

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



**PROCEEDINGS OF THE XVII SYMPOSIUM OF THE SCANDINAVIAN  
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# BULLETIN OF THE SCANDINAVIAN SOCIETY FOR PARASITOLGY

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The Bulletin is a membership journal of the Scandinavian Society for Parasitology. Besides membership information, it also presents articles on all aspects of parasitology, with priority given to contributors from the Nordic countries and other members of the Society. It will include review articles, short articles/communications. Comments on any topic within the field of parasitology may be presented as Letters to the Editor. The Bulletin is also open for a short presentation of new projects. All contributions should be written in English. Review articles are commissioned by the editor, however, suggestions for reviews are welcomed.

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**Cover:** In Norse mythology, the giant ash tree - Yggdrasill - 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 Níðhogg is ceaselessly gnawing at the roots of the ash. The second spring - Mímisbrunn - is the source of wisdom and is guarded by Mimir. The third spring - Urðarbrunn - is guarded by three women, the Norns, which mete out man's thread of life.

***PROCEEDINGS***  
***of the***  
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**Introduction by the editors**

The present Proceedings comprise the Welcome words of the President of the Scandinavian Society for Parasitology, the abstracts of six invited speakers, and the abstracts of 55 oral presentations and 64 poster presentations at the 17th Scandinavian Symposium of Parasitology. On behalf of the Organizing Committee we would like to thank all contributors for their interest in the symposium.

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## Welcome by the president of the Scandinavian Society for Parasitology

Inger Ljungstrom

Swedish Institute for Infectious Disease Control, Stockholm, Sweden

Dear friends - on behalf of the Scandinavian Society for Parasitology - I wish to welcome you all to this, the 17th symposium of our Society. A special welcome to our friends from our Eastern neighbour countries, the Baltic states and Russia. As you all remember we participated last autumn in a very successful meeting held in Vilnius. Another success was the meeting regarding "Parasites of biological and economic significance of the aquatic environment" held in Iceland in the summer 1994.

We are a Society with members from the Nordic countries, which is reflected in participation in this symposium. But you will also find members from many other countries. We all have different background in our training like biologists, microbiologists, veterinarians and medics. Our research interest covers areas such as epidemiology, treatment, genetics, molecular biology and many others. However, one thing brings us together - the parasites. The subjects we will discuss during these three days are parasites from a different point of view, but I hope you all have an open mind, as this diversity is reflected in the scientific interest of the members. I hope the various subjects will give you all inspirations for your future research.

A special welcome is directed to the invited speakers. This afternoon we will hear about "*Toxoplasma*-like organisms - a hazard to animals and man" and "Cell-to-cell interactions of importance for the development of severe malaria". Tomorrow two other items will be presented "Co-evolution of microparasites and a facultative sexual host" and "Plant allelochemicals and the control of parasites".

Finally, again two other topics are dealt with the last day "Through the looking glass: host-parasite coevolution and sex" and "Antiparasitic treatment - potential risk to animal production and human beings". I really look forward to fruitful discussions. The workshops "Parasitism and biodiversity; a parasitized ecosystem is a healthy one?" and "Novel, non-chemical approaches to helminth control" will also be of great interest.

Dear friends, without you as dedicated scientists, who are going to present exciting results, this symposium would not have been possible. I hope, you all will bring new ideas back home.

Finally, I will thank the local organizing committee headed by Associate Professor E. Tellervo Valtonen (Jouni Taskinen, Hans-Peter Fagerholm, Voitto Haukisalmi, Sven Nikander, Antti Oksanen) having performed a formidable task in arranging this symposium in the heart of the beautiful Finnish lake district. It is an honour and privilege to be able to thank them on your behalf for the successful effort. We are also indebted to agencies and sponsors which have believed that this symposium will be a success both scientifically and socially.



## ***Toxoplasma*-like organisms - a hazard to animals and man**

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*Toxoplasma*-like organisms covered by this review are *Toxoplasma gondii*, *Neospora caninum*, *Hammondia heydorni*, *Hammondia hammondi* and *Hammondia pardalis*. *T. gondii*, *H. heydorni* and *H. hammondi* are generally accepted as valid species whereas the validity of the other two parasite species is under discussion. Our knowledge on *T. gondii* has consolidated. It is a health hazard to the unborn child and to immunocompromised persons. It also causes abortions in sheep. Acute disease in pigs, so far reported mainly from Japan, has recently also been reported in Europe. However, it remained unclear whether the outbreak was due to a concurrent infection with an immunosuppressive effect, the uptake of an overdose of oocysts or the presence of a strain of high virulence. The prevalence of *Toxoplasma* infections in fattening pigs in Europe is generally decreasing. Cattle do not play a significant role in the epidemiology of toxoplasmosis. *Toxoplasma*-like organisms in cattle have been identified as *N. caninum*. This parasite may cause abortion in cattle and clinical disease in dogs. However, it was found that healthy cattle and dogs may also have antibodies against *Neospora*. Experimentally, intrauterine infections have also been induced in non-human primates. A detailed comparison between *N. caninum* and *H. heydorni* will be presented with the conclusion that, thus far, there is no published proof that these two organisms are not identical. *H. hammondi* has a cat-rodent cycle. This parasite is closely related to *T. gondii*, but is clearly a different species as shown by comparison of phenotypic and genotypic characters. *H. pardalis* is obviously a mixture of developmental stages of two already known other parasites, i.e. the oocysts of *Cystoisospora felis*, the dormozoites of *C. felis* and the endozoites of *N. caninum*.

## Cell-to-cell interactions of importance for the development of severe malaria

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The capacity of *Plasmodium falciparum* infected erythrocytes to form rosettes has been found to be associated with the virulence of the parasite. In search of a parasite associated rosetting ligand we found a set of clone, strain- or isolate specific, SDS-soluble, low molecular weight polypeptides of Mr 22,000- Mr 45,000 expressed at the surface of the malaria infected erythrocytes. A Mr 35,000 polypeptide was strictly associated with rosetting of the Palo Alto strain. The rosette-associated polypeptides were stably expressed after long-term *in vitro* cultivation only if the parasite was continuously enriched for the rosetting phenotype. PfEMP1 's of different sizes were also detected. We have found the infected erythrocyte to bind either immunoglobulin M or IgM and IgG which the parasite seems to use to form electron-dense fibrillae that mediate binding to host cells. The ABO-blood group oligosaccharides ( $(\alpha\text{galNAc}(1-3)\beta\text{gal}(1-3)\alpha\text{fuc})$  or  $(\alpha\text{gal}(1-3)\beta\text{gal}(1-3)\alpha\text{fuc})$ ) were in inhibition experiments also found to be involved as rosetting receptors and blood group O RBC formed smaller and weaker rosettes than the blood group A, B, or AB RBC. The blood group O phenotype has in epidemiological studies been implicated to protect against cerebral malaria. In conclusion, rosetting seems to be mediated by low- to medium sized molecular weight polypeptides and immunoglobulins on the surface of the infected erythrocyte and the ABO blood group sugars on the uninfected counterpart.

**CO-EVOLUTION OF MICROPARASITES AND A FACULTATIVE SEXUAL HOST. Dieter Ebert, NERC Centre for Population Biology, Imperial College at Silwood Park, Ascot, UK.**

For most host-parasite interaction is a reduction of host survival and fecundity, which are - everything else being equal - neither beneficial for the parasite nor for its host is typical. I use 3 examples from the facultative sexual crustacean *Daphnia magna* and its microparasites to demonstrate that the level of parasite virulence and the expression of different disease symptoms can be explained from an adaptationist point of view.

1) Mathematical modelling showed that trade-offs between virulence and parasite spore production can contribute to the maintenance of virulence. Such a trade-off was found across 3 strains the microsporidium *Pleistophora intestinalis* infecting different host clones. The more spores the parasite produces, the higher the chance that its host will die. The evolutionary consequences of this correlation are further demonstrated with results from a 14 month selection experiment.

2) Using the same host-parasite system I found that parasite strains which are adapted to their local host population are, on average, more virulent than parasite strains introduced into host populations which previously had no experience with this parasite strain. This result contradicts the previous belief that adapted parasite strains are less virulent than novel strains.

3) Parasitic castration and gigantism have often been implied to benefit the parasite. Results from experiments with the spore-forming bacterium *Pasteuria ramosa* supports this hypothesis.

## Plant allelochemicals and the control of parasites

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### INTRODUCTION

The secondary metabolites synthesised by plants are organic molecules which are not parts of the fundamental anabolic and catabolic pathways that are common to all plant species. Instead, the secondary metabolites consist of an immensely wide spectrum of compounds which are often species- or family-specific in their taxonomic distribution. This taxonomically patterned distribution of individual metabolites or categories of them has led to their frequent use as phytochemical markers for taxonomical relationships between plant species.

The functional significance of secondary metabolites in the plants that produce them is usually imprecisely understood. In a few cases evidence exists to explain the link between taxonomical distribution and function. Many of the flavonoids characteristic of the 15,000 species of leguminous plants are now known to be part of a reciprocal set of chemical signals between legumes and their nitrogen-fixing, root nodule-producing symbiotic bacteria in the genus *Rhizobium*. The symbiosis is established when flavonoids are exuded from the roots into the rhizosphere and induce the expression of bacterial genes called *nod* (nodulation) genes in *Rhizobium*. The otherwise free-living bacteria in the soil employ the secondary metabolites as specific triggers for switching to a symbiotic metabolism with particular leguminous partners (Dénarié et al, 1992).

Although, in most other cases, the precise functional role of secondary metabolites is not clear, a working hypothesis has often been that they function as chemical defences. Secondary metabolites often seem to act as permanent or responsive (phytoalexin) protective molecules against pathogenic viruses, bacteria, fungi and nematodes as well as herbivorous insects and vertebrates.

Given this potentially adaptive role, it is not surprising that many plant secondary metabolites are allelochemicals, that is, compounds produced by one species that have profound bioactivity with respect to a range of other species. In this context there is increasing interest from researchers both outside and within the pharmaceutical industry in searching among the high biodiversity of plants and their secondary metabolites for novel pharmaceuticals and pesticides. Antiparasitic drugs and pesticides for parasite vector control are important facets of the target range of this search (Mott, 1987; Borris & Schaeffer, 1992). This short review provides a selective overview of this area of research activity.

## **HISTORICAL PRECEDENTS**

The past 350 years have provided ample evidence of the, often crucial, role of plant allelochemicals in the battle against human parasitic disease. Two examples, both linked with malaria, exemplify both the potency of natural plant allelochemicals and their secondary role as progenitor compounds for the development of increasingly efficient synthetic chemical analogues. The examples are those of quinine and pyrethrins

Quinine (Fig. 1a) is one of a series of antimalarial alkaloids sequestered in the bark of trees in the genera *Chinchona* and *Remigia* (Rubiaceae) from the Andes. The powdered bark of the trees was undoubtedly used by the indigenous peoples of South America against malarial infections before the coming of Europeans. During the mid-1600's its efficacy was recognised by colonists in what is now Peru and Ecuador and the medicine became known as 'Jesuit's bark' or 'Jesuit's powder' and was soon being used in Europe (Joyce, 1994). By 1820

Pelletier and Caventou in France had isolated the active principle, quinine. The molecule was first produced synthetically in 1944 and this led the way for the production of useful synthetic analogues such as chloroquine (Fig. 1b). Both quinine and chloroquine can interfere with nucleic acid synthesis in cells of *Plasmodium* but can probably also damage the parasite by the inhibition of malarial haem polymerase which normally allows *Plasmodium* to detoxify the haem products of haemoglobin digestion (Wellems, 1992).

The dried and powdered flower heads of *Chrysanthemum cinerarifolium* (Compositae) are known as “pyrethrum” or “insect powder”. The insecticidal activity of this natural product has been known for about two thousand years (Davies, 1985). The active ingredients of the powder are six esters between two carboxylic acids, chrysanthemic acid and pyrethric acid and three cyclopentenolones, pyrethrolone, cinerolone and jasmolone. One of these esters, pyrethrin 1, is illustrated in Fig. 2a. The plant-produced esters such as pyrethrin 1 are insecticidal but suffer from being photolabile and having some mammalian toxicity. Synthetic analogues, known collectively as pyrethroids, have subsequently been developed which have enhanced insecticidal activity, chemical stability in sunlight and low mammalian toxicity. Deltamethrin (Fig. 2b) is such a pyrethoid and has found considerable use as a persistent insecticide for treating mosquito nets as part of integrated control strategies for malaria.

It should be noted that in both the quinine and pyrethrin case histories pre-existing ethnopharmacological evidence pointed to a likely source of a strongly bioactive plant allelochemical. For a researcher today, confronted with the immense biodiversity of flowering and non-flowering plants, such evidence remains a highly effective means of targeting investigations.

## RECENT AVENUES OF INVESTIGATION

Several recent studies on plant allelochemicals have produced exciting results which relate to different modalities of parasite treatment and control. A few lines of investigation have

generated clinically-useable new drugs. In most others, though, developmental work is at a much earlier stage. The following examples illustrate some of the principal areas of activity:

**(a) New antiprotozoal drugs**

One of the most important new antimalarial drugs is the sesquiterpene endoperoxide, artemisinin (Fig. 3). This terpenoid, a plant allelochemical isolated from *Artemisia annua* (Asteraceae) is the basis of a herbal medicine long used in China for malaria therapy. Much development work is continuing with analogues of this compound in order to improve its solubility and stability and it seems likely that one of these will find a clinical place as a possible substitute for primaquine. The mode of action of artemisinin is unknown but a free radical mechanism is suspected (Meshnick *et al.*, 1989).

**(b) New molluscicides for schistosomiasis control**

After a period in the mid 1980's when it was thought that chemotherapy alone with drugs like praziquantel might provide an adequate control strategy for schistosomiasis, that perception is now generally regarded as over-optimistic (Webbe & Jordan, 1993; Perrett & Whitfield, 1995a). Several recent field studies have shown that control programmes based on chemotherapy alone can provide only temporary curtailment of schistosome transmission. Other investigators have shown that schistosomes have the potential to develop heritable resistance to praziquantel (Fallon & Doenhoff, 1994). Findings like these and the lack, to date, of a schistosomiasis vaccine have refocused attention in the role of molluscicides in the integrated control of this important helminth disease.

The synthetic molluscicide, niclosamide is the present molluscicide of choice in control programmes (WHO, 1993). It is highly effective against schistosome-transmitting snails and their eggs but causes worrying levels of fish mortality and is expensive. Such problems have meant that attention has been given to the search for molluscicides based on plant

allelochemicals in the hope that they might be cheap, biodegradable and locally-produced control agents. To this end over 1000 plant species have been screened for molluscicidal bioactivity (Kloos & McCullough, 1987). By the late 1980's it seemed that of the numerous plant allelochemicals with molluscicidal activity, the saponin triterpenes from species like *Phytolacca dodecandra* (Phytolaccaceae) and *Swartzia madagascariensis* (Leguminosae) showed the greatest promise (Hostettmann & Marston, 1987). Some of these saponins like lemmatoxin (Fig. 4) from *P. dodecandra* had specific activities towards snails almost as high as those of synthetic molluscicides and were present in the plant materials at high concentrations. An important gap in the activity spectrum of these triterpenes, however, is their ineffectiveness towards developing snail embryos in underwater egg masses. This drawback exists for both *P. dodecandra* molluscicide (Lemma, 1970) and that produced from *S. madagascariensis* pods (Suter *et al.*, 1986). This lack of ovicidal activity is serious because it means that snail embryos and juveniles in egg masses can withstand a single molluscicidal treatment and then emerge as hatchlings when molluscicidal concentrations have diminished after the application. The lack of potency seems to be due to the inability of the saponins to traverse diffusion barriers around the embryos. An inner proteinaceous membrane of the egg capsules is known to have a porosity which excludes molecules above a molecular weight of about 350 (Beadle, 1969). As the bioactive triterpenes of *Phytolacca* and *Swartzia* have molecular weights above 700 they are unlikely to be able to gain access to the snail embryos.

*Millettia thonningii* (Leguminosae) is a more recently discovered molluscicidal plant from West Africa. Aqueous extracts of the ground dried seeds are molluscicidal (Evans *et al.*, 1986) while chloroform and dichloromethane extracts are even more potent (Lam *et al.*, 1989). The bioactive allelochemicals in the seeds are not saponins but the isoflavonoids alpinumisoflavone, 4'-methylalpinumisoflavone and robustic acid (Fig. 5 a, b and c) (Perrett & Whitfield, 1995a). Importantly the low molecular weights of these molecules seem to allow them access to developing snail embryos. A dichloromethane extract, rich in isoflavonoids, is very effective at aborting embryonic development of *Biomphalaria glabrata* and killing the embryos (Tang *et al.*, 1995). At concentrations as low as 5mg/L, the extract is successful in curtailing embryonic



snail development at the gastrula to trochophore transition stage. It seems likely that the mode of action of the molluscicidal isoflavonoids in *Millettia* is site I electron transport inhibition in mitochondria (J.Lyddiard and P.J.Whitfield : unpublished observations).

An additional beneficial feature of *Millettia* molluscicide is its relatively low toxicity towards freshwater fish (Squire *et al.* , 1989). At concentrations which are molluscicidal to *Biomphalaria* , aqueous extracts of *Millettia* are not toxic to grass carp (*Ctenopharyngodon idella*) and *Tilapia nilotica/aurea* hybrids, both important food fish in Egypt.

*Millettia* molluscicide has an appropriate level of susceptibility to biodegradation in non-sterile, tropical, aquatic conditions. Its bioactive components are degraded with time but a useful level of toxicity persists for over three weeks (Perrett & Whitfield, 1995b).

#### (c) New schistosomal larvicides and cercarial repellants

Many molluscicides, both synthetic and allelochemical, demonstrate additional toxicity towards the miracidial and cercarial larvae of schistosomes. This bioactivity must enhance their overall depressive effect on schistosome transmission potential. The isoflavonoids in *Millettia* molluscicide share this enhanced capacity. Chloroform and dichloromethane extracts show a dose-dependent capacity to immobilize and kill free-swimming miracidia and cercariae (Squire & Whitfield, 1989; Perrett, 1994). Even sub-lethal doses which have no overt impact on swimming activity profoundly reduce larval infection success towards experimental snails and mice.

The susceptibility of *Schistosoma mansoni* cercariae to *Millettia* molluscicidal preparations has prompted a series of experiments in which dichloromethane extract was applied to mouse skin to investigate its potential as a topical cercarial repellant (Perrett *et al.*, 1995). These experiments have shown that the extract, when applied in an ethanolic carrier which is then

evaporated, is capable of greatly reducing cercarial infectivity even when applied 24 hours before cercarial challenge.

#### (d) **New anthelmintics**

No anthelmintics based on plant allelochemicals are part of our present chemotherapeutic armoury. Several interesting recent studies, though, have identified novel plant secondary metabolites which show intriguing levels of activity towards different types of parasitic worms.

Acetogenins from the tropical plant family Annonaceae show remarkably high levels of bioactivity towards model nematodes like *Caenorhabditis elegans* and *Molinema dessetae*. Several individual acetogenins including annonacin (Fig. 6b) from *Annona muricata*, the sour sop or guanabana, possess a higher specific activity towards *Molinema* than the commercial anthelmintic ivermectin (Bories *et al.*, 1991). Acetogenins as a group have a very wide spectrum of activity, showing toxicity towards parasitic protozoans and insects as well as nematodes (Fang *et al.*, 1993). These allelochemicals seem to be inhibitors of site I electron transport in mitochondria (Londerhausen *et al.*, 1991).

Chinese traditional medicinal plants have been a fertile source of antiparasitic allelochemicals. Two examples from recent studies on these plants, those on *Evodia rutaecarpa* (Rutaceae) (Perrett & Whitfield, 1995c) and *Acorus gramineus* (Araceae) (Perrett & Whitfield, 1995d) show the utility of ethnopharmacological leads but also the care that must be taken in investigating potent allelochemicals.

The dried unripe fruits of *Evodia rutaecarpa* form the basis of the Chinese preparation “wuzhuyu” which is used for a variety of human diseases and is known to kill pig ascarid nematodes *in vitro*. Activity-guided fractionation of an ethyl acetate extract of the fruits eventually yielded minute quantities of the alkaloid atanine (Fig. 7). Atanine represents only 0.04% of the original dried fruits. It demonstrates considerable activity towards both nematode

targets and larval schistosomes and certainly merits further investigation as a progenitor compound for new anthelmintics. It appears to possess pharmacological action at the neuromuscular junctions of smooth muscles.

Dried rhizomes of *Acorus gramineus* are the source of the Chinese medicine “shishampu” used to treat convulsions and epilepsy. Hexane extracts show very high activity towards a range of bioassay targets including the parasitic nematodes *Nippostrongylus brasiliensis*, *Ostertagia circumcincta* and *Trichostrongylus colubriformis*, the free-living nematode *Caenorhabditis elegans*, larvae of the sheep blowfly *Lucilia sericata* and of the tick *Boophilus microplus* and *Ornithodoros moubata* adults. Activity-guided fractionation eventually identified the bioactive principles in the extract as the phenylpropanoids,  $\alpha$  and  $\beta$  asarone (Fig. 8 a and b). These are dangerous cytotoxins with known teratogenic effects in experimental mammals. It is evident that not all plant allelochemicals with toxicity towards parasites are potentially usable therapeutically.

A final fascinating example shows that ethnopharmacological clues for the discovery of possible anthelmintics need not be restricted to human ones. Wild chimpanzees selectively gather and swallow whole, unchewed leaves of *Aspilia mossambicensis*, *A. plurisetata* and *A. rudis* (Asteraceae). Rodriguez *et al.* (1985) have isolated the polyacetylene compound, thiarubrine A (Fig. 6a) from the leaves of *Aspilia* spp. and demonstrated that it has nematocidal activity in a *Caenorhabditis elegans* bioassay. It is speculated that the apes may be regulating intestinal nematode parasites by their use of these leaves in their diet.

## CONCLUSIONS

More than 200,000 species of flowering plants exist. It is perfectly plausible to suggest that individual plant-produced secondary metabolites may occur in numbers an order of magnitude greater than this. Only a minute fraction of the species and their allelochemicals have been identified and assayed for antiparasitic and anti-vector biological activity. There is no doubt

that, as Borris & Schaeffer (1992) have put it “.....a substantial number of potentially interesting active compounds may still remain to be discovered, representing a fertile area for investigation in years to come.” This might be an understatement.

## ACKNOWLEDGEMENTS

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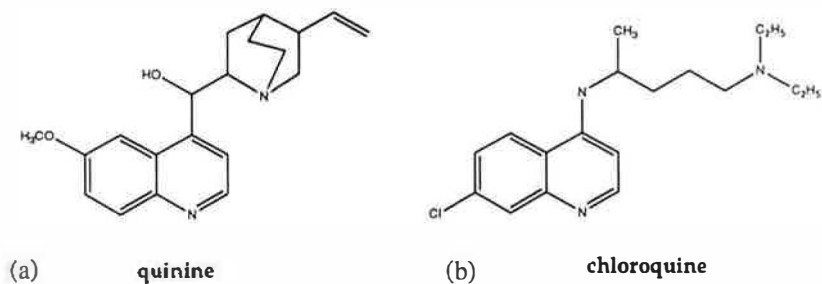


Fig. 1

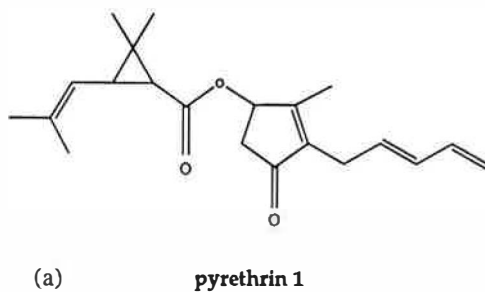
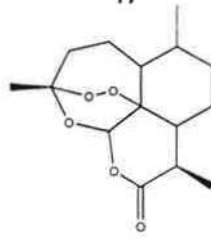


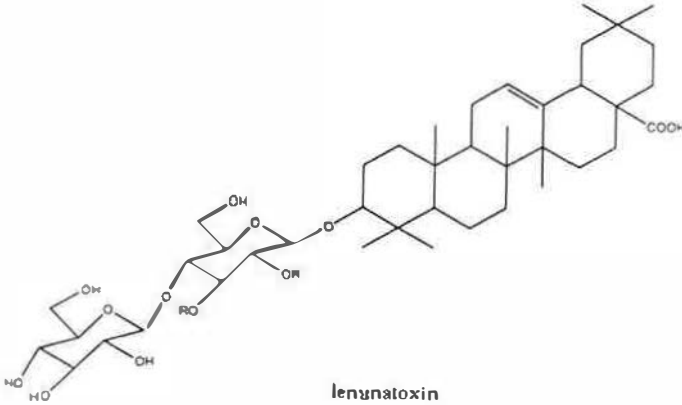
Fig. 2

17



artemisinin

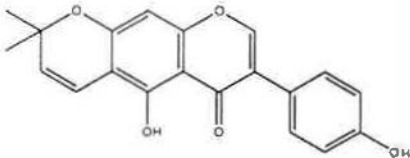
Fig. 3



lenunatoxin

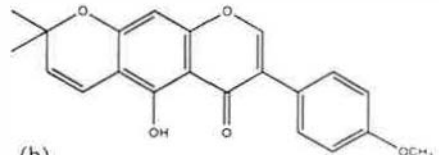
R=galactose

Fig. 4



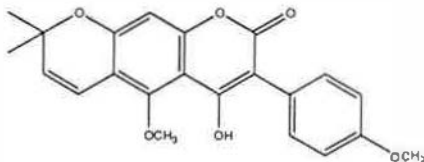
(a)

alpinumisoflavone



(b)

4'-methyalpinumisoflavone

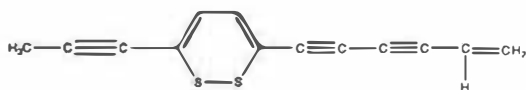


(c)

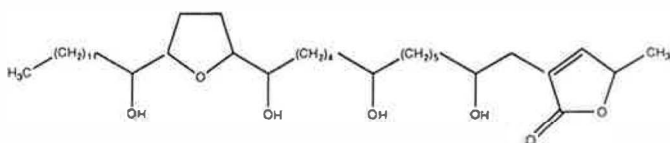
robustic acid

Fig. 5





(a)

**thiarubrine A**

(b)

**annonacin**

Fig. 6

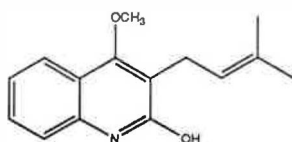
**atanine**

Fig. 7

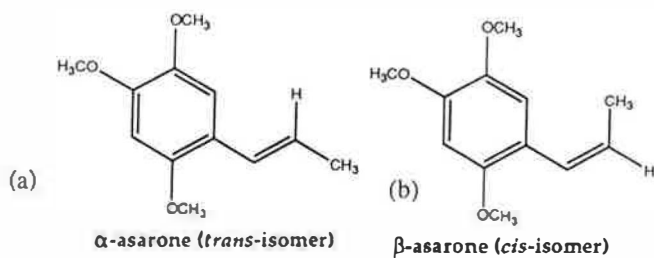


Fig. 8

## **Through the Looking Glass: Host-Parasite Coevolution and Sex**

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Most eukaryotic organisms reproduce by cross fertilization, at least during some part of their life cycle. That this is true in spite of the costs incurred by biparental reproduction indicates that there is some very general advantage associated with meiosis and genetic exchange. Conversely, it would seem that there must be some severe disadvantages associated with the various kinds of uniparental reproduction, like parthenogenesis and self fertilization. The principal goal of this talk is to flesh out some of the recent ideas on parasite-mediated selection for cross fertilization. At the heart of this subject is the concept of time-lagged, frequency-dependent selection. Natural selection of this kind is just as the name implies --selection against common genotypes, but with a delay. It is expected to be especially common in coevolutionary interactions between hosts and their parasites. The reason is that parasites are under strong selection to infect the most common host genotypes; but because they cannot instantaneously track genetic changes in the host population, there will be a lag in time. This kind of interaction can easily lead to sustained oscillations in host and parasite gene frequencies; and hence the maintenance of genetic variation. It might also lead to selection for cross fertilization and mate choice.

**"ANTI-PARASITIC TREATMENT-  
A POTENTIAL RISK TO ANIMAL PRODUCTION AND HUMAN BEINGS"**

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**Introduction**

Anti-parasitic treatment has brought great benefits to animal health and production and the health of human beings. However, every coin has its other side. Uncontrolled treatment has sometimes consequences which were not foreseen or underestimated. With regard to using chemicals there is a historical lesson to be learned from the consequences of the use of antibiotics, insecticides, anti-protozoan drugs, fungicides and herbicides. But, as in many other disciplines, lessons of history are often ignored and the same mistakes are made again and again.

In this contribution the other side of anti-parasitic treatment will be discussed with emphasis on anthelmintic treatment. This does not mean that the other parasitic treatments, mainly anti-protozoan and anti-arthropod treatments, are not important, and have no adverse effects. Far from that. The main reason for choosing anthelmintic treatment is that, particularly in animal production, it is used widely all over the world in temperate, subtropical and tropical climates.

The introduction of the first broad-spectrum anthelmintic with wide safety margins, thiabendazole, in 1961 was a milestone in the history of anthelmintic treatment. The drug group of which thiabendazole was the first - the benzimidazoles (BZ's) -, has expanded exponentially. New derivatives have been discovered which were more effective in lower doses to more nematode species and even other groups of helminths, such as trematodes and cestodes. Other drugs with different mode of action were developed, such as levamisole (LEV), pyrantel (PT) and morantel (MT), and at the end of the seventies the avermectins (AVM's) were introduced. This new group of anthelmintics had not only a very strong effect on nematodes in extremely low doses, but had also a lethal effect on arthropods.

However, as shown by history, when mankind uses chemicals, "nature" strikes back and resistance may develop.

The adverse effects of antiparasitic treatment can be observed with respect to the environment, in animals and as a consequence in animal production and in human beings.

## Environment

Anti-parasitic drugs, their residues or metabolites may reach the environment and exert a deleterious effect:

1. The unaltered drug or metabolites can be excreted by the living animal/human in urine or faeces (important)
2. Anti-parasitic drugs may be spilled at treatment, or used dipping fluid may be drained off in ditches (important)
3. Orally dosed drugs may be regurgitated and vomited (less important)
4. The carcass of a recently treated animal may contain the drug or residues of the drug and thus act as a potential source of contamination for predators (theoretically)

The effects of excreted anthelmintics on the environment have been poorly investigated. With regard to the most widely used group of anthelmintics, the BZ's, no adverse effects have been described on dung or soil ecology. The same can be said for LEV, PT and MT. However how much attention has been paid to the environmental impact of usage of these drugs? We know more about the effects of avermectin usage in livestock (1). It has been shown that usage of ivermectin has insecticidal effects on both pest and non-pest arthropod populations in animal dung. The influence on pastureland ecology remains still unanswered.

The need for alternatives for drugs has stimulated research for biological control systems. Much work has been done in Denmark on the use of nematode-trapping fungi to control gastrointestinal helminths in livestock (2). The results are promising, but the influence on free-living soil and dung nematodes is not yet clarified.

It may be clear that spilling of anthelmintics at treatment or draining dipping fluid in ditches can have great influence on the local fauna. This is particularly the case if chlorinated carbohydrates such as lindane are used with their long persistence in nature. Fortunately these are now forbidden in many countries.

## Animals and animal production

The risks of antiparasitic treatment are

1. (embryo)toxicity (less important)
2. effects of residues (less important)
3. anaphylactic reactions (less important)
4. development of resistance (important)
5. impaired build up of immunity (important)

## (Embryo)Toxicity

The BZ's have a very high safety index (max. tolerated dose/ min. effective dose) of  $\geq 10$ . There are reports of teratogenic effects on the offspring due to the use of parabendazole (PBZ), oxfendazole (OFZ)(3), albendazole (ABZ) and oxibendazole (OXZ). Besides these reports there are

publications on fenbendazole (FBZ) causing drop of feathers in young pigeons (4). No teratogenic effects are reported from LEV, PT, MT or the avermectins. However, the safety index of LEV is much lower and particularly in fibre-producing goats there may be adverse reactions in individual cases (5).

In most animals, no problems occur with toxicity of IVM, but there are some exceptions: Collie dogs are highly susceptible to IVM treatment (6).

### **Residues**

Residues of anthelmintics do not cause adverse effects in animals or animal production. It is, however, a consumer's right that one receives products without residues. Therefore the minimum time between treatment and slaughter has to be exactly defined. Residues may cause other troubles. It has been reported that cheese processing is influenced by drug residues in milk. This has probably to do with effects of the benzimidazoles on lactic acid bacteria and, in Roquefort cheese production, the fungicidal effect on *Penicillium roquefortii* (7).

### **Anaphylactic reactions**

Anaphylactic reactions may occur if large numbers of worms are killed by treatment and the animal is not able to get rid of the dead worms. Material from the decaying worms will cause a huge quantity of antigens and, as a consequence, problems for the host. This phenomenon is well known from treatment of seal lungworms in rehabilitation centres and in dogs treated with diethylcarbamazine for an infection with *Diriofilaria immitis*.

### **Anthelmintic resistance**

The first cases of anthelmintic resistance (AR) were described soon after the introduction of broad-spectrum anthelmintics. Today AR is found in all classes of anthelmintics in all important farm animals: it is widespread in sheep, goats and horses, and occurs locally in cattle and pigs. A recent survey of AR in Europe can be found in an EU-report of a conference held in Brussels in November 1993 (8). A serious threat is the import of animals from countries in which AR has been common to countries where it is absent or rare.

### **Impaired build up of immunity**

During the sixties and seventies our knowledge of the epidemiology of helminth infections increased rapidly. As a consequence, the control of the helminth diseases changed from curative to preventive. Anthelmintic treatment in farm animals, particularly in cattle, has been very successful in protecting first grazing season calves against the adverse effects of parasitic infections on growth. However, the overprotection of these calves has led to impaired build up of immunity. Worm problems have now been delayed to the second year on pasture or even to a later stage. This may explain the increasing number of reports on worm problems, particularly lungworms, in milking

cows. The effect of chemoprophylaxis on immunity to gastrointestinal nematodes in cattle has been recently reviewed (9).

### **Risks for human beings**

Basically the effects of anti-parasitic treatment of human beings is not different from the effects in animals. There may be a toxicity problem, there are environmental aspects and there may be an impaired build up of immunity. As consumers, we may ask for residue free animal products like meat, cheese, eggs etc. However, the greatest risk of anti-parasitic treatment in humans are the development of resistant strains (malaria) and antigenic variation (trypanosomes).

### **Conclusions**

Anti-parasitic treatments have brought welfare and health, but, as with all chemical treatments they have their adverse effects and induce a reaction in the attacked organism. Therefore they are only temporarily useful. New control methods have to be developed which lack chances for the parasite to make an evolutionary escape. For animal production this includes integrated management systems with a balance between parasite and host without economic losses. Candidates for alternative control systems are biological control and vaccination. The fast development of our molecular biological knowledge will certainly have a spin off towards antiparasitic treatment.

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## PARASITISM AND BIODIVERSITY: A PARASITIZED ECOSYSTEM IS A HEALTHY ONE?

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Biodiversity decline is a major global crisis since it is irreversible. Biodiversity still tends to be equated with free-living organisms, especially mammals, birds and plants and parasites are ignored. Parasites may represent 50% of all living organisms and we should be just as concerned about the decline in their biodiversity and their extinction: there is no moral or ethical right to except them.

Parasite diversity may be as high as that of many free-living communities e.g. in aquatic birds, kangaroos and equids and tropical fish. They are important in evolutionary time in providing selection pressure for heterogeneity, immunity and sex. In contemporary time they interact significantly with free-living animals at population, community and ecosystem levels: the extent and impact of this is only now starting to be appreciated.

It is thus reasonable to suggest that a parasitized ecosystem is a normal and healthy and natural one. Parasites may provide indications of, rather than threats to, free living biodiversity. We should be concerned about loss and extinction of parasites. J.E.A. Sprent has recently and pessimistically drawn our attention to this: A.O. Bush and C.R. Kennedy have optimistically suggested how parasites may escape from extinction. However, we still cannot realistically evaluate the real threats from habitat and host population ecosystems. Can parasites escape the threat from fragmented ecosystems, and do stressed ecosystems result in lower parasite diversity? Empirical evidence is so far equivocal.

We should thus adopt a conservative approach, assume loss of parasites will occur and will be deleterious, and thus seek to preserve them and protect the maximal biodiversity on earth.

## BIOLOGICAL CONTROL OF PARASITIC NEMATODES OF LIVESTOCK BY MEANS OF MICROFUNGI. A SURVEY

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A number of nematode-killing microfungi may grow in faecal material where they produce trapping devices that capture live nematode larvae. *In vitro* and *in vivo* screening procedures have been developed for selecting suitable fungal candidates which both have a strong nematode-killing effect and the ability to maintain viability after passage through the gastro-intestinal tract of host animals. *Duddingtonia flagrans*, possibly due to its high ability to produce chlamydo-spores, is at present a particularly promising candidate for biological control of nematodes, since it survives gut passage of both cattle, sheep, horse and pig and subsequently destroys nematode larvae in the faeces of these species. Apparently, the entrapment of larvae is not very discriminative, since many different nematode species are killed, provided they are present as larvae at the time when the fungus is operative. The nematode-killing effect is dependent on fungus dose level, but also on the number of developing parasitic nematode larvae in the faecal material. The simultaneous presence of free-living soil nematodes seems to enhance killing of the parasite larvae.

The paper discussed the potential use of microfungi for controlling animal parasitic nematodes under practical conditions. A number of technical obstacles and limitations for general nematode control are encountered, but for some parasites and epidemiological situations there may be prospects for practical implementations in the not too distant future.



## **NON-CHEMICAL APPROACHES TO NEMATODE CONTROL - HOW MODELS CAN HELP**

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The widespread incidence of anthelmintic resistance and environmental and public health concerns have prompted research into non-chemical control of parasites in livestock. For nematode parasites of small ruminants this has mainly focussed on the development of vaccines and selection of resistant hosts, and to a lesser extent on the use of nematophagous fungi and rotational grazing of pastures.

Mathematical models that simulate parasite populations can help predict the benefits of such control methods before research has even commenced, and thus may help to attract funding. They can help determine the level of control required for the method to be useful. When the method does become available, the model can help determine how best to use it in conjunction with other control measures and farm management practices. Cost-benefit analyses may help convince potential users of the method's value by demonstrating its likely benefits relative to the cost of parasitism or of alternative control methods.

Examples of such applications will be demonstrated with a mathematical model of populations of Trichostrongylus colubriformis, a nematode parasite of sheep.

## *DUDDINGTONIA FLAGRANS* - A POTENTIAL CANDIDATE FOR BIOLOGICAL CONTROL OF HORSE STRONGYLES.

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Research performed over the last 5-10 years in an attempt to find candidates to be used in future biological control strategies has clearly demonstrated that we have some very promising candidates within the group of nematode-destroying microfungi. Both *in vitro* and *in vivo* experiments uncovering the possible control of gastrointestinal nematodes of cattle and sheep have been performed successfully, all but a few using *Duddingtonia flagrans*, a netforming microfungus. In 1992 a survey conducted in Australia showed that apart from being able to isolate nematode-trapping fungi (*D. flagrans* and *Arthrobotrys* spp.) from sheep and cattle faeces these fungi also appeared in fresh faeces from horses. An American study demonstrated that two fungi - *A. oligospora* and *A.* (= *Duddingtonia*) *flagrans* - were able to significantly reduce the number of infective cyathostome larvae in faecal cultures when more than 10 spores were added per egg present in the faeces. Two very recent Danish experiments have shown that when a sufficient dose of *D. flagrans* was fed daily to horses the fungus survived the passage through the gastrointestinal tract and when the fungus was feed to grazing horses during the first part of the grazing season it significantly reduced the number of infective strongyle larvae which developed in the excreted faeces. Due to the effect on larvae in the faeces the number of larvae which were transmitted to the herbage was significantly reduced. Subsequent to this reduction in grass infectivity, the acquisition of strongyles in grazing horses was also significantly reduced. Further experiments will be needed to optimize long term control of both large and small strongyles in grazing horses.

## **The evolution of heteroxeny by parasitic nematodes of mammals**

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Why have some parasites included an intermediate host in their life cycle? Heteroxeny is apparently a risky strategy: if there is a small chance of locating one host, there is an even lower probability of finding two. The ecological conditions selecting for heteroxeny are therefore not obvious, especially in parasites which do not replicate within the intermediate host.

One way to identify the selective advantages of heteroxeny is to look for correlated changes in life cycle and ecology throughout the phylogenetic tree. Using data from more than 700 mammalian nematodes we examined whether the use of an intermediate host is associated with enhanced growth or development. In addition, we report that life cycle and adult breeding habitat are highly correlated. Changes from monoxeny to heteroxeny are associated with changes from gastrointestinal to tissues habitats. This previously unsuspected association will be discussed in relation to host immunity and parasite survival.

PARASITES AND FLUCTUATING ASYMMETRY IN REINDEER (*Rangifer tarandus tarandus* L.)

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*Objectives*

Current theory suggests that parasite resistance is of particular importance in sexual selection. Additionally, preferences for symmetrical ornaments have been shown in several species. We investigated whether parasite load is more related to asymmetry in antlers than to asymmetry in non-ornamental traits in reindeer.

*Methods*

Male reindeer, slaughtered just before rut, were examined for body size, condition, antler size, parasites and asymmetry in both antler size and jaw length. A parasite index was constructed based on abomasal and faecal parasite counts.

*Results*

Parasite index showed no relationships to body size, condition or antler size. Additionally, there were no relationship between antler asymmetry and asymmetry in jaw length, an indicator of overall body symmetry. Parasite index was also not related asymmetry in jaw length. There was, however, a positive relationship between parasite index and asymmetry in antler size.

*Conclusions*

Parasite load is reflected in asymmetry of ornamental characters, but not in asymmetry non-ornamental traits in male reindeer just prior to rut. We discuss possible implications for parasite mediated sexual selection.

## OPTIMAL BODY SIZE AND MATURATION IN TAPEWORMS

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Tapeworms (Cestoda) display considerable intra- and interspecific variation in body size even within one host individual. We study the variable body size as an adaptation to different levels of mortality and environmentally determined growth rates. Determining optimal adult body size is equal to determining optimal age-at-maturity, if body size do not increase after maturation. We assume that natural selection adjusts age-at-maturity so as to maximize fitness, which is measured as the expected lifetime production of eggs. We assume that the individuals do not differ in other respects than maturation strategy. Hence the model is best interpreted as describing intra-species variation in body size. However, we believe that it also gives some insight in community level size distributions.

We assume that the parasite does not affect survival of the host. The mortality rate is constant and is due to accidental events killing the host or all parasites within a part of intestine. The growth rate is proportional to surface area of the animal, and to the number of competitors present (*i.e.* intensity of intraspecific competition), which is explicitly included into the model.

The model predicts that optimal body size (and age-at-maturity) should be positively correlated with environmentally determined maximum growth rates and negatively correlated with mortality rate and number of competitors. Note that these results are also valid if only one competitor is present, thus competition is not necessary for considerable variation in body size to occur.

The intestinal environment is the most nutritive in midgut, followed by foregut, while hindgut is much poorer. Different parts of intestine are typically inhabited by parasites with different body sizes. The model correctly predicts that midgut parasites should have large body size, and hindgut parasites small body size. We propose that for species which have solved the problem of mortality caused by peristaltic movements in foregut, it is low mortality area and large body size is thus expected. However, it is possible that small opportunistic species can also occur, which would explain the higher variability in sizes of the foregut species. We are seeking for a suitable data for quantitative testing of our model.

## GENETIC POLYMORPHISM AND HOST PARASITE COEVOLUTION

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The relationship between parasite and host is stipulated by many factors. The last years studies have revealed that this relationship is genetically controlled. In assumption of host and parasite is the cause of various infection rate of helminth.

Electrophoretic studies on the variation of enzymes within populations of helminth reveals that different populations and species of parasites may have different values of polymorphism and mean heterozygosity. For examples the levels of genetic variation of trematode varied from  $P=0.0$  and  $H=0.0$  for *Schistosoma japonicum*, *S. mekongi* (Woodruff et al, 1987) to  $P=0.52$  and  $H=0.185$  for *Notocotylus ephemera* (Paulauskas, 1991) and of Nematode from  $P=0.03$  and  $H=0.015$  for *Parascaris univalens* to  $P=0.62$  and  $H=0.21$  for *Contracecum rudolphii B* (Bullini et al, 1986).

On the other hand the levels of genetic variation among host species and population have also different values: these values of intermediate host (for example mollusca) ranged from  $P=0.0$  and  $H=0.0$  for *Arion sp.* to  $P=1.00$  and  $H=0.63$  for *Cerithium caeruleum*, and of definitive host (for example birds) - from  $P=0.06$  for *Larus marinus* to  $P=0.58$  for *Coturnix coturnix* (Nevo et al, 1984) and  $P=0.54$  and  $H=0.169$  for *Anas platyrhynchos* (Paulauskas, Sruoga, 1993).

Enzyme polymorphism analysis of host-parasite system show that the infectivity of *Notocotylus sp* strain in ducks was different, depended on both the and the host genotype. The *Notocotylus sp.* with high heterozygosity possessed high infections rate and with low - low infections rate. Nevo et al (1984) reported a positive correlation between the width of the niche of a species (generalist vs specialist) and genetic heterogeneity.

In the case of maximum specificity for a host species and population the parasite genome is strictly bound to one host genome and must imperatively find it from generation to generation. Evolution of the host polymorphism is thus determinant with regard to that of the parasite. The existence thus large polymorphism on both the parasite and the host have been decided the parasite adapt to new conditions and to modifications to its biotope. The rate of molecular evolution in the parasite has been much higher than in the host, because the rate of generation of the parasite is higher than that of the host. The parasite and the host genotype is in the evolutionary connection.

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## ASSOCIATION BETWEEN HAEMATOZOAN INFECTIONS AND REPRODUCTION IN THE PIED FLYCATCHER

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### Objectives

Parasites may affect breeding success of their host since they compete for the same resources as their hosts. The reproduction may also increase the susceptibility of a host to parasite infections due to lowered resistance to parasites during breeding. We studied the association between haematozoan parasite infection and breeding performance of the pied flycatcher (*Ficedula hypoleuca*)

### Methods

We used both natural data on reproduction of the pied flycatcher and data from clutch size manipulation. We captured birds and collected blood samples with a smear technique (Bennett 1970). Blood parasites were quantified by GFB at the International Reference Centre for Avian Haematozoa.

### Results

The most frequently occurring blood parasites of the pied flycatcher in the central Finland were *Haemoproteus balmoralis*, *Haemoproteus pallidus* and trypanosomes belonging *Trypanosoma avium* complex. There was no difference in either breeding parameters or survival between infected and healthy pied flycatchers. The variation in breeding effort did not seem to influence susceptibility to new blood parasite infections. However, we found that the intensity of *Haemoproteus balmoralis* infection increased in infected males as the brood size was artificially enlarged. Also, in females intensity of *H. pallidus* infection tended to increase with the level of clutch size manipulation.

### Conclusions

We did not find evidence that these haematozoan parasites have any debilitating effects on either the reproduction or survival of the pied flycatcher. However, there was some evidence that increased breeding effort impaired the ability of pied flycatchers to control chronic infections.

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## THE EFFECT OF A LONG-LIVED PARASITE ON THE REPRODUCTIVE EFFORT OF ATLANTIC COD

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Life history theory predicts that an individual should maximise reproduction over its entire lifetime. In iteroparous species a decrease in future reproductive value should therefore be adjusted by an increase in current reproductive effort. Long-lived parasites have the potential to decrease the future reproductive value of a host. We would therefore expect an increased allocation to reproductive tissue in hosts infected with such parasites.

We have tested this prediction using caged Atlantic cod, *Gadus morhua*, infected with ascaridoid nematodes. Based on the 25th and 75th percentiles of the parasite frequency distribution, cod were divided into three groups with low, medium and high parasite intensity. In order to test the specific effect of parasites, we had to correct for cod weight and cod age, since both of these variables may affect reproductive allocation. As predicted, the weight of gonadal tissue relative to body weight and age was highest in cod with high parasite-intensities, and lowest in fish with few parasites. This effect was observed in both male and female cod.



SPATIAL VARIATION FOR INFECTION BY DIGENETIC TREMATODES IN A  
POPULATION OF FRESHWATER SNAILS (*POTAMOPYRGUS ANTIPODARUM*)

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**Objective.** Larval digenetic trematodes commonly castrate their first intermediate hosts, and should therefore impose strong selection on the timing and mode of host reproduction. Here we examine spatial variation for infection by trematodes in the freshwater snail *Potamopyrgus antipodarum*.

**Methods.** Snails were collected at eleven different sites at Lake Alexandrina on the South Island of New Zealand from transects that ran perpendicular to the shore and across several different habitat types (from 0 to 8 meters deep). Logistic regression was used to analyze the relationships between the frequency of trematode infection and snail size, habitat type, and transect location.

**Results.** On average, the probability of infection increased 3.3 times with each 1 mm increase in shell length. Prevalence of infection by the most common species of trematode, *Microphallus* sp., was highest in the shallow-water habitats where its final host (waterfowl) spend most of their time. Prevalence of infection by another parasite, *Telogaster ophiorchis* (final host: eels) increased with depth, but because *Microphallus* was much more common, total infection by all trematodes decreased with depth. The effects of transect location were minor for *Telogaster*, but there was significant variation in *Microphallus* prevalence among transects, especially in the shore-bank habitat.

**Conclusions.** Taken together, these results suggest that the risk of infection is spatially variable, but generally higher in shallow-water habitats, which may explain the greater frequency of sexual individuals as well as earlier reproduction among individuals near shore.

## PREVALENCE OF PARASITIC DISEASES OF GOATS ON SMALL SCALE FARMS IN EMBU DISTRICT - KENYA

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### **Introduction:**

Goats are well suited for small holder farms in the arid and semi arid areas as they are able to utilise the limited feed resources.

### **Objective:**

To study diseases that limit goat production among small scale farmers in Gachoka Division, Embu District.

### **Methods:**

A survey was carried out in a semi-arid area of Embu District, Kenya. 25 farmers each with minimum heard size of 10 goats were interviewed on disease problems affecting their goats using a standard questionnaire. Faecal and blood samples were collected from a minimum of 10 goats (5 kids and 5 adults) from each farm and analysed for helminth eggs, blood parasites and serology for cowdriosis (Western blot) in October 1994 during the short rains.

### **Results:**

All farmers were keeping goats of the Small East African breed. Mean herd size was 25 goats (range 10-90) structured as 4 entire males, 13 females, and 8 kids per farm.

Eight diseases were identified as the most prevalent by the farmers: helminthiasis, cowdriosis (heartwater), pneumonia, ticks and fleas, mange, orf, footrot and trypanosomiasis in order of priority. Helminthiasis was reported by the farmers to have caused 46% of the deaths in kids and 20% in adults during the year. This assumption was based on clinical signs of emaciation and diarrhoea observed before death. 22 farms were using levamisole on a regular basis (2-3 times a year) while the other 3 were using local herbs whenever clinical signs appeared.

Faecal sample analysis showed that all goats were shedding nematode eggs with egg counts 400-900 epg and 600-4200 epg in adults and kids, respectively. Faecal culture showed that the main nematodes were *Haemonchus contortus* (80%) and *Trichostrongylus* spp. (13%). No blood parasites were detected. 9 out of 13 serum samples from adult goats were serologically positive for *Cowdria ruminantium*.

**Conclusion:** Helminthiasis caused by *Haemonchus contortus* was thought to be one of the important diseases that may limit goat production among small scale farmers in this area.

## **SCHISTOSOMA BOVIS IN GOATS. CONSEQUENCES OF CHALLENGE INFECTIONS**

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### **Objective**

The objective of the study was to elucidate the mechanism of developed resistance seen in goats infected with *Schistosoma bovis*. This was sought by quantifying worm burdens and relating those to relevant host-parasite parameters at different times following primary and challenge infections.

### **Methods**

Forty four West African Dwarf goats were included in the experiment. Groups A (15 goats) and B (10 goats) received a primary infection (1,000 cercariae /goat) week 0. Week 16 groups B and C (10 goats) got a challenge infection (1,000 cercariae/goat) whereas group D (9 goats) served as a negative control. Subgroups of groups A and D were necropsied week 16 and week 22 and 32 subgroups of all groups were necropsied. Following necropsy all infected goats were perfused for schistosome recovery and tissues were collected for tissue egg determination (liver and intestines). Body weight was measured and faecal samples were collected every two weeks during the study.

### **Results**

The mean number of worms recovered 22 weeks post primary infection was comparable in groups A and B but group C had significantly higher number of worms than group A. Thirty two weeks post primary infection groups B and C had significantly higher mean worm counts than group A. Tissue egg counts were comparable in groups A and B both 22 and 32 weeks post primary infection in both the liver and the intestines. Group C had significantly higher tissue egg counts in parts of the intestines week 22 but not week 32 compared to groups A and B. With regards to faecal egg counts no significant difference was observed between groups A and B at any time, whereas group C was significantly higher than groups A and B week 24. Reduced weight gain was most prominent in group B, but was also seen in groups A and C compared to group D.

### **Conclusion**

The number of worms established in the primary+challenge group did significantly exceed that of the primary group, (though the establishment was apparently delayed compared to the challenge control group), but since no additional tissue or faecal egg counts were observed, an anti-fecundity effect had developed. However, despite the anti-fecundity effect, the primary+challenge group showed the poorest weight gain, thus being clinically affected by the double infection.

## CHARACTERIZATION OF THE ANTIGEN EMPLOYED IN THE ELISA FOR THE DIAGNOSIS OF SARCOPTIC MANGE IN DOGS.

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The development of a unique serological test for the demonstration of antibodies to *Sarcoptes scabiei*, a common ectoparasite of more than 40 mammals, including many domestic species and man, has been reported earlier. Characterization of the assay antigen, isolated from naturally infected red foxes (*Vulpes vulpes*), was made by SDS-PAGE, Western blot and isoelectric focusing.

SDS-PAGE of the crude mite antigen preparation revealed polypeptide bands of molecular weights ranging between 14 and 164 kDa. In Western blots probed by sera from dogs and red foxes experimentally and naturally infected with *S. scabiei*, a range of antigens were recognized ranging from 38 to about 300 kDa. Particularly dominant were antigens of 164 and 147 kDa. These were found to have isoelectrical points between 5.7 and 6.5.

## MOLECULAR-GENETIC MARKERS FOR PARASITIC HELMINTHS

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Research on helminth parasites has focussed on chemotherapy and host reactions to parasites, but in comparison, less attention has been paid to the population biology and bionomics of the parasites themselves. This lack of balance constitutes a major obstacle to a closer insight into basic parasite/host relationships and to the development of effective drugs and vaccines. A lack of knowledge of the basic transmission biology of parasites makes effective and sustainable control difficult. Furthermore, current methods for parasite control rely almost entirely on the use of anthelmintics, which has had serious consequences such as drug resistance in some parasite groups and adverse effects of these drugs on the environment. To understand and prevent these problems, an insight into the dynamics of helminth populations is essential.

Accurate identification of a parasite at any stage of its life cycle is central to studying its population biology and epidemiology. However, this is frequently not possible for many parasites with current diagnostic techniques. The advent of DNA technology has provided useful techniques to rapidly and reliably identify species and assess genetic diversity. Ribosomal and mitochondrial genes are among the most useful targets, and we have recently established molecular techniques (rapid DNA cycle sequencing and RFLP strategies) based on ribosomal DNA that can delineate even among very closely-related parasitic helminths and identify single parasite eggs/larvae. Several examples of veterinary importance will be presented and the potential of such molecular approaches discussed.

# The occurrence of *Babesia divergens* and *Anaplasma marginale* in Austrian cattle

Renate Edelhofer and W. Baumgartner

The parasite *Babesia divergens* occurs in certain parts of Austria and causes significant economic losses each year. Anaplasmosis of cattle is an infectious and transmissible disease caused by the rickettsia *Anaplasma marginale*. In 1987, during the examination of cattle destined for export to China, *A. marginale* could be diagnosed in Austria the first time. The most prominent symptoms of *Babesia* and *Anaplasma* infections are high body temperature and haemolytic anaemia associated with the presence of intraerythrocytic inclusion bodies. But only *Babesia* infections cause haemoglobinuria.

In order to obtain informations on the prevalence of antibodies against *B. divergens* and *A. marginale* in cattle, 5076 blood samples of 8 districts of Austria were collected. In order to achieve a fairly even distribution of samples in Austria, one serum from every 25<sup>th</sup> cattle farm out of altogether 140 081 farms in the whole country was taken for testing in the course of the brucellosis-leucosis-IBR / IPV control program of the years 1988 / 1990. 2309 (45.7 %) of 5054 animals tested by an ELISA showed positive titres against *B. divergