the quantity of pseudoaeosinophilic leucocytes, pseudobasophilic leucocytes and monocytes were related with infestation of nematode larvae. Quantity of vacuolated leucocytes in bream blood was highest in fish with pigmented epithelioma on skin surfaces.

Detected parasitic organisms of the bream probably can cause damages of infested tissue and be able to influence some blood characteristics.

EFFECTIVE PROTECTION OF HORSES AGAINST BITING AND NUISANCE INSECTS

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An insecticide-treated net was used for the protection of horses kept on pasturage in Brandenburg, Germany during June – September 2004. The height of the net was 100 cm from ground level. It consisted of polyester with 150-denier strength, which had been pre-treated with the pyrethroid deltamethrin. An incorporated UV protection ensured a sufficient insecticidal activity of the pyrethroid throughout the trial period as was regularly confirmed during bio-assays. Three areas with comparable fly pressures were selected for the trial. Fly densities were assessed with twelve *Nzi*-traps (four per area) and measured at weekly intervals for a period of 30 h. Digital photographs were also taken at weekly intervals from horses with comparable size and colour. The pictures were taken during 30 min from five different anatomical regions: eye, cervical, dorsal and sternal regions, as well as lateral chest and abdominal wall. Complete protection of a paddock resulted in an immediate and significant reduction of tabanids and nuisance insects when compared to the horses, which were kept on the unprotected pasturage. Incomplete protection, i.e., 126 m out of another pasturage with a perimeter of 941 m, still helped to significantly reduce fly numbers in comparison to the horses kept on the unprotected pasturage. In conclusion, the protection of pasturage with insecticide-treated netting material offers a simple and affordable solution for what is rightly perceived as a considerable constraint to horse keeping in much of north-western Europe.

VALIDATION OF MOLECULAR-DIAGNOSTIC TECHNIQUES FOR THE ACCREDITED LABORATORY

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Routine diagnostic laboratories today usually operate according to standard quality management procedures such as ISO 17025. This requires that only validated methods are used. Validation procedures help to document that a particular protocol used by the accredited laboratory has a guaranteed performance in this particular laboratory. This implies that each method must be validated in each accredited laboratory.

Several study designs exist for validation procedures. Computer programmes are available to help with the statistical analysis of validation results (e.g. WinEpiscope; free download from <http://www.clive.ed.ac.uk/winepiscope/>)

The agreement beyond chance of results obtained in the protocol that is to be validated can be compared those achieved in an already established test (test agreement, kappa statistics). When the performance of molecular-diagnostic techniques is described, researchers often rely exclusively on the analytical sensitivity and specificity of a technique, i. e. the minimum DNA copy number detectable by a polymerase chain-reaction (PCR) is determined or cross-reactions with a specified number of other organisms are excluded. However, for a method that is used under routine conditions or for epidemiological studies, it is usually necessary to assess the diagnostic sensitivity and diagnostic specificity of the technique. These parameters can be estimated by comparing the method that needs to be validated with an existing reliable method ('gold standard') by testing a standard set of well-documented samples in parallel using both techniques. Approaches using Bayes' theorem are used to perform gold standard-free validations.

Many PCR-based methods are characterised by an excellent analytical sensitivity and are thus good candidates for diagnostic tools with the required diagnostic sensitivity. However, the high level of analytical sensitivity can also make molecular techniques susceptible to cross-contamination problems leading to false-positive results. Moreover, the presence of inhibitors can cause to false-negative results. Techniques will be illustrated which allow the detection of contamination problems during the processing of samples and recognising the potential presence of inhibitors.

After an initial validation, test performance should be continuously monitored and results compared to those obtained by other laboratories, e.g. by participating in ring trials.

SPATIAL AND TEMPORAL VARIATIONS IN THE PARASITE FAUNA OF
JUVENILE HERRING CAUGHT WEST OF THE BRITISH ISLES AND IN
NORTH NORWAY

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Juvenile herring *Clupea harengus* L. were examined for parasite infections as part of a multidisciplinary stock identification project (WESTHER) funded by the European Union. Herring were caught by commercial and research vessels from six locations: east and west coasts of the Irish Sea, Stanton Bank, sea lochs on Scotland's west coast, the Minch and an outlier from Northern Norway. Their internal viscera and mesenteries were removed and preserved in ethanol for dissection and observation of parasitic infection.

Thirteen parasite species were identified: seven Digenea, two Nematoda, two Myxosporaea, one Protozoa and one Cestoda. Preliminary findings show significant variations between six of the parasites over the whole study area (Kruskal-Wallis Test, $P < 0.001$). The six juvenile herring samples were divided into five groups using the similarity ratio as a measure of association. Only the west coast of the Irish Sea and The Minch samples were associated.

The Irish Sea contains a mixture of herring potentially from several spawning stocks. Juvenile herring from the east and west coast of the Irish Sea exhibited a significant difference in the levels of infection of two species of digenean metacercariae (*Cercaria pythionike* and *C. doricha*) and an adult digenean (*Hemiurus luehei*) (Mann-Whitney U test; $P = 0.003$, 0.006 and < 0.001 , respectively). *Cercaria pythionike* and *C. doricha* only infect herring during their first twelve months of life although the metacercariae remain viable on the pyloric caeca for the rest of the life of the fish. Differences between the two sites were related to the physical and biological environment of the nursery grounds.

Cercaria pythionike and *C. doricha* prevalences in juvenile herring from inshore nursery grounds situated on the west coast of Scotland showed little temporal fluctuation. A comparison of data from an earlier study in the 1970's and from three consecutive years between 2002 and 2004 suggests a relatively stable host-parasite relationship.

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EIMERIA ZUERNII COCCIDIOSIS IN A LARGE DAIRY HERD

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A large dairy farm had been experiencing problems with catarrhal to haemorrhagic diarrhoea in their 3-4-month-old calves, in some cases with a fatal outcome, for about one year. The farm profile is as follows: 1. Dairy unit with about 1,800 dairy cows, nursery (newborn calves up to 10 days of age housed in single pens) and starter section (calves up to 12 weeks of age penned in groups of 25 on deep litter); 2. Calf rearing unit (grower section: calves to 24 weeks of age, loose-housed in groups of 12 – 25); 3. Heifer unit. From April to June 2002, 20% of the calves were clinically examined in groups to ascertain their parasitological status. *E. ellipsoidalis* was the only *Eimeria* species encountered in the nursery. Scouring began 3 to 4 weeks after the calves had been moved from the starter to the grower section and was associated with increasing coccidial excretion. The infection spread rapidly throughout the herd, affecting up to 100 % of calves within this time. *Eimeria zuernii* was diagnosed as the primary cause of the clinical coccidiosis cases. Five further species were identified in the grower section: *E. ellipsoidalis*, *E. bovis*, *E. auburnensis*, *E. subspherica* and *E. cylindrica*. Following the appearance of clinical cases, the entire group of calves was treated with Vetoprim® 900-N via the feed; severely ill calves were given the drug by injection. In July 2002 the calf rearing unit was evacuated, thoroughly cleaned and disinfected with Lomasept®. However, continued tests following restocking in September 2002 revealed no change in the herd's health status. The recurrence of diarrhoea and mortalities with excretion levels for *E. zuernii* of up to 700,000 opg demonstrated that the measures taken (litter hygiene, thorough disinfection, treatment of the group post infection) failed to control the problem of *E. zuernii* coccidiosis in the herd.

MOLECULAR DIAGNOSIS OF TAENIID INFECTIONS

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A number of molecular techniques (coproantigen detection by ELISA, copro-DNA by PCR) are available or are being developed for the diagnosis of taeniid infections of carnivores. Depending on the question addressed (e. g. diagnosis in individuals, mass screening in epidemiological investigations, surveillance of control programmes, molecular epidemiological studies), equipment available and expertise of the investigators, different methods and diagnostic strategies may be applied. PCR, which

is a laborious technique, allows to differentiate eggs of *Echinococcus multilocularis* and *E. granulosus* from eggs of *Taenia* spp. Furthermore, PCR-based approaches enable to identify the various strains of *E. granulosus* which has important implications in the understanding of the epidemiology. New approaches allow to identify of taeniid eggs in environmental samples and facilitate studies on zoonotic transmission. Finally, in cases where metacestode morphology is not typical in intermediate and aberrant hosts, the parasites are more accurately identified by PCR.

DYNAMICS OF THE ANTIGEN HIPODERMIN C IN SERA FROM CATTLE IN DIFFERENT GRAZING SEASONS

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The evolution of the antigen hypodermin C (HyC), secreted by first instars of *Hypoderma* sp. during their intraorganic migration, has been studied in 49 Frisian cows maintained in a farm with a high prevalence of hypodermosis. Animals were divided into three groups taking into account their grazing year at the beginning of the study: Group A (G-A) composed by 29 animals in their third grazing season (with two previous infections); G-B integrated by 16 cows in their second grazing year (reinfected for the first time) and G-C, 10 heifers in their first grazing season (primoinfected). Cattle were bled monthly during a complete life-cycle of the parasite in this area. The hypodermin C was detected in sera by means of a sandwich ELISA test, using a monoclonal antibody as primary antibody.

The dynamics of the HyC was similar in all the animals, but in reinfected groups (G-A and G-B) antigen levels were more constant than in the primoinfected one (G-C). In all groups antigen increased significantly at the beginning of the study (My-JI) coinciding with the migration of first instars towards the oesophagus (*H. lineatum*) and/or epidural fat (*H. bovis*). A second peak was detected two-three months before the apparition of first grubs on the back (October). Levels of antigen fell quickly following arrival of larvae at the back and completion of the moult to second instar. At the end of the study, in all groups HyC levels increased significantly because probably a new infection had occurred. No differences were found in the intensity of parasitation (grubs on the back) in the three groups. No correlation was found between the number of grubs and the HyC levels throughout the study in groups A and C. However in G-B there were a negative correlation between the number of grubs and the average and maximum levels of HyC registered throughout the study that could be related with the mortality of larvae during their migration.

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VACCINE ADJUVANT DEVELOPMENT FOR CUTANEOUS LEISHMANIOSIS

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Leishmaniosis is a public health problem in many parts of the world (endemic in 88 countries) with approximately 90% of the cutaneous form of the disease occurring in the Mediterranean region, South America, Africa and Asia. Leishmaniosis is also emerging as an indicator infection of acquired immunodeficiency. Vaccine development remains a high priority in attempts to reduce the incidence.

Complex glycans derived from lipid, nucleic acid and protein free extracts of *Taenia crassiceps* metacestode larvae were found to have adjuvant effect against *Leishmania mexicana* antigens in BALB/c mice experimentally infected with *L. mexicana*. A single intraperitoneal or subcutaneous injection of *Taenia* glycans altered the Th-1/Th-2 balance in experimentally infected mice as determined by Western blot analysis of IgG 1 and IgG 2a antibodies to *L. mexicana* antigens. *Leishmania* antigens, which were immunogenic in *Taenia* glycan vaccinated mice, were different from those of non-vaccinated mice. Vaccination induced *Leishmania* antigen specific IFN- γ expression in *in vitro* culture of spleen cells from *Taenia* glycan vaccinated-*Leishmania* infected mice and not from mock-vaccinated-*Leishmania* infected BALB/c mice. The responsive cell in vaccinated animals appears to be the macrophage. By molecular sieving on Sephacryl 4B, it was possible to fractionate the IFN- γ inducing glycans and this activity could be enhanced by glycanase digestion with α -fucosidase and mannosidase. *Leishmania* lesions in vaccinated mice were smaller in size and resolved earlier than in non vaccinated mice.

We conclude that *T. crassiceps* glycans have immunoadjuvant effects against *Leishmania mexicana* and may be developed as adjuvants in anti-*Leishmania* vaccines.

THE INFLUENCE OF STUDY SITE, AGE AND FEEDING STYLE ON *ISOSPORA* SPP. (PROTOZOA: COCCIDIIDA) INFECTION IN PASSERINE BIRDSO. V. DOLNIK^{1,2} & F. BAIRLEIN¹¹*Institute of Avian Research "Vogelwarte Helgoland", Germany*²*Zoological Institute, Russian Academy of Science, Russia*

Coccidians of the genus *Isoospora* are widespread intestinal parasites of wild passerine birds. The aim of our study was to check if (i) the feeding style, (ii) study site and (iii) the age of the birds has any influence on prevalence or intensity of *Isoospora* spp. infection.

Birds were trapped in the afternoon and their faeces were checked for *Isospora* spp. oocysts after flotation centrifugation. For experiments in captivity, a group of Blackcaps *Sylvia atricapilla*, naturally infected with *Isospora sylvianthina*, was kept under controlled laboratory conditions and their faeces were also checked for *Isospora* oocysts. Some of these birds were re-infected artificially with *I. sylvianthina*, and their body mass as well as oocyst output were controlled daily.

In the wild, the prevalence and average intensity of infection varied in different bird species. From 56 screened bird species, ground feeders were more frequently and intensively infected than species feeding in the air.

Comparison of intensity and prevalence of infection in five bird species on the Curonian Spit and on the island of Helgoland during autumn migration showed no difference in prevalence of infection but the intensity of infection was lower on the island than on mainland.

In the wild, young birds appeared to be more intensively infected than adult birds. Under controlled laboratory conditions the intensity of chronic infection of Blackcaps decreased continuously during the first year of life. Re-infection of chronically infected birds caused an increase in oocyst output for some days. It took much longer to return to the low chronic infection level in most young birds than in at least one year old birds. Subsequent re-infections weakened the birds so that the second re-infection led to pronounced body mass loss in some individuals. The decrease in body mass occurred some days after the maximum of oocyst output.

Our results show that prevalence and intensity of *Isospora* infection in wild birds can depend on the study site, feeding style and the age of the host. Under natural circumstances most birds are likely to be able to tolerate *Isospora* infection. However, in very young birds, by repeated re-infection, or in particular environmental constraints the infected birds may face profound effects on fitness and survival.

ISOSPORA (PROTOZOA: COCCIDIIDA) INFECTION AND HIERARCHY RELATIONS IN HOUSE SPARROWS *PASSER DOMESTICUS*

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The colonial house sparrow *Passer domesticus* is known to suffer a high infestation with different *Isospora* species. In the wild up to 100% of individuals of a population can be infected. Many aspects of the life cycle of *Isospora* spp. were in fact studied on house sparrows, but the influence of these parasites on bird behaviour has been insufficiently investigated. The aim of our study was to find out, whether the infection

with *Isospora* spp. has any effect on the hierarchy of male sparrows and how the infection influences the behaviour and fitness of these birds.

Adult male house sparrows, naturally infected with *Isospora* spp., were trapped in the wild, colour-ringed and kept in four rooms, eight birds in each. The dominance rank in each room was revealed during observations. The faeces of all the birds were checked for *Isospora* spp. oocysts using flotation centrifuging. The birds from one room were kept as a control, in two other rooms the dominant males were artificially re-infected with *Isospora michaelbakeri*, and in the fourth room all males were re-infected. Body mass as well as oocyst output of all the birds were recorded daily. After infestation, aggressive behaviour of birds was observed and the attacks of every individual were documented. We also estimated the dimension of the black breast patch, some blood parameters such as haematocrit, erythrocyte sedimentation rate and cell-mediated immune response (phyto-haemagglutination test).

The results of our study showed that oocyst output increases after infection, followed by a decrease in body mass. The dominance relationship in a group changes when infecting the dominant birds. Only the "fittest" dominants, however, did not lose their dominant position. After infection male aggressiveness is significantly related to the size of their black breast patch and to the sedimentation rate.

We conclude that the infection of house sparrows with *I. michaelbakeri* can change the hierarchy in a flock, and the relationship between aggressiveness and fitness parameters of the bird can be observed especially clear during the acute phase of infection. Almost all house sparrows in the wild are infected with *Isospora* spp. and under "normal" condition the infection hardly reveals an effect on individual fitness. However, in case of re-infection in particular environmental constraints even dominant birds may face profound effects on fitness and survival.

DISTRIBUTION OF *ECHINOCOCCUS MULTILOCULARIS* IN POLAND-SLOVAK BORDER AREAS

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The aim of this study was to assess the current situation in the occurrence of *Echinococcus multilocularis*, a causative agent of alveolar echinococcosis, in Poland-Slovak frontier zone of the Carpathian region and compare it with the situation in adjacent regions in both countries.

The areas surveyed were divided into two zones. Zone I included the contiguous frontier districts of Poland and Slovakia, whose territories are up to 50 kilometres away from common border. The infection prevalence rates were compared in bordering districts of this zone in West-East direction. Zone II included Polish and Slovak adjacent districts situated from 50 to 140 kilometres from the common state border. *Echinococcus multilocularis* presence in red foxes was detected by necropsy of small intestine and coproantigen detection in faeces. The infection prevalence rate and its range were determined using the General linear model (GLM). Non-parametrical Chi-square test was used to determine the statistical significance of the differences observed.

In all Polish districts of Zone I, except district Jasło, the prevalence was higher (with average rate 45.7 ± 18.6 %) than in bordering Slovak districts, where average prevalence rate reached 25.0 ± 10.7 %. In both countries, *E. multilocularis* prevalence in Zone II in comparison to Zone I was considerably lower (Poland 20.8 ± 9.0 %, Slovakia 18.9 ± 9.2 %). Mean worm burden on the Slovak territory was 878 tapeworms; it was considerably higher in the frontier Zone I (1026 specimens) than in the adjacent Zone II (659 specimens). Mean worm burden in the surveyed Polish regions was 332 in Zone I and 300 in Zone II. Massive worm burden (more than 1000 specimens) was recorded in 20.2 % of Slovak foxes and 12.1% foxes in Poland.

Echinococcus multilocularis in Carpathian region of Slovakia was for the first time recorded in 1999. Localisation of endemic areas in the Northwest and Northeast of the country may point out the parasite spread into this territory from Poland. The outcomes of four-year-project show that the values of the infection prevalence and the infection rate in both countries are significantly increasing towards the common state border and are higher in the districts of the Carpathian region than in adjacent areas. The differences are due to the geomorphological and ecological factors, which seem to be crucial for the survival of the tapeworm eggs and subsequent, spread of infection.

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METAZOAN PARASITE FAUNA OF FISH FROM THE LAKE KORTOWSKIE, POLAND

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Some fish parasites from Kortowskie Lake, which is located in north-eastern Poland, in Olsztyn, were studied. This is the eutrophic lake. Since 1959 it has been purified, waters from hypolimnion have been removed by special pipeline to the river Kortówka. This experiment is carrying out until now. From full year's seasonal

research sanitary-epidemic station results, that waters are classified to II class purity, and in spring season even to I class (Endler, personal inf.). The aim of the present study was to recognize parasite fauna of fish species from this lake and to determine prevalence and intensity of infection.

Parsitological research of fish parasites has been carried out since April to June during the years 2001-2004. A total of 381 specimens, representing 10 fish species were studied. They were: perch (*Perca fluviatilis* L.), bleak (*Alburnus alburnus* L.), white bream (*Abramis bjoerchna* L.), ruff (*Gymnocephalus cernuus* L.), pike (*Esox lucius* L.), gudgeon (*Gobio gobio* L.), rudd (*Scardinius erythrophthalmus* L.), tench (*Tinca tinca* L.), bream (*Abramis brama* L.) and roach (*Rutilus rutilus* L.). The examination included: the skin, fins, gills, eyes, stomach, intestine, liver, spleen, kidney, brain and swimbladder. Preparations were made according to methods using in parasitology.

A total of 50 parasite species (30 ectoparasite and 20 endoparasite species) belonging to different systematic groups were found: Monogenea (26 species), Digenea (6), Cestoda (7), Nematoda (4), Acanthocephala (3) Crustacea (3), Glochidium larvae (1). The most parasite species were found in roach (16), in perch, bleak, white bream (13), in bream (11), ruff, pike (10), but in gudgeon, tench, rudd (3 and 2 respectively). The most abundant were metacercarie of *Diplostomum* sp., *Tylodelphys clavata*, tapeworms *Triaenophorus nodulosus*, *Proteocephalus percae*, *Caryophyllaeus laticeps*, nematode *Rhapidascaris acus*, monogenea *Tetraonchus monenteron*, copepods *Ergasilus sieboldi* and *Glochidium* larvae. The highest prevalence of infection with *Diplostomum* sp. were noted in ruff (76,54%, mean intensity 8.68), rudd (60%, 2.66), white bream (52,63%, 19.92), with *Tylodelphys clavata* in ruff (70,90%, 13.35), with *Caryophyllaeus laticeps* in bream (58,33%, 12), with *Proteocephalus percae* in ruff (69,09%, 3.97) and with *Triaenophorus nodulosus* (83,33%, 27.6), *Rhapidascaris acus* (50%, 1.33), *Tetraonchus monenteron* (83,33%, 24.8), *Ergasilus sieboldi* (83,33%, 43.8) and *Glochidium* larvae (83,33%, 14.2) in pike. The remaining parasites were found less frequently and rarely.

THE NIDICOLOUS INHABITANTS OF BIRDS IN DIFFERENT BIOTOPS OF NATURAL AND URBAN LANDSCAPE

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Birds have the certain complex of parasitic species of the arthropods - ectoparasites of birds and inhabitants of their nests. Many species of them are transmitting the agents of diseases of the man and animals.

The analysis of microbiocenose structure of nidicolous inhabitants in natural (Berezinsky biosphere reservation, 70 nests of birds) and transformed (Minsk area,

130 nests) territories has shown that among the nidicolous inhabitants there are more than 112 species of the arthropods [the relative abundance (RA) was 503.6 invertebrates per nest]. 22 arthropod species are considered to be the nest-burrow parasites (66.5 % from the total number of the nidicolous inhabitants, RA = 335.1). The hematophages are represented by gamasina mites (40.7 % from all nidicolous inhabitants), ixodinae ticks (0.5 %), fleas (22.2 %) and bugs (3.1 %).

The average density of arthropod micropopulations of the nests, collected from various type of biotopes in natural biocenoses, considerably differs from that of nests, collected in urban landscape. The number of arthropod micropopulations of the bird nests in wood biotopes on the transformed territory was higher, than in natural, and for spruce forests these differences were significant. The quantification of micropopulation number of the parasitic species of gamasina mites and fleas in bird nests of wood biotopes has shown, that the greatest density of the hematophage micropopulations is recorded in pine forests of natural territory (RA = 296.6). For bird nests, collected in various wood biotopes of natural biocenoses (pine forests, RA = 383.1; alder forests, RA = 166.2, and etc.), the dominance of parasitic arthropod species is characteristic, while in spruce forest (RA = 35.3) 56.7 % of invertebrates was represented by oribatoid mites. The quota of gamasina mites was 19.8 %. In similar biotopes in the transformed territory, the majority of the nidicolocenes also were the parasitic species (gamasina mites and fleas), but their share in assemblage of invertebrates was a little bit lower. In urban biotopes, the most numerous micropopulations create the parasitic species of the arthropods in nests, collected in the following biotopes: parks (RA = 262.0), one-storeyed wooden building (RA = 534.1) and multi-storey brick buildings (RA = 5368.9). This is due to high density of bird nests in these biotopes, large species diversity of birds and a prevailing of synanthropic bird species. Numerous micropopulations of hematophages belonging to the families Dermanyssidae, Ceratophyllidae, as well as bugs *Cimex lectularius*, *Oeciacus hirundinis*, and also plenty of facultative hematophages such as *Androlaelaps casalis* and louse flies, which are dangerous from the point of view of some diseases epidemiology, were recorded.

NEOSPORAS CANINUM AND TOXOPLASMA GONDII INFECTION IN DOMESTIC ANIMALS IN LATVIA

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The epizootic situation of toxoplasmosis and neosporosis infection in Latvia and the distribution of neosporosis in some herds with reproduction problems were studied. The work was carried out in the State Veterinary Diagnostic Center from 1993 to 2004. The *Toxoplasma gondii* antibodies were determined in the blood serum of domestic animals obtained from different regions of Latvia. A complement fixation

test (CFT) was used for 1606 blood serum samples, and 630 serum samples were screened for antibodies employing latex agglutination test (LAT). In all, 166 blood serum samples of cows and 2 blood serum samples of dogs as well as bulk milk samples of 11 herds were examined for antibodies to *Neospora caninum* with ELISA test. In 2 herds a relationship was detected among animals seropositive to neosporosis in order to find out if the infection can be transmitted from generation to generation. Part of these samples was examined in the Swedish National Veterinary Institute laboratory.

The toxoplasmosis was diagnosed in 0.8 % of cows, 5.6 % of sheep, 3.7 % of goats, 4.9 % of pigs, 9.7 % of dogs and 6.6% of cats by means of CFT. Employing the LAT, infection was detected in 45.0 % of sheep, 43.5 % of goats, 30.4% of pigs, 46.7 % of dogs and 29.1 % of cats. In 10 examined cows, antibodies to *T. gondii* were not recorded. According to the statistics of the State Health Agency, there were 52 cases of toxoplasmosis in humans in Latvia in 1999 – 2004.

The first serological examinations for neosporosis in Latvia were carried out in 2001. In the period till 2004, antibodies to *N. caninum* were found in 16.2 % of the aborted cows and in one of two tested dogs. Also, positive tests to neosporosis were obtained in 4 (36.4 %) bulk milk samples from herds with reproduction problems.

Neospora caninum antibodies were detected in daughters of seropositive cows and further in the female progeny. This supports a possibility of vertical transmission of neosporosis.

The investigation provide evidence that toxoplasmosis and neosporosis are distributed among domestic animals in Latvia; human health is endangered. These parasites can cause remarkable losses in animal husbandry.

MONITORING AND FISH HEALTH CONTROL IN AQUACULTURE OF KARELIA

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The role of salmon fish in aquaculture grows during the last years in Karelia. Commercial fish culture in cage farm develops especially successfully. Control and observation of fish diseases is fulfilling by veterinarians and ichthyopathologists from Karelia and St. Petersburg. Some investigations are held by a researcher from Finland.

The object of research was the rainbow trout from lake cage farms of middle and south Karelia. In observation of farmed fish parasitological and microbiological methods were used.

Among the infectious diseases, the most widespread were conditional-pathogenic bacterioses. As a result of microbiological research agents of pseudomonosis (*Pseudomonas fluorescens*) and flavobacteriosis (*Flavobacterium psychrophilum* and *F. columnaris*) have been revealed. The agent of bacterial cold water disease (*F. psychrophilum*) was detected in the trout of different age, thus disease proceeded both in sharp, and in the chronic form. The flavobacteriosis developed in most cases as a result of the stress caused by transportation of fish from nursery.

The list of causative organisms of parasitic diseases includes 10 species of which protozoans make up half. The most important parasites were *Ichthyophthirius multifiliis*, *Costia necatrix* and *Capriniana piscium*. Among metazoan parasites *Diplostomum* sp. and *Argulus foliaceus* were most frequently recorded.

The fungal disease caused by *Branchiomyces* sp. has been recorded in two lake cage farm of Karelia in summer of 2003. The first case of occurrence of branchiomycosis in trout in Karelia is connected, most likely, to the general recent warming of a climate and increase of organic in northern water bodies under influence of trout fish farms activity.

Virus diseases in aquaculture in Karelia are not recorded, however, there is a real threat of their occurrence due to purchase trout fry from Finland.

The most important factors promoting occurrence and course of diseases, are conditions of cultivation, temperature of water and transportations between fish farms. The intensification of fishery in Karelia demands the constant control of fish health situation.

SPECIES PARASITIZING YOUNG ATLANTIC COD *GADUS MORHUA* IN ICELANDIC WATERS

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The objective of this study was to investigate when parasites start to infect wild cod *Gadus morhua* L. in the first and second year of life in Icelandic waters and to analyze the prevalence, the intensity and the progress of infections.

During 1998-2000 approximately 1000 juveniles of two year classes, aged approximately 2, 4, 6, 10, 18 months, were sampled, mainly from one big fjord in NW Iceland. They were examined by standard parasitological methods, histology and haematology.

A total of at least 28 parasitic species were found. Ectoparasites, Protozoa: *Trichodina* spp.; Monogenea: *Gyrodactylus* spp.; Crustacea: *Caligus* sp., *Clavella adunca*, *Lernaeocera branchialis* and *Holobomolochus confusus*. Endoparasites,

Protozoa: *Ichthyophonus hoferi*, *Goussia* sp. and pseudobranch tumors reported to be caused by a protozoan; Microsporidia: *Loma* sp. and *Pleistophora* sp.; Myxosporea: *Myxidium gadi*, *Zschokkella* sp.; Digenea: *Brachyphallus crenatus*, *Derogenes varicus*, *Lepidapedon elongatum*, *Podocotyle atomon*, *Proisorhynchoides gracilescens*, *Stephanostomum* sp.; Cestoda: Pseudophyllidea larvae; Nematoda: *Anisakis simplex*, *Cucullanus cirratus*, *Hysterothylacium aduncum*, *Pseudoterranova decipiens*; Acanthocephala: *Corynosoma* sp., *Echinorhynchus gadi*. No blood parasites were found.

One parasitic species (metacercariae) was detected in a 2 months old juvenile. In 4 months old cod digeneans, anisakid nematodes and *Caligus* larvae were found. At the age of 6 months many additional parasites were found, including monogeneans, various helminths, protozoans, Myxosporea, *I. hoferi*, a protozoan causing pseudobranch tumor and *Trichodina* ciliates. In 10 months old juveniles *Loma* sp. was first detected, and at the age of 18 months *Pleistophora* sp. and *L. branchialis* were first found. The cumulative number of species reached 9 in 4 months old cod, 21 in 6 months, 22 in 10 months and 28 in 18 months old fish. Prevalence varied, but in general both prevalence and intensity of metazoan parasites increased with age. For 8 species the highest prevalence ranged from 55% to 99%, prevalence for other species was lower. Highest mean intensities for metazoans among these 8 species ranged from 2 to 67.

One *Gyrodactylus* species and *Stephanostomum* sp. metacercariae found during this study have apparently not been reported previously from Atlantic cod.

Infections caused by *L. branchialis* and the protozoan causing pseudobranch tumors had severe effects on the organs affected. As the prevalence of these infections reached 17% and 8% respectively, one can assume that these infections could lead to mortality among affected fish and have a significant influence on the stock size of these year classes in the survey area.

POPULATION DIVERGENCE AND SPECIATION IN DIGENETIC
TREMATODES WITH TWO-HOST LIFE CYCLES – THE “PYGMAEUS”
MICROPHALLIDS

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The “*pygmaeus*” microphallids are common and widespread parasites of migrating marine and coastal birds of Eurasia and North America. They all have a similar two-host life cycle involving one intermediate host (a littoral or sublittoral gastropod). The metacercariae that are invasive for birds develop inside daughter sporocysts. The group is comprised of six distinct species that differ from each other morphologically and genetically (according the UP-PCR analysis and preliminary phylogenetic study of 3'end region of ITS1 ribosomal spacer).

In this study we applied Universal Primed PCR (UP-PCR) to investigate infraspecific variability among isolates of 4 species of metacercariae collected from molluscs on the coasts of Iceland, Norway, Arctic and Far East Russia.

It seems that the rate of genetic variability correlated with host specificity of given species. Least variability was reordered for *Microphallus pygmaeus* and *M. triangulatus*, which use only common eider and some other sea ducks as final hosts and *Littorina* spp. as intermediate hosts. *Microphallus piriformes* and especially *M. pseudopygmaeus* were more genetically heterogeneous. Even though *M. piriformes* intramolluscan stages develop only in *Littorina* spp. the list of the final hosts for this species is much broader and includes ducks, gulls and waders. The final hosts for *M. pseudopygmaeus* are the same as those of *M. pygmaeus* and *M. triangulatus*. However, *M. pseudopygmaeus* is unique for trematodes in that it develops in numerous species of gastropods belonging to different genera, families and orders. Comparison of UP-PCR banding profiles among and between isolates of each species using GelCompare software gave distance and cluster estimates which showed that geographical region did not significantly influence infraspecific genetic variability. This can probably be explained by extensive gene flow between parasite populations in different geographical regions provided migrating birds. Most probably the groups of *M. piriformes* isolates resolved reflect specialization to final host species or group of species. Significantly *M. pseudopygmaeus* isolates from the same molluscan host species (genus), but from different geographical regions, were shown to form clusters in phylograms. It is conceivable that strengthening of differences between such groups

could result in the appearance of new species. If that is the case, we are faced with a version of host-parasite co-evolution in trematodes passing in our sight. It would appear then that final host selection, gastropod host selection and possibly geographical isolation have all played a role in the evolution of "pygmaeus" microphallids.

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STUDY OF *SOMATERIA MOLLISSIMA* PARASITE FAUNA: SPECIES OF THE GENUS *MICROSOMACANTHUS* (CESTODA: HYMENOLEPIDIDAE)

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Type species of the genus *Microsomacanthus*, *M. microsoma* (Creplin, 1829), was described from eider duck *S. mollissima* and since that time 13 other *Microsomacanthus* spp. were mentioned for this host (excluding its new Black Sea isolated population). Vague differential diagnoses of some species often caused misdefinitions. The general review of the group based on slide collections (Geneva, Moscow, St. Petersburg), original materials both from Atlantic and Pacific regions and literary data is presented here.

As a result of the redescription of syntypes of *M. jaegerskioeldi* (Fuhrmann, 1913) and *M. diorchis* (Fuhrmann, 1913) their reliable recognizable features were detected and it became obvious that the former was mentioned in Newfoundland (Canada) as *M. microskrjabini* Spassky et Jurpalova, 1965 and the latter – as *M. somateriae* Ryjikov, 1965. Owing to Regel's more than 20 years long investigations in North-West Pacific, *M. paraparvula* Regel, 1994 and *M. parasobolevi* Regel, 2005 (syn. *M. formosoides* Spasskaja et Spassky, 1961 sensu Bishop, Threlfall, 1974) are described, *M. minimus* Ryjikov, 1965 and *M. somateriae* are shown to be common parasites of *S. mollissima*, *Microsomacanthus* sp. Ryjikov, 1965 (part) is identified as *M. recurvata* Spasskaja et Spassky, 1961. One species, very similar to *M. ductilis*, needs supplementary study; we designate it here as *M. ductilis* sensu Galkin et al., 1999. *Microsomacanthus heterospinus* Spassky et Jurpalova, 1965 is synonymized with *M. microsoma*. The finding of *M. paramicrosoma* on the Wrangel Island is very dubious as in *S. mollissima* as in principle. Registration of *M. formosa* (Dubinina, 1953) in eider ducks we assess as an error and the species itself must be excluded from the genus *Microsomacanthus* as it has *sacculus accessorius*.

Thus *Microsomacanthus* fauna of *S. mollissima* consists of nine species. *Microsomacanthus microsoma*, *M. jaegerskioeldi*, *M. parasobolevi*, and *M. ductilis* sensu Galkin et al., 1999 are common for both Atlantic and Pacific populations of the

host; *M. diorchis* is widespread in Atlantic region but its occurrence on the Wrangel Island needs confirmation, while *M. somateriae*, *M. minimus* (a new host record), *M. recurvata* and *M. paraparvula* are restricted to Pacific region. The most of the above mentioned species parasitize several species of eiders or even other diver ducks but *M. diorchis* and *M. somateriae* are specific to *S. mollissima*. The postembrional development of the majority of the species discussed is connected with marine or brackish-water amphipods. The only exception is *M. paraparvula* whose intermediate hosts are caddisworms. Consequently, *S. mollissima* is its facultative or even accidental host. Evidently the same case is with *M. recurvata* which finding is single.

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MOLECULAR TOOLS – ADVANCES, OPPORTUNITIES AND PROSPECTS

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DNA technology is having a major impact in many areas of veterinary parasitology. In particular, polymerase chain reaction (PCR)-based approaches have found broad applicability because their sensitivity permits the enzymatic amplification of gene fragments from minute quantities of nucleic acids from tiny amounts of parasite material. Also, micro array methods and high resolution electrophoretic are finding increased utility.

This paper discusses some developments and applications of DNA methods to parasites and highlights their usefulness or potential for those of veterinary importance. The focus is on tools for the accurate identification of parasites, the diagnosis of infections and the detection of genetic variability using mutation scanning technology.

INFECTION OF HORSES WITH INTESTINAL PARASITES BASED ON NECROPSY AND FECAL SAMPLES EXAMINATION

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To assess the influence of anthelmintic treatment on intestinal helminth fauna in horses, necropsy investigations on slaughtered horses and fecal egg examinations in riding horses were carried out.

The digestive tracts of 14 working horses slaughtered for meat, and fecal samples for egg counts with larvae differentiation of 170 horses from arabian horse farm and 40 half-breed horses from 5 riding clubs were examined. Twenty-one nematode species, one tapeworm species and one species of botfly were recovered in horses autopsied. Among nematodes were four species of large strongyles, fourteen species of small strongyles, *Parascaris equorum*, *Oxyuris equi* and *Probstmayria vivipara*. *Strongylus vulgaris* was the most common strongyle parasite, with 64.3% prevalence. The most prevalent cyathostome species were *Coronocylus coronatus* (57.1%), *Cylicocylus nassatus* (50%), *Cyathostomum catinatum* (42.9%), *Cylicostephanus calicatus* and *C. minutus* (both 28.6%). Tapeworm and botfly infections were not prevalent (*Anoplocephala perfoliata* and *Gasterophilus intestinalis* 7.1% each).

Based on the larvae differentiation no large strongyle infection was found in horses from arabian farm and riding clubs. The prevalence of cyathostomes was similar in the farm (69.2%) and in riding clubs (36.3-87.1%), while a mean fecal egg counts were higher in the arabians (1018) than in half-breed horses (302-515). *Parascaris equorum* was low prevalent in adult arabian horses (0.5%), while 3.7-21% of horses in clubs were found infected. *Anoplocephala* spp. were more prevalent in arabian farm (6.7%) than in riding clubs (0-1.8%). Horses in arabian farm and riding clubs were routinely dewormed twice a year. It was stated that no anthelmintics had been used in slaughtered horses.

These results revealed that despite of regular anthelmintic treatment small strongyles (cyathostomes) are the most common intestinal parasites in the horse. It was stated that in diagnosis of the abdominal pain, i.e. colic in not dewormed horses (working or those kept in organic farming system), *S. vulgaris* infection should be taken into account.

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THE STUDY OF INTESTINAL STRONGYLE PARASITES IN WORKING HORSES IN SOUTHERN POLAND

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The objective of the study was the investigation of strongyle fauna and its prevalence in working horses from southern Poland.

Fourteen horses from Tarnow county slaughtered for meat were examined. Fragments of intestinal wall and separate portions of ventral colon, dorsal colon and

caecum content were taken. Regarding that strongyles and cyathostomins are attached to or localized close to mucosa, 3-5% of contents near the wall of those parts of the intestine were collected. From each part approximately 200-300 strongyles were identified.

Totally 18 species of strongyles were found. Maximum number of species per one host was 14. Six species have prevalence more than 20%: *Strongylus vulgaris*, *Coronocylus coronatus*, *Cylicocylus nassatus*, *Cyathostomum catinatum*, *Cylicostephanus calicatus* and *C. minutus*. Six species were localized mainly in the caecum: *S. vulgaris*, *S. equinus*, *Triodontophorus serratus*, *C. coronatus*, *C. calicatus* and *Petrovinema poculatum*, two species in the ventral colon: *C. catinatum* and *Poteriostomum imparidentatum*. Finally, 9 species were localized in the dorsal colon: *T. brevicauda*, *Cyathostomum pateratum*, *Coronocylus labiatus*, *Cylicostephanus longibursatus*, *C. goldi*, *C. minutus*, *Cylicocylus ashworthi*, *C. leptostomus* and *Gyalocephalus capitatus*.

A quantitative analysis of horse parasitofauna in Poland and in Ukraine revealed 32 and 35 strongyle species, respectively. The most abundant cyathostome species in this study are the same as in previous reports. Two species, *C. insigne* and *C. labratus*, previously recorded as fairly common, were not found in this study. It was stated that no anthelmintics had been used in horses examined in this study, similarly as in earlier reports.

The results of this study confirm that few species make the main part of worms community of horses. Other species present so called "tail" of community. Some species are localized just in certain parts of large intestine.

ELAPHOSTRONGYLUS ALCES – A NEW PARASITE OF MOOSE IN POLAND

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Elaphostrongylus alces belong to the family Protostrongylidae. These nematodes have a clinical importance because a lot of species are significant pathogens of domestic and wild animals. Adult worms occur in the epidural space of the spinal canal and beneath the fascia of muscles of the chest and limbs. Larvae of *E. alces* migrate through the respiratory system. These parasites have been described only from the moose in Scandinavia. Because the parasites localize in the CNS, the neurological symptoms occur, such as: an unsteady gait, leaning on the front limbs and in the end paralysis of the hind legs. In pathologic picture develop edema, hemorrhage and yellowish-brown discoloration in epidural space of the vertebral canal. In life cycle the intermediate host occurs – terrestrial and aquatic snails. The final hosts are infected by ingesting snails while browsing.

The research material was obtained from the Kampinoski National Park, in Poland in 2004 – 2005. The 75 faecal samples (5 g.) were examined by Baermann method. Twenty first stage larvae were measured. Dimensions are as follows: length (368 – 451 μm), width (16 – 18 μm), nerve ring – distance from the anterior end (75 – 107 μm), excretory pore - distance from the anterior end (98 – 140 μm), oesophagus length (167 – 192 μm), genital primordium - distance from the posterior end (81 – 158 μm) and tail length (32 – 50 μm). These morphometric features show that these larvae are larvae of *E. alces*. The prevalence of infection of moose by *E. alces* larvae was 37%. The maximum intensity reached 294 larvae. This is the first record of *E. alces* larvae in faeces of moose in Poland.

OCCURRENCE OF *FRENKELIA* SPP. (COCCIDIA: SARCOCYSTIDAE) IN SOME SMALL RODENT SPECIES IN LITHUANIA

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Protists of the genus *Frenkelia* Biocca, 1968 are obligatory heteroxenous coccidian parasites forming tissue cysts in the brains of rodents and oocysts/sporocysts in the small intestine of buzzards (*Buteo* spp.). The aim of this report is to present the data on investigation of intermediate host spectrum and species composition of *Frenkelia* in some mouse-like rodents living in Lithuania. Attempts to transmit cyst-forming coccidians from natural definitive host (buzzard) to rodents are discussed too.

In all, 2213 rodents belonging to 13 species of the families Arvicolidae and Muridae were examined from different habitats in various localities in 1995-2003. In order to determine *Frenkelia* infection, brain samples were examined by compressor microscopy method in stained with methylene blue and unstained squash preparations as well as histologically.

Natural infection with cysts of *Frenkelia* were detected in the brain of four rodent species: bank vole *Clethrionomys glareolus*, short-tailed vole *Microtus agrestis*, root vole *M. oeconomus* and yellow-necked mouse *Apodemus flavicollis*. The prevalence of infection was greatest (24.0%, n=1143) among bank voles and it was strictly age-dependent. Experimentally these parasites were found in the brain of three individuals of sibling vole *M. rossiaemeridionalis* after infecting them with sporulated oocysts/sporocysts from buzzard *Buteo buteo*. Based on morphology of cysts and intermediate host-specificity, two *Frenkelia* species known in Europe were identified: *F. glareoli* in *C. glareolus*, and *F. microti* in all other vole species. In addition, *F. microti*-like cysts were detected in two bank voles and *F. glareoli*-like cysts in one yellow-necked mouse.

These results add to our earlier findings that represented the first contribution to the investigation of *Frenkelia* spp. fauna, as well as these parasites prevalence and distribution among rodents in the Baltic region.

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PRELIMINARY FINDINGS ON PROTOZOAN PARASITES OF ICELANDIC REINDEER *RANGIFER TARANDUS*

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Reindeer were imported to Iceland from Finnmark in Norway in the late 18th century. After rapid growth in the first half of the 19th century, marked population fluctuations were noted. In the 1940s, only 100 – 300 animals occurred in mountainous area in eastern Iceland. Hunting ban prevented the extinction of the population. At present approximately 4000 animals occur in the eastern and south-eastern part of Iceland. In the past, parasitological examinations on Icelandic reindeer mainly focused on helminths, but investigation on protozoan infections were neglected. The present study focuses on protozoan parasites.

Overall, 219 fecal samples of reindeer were examined; 193 were from reindeer calves and collected from the ground in three distinct areas in eastern Iceland in summer 2003 and 26 were from adult reindeers hunted during 2003 and 2004. HE stained tissue samples have already been examined from the heart, masseter, diaphragm and oesophagus of 14 animals. Fecal samples were examined, 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.

So far, six protozoan parasites have been found. Three morphologically distinct *Eimeria* species were recorded. All rarely occurred; prevalence of infection varied between 0.4 and 2.3%. Opg values ranged from 150 to 167.700. One of the species, *Eimeria mayeri*, was improperly described in Russia in 1936. We have already redescribed this coccidian. The other two were previously undescribed species. One has already been described with the name *Eimeria rangiferis*, but the description of the other one is in progress. Cysts of *Giardia* sp. were only found in reindeer calves (prevalence 13.5%), but *Cryptosporidium* sp. was not detected. *Entamoeba* sp. was commonly found (in 45.8% of the examined samples), both in calves and adult animals. Tissue cysts of *Sarcocystis* sp. were found in two adult reindeers (14.3%).

Reindeer imported from Norway to Iceland more than 200 years ago, must have been infected by the host-specific reindeer parasites (the three eimerids and *Sarcocystis* sp.). *Eimeria mayeri* was originally described from the Murmansk region in Russia. Therefore, at least it is likely to occur across the Palearctic. The definitive host of the *Sarcocystis* species found is unknown. Most probably it is the Arctic fox *Alopex lagopus* but the domestic dog could also be a candidate. The origin of the *Giardia* sp. and *Entamoeba* sp. infections in reindeer remains unknown but we point out that both species are extremely common in sheep grazing in the same area as the reindeer.

THE NITRERGIC, AMINERGIC AND PEPTIDERGIC NERVOUS SYSTEMS IN PARASITIC FLATWORMS

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During ten years we have studied the nitrergic nervous system (NNS) in parasitic flatworms. The NNS communicates through nitric oxide, which represents a new category of neuronal signal substances: a transmitter gas. So far the NNS have been described in less than 15 species of parasitic flatworms. Our goal is to provide basic data about the NNS and its spatial relationship to the aminergic and peptidergic NS.

NADPH-diaphorase staining, radiometric assays, immunocytochemical staining for cyclic-GMP, 5-HT, FMRFamide and TRITC-labelled phalloidin staining have been used.

NADPH-diaphorase positive nerve cells and fibres were observed in the CNS and PNS of tapeworms and flukes, larval stages and adults. The nerve fibres are closely associated to muscle fibres. The radiometric assay shows that neuronal nitric oxide synthase is active. The presence of cGMP-IR indicates that the NO-cGMP signalling is present in the worms. The NADPH-d reaction and the 5-HT-IR and FMRFamide-IR occur in separate set of nerves.

LOA LOA MICROFILARIAE EVADE COMPLEMENT ATTACK BY ACQUIRING REGULATORY PROTEINS FROM HOST PLASMA

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Loa loa is one of the filarial nematodes that infect humans. Although majority of infected individuals are asymptomatic this parasite is responsible of several clinical features such as calabar swelling, pruritus and ocular pathology. The adult worm lives in human subcutaneous tissue and produce microfilariae (mf) that circulate in peripheral blood. This study was undertaken to understand the mechanisms by which the microfilariae survive in constant contact with plasma for several weeks, i.e. are able to evade complement-mediated destruction before transmission to the dipterian vector. Complement (C) is an arm of innate immunity that consists of plasma proteins. It attacks foreign targets either after binding of host antibodies or using antibody-independent recognition of non-self structures that lack specific regulators. Complement is activated through three pathways (classical, lectin and alternative pathways) and is regulated by both membrane-bound regulators and plasma complement regulatory proteins such as factor H (FH) and C4b-binding protein (C4bp). Our hypothesis was that *Loa loa* mf is able to avoid C attack and acquire C resistance by binding host FH and/or C4bp from plasma.

In this study we isolated *Loa loa* mf from EDTA- and heparin-anticoagulated venous blood samples of *Loa loa* -infected Caucasian male. We could not detect any C activation products from the isolated mf indicating that mf did not significantly activate C *in vivo*. Minor amounts of C3b depositions could be generated on mf surface *in vitro* by incubation with serum but the C3b depositions quickly turned to cleaved and thereby inactivated iC3b or C3d fragments. The microfilariae did not cleave C3b themselves but were found to bind host C regulators FH and C4bp in specific and dose-dependent manner. The interactions were verified using direct binding assays with purified ¹²⁵I-labeled C regulatory proteins, immunofluorescence microscopy and Western blotting (WB). Finally we showed that mf-associated FH and C4bp lead to inactivation of C3b/C4b by human factor I.

In conclusion, our study shows that *Loa loa* mf acquire host complement regulators FH and C4bp and in this way block otherwise destructive activation of the classical and alternative pathways of complement. We suggest that C evasion might be essential for survival of filarial parasites in host and therefore an attractive target for vaccine development.

VARIATION IN PREVALENCE OF APICOMPLEXAN PARASITES IN THE
GREAT SNIPE (*GALLINAGO MEDIA*) IN RELATION TO AGE AND MHC
GENOTYPE

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Occurrence of avian blood parasites can vary among individuals and they may have a regulatory effect on populations of wild birds. The prevalence can vary between years and populations and be related to an individual's age and genotype. In this study we examined 1113 adults and 17 nestlings of the migrating Great snipe (*Gallinago media*) from 11 European breeding populations for presence of *Plasmodium/Haemoproteus* and *Leucocytozoon* spp. A nested polymerase chain reaction (PCR) was used to amplify a part of the parasite mitochondrial cytochrome *b* gene. Using this method, parasites belonging to the genera *Plasmodium* and *Haemoproteus* could not be discriminated but they could be separated from *Leucocytozoon* spp. in a second PCR step. In total, blood parasites belonging to the genera *Plasmodium/Haemoproteus* and *Leucocytozoon* spp. were found in 29% and 4.9% of the birds, respectively. The prevalence of *Plasmodium/Haemoproteus* infection showed no difference in 10 of 11 investigated populations; it was 100% in one population. The overall prevalence lies within the range reported from other bird studies. In one population we could test for temporal variation among the years 1993-2001. Temporal variation in prevalence was found for both *Plasmodium/Haemoproteus* and *Leucocytozoon* spp. and they showed a similar temporal pattern. None of the nestlings were infected with any of the parasites investigated, thus we found no evidence of local transmission. Previously collected information on the major histocompatibility complex (MHC) genotype variations in the Great snipe (n=217) was examined for relationship between *Plasmodium/Haemoproteus* and *Leucocytozoon* spp., respectively. However, infection prevalence was similar for all genotypes. We found a significant difference in prevalence in birds of different age classes, where young individuals had higher prevalence of *Plasmodium/Haemoproteus* parasites than old individuals. This could be due to young birds being more susceptible to infections or infected birds having higher mortality rate.

REINDEER DENSITY, CLIMATE, AND LANDSCAPE, AND THE ABUNDANCE OF *ELAPHOSTRONGYLUS RANGIFERI* (NEMATODA: METASTRONGYLIDAE)

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The occurrence of infection with *Elaphostrongylus rangiferi* in reindeer calves at an age of about 10 months was studied in two neighbouring reindeer herds for 12 years from 1974 to 1985 in Herd 1 and 10 years from 1976 to 1985 in Herd 2. Prevalence, mean intensity, and abundance of infection were based on the density of first-stage larvae in rectal faecal samples from the calves (lpg), and these statistics of infection were examined for correlation with indexes of climate, summer temperature, precipitation, and reindeer density. For Herd 1 the density index varied from 2.3 to 6.5 (reindeer per km²), for Herd 2 from 6.5 to 12.2. Abundance of infection ranged from no infection to 42.0 (lpg/reindeer) and for Herd 2 from 17.4 to 551.5. For Herd 1 abundance of infection correlated to the same extent with temperature and density (Spearman coeff. 0.487 and 0.413). For Herd 2 abundance showed better correlation with density (coeff. 0.571) than with temperature (coeff. 0.097). For Herd 2 the data fitted significantly to the regression model $\text{Abundance} = \text{Constant} + \text{Density}$ ($P = 0.017$). Habitats for gastropod intermediate hosts made up about 7% of the area in the range of Herd 1. In the range of Herd 2 this fraction was 38% prior to and 21% after an extension of the range during the winter of 1980 - 1981. This higher fraction of gastropod habitats explains the higher abundance of infection and the higher importance of reindeer density for explaining the variance in the abundance of infection in Herd 2 than in Herd 1.

ECHINOCOCCUS MULTILOCULARIS IN BELGIUM: INCIDENCE IN THE RED FOX *VULPES VULPES* AND IN DIFFERENT INTERMEDIATE HOSTS IN 2003 AND 2004

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The main objective of this study was to assess the prevalence of *Echinococcus multilocularis* carriage in the red fox in different areas of Wallonia (South of Belgium). Additionally, the role of different species of rodents or insectivores as intermediate hosts was evaluated.

The gut of foxes were frozen at -80°C for at least 10 days before further examination. For each animal, 15 gut scrapings were performed at different levels of the small intestine and examined under a dissecting microscope. The parasite was identified according to morphological criteria (size of the worm and aspect of the last mature segment). Small rodents and insectivores were captured using appropriate traps

whereas large rodents (musk rats *Ondatra zibethicus*) were trapped by the appropriate staff of the Regional Ministry of Agriculture and Environment. All animals were necropsied and the presence of compatible hepatic or extra hepatic lesions was recorded. In doubtful cases, the lesions were submitted to a histopathological examination.

A total of 990 foxes were available during the study period. The prevalences were found to be highly variable from one province to another (from 1.92% in Walloon Brabant to 41.67% in Luxembourg). During the same period, 914 *Microtus arvalis*, 39 *Microtus agrestis*, 55 *Apodemus sylvaticus*, 215 *Sorex araneus*, 1 *Sorex minutus*, 23 *Clethrionomys glareolus* and 2 *Arvicola terrestris* were captured in a highly endemic area (Luxembourg). Only one *M. arvalis* (0.11%) and 1 *C. glareolus* (4.34%) were found to be infected by the metacestode. As far as the musk rat was concerned, 1728 animals originating from Wallonia were available in 2003 and 2004. Four hundred and ninety five (28.6%) were found to be infected by the metacestode.

The results confirm that *E. multilocularis* is widely distributed in Wallonia although the prevalence is largely variable from one place to another. Small rodents and insectivores seem to play a minor role in the completion of the life cycle whereas the musk rat is highly susceptible and could play a major role in the epidemiology of this zoonotic infection at least in the investigated region.

EVOLUTION OF THE INTERGENIC SPACER REGION IN *GYRODACTYLUS SALARIS* AND *G. THYMALLI*: IMPLICATIONS FOR TAXONOMY

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The species status of the pathogenic salmon parasite *Gyrodactylus salaris* and the benign grayling parasite *G. thymalli* is challenged due to application of new methods in molecular taxonomy. Host specificity and different pathogenicity of *G. thymalli* and *G. salaris* are the major arguments for still considering them different species. The ribosomal internal transcribed spacer regions (ITS), a powerful marker for species discrimination of the *Gyrodactylus*, are identical between almost all studied isolates of the two species. In contrast, substantial genetic diversity was detected for the mitochondrial cytochrome oxidase I (COI). These sequences group into up to six well-supported clades each consisting of haplotypes specific for either species. However, the COI sequences do not support monophyly of the haplotypes of either species, but may be powerful for delimiting pathogenic and non-pathogenic strains.

Based on molecular data it has been put forward that *G. salaris* and *G. thymalli* represent two polytypic species or one polytypic species, or refer to a complex of more than two sibling species. The repetitive regions of the ribosomal intergenic spacer (IGS) region have also been applied to discriminate *G. salaris* from *G.*

thymalli. Each of the two repetitive IGS regions consists of a varying number of 23 bp repeats that obviously evolved from a common ancestral 23 bp repeat. Previous studies showed that IGS sequences allowed the discrimination of *G. salaris* specimens infecting Atlantic salmon and *G. thymalli* specimens infecting grayling. *Gyrodactylus salaris* specimens from rainbow trout shared sequence features with the IGS of specimens of both *G. salaris* on salmon and *G. thymalli* on grayling. As the different IGS sequences differ first of all in the number and order of different repeat motifs that differ frequently by just one nucleotide, sequence alignments may be biased and arbitrary, which impedes meaningful phylogenetic analyses.

Here we present a comparative analysis of IGS and COI sequence variation of *G. salaris* and *G. thymalli* specimens from several populations across the distribution range. We suggest an evolutionary history for the 23 bp IGS repeat motifs and analyze whether or not the mitochondrial COI diversity is reflected in the IGS region. Particularly we focus on the previously published hypothesis that some strains of *G. salaris* such as e.g. the specimens infecting rainbow trout represent hybrids of *G. salaris* and *G. thymalli*.

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MITOCHONDRIAL DNA VARIATION IN THE FISH PARASITE GENUS *GYRODACTYLUS*

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Mitochondrial DNA (mtDNA) sequences are frequently used markers for studying phylogeographic patterns, invasion history, and systematic and taxonomic questions. Recently, cytochrome oxidase I (COI) mtDNA sequences have been published for the monogenean fish parasites *Gyrodactylus salaris* and *G. thymalli*. *Gyrodactylus salaris* infects Atlantic salmon parr *Salmo salar* and has caused severe biological and economical damage in Norwegian rivers, and has a wide host range. In contrast, *G. thymalli* infects grayling *Thymallus thymallus*, apparently causes no severe harm, and has a more restricted host range. Both species are morphologically very similar and molecular markers, such as the ribosomal internal transcribed spacers (ITS1 and 2) cannot discriminate them. Host preference is the primary argument for treating them as two separate species. The sequence similarity of the species' rDNA is remarkable since ITS serves as a reliable barcode for identifying all other *Gyrodactylus* species. COI sequences from populations of *G. salaris* and *G. thymalli* covering a large part of the species' ranges revealed substantial genetic diversity. The COI sequences cluster

according to geography and host specificity with approximately 2% divergence between well-supported clades but did not support monophyly of either species. Nevertheless, phylogeographic patterns, such as the invasion history of *G. salaris* into Norway from the Baltic, could be resolved.

Based on COI variation, it has been proposed that *G. salaris* and *G. thymalli* could represent two polytypic species, one polytypic species, or a complex of more than two sibling species. Assuming a very high evolutionary rate for mtDNA in *Gyrodactylus* spp., the current data fits best the single polytypic species concept. Unfortunately, gyrodactylid COI sequence data is only available for *G. salaris* and *G. thymalli*. To test for the generality of the results obtained, COI was sequenced for captive and wild populations of *G. turnbulli*. *Gyrodactylus turnbulli* is a common parasite of the guppy *Poecilia reticulata*, and is reportedly host specific. Surprisingly, we detected similar levels of genetic differentiation between populations of *G. turnbulli* as for *G. salaris* and *G. thymalli*. We discuss the implications of this comparative study for the understanding of the evolution of mitochondrial COI sequences in the genus *Gyrodactylus* and the application of this molecular marker for taxonomic and systematic purposes.

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EQUINE LARVAL CULTURE WITH FOCUS ON *STRONGYLUS VULGARIS*: QUALITY EVALUATION AND THE INFLUENCE OF VARIOUS FACTORS

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The prevalence of *Strongylus vulgaris* was examined in 105 horse farms on Zealand, Denmark, all of which participated in a worm control program based on selective treatment.

Two larval culture procedures based on examination of individual and pooled samples, respectively, were compared. The individual procedure revealed *S. vulgaris* prevalences of 5.7% and 26.7% at individual and herd levels, respectively, whereas the pooled-sample procedure revealed a herd prevalence of 15.3%.

The relationship between EPG (strongyle eggs) and the occurrence of *S. vulgaris* was evaluated, showing that the likelihood of diagnosing *S. vulgaris* infection was generally very low, but increasing at high EPG levels, yet only 50% at 7,000 EPG.

The results of the prevalence study showed no systematic correlation between the occurrence of *S. vulgaris* and herd size.

The effect of light conditions during incubation on the strongyle larval recovery was studied by subjecting the larval cultures from three horses to different light intensities. In comparison with shady conditions, incubation in direct sunlight resulted in significantly decreased larval recovery, for total counts as well as for specific *S. vulgaris* larval counts.

A study on the role of presence/absence of soil nematodes in otherwise identical faecal cultures showed that presence of soil nematodes cause a significant decrease of larval detection.

COSTS OF PARASITE RESISTANCE FOR FEMALE SURVIVAL AND PARENTAL CARE IN A FRESHWATER ISOPOD

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Parasite resistance is expected to be costly because activation and maintenance of immune system requires energy that will not be available for other fitness related functions. In the wild, free living organisms are continuously exposed to parasites and pathogens, requiring continuous activity of the immune system. Experimental work on the costs of parasite resistance is still limited and our knowledge of the possible magnitude and expression of costs of resistance comes from a few well-studied model systems. Here, we experimentally exposed isopods *Asellus aquaticus* to trophically transmitted acanthocephalan parasite *Acanthocephalus lucii*. Successful establishment of the parasite requires penetration to body cavity; therefore, it is likely to induce immune response. We assessed the effect of parasite resistance and infection on reproductive life-history traits and female mortality. We exposed gravid females of a parasite-free population and a population having history with the parasite in a long-term experiment where we assessed female mortality, brood dumping, and offspring growth rate. Females from parasite-free population were slightly more susceptible to infection than females from population with history with the parasite, but the difference was not statistically significant (68% vs. 54%, respectively). Resistant females from both populations experienced higher mortality than susceptible or control females. Parasite exposure reduced the offspring size at birth in both susceptible, but especially, in resistant females, suggesting that females fighting the parasite had less resource to direct for parental care. Parasite exposure had less effect on brood dumping and offspring growth rate after birth. Hence, our results reveal costs of resistance in parental survival and parental care of offspring.

PUBLIC KNOWLEDGE ABOUT THE PREVENTION OF *ECHINOCOCCUS*
MULTILOCULARIS INFECTIONS IN FOUR EUROPEAN COUNTRIES

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Public attitudes towards zoonoses play a major role in the successful implementation of prevention, control and management measures. Therefore, the development of strategies to inform the public about risk and prevention of zoonoses has to be based on the problem as perceived by the public. Furthermore, public attitudes towards zoonoses can differ from region to region, and communication about prevention measures should be adopted based on regional and population-specific demands. Due to increasing fox populations in Europe and the recent invasion of this species into urban habitats, human alveolar echinococcosis (AE), caused by larval stages of the small fox tapeworm *Echinococcus multilocularis*, is helminthic zoonosis of increasing concern. In order to plan effective information campaigns about human AE, current public knowledge of *E. multilocularis* was assessed by means of telephone interviews in the Czech Republic, France, Germany and Switzerland.

A total of 2041 interviews were carried out according to the "Random-Quota" method. For all five questions of the inquiry, significant country-specific differences were found. The portion of people that had heard about the small fox tapeworm varied between 13.7% (Czech Republic), 17.6% (France), 62.6% (Germany) and 69.4% (Switzerland). In the Czech Republic, Germany and Switzerland between 50% and 60% of the population who had heard of the small fox tapeworm felt that they are reasonably informed, whereas this portion was only 17% in France. The percentage of informed people that believed that this parasite represents a high risk ranged from 12% in Switzerland to 43% in France.

Individuals who had some knowledge of the small fox tapeworm were asked to choose one or several answers among six randomly presented options on how to protect themselves against the parasite (A: cook food, B: wash food, C: consume no wild berries, D: avoid fox droppings, E: deworm pet, F: treat foxes). In the Czech Republic and France, answers B and D were the most and answers C and F the least selected options. In Germany and Switzerland, C and D were the most and A and F the least favoured answers.

Our results and the actual epidemiological situation with regard to *E. multilocularis* call for pro-active public information programs about this zoonotic parasite, especially in countries or regions where this parasite is prevalent, but rather unknown. We suggest that public information should (a) enable the public to achieve a realistic risk perception, (b) prevent overreaction or panic with respect to alveolar echinococcosis, (c) prevent carelessness when dealing with foxes, pets and other risk factors (such as food handling) and (d) give clear information on how people can minimise their infection risk.

THE REVEALING OF PARASITE-HOST-VECTOR INTERACTIONS USING A SINGLE SAMPLE AND MOLECULAR METHODS

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To understand geographical patterns in transmission and distribution within different host species of vector transmitted blood parasites (in this case heamosporidian parasites of the genus *Leucocytozoon*) it is essential to know the three-way association in the system. This is suggesting that the distribution of the parasites is not only determined by associations with the vertebrate host and the vector, but also by the association between the vector and the vertebrate host.

To investigate the three-way associations in wild populations for single parasite species we applied a method of molecular identification of the gut content in simuliid flies. The sample consisted of simuliid flies randomly collected in the wild, of which a small fraction (0,003%) of the females had an undigested blood meal in the belly. The species identity of the blood meals in a total of 200 individuals, belonging to 16 different species of simuliid flies, were analysed using standard PCR and sequence based methods. From the same samples we detected and identified of *Leucocytozoon* spp. that the vertebrates were carrying when the fly took its blood meal. In addition, we also used the sample one more time to conduct a sequence-based identification of the simuliid flies. Hence, from one sample we could identify and create a molecular based phylogeny of the simuliid flies, identify the vertebrate it had taken its blood meal from, and identify and construct a molecular phylogeny of the parasites that were in the vertebrate when stung by the vector. This provided unique possibilities to explore the associations between different species of vectors, parasites and hosts. The results revealed host-specificity between the host and the vector. The result indicated that the preferences of the vector to a large extent could influence the distribution of the parasites and act as an ecological barrier for transmission between different groups of vertebrate hosts.

MOLECULAR DIAGNOSIS AND EQUINE PARASITOLOGY

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Knowledge of the biology and epidemiology of equine parasitic infections together with accurate diagnosis represent the greatest challenges in the implementation of parasite control strategies. In order for alternative approaches to helminth control to be sustainable a greater insight is required into the basic biology, epidemiology and pathogenicity of the major equine parasites. In particular, strongyle parasites are

recognised as important pathogens of horses and reducing infection with these parasites has been the aim of current control methods. Control has relied largely on anthelmintics whose widespread use has led to drug resistance in one particular group of parasites, the cyathostomins, bringing them to prominence as the major parasitic pathogen of equids.

This paper will provide a review of the current molecular methods available for the detection of equine parasites and the current application of these techniques. In particular, the major advances made in the study of strongyle parasites at the single species level will be addressed. These studies have focussed on various regions of the parasite genome to study individual species within mixed infections. In this way it has been possible to look at the role of a particular parasite species in natural infection, disease and drug resistance. It is not the intention of this paper to look in detail at the molecular diagnosis of anthelmintic resistance but the development of molecular tools to detect benzimidazole resistance in cyathostomins will be reported. In addition, the need to employ existing techniques and develop new tools to aid diagnosis and control in the future will be highlighted.

ANALYSIS OF THE EPIDEMIOLOGICAL FACTORS OF VULPINE TRICHINELLOSIS IN ECOLOGICALLY DIFFERENT REGIONS

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Natural ecosystem plays an important role in the maintenance of the sylvatic trichinellosis in the nature and human behavior influences the ecology of trichinellosis in both domestic and sylvatic habitat. As ecosystem become more fragmented and human – wildlife interactions intensify, a clear understanding of what constitutes a truly natural distribution of *Trichinella* genotypes and species is needed.

In the Slovak Republic, trichinellosis circulates almost exclusively in the sylvatic cycle, with main reservoir host red fox *Vulpes vulpes* and wild boars and outbreaks in humans also sporadically occur. The substantial increase of the red fox population in recent provides a potential for spread of this parasite in the wildlife, that is the main reason for focusing the study on the epidemiology of trichinellosis in the red fox that represents most important reservoir of the parasite in the Slovak territory. Reassessing the results of longitudinal study (2000-2004) on trichinellosis in the red foxes, which pointed out substantial geographical stratification of occurrence, a more detailed study was performed in five ecologically different regions of eastern Slovakia with more profound regard to ecogeographical and anthropogenic influences to natural red fox habitat. In total of 689 red foxes originating from selected regions were examined using artificial digestion method. The zoogeographical, climatic, and landscape characteristics and several parameters with respect to human impact on the red fox

habitat – human population density, the intensity of human activity and type of human intervention to the country, were followed.

The results showed that vulpine trichinellosis was most prevalent in the red foxes hunted in mountains where elevated population density of the red foxes followed by reduction of their operating territories implied frequent cannibalism within the fox population, which eventuated the increase of the parasite prevalence. In the contrary, in region of the Východoslovenská Nížina lowland, with high ratio of anthropogenic influence, the relatively low infection prevalence rate was found. Human impact markedly affected the red fox habitat thereby the biotope becomes not sufficient for the foxes and they are attracted by abundant human dumps and domestic animals and the risk of transmission of sylvatic trichinellosis due to cannibalistic fox behaviour decreased.

Natural ecosystem plays an important role in the maintenance of the sylvatic trichinellosis in the nature and human behavior influences the ecology of trichinellosis in both domestic and sylvatic habitat. Land cover and land use integrated with georelief characteristics, provide the background for observing the intensity of landscape processes, changes in the landscape and identification of human influences which might contribute to the better understanding of potential interacting factors affecting the epidemiology and ecology of trichinellosis in the Slovak Republic.

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QUANTITATIVE DYNAMICS AND SPATIAL STRUCTURE OF POPULATIONS OF SMALL MAMMAL PARASITES

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Material for this paper was collected during studies of the biology of small mammals and their parasites from southern Karelia (62°04' N, 33°55' E) conducted in the summer-autumn seasons in 1994–2003. In all 1200 animals from 53 sample plots were subjected to parasitological dissection. The specimens represented 5 species of Rodentia: birch mouse *Sicista betulina* (37 individuals), harvest mouse *Micromys minutus* (13), bank vole *Clethrionomys glareolus* (1098), field vole *Microtus agrestis* (107), root vole *M. oeconomus* (45).

The parasite abundance was found to vary notably over years. The prevalence of most parasites was the lowest when the host abundance peaked, whereas the infestation rate in the following year, when small mammals experienced a decline, increased sharply. The species diversity of the parasite fauna and the total parasite load changed asynchronously in different years. Thus, in years of depression of the

host abundance, the parasite diversity was the lowest, whereas the parasite burden reached a maximum.

For most parasites, the frequency distribution across the host population is described by the negative binomial distribution. The best fit to this theoretical model was reported for ectoparasites ($0.05 \leq P < 0.90$). The distribution of endoparasites conforms to the negative binomial and Gamma distributions ($0.05 \leq P < 0.15$). Analysis of the parasite distribution across the host population and the dynamics of its parameters suggest that stability is maintained in the system of host-parasite relationships.

The nematode *Heligmosomum mixtum* was used as an example to investigate patterns in the spatial distribution of parasites. Linear multiple regression equations were built to describe the relationship between the nematode abundance in the biotope and I- and II-order environmental factors (48 factors in total). Early summer was the time with the greatest number of significant correlations, the weightiest factors being host abundance ($r = 0.84$), the proportion of juvenile individuals ($r = -0.28$) and mature males ($r = 0.80$) in the host population, soil moisture ($r = 0.12$). Calculated values of *H. mixtum* abundance correlated well with empirical data ($R^2 = 0.99$). *Heligmosomum mixtum* distribution across biotopes in August followed a random pattern. None of the investigated factors had a significant effect, and the regression model explained only 37% of *H. mixtum* abundance observation events. The presumable reason for that is that active migrations of the host in this period, related to the dispersal of juveniles, level off any distinctions between biotopes.

EXPERIMENTAL DATA ON HOST SPECIFICITY OF AVIAN MALARIA PARASITES OF THE SUBGENUS *NOVYELLA*

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Avian malaria parasites (Plasmodiidae, *Plasmodium*) of the subgenus *Novyella* are some of the most common hematozoa, found particularly in passeriform birds. Although numerous surveys report *Novyella* species in passeriform birds all over the world, experimental studies on their specificity are few. In the present paper, we present results of experimental transmission of 2 malaria parasites of the subgenus *Novyella*, i. e., *Plasmodium nucleophilum* and *P. vaughani*.

Susceptibility of wild-caught European passeriform birds to naturally isolated malaria parasites was studied by means of intramuscular subinoculation of infected citrated blood. The work was carried out at the Institute of Avian Research 'Vogelwarte Helgoland', Wilhelmshaven, Germany. All birds were caught in the environs of the institute in June 2002. One great tit, *Parus major*, and 1 robin,

Erithacus rubecula, naturally infected with *P. nucleophilum* and *P. vaughani*, respectively, and 9 uninfected birds of 4 species were used during this study. The infected birds with active parasitemia were used as donors of malaria parasites to infect the recipient birds, which were 3 great tits, 1 robin, 4 blackcaps, *Sylvia atricapilla*, and 1 dunnock, *Prunella modularis*. Blood from the recipient birds was taken once per day during 5 days before subinoculation. The blood smears from each recipient bird were examined for hematozoa at high magnification for 3 hr. Malaria parasites were not seen in them.

Plasmodium nucleophilum of the great tit was transmitted to 3 great tits, but 3 blackcaps were not susceptible. *Plasmodium vaughani* of the robin was transmitted to 1 robin and 1 blackcap, but 1 dunnock was not susceptible. The prepatent period was between 8 and 10 days in all experimental infections. Maximum experimental parasitemia (3.4% of red cells) was detected in great tits infected with *P. nucleophilum* 23 days post-exposure. No obvious clinical signs or mortality occurred in any avian host during this study, which agrees with low virulence recorded formerly for the majority of *Novyella* species during their development in wild birds.

This study is in accord with former experimental observations on host specificity of *P. nucleophilum* and *P. vaughani*, which are characterised by a wide, but selective, range of avian hosts. It is probable that there is a marked intraspecific divergence of specificity of avian malaria parasites, which is in accordance with information on huge genetic divergence of avian malaria parasites and other hemosporidians recorded using PCR-based techniques.

ECDYSIS OF *OESOPHAGOSTOMUM* SP.: POSSIBLE INVOLVEMENT OF EICOSANOIDS AND DEVELOPMENT OF A BIOASSAY

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Bioassays were developed and applied to test the role of eicosanoids and pH changes in ecdysis of *Oesophagostomum dentatum*.

Exsheathment (80-100 %) was achieved by subjecting third-stage larvae either to chlorine (hypochlorite assay) for 5 min or by incubating them in HCl followed by addition of NaHCO₃ (pH-change assay) with subsequent cultivation at 38.5 °C/10 % CO₂ for one week. Addition of the lipooxygenase (LOX) inhibitor diethylcarbamazine (DEC) to the larvae resulted in reduction of the exsheathment rates which could be restored by addition of leukotrienes (LT)B₄, LTC₄, LTD₄ and LTE₄. Addition of the cyclooxygenase (COX) inhibitor acetylsalicylic acid (ASA) also resulted in decreased exsheathment rates both in the hypochlorite and in the pH-change assays in a dose-

dependent manner. However, the primary COX products (prostaglandins) were not able to reverse this effect, in contrast to LTC₄.

It was concluded that (a) both tests are suitable for bioassaying the effect of substances on exsheathment and (b) eicosanoids involved in the control of exsheathment of L3 of *O. dentatum* are primarily LT.

THE DEPENDENCE ON THE TYPE OF HOST MUSCLE TISSUE FOR THE FREEZE TOLERANCE OF NINE *TRICHINELLA* SPECIES

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Infected muscle tissue was obtained from a series of experimental infections in pigs, wild boars, horses, mice, seals, cats and foxes, with the aim to evaluate the cold and freeze tolerance of nine well-defined genotypes of *Trichinella* spp. The muscle tissue samples originated from necropsies performed 5, 10, 20 and 40 weeks post inoculation (pi) of the respective host species. Sub-samples of 100 g were stored at 5, -5 and -18 °C. After storage for 1, 4 and 8 weeks, one sub-sample was taken from each temperature treatment and digested; recovered larvae were inoculated into 5 mice per sample (up to 500 larvae per mouse). Five weeks pi mice were killed and digested for recovery of muscle larvae. In meat of pigs and wild boars, no *Trichinella* spp. were able to survive at -18 °C for 1 wk; most species showed some survival at -5 °C, and all survived at +5 °C for 4 wks. In mice, only *T. nativa* and *Trichinella* sp. T6 survived at -18 °C for 1 wk, but *T. murrelli* and *T. britovi* showed good tolerance at -5 °C. In the carnivores, *T. nativa* and *Trichinella* sp. T6 survived at -18 °C for 4 wks; *T. murrelli* and *T. britovi* survived 1 wk at this temperature. In horses, which were inoculated with *T. spiralis*, *T. britovi* and *T. pseudospiralis* only, all three species survived at both -5 °C and -18 °C for 1, 4 and 8 wks. The results clearly show that freezing at -18 °C is an effective way to inactivate *Trichinella* spp. in pork and wild boar meat, but that some *Trichinella* spp. genotypes survive freezing in meat of carnivores. Further, it appears that horse meat most likely contains substances that effectively prevent freezing of *Trichinella* spp. muscle larvae.

VARIETY OF HORSE CYATHOSTOMES IN LITHUANIA AND THEIR RESISTANCE TO FENBENDAZOLE

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The aims of the present study were to investigate establishment and variety of horses cyathostomes in Lithuania and to estimate their resistance to Fenbendazole. In addition, the correlation of FECR with the reduction of worm burden was performed.

The experiment was conducted at Lithuanian horse breeding farm "Vilniaus žirgynas", where horse strongyles resistance to fenbendazoles was earlier detected by FECR test. The experiment included 4 horses, naturally infected with strongyles. The following anthelmintics were administered to three experimental horses: Fenbendazole (7.5 mg/kg BW) and after 13-14 days – Ivermectin (0.2 mg/kg BW). One (control) horse was not treated. After treatment with Fenbendazole strongyle eggs were found in faeces of all horses, meanwhile on the third day after treatment with Ivermectin strongyle eggs were not detected. The treatment with Fenbendazole in each experimental horse reduced strongyle FECs to 86.3%, 94.5% and 83.1% (mean 90.7%), and strongyle worm burdens on 81.4%, 67.1%, 48.1%, respectively (mean 65.5%).

The total of 5491 cyathostomes were collected and identified during the experiment. In investigated horses it was determined 13 species of cyathostomes. *Cylicocycylus nassatus* and *Cyathostomum catinatum* (42.2%, 22.7%) were the dominant species though *Cylicocycylus leptostomus*, *Cylicostephanus longibursatus*, *Cylicocycylus ashworthi*, *Cylicostephanus goldi*, *Cyathostomum pateratum* (13.6%, 7.8%, 7.8%, 3.2%, 2.2%) were also frequently present. The other six species (*Coronocycylus labiatus*, *C. coronatus*, *C. labratus*, *Cylicostephanus calicatus*, *C. minutus*, *Cylicocycylus insigne*) were found only as solitary individuals. Some horses were hosts of 8 to 10 species of cyathostomes. It was determined that *Cylicocycylus ashworthi*, *C. nassatus*, *Cylicostephanus goldi* had developed strong resistance to Fenbendazole (54.7%, 53.0%, 34.1%, respectively). *Cyathostomum catinatum* was less resistant (16.8%). *Cylicostephanus longibursatus* and *Cyathostomum pateratum* had light resistance, whereas, *Cylicocycylus leptostomus* performed no resistance at all. The small number of detected other species is an insufficient basis for judgement about their resistance.

The results from this study indicate that use of two anthelmintics have potential value in detecting of anthelmintic resistance in horse strongyles and could be used as alternative to the necropsy, particularly taking into account the price of studhorses. However, this suggestion requires further investigations.

DYNAMICS OF THE SPREAD OF ENDOPARASITOSEs IN SHEEP AND GOATS IN LATVIA

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The dynamics of the spread of endoparasitoses in sheep and goats in Latvia was investigated as well as the tendency of their distribution was detected depending on the animal species. The results of coprological examinations and helminthological necropsies carried out in the Laboratory of Parasitology, Faculty of Veterinary Medicine, as well as the survey data of the State Veterinary Laboratories and slaughterhouses were analysed. The following standard methods were used: Fuelleborn, Demidov, Vaid or Mc Master. To diagnose skrjabinemosis, a method of scraping of the perianal folds or transparent sticky tapes was used.

It was found out that in the 1950's – 1960's in Latvia most sheep, between 43.5 and 100% of the investigated animals, were infected with gastro – intestinal strongylatoses (*Trichostrongylus* spp., *Ostertagia* spp., *Nematodirus* spp., *Haemonchus contortus*, *Chabertia ovina*, *Oesophagostomum venulosum*, *Bunostomum trigonocephalum*) and fasciolosis (*Fasciola hepatica*, prevalence of infection is 29.0 – 35.9 %). Also, monieziosis (*Moniezia expansa*, *M. benedeni*, 6.8 – 20.0%), protostrongylidoses (*Muellerius capillaris*, more rarely *Protostrongylus* spp., 13.5 – 14.4 %), dictyocaulosis (*Dictyocaulus filaria*, 8.4 – 8.5%), strongyloidosis (*Strongyloides papillosus*, 3.8 – 10.7 %) and cysticercosis (*Cysticercus tenuicollis*) were distributed here. Eimeriosis (*Eimeria* spp., 5.7 – 6.1%), trichuriasis (*Trichuris ovis*, 1.8 – 3.4 %), echinococcosis (*Echinococcus granulosus* larvae, ~ 0.8 %) paramphistomosis (*Paramphistomum cervi*, ~ 0.2 %), dicrocoeliosis (*Dicrocoelium lanceatum*, ~ 0.02 %) and cenurosis (*Coenurus cerebralis*) were found more rarely in sheep. With years, starting from the 1970's, the prevalence of infection of the sheep with fasciolosis, dictyocaulosis and echinococcosis has decreased several times. In the 2000's eimeriosis and strongyloidosis were diagnosed in sheep more often. The prevalences of the rest of infections were not changed significantly.

The distribution of endoparasitoses in goats was started to analyse in 1994 as the goat keeping is one of traditional branches of animal husbandry in Latvia, and systematic examinations were not carried out before. The investigations provided evidence that the prevailing endoparasitoses in goats in Latvia are protostrongylidoses (prevalence of infection 20.5-76.5 %), gastro – intestinal strongylatoses (28.7-86.9 %), eimeriosis (3.0-71.0 %), strongyloidosis (0-16.9 %), trichuriasis (0 – 9.1 %), while dictyocaulosis (0 – 2.4 %), monieziosis (0 – 9.4 %), fasciolosis (0 – 1.7 %), echinococcosis and cysticercosis were reported more rarely. In 1998, skrjabinemosis (*Skrjabinema ovis*) was diagnosed in goats on one farm in Madona region. This infection was not recorded in Latvia before. In 2004 and 2005, skrjabinemosis in goats was also found on some farms in Rīga and Talsi regions. Paramphistomosis and dicrocoeliosis in goats were not diagnosed.

Both coprological examinations and helminthological necropsies showed that in most cases animals were infected with two, three or more species of parasites at the same time. The prevalence of infection and the character of associations in regions were different and varied yearly. Goats in comparison with sheep were more infected with *Eimeria* and nematodes, but less with *Moniezia* and trematodes.

ENDOPARASITES OF RED FOXES IN LATVIA

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Between 1995 and 2005, material has been collected from various regions of Latvia in order to detect the endoparasites of red foxes *Vulpes vulpes* that could endanger human and domestic animal health. Red foxes are important animals for hunting the number of which has markedly increased in Latvia in the last years. For example, in 1980 there were 9141 foxes registered in Latvia, in 1990 – 11817, in 1995 – 18466, but in 2001 there were 27649 foxes of which 8399 were hunted down.

In the studies of the trichinellosis, a digestion method with artificial gastric fluid employing the magnetic stirrer was used. The total number of the examined muscle samples of foxes was 1343. In the Danish Center for Experimental Parasitology, Copenhagen, *Trichinella* larvae were identified from 129 foxes by means of polymerase chain reaction – multiplex PCR. Postmortem examinations of foxes were carried out in a random manner by means of academician K.I. Skrjabin's method and coprological examination with standard methods such as Fuelleborn, Demidov, Baermann or McMaster. It was found that 28.4 % of foxes had been *Trichinella* infected in Latvia. Trichinellosis was found in all regions, its prevalence varied between 16.4 % and 60.0%.

Three *Trichinella* species were identified in foxes: *T. spiralis* (Owen, 1835), *T. nativa* (Britov, Boev, 1972) and *T. britovi* (Pozio et al., 1992). *Trichinella britovi* (the prevalence of infection is 77.7 %) was spread in almost all regions of the country; *T. nativa* (8.5%) and *T. spiralis* (2.3%) were recorded more rarely. In several cases (11.7%), two *Trichinella* species were found in foxes at the same time.

Several species of helminths were detected in postmortem and coprological examinations. These are *Toxocara canis* (Werner, 1782), *Toxascaris leonina* (Linstow, 1902), *Uncinaria stenocephala* (Railliet, 1884), *Trichuris vulpis* (Froehlich, 1789), *Crenosoma vulpis* (Dujardin, 1845), *Strongyloides* spp., *Capillaria* spp., *Dipylidium caninum* (Linne, 1758), *Mesocostoides lineatus* (Goeze, 1782), *Echinococcus granulosus* (Batsch, 1786), *Taenia* spp., *Alaria alata* (Goeze, 1782), as well as unicellulars *Cystoisospora* spp. and mange ticks *Sarcoptes canis* (Gerlach, 1857). Investigations are continued.

GEOGRAPHIC DISTRIBUTION OF HUMAN ALVEOLAR ECHINOCOCCOSIS IN GERMANY: RECENT DATA FROM THE EUROPEAN ECHINOCOCCOSIS REGISTRY AND HISTORIC DATA FROM PUBLICATIONS SINCE 1855

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The European Echinococcosis Registry, founded in 1998, aims at achieving a comprehensive case collection of human alveolar echinococcosis (AE) in order to estimate the true prevalence of AE, to identify risk areas and to improve knowledge on this potentially fatal disease. In Germany, the disease was first identified in 1855 by Virchow; further cases were hence diagnosed regularly, but the disease remained rare. Only in the 1950s, the life cycle of the *Echinococcus multilocularis*, including canids and small mammal species, was finally demonstrated. Intensive research on *E. multilocularis*-prevalence in regional fox populations began after 1975. In this study, we present data on human infections from the registry, complemented by data gained from a literature search including all available publications on the disease.

In the German registry, 198 AE-patients were notified until December 2004. The literature search is still in progress; of 70 publications (monographs, reviews, doctoral theses and case reports), 38 are currently analysed, yielding 415 AE-cases. The origin and a detailed case history were available for 295 cases. For historic cases, the potential place of infection was determined to be the place of longest residence over the whole lifetime; for recent cases, the place of longest residence during the 10 years before diagnosis was taken.

The geographic distribution of human AE shows a similar pattern throughout all periods: the majority of cases comes from villages in a "core area" in southern Germany, however, sporadic cases have consistently been diagnosed in a rather large border area. The data complement recent findings on *E. multilocularis*-prevalence in foxes: the core area roughly corresponds to areas of high AE-endemicity in fox populations (> 30%).

The combined data of recent and historic investigations will help to evaluate possible changes of exposure risks in new areas which can arise in future from increasing disease prevalence in foxes and growing fox populations in towns and cities.

The European Echinococcosis Registry is a partnership project in 15 European countries. In Germany, it received financial support by Paul-Ehrlich-Gesellschaft für Chemotherapie (PEG), Glaxo SmithKline GmbH, and the medical faculty of the University of Ulm.

GEOGRAPHIC DISTRIBUTION OF *PLASMODIUM* LINEAGES IN A NORTH AMERICAN PASSERINE HOST

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Avian haematozoa are cosmopolitan in distribution, but the geographic limits to their distribution remain poorly understood. Recent molecular studies have shown that the same haematozoan lineages can occur in diverse avian hosts separated by vast geographic distances. A parasite with a broad avian host range might thus be expected to exhibit no genetic structure across space.

Plasmodium species infect a broad range of avian hosts around the world. We examined cytochrome *b* sequence of *Plasmodium* in several populations of the house finch (*Carpodacus mexicanus*), a common passerine in North America. We found five unique cytochrome *b* haplotypes in 76 infected individuals. One haplotype was common and widespread at all sites, a second was detected only in the eastern populations, and three others were detected in the western populations only. The ubiquitous haplotype differed from the others by 29-34 nucleotide changes, the western haplotypes differed from each other by 1-13 changes and the eastern haplotype differed from the western by 4-12 changes. These data indicate that *Plasmodium* lineages represented by cytochrome *b* haplotypes in a single avian host species differ in their distribution patterns. We find mixed support for our expectation of random distribution of haplotypes across North America: one haplotype is found in all populations in high frequencies while the others are restricted to eastern or western birds.

The genetic differences between parasites in eastern and western host populations are unlikely to be due to host genetic divergence because eastern house finches are derived from a small number of recently introduced western individuals.

PRESENT SITUATION OF PARASITE FAUNA OF RIVER LAMPREY *LAMPETRA FLUVIATILIS* DURING SPAWNING MIGRATION

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The river lamprey is a popular foodstuff for inhabitants of Latvia. From 2003 to 2005 we have studied 165 river lampreys from the mouth of Daugava near Riga Gulf (in September) and from Daugava river near Ogre (in January). Fish were studied by the method of total parasitological examination.

Totally we have detected 10 parasites species. *Corynosoma strumosum* was found in lamprey body cavity in the waters of Latvia for the first time. The river lamprey leads a predatory way of live during its marine period. In this time the lamprey is infected by larvae stages of parasites belonging to Cestoda, Nematoda and Acanthocephala. Some of these parasites can be transit. That is why the lampreys, which were fished out in autumn, had such a various parasite fauna (10 parasite species). Infection by trematodes metacercarians occurs during the fattening in costal zone of Riga Gulf and during the entrance in the rivers. During spawning migration in the rivers the lamprey stops the feeding and its intestine atrophies. We have ascertained 7 parasites species in the lamprey from river.

Our investigation has displayed that lamprey parasite fauna has changed in quantitative and qualitative ratio at present. Approximately 50% of the lamprey from river were free from parasites, and at the same time 30.2% of fish in the beginning of spawning migration were not infected. In the brain of 65.1% of the river lamprey from mouth trematode metacercarian *Diplostomum petromyzifluviatilis* were detected with abundance index 2.8. The lamprey from the mouth had higher prevalence of infection with *Proteocephalus* sp., *Eubothrium* sp., *Corynosoma semerme*, *C. strumosum* and *Cystidicola farionis* than the lamprey from river. The nematode *Cucullanus truttae* was found in each third fish from the mouth with abundance index 3.35. Larva stages of these nematodes were determined more often than imago. Approximately 59% of examined lampreys from river were infected with *D. petromyzifluviatilis* metacercaria and abundance index was the same – 2.7. Nematodes *C. truttae* were determined in the intestine of 22.7% of the river lampreys. Dead incised nematodes' larvae were detected often. Most of the parasite species were found rarely and in low intensity. The corynosomes were detected in intestine in half-digest condition, but there was a case when corynosomes were found in body cavity and also percolate in intestine wall from body cavity side. Grey seal (*Halichoerus drypus*) is the main definitive host of corynosomes in the Baltic Sea.

Prevalence of infection with *D. petromyzifluviatilis* is increased. This study contributes to the information about river lamprey parasite fauna in Latvian waters.

OCULAR TOXOCAROSIS – OPHTHALMOLOGICAL AND IMMUNOSEROLOGICAL EVALUATION IN LATE PERIOD OF INVASION

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Toxocarosis exhibits complex pathogenesis and multiorgan pathology. Frequently, organ of sight is involved and, thus, late detected and untreated toxocarosis leads to inadvertent, stable lesions to ocular bulb. Present report aimed at describing late

pathology in organ of sight due to old time invasion with *Toxocara* spp., immunoserological appraisal and long term observation of the effects of treatment.

In 5 patients multispecialistic clinical evaluation was performed including oculistic USG and electrophysiological tests, ELISA detection of IgG anti-*Toxocara canis* antibodies (E/S antigen, Bordier Affinity Products, Switzerland). Abs titres above 1,200 were accepted to represent positive result. The patients were treated with several courses of albendazol (Zentel, Smith Kline and Beecham, UK). All the patients reported late after vision disturbances had appeared: 10-year-old PD male after 9 months, three patients (38-year-old MM male, 34-year-old DzJ female, 36-year-old TA male) after 1 year and one female patient, SzI, after 2 years. No significant lesions were noted in other organs and no eosinophilia was noted. Preliminary ELISA tests demonstrated in the individual patients antibody titres of 1,750, 1,180, 1,300 and 2,300. Ophthalmologic lesions included retinal detachment, choroiditis, presence of connective tissue membranes, inflammatory reaction in vitreous humor, neuropathy of optic nerve. The lesions were unilateral. In course of three-year observation the patients were treated with 2 to 4 five-day courses of albendazole (10 mg/kg b.w. in the child and 15 mg/kg b.w. in adults), with ophthalmological and anti-inflammatory drugs, including encorton. A slow, irregular decrease of serum anti-*Toxocara* antibody titres was noted with no parallel sight improvement or regression of pathology. In 3 patients surgery is planned due to secondary cataract and in 2 patients vitrectomy will be performed due to pronounced lesions to vitreous humor.

In conclusion, at the late stages of ocular toxocarosis lesions to various structures of the organ of sight were detected. Three-year monitoring and repeated treatment resulted in lowered ELISA titres but no improved vision or regression of late ocular pathology was noted.

AVIAN SCHISTOSOMES OF THE GENUS *TRICHOBILHARZIA* IN FINAL HOSTS IN EUROPE

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Trichobilharzia is the largest genus of the family Schistosomatidae covering over 40 species of avian flukes. Larval stages of the schistosomes represent the most important causative agent of human cercarial dermatitis, which is regarded as an emerging disease. The occurrence of the disease is delineated by the distribution of intermediate hosts (water snails) infected by the schistosomes. The appearance of

infected snails in a particular water reservoir depends essentially on the presence (even transient) of final hosts. However, compared to the data from snails limited information on the distribution of the schistosomes in birds are available.

During 2002 - 2004, various anseriform birds, originating from two European countries with a risk of cercarial dermatitis, were examined for schistosome infections. Parasitological examinations revealed *Trichobilharzia* spp. flukes and eggs.

In Iceland, 20 (41.7%) out of 48 examined birds were found to be infected with visceral species; the parasites were detected in 6 (out of 19 examined) *Cygnus cygnus*, 4 (11) *Anser anser* and 10 (14) *Anas platyrhynchos*. No schistosomes were found in *A. penelope*, *Somateria mollissima*, *Bucephala islandica* and *Mergus serrator* (of each species only 1 bird examined). Nasal schistosomes were exclusively detected in *A. platyrhynchos*; the overall prevalence was 20.8%. Concurrent infections by both types of schistosomes were observed in all infected mallards.

In France, birds were investigated in recreational lakes. In the first one, the Lake of Annecy, a total of 41 (74.5%) out of 55 individuals examined for nasal *Trichobilharzia* spp. revealed eggs and flukes in the nasal mucosa: 2 (out of 2 examined) *C. olor*; 1 (2) *Aythya ferina* and 38 (51) *A. platyrhynchos*. Only 5 out of 51 mallards have been studied for visceral *Trichobilharzia* spp. and one of them was positive. In the Lake Der-Chantecoq and surrounding areas, a total of 99 aquatic birds were examined for nasal or visceral schistosomes. Nasal schistosomes were found in a total of 22 (22.22%) individuals: in 19 (out of 57 investigated) *A. platyrhynchos*, 2 (4) *A. clypeata* and 1 (7) *C. olor*. And, in some of the positive hosts also visceral species were found: 4 (5) *A. platyrhynchos*, 1 (3) *A. clypeata* and 2 (4) *C. olor*. Examinations of the other birds (6 *A. crecca*, 3 *A. strepera*, 3 *Aythya ferina*, 6 *Phalacrocorax carbo*, 3 *Larus ridibundus*, 2 *Anser anser*, 1 *Branta bernicla*, 1 *Podiceps cristatus*, 1 *Fulica atra*, 1 *Gallinula chloropus*, 1 *Ardea cinerea*) were negative.

The results confirm that under natural conditions a number of waterfowl birds play an important role in the transmission of bird schistosomes and if migratory birds are infected they are able to transport different species of the parasites to and from the wintering areas.

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A STUDY OF THE CESTODE FAUNA OF THRUSHES ON THE CURONIAN SPIT

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The information available on the cestode fauna of thrushes on the Curonian Spit is incomplete. The aim of the present study is to obtain additional information on the cestode fauna of these birds.

In all, 142 individuals of birds belonging to 8 species of the Turdidae (Passeriformes) were investigated for the occurrence of helminths by means of the total helminthological dissection and the light microscopy of haematoxylin stained specimens. The material was collected on the Curonian Spit (Biological Station of the Zoological Institute, Russian Academy of Sciences) during spring and autumn migrations in 2003 and 2004. The following cestodes belonging to four families of the order Cyclophyllidea were recorded: Ophryocotylidae (*Fernandezia spinosissima*), Paruterinidae (*Biuterina cordifera*, *Spasskyterina trianguloides*), Hymenolepididae (*Monorcholepis dujardini*, *Passerilepis crenata*, *Variolepis farciminosa*) and Dilepididae (*Dilepis undula*, *Monopylidium passerum*, *Ptilotolepis raymondi*, *Sobolevitaenia moldavica*, *Sobolevitaenia verulamii*).

Fifty birds belonging to four species of the genus *Turdus*: blackbird *Turdus merula*, song thrush *T. philomelos*, redwing *T. iliacus* and fieldfare *T. pilaris*, were investigated; 44 (88%) of them were infected with cestodes. The most common cestode species in the thrushes studied was *D. undula*. In all, 37 (74%) thrushes were infected with this parasite. Such high prevalence of *D. undula* can be explained by the nutrition of thrushes. One of the basic components of their nutrition is earthworms, which are the main intermediate host of *D. undula*. Besides, all four species of thrushes were infected with *P. crenata*. *Monorcholepis dujardini* was found in three species of thrushes (*T. merula*, *T. philomelos* and *T. iliacus*). Two blackbirds were infected with *F. spinosissima*. Six blackbirds were infected with *M. passerum*. One song thrush was infected with *P. raymondi*.

Robins *Erithacus rubecula* (82 birds) and thrush nightingales (*Luscinia luscinia* (3 birds) were mostly infected with cestodes of the family Paruterinidae. *Biuterina cordifera* and *S. trianguloides* were recorded to have low intensity of infection. Robins and thrush nightingales were also infected with *D. undula* and *V. farciminosa*, respectively. It should be noticed that the prevalence of cestodes in robin was much lower (8.5% birds were infected) than in birds of the genus *Turdus* (88%) or thrush nightingales (all 3 investigated birds were infected). The common redstart *Phoenicurus phoenicurus* was infected with *S. trianguloides*. Cestodes were not found only in the whinchat *Saxicola rubetra*, however a few of them were investigated.

In the present study, eleven species of cestodes were recorded. Two species (*F. spinosissima* and *M. passerum*) were recorded in thrushes on the Curonian Spit for the first time. The results of the present study confirm the findings of other authors that the most common cestode species in thrushes on the Curonian Spit are *D. undula* and *P. crenata*. Other cestode species are much rarer.

HOST SPECIFICITY OF AVIAN HAEMOSPORIDIAN PARASITES: LINKAGE BETWEEN MOLECULAR BIOLOGY AND TRADITIONAL DATA

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In spite of numerous records and often high prevalences of avian haemosporidian blood parasites (Sporozoa, Haemosporida) all over the world, only few studies have addressed the level of their natural specificity in birds. However, such studies are important to test evolutionary hypotheses about host immunocompetence, sexual selection, and evolution of virulence. The objective of this study was to combine the PCR-based techniques and light microscopy to determine the distribution of specific lineages of *Haemoproteus* and *Plasmodium* spp. in different passerine birds at the same study site.

The work was carried out on the Curonian Spit in the Baltic Sea (55° 05' N, 20° 44' E) in May-July 2003 and 2004. Birds were captured and blood was taken by puncturing the brachial vein. Blood films were examined microscopically. Approximately 50 µl of whole blood for molecular assays were collected in SET buffer. The nested - PCR protocol was used for amplifying and sequencing a fragment of approximately 480 nucleotides of the cytochrome *b* gene of the mitochondrial DNA of *Plasmodium* and *Haemoproteus* spp. Samples from 243 birds, which were recorded to be positive both by microscopic examination and mtDNA amplification, were used in this study.

We found that *Haemoproteus majoris* (lineages PHISIB1, WW2, and PARU1), *H. fringillae* (CCF5), *Haemoproteus* sp. (WW1), *Plasmodium* (*Haemamoeba*) sp.1 (SGS1) and *Plasmodium* (*Haemamoeba*) sp.2 (GRW11) repeatedly completed their life cycles in birds belonging to different families of the Passeriformes. The lineage PHISIB1 was recorded in warblers *Phylloscopus trochillus* (1 case), tits *Parus major* (3), *P. caeruleus* (1) and flycatchers *Ficedula hypoleuca* (1); WW2 in warblers *Sylvia atricapilla* (8), *S. borin* (3), *S. curruca* (4), *S. communis* (1), *Phylloscopus collibita* (1), *Acrocephalus palustris* (1) and tits *P. major* (2); PARU1 in warblers *Sylvia*

atricapilla (1), *S. borin* (1) and tits *P. major* (29), *P. caeruleus* (13), *P. atrer* (1), *P. palustris* (3), *P. montanus* (2); CCF5 in tits *P. cristatus* (1) and finches *Fringilla coelebs* (10); WW1 in warblers *Acrocephalus scirpaceus* (1) and flycatchers *Ficedula hypoleuca* (1); SGS1 in warblers *Sylvia curruca* (1), *S. communis* (2), tits *P. major* (1), flycatchers *F. hypoleuca* (1), and finches *F. coelebs* (1), *Carpodacus erythrinus* (1); GRW11 in warblers *Sylvia borin* (1), *S. curruca* (1), *S. communis* (1) and in finches *F. coelebs* (1).

Thus, several lineages of *Haemoproteus* and *Plasmodium* spp. are transmitted between birds belonging to different families of the Passeriformes. It seems probable that these parasites may play a complex role in the evolution of their avian hosts, because the host shifting is often associated with a change in virulence. Furthermore, the natural host range cannot be used as a valid taxonomic character in the systematics of haemosporidians.

GENETIC DIVERSITY OF MALARIA PARASITES AND OTHER HAEMOSPORIDIANS (HAEMOSPORIDA, HAEMOPROTEIDAE) FROM BIRDS OF PREY AND OWLS: PRELIMINARY RESULTS

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Only recently molecular methods have been applied to the study of avian haematozoa. With the sensitive PCR techniques, it is easier to detect haematozoan infections than with traditional microscopic examination of blood films, especially at low intensity of parasitaemia. The aim of this study was (i) to examine if the number of morphological described haematozoan species from birds of prey and owls is identical to the number of strains of the parasites detected by the PCR method, (ii) to establish the prevalence of different haematozoan strains, and (iii) to determine if the same strain occurs in more than one host species.

We analysed blood samples from 92 birds of prey of 11 species and 9 owls of 2 species with both PCR and microscopic examination. A nested PCR protocol was used to amplify a sequence of the cytochrome *b* gene of the mtDNA of avian *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* spp.

We found 16 individuals (15.8%) ($n = 101$) positive for haematozoa in the blood films, but 22 individuals (21.8%) were positive using PCR. Fourteen mtDNA-strains of the parasites were recorded.

In the birds of prey, four *Leucocytozoon* lineages were found; one each from Marsh harrier *Circus aeruginosus*, Sparrowhawk *Accipiter nisus*, and two from the

Common Buzzard *Buteo buteo*. In owls, we found three *Leucocytozoon* lineages, one from Tawny Owl *Strix aluco* and two from Long-eared Owls *Asio otus*. Only two *Haemoproteus* lineages were detected, one in the Tawny Owl and one in the Eurasian Kestrel *Falco tinnunculus*. Two new *Plasmodium* lineages were found, from a Marsh Harrier and from a Common Buzzard. Apart from these, we found three *Plasmodium* lineages already known from passeriform birds. These *Plasmodium* lineages were detected in Rough-legged Buzzard *Buteo lagopus*, Common Buzzard, Sparrowhawk, Goshawk *Accipiter gentilis*, and Tawny Owl, indicating that some *Plasmodium* strains span large host ranges.

Our results indicate much higher diversity of the haematozoa in birds of prey and owls than was thought previously.

PRELIMINARY STUDIES ON THE LIFE CYCLE OF *SARCOCYSTIS* SP. FROM THE WHITE-FRONTED GOOSE *ANSER ALBIFRONS*

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Birds belonging to the Anseriformes are intermediate hosts of protists of the genus *Sarcocystis*. From these birds ducks have been investigated best. Little is known about *Sarcocystis* spp. of geese.

The aim of our work was to investigate life cycle of *Sarcocystis* sp. (cyst type III) from the white fronted goose *Anser albifrons*.

Two white fronted geese were hunted on the coast of the Baltic Sea near the Curonian Bay in April 2004 and investigated for the presence of *Sarcocystis* spp. cysts. The intensity of infection was determined by counting cysts in 28 methylene blue stained sections of leg muscles of geese. Morphometric analysis of cysts and cystozoites was made with light microscope. Three 25-30 day old young arctic foxes *Alopex lagopus* were infected by feeding them with leg muscles of the above mentioned geese. Faeces of the foxes were examined daily for the presence of oocysts/sporocysts of *Sarcocystis* spp. and other coccidia. For this purposes flotation with concentrated NaCl solution and direct microscopical examination of faecal material were applied.

The cysts are ribbon-shaped up to 4 mm long and 750 µm wide. On the surface of the wall (up to 2.4 µm) they have teat- or finger-like protrusions. They measured 11.4 x 1.7 (10.0 - 13.5 x 1.5 - 2.5) µm. Cystozoites are almost straight and shuttle-shaped. The foxes started shedding typical 12.0 x 8.0 (10.0 - 12.8 x 6.8 - 8.6) µm *Sarcocystis* sp. sporocysts on 13-14 days post infection. The patent period lasted 19 days.

The arctic fox is a definitive host of *Sarcocystis* sp. (cyst type III) from the white-fronted goose.

ASHWORTHIIUS SIDEMI IN WILD RUMINANTS IN BIALOWIEZA FOREST

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Ashworthius sidemi Schulz, 1933 – a blood-sucking abomasal nematode is a typical, natural parasite of Asiatic deer *Cervus (Rusa) unicolor*, but also for sika deer *Cervus nippon* via which it was introduced into many countries of the former Soviet Union, including Belarus and Ukraine, as well as Slovakia, the Czech Republic and France. As a result of the introduction of sika deer into these countries *A. sidemi* has colonized local ruminant species like maral deer, red deer, roe deer, elk and mouflon. In Poland *A. sidemi* was found for the first time in 4 European bison in Bieszczady Mountains (south-east Poland) in 1997. Farwell examinations showed that all examined wild ruminants (European bison, red deer and roe deer) in Bieszczady are infected with this parasite.

For the investigations abomasum and duodenum were used from European bison shot during selection from free-living herd in Bialowieza Forest. Content of them was decanted in laboratory, and all specimens of *A. sidemi* were taken out and conserved in 70% ethanol with 5% glycerol.

Untill 1999 *A. sidemi* has never been found in any animal in Bialowieza Forest. The first finding of *A. sidemi* infection in European bison in Bialowieza Forest has been noted in all 6 examined bison from Belorussian part of it in 1999 – 2001. In Polish part *A. sidemi* was found for the first time in one bison in 2000. In 2000 – 2002, there were found 7 infected bison of 25 examined (28%), however in 2003, 11 of 12 (92%), and in 2004 all 12 examined bison (100%) were infected. Also all shot this year bison are infected with *A. sidemi*.

It is interesting that intensity of infection is still increasing. In the first group from Belarus the mean intensity of infection was 115 specimens (max. 416), and in Polish part in successive years the mean intensity raised: in 2000 – 2002 it was 29 (up to 80), in 2003 - 1176 (up to 7053), in 2004 - 2374 (up 11913) specimens, respectively. Preliminary results of this year investigations show that *A. sidemi* occur also in 100% of examined animals, and the intensity of infection is still rising about up to 20000 specimens.

PHYLOGENY OF THE FENNOSCANDIAN CERVID STRAIN OF
ECHINOCOCCUS GRANULOSUS

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Echinococcus granulosus is made up of a number of biologically, epidemiologically and genetically distinct entities. These variants have been categorised into genotypic strain groups (genotypes G1-10). Some of these strains will probably be classified as distinct species, and already the horse strain (G4) has been proposed to be a distinct species, *E. equinus*.

In North-Eastern Finland, *E. granulosus* occurs in the life cycle between wolves and cervids. In Sweden, cysts have been sporadically found from reindeer and elk. The *E. granulosus* strain occurring in Fennoscandia was earlier believed to belong to the so-called cervid strain (G8), which has been described from the North-American moose. However, the Finnish *Echinococcus* was recently found to represent a distinct genotype (G10), the Fennoscandian cervid strain. The aim of this study was to genotype a Swedish *E. granulosus* isolate of reindeer origin and to define the phylogenetic relationships of the Fennoscandian cervid strain.

In this study, regions of four mitochondrial genes were sequenced from Finnish and Swedish reindeer hydatid cysts. Phylogenetic analysis of the genes was performed using maximum likelihood (ML), neighbour-joining and maximum parsimony methods.

The mitochondrial sequences of the Finnish and Swedish isolates were found to be identical. In the phylogenetic analysis, the camel (G6), pig (G7), cervid (G8) and Fennoscandian cervid (G10) strains clustered in a well-supported monophyletic group. Inside this group, G6 and G7 formed an independent evolutionary lineage that seems to have diverged quite recently from the G8 and G10 genotypes. In the ML tree, G10 was found to be evolutionary very close to the hypothetical ancestor of this group. The G6-7-8-10 group differs clearly from the common sheep (G1) and horse (G4, '*E. equinus*') strains. Thus, it obviously constitutes a distinct taxonomical unit. Based on morphological descriptions from the 1960's, concerning forms of *Echinococcus* found in cervids, the correct name for this G6-7-8-10 species would be *E. canadensis*.

IDENTIFICATION OF *GIARDIA* GENOTYPES IN SWEDISH ANIMALS USING A PCR-RFLP ASSAY

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Giardia lamblia is a major cause of diarrhea in humans and animals worldwide. Molecular techniques are useful for studying the zoonotic potential of *Giardia* isolated from animals. In the present study a PCR-RFLP assay based on the beta giardin gene was used to characterize the *Giardia* isolates. Subsequent sequencing of the PCR product was performed on a limited number of samples. *Giardia* cysts were detected by microscopy performed on fecal samples from different Swedish animals (cats, dogs, cattle, sheep, one elk and one pygmy marmoset monkey, n=63) and the DNA was extracted after cyst concentration on a sucrose gradient. Five dog isolates revealed the dog specific Assemblages C (n=1) and D (n=4). Two of the investigated cats had the feline specific Assemblage F, while two had Assemblage A. The only investigated monkey had Assemblage B. Typical RFLP banding patterns for Assemblage E were found in all 13 isolates from cattle and in 19 isolates from sheep. Four isolates from sheep demonstrated patterns indicating mixed infections. Among the remaining sheep isolates the human genotype A1 was detected in one case, while 15 isolates showed two different atypical banding patterns. DNA sequencing analyses revealed that these new patterns were consistent with Assemblage A (n=10) or E (n=5). The investigated elk sample also showed this new Assemblage A pattern. Only four isolates displayed patterns identical to human Assemblages A or B. The novel A subgenotype here identified in 10 sheep and one elk should be further investigated and its zoonotic potential determined.

THE SEASONAL DYNAMICS OF FISH TREMATODA FAUNA IN LADOGA LAKE

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Investigations of dynamics of helminths in fishes are significant for understanding of the parasite transmission in the life cycle. It is also important for knowledge on the population biology of the parasite within the host. The seasonal occurrences of many species of helminths in freshwater fishes have been described.

The aim of present paper was to study seasonal dynamics of population abundance and age structure of trematoda *Sphaerostomum globiporum* Rudolphi, 1802 in roach, *Rutilus rutilus* in Ladoga Lake.

Fish, 313 specimens in total and minimum 21 specimen monthly, were caught in gill nets in south-eastern part of lake during 2002-2003. Maritas collected were divided into 6 age stages depending on development of reproductive system.

Sphaerostomum globiporum occurred in more than 50 % of roach. The data on this species occurrence indicated clearly seasonal character of recruitment and maturation. The curve of *S. globiporum* incidence in roach was described as: lack of parasites in August and September, then increase of infestation until spring and decrease in infestation in June and July. The invasion of fluke begun in October, and only young stage worms were founded. Eggs production started in the end of January. Gravid trematodes with a few eggs in uterus were appeared. Fully mature parasites composed a big part of the samples collected in April and May. Number of trematodes was the highest in spring and minimal population density was observed in summer. Extinction of subpopulation of adult digeneans occurred in July although single specimens could survive longer. Such old trematodes with a few eggs in uterus and destroyed reproductive system were recorded in October.

Results obtained are similar to data on parasite seasonal dynamics in Northern Finland water bodies. The reason is likeness of Ladoga Lake hydrology to Finnish ones because all of them are large, oligotrophic and coldwater pools.

Thereby, we have concluded that *S. globiporum* life cycle in Ladoga Lake took up about one year. Both trematoda maturing and reproduction period were about 5 months. Although these two periods of life cycle were not divided obviously in time, there was not overlapping of two different generations in roach.

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KUDOA HISTOLYTICA CAUSING *POST MORTEM* MYOLIQUEFACTION (SOFT FLESH) IN NORTH SEA MACKEREL

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Many myxosporidian species are known from commercially important fish of the Northeast (NE) Atlantic. While most species seem to be rather non-pathogenic and apparently do not affect the quality of the fish flesh, the multivalvulide species *Kudoa histolytica* may strongly reduce the fillet quality of North Atlantic mackerel. However,

information on the occurrence and distribution of *K. histolytica* in the NE Atlantic is scarce.

During routine parasitological examination of North Sea mackerel in 2003, the flesh of several specimens had a soft, jelly-like appearance, which was initially attributed to poor storing conditions onboard of the fishing vessels. However, upon microscopic investigation of fresh muscle smears, innumerable spores of a *Kudoa* species were found. Based on the morphology and morphometry of taxonomically important spore characters, the species was identified as *Kudoa histolytica*. Subsequent histological examination revealed numerous polysporic plasmodia in both the white and red muscle of heavily infected mackerel.

During September and October 2004, 215 newly landed mackerel caught in the northeastern North Sea were examined for *Kudoa* spp. Only 5 fish which all belonged to the largest size group (> 600 g, $n = 62$) were found to be infected, representing 2.3 % and 8.1 % of the total sample and the particular size group, respectively. However, not all of the infected mackerel showed the typical soft flesh symptoms. Thus, as light infections are easily overlooked during routine investigations, a higher prevalence of *K. histolytica* in North Sea mackerel than found in this study seems to be likely. Preliminary analyses indicate that the parasite's proteolytic enzyme release is strongly correlated with the intensity of infection and the temperature during storage and transport of the fish. Thus, when deep-freezing infected mackerel fillets for several months, the enzymatic degradation process seems only to be dormant as it restarts immediately after thawing. Further aspects of the taxonomy and proteolytic enzyme release of *K. histolytica* will be discussed.

WEB-BASED VIRTUAL MICROSCOPY FOR THE DETECTION OF HELMINTH EGGS AND PROTOZOA: A NOVEL TOOL FOR EDUCATION AND QUALITY ASSURANCE IN THE PARASITOLOGY LABORATORY?

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Virtual microscopy, recently introduced as a tool for education in histopathology, has now been adapted for the purpose of making available specimens containing commonly encountered stool and blood parasites (<http://www.webmicroscope.net/parasitology>). The study was motivated by the observed extensive over-diagnosis of *Entamoeba histolytica* /*dispar* at health centers in León, Nicaragua. These virtual

specimens, containing cyst and trophozoite forms of protozoa as well as helminth eggs, may be used as a teaching aid for self education and educational seminars at the basic level, identification parasites in stool and blood samples at health centers by microscopy.

We also see the web microscope as an educational tool for distribution of rare parasitological specimens and a method overcoming several problems encountered in large-scale quality control in diagnostic parasitology.

PARASITES IN PARADISE: THE PARASITE FAUNA OF SMALL GROUND FINCHES (*GEOSPIZA FULIGINOSA*) IN FOUR ISLAND POPULATIONS

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Introduced infectious diseases can cause population declines and species extinctions in endemic island populations. This has become clear from studies performed on Hawaii, where introductions of avian pox and *Plasmodium* sp. have contributed to the extinction of endemic bird species. This study was performed on a similar island ecosystem, the Galapagos islands, where few studies have focused on avian parasites.

The objective of this study was to identify some of the present parasite fauna of small ground finches (*Geospiza fuliginosa*) and to investigate how parasites were distributed across island populations. We sampled 400 live small ground finches from four island populations, investigated birds for the presence of ecto-, endo- and blood-parasites. From each bird, we visually estimated the load of ecto-parasites, recorded the presence of avian pox symptoms and collected blood samples. For a subset of birds, we used a dust-ruffling technique to collect ectoparasites, and we collected fecal samples to identify intestinal parasites.

Avian pox infections were common, and in the most exposed population (70 %) of the birds showed symptoms of previous infections. We screened blood samples of 40 individuals from the most parasite rich population for presence of *Plasmodium*, *Haemoproteus*, *Leucocytozoon* and *Atoxoplasma* spp. with molecular markers. None of the 40 individuals tested were positive for *Haemoproteus*, *Plasmodium* or *Leucocytozoon* spp., but we found a high (81 %) prevalence of *Atoxoplasma* infections. From the dust-samples we identified four common species of avian mites, and an additional four species represented by single collections. From the fecal

samples we recovered three species of *Isospora* sp. *Isospora* sp. oocysts were almost exclusively secreted in the afternoons, and the infection prevalence of *Isospora* sp. in these samples was high (61%).

Our data showed that abundance of the most common parasites increased with the size of the study islands. This could be because the transmission of contact-transmitted diseases increased with host population size. The parasite load of the most abundant avian mite also increased with host body size. The apparent absence of *Plasmodium* sp. could indicate that vector-transmitted avian disease has not been established on the Galapagos. Still, since several of the parasites that were found in this study could have negative impact on their avian host population, further research is needed to evaluate the demographic effect and colonization history of these avian parasites.

DISTRIBUTION OF *ANISAKIS SIMPLEX* LARVAE IN NORWEGIAN SPRING SPAWNING HERRING AND NORTH SEA MACKEREL

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Norwegian spring spawning (NSS) herring *Clupea harengus* and mackerel *Scomber scombrus* are among the commercially most important fish species in the Norwegian economic zone. During recent years there has been increased focus on the presence of nematode larvae in several important export markets for both fish species. Thus, it is important for the pelagic industry as well as the Norwegian Food Safety Authority to continuously obtain knowledge on the occurrence and distribution of such larvae through the entire fishing season. Such data were obtained by NIFES during 2003 and 2004, some of which will be presented here.

NSS herring were sampled each year at the wintering area in the Vestfjord basin in October, and at the southern spawning grounds off the coast of Møre og Romsdal in February. Several lots of mackerel were caught in the north-eastern North Sea in the period from August to November both years. Examinations were carried out on fresh samples, i.e. unfrozen and newly landed fish. Separately for each species, the fish were measured, weighed and categorized by size. Subsequently, the presence and site distribution of the nematode larvae were recorded. The visceral organs including the swim bladder (which is absent in mackerel) and the mesenteries were removed and visually examined for nematode larvae under a dissecting microscope. The remaining of each fish was examined by visual inspection followed by complete degradation of the soft tissue in an acidic pepsin solution.

All larvae found in this survey belonged to the *Anisakis simplex* complex. In herring, a significant increase in total *Anisakis* burden with increasing body size was found. In contrast, a weak but significant decrease in total *Anisakis* burden with increasing body size was observed in mackerel. For both fish species the majority of

larvae resided on the visceral cavity organs. However, the prevalence of *Anisakis* larvae in the flesh varied considerably between the size groups of each species, i.e. from 15 to 60 % in herring and 32 to 77 % in mackerel. The inter- and intraspecific differences in *Anisakis* infection may be due to physiological, immunological and/or ecological characteristics of each species. Some possible fish host-parasite regulatory mechanisms in each of the two species will be discussed.

PREVALENCE OF *CRYPTOSPORIDIUM* AND *GIARDIA* SPP. IN DIFFERENT AGE GROUPS OF CATTLE AND SWINE IN DENMARK

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Until recently, little was known about the prevalence of *Giardia* and *Cryptosporidium* spp. in Danish livestock, except that both parasites occurred in Danish cattle, whereas minimal information was available regarding pigs. Similarly, little knowledge existed concerning the possible influence of different management systems on the occurrence and intensity of infection. Hence, in 2003 an epidemiological survey was initiated including 50 dairy farms and 50 sow herds, which each were visited once for the collection of faecal samples and for registration of basic management parameters as well as hygiene levels in the surroundings of the examined animals.

The farms were selected at random via a central farm registration system and are located throughout Denmark. On each farm faecal samples were collected from different age groups of animals, i. e. 5 sows/cows, 10 calves/piglets less than 1 month and 10 calves 3-9 months or 10 pigs 8-30 kg. The faecal samples were purified by a flotation/centrifugation technique and the number of cysts/oocysts quantified microscopically following staining with Crypto-Giardia CEL (Waterborne, Australia) immunofluorescence kit. Subsequently, DNA was extracted from the (oo)cysts from 5 animals per herd for genotype determination to enable assessment of the zoonotic potential of the protozoans, results of which are reported elsewhere.

The results show a herd prevalence of 98-100% and individual prevalence of approximately 33% for *Cryptosporidium* sp. in both animal species and for *Giardia* sp. in cattle, but 85% herd prevalence and 20% individual prevalence of *Giardia* sp. in swine. Management parameters, e. g. in relation to intensity of parasite excretion and occurrence of diarrhoea, are being analysed statistically while still awaiting the final results of genotyping. However, results from initial regression analyses demonstrate a very large variation in excretion (levels) of *Cryptosporidium* and *Giardia* spp. between individuals in the same herd. This implies that individual factors have great influence on the excretion from each animal, whereas herd related factors may have less influence. The widespread occurrence of both parasites in both livestock species

gives rise to further questions regarding importance in relation both to animal health and zoonotic potential, which we hope to examine in the future.

ETHNOVETERINARY MEDICINE PRINCIPLES IN ANIMAL PARASITE CONTROL

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Integrated animal healthcare takes into consideration the diversity of livestock keeping and the environment. It combines animal treatment with disease prevention and improved management. Herbal medicine is one of the alternative therapy disciplines and is one of the areas of ethnoveterinary medicine. As herbal medicine is the oldest form of healthcare known to mankind, several plants with insecticidal or nematicidal properties have been used for medical purposes throughout history. Most insect repellents and parasite reproductive inhibitors have been extracted from certain plants in last decade and used for treatment of parasitic diseases. These extracts affect the feeding behaviour and life cycle of parasites and are safe both for human beings and animals. Moreover, using extracts of medicinal plants occurs to be economical because most of them can be made at home.

To test antiparasitic activities of several domestic plants against the swine digestive tract nematodes *Oesophagostomum dentatum* and *O. quadrispinulatum*, investigations were made in swine trial groups to compare the effect of pumpkin (*Cucurbita pepo*) seeds, tansy (*Tanacetum vulgare*) and lambsquarter (*Chenopodium album*) herbs, sweet flag (*Acorus calamus*) rhizome and garlic (*Allium sativum*) bulbs. Clinical evaluations and faecal analyses were done weekly, using the modified McMaster egg count method and observations continued up to 2 months after treatment. Individual faecal samples from each pig were examined microscopically to determine the number of parasite eggs present. Farm trial was finished in autumn with worm recovery: the agar-gel migration technique was applied to determine the number of parasites in the intestinal contents.

Post-treatment examination showed that the number of excreted eggs (EPG) in faeces and the final infection level (the total number of worms) were significantly lower (up to 75 – 97%) in treated pigs, and a great improvement of pigs was noticed during the experimental period. Sweet flag rhizome occurred to be the most effective natural drug in farm conditions against swine parasites of *Oesophagostomum* spp.

The results of our investigations in pig herds on the Estonian small and large-scale farms led to suggestion that plant products may be used in future as alternatives to synthetic drugs against internal swine parasites. On the basis of such studies it would

be possible to work out new biological parasite control measures and plants, containing antiparasitic compounds require more attention and further investigation.

EPIDEMIOLOGY OF TRICHINELLOSIS IN LITHUANIA DURING 1990 - 2004

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In Lithuania, the annual incidence of human trichinellosis decreased from 21.8/100000 inhabitants in 1992 to only 0.6/100000 during recent years, but still the disease remains an important food-borne parasitic zoonosis. Considerably increased findings of *Trichinella* spp. infections in domestic pigs and wild boars in Lithuania coincided with marked socio-economical changes in the country during late 1980s and the beginning of 1990s. Prevalence of pig trichinellosis increased from 0.0027% during 1981-1985 to 0.1% in 1993 and outbreaks in big commercial pig farms did occur. Infected pork became the main source of human trichinellosis causing approx. 80% of all cases. Situation improved in late 1990s, when trichinellosis started gradually decreasing and reached the present level (0.004% approx. 1 mill. pigs slaughtered and examined). Presently, trichinellosis in pigs occurs only in small households where usually only few pigs are kept and such farms are identified to be the ones where along with *T. spiralis* transmission the sylvatic *T. britovi* is also present. Despite improved situation infected pork remains the main source of human trichinellosis, responsible for approx. 55% of all human cases. Wild boar meat is less important source of human trichinellosis, although after wild boar trichinellosis became more prevalent in beginning of 1990s it remained at fluctuating but high levels (0.7 – 1.8%) until today and human outbreaks (e.g. 69 cases or 65% of all human cases in 2001) due to wild boar meat still occur. Unfortunately, each year the source of infection for 10 – 42% of human cases remains unknown.

Recent wildlife surveys in Lithuania showed very high prevalence (40 – 50%) of *Trichinella* spp. in red foxes *Vulpes vulpes* and raccoon dogs *Nyctereutes procyonoides*. This high prevalence most likely is linked to increased wildlife populations and improper hunting practices. Importantly, it appeared that raccoon dogs have significantly higher larval burdens than foxes indicating its important contribution to the overall transmission of *Trichinella* spp. biomass. Future spreading of these omnivore animals westwards may have considerable implications on epidemiology of *Trichinella* spp. in Europe. Molecular analysis of *Trichinella* species revealed transmission of the domestic *T. spiralis* to wildlife in areas where domestic

trichinellosis is present. In Lithuania in contrast to central and southern Europe wild boars *Sus scrofa* are more frequently infected with sylvatic *T. britovi* than *T. spiralis* indicating important impact of cold climate on ecology of the later.

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IMPORTANCE OF STUDIES CARRIED OUT IN THE ECHINORISK PROJECT FOR THE RECOGNITION OF THE EPIDEMIOLOGICAL SITUATION OF ALVEOCOCCOSIS IN SCREENED AREAS IN POLAND

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The recognition of an increasing prevalence of *Echinococcus multilocularis* in red foxes *Vulpes vulpes* in Poland has stimulated interest in alveolar echinococcosis (AE) in humans. The registration of AE in human in Poland started in 1992. At that time *E. multilocularis* was not known as parasite of the red fox in the country. Till 2002, 20 well documented human cases were recorded. To date, 32 patients are recognized to be infected. The first case of *E. multilocularis* in red foxes was detected in Poland in 1994. During further studies in 1995–2000 among about 3500 foxes examined in approximately of 90% territory of Poland, 2.8% were found to be infected. The highest prevalence was revealed in the north-east and the south-east of the country.

According to the program of the project (grant QLK2-CT-2001-01995 - "Risk Assessment and Prevention of Alveolar Echinococcosis" – EchinoRisk) red foxes from three regions of Poland: Pomerania, Warmia-and-Mazuria (north-east) and Carpathian Foothill (south-east) were examined. Between October 2001 and June 2004, a total of 1514 foxes were investigated by the intestinal scraping technique (IST). The mean prevalence of *E. multilocularis* infection was 23.8%. The prevalence in Pomerania, Warmia-and-Mazuria and Carpathian Foothill was 8.0%, 39.6% and 36.8%, respectively. In some counties more than 70% of examined foxes were found infected.

Comparison of the results in the EchinoRisk project with those achieved previously has shown a strong increase of *E. multilocularis* infection in foxes in the screened regions. It is important to mention that during last ten years the population of foxes in Poland grew up rapidly from 67,000 (in 1995) to 174,000 (in 2004).

The obtained data emphasize the importance of investigations carried out in the EchinoRisk project on the recognition of AE risk regions for human in Poland. There is a need for cooperation of veterinary and medical services in order to define the areas for screening action in human. An increase in the prevalence of infection in foxes needs careful considerations and further studies.

SOME ASPECTS OF *ECHINOCOCCUS MULTILOCULARIS* INFECTION IN HUMANS IN LITHUANIA

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For a long time alveolar echinococcosis (AE) has been a very rare human disease with various clinical syndromes mostly observed only in outcome stages in Lithuania. In last years national CDC represents an incidence rate of this infection of 0.01/10,000 population. The aim of this research is to analyze and present the epidemiological, clinical, laboratory and therapeutic characteristics and outcomes of alveolar echinococcosis in Lithuania during the period of 1998-2005.

Patients with confirmed alveolar echinococcosis were analyzed. Diagnosis was established by immunoenzyme assay, WB, morphological and instrumental methods. Statistical analysis of epidemiological and clinical data was performed, and diagnostic and treatment peculiarities were analyzed.

In all, 47 new cases of AE were diagnosed and confirmed during this that period. High rate of hydatid disease caused by *E. multilocularis* has been observed in northwest (three cases were diagnosed even in the same village) and northeast of Lithuania. Most probably they have been accidentally infected with contaminated vegetables and berries and during contact with infected animals obtained by hunting. Data about prevalence the *E. multilocularis* in foxes in Lithuania are absent.

AE primarily affected the liver and typically demonstrated characteristic imaging findings in all cases; only renal involvement together with hepatic localization was diagnosed in two clinical cases. Results of routine laboratory tests were not helpful. Eosinophilia was not detectable. Echinococcal antibodies were positive in mostly cases. CT and ultrasonoscopy have proved to have good sensitivity and specificity in the detection of AE. AE diagnosis always was confirmed by morphological methods too.

Two thirds of patients were operated due to local complications, 1/3 of patients were operated radically and antiechinococcal antibodies on 2-3 months after operation were not detectable. New surgical methods in the treatment of AE are discussed.

The present data suggest a new situation in suspected endemic morbidity of AE in Lithuania. AE appears to be an important social and medical problem required essential efforts for sanitation of humans and animals. Early diagnosis of AE accompanied with radical surgery may lead to complete recovery from this lethal

parasitosis. According to the European Echinococcosis Registry requirements, we established the Lithuanian variant of Echinococcosis Registry.

HATCHING OF PARASITIC NEMATODES OF GENUS *GLOBODERA* IN POTATO ROOT DIFFUSATES UNDER DIFFERENT INFECTION CONDITIONS

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Potato cyst-forming nematodes (PCN) are included in a list of 10 highly harmful nematode genera caused significant losses of agriculture in many world countries. PCN life cycle is closely synchronized with potato growth. Mass juvenile emergence from cysts is possible only after stimulation with plant root diffusates. Because stage of hatching and penetration into young host roots is the most vulnerable in nematode life cycle attention to search on control measures for nematode abundance must be concentrated especially on this stage.

In 1998-2003 investigations on juvenile hatching of two PCN species *Globodera rostochiensis* (Wollenweber, 1923) Behrens, 1975 and *G. pallida* (Stone, 1973) were carried out. Data on influence of cultivar resistance, plant age, presence of other pathogens, heavy metal salts, wastes of paper-making industry on hatching process were obtained.

Different concentrations of one cultivar root diffusates determined the changes of hatch terms and juvenile abundance. Peculiarities of cultivar root diffusates in their influences on hatching process and different behavior of local PCN populations in root diffusates of the same potato cultivars were pointed out. Potato resistance to PCN did not affect the ability of cultivars to stimulate hatching of juveniles.

Under optimal conditions only 2/3 of juveniles emerge from cysts, the rest of them remain in dormancy and are able to hatch in next year. It was established that even small doses of root diffusates from young plants caused increase in hatch due to the activation of dormant juveniles. The highest effect was revealed for root diffusates of cultivars possessed both fully (*G. rostochiensis*) and partly (*G. pallida*) resistance.

Freshly-crushed conifer barks and wastes of paper-making industry influenced hatching negatively. Both delay of the process coming and decrease in total juvenile abundance were observed. Results obtained allow considering wastes of logging and wood industry as effective methods of *Globodera* spp. control.

Investigation on influence of heavy metal salts [$Pb(NO_3)_2$ and $ZnSO_4$] in combination with root diffusates has shown that low concentrations (Pb - 0.1 mg/l, Zn - 1, 10 mg/l) stimulated juvenile hatch: nematode quantity exceeded control 2 folds while high concentrations (Pb - 2.5 mg/l, Zn - 25 mg/l) did not activate nematode hatch.

EXPERIMENTAL INVESTIGATIONS ON SURVIVAL OF PHYTOPARASITIC NEMATODES UNDER INFLUENCE OF DIFFERENT FACTORS

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Survival of potato cyst-forming nematode (PCN) *Globodera rostochiensis* (Wollenweber, 1923) Behrens, 1975 was investigated experimentally under various initial parasite quantity, infestation of different on resistance potato cultivars, combination with other pathogens and applying of wastes of logging and wood industry.

It was established that initial nematode population density determined the host response to the invasion and the level of intrapopulation regulators of parasite abundance. The survival and nematode reproduction capacities were maximal under low infection level (2500 eggs and juveniles per plant). Under high infection levels (12500-25000 eggs and juveniles per plant) multiplication rate, female sizes and total number of eggs and juveniles inside newly formed cysts were considerably reduced. It was shown an availability of intrapopulation regulators of parasite abundance, which depends on initial infection dose and leads to decrease in nematode survival, reproduction potential and fecundity under high infection density.

Under investigations on interspecies relations between potato cyst-forming nematode and virus and fungus infections it was revealed that X and Y viruses, fungus *Rhizoctonia solani* Kühn in different combinations negatively influenced nematode development. Female quantity, total number of eggs and juveniles per 1 cyst and multiplication rate had the lowest values under complete pathogen complex due to the concurrent interactions and decline of plant-host state. Effect of fungus on nematode was the same on susceptible and resistant potato cultivars.

Applying of freshly-crushed conifer barks and wastes of paper-making industry in soil caused decrease in the number of cysts, total number of eggs and juveniles per 1 cyst and their viability. Moreover change of sex structure of the *Globodera* population such as increase in percent of male was recorded. It is possible that this change is consequence of decline of parasite development conditions.

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RESULTS OF APPLICATION OF THE INFORMATION SYSTEM IN PARASITOLOGICAL RESEARCH

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The further development of faunal and taxonomic researches depends to a large extent on efficient means of analysis of the ever growing data array. The new possibilities in the study of correlative relations of the structural characteristics, distribution and host-parasites relations of fleas can be realized by means of the information analytical system (IAS) PARHOST1 on the world fauna of the order (address in the Internet //www.zin.ru/ Animalia/Siphonaptera/). This system has been developed in the Laboratory of Parasitology of the Zoological Institute of the Russian Academy of Sciences since 1995. Using the IAS PARHOST1 system distribution of 1835 flea species in 8 regions and 26 subregions has been studied; their host relations with 1644 species of mammals and 544 species of birds have been elucidated. The results of studies of the distribution patterns and host-parasite relations of the flea order were published in a series of articles.

On the basis of the data of morphological studies of structures of the different tagmae of flea body a multilevel classifier of flea skeletal structures and their elementary (irreducible) structural characters was developed for the first time. A table of complex characters and a table of distribution of their states by flea taxon were created. The latter table contains to date 123,000 records comprising 20.2 Mb. A study was performed on distribution of 1) different states of characters of structure of three tagmae of body by flea taxa, 2) carriers of these states of flea species and genera by species and genus of hosts, and also by zoogeographic region. The methods of non-parametric statistics were used to assess the relationship of structural characters in taxonomy of fleas and their hosts, geographic distribution and also the influence of the structural characters on the expansion to taxa of hosts.

The indices of adaptive value of the characters (in terms of expansion of a flea taxon to host species and genera) are higher than the chi-square values, assessing the interrelationship between the same characters and taxonomy of fleas. Geographic distribution is also related to the morphological characteristics, but to a lesser extent than the influence of hosts. Some groups of states of the head, thorax, and abdomen having the highest values of chi-square, irrespective of the methods of calculation, have been revealed. In all probability their formation was determined to a greater extent by the co-evolutionary relationships.

BINDING OF COMPLEMENT CLASSICAL PATHWAY REGULATOR C4BP BY
ADULT WORMS OF *SCHISTOSOMA MANSONI*T. MERI¹, T.S. JOKIRANTA¹, A. BLOM², C. THORS³, E. LINDER³ & S. MERI¹¹*Haartman Institute, University of Helsinki and University Central Hospital of Helsinki, Finland*²*University Hospital Malmö, University of Lund, Sweden*³*Swedish Institute for Infectious Disease Control (SMI), Sweden*

Schistosoma mansoni is a trematode worm that lives in human mesenteric and portal veins and causes schistosomiasis. The adult worms can survive intravascularly in direct and continuous contact with the human defense mechanisms for years. The complement (C) system is an important part of innate immunity and can opsonize targets for phagocytosis and cause damage by making cytolytic pores to nonprotected surfaces. The activation is initiated either through surface recognizing antibodies (classical pathway; CP) or through nonspecific binding of C3b to the surface (alternative pathway). *Schistosoma mansoni* worms have previously been shown to activate both the classical and the alternative pathway.

In this study we found that adult *S. mansoni* worms are able to acquire the main host CP regulator C4b-binding protein to their surfaces. Live worms were incubated with ¹²⁵I-C4BP and the amount of bound protein was quantified. ¹²⁵I-C4BP bound clearly to the worms, ¹²⁵I-FH, the main regulator of the AP, did not bind at all. Localization of the bound ¹²⁵I-C4BP was analyzed by in situ-type of autoradiography from 5 µm frozen sections of the worms. C4BP bound to the surface tegument of the worms after a 30 min incubation. Localization of the acquired C4BP to the worm surface was verified by immunofluorescence-staining after incubation in non-immune serum or purified C4BP. The worm-bound C4BP was shown to be functionally active in a cofactor-assay.

In conclusion, *S. mansoni* was shown to bind human C-regulator C4BP to its surface tegument from serum and also when purified protein was used. The bound C4BP could promote inactivation of C4b and thus downregulate CP activation. These results suggest that *S. mansoni* worms exploit host C4BP to evade C attack inside blood vessels.

TRICHINELLA INFECTION IN FARM FUR-ANIMALS IN ESTONIA

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Trichinellosis is widely distributed in wild carnivorous animals in Estonia. The objective of the present study was to evaluate the situation in farm fur-animals.

Material was collected from 8 fur bearing farms. Total number of fur-animals examined for *Trichinella* spp. was 281: 103 blue foxes *Alopex lagopus*, 128 silver foxes *Vulpes vulpes fulva*, 35 minks *Mustela vison* and 15 raccoon dogs *Nyctereutes procyonoides*. Eighteen brown rats *Rattus norvegicus*, caught on the territory of Hiiumaa farm, were examined too. Samples (5 - 10 grams) from different muscles were taken and examined using HCl-pepsin digestion. The prevalence of infection and the number of *Trichinella* larvae per gram of muscle tissue (LPG) were evaluated. Species of *Trichinella* were identified by RAPD analysis in the *Trichinella* Reference Centre. An investigation of 190 samples from feed-briquettes for fur-animals was carried out too.

In 2 (on the island of Hiiumaa and on the mainland) out of 8 fur bearing farms *Trichinella* spp. were detected. The overall prevalence of infection in fur-animals was 7.5% (21 out of 281). Only basic blue foxes, 11 out of 21 examined were infected in the island farm with average rate of 5.6 LPG (range 1.0—19.0). The higher worm burden was found in front limb muscles: 8.6 LPG (0.1—19.0), followed by the masseter muscle: 6.0 LPG (4.0—8.0), the temporal muscle: 3.8 LPG (3.0—7.0) and diaphragm: 3.6 LPG (2.0—6.0). Two brown rats out of 18 were infected; the worm burden was high (33.0 and 90.0). No *Trichinella* larvae were found in the pork leavings briquettes. In one farm on mainland *Trichinella* spp. were diagnosed in basic blue foxes, in one basic silver fox and basic minks. Four blue foxes out of 12 were infected with an average intensity of 4.0 LPG (1.0—10.0), 5 minks out of 15 4.8 LPG (1.0—9.0) and the silver fox 2 LPG.

Trichinella spiralis was identified in blue foxes and brown rat in the farm on the island. In blue foxes and silver fox in the mainland farm *T. nativa* was determined. Formerly only *T. britovi* was found in wild animals on Hiiumaa and *T. britovi* and *T. nativa* on mainland. Probably blue foxes in the island farm have got infection from rats. Infected rats may drop into feed breaker and skinned carcasses of fur-animals are sometimes easily accessible to rats. Often skinned carcasses are fed to animals of the same farm. The finding of *T. nativa* in farm fur-animals in the mainland farm demonstrates the transmission of sylvatic species into domestic cycle. The reason for this is the use of raw or undercooked wild animal carcasses as food for farm fur-animals.

Fur-animals in some farms were infected with *Trichinella* spp. due to human improper behaviour and insufficient measures for avoiding the infection.

HELMINTHS OF BEAVER (*CASTOR FIBER*) AND THEIR POPULATION REGULATION IN LITHUANIA

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Seventy years ago beaver *Castor fiber* was an endangered or even extinct species in many countries of Europe and Asia due to overhunting. The last of the beavers of the original Lithuanian stock was extirpated in 1938. Between 1947 and 1959 beavers were reintroduced and/or translocated in Lithuania. The population of Eurasian beaver currently is abundant in Lithuania.

Beaver helminths were investigated in Lithuania in 2001-2004. We dissected 37 beavers. The beavers were hunted in: Molėtai, Šilutė, Klaipėda and Jurbarkas districts.

All investigated beaver individuals were infected by two parasite species: fluke - *Strichorchis subtriquetrus* [infection intensity ($I_{\min} - I_{\max}$) – 6 – 314; abundance of helminths (\bar{X}) – 82.54; infection prevalence (E (%)) – 100%; variance (S^2) – 6675.81] and nematode - *Travassosius rufus* [($I_{\min} - I_{\max}$) – 2 – 1671; \bar{X} – 319.2432; E (%) – 100%; S^2 – 152256.98]. Both species of helminths are specific parasites of the beaver.

We analyzed quantitative dependence between fecundity – mean eggs number per female of *T. rufus* and number of the parasite in beaver individuals. The negative relation ($r = -0.52$, $p = 0.0092$) between these variables indicates the regulation of *T. rufus* population at beaver individual level.

This study was supported by the Lithuanian Science and Studies Foundation, project Nr. K-041.

LONG-TERM SURVEY OF THE *ECHINOCOCCUS MULTILOCCULARIS* DISTRIBUTION IN THE SLOVAK REPUBLIC

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Detailed epidemiological survey of the *Echinococcus multilocularis* tapeworm occurrence and distribution in red foxes in the Slovak Republic was carried out in the period from 2000 to 2004. The main aim was to find out the rate of prevalence and intensity of infection in individual districts and regions of the country. The second goal was to identify potential risk areas and to determine risk factors influencing the disease spread and the transmission.

More than 3000 red foxes from all regions of Slovakia were captured and examined for infection with *E. multilocularis* using necropsy and coproantigen detection. Chi-squared test was used for compare prevalence between years. Climatic and environmental factors were taken into account in explanation of great regional differences and between-year prevalence fluctuation.

The prevalence of infection increased from 24.8 % in 2000 to 32.8 % in 2002. During the year 2003 and spring/summer season 2004 significant decrease of the prevalence together with decrease of the infection intensity was recorded in the whole territory of the country. Consequently, prevalence repeatedly increased from 13.8 % in spring/summer season 2004 to more than 30.0 % in winter 2004. During the whole surveyed period, the highest prevalence rates as well as the highest mean worm burden were always recorded in northern parts of the Slovak Republic, in mountainous areas with low mean annual air and soil surface temperature and high mean annual rainfall. Some relations between the infection rate of *E. multilocularis* in red foxes and the small mammals' abundance were observed. Changes of the climatic factors seem to be also very important for the prevalence fluctuation.

Our findings show that areas with permanently high prevalence and intensity of *E. multilocularis* infection exist in the territory of the Slovak Republic. The contamination of environment with parasites' eggs is considerable in these areas and represents high risk infection for human.

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COMPARATIVE DYNAMICS OF THE ANTIGENS AND ANTIBODIES IN SHEEP EXPERIMENTALLY INFECTED AND REINFECTED WITH *FASCIOLA HEPATICA*

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The aim of this study was to analyze dynamics of antigens and antibodies in sheep infected and reinfected with metacercariae (mc) administered by a trickle system (small repetitive infections with 25 mc per animal during 7 consecutive days), which are similar to those that often occur in natural infections.

Twenty eight three-months old Gallega autochthonous breed which were divided into 4 groups of 7 animals in each: G-I trickle infected with a total of 175 mc; G-R trickle infected with a total of 175 mc and reinfected 18 weeks after primary infection (WAPI) with another 175 mc administered by the same trickle system; G-Tbz trickle infected with a total of 175 mc and treated with Triclabendazole (10 mg kg⁻¹ b.w.) 4

weeks after infection and reinfected 18 WAPI with another 175 mc administered by the same trickle system; G-C was left uninfected as control. Individual faecal and blood samples were obtained weekly from each sheep. *Fasciola hepatica*-egg counts were estimated by means of the coprological sedimentation technique. The dynamics of antigens was determined by a direct-ELISA with a polyclonal IgG anti-*F. hepatica* and the kinetic of antibodies was studied by an indirect-ELISA and excretory/secretory antigens of *F. hepatica*.

All sheep, except G-C, eliminated eggs of *F. hepatica* and egg output started from the 10th WAPI to the end of the study. The first *F. hepatica*-antigen was detected as soon as the 1st WAPI in the three infected groups. At 2 weeks after treatment with Triclabendazole levels of antigens were negative but it increased again after reinfection in the sheep of this group and in the animals of G-R. The IgG response increased significantly from the 2nd WAPI in the three infected groups; however, in the animals of G-Tcbz lower levels were reached after treatment although they still remained positive.

We concluded that the direct-ELISA provides an adequate test of the early diagnostic of *F. hepatica* in experimentally-infected sheep and it is able to evaluate the efficacy of a fasciolicide treatment.

This study was supported by the Research Project PGIDIT04RAG261009PR (Xunta de Galicia, Spain).

SHEEP-DOGS, THE MAIN RESERVOIR OF VISCERAL LEISHMANIOSIS IN CHAHARMAHAL AND BAKHTEYARI PROVINCE OF IRAN

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Chaharmahal and Bakhteyari province, the west part of Iran is one of the foci of ZVL caused by *Leishmania infantum*. Because in the rural and nomad populations of the area contact with dogs is inevitable, the present study was carried out to screen the sheep-dogs against *L. infantum* infection and to reveal the relationship between the infection and the variables, sex, age, location, clinical manifestations and type of tribal migration.

In different areas of the province 301 sera were collected from sheep-dogs and examined by Direct Agglutination Test (DAT) using *L. infantum* /IR/LON.49 antigen. Clinical examination of all of the subjects was accomplished by a veterinarian, and the Chi-square test was applied for analyzing the data.

Thirty-three samples (10.96%) out of 301 showed some levels of anti-*leishmania* antibodies ranging 1/320-1/5120. The highest and the lowest rates of infection had occurred in Chelgerd (14.3%) and Boroujen (1%) areas of the province and there was

a significant correlation between the infection and geographical location ($P < 0.05$). The study also showed a significant correlation between the sero-positivity and age and the type of migration of animals. The highest rate of infection had occurred in sheep-dogs under 3 years and in the animals which had been migrated to the neighboring province, Khozestan ($P < 0.05$). Only 5% of the infected animals had showed some clinical manifestations of ZVL, such as alopecia, chacexia or eye lesions.

ZVL is a relatively prevalent infection among the sheep-dogs in this area and humans sharing environment with infected dogs are at the risk of infection. Since, these animals are the main animal reservoir of the infection, control of infection among humans depends on identification of infected dogs, eliminating them and vaccination of healthy dogs.

POPULATION STRUCTURE IN *ASCARIS SUUM* AMONG DOMESTIC SWINE IN DENMARK

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The population structure of the pig roundworm, *Ascaris suum*, in domestic pigs in Denmark, has been described using a whole-genome DNA fingerprinting technique, "amplified fragment length polymorphism" (AFLP) analysis, from which we have extracted absolute gene frequency variance components and G-statistics for 135 independent nucleotide polymorphisms. We have found virtually no restriction in gene flow between the two major regions in Denmark, Jutland and Zealand, since the average proportion of total variance partitioned between regions is less than 3% of the total. The difference between two farms widely separated in Jutland accounted for only 5% of the total genetic variance of these two farms. Conversely, worms from different hosts within these farms are more subdivided, with about 12% of the total variance within each farm being distributed between hosts, suggesting substantial effect of the individual pig host, perhaps caused by single generation inbreeding due to founder effects in the colonization of single hosts. Absolute variance components extracted from the gene diversities also showed significant differences, with the among-host variance being greater than the between-farm and between-region values. Comparison of our results with other studies on *A. suum* and *A. lumbricoides* suggests that patterns of host dispersal effectively control patterns of worm gene flow. The results indicate that the potential spread of developing anthelmintic resistance among *A. suum* is most likely to be rapid, due to the continuous mixing of domestic swine stocks in Denmark.

MOLECULAR EVIDENCE FOR THE INFECTION OF ZOO CHIMPANZEES BY PIG *ASCARIS* SP.

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We here describe the transmission of pig *Ascaris* sp. to chimpanzees maintained in the Copenhagen Zoo, Denmark. The worms from the chimpanzees were compared with ascarids obtained from different putative sources of infection (human, pig and polar bears) using a technique for whole genome fingerprinting, amplified fragment length polymorphism (AFLP) and PCR linked restricted fragment length polymorphism (PCR-RFLP) of the internal transcribed spacer region (ITS) of the nuclear ribosomal DNA. By the use of different distance and clustering based methods on the AFLP data set, the worms from the chimpanzees were assigned to the same cluster as that of the worms from pigs. The PCR-RFLP analysis supported the AFLP results. Therefore, the zoo chimpanzees appear to have required *Ascaris* infections by contamination of either food or their environment with eggs from infected pigs.

THEORETICAL ASPECTS OF ECOLOGICAL NICHE AND LIFE CYCLE STRUCTURE IN HELMINTHS

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In the modern ecological niche theory, each life cycle stage of a population has its' own separate ecological niche. However then we lose sight of the fundamental characteristic of population and its life cycle - their holistic nature. In life cycles of parasites, the situation is complicated by presence on the one hand of two types of habitat – host organisms and environment, and on the other hand of multiple changes of hosts (in most helminths). The niche notion for parasite life cycle has intricate hierarchic character and it not developed in framework of Hutchinson's hypervolume niche concept.

The aim of this report is an attempt of system synthesis of basic ecological notions (population, its life cycle, ecological niche, life form, community) regarding helminths.

The final goal of population life cycle is optimal reproduction, and it is elementary unit of selection, evolution and functioning in ecosystem i. e. it is a single whole. At

the same time the multistage is fundamental characteristic of helminths life cycle. It includes some morpho-ecological specific stages that inhabit very different habitats. Every of these stages is an elementary functional and adaptive subunit, an elementary life form, and it realize own special role in the "chain" of a life cycle. The holistic population interpretation of ecological niche notion is proposed for consistent system synthesis of basic ecological notions. It is the multidimensional space of environmental factors at what population life cycle is realised. In this case the population niche is characterised by its extension during a life cycle, which determines the degree and character of genetically and phenotypically determinated variations of niche width during the life cycle. The number of life cycle stages, and accordingly - the specific parts (levels) of population niche's "channel" is its integral parameter. Therefore each stage has own subniche in total population niche "channel". The community is considered within the space of environmental factors as system of population niches that at every instant interacting and conjugated on the level of stage subniches. The situation for endoparasites is complicated due to possibility in given larval stage change of paratenic hosts some times. Accordingly the "space" of given subniche must include some subdivisions respective the specific and number of paratenic hosts that change each other. They may be named as "hostal subphase of subniche" of given life cycle stage. This approach may be used to optimize the planning of the population investigations of structure of helminths life cycles and comprehend its results in synecological context.

THE TRANSIT FOOD RELATIONS AS A POTENTIAL SOURCE FOR PREDATORS INFESTATION BY HELMINTHS

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Most endoparasitic helminths have complex life cycles with change of several hosts. This is mainly the result of the multiple acts of predation of one host by another. In other words, the realisation of life cycles of these helminths occurs via stable trophic chains. That is why, for decoding of helminths life cycles, it is very important to know the true trophic structure of given ecosystem. However, although in last three decades the nature and dynamic of trophic webs attracted ascending attention, it was studied and theorised on the proper food relations, i. e. the cases of direct eaten up each other or detritus. There is no data on another type of trophic relations – a transit food phenomenon.

The goal of this report is brief description of this phenomenon and of working hypothesis on it possible significance in the realisation of life cycles of helminths.

The transit organisms (secondary food) are not eaten directly by predator, but they get into predator's stomach after eating of the proper food organism with viscera. After digestion of prey's stomach or intestine integuments, these transit organisms or their remains fall into stomach cavity of predator and they are utilized here. This phenomenon is very common among various water predators and it was observed in copepods, shrimps, squids, sharks, teleosts and mammals. It is undoubtedly also characteristic for terrestrial predators that eat up their preys wholly. From point of view of energy metabolism of organisms and energy flows in ecosystems this type of food relations is mainly insignificant. It may be significant, however, from point of view of numbers of food organisms in stomachs of predators. For example, in stomachs of oceanic nektonic squids, lancet fishes (*Alepisurus* spp.) and tunas, there were found tens and even hundreds small transit plankton organisms, mainly different crustaceans and fish. Their number and volume are especially high in stable food chains when the peaks of daily feeding activity of two-three trophic interrelated populations are synchronised. The role of transit food relations in deep-sea communities and in many other situations in different ecosystems is probably significant also. For correct description of proper and transit food organisms, the taxonomic and size structure of these organisms needs to be taken into account. Such information may reveal the real members of the food chain that take part in transmission of helminths from one host to another.

Thus, the transit food organisms that are infested by larvae of helminths may potentially act as transmitters of the helminths between different trophic levels.

TRICHINELLA PREVALENCE AND FOODBORNE DISEASES IN EUROPE: ASPECTS OF A MODERN RISK ANALYSIS

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Human trichinellosis poses a threat for human health. Presently, four autochthonous *Trichinella* species, namely, *T. spiralis*, *T. britovi*, *T. nativa* and *T. pseudospiralis* were discovered in domestic and silvatic animals in Europe. Every year human trichinellosis cases occur in many European countries due to the consumption of raw or improperly treated pork, wild boar or horse meat which could pass the food chain because of a failure in meat inspection.

Epidemiological aspects of *Trichinella* prevalence in the domestic and silvatic cycle are explained and natural and artificial factors for the maintenance, as well as risk factors to acquire trichinellosis are presented.

Conclusions refer to the current needs for improving consumer protection according to the "White Paper on Food Safety" of the European Commission which

gives emphasis on a modern risk analysis with its three components: risk assessment, risk communication and risk management.

THE DYNAMICS OF COREGONIDAE FISH PARASITE FAUNA IN THE CIRCUMSTANCES OF TROPHIC WATERBODY CHANGES

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The Syamozero is one of the largest water bodies of South Karelia (Russia). Complex monitoring investigations are doing on the lake during the last 50 years. It was established that considerable changes in natural conditions of the lake took place. In 1970-80th the water body trophic status had been changed due to anthropogenic pressure. In the same time a new fish species – smelt *Osmerus eperlanus* had been introduced. Processes mentioned above led to considerable changes of Coregonidae fish parasite fauna.

The aim of our research was to study the parasite fauna of Coregonidae fish [vendace (*Coregonus albula* (L.) and whitefish (*C. lavaretus pallasii* (Val.))] under changes of trophic status and ichthyocoenosis structure of this water body.

The work was carried out according to standard methods. During the period of 2003 – 2004 vendace specimens (15) and whitefish specimens (20) were studied.

The parasite fauna of whitefish includes 17 species: Myxosporidia – 1, Ciliophora – 2, Cestoda – 3, Trematoda – 7, Nematoda – 2, Crustacea – 2. Parasite fauna of vendace consists of 17 species: Ciliophora – 3, Cestoda – 2, Trematoda – 6, Nematoda – 2, Crustacea – 3.

In 70-80th eutrophication led to considerable changes of Coregonidae fish parasite fauna. Fish infection by parasites, life cycle of which includes zooplankton organisms, increased significantly. Cestodes *Proteocephalus longicollis* and *Diphyllbothrium dendriticum* were the dominant species. A share of Salmonidae fish parasite species had been decreased: myxosporidia *Chloromyxum coregoni* and trematoda *Crepidostomum farionis* disappeared, monogenea *Discocotyle sagittata* rarely occurred.

At present an alteration of dominant species in Coregonidae fish parasite fauna is revealed. Metacercaria *Ichthyocotylurus erraticus* appear again as dominant species how it was revealed in 1950 - th. The decrease in number of Salmonidae parasite species was noted. However, during the period of investigations (from 50 - th) Salmonidae fish parasites such as *D. ditremum*, *P. longicollis*, *I. erraticus*, and *Triaenophorus crassus* for whitefish and *Phyllodistomum conostomum* for vendace, remain constant components of parasite fauna. The increase in total number of parasite species was recorded. New species are mainly protozoans and crustaceans that characterize habitat conditions of whitefish and vendace.

The changes of parasite fauna reflect the processes occurring in the lake ecosystem in whole.

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TRANSMISSION OF *GYRODACTYLUS SALARIS* (MONOGENEA: GYRODACTYLIDAE), WITH EMPHASIS ON DEAD HOSTS AS A RESERVOIR OF INFECTION

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Economically, one of the most important platyhelminths to have successfully invaded novel fish populations is *Gyrodactylus salaris*. This ectoparasitic monogenean has devastated stocks of wild Atlantic salmon in Norway since its introduction from the Baltic during the 1970s. The parasite with its high fecundity and transmission potential can rapidly colonize an entire river system. Having no specific transmission stage, *G. salaris* utilizes four different routes of transfer to new hosts: via contact with live hosts, via dead hosts, by detached parasites drifting in the water column, and by parasites attached to the substrate. However, the relative importance of these different routes is largely unknown.

In the current study, we evaluate the transmission strategies of *G. salaris* focusing on the importance of dead hosts, by assessing the survival and infectivity of detached worms and those removed from dead hosts. Worms were also aged to determine whether transmission was influenced by life span. At 18°C, survival off the host is 1d, but at 3°C parasites survive for 4d. Surprisingly, however, most parasites remain with their host following its death. Such worms survive despite being wrapped in decaying host tissue, their life span being doubled compared with individuals maintained *in vitro*, probably sustained by feeding on the dead host. *G. salaris* can actually survive for up to 6d at 12°C on a dead host and, importantly, are still viable, at least up until 72h post-host death. This survival strategy is in stark contrast to that previously observed for individuals of *G. turnbulli* in which parasites actively leave a dead host. For *G. salaris*, dead hosts may serve as an important infection source both in rivers and hatcheries. From the age-distribution data, it appears that worms that have given birth are more likely to transfer to a new host than those that have not given birth. Thus, although having no specific transmission stage, gyrodactylids apparently do display a non-random timing of transmission.

The present results highlight the importance of dead hosts as a significant reservoir of infection. Taken the significant threat that *G. salaris* constitutes to populations of

Atlantic salmon, this is an aspect which should be incorporated into future management policies and risk assessments.

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FIRST DATA ON OCCURRENCE OF AVIAN BLOOD PARASITES IN EKATERINBURG AND IRKUTSK DISTRICTS OF RUSSIA

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In spite of numerous records and often high prevalences of blood parasites reported in birds all over the world, the fauna and distribution of the haematozoa has been studied irregularly in all zoogeographical regions. There is no information about distribution of these parasites in vast territories of western Russia, including the Ural Mountains, Siberia, and Far East regions. Our objective was to obtain first information on occurrence of blood parasites in Ekaterinburg and Irkutsk districts of Russia.

The material was collected at two sites in Ekaterinburg district on the European slip of the Ural mountains (57° 20' N, 59° 49' E, and 56° 50' N, 58° 36' E) and at one site in Irkutsk district near the Baikal Lake (51° 34' N, 103° 54' E) in the beginning of breeding period of birds between 18 May and 7 June 2004. Birds were caught with mist nets, collected from nest boxes, and sometimes gone-short. In all, 119 individual passeriform birds belonging to 5 families and 11 species were investigated. Among them were birds belonging to the Fringillidae (19 individuals), Muscicapidae (31), Paridae (29), Sylviidae (17), and Turdidae (23). Blood films were air-dried, fixed in methanol, stained with Giemsa, and examined microscopically at a low (× 400) and then at high magnification (× 1,000).

Blood parasites were common at each study site. The overall prevalence of infection was 74.8%. Prevalences of *Haemoproteus* spp. (34.7%), *Leucocytozoon* spp. (34.7%), *Plasmodium* spp. (22.8%), *Trypanosoma* spp. (21.0%), *Hepatozoon* spp. (0.8%), and microfilariae (12.7%) were recorded. Representatives of all genera were found in resident (non-migrating) bird species, indicating active transmission at the study sites. The overall prevalence of infection varied between 46.1% and 100% in different species of avian hosts. All investigated thrushes (*Turdus iliacus*, *T. philomelos*, and *T. pilaris*) were infected. The prevalence of *Haemoproteus*, *Plasmodium*, *Leucocytozoon*, *Trypanosoma* spp. and microfilarial infections in the thrushes was 70.0%, 30.4%, 87.0%, 39.1%, and 47.8%, respectively.

Haemoproteus balmorali, *H. belopolskyi*, *H. fringillae*, *H. majoris*, *H. minutus*, *H. pallidus*, *Leucocytozoon dubreuilii*, *L. fringillinarum*, *L. majoris*, *Plasmodium (Haemamoeba)* spp., *Trypanosoma avium*, *T. everetti*, *Hepatozoon parus* were identified to the species level. It seems probable that these parasites are cosmopolitan in the Palearctic.

The present study indicates that there is an active transmission of representatives of the great majority of genera of avian blood parasites in eastern Russia. Further studies, including studies of DNA of the parasites, are needed to fully understand the peculiarities of the formation of fauna of avian haematozoa in certain regions of the Palearctic.

STUDIES OF BLOOD PARASITES IN MIGRATING BIRDS IN ISRAEL

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Israel is located on the major flyway of bird populations that migrate between West Eurasia and Africa, with over 500 million birds (of more than 200 species) passing through. This makes Israel a hot-spot of bird biodiversity and potentially for their associated parasites. During the springs and autumns of 2002-2003 blood was collected from birds caught for ringing in the Jordan valley and examined for parasites. During the springs a total of 438 birds of 31 species (338 migratory birds of 21 species) were studied and during the autumns 615 birds of 44 species (411 migratory birds of 31 species). Since 2004, studies are on-going in Eilat, located on the northern end of the gulf of Aqaba. In Eilat 980 birds (843 migrants) of 98 species (86 migrant species) were examined during spring 2004, but only 266 (163 migrants) of 43 species (35 migrants) were sampled for blood during the autumn. Recovered infection included *Haemoproteus* spp., the most ubiquitous, species of *Leucocytozoon* and *Plasmodium*, and more rarely trypanosomes and microfilaria. Sporozoites of *Lankesterella* were particularly common in *Acrocephalus scirpaceus*. In most birds parasitaemia was moderate to low, while recrudescence led sometimes to heavy infection (12-15% parasitaemia in species of *Phylloscopus*). In Eilat, infection prevalence with *Haemoproteus* and *Leucocytozoon* species (but not with *Plasmodium* spp.) gradually increased in later-arriving migrants, peaking by May or even June. The same trend was maintained when analyzed data were segregated to the more readily infected warblers (Sylviidae). Among autumn migrants infection was traced among fledglings (born this year) as well as birds of older age class.

Data from the birds caught in the Jordan valley demonstrated a fairly high consistency in prevalence from one year to the next, with higher infection prevalence during spring migration. Same trend of higher infection prevalence in spring was

maintained among migrants passing at Eilat, although they apparently comprise migratory populations from separate geographical distributions.

COMPARATIVE ANALYSIS OF DIFFERENT MODIFICATIONS OF MCMASTER METHOD

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The efficiency of seven modifications of McMaster method was compared. Investigations were based on analysis of horse faeces infected with strongyle eggs. Each modification was evaluated after examination of 30 samples of faeces. The evaluation was based on the number of positive samples, total number of eggs, variation of EPG and complexity of modifications. The positive samples were identified by count of strongyle eggs in one, two or three compartments of McMaster chamber. Neither of the modifications was sensitive enough for egg count in a single chamber. Examination by Henriksen & Aagaard and Urquhart modifications in two chambers have shown positive results. Only two modifications: Gronvold, using saturated salt and glucose mixture and Thienpont were insufficiently sensitive to samples examination in three compartments. The modifications were evaluated by the results of sample examination in two chambers and calculating the average EPG. The highest EPG was obtained by Urquhart modification; to this result was given the grade of highest efficiency equalled to 1. Other modifications were evaluated dividing results in EPG by EPG obtained by Urquhart modification. Further, the efficiency coefficient of each modification was calculated. It varied from 0.98 to 0.66. Investigation performed using any of the described modifications and the calculated coefficient provides a possibility to recalculate and unify the results of faeces examination and to interpret the coproscopical examinations of other authors. The smallest variations of egg count were obtained by Henriksen & Aagaard modification. The Thienpont is the simplest and the Henriksen & Aagaard modification is the most complex. Though the last modification is the most sophisticated, it demonstrates the highest performance. Henriksen & Aagaard modification of McMaster method had a lower efficiency coefficient according to the egg counts, and was most suitable for scientific investigation as the results obtained by this modification are more reliable.

DISPERSAL INCREASES LOCAL INFECTIVITY OF MALARIAL PARASITES

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The relationships between dispersal and local infectivity of parasites are essential to understanding host-parasite coevolution and the emergence and spread of novel disease threats. Dispersal of parasites among host populations may hamper parasite local infectivity if immigrants are poorly adapted to local hosts. However, dispersal may increase parasite local infectivity if genetic variation helps parasite populations to track susceptible hosts. These two alternative hypotheses remain poorly investigated, and still less is known of the potential of parasites to evolve different dispersal mechanisms.

We analysed the relationships between dispersal potential and local transmission of haemosporidian parasites (*Plasmodium* and *Haemoproteus* spp.) in a migratory bird, the blackcap *Sylvia atricapilla*. We studied parasites infecting breeding birds from six populations widely distributed in Western Europe, and wintering birds from one of these sites (southern Spain). We detected parasites using a PCR technique for amplification of a fragment of the cytochrome *b* gene, and identified different parasite lineages by DNA sequence differences. A parasite phylogeny estimated from mtDNA sequences was used in comparative analyses of the relationships between opportunities for dispersal among populations, size of the geographic range, and local prevalence of parasites.

Our results show that year-round transmission, as opposed to summer transmission, has repeatedly evolved in malarial parasites of blackcaps. Year-round transmission allows parasites to spread in sympatric host's wintering areas, and hence to colonise distantly located host's breeding areas connected by host migration movements. Widespread parasites had higher local prevalence, revealing increased transmission, than geographically restricted parasites. These results show a positive relationship between dispersal and local infectivity of malarial parasites that is apparently mediated by frequent evolutionary changes in parasite transmission dynamics. In addition, our results also reveal an important role of host migration in the epidemiology of blood parasites. These phenomena have important implications for the ecology and evolution of infectious diseases; however, they would have remained hidden to our eyes without the help of molecular methods.

THE EFFECT OF INULIN ON NEW AND ON PATENT INFECTIONS
OF *TRICHURIS SUIS* IN GROWING PIGS

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The objective of this experiment was to investigate the potential influence of inulin on the establishment of new and patent infections of *Trichuris suis* in growing pigs. Two experimental diets were formulated based on barley flour with added either insoluble fibre from oat husk (Diet 1) or a pure inulin (16%) supplementation (Diet 2). Twenty-eight 10-week old pigs were divided randomly into 4 groups (Groups 1-4) each of 7 pigs. After three weeks adaptation to the experimental diets all pigs were infected with a single dose of 2,000 infective *T. suis* eggs. Group 1 was fed Diet 1 until 7 weeks post infection (PI) and Group 3 until 9 weeks PI. Group 2 was fed Diet 2 until 7 weeks PI. Group 4 was fed Diet 1 until week 7 PI and was switched-over from Diet 1 to Diet 2 until week 9 PI. Seven weeks post infection pigs in Groups 1 and 2 were slaughtered, and pigs in Groups 3 and 4 were slaughtered at 9 weeks PI. *Trichuris suis* worm burdens were determined for all pigs.

Inulin fed pigs (Group 2) exhibited an 87% reduction in EPG, compared to the pigs on standard diet (Group 1) ($P < 0.0001$). The number of worms recovered at week 7 PI from pigs on the inulin diet (Group 2) was significantly reduced by 71%, compared to the pigs on standard diet (Group 1) ($P < 0.01$). At week 9, worm recovery in pigs on the inulin diet switch protocol (Group 4) was reduced by 47% compared to the control pigs in Group 3 ($P < 0.01$). Further, the inulin-fed pigs exhibited a significant reduction in female worm fecundity and worm large intestine location was more distal compared to those from pigs on standard diet. These results demonstrate that inclusion of the highly degradable fructose polymer inulin in the diet leads to significant reductions in *T. suis* establishment, egg excretion, and female worm fecundity and can be used as a treatment for patent infections.

POLYMORPHISM OF *BUNODERA LUCIOPERCAE* (TREMATODA, ALLOCREADIIDAE) EMERGED FROM CHROMOSOME ANALYSIS

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Bunodera luciopercae (Müller, 1776) is Holarctic species with considerable morphological variability both in the Nearctic and Palearctic. It is common parasite in perch, pike, ruff, pike-perch, burbot and other freshwater fish in Lithuania. Typical ophthamoxiphidiocercariae with eyespots and stylet develop in *Sphaerium* and *Pisidium* spp. The existing data on the chromosome set structure of *B. luciopercae* are controversial. Seven or six meiotic chromosomes were revealed in the cells of gonads by different authors and the possibility of the existence of geographic races of these worms was presumed; 26 chromosomes were described in diploid complement.

The aim of this study was to examine karyotype of the Lithuanian population of *B. luciopercae* and to compare its chromosome set structure with the literature data.

Mitotic chromosome preparations were made from cells of parthenitae obtained from naturally infected *Sphaerium corneum*. The mollusks were collected in the water reservoir of the Lithuanian Power Station. Microscope preparations were made using air-dried method and conventional Giemsa staining. The karyotype consists of 7 chromosome pairs, $2n=14$. The chromosomes are large and measured from 12.8 μm to 2.3 μm . The first two pairs of homologues are markedly large than the remaining elements and constitute 51.7% of the total chromosome complement length. According to the centromere position, chromosome pairs 1 and 2 are metacentric, pair 3 is submeta-subtelocentric and the remaining elements are acrocentric.

Polymorphism emerged in chromosome set structure of *B. luciopercae* from different localities, is a unique phenomenon among trematodes, presuming the existence of cryptic species. In some trematode species polyploid, most often triploid, populations and specimens were revealed. Chromosome number variation due to the presence of B chromosomes was detected in some species. But these mechanisms could not explain the variation in chromosome set structure of *B. luciopercae*. It is possible that *B. luciopercae* includes more than one (host-specific) species. Cryptic speciation, i.e. speciation without obvious morphological divergence, is expected to be common in endoparasites, because host divergence is unlikely to exert strong selection pressures for morphological adaptations.

BIOGEOGRAPHY OF MONOGENEAN PARASITES OF BUTTERFLYFISHES (CHAETODONTIDAE) WITHIN THE INDO-WEST PACIFIC OCEAN

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The biogeography of butterflyfish monogeneans (Platyhelminthes) is studied within the Indo-West Pacific Ocean.

Most marine organisms, including butterflyfishes (Chaetodontidae), show a typical east to west biodiversity gradient with the centre of biodiversity located in the Philippines - Indonesia - Papua New Guinea area. The study of monogenean parasites of butterflyfishes reveals, contrary to their hosts, a weak diversity gradient from east to west. However, in order to understand the processes of island colonisation that gives rise to general biodiversity patterns, the phylogeography of two monogenean species, *Euryhaliotrematoides grandis* and *Haliotrema aurigae*, parasitizing butterflyfishes, are more closely investigated. Bayesian analyses were conducted using intraspecific molecular sequences (mitochondrial cytochrome c oxidase subunit I) of parasites collected on a single host species from a range of localities.

The results demonstrate that the genetic structure of *H. aurigae* is not correlated with host location, whereas that of *E. grandis* forms highly structured populations linked with their spatial distribution in the investigated areas within the Indo-West Pacific Ocean. The early branching position of individuals from Moorea (French Polynesia) indicates that this locality represents possibly the centre of origin for *E. grandis*. The present hypothesis of Moorea being a centre of origin for the monogenean parasites of the Chaetodontidae and the strategies for remote islands colonisation will be discussed.

PARASITE COMMUNITIES AND ENVIRONMENT QUALITY

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There are numerous publications dealing with the use of parasitological data for environment quality assessment. The results can be briefly summarized in the following way: under pollution reduction of parasite fauna is to be observed; under pollution reduction of parasite fauna is not to be observed; under pollution some parasite species demonstrate a decrease of infection; under pollution some parasite species demonstrate an increase of infection. On the one hand, pollution can affect negatively the parasite larval stages and/or the intermediate hosts, which leads to a

decrease of infection. On the other hand, pollution can cause host immunity depression, which leads to an increase of infection. On the face of it, the results that are so contradictory make doubtful the idea to use parasitological data for environment quality assessment. It has to be emphasized that the pollution acts against a background of natural changes in parasite communities such as seasonal and age dynamics, migrations etc. Thus, the search of an integral test of assessment of parasite community state is needed.

Two hundred and twenty-one parasite component communities of 34 fish species from North Asia water-bodies were used in this study, as well as data on parasite communities were compiled from previously published studies. Berger-Parker, Shannon's and evenness indices were used as integral tests.

Parasites in the component community are not interacting. Saturation of this community by parasites is unlimited or limited by the number of host individuals and environmental conditions. Infracommunity state is defined by interaction between parasites as well as between parasites and host organism, component community state is defined by environment and host ecology. Values of indices sharply react to sample heterogeneity, changes in host ecology, etc. No significant correlations between indices and geographic coordinates have been revealed. Taking into account irregular distribution of environmental conditions through North Asia territory one can expect some general tendencies (nonlinear dependences). However, such tendencies have not been revealed. One was obtained when fish species were grouped in accordance with their ecological characters (faunal complexes). These tendencies reflect optimum – pessimum continuum of environmental conditions for fish ecological groups and for single fish species. Pollution will cause disturbances in parasite community and adequate changes of indices. Optimum indices can be determined by analyzing series of their values through host area except when its deviation is brought about by natural causes or sample heterogeneity.

THE EFFECT OF SIZE-SELECTIVE REMOVAL ON HOST AND PARASITE PERSISTENCE IN EXPERIMENTAL POPULATIONS

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Predators have the potential to limit the spread of pathogens not only by selecting infected prey but also by shaping prey demographics. We tested this idea with an epidemiological experiment where we simulated variable levels of size-selective predation on zooplankton hosts and monitored the persistence of host and parasite populations. In the absence of simulated predation, the highly virulent protozoan

Caullerya mesnili frequently drove its host, *Daphnia galeata*, to extinction. Control populations showed lower extinction rates and higher average densities than infected populations in the absence of simulated predation. With a weak removal rate of the largest hosts, the proportion of populations where the parasite drove the host, and consequently itself, to extinction decreased. Host-parasite coexistence was also observed in some cases. However, with intermediate levels of removal, most of the parasite populations went extinct, while the host populations persisted. With an even higher removal rate, *Daphnia* were driven to extinction as well. Thus, variation in one factor, size selective mortality, resulted in four different patterns of host-parasite dynamics. Our results highlight the potential role of predation for shaping the epidemiology and community structure of host - parasite systems.

INVESTIGATION OF *ECHINOCOCCUS MULTILOCULARIS* IN MEURTHE ET MOSELLE DEPARTMENT, FRANCE

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The cycle of *Echinococcus multilocularis* in red fox *Vulpes vulpes*, its classical host, was investigated during 3 years in an endemic area of alveolar hydatid disease in Lorraine in North Eastern France.

The technique used for the diagnosis is the sedimentation and counting technique. The age determination of foxes was assessed by two ways. Macroscopically for 30 foxes: juvenile foxes (< 1 years) have three-lobed incisors without any sign of wear; other foxes are identified as adults. In the second group of 44 animals, age has been determined by counting cementum lines in a cut of canine teeth.

In all, 74 wild foxes originating from an endemic area located in North East of France were trapped between March 2001 and November 2004. *Echinococcus multilocularis* was observed in 44.6 % of animals. The foxes harboured a total number of parasites of 340,561 (infection intensity ranging between 2 and 135,565). Three (9.09%) most heavily infected animals harboured 86.38 % of the total number of parasites. In all, 36.36 % of the foxes had a worm burden > 1,000 parasites, 24.24 % ranging between 100 and 1,000 and 39.4 % of infected foxes harboured less than 100 parasites. Approximately 51 % of infected foxes are juvenile (0-1 years) and the highest worm burden is recorded in this class.

Echinococcus multilocularis is fairly common in urban and peri-urban foxes in France. The risk of transmission to humans is important in this department. The novel epidemiological and infectiological questions are discussed.

ARCTIC CHARR *SALVELINUS ALPINUS* AS NATURAL HOST FOR *GYRODACTYLUS SALARIS* (MONOGENEA) IN NORWAY

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Arctic charr *Salvelinus alpinus* represent the only *Salvelinus* species with a natural distribution in Norway ranging from the North-Norway to southern parts of the country. Anadromous charr are co-occurring with Atlantic salmon in North-Norwegian rivers and resident populations occur in lakes draining into salmon populated river systems in southern Norway. Charr has the potential for disseminating *G. salaris* to wild salmon, as charr has been found susceptible to *G. salaris* experimentally in the laboratory. However, reports of gyrodactylid infections on charr in Norway are scarce, only on three occasions has charr been reported infected: during the extermination of *G. salaris* with the biocide rotenone in River Skibotnelva, North Norway in 1988, during an inventory investigation of gyrodactylids in Signaldalselva nearby Skibotnelva, and in Buskerud County, the southern part of Norway. The species recovered are respectively, *G. salaris*, probably *G. salaris*, and *G. birmani* Konovalov. During extensive sampling we recovered gyrodactylid infected charr both in River Signaldalselva, North-Norway, and Lake Pålbufjorden, southern Norway above the salmon populated areas of the Numedalslågen river system. A taxonomical study was therefore initiated with the following aims: (i) to characterize morphologically and genetically *Gyrodactylus* sp. on Arctic charr from River Signaldalselva, North-Norway and to compare the result with concurrent infection on Atlantic salmon; (ii) to characterize morphologically and genetically *Gyrodactylus* sp. on Arctic charr from Lake Pålbufjorden, South-Norway and to compare the results with two *G. salaris* populations on salmon and rainbow trout *Oncorhynchus mykiss* selected on basis of genetic similarity (ITS, CO1). The results of the morphometric and genetic analyses will be discussed.

This study was supported by the NRC Wild Salmon Program (project 145861/720) and the National Centre for Biosystematics (project 146515/420), co-funded by the NRC and the NHM, University of Oslo, Norway.

INFESTATION OF HERRING WITH *CONTRACAECEUM OSCULATUM* LARVAE (NEMATODA: ANISAKIDAE) IN THE SOUTH BALTIC

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The third-stage larvae of the nematode *Contracaecum osculatum* (Rudolphi, 1802) (Ascaridoidea, Anisakidae) occur in different fish species in the Baltic Sea. The life cycle of these nematodes involves invertebrates as intermediate hosts and seals as final hosts.

In this study we analyzed length-age, geographical, long-terms, and herring population variations on infestation of the Baltic herring, an abundant pelagic fish.

Contracaecum osculatum larvae were collected from 5414 herring specimens (total length 9.0-41.5 cm) in 1996-2003. Samples of the herring were taken from coastal and off-shore grounds in 26 and 28 ICES-Subdivisions. The fish were classified as belonging to the spring coastal, spring open sea herring and autumn herring populations according to Kompowki based on otolith structure. Worms were taken from frozen and fresh fish. The larvae were studied under light microscope after clearing in glycerol combined with lactic acid.

Total herring infestation was low – 1, 4% (intensity 1-4 specimens). The majority of nematodes were found in serous membrane of the internal organs (32. 2%), in intestine (27.4%) and in pyloric caeca (27.4%). Larvae length was 1.65 – 16.67 mm. Nematodes were found in fish whose length varied from 15.7 to 35.0 cm, and age from 1 to 12 years. The infection indexes (prevalence and abundance) had the positive correlation with fish length and age. *Contracaecum osculatum* larvae were found in all regions of our investigation. They were most common in coastal grounds in 28 ICES-Subdivision. Infection prevalence of spring open sea herring was higher than of spring coastal fish (2.1% and 1.4%). These nematodes were absent in autumn herring. Larvae were found in herring in different seasons. They have been seen more often (3.7%) in September. The long-term changes of herring infestation had the periods of its increase and decrease. Maximum indexes of prevalence and abundance were recorded in 1998 (2.8% and 0.05) and in 1999 (3.0% and 0.03).

The analysis of infection of the Baltic herring with *C. osculatum* 1. has revealed dependence of parasitological indexes on the fish length and age, season, sea region and herring population structure. These data are important to understand the structure of parasite fauna of the Baltic Sea ecosystem.

HELMINTH EGGS FROM AN ARCHAEOLOGICAL EXCAVATION OF A VIKING AGE BUILDING IN VIBORG, DENMARK

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A small building has been excavated at the bank of the lake Viborg Søndersø in the central Jutland, Denmark. Contexts of deposits were analysed by traditional archaeological techniques as well as by various technical methods. Thus, a total of 46 and 18 contexts were analysed by flotation and sedimentation, respectively.

Eggs of *Trichuris* sp. (whipworm) were found in 72% of the samples (1-3432 eggs per 5 g), and due to the small size of these eggs, they may all be regarded as having a human and/or porcine origin. Eggs of the large roundworm of man and/or pigs, *Ascaris* sp., were found in 41% of the samples (1-1386 eggs per 5 g). All other parasite eggs were found only sporadically: *Fasciola hepatica* in 17% of the samples (1-6 eggs per 5 g), *Capillaria* sp. in 11% of the samples (1-6 eggs per 5 g), and *Toxocara canis* in 1 sample (27 eggs per 5 g). All these eggs were thick-shelled and no thin-shelled helminth eggs were observed. One sample contained coccidial oocysts, however, due to their appearance, they were regarded as recent contamination.

Dendrochronological analyses showed that the walls were built in or shortly after year 1018 (the Viking Age). Archaeological and natural scientific evidence indicated that the building housed a silver smith and that the surrounding area had been used for handcraft (leather, bone/antlers) with a latrine west of the building. The parasitological findings contributed considerably to this interpretation, as *Trichuris* and *Ascaris* spp. eggs from humans (and/or pigs) were dominating with the highest concentrations in and around the latrine west of the building. A few faecal deposits, mostly of human/porcine origin were observed north of the building while contexts inside and south of the building generally had weak traces of faeces. As the ground was wet and soft and the sediment layers were largely intact, a human origin of *Trichuris* and *Ascaris* spp. eggs is most likely.

At other archaeological excavations, parasitological analyses have mostly been conducted to verify a faecal origin of a few deposits, while not on larger number of different layers. The present results indicate that a systematic analysis for parasite eggs may be an important analytical tool in future archaeological studies.

THE EUROPEAN UNION PROJECT ECHINORISK: NEW DATA ON SPATIAL AND TEMPORAL EPIDEMIOLOGY OF *ECHINOCOCCUS MULTILOCULARIS*

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To recognize the changing risk of infection, the present status and dynamics of *Echinococcus multilocularis* transmission was assessed on a European scale between 2001 and 2004.

Data of *E. multilocularis* prevalence in foxes were obtained in 9 European countries: France, Switzerland, Germany, the Netherlands, Austria, Czech Republic, Poland, Slovak Republic and Italy. Results include the first records of *E. multilocularis* in foxes from northern Italy, Carinthia in Austria, southern Slovakia and the north of the Czech Republic. In France, a western and southern extension of the known distribution of the parasite was demonstrated. Prevalence increases – compared to earlier surveys – were recognized in Slovakia, parts of Austria, southern Germany and Poland, while the situation appeared stable in the Netherlands and the investigated part of Switzerland (south of the Alps). Decreased prevalences were not found anywhere.

To recognize correlations between landscape parameters and *E. multilocularis* transmission, geographical data banks (mainly CORINE land classes - CLC) were used for analyses. In southern Germany, communes with predominantly agricultural and grassland structures were at higher risk for *E. multilocularis* transmission than those with predominantly forested or urban environments. However, CLC data did not explain an observed prevalence gradient in the southwest of the Czech Republic, and comparative studies in Slovakia and Austria were inconclusive. A detailed study in eastern France found an insufficient fine-grain resolution of CLC in comparison to IRS satellite imaging. This was confirmed by a comparison of CLC with a ground survey in southern Germany, which found CLC to considerably underestimate transmission-relevant landscape types (e.g. grassland) due to patchy distribution. In a different approach in Switzerland, data were specifically collected at the southern border of the known range of *Echinococcus multilocularis*, and the presence and prevalence of *E. multilocularis* in foxes was compared with the density of suitable intermediate hosts and with the predation rate of foxes on these species. The prevalence correlated significantly with the proportion of fox stomachs containing voles of the group *Microtus/Pitymys*.

As a tentative conclusion, the distribution of *E. multilocularis* in foxes appears to reflect the availability of suitable intermediate hosts, especially *Microtus arvalis*.

NEW RECORDS OF PLANT PARASITIC NEMATODES IN ANTARCTICA

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One new, four known and one unidentified species of tylenchid nematodes are described from samples collected on the Dronning Maud Land, East Antarctica.

Apratylenchoides sp. nov. differs from the only other known species of *Apratylenchoides*, *A. belli* Sher, 1973, in having a pumpkin-like spermatheca, shorter dorsal gland lobe, longer tail, and crenate tail tip. *Pratylenchus andinus* Lordello, Zamith et Boock, 1961, *Tylenchorhynchus maximus* Allen, 1955, *Aglenchus agricola* (de Man, 1884) Meyl, 1961 and *Paratylenchus namus* Cobb, 1923 were also recorded for the first time in Antarctica.

The rather unexpected presence of plant parasitic nematodes in habitats devoid of vascular plants and some biogeographical implications of the findings are discussed.

REVIEW OF THE GENUS *BURSAPHELENCHUS* FUCHS, 1937 (NEMATODA, APHELENCHIDA) WITH SOME CONCLUSIONS ON THE HOST-PARASITE AND VECTOR-PARASITE EVOLUTIONA. RYSS¹, P. VIEIRA², M. MOTA² & O. KULINICH³¹*Zoological Institute, Russian Academy of Sciences, Russia*²*University of Evora, Portugal*³*Institute of Parasitology, Russian Academy of Sciences, Russia*

Genus *Bursaphelenchus* includes several pests of the world importance for the rural economy. The most dangerous are the *Bursaphelenchus xylophilus* (the pinewood nematode caused decline of the pine trees in south Asia and in one spot area in Europe, Portugal, Peninsula de Setubal) and the *B. cocophilus*, causing the decline of coco-palm plantations in Carribean and Latin American regions. The peculiarity of the host-parasite association in the genus is that the nematode life cycle includes three trophic components: plant (mostly a tree), insect vector and a fungus. Goals of the presentation is to list all species of the world fauna and all efficient diagnostic characters, then create the identification tool and analyze the similarity of species and possible ways and causes of the host-parasite evolution of the group.

Complete list of species with synonymy and a catalogue of all efficient diagnostic characters with their states, selected from papers of the most experienced taxonomists of the genus, are given for the genus *Bursaphelenchus*. List of known records of *Bursaphelenchus* species with names of natural vectors and plants and their families is given (for world pests the most important groups of trees and insects are listed). The

tabular, traditional and computer-aided keys are presented. Dendrograms of species relationships (UPGMA, standard distance: mean character difference) based on all efficient taxonomic characters and separately on the spicule characters only, are given. Discussion whether the species groups are natural or purely diagnostic ones is based on the relationships of dendrograms and the vector and associated plant ranges of *Bursaphelenchus* species; the *xylophilus* species group (*B. xylophilus*, *B. abruptus*, *B. baujardi*, *B. conicaudatus*, *B. eroshenkii*, *B. fraudulentus*, *B. kolymensis*, *B. luxuriosae*; *B. mucronatus*), the *hunti* group (*B. hunti*, *B. seani*, *B. kevinii* and *B. fungivorus*) are probably the natural ones.

The parasitic nematode association includes three trophic components: plant, insect vector and fungus. The initial insect-plant complex Scolytidae-Pinaceae is changeable and only in rare occasions the change of the preferred vector to Cerambycidae (the *xylophilus* group), Hymenoptera (the *hunti* group) led to formation of the natural species-groups. From the analysis it is clear that although the vector range is changeable it is comparatively more important for the evolution of the genus *Bursaphelenchus* than associations with plants at the family level. Data on the fungi species (3rd component in natural *Bursaphelenchus* associations) are insufficient for the detailed comparative analysis.

NEW INTERESTING RECORDS OF THE SPECIES OF THE PLANT PARASITIC GENUS *BURSAPHELENCHUS* IN CHINA AND THE USE OF MORPHOLOGICAL AND MOLECULAR APPROACHES FOR THE SPECIES IDENTIFICATION

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During the survey in the South China pine forest some interesting nematodes belonging to the genus *Bursaphelenchus* were recorded in Jiangxi province, Forestry Station near Jingdezhen.

One species was identified as the *Bursaphelenchus glochis* Brzeski & Baujard, 1997, which was earlier recorded only in Europe. Another species *Bursaphelenchus* sp. n. was similar to the first one but differs in the head shape, male spicules structure and the female tail. Presence of the *B. glochis* (belonging to the *peniperdae* species group within the genus) in the native forest of China shows the possible Asian origin of this European species, which confirms the similar conclusion on the European species of the *xylophilus*-group of the *Bursaphelenchus*. The species was cultured on the fungus *Botrytis cinerea* and used for molecular analysis (it is the first molecular

study of this species). RFLP pattern shows a difference of *B. glochis* from other *Bursaphelenchus* species.

Tabular, text and electronic identification keys for the *Bursaphelenchus* species recorded in China and Russia are given.

CANINE BABESIOSIS: NEW ENDEMIC AREAS IN SWITZERLAND

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Canine babesiosis is mainly caused by *Babesia canis* and *B. gibsoni*. Endemic regions in Europe were found in Mediterranean areas with mild climate, allowing the survival and proliferation of transmitting vectors. For *B. canis* the two tick species, *Rhipicephalus sanguineus* and *Dermacentor marginatus*, are responsible for transmission of the disease. Within the last decades, autochthonous transmission of canine babesiosis has been reported in several mid and northern parts of Europe. In Switzerland endemic areas were mainly found in the southern (Ticino) and western part (surrounding of lake of Geneva) of the country.

Within few months five cases of canine babesiosis were recorded in a small animal clinic in the central part of Switzerland. Parasites were detected by blood smear in all five cases, while only three were positive in the first serological analysis by immunofluorescence. A second serology at a later timepoint showed seroconversion and confirmed the diagnosis of canine babesiosis. The blood samples of two parasitized dogs were used for DNA-isolation and were tested with a *Babesia*-specific PCR, detecting the 18S rRNA-gene. Sequencing of the amplified products revealed a 100% identity with the sub-species *B. canis canis*.

None of the presented dogs had been abroad or had visited known endemic areas in Switzerland, but all were walked in a well-defined recreation-area. A total of 152 ticks was collected from dogs and from vegetation and was specified by morphological criteria. In one case, a female *R. sanguineus* could be identified. However no *Babesia* could be detected in this tick by PCR.

Although the final proof for the complete life cycle is lacking, it is probable that *B. canis* has become autochthonous in the central and northern part of Switzerland.

USE OF A TRITRICHOMONAS FOETUS-SPECIFIC PCR IN ROUTINE DIAGNOSIS: FIRST EXPERIENCES

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Trichomonas foetus is the causative agent of bovine tritrichomonosis, a sexually transmitted disease leading to infertility and abortion in cattle. Due to the widespread use of artificial insemination and intensive testing of breeding animals, effective control but not eradication of tritrichomonosis can be achieved. So far, diagnosis of bovine tritrichomonosis was based mainly upon light microscopical examination of preputial washings from bulls and cervicovaginal secretions from female cattle. Previous in vitro cultivation of parasites from diagnostic samples increases sensitivity. However, this technique is time consuming and rather expensive. Additionally, morphological diagnosis is hampered by contamination of samples with intestinal or coprophilic (tri-) trichomonadid protozoa, which might be mistaken for *T. foetus*. A critical point is also viability of the parasites due to the sampling procedure and transportation. Therefore, in vitro cultivation may be hampered by conditions negatively selective for certain isolates. Consequently, especially when only low parasite numbers are present, PCR can offer a valuable alternative for the sensitive detection of *Trichomonas* spp.

A PCR for diagnostic use was tested on single and pooled preputial washings from bulls. In a blind study, individual samples were spiked with defined numbers of *T. foetus*. It was possible to detect 8 to 10 trophozoites in a pool of 10 wash fluids by PCR, while the InPouch TF-Test culture system reached a sensitivity of 10 parasites per individual sample.

Within the last 3 years, we routinely used PCR for detection of *T. foetus* in preputial washings from bulls selected for artificial insemination during quarantine. This analysis was carried out in addition to the prescribed InPouch TF-Test culture system. From 644 bulls, none was positive, neither in PCR nor in the culture system.

The conclusion is that pooling samples for PCR does not result in a lack of sensitivity when compared to the time-consuming individual testing with the InPouch TF-Test. On the other hand, due to high specificity of PCR, no false positive results were observed. We therefore claim the *T. foetus*-specific PCR as an internationally accepted method for the detection of this parasite.

ANTIBODIES TO *OESTRUS OVIS* IN SHEEP FROM GALICIA, NW SPAIN

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Larvae of *Oestrus ovis* cause myiasis in small ruminants maintained under semi-extensive or extensive management in hot and dry regions, such as Mediterranean countries. Likewise, it has been also detected in the warmer southern counties of several non-Mediterranean countries, like South-western Germany.

The aim of this study was to determine the presence of antibodies against *O. ovis* in sheep grazing in the province of Lugo (Galicia, northwest of Spain) during 1 year period (2001-2002).

In previous works an indirect-ELISA with metabolic antigens excretory/secretory obtained from 2nd stage larvae was developed. We collected 270 sera of sheep randomly selected in different flocks. Two zones were considered: Coastal, with a Maritime climate (18.9 °C maximum average temperature, 8.2 °C minimum average temperature, 1304 cm³ rainfall, 1899 sun hours and 0-100 m height); Interior, with a wet climate (14.4 °C maximum average temperature, 5.9 °C minimum average temperature, 1544.7 cm³ rainfall, 954 sun hours and 500-700 m height). We collected 100 samples from the Coast and 170 from the Interior.

A total of 68.1 % of the sera evaluated had positive values of antibodies against *O. ovis*. A greater seroprevalence was obtained in the Coast (76 %) than in the Interior (68.1 %). When considering the time when the samples were collected, the highest prevalence was obtained in summer (87.5 %) and the lowest in the spring (27.7 %). By taking into account both the zone where the samples were collected and the period of investigation, the highest percentage of antibodies was observed in the Coastal in summer (92.3 %) and the lowest in the Interior in spring (22.2 %). These results indicate that climatic conditions favour the development of the *O. ovis* life-cycle in the Coastal region, probably due to the warmer temperatures.

This is the first study about the presence of antibodies to *O. ovis* in sheep grazing in Galicia. We concluded that an elevated percentage of ovine had been in contact with larvae of this fly; however, further investigations are required to estimate the importance of this myiasis in the ovine livestock in areas with wet climate.

EFFECTS OF DEFORESTATION ON THE PREVALENCE OF BLOOD PARASITES IN AFRICAN RAINFOREST BIRDS

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The effects of deforestation on health are diverse, and are becoming increasingly apparent with the highly publicized recent outbreaks of several diseases spread to humans by animals. Here, we study the effects of deforestation on the spread of infectious diseases in African rainforest birds.

Over the past 13 years, a unique set of more than 4000 individual blood samples has been collected from over 200 rainforest bird species in a variety of habitats across Cameroon, the Ivory Coast and Uganda. Significantly, the samples were collected from sites both before and after habitat degradation, permitting a unique examination into the direct effects of human induced habitat alterations. Using complementary techniques of blood smear analysis, and molecular biology, samples have been assayed for *Haemoproteus*, *Plasmodium*, *Leucocytozoon*, *Trypanosoma* spp., and microfilariae. In addition, we implement satellite imagery data to predict how changes in forest composition may affect the spread of diseases in the future. Results show that prevalence rates differ between small forest fragments and large contiguous rainforest sites. In addition, degrees of host-specificity vary among parasites. Filariid nematodes appear to have a high degree of host specificity and are highly prevalent in one host species, the Fire-crested Alethe *Alethe diademata*. Trypanosomes, on the other hand appear to lack both host and geographic specificity. We also begin to link morphologically defined species with molecular DNA sequences and show that significant mitochondrial sequence divergent exists within the morphologically well-defined chicken parasite *Leucocytozoon schoutedeni*.

The goal of the research is significant because it will lead to the development of models that could predict how deforestation may influence future disease outbreaks.

DISTRIBUTION OF *TRICHINELLA* SPP. AMONG SOME MAMMALS IN LITHUANIA AND THE INFECTIVITY OF THEIR ISOLATES TO THE LABORATORY RAT

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The aim of this report is to generalise the original data on the distribution of *Trichinella* spp. among some sylvatic, synanthropic and domestic animals in Lithuania and to present the experimental data on the infectivity and persistence of some *Trichinella* isolates in laboratory rats.

Muscle samples of more than 2000 individual mammals belonging to 15 species were collected in 1987-2004. Using the methods of compressor microscopy and the staining the muscle samples in methylene blue, the prevalence and intensity of infection were determined. The highest prevalence of infection with encapsulated *Trichinella* larvae was recorded in pine martens *Martes martes* (6/9), red foxes *Vulpes vulpes* (10/28) and raccoon dogs *Nyctereutes procyonoides* (4/15). These parasites were also detected in the muscles of wolf *Canis lupus* (1/7), European polecat *Putorius putorius* (1/12), stray cat *Felis domesticus* (4/88) and wild boar *Sus scrofa* (2/160).

Response of Wistar rats to the infection with four *Trichinella* isolates (the adapted laboratory one and isolated from naturally infected pig, wild boar and cat) was examined. The body mass, clinical symptoms, peripheral blood cell picture, adaptation to the laboratory rat were considered.

Having infected the rats with the adapted laboratory isolate of *T. spiralis*, the strong dehydration, loss of the body mass or slowing down of its increase were recorded at the acute stage of infection. Among the rats infected with the natural isolate of *Trichinella* sp. from wild boar, the clinical symptoms of trichinellosis were manifested earlier (between the 7th and 14th days of infection).

Having infected the rats with *Trichinella* larvae from domestic pig, their development and incapsulation in the muscles of the new host took place more slowly. However, the larvae remained viable and were successfully repassed to the laboratory rats. The intensity of infection during the first passage was by 77 % lower and during the second passage slightly higher than in case of infection with the adapted laboratory isolate. The infection provoked the degenerative changes in the cells of peripheral blood.

When the rats were infected with *Trichinella* sp. from cat, larvae in the muscles of the new host developed slowly. Even several months after infection, the larvae did not reach the adult stage. The capsule around helminths did not develop, and the larvae were not viable. Therefore, their repassage was not successful.

Intensity of infection in the rats infected with a natural *Trichinella* isolate from wild boar was considerably higher than in rats infected with other isolates.

THE STRUCTURE OF HELMINTHS' COMMUNITY OF *APODEMUS FLAVICOLLIS* MELCHIOR, 1834 ON THE TERRITORY OF MINSK, BELARUS

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The aim of this study was to determine the species structure of helminths of *Apodemus flavicollis* and to estimate its seasonal infestation on urban territory.

The data on structure of helminths community of *A. flavicollis* were collected monthly from April to October. In all 330 *A. flavicollis* from parks with a various type of vegetation (coniferous and deciduous breeds) were investigated by total helminthological method.

Apodemus flavicollis is widely distributed in territory of Minsk. In different habitats this species occupies a place from background (in foliage tree parks, 59 % from total number of rodents), minor kind in coniferous parks (31 %), and with low frequency in inhabited and industrial buildings (0.5 %). In urban territory 12 parasite species of *A. flavicollis* were recorded (Cestoda: *Aprostotandria macrocephala* (Douthitt, 1915), *Catenotaenia cricetorum* Kirschenblatt, 1949, *C. pusilla* (Goeze, 1782), *Skrjabinotaenia lobata* (Baer, 1925), *Hymenolepis diminuta* Rudolphi, 1819, *H. horrida* (Linstow, 1901) and Nematoda: *Heligmosomoides glareoli* (Baylis, 1928), *H. polygyrus* (Dujardin, 1845), *H. laevis* (Dujardin, 1845), *Heligmosomum mixtum* (Schulz, 1952), *Syphacia obvelata* (Rudolphi, 1802), *S. frederici* Roman, 1945). In all, 18.7% of *A. flavicollis* were parasitized by worms. The clearly predominant species is *H. polygyrus* (47.0 %). The analysis of seasonal dynamics of infection has shown, that highest prevalence of infestation by helminths is observed in summer months (21.6 %); in the spring it is 15.4 % and in autumn – 14.5 %. It is necessary to note, that during one year changes the structure of species of helminths. So, in the spring we have found out 5 species of the parasites, from which 4 are cestodes (*A. macrocephala*, *C. pusilla*, *S. lobata*), in the summer - 11 species of worms (5 cestodes - *A. macrocephala*, *C. pusilla*, *S. lobata*, *H. diminuta*, *H. horrida*) and in autumn 7 species were recorded (5 cestodes - *A. macrocephala*, *C. cricetorum*, *S. lobata*, *H. diminuta*, *H. horrida*). Besides cestodes, we found the predominant species *H. polygyrus* within all year.

Thus, cestodes are the most common species of parasites of *A. flavicollis* on the territory of Minsk. This is probably due to the distribution of their intermediate hosts, which are arthropods inhabiting burrows of these rodents.

PARASITES OF HORSE MACKEREL *TRACHURUS TRACHURUS* FROM THE ATLANTIC COAST OF THE NORTHERN AFRICA AND THE AZORES ARCHIPELAGO BANKS AS POTENTIAL BIOLOGICAL TAGS FOR STOCK IDENTIFICATION

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The parasite fauna of horse mackerel *Trachurus trachurus* (L., 1758) was studied in different areas of the Atlantic coast of the northern Africa and the Azores Archipelago banks.

Samples of the horse mackerel were collected in neritic areas of Morocco, Western Sahara, Mauritania and from the banks of the Azores Archipelago (the Great Meteor Bank, the Hyeres Bank and the Irving Bank) in 1994-2003. In all 296 specimens of horse mackerel (TL = 16-45 cm) were examined by the total parasitological dissection method.

Twenty-one species of parasites of following groups have been found: Myxosporidia (4 species), Monogenea (3), Cestoda (3), Trematoda (5), Acanthocephala (2), Nematoda (3) and Crustacea (1). Total infection rates in all six areas ranged between 35% and 98%. Fauna of parasites and quantitative infection indexes were not similar in different areas. Nineteen species of parasites were found in horse mackerel from neritic areas of Morocco. The rates of infection were considerably higher for monogenean *Gastrocotyle trachuri* (Prevalence – 50.4%, Intensity - 1-31 specimens) and larval nematodes *Anisakis simplex* (P=57.4%, I=1-320). Eight species of parasites were common in fish from neritic areas of Western Sahara. Monogeneans *G. trachuri* and *Pseudaxine trachuri* had the highest indexes of infestation (P = 91%, I = 1-14 and P = 52%, I = 1-8, respectively). There were found six parasites in the horse mackerel from the Mauritania waters. The parameters of infestation by main monogeneans *G. trachuri* (P = 34%, I = 1-16) and *P. trachuri* (P = 33%, I = 1-7) and larval nematodes *A. simplex* (P = 24%, I = 1-3) and *Hysterothylacium* sp. (P = 24%, I = 1-2) were lower than in areas of Morocco and West Sahara. Myxosporidia and trematodes were absent in the horse mackerel from the banks of the Azores Archipelago. Six species of parasites were found in the horse mackerel from the Great Meteor Bank. The prevalence and intensity data for the main parasite species (*G. trachuri*, *P. trachuri* and *A. simplex* 1.) were similar to these of fish from Morocco. Five parasites were marked in the horse mackerel from the Irving Bank. Myxosporidia, monogeneans and trematodes were absent there. Larval nematodes *A. simplex* (P = 79%, I = 1-2) had the highest parameters of infestation. The poorest parasite fauna was in fish from the Hyeres Bank. Only one species, *A. simplex* 1. (P = 40%, I = 1-4) was recorded.

The differences between horse mackerel parasite fauna in the neritic areas and in the Azores Archipelago banks were found. Peculiarities of mackerel parasite fauna in

two areas (Morocco - Western Sahara and Mauritania) support the hypothesis on the existence of isolated populations in these two regions.

WATER BIRD DIGENEANS IN COASTAL ECOSYSTEMS OF SW ICELAND: FAUNAL COMPOSITION, DISTRIBUTION AND TRANSMISSION PATTERNS

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In Iceland, water birds such as ducks, waders and gulls are trophically connected with coastal ecosystems and a rich digenetic trematode fauna occurs in prosobranch molluscs in the intertidal zone. Extensive studies have revealed intramolluscan stages of 29 digenean species belonging to the families Microphallidae, Echinostomatidae, Notocotylidae, Heterophyidae, Gymnophallidae, Psilostomidae and Philophthalmidae. Species composition and prevalence significantly vary from one littoral site to another as a result of different habitat use and distribution patterns of the final hosts, the birds. Highest prevalence indices have been found in areas where birds accumulate, e.g. in and around fishing ports which attract large and small gulls as well as in harbour areas which are temporarily visited by flocks of eiders or some waders searching for food. Microscale heterogeneity in trematode infections has often been recorded at a distance of just few meters.

Studies performed in the Melabakkar saltmarsh area (peninsula of 0.6 km², dotted with dozens of brackish ponds) showed that digenean species composition and prevalence in the mudsnail *Hydrobia ventrosa* significantly varied from one pond to another. Correlation analysis of different biotic and abiotic variables and the trematode infections showed that the variables indirectly affected the trematode infections in the ponds because some of them determined how attractive the ponds were for the birds. We concluded that the clear correlation between infection parameters and the composition, abundance and behaviour of the final hosts (the birds) was determined by the life cycle patterns of the trematode species occurring in the area.

The first intermediate hosts of trematodes, the gastropods, are not mobile animals. There are a few mechanisms by which invasive stages of water bird digeneans can spread from snails in Iceland. In microphallids of the "pygmaeus" group, *Microphallus brevitatus*, and *M. pirum* only a gastropod is involved in the life-cycle whereby the metacercariae which are invasive for the final hosts develop inside daughter sporocysts. In the notocotylids and the philophthalmids cercariae rapidly encyst on the surface of various underwater substrates such as grass and invertebrate shells after a short swimming phase and become invasive for the definitive hosts immediately after their encystment. In the echinostomatids, psilostomatids and gymnophallids the role of the second intermediate host is played by immobile bivalves

and gastropods. In microphallids with three-host life cycles the metacercariae develop in amphipods or isopods but in the case of the heterophyids – in littoral fishes such as sticklebacks. However, none of these intermediate hosts is able to travel for long distances. Thus, in general, the restricted distribution possibilities of intermediate hosts harbouring invasive stages of Icelandic water bird digeneans promote intensive infections in areas where final hosts gather, for example in areas which offer birds shelter or where food is abundant.

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NEW FINDINGS ON *TRICHOBILHARZIA* SPP. AND SWIMMER' S ITCH IN ICELAND

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Swimmer's itch (SI) was first reported in Iceland in 1997. Until now, *Trichobilharzia* cercariae have been confirmed in nine lakes or ponds where bird life is rich and the snail *Radix peregra*, the only potential intermediate host of *Trichobilharzia* spp. in Iceland, is abundant. Morphologically distinct, species-specific egg forms and/or adult worms suggest that at least four *Trichobilharzia* species occur in Iceland; three are representatives of visceral schistosomes (found in swans *Cygnus cygnus*, geese *Anser anser* and mallards *Anas platyrhynchos*, respectively) and one was found in the a nasal area of mallards. Morphological and molecular work on the taxonomy gradually steps forward.

The inland hot spring area of Landmannalaugar annually attracts approximately 150,000 tourists. Until recent years, roughly half of the visitors used to bath or swim in a brook in the area filled with slowly streaming, geothermally heated groundwater. Starting in the second half of August SI was frequently reported after bathing in the brook until October when the number of cases dropped but occasional infections were reported in December 2003 and in March and April 2004. In spring and early summer 2004 no SI cases were recorded but, as in the previous year, from the middle of August until October 2004 SI was frequently reported among persons who ignored warning signs in the area. The prevalence of snails shedding *Trichobilharzia* cercariae on the bathing site on three occasions during autumn 2003 and in September 2004 never exceeded 1%. Several wardens and visitors who have frequently bathed in the hot spring area in past decades were asked if they ever acquired dermatitis in the area. Some of them recall to lesions from the 1980s and 1990s which resemble SI but a general outbreak as described in past two autumns obviously not occurred in past decades. Mallards seem to be responsible for the recent introduction of the parasites to

the area. In past two years one or two mallard females bred and raised their ducklings close to the bathing area. Attracted by thousands of snails per m² the mallard families were repeatedly observed by searching for food on the bathing site and in the upper part of the brook. Post mortem examination of the ducklings (five collected by the end of August 2003, five collected by the end of September 2004) revealed both visceral- and nasal *Trichobilharzia* infections in all birds. Considering the generation time of *Trichobilharzia* spp. and the fact that SI already appeared by the middle of August in both years the ducklings apparently acquired the infections soon after being hatched in June.

Severe SI started in Lake Botnsvatn (NE-Iceland) by the end of July 2004. Two weeks later a warning sign dissuaded guests from wading or swimming in the lake. Unusually high infection prevalence (9.4%) was reported in snails in the lake and 88% of the 25 humans known to expose themselves to the cercariae got SI. Visitors at the lake report that SI has been acquired in the lake in late summer since 2001.

BIODIVERSITY OF THE PARASITE FAUNA OF FISH FROM THE SZCZECIN LAGOON AND THE POMERANIAN BAY, POLAND

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The Pomeranian Bay is the area where the waters discharged by the Odra River mix with the waters of the Baltic Sea. The Szczecin Lagoon is inhabited by freshwater fish, while the Pomeranian Bay is the destination place of their feeding and reproductive migrations. The latter body of water is also inhabited by Baltic fish. The aim of the present study was to determine the composition of parasite fauna of three most common fish species in both areas. The targeted fish species differ in their life styles and food preferences, and therefore it was interesting whether they may host any "common" parasite species. Another puzzling question was whether the parasite numbers, their specific composition, dominance or absence of certain parasite species may affect the condition factors of the fish studied.

Bream (60 individuals), perch (55), and cod (41) were acquired from commercial catches. The average weights of the fish were: 779.98 g, 199.27 g, and 596.22 g, respectively, while their average lengths—32.5 cm, 21.6 cm, and 36.9 cm, respectively. During necropsy the parasites were identified from wet mounts or they were fixed and preserved in 75% ethyl alcohol. The following parameters were calculated: the mean intensity of infection, abundance, prevalence and Fulton's condition coefficient (of fish studied).

All studied fish, representing three species, were infected by parasites. The prevalence values of bream parasites ranged from 1.67% (*Caspiobdella fadejewi* and

glochidia of *Unio* sp.) to 73.34% (*Diplostomum* spp.). This parameter for perch parasites ranged from 1.82% (*Piscicola geometra*, glochidia of *Unio* sp., and *Pomphorhynchus tereticolis*) to 47.27% (*Bunodera luciopercae*). The lowest prevalence for cod parasites was for *Ascarophis arctica*, *Pseudoterranova decipiens*, and *Diphyllbothrium* sp., while the highest - for *Echinorhynchus gadi* (95.12%). In bream, the highest abundance (11.18) represented metacercariae of *Diplostomum* spp. In perch - *Trianenophorus nodulosus* (10.36). Leaches *Piscicola geometra* and glochidia of the Unionidae species were common parasites of bream and perch. *Echinorhynchus gadi* in cod represented the highest mean intensity of infection (61.1). Tapeworm *Diphyllbothrium* sp. was a common parasite of perch and cod. There was a negative correlation between the number of *E. gadi* found in cod and the condition of the host fish. No such correlation was observed between other parasites and their hosts.

OBSERVATIONS ON PIGLET ISOSPOROSIS IN RELATION TO TOTRAZURIL TREATMENT

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Coccidiosis caused by *Isospora suis* is an important cause of suckling piglet diarrhoea reported from all types of farrowing facilities. The aim of this study was to investigate the impact of a short term treatment with totrazuril to the spread of isosporosis in a naturally infected with the parasite pig farm. The trial took place under regular farm farrowing conditions, on a farm where anti-coccidial compounds were not used before and the overall prevalence of isosporosis among litters was approximately 90%, diarrhoea being present in 54% of those. The farrowing house of this farm was divided in 4 separate rooms with 20 pens each. The farmer was farrowing in a different room each week in "all in all out" per room system.

Toltrazuril (BAYCOX 5%) was administered orally (at one single administration at a dose of 20 mg Toltrazuril/Kg BW) 3 days after farrowing to piglets in all 4 houses for 2 consecutive usages of the farrowing houses (8 continuing weeks). After that the litters in the farm were left without treatment for 1 usage of the houses (4 continuing weeks).

Throughout the trial period faeces consistency (score: 1 formed to 4 liquid) and oocyst excretion (opg: oocysts per gram of faeces) were recorded from faecal samples taken individually and then pooled from half of each litter population on days 10, 14 and 18 after farrowing. The samples were examined by a concentration flotation technique using saturated sodium chloride solution with glucose and counted in a McMaster chamber under a fluorescence light source. In addition, data on farrowing date and the weight of all piglets at farrowing and weaning were also

recorded. In total there were examined: 40 litters (415 piglets) before treatment applied, 160 litters (1680 piglets) during treatment and 80 litters (848 piglets) after treatment stopped.

The presence of *I. suis* oocysts in faeces was greatly reduced while the animals were treated being present in only 12% of the litters at the same time as diarrhoea was recorded in 5.3% of the piglets treated. Piglets treated with totrazuril had a notably higher growth rate than before treatment (mean piglet bw before treatment 7.3 kg, after treatment 8.1 kg). With the interruption of the treatment the disease was gradually re-introduced in the farm. Oocysts of *I. suis* were detected in 25% of the first after treatment farrowed litters and in 100% of the last ones. Likewise, the prevalence of diarrhoea in piglets ranged from 9.8% the 1st week to 41.9% the 4th week after treatment was interrupted.

DOES HELMINTH INFECTION INCREASE THE RISK OF OTHER ENTERIC PATHOGENS? A COMMUNITY STUDY OF SCHOOL CHILDREN IN PERI-URBAN GUINEA-BISSAU

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Concomitant helminths and bacteria may affect the course, and the resulting disease outcome, of the individual infections. The aim of the present epidemiological study from Guinea-Bissau was to explore correlations between intestinal helminth infections and other gastrointestinal pathogens in schoolchildren from a poor semi-rural area of the capital Bissau, while adjusting for certain socioeconomic risk factors. A total of 705 children were examined and helminthes were detected in 312 children (44.2%), enteropathogenic bacteria in 97 (13.7%), protozoans in 361 (51.2%), and rotavirus in 42 (6%). In all, 537 (76.1%) had an infection of some sort, and 41.6% were concomitantly infected with more than one and up to five gastrointestinal pathogens. In the final model for helminth risk factors, only an association to *Entamoeba histolytica* remained significant. No associations were seen between egg counts and infection with any of the other pathogens. Other risk factors in relation to helminths included increasing age, male sex, and chicken husbandry in the neighbouring family. Maternal school attendance, belonging to a Muslim family, or having electricity decreased the risk of helminth infection. It cannot be ruled out that a higher intensity of hookworm or more infections with more pathogenic helminths, may have revealed associations with other pathogens, as has been shown in other studies.

IDENTIFICATION OF BLOODMEALS FROM TSETSE FLIES BY PCR-RFLP ANALYSIS

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Tsetse (*Glossina* spp.) are the primary vectors of animal and human trypanosomosis in tropical Africa, and are a continuing threat to livestock production, human health and agricultural development over much of the continent. Knowledge on the feeding behaviour of tsetse flies is of importance in improvement of vector control and disease eradication strategies. Serological techniques using host-specific antisera were developed several decades ago to identify the source of vertebrate blood from the intestinal tracts of wild-caught tsetse flies. Up to now, however, some problems exist with the accurate identification of phylogenetically closely related species due to interfering cross reactivity between species leading to a considerable percentage of bloodmeal hosts only classified down to the family level. The aim of this study was to adapt the restriction fragment length polymorphism (PCR-RFLP) analysis technique for host species identification of tsetse flies.

Blood samples from ten species of the family *Bovidae* were enrolled in the study. A variable 359 bp region of vertebrates cytochrome *b* gene (*cyt b*) was amplified using universal primers and subjected to PCR-RFLP analysis. To obtain sequence information on the *cyt b* gene of the different bovid species tested, the retrieval tool of the National Centre of Biotechnology Information (NCBI) was utilized. Search for appropriate restriction enzyme sites were supported by means of the free available programme, NEB cutter V2.0.

PCR-RFLP analysis largely yielded the predicted fingerprints and the bovid species under investigation were accurately identified. Unexpected patterns with a surplus of additional bands after digestion were noticed in some samples tested but did not readily hamper species identification in our study. Such additional bands are commonly attributed to the co-amplification of *cyt b* pseudogenes. It is thought that they are evolutionarily incorporated into nuclear genome during repair of chromosomal breaks by non-homologous recombination. In tsetse flies, adequate PCR amplification of the animal host DNA for subsequent RFLP analysis amounted to 100%, 80%, 60% and 40% at 24, 48, 72 and 96 h after feeding, respectively. In case of subsequent feeding on different hosts animals 48 h apart, generally the last bloodmeal could be identified.

It is concluded that the PCR-RFLP analysis is a promising method for host species identification in tsetse flies and most probably other haematophagous arthropods.

ECHINOCOCCUS MULTILOCULARIS IN THE SIBLING VOLE – ARCTIC FOX SYSTEM AT SVALBARD

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In 1999, the cestode *E. multilocularis* was detected in an isolated population of sibling voles *Microtus rossiaemeridionalis* at the high arctic island of Spitzbergen in the Svalbard archipelago. At Svalbard, the sibling vole and arctic fox are the only available intermediate and definitive host species for *E. multilocularis*. The sibling vole exists only in a geographically very restricted area and long term studies have shown considerable temporal and spatial variation in vole densities within this area. Since 2002 the causes of observed variation in vole densities and effects of this variation on the transmission dynamics of *E. multilocularis* have been investigated using snap-trapping and mark-recapture studies of the vole population, estimation of scat densities and *E. multilocularis* coproantigen prevalence in the scats from the fox population, and by detailed monitoring of local climate. Between years variation in vole densities is mainly determined by variation in winter conditions, but has delayed effects on the transmission dynamics of *E. multilocularis*. The results are discussed with focus on the impact of seasonality in arctic fox diet and demographic delays in the life-cycle of *E. multilocularis*.

DISPERSION PATTERN AND PREFERENCE FOR FISH LIPIDS IN *ANISAKIS* *SIMPLEX* THIRD-STAGE LARVAE: AN *IN VITRO*-STUDY

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In nematodes behaviour in response to chemical stimuli has been extensively investigated in some free living- and plant parasitic species. Regarding this matter in animal parasitic species little is yet known, especially in the marine forms. However, some results indicate that *Anisakis simplex* L3-larvae tend to prefer fish tissue with a high content of lipids. The intention of this study was to consider this situation in further details by investigating the behaviour of *A. simplex* L3 in response to different concentrations of fish lipids. This was done by use of an *in vitro*-study based on material from cod *Gadus morhua*, Northern-Norway 2002. For 2 weeks samples of 10

larvae was placed in each of the culture containers (agar based on distilled water and 0.9 % NaCl). The agar in all containers was separated in 3 segments of equal size. Three categories of agar were used: (A) 0 %-, (B) 2 %-, and (C) 7 % cod liver oil. A total of 900 larvae were included.

The study consisted of three experiments. Experiment I was intended to establish whether different lipid concentrations influenced the migration pattern at all. Three different groups of containers were filled with agar from one of the agar categories A, B or C. In this experiment the larvae were put along the mid-line in the central segments. Experiment II was intended to examine whether *A. simplex* L3-larvae are able to actively search for lipids. The agar sequences in the two groups included in experiment II were respectively: A B C and A C B. To gain further information on the short-distance dispersion of the L3-larvae, experiment III was set up with the segment sequence: B A C. In experiment II and III the larvae were put along the mid-line of the A-segment.

Experiment I indicated that the L3-larvae move- but do not *stop* randomly since the tendency to move out of the start segment was inversely correlated with lipid concentration. Experiment II indicates that the larvae are almost unable to select areas of high lipid concentrations when there is any distance involved. Experiment III showed a significantly higher number of larvae in the C- than in the B-segment, indicating that the L3-larvae prefer a high fat content and can seek it out over short distances. The pattern of dispersion demonstrated in this study could be accounted for by applying a modified version of the mathematical model "random walk."

WHAT ARE HOSTS OF *PHYLLODISTOMUM FOLIUM* (TREMATODA, GORGODERIDAE)? THE COMPARISON OF RIBOSOMAL DNR SEQUENCES OF TREMATODES FROM INTERMEDIATE AND FINAL HOSTS

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A wide range of fish species has been recorded as hosts of adult *Phyllodistomum folium* and obvious disagreement exist regarding the degree of specificity of this parasite. The first description of *P. folium* was made on material from pike *Esox lucius*. According to some authors *P. folium* is mainly a parasite of three-spined sticklebacks *Gasterosteus aculeatus*. Cercaria of *P. folium* was described from *Dreissena polymorpha*. The figures presented as *P. folium* in the literature clearly indicate that the species is extraordinary variable or probably several species have been described under the name *P. folium*. We have used ribosomal 28S and ITS DNA sequences to compare adult and larval stages to avoid the information noises of the morphological data.

Adult and larval phyllodistomes were collected in fish (*G. aculeatus* and *E. lucius*) and bivalves (*D. polymorpha*, *Sphaerium corneum*, and *Pisidium amnicum*) from different water bodies in Lithuania and Belarus.

Sequence differences of larvae from *D. polymorpha* revealed existence of at least 2 intraspecific variations differing in ITS1 fragment which was longer by 5 bps in one variation and has 10 different bps; ITS2 and 28S fragments had 3 and 9 different bps, respectively.

The 28S and ITS2 sequences were identical in phyllodistomes from *S. corneum*, *G. aculeatus* and *E. lucius*. However, comparison of ITS1 sequences from these species indicated, that pike's trematode ITS1 fragment had 7 different bps and was longer by 2 bps. DNA sequences of larvae from *D. polymorpha* and *P. amnicum* were different and differ from above mentioned sequences.

Sequence identity matrixes of phyllodistomes from *S. corneum* and *G. aculeatus* (identical sequences), *D. polymorpha* and *P. amnicum* shown substantial differences: an alignment of ITS1-ITS2 sequences (727 bps) had 13 or more gaps in each sequence. The sequences of phyllodistomes from *D. polymorpha* and *P. amnicum* were most similar (identity index 0.932); while phyllodistomes from *G. aculeatus* and *S. corneum* compared with larvae from *D. polymorpha* were most genetically different (0.757). The sequence identity matrix of 28S sequences (1178 bps) of these parasites has shown the similar results.

These data clearly indicate, that intermediate host of *P. folium* is *S. corneum* and final hosts are *E. lucius* and *G. aculeatus*. Minor differences in ITS1 sequence of *E. lucius* could be estimated as intraspecific differences. Trematodes from *D. polymorpha* and *P. amnicum* are different *Phyllodistomum* species and further investigations to determinate their final hosts are required.

NORMAL MICROFLORA OF THE DIGESTIVE TRACT OF HYDROBIONTS AS INDICATIVE OF HEALTH

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Complex investigations of the abundance, species composition of bacteria in the digestive tract of hydrobionts, as well as the activity of intestinal bacteria in biosynthesis of vitamins, ferments and amino acids have been carried out during different periods of hydrobionts feeding. Simultaneously biochemical investigations of hydrobionts have been performed. Microbial communities in the digestive tract of fishes, mollusks and crayfish are not accidental of transit. It has been established that the main factors controlling the quantitative and qualitative composition of microbial populations in the digestive tract of hydrobionts and also the biochemical and

biosynthesical activity of intestinal bacteria are considered to be the feeding spectrum, environmental conditions and physiological state of macroorganism.

It has been determined that intestinal microflora of these animals possesses the elements of constancy. In the comparative aspect the functions of microorganisms of the digestive tract of hydrobionts have been analyzed. The microorganisms were regarded not as food but as suppliants of enzymes taking part in the decomposition of food substrate.

It has been proved that digestion of these animals is marked not only by the qualitative composition of food and enzymatic systems of digestion organs but by the character of endosymbiotic interrelationship between macroorganisms and microorganisms inhabiting its intestines and their capability to form and to provide the host organism with vitally indispensable metabolites as well.

Intestinal bacteria from the digestive tract of fish, mollusks and crayfish are notable for being highly active producers of valuable compounds serving as vitally necessary metabolites for these animals. In some cases these bacteria produce up to 94% of essential amino acids of the amount of free amino acids detected in the digestive tract of these hydrobionts.

PELODERA STRONGYLOIDES AS A CAUSE OF DERMATITIS IN A LABRADOR BREED DOG

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Pelodera strongyloides (Rhabditida: Nematoda) is a free-living saprophytic soil nematode. There are reports about skin invasions of the third stage larvae in rodents, dogs, cattle, horses, sheep guinea pigs and even humans. Exposure to the larvae occurs through direct contact with infested material or soil. It is believed that the larvae are not able to penetrate healthy skin and the infection is self-limiting when the exposure to the larvae ends.

In April 2003, a 5 years old female Labrador came to the Small Animal Clinic with signs of severe pustulous dermatitis and alopecia on lateral thorax and abdomen and on outer sides of thighs. The dog suffered from severe itch. Alive worms of 500-600 μm of body length were found from skin scrapings. Larvae were cultivated on agar plates and free-living females and a few male worms were obtained after one day. The worms were identified as *Pelodera strongyloides* (Schneider, 1860). Subcutaneous ivomec treatment at recommended dose was applied three times with 10 days intervals between treatments without seeming clinical and parasitological

improvement. Skin scrapings still contained alive larvae. Then tea tree oil treatment of damaged skin was carried out three times. Itch disappeared and hair started to grow, although a few alive larvae were still found. By the end of July all skin scrapings remained negative and the dog had totally improved. It is not clear whether the dog had some kind of skin damage before the *Pelodera*-infestation or not. Ivermectin seemed to be not effective against *Pelodera* 3rd-stage larvae in the skin. These larvae are called also “dormant” and probably their metabolism is too low to ensure sufficient intake of the drug.

In our study tea tree oil direct application to the skin appeared to be the most effective therapy. Seemingly certain *Pelodera* strains may be more pathogenic than others and more evolved towards parasitic mode of life.

MANAGEMENT AND CONTROL OF PARASITIC GASTROENTERITIS IN SHEEP USING COMPUTER MODEL SIMULATIONS

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Parasitic gastroenteritis (PGE) is a complex helminthological disease syndrome resulting from the interplay of a large number of factors, including climate, nematode bionomics, animal management and husbandry practices. Computer models have been reported that both predict the rate of development of anthelmintic resistance, and the effectiveness of control strategies for species of sheep nematodes. This paper provides an overview on the development of a computer model for use in the predictive design of integrated control strategies for PGE in sheep that takes into account factors such as host-parasite interactions, parasite biology and bionomics, farm managements and husbandry systems, regional climatic variations and climate changes, and patterns of anthelmintic usage.

The model is built around extensive data for the major species of parasitic nematodes of sheep found in the UK (*Ostertagia* spp., *Haemonchus* spp, *Trichostrongylus* spp. and *Nematodirus* spp.) and regional sheep management, each of which has been collated and incorporated into equations that drive information around the model. Farm management systems can be defined using a variety of specific input options via a user interface that sits above the modelling layer. The model allows the user to carry out predictive simulations and plot the dynamics of infection over time using a range of different user-defined options. A further sector has been built into the model to describe the dynamics of anthelmintic treatments with the 3 major anthelmintic classes. The interaction between treatments and resistant and susceptible worms within the parasite life cycle model are described using equations again derived from published data. The user is then able to input values for any treatments implemented.

The model can be used to examine the effects of different treatment strategies on levels of infections and predict the effect that they have on the development of resistance. The model is unique in that it has been constructed to allow a wide range of user inputs that can be used to define flock management systems and treatment strategies for individual farms in differing regions of the UK.

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FASCIOSIS IN DANISH CATTLE: A RE-EMERGING PROBLEM?

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The liverfluke, *Fasciola hepatica*, may cause severe disease and production loss in small and large ruminants. Fasciolosis in cattle can lead to reduced milk production, lowered fertility, reduced weight gain and condemnation of infected livers at slaughter. In Denmark in 1973, Riising *et al.* reported an overall prevalence of 16.4-16.6% based upon slaughterhouse registrations (1969-1972). In a later study by Henriksen & Pilegaard-Andersen it was found that the prevalence decreased and it was speculated that improved drugs for treatment as well as drainage of land contributed to this. Since the early 90th wetlands have increasingly been recognized as important for the environment, and farmers have been encouraged to graze these areas with cattle. This change in practice, or re-adoption of old practices, together with a change in climate may have increased the risk for grazing animals to become infected.

An epidemiological study of fasciolosis in Danish cattle based on recordings of liver condemnations at the abattoirs from 2000 to 2003 was performed. The study included a total of 1.4 million heads of cattle (or 85-90% of all cattle slaughtered during that period) on approximately 16,000 farms. The prevalence data were analysed with regard to production type (beef or dairy), years and animal category defined by age and sex.

A significant difference between the prevalence in dairy and beef cattle was found for all animal categories ($P < 0.001$) except heifer calves ($P = 0.06$). The Odds Ratio (OR) for beef cattle versus dairy cattle varied from 2.5 for steers to 11.1 for slaughter calves. An overall increase in prevalence from 2000 to 2003 from 3.2% to 8.3% and 0.9% to 2.7% for beef and dairy cattle, respectively, was observed. This included significant increases for all animal categories except heifer calves. In both beef and dairy cattle, the highest prevalences were found in older cattle (adult bulls and cows) or cattle that are often grazing marginal land (2 year-old steers and old heifers). In the same period the farm prevalence increased from 11.5% to 22.3% and from 11.7% to 25.7% of beef and dairy farms, having one or more infected animals. Regional maps

of prevalences for dairy and beef cattle supported earlier studies showing large regional differences with the overall highest prevalence in Jutland. The more than doubling in prevalence in this 4-year period could be a result of changes in management (more grazing of wetlands), reduced use of flukicides and/or climatic changes but an effect of better level of recording at abattoirs cannot be ruled out.

IMMUNOGLOBULIN UPTAKE AND PROCESSING BY *SCHISTOSOMA MANSONI*

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Schistosoma mansoni worms in experimentally infected mice were studied for the localization of *in vivo* bound host immunoglobulins at the light and ultrastructural level. Furthermore, immunoglobulin uptake by intravascular worms was demonstrated after passive administration of pooled human immunoglobulins labeled with radioactive Indium.

The results show that host immunoglobulins are located in parasite organelles identified as "elongated bodies" or "discoid bodies" subtegumentally in the parasite corresponding to a fine granular staining at the light microscopical level. Indium labeled immunoglobulins were shown to localize as fine strands running perpendicular to the parasite surface. The results are consistent with studies showing presence of immunoglobulin Fc-receptors at the schistosome surface. Our results further support the observation that paramyosin may function as such a Fc-receptor since this parasite protein has been localized to "elongated bodies".

Taken together these results suggest that host immunoglobulins are taken up by intravascular schistosomes as part of an unknown process involving transportation into the parasite.

MOLECULAR EPIDEMIOLOGICAL SURVEY ON THE EQUID GASTRIC HABRONEMOSIS IN CENTRAL ITALY

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Gastric habronemosis is a parasitic disease of equids caused by *Habronema microstoma* and *H. muscae* (Nematoda, Spirurida) and is characterized by gastritis,

diarrhoea, progressive weight loss and ulcers. Despite the importance of gastric habronemosis in equine practice there are still gaps in our knowledge on the epidemiology, mainly due to diagnostic constraints. In fact, the clinical diagnosis is impossible and the coprology (i.e. flotation procedure, Baermann technique and coproculture) has a very low sensitivity. Therefore, a reliable diagnosis can be achieved only by necropsy. Recently, a species-specific PCR-based assay using markers in the second internal transcribed spacer (ITS2) of ribosomal DNA (rDNA) allowed the accurate identification of *H. microstoma* and *H. muscae* irrespective of their biological stage. This assay has been later modified into a two-step semi-nested PCR assay, powerful for the detection of *H. microstoma* and/or *H. muscae* DNA in equine faeces with high sensitivity and specificity (96.7 and 100% respectively), thus allowing the molecular diagnosis *in vivo* of gastric habronemosis. To evaluate the efficacy of such diagnostic assay in the field, a molecular epidemiological survey was carried out on the equid gastric habronemosis in central Italy.

From April to September 2004, 131 individual faecal samples were collected from the rectum of native horses bred in a *Habronema*-infected area in central Italy. All 131 faecal samples were subjected to the coprological examination (i. e. flotation procedure) and to the diagnostic semi-nested PCR.

Out of 131 horses, 59 were positive for strongyle eggs, 4 for ascarid eggs, 10 for both strongyle and ascarid eggs, 1 for both strongyle and oxyurid eggs, and 1 for both ascarid and oxyurid eggs. No horses (0%) were found positive for *Habronema* spp. larvated eggs. Regarding the results of the semi-nested PCR assay, 49 horses (37.4%) were positive for *H. microstoma*, 10 for *H. muscae* (7.6%) and 13 for both species (9.9%). Thus, the related prevalence rate of gastric habronemosis detected by the molecular assay is 54.9%.

This study demonstrates that the two-step semi-nested assay previously developed for the detection of *Habronema* spp. DNA in stool samples clearly overcomes the major constraints of traditional diagnostic techniques. Thus, this technique both confirms the presence of habronemosis in horses in central Italy and provides a powerful approach for field *ante mortem* surveys of the prevalence of *H. microstoma* and *H. muscae* infections in equids, which is likely to have been underestimated to date due to the inherent copro-diagnostic limitations.

DIVERSITY OF *BORRELIA BURGDORFERI* SENSU LATO GENOSPECIES IN *Ixodes ricinus* - LIKE TICKS IN LITHUANIA

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Transpalearctic species *Ixodes persulcatus* is overlapped in the north of Lithuania by *I. ricinus*, which is typically European in distribution. Sympatry of these two species is possible, nevertheless *I. persulcatus* was found only once in northern Lithuania in 1972. Ticks of the *Ixodes ricinus* group are known as major vectors of the causative agents of Lyme borreliosis in European countries. Recent studies have demonstrated that the Lyme disease agent actually represents a bacterial species complex comprising 11 known genospecies, which are structured ecologically into distinct clusters that are host specific.

The aim of this study was to determine whether *I. ricinus* - like ticks collected in north regions of Lithuania were infected with Lyme borreliosis, to identify genotypes and to identify the species of *I. ricinus* - like ticks by molecular methods, unless morphological identification is rather controversial. *Ixodes ricinus* - like ticks collected from south – east regions of Lithuania and in Norway were included in this study also. All ticks were analysed individually.

Ixodes ricinus - like ticks were identified by molecular analysis of already known markers. 150 bp segment of the 5.8S rRNA gene, which is specific for *I. ricinus*, was amplified. All the ticks from the north part of Lithuania (53 samples) and Norway (27 samples) were confirmed as *I. ricinus* species. Hypothesis about only one *I. ricinus* species presence in Lithuania will be discussed.

Molecular analysis of *Borrelia burgdorferi* genospecies showed rather low diversity, especially for *B. burgdorferi* s. s. *Borrelia* genospecies were identified as follows: 66% (25/38) *B. afzelii*, 31% (12/38) *B. garinii* and 3% (1/38) *B. burgdorferi* s. s. across all the territory of Lithuania. Most prevalent genospecies in the northern Lithuania was *B. afzelii* 13 % (9/67), followed by *B. garinii* 1% (1/67) and *B. burgdorferi* s. s. was absent. The distribution of genospecies is similar to that in Europe except *B. garinii* (21.2%). Most abundant genospecies in west Europe is *B. afzelii* (39%) and *B. burgdorferi* s.s. is rare (1.5%). Different genospecies may have varying predilections for different reservoir hosts, as *B. afzelii* genospecies has a special association with rodents, *B. garinii* – with birds. Further studies required identifying the presence of particular reservoir hosts in a habitat and also their role in generating infected ticks.

TERMINOLOGY OF MALARIA: WHAT IS THE DIFFERENCE BETWEEN MALARIA PARASITES AND OTHER PIGMENTED HAEMOSPORIDIANS?

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In numerous current publications in molecular biology, the term 'malaria parasites' has been used in its earliest meaning, i.e. it has been employed for all pigmented (and even some non-pigmented) haemosporidian parasites (Sporozoa, Haemosporida) inhabiting red blood cells (and sometimes white). It is difficult to agree with this terminology.

When life cycles of haemosporidians were studied in the 20th century, the clear differences in epizootiology of diseases caused by representatives of the different genera of pigmented haemosporidian parasites (*Plasmodium*, *Haemoproteus*, *Hepatocystis*, *Nycteria* and others), and non-pigmented, have been merged. The *Terminology of malaria and of malaria eradication* (WHO, 1963) recommend restricting the term 'malaria parasites' to the haemosporidians belonging to the genus *Plasmodium*. P.C.C. Garnham (1966) followed this recommendation in his outstanding monograph *Malaria parasites and other haemosporidia*. Subsequent studies and reviews of haemosporidians continued to apply the term 'malaria parasites' to those pigmented parasites that multiply in the blood stream, i. e. *Plasmodium* species. The species of *Haemoproteus*, *Hepatocystis*, and other haemoproteids are excluded from this definition because they do not multiply in the blood even though they sometimes give rise to outward clinical signs and symptoms characteristic of malaria. It is worth noting that malaria is characterised by recrudescence and is transmitted by mosquitoes, whereas species belonging to the genus *Haemoproteus* and closely related genera are not.

Certainly, molecular genetics provides inexhaustible opportunities for investigations into the phylogenetic relationships of haemosporidian parasites, but it is limited in what it can tell us about the basic life history strategies of these organisms. Much information would be omitted from consideration if the data on species of the

Plasmodiidae and Haemoproteidae were combined under the name 'malaria parasites' during ecological, epizootiological and molecular biology studies, which is currently a frequent occurrence in publications in molecular biology.

The epidemiology of diseases caused by species belonging to different genera of the Plasmodiidae and Haemoproteidae differ from each other. We feel that traditional terminology of malaria should be retained until we have a better understanding of how the genetics of this group of parasites reflects their diverse life history strategies. It would be preferable to restrict the term 'malaria parasites' to species belonging to the genus *Plasmodium*. Diseases caused by *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* spp. may be named as malaria, haemoproteosis, and leucocytozoonosis, respectively.

NESTED CYTOCHROME *B* PCR DIAGNOSTICS UNDERESTIMATE SIMULTANEOUS INFECTIONS OF AVIAN HAEMOSPORIDIAN PARASITES: A CALL TO IMPROVE THE METHOD

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Numerous PCR-based methods were developed and used increasingly to screen blood samples from wild vertebrates for diagnosing haemosporidian blood infections. PCR tests using highly conservative primers offer an inexhaustible reserve for future investigations into determining host-parasite relationships. However, a critical evaluation of sensitivity of these methods in estimation of simultaneous infections caused by different species belonging to the same and different genera of haemosporidians (Sporozoa, Haemosporida) has not been done, but is important to estimate a diversity of these parasites in natural host populations. Our objective was to link the information obtained by traditional microscopy and nested cytochrome *b* (*cyt b*) PCR in determining simultaneous haemosporidian infections in naturally infected bird.

Blood samples from seven species of birds belonging to five families of the Passeriformes were collected on the Curonian Spit (Russia) and in Ottenby (Sweden) in May-July 2003. The nested PCR protocol was used for amplifying and sequencing a fragment of approximately 480 nucleotides of the *cyt b* of the mitochondrial DNA (mtDNA) of *Plasmodium* and *Haemoproteus* spp.

Samples from 33 individual birds with single infections of *Haemoproteus* or *Plasmodium* sp., as determined by mtDNA amplification (no "double base calling" in electropherogram), were investigated by microscopic examination of stained blood films. Seventeen samples (approximately 50% of the investigated birds) were

determined to harbour simultaneous *Haemoproteus* or *Plasmodium* spp. infections or both as determined in blood films. Thus, the PCR method underestimates simultaneous infections of haemosporidian parasites in naturally infected birds.

The current PCR diagnostics of haemosporidian infections predict that the used primers are highly conserved regions of the parasites' DNA for all species belonging to the genera *Haemoproteus* and *Plasmodium*. The present communication, however indicates that this is probably not because the amplification is often highly selective during simultaneous haemosporidian infections. To determine true species composition of haemosporidian parasites in each individual host, PCR diagnostics should be improved. Specific primers for *Haemoproteus* and *Plasmodium* spp. should be developed. A combination of approaches of microscopy and PCA-based methods is recommended for this purpose.

ECHINOCOCCUS MULTILOCULARIS: INCREASING PRESENCE IN THE SOUTHERN BORDER AREA IN THE NETHERLANDS.

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As part of the Echinorisk project between January 2002 and March 2003, a base line prevalence study was carried out to get a better insight in possible spread of the parasite after its first recognition in 1996 in the Netherlands. In an area of approximately 800 km², with an average prebreeding fox population of 2000 animals, a total of 196 animals were investigated for *E. multilocularis* by microscopical examination of the jejunum and PCR method of colon contents. In addition, the same foxes were examined for ecological factors, such as sex, age, condition and stomach contents, to get a better insight into the interaction between the parasite and the fox population dynamics. Of the 196 foxes examined, 25 were positive by either microscopy or by PCR. Most positive foxes were identified during the winter period of January and February 2003. Worm burdens were significantly higher in this study compared to the previous study. In addition, spatial analysis using GIS and kriging shows that the regional parasite prevalence increased in the period between 1996 and 2003. This is the first time an increased infection pressure has been described for humans in the Netherlands.

SURVEY OF ANTHELMINTIC RESISTANCE ON SHEEP FARMS IN SLOVAKIA

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This survey was conducted to determine the prevalence of benzimidazole-resistant nematode in sheep flocks in Slovakia.

Twenty-eight sheep farms were investigated mostly in the middle and northern Slovakia, where the sheep industry is concentrated. Flocks consisted of approximately 100-1600 animals. Animals had to be left untreated at least 10 weeks prior to the study. The efficacy of albendazole and ivermectin was tested by a faecal egg count reduction (FECR) test. On each farm, 45 sheep were randomly distributed into three groups of 15 animals: albendazole group (5 mg/kg BW), ivermectin group (0,2 mg/kg BW), and untreated control group. Benzimidazole resistance was additionally examined using an *in vitro* egg hatch assay and larval development assay with thiabendazole. A questionnaire survey to obtain information on endoparasite control practices and management has also been included. FECR were evaluated between day 10 and day 14 post-treatment and percentage reduction and 95% confidence intervals were determined. Anthelmintic resistance was declared when the percentage reduction was <95% and the 95% confidence interval was <90%. Resistance was suspected when only one of the two criteria was met.

The survey indicated that benzimidazole resistance occurred in one farm and was suspected in one of the farms. Ivermectin (27 farms) was effective (96.6 -100 %) on 11 farms examined with this drug. Resistance was declared as suspected on 4 farms and 12 farms exhibit the efficacy ranging from 55.3 to 94.9 %. Results from *in vitro* egg hatch assay indicated that mean of concentrations of thiabendazole that inhibited hatching in 50 % of eggs (ED_{50}) in resistant populations were over 0.1 ug.ml^{-1} of thiabendazole. LD_{50} values (thiabendazole concentration inhibiting development of 50 % of eggs to L3 infective larvae) in larval development assay for susceptible population varied from 0.00181 to 0.01 ug.ml^{-1} . Both *in vitro* assays showed comparable results, which are fairly reliable for detection of benzimidazole resistance under field conditions. When the level of resistance is high, they provide an effective method to detect resistance. To increase the sensitivity we recommend using delineation dose (ED_{99}/LC_{99} , MIC), which could reveal relatively small proportion of resistant worms in the population.

FIRST DATA ON OCCURRENCE OF AVIAN BLOOD PARASITES
IN VILNIUS DISTRICT OF LITHUANIAI. VENSĖSKUTĖ^{1,2}, T. A. IEZHOVA¹, S. BENSCH³, L. RAUDONIKIS¹
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Blood parasites are common in birds all over the world. However, some regions of Europe, especially areas located close to prominent scientific centres and thus being convenient sites for experimental and field investigations of the host–vector–haematozoa interactions, often have been rather neglected in regard of studying these infections. The objective of the present study was to determine occurrence of blood parasites in birds in the environs of Baltoji Voke (54° 36' N, 25° 11' E), the area located approximately 25 km south of Vilnius. This locality is of particular interest due to a high density and variety of birds and blood-sucking dipteran insects.

In the beginning of June and in July 2002, 113 birds belonging to 18 species and 5 families of the Passeriformes were caught with mist nets and investigated by microscopic examination of stained blood films. The majority of birds (98 individuals) belonged to the Sylviidae.

Haemoproteus belopolyskyi, *H. majoris*, *H. fringillae*, *H. lanii*, *H. minutus*, *Leucocytozoon fringillinarum*, *Trypanosoma avium* were identified. *Haemoproteus belopolyskyi* and *H. majoris* were most frequently found. The overall prevalence of blood parasites was 36.3%. The prevalences of *Haemoproteus* spp. (28.3%), *Plasmodium* spp. (3.5%), *Leucocytozoon* spp. (2.7%), *Hepatozoon* spp. (1.8%), *Atoxoplasma* spp. (0.9%), and *Trypanosoma* spp. (0.9%) were recorded. Only 10% of all infections were mixed infections with parasites from two to four genera present in each blood film. Of all recorded infections, 51.2% were of low intensity (<0.1% of infected erythrocytes and a few trypomastigotes present in blood films) and thus could be classified as chronic.

The prevalence of *Haemoproteus* spp. infection was greatest in Common Whitethroat *Sylvia communis* (61.1%), Willow Warbler *Phylloscopus trochilus* (35.3%), and it was much less in other avian hosts. Species of *Haemoproteus* and *Plasmodium* were found in juvenile birds, indicating that transmission of the parasites takes place at the study site. The prevalence of *Haemoproteus* spp. infection was significantly greater in adult birds than in juvenile ones ($P < 0.001$).

The present communication indicates that blood parasites are common in birds in Baltoji Voke. Furthermore, species of *Haemoproteus* and *Plasmodium* are certainly transmitted there. Additional studies are needed to fully understand the epizootiology of avian blood parasites in this area, which is a convenient site for future

investigations of host–vector–haematozoa interactions. This is a first study on the occurrence of avian blood parasites in Vilnius district of Lithuania.

MOLECULAR DIAGNOSIS OF ANTHELMINTIC RESISTANCE

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Recent findings confirmed that the spread of anthelmintic resistance is increasing in sheep, horse and also cattle parasite species. The nematode species are currently most severely affected by this problem, although a number of investigations have also demonstrated resistance in trematode and cestode species. In the past it has become clear that highly sensitive tests for the presence, or the beginning of the development, of anthelmintic resistance are urgently needed for all strategies aiming at the control of anthelmintic resistance. It was shown that molecular tests will be suitable for the diagnosis of resistance even at its very early stages. However, currently such tests are only available for the analysis of benzimidazole resistance in a few sheep gastrointestinal nematode species. They are generally based on the information that benzimidazole resistance in these organisms correlates with a specific single nucleotide polymorphism in the beta-tubulin codon 200. A further correlation between a single nucleotide polymorphism in an anthelmintic target gene was recently described for ivermectin resistance in the cattle nematode *Cooperia oncophora*. However, until now no respective molecular assays have been published. The molecular benzimidazole-resistance tests are PCR-tests which use allele-specific primers by which the presence or absence of the resistance related allele in DNA samples of single parasites can be investigated. The assessment of the resistance status of a given field sample thus requires the analysis of a representative number of individuals, such as larvae or adult worms. This is costly and laborious, preventing these methods from being used for routine diagnosis. Therefore, attempts were made to achieve the molecular assessment of resistance-related allele frequencies in samples of pooled larvae or adults. The quantitative real-time PCR and pyrosequencing technologies offer the opportunity to achieve this goal. Data will be presented on the use of both systems for the detection of benzimidazole resistance in sheep trichostrongyle and horse cyathostomin species. The preliminary findings indicate that these systems may be readily applicable for the routine molecular diagnosis of benzimidazole resistance.

EXPERIMENTAL INFECTIONS WITH *TRICHINELLA* SPP. IN RED FOXES *VULPES VULPES*

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Although carnivores are considered to be worldwide reservoir hosts of most *Trichinella* species, experimental studies in carnivores are rare. The present series of studies aimed to provide data on the infection biology of *Trichinella* spp. in foxes. The infectivity and persistence of nine genotypes of *Trichinella* spp. and the host antibody response was compared in 108 red foxes, each inoculated orally with 10,000 larvae. Muscle larvae burden and antibody response was determined at necropsy. The muscle distributions of the different *Trichinella* genotypes were evaluated to determine the predilection sites. In another study, 138 foxes were inoculated orally with 7000 *Trichinella* larvae with the aim to evaluate the intestinal establishment and reproduction of four *Trichinella* species in mono- and mixed species infections. The intestinal worm burdens and the muscle larvae burdens were determined and typed to species using a multiplex PCR.

In the first study, both the encapsulating and non-encapsulating *Trichinella* spp. had comparable, high infectivity and good persistency. Larvae of all genotypes isolated from fox musculature were infective to mice. The encapsulating species were primarily found in the tongue, extremities and diaphragm, whereas the non-encapsulating species had predilection site in the diaphragm. High muscle larvae burdens appeared to be more evenly distributed with less obvious predilection than seen in light infections: predilection muscles harboured a relatively higher percent of the larval burden in light infections than in heavy infections. This probably reflects increasing occupation of available muscle fibres. The foxes showed a clear antibody response from 3-5 wpi, which persisted for at least the duration of the experiment.

In the second study, muscle larvae burdens in the foxes were again at comparable levels for all *Trichinella* species. The intestinal phase of *Trichinella* spp. in foxes appeared to be short and simultaneous infection with two species lead to establishment of muscle larvae from both species. Interaction between the species is suggested since PCR typing revealed, that given in equal doses, the two species established in different numbers and that this difference from an equal distribution was highly significant in the muscles. The recovery of all *Trichinella* species in both mono species and mixed species infections from the same intestinal location suggests reproductive isolation rather than a physical intestinal separation as a mating barrier. In conclusion, these results establish that foxes are suitable indicator animals for epidemiological monitoring and appropriate hosts for comparative studies on biological characteristics of both sylvatic and domestics genotypes of *Trichinella* spp.

TAXONOMY AND SYSTEMATICS OF *GYRODACTYLUS* (MONOGENEA)
POPULATIONS INFECTING RIVERINE ALPINE BULLHEAD *COTTUS*
POECILOPUS IN NORWAY AND SLOVAKIA

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Only three species of *Cottus* are widespread in Eurasia: the cold-adapted Alpine bullhead *C. poecilopus*, the common bullhead *C. gobio*, and the Siberian *C. sibericus*. In Europe, *C. poecilopus* and *C. gobio*, which both occur in Norway, have various phylogeographic sub-divisions. After the last ice age, displaced populations most likely re-colonized the south-eastern part of Norway from the south of the country and into northern Norway from the north east. Today the Alpine bullhead occurs in both northern Norway and south-eastern Norway whereas the common bullhead occurs only in a restricted area in the south east of Norway. Occasionally, both species occur sympatrically. Gyrodactylid species have been recovered from cottids worldwide. In Fennoscandia, however, there is only one report of a *Gyrodactylus* specimen recorded on brown trout *Salmo trutta* but is supposed to have originated from Alpine bullhead. Morphologically, this specimen was considered to be a close relative, if not identical, to *G. hrabei* Ergens, 1957 described from common bullhead *Cottus gobio* in Slovakia. This species has also been recovered from Alpine bullhead in Central Europe. An examination of electro-fished Alpine bullheads from both northern Norway and south eastern Norway revealed a *Gyrodactylus* infection on the skin and fins of the fish. A detailed taxonomical study based on both morphometry and nucleotide sequences (mtCO1) were performed over a wider range of host populations. The results were subsequently compared with the morphological data of the type material of *G. hrabei* from the common bullhead in Slovakia, and the morphological and molecular data of topotypes from Alpine bullheads.

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THE INFESTATION OF THE BALTIC COD *GADUS MORHUA CALLARIAS* AND FLOUNDER *PLATICHTHYS FLESUS* WITH ANISAKID NEMATODES IN THE SOUTH BALTIC IN 2002 - 2004

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The infestation peculiarities of the cod and flounder with anisakid nematodes (*Hysterothylacium aduncum* larvae and adults, *Contracaecum osculatum* l. and *Anisakis simplex* l.) were studied.

In all 225 specimens of the cod (10.2 - 54.0 cm) and 288 of flounders (13.2 - 40.5 cm) were investigated in the South Baltic in 2002 - 2004. Nematodes were studied under light microscope after clearing in glycerol combined with lactic acid.

Three nematode species were found: *Hysterothylacium aduncum* (larvae and adults), *Contracaecum osculatum* l. and *Anisakis simplex* l. The parasites usually localized in the digestive tract and in the visceral cavity of fish. *Hysterothylacium aduncum* l. was prevailed in the cod (Prevalence = 34.7 %, Intensity = 1-27 specimens) and in the flounder (P = 8.7 %, I = 1-13). Adult *H. aduncum* were recorded only in the cod (P=1.3 %, I=1-3). Prevalence of infection of the cod with *C. osculatum* l. was 8.4 % (I = 1-3), flounder - 0.3 % (I = 1). Larvae *A. simplex* were found only in two flounders (length 28.0 and 32.0 cm) in October 2002 (1 and 5 specimens). Parameters of the cod infestation with *H. aduncum* l., *H. aduncum* and *C. osculatum* l. were variable in the different size groups. Larvae *H. aduncum*. were found in all size groups of fish, adults *H. aduncum* were only in the cod more than 40 cm in length. Larvae *C. osculatum* were recorded in the cod ranging 25.5 - 51.0 cm in length. Index of infestation of the cod with larvae of *H. aduncum* and *C. osculatum* increased directly proportionally to sizes of fish. The highest prevalence of infection of the cod with anisakid nematodes were recorded in fish ranging 50.1-55.0 cm in length: *H. aduncum* l. (50 %), *H. aduncum* (12.5 %), *C. osculatum* l. (37.5%). Anisakid nematodes were found in the flounder ranging 15.0 - 40.0 cm in length. Prevalence of infestation with *H. aduncum* l. decreased from 16.2 to 9.5 % with increase of the fish size. *C. osculatum* l. were recorded in flounder ranging 20.1-25.0 cm in length very seldom (P = 1.2%, I = 1). Seasonal variabilities of the cod infestation were revealed. The highest prevalence of the cod infestation with *H. aduncum* l. and *C. osculatum* l. were recorded in October-December (64.9 % and 16.7 %, respectively). Parameters of flounder infestation with *H. aduncum* l. were similar during different seasons.

The positive correlation of the cod infestation parameters with anisakid nematodes and the fish sizes was revealed. The indexes of flounder infestation decreased with the fish length. Seasonal differences of parameters of infestation were recorded for the cod only.

SEASONAL DYNAMICS OF *IXODES RICINUS* L., TICKS PARASITING ON SMALL MAMMALS IN MINSK AND ITS SUBURBS, BELARUS

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City territory is a zone of severe anthropogenic pressure, which causes biota change including species variety of small mammals, which are the basic sustenance of preimaginal phases of *Ixodes ricinus* ticks. Seasonal dynamics evaluation of the numbers of satiated larvae and nymphs of the ticks is essential for prognosing the numbers of imagoes in the years to come.

As a result of monthly registration from April to October, 699 animals were trapped in forest biocenosis of the suburban zone (Priluksky reserve) and on the territory of Botanical Gardens (Minsk) and 287 larvae and 46 nymphs of *I. ricinus* were collected from 7 species of small mammals.

In the suburban zone the average number of small mammals was 5.6 specimens per 100 trapping days, and average tick infection was 0.17 specimens per one animal. The bulk ticks number (87%) parasitizes *Clethrionomys glareolus*, 15% of the ticks parasitize on *Sorex araneus* and 7% on *Apodemus flavicollis*. Ticks were detected on animals from April to August. The highest ticks number (0.32 per one animal) were recorded in May.

On the territory of Botanical Gardens the number of rodents is higher, it reach 7.2 specimens per 100 trapping days and average ticks infection is also higher – 1.12 specimens. *Apodemus agrarius* and *A. flavicollis* are the basic sustenance for *I. ricinus*, 53% and 43% respectively. Ticks on animals were detected from April to October. In the city parasitizing terms extended for two month. This is due to higher average temperature. Maximum ticks number was recorded in May (4.16 per animal).

Thus, seasonal dynamics of *I. ricinus* ticks parasitizing on small mammals in the suburbs and in the city has one peak of activity in May. On the territory of the city higher number of hosts and parasites and extension of parasitizing season have been recorded. This fact aggravates parasitological situation due to *I. ricinus*, which is the main carrier of transmissive infections in Belarus.

TICK-BORNE DISEASES IN LITHUANIA

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The main vector of tick-borne diseases (TBD) in Lithuania is *Ixodes ricinus*, which is a tick of temperate regions in Europe. Between 1969 and 2004, a total of 5288 cases of Tick-borne encephalitis (TBE) were reported in Lithuania. A significant rise of morbidity has been reported in the last 12 years (1993-2004). The highest annual number of cases (763) was recorded in 2003. Each year 656-1740 cases of Lyme disease have been recorded; however, the number of this disease cases reached 3688 in 2003. TBE virus has been found in ticks collected from 221 localities and *Borrelia burgdorferi* s. l. from 150 localities in Lithuania. Besides TBE virus and *B. burgdorferi* s.l., *Anaplasma* bacteria and two species of protozoa belonging to the genera *Babesia* and *Trypanosoma* were detected in *I. ricinus* ticks.

Ixodes ricinus ticks were sampled from woodlands by dragging flannel over the vegetation and examined for the presence of *B. burgdorferi* and *Trypanosoma* sp. in bowel smears. From 1970 till 1989 TBE virus isolation was performed in suckling mice, later SPEV- pig embryo kidney – cell culture was applied and the identification was performed by indirect immunofluorescence method. In 2001, the ticks were tested by polymerase chain reaction (PCR). The prevalence of the TBE virus in tick pools was 1.38%; it was 1.03% in Panevėžys, 3.33% in Šiauliai, and 16% in Radviliškis. The sequence analysis showed that TBE virus belong to the Western TBE lineage. PCR was used for identification of *Borrelia* parasites and genospecies of *Anaplasma* and *Babesia*.

The mean infection prevalence of *I. ricinus* ticks by *B. burgdorferi* s.l. in Lithuania was 13.4%, ranging from 1 % to 35% in different locations. Three *Borrelia* genospecies were detected in ticks in Lithuania: *B. afzelii* (9-10%), *B. garinii* (2.5-5%) and *B. burgdorferi* s.s.(0,4%). The ticks were detected (5%) to be positive for *Ehrlichia/Anaplasma*, 0,4% *B. burgdorferi* s. s., and 2% for *Babesia divergens*. In 1992-1997 among more than 3000 *I. ricinus* examined *Trypanosoma* sp. (amastigotes, promastigotes and epimastigotes) were detected in 3 individual ticks (0.1% prevalence).

In 2003, the first patient with recognized manifestation of ehrlichia infection was reported.

The epidemiological importance of TBD in Lithuania is the similar to that in other countries of Central Europe. Agents of infectious diseases transmitted by ticks, such as TBE virus, *B. burgdorferi* s. l. are serious hazards of human health. Ongoing research

on tick-borne diseases will help to assess reasons of the wide distribution of these zoonoses.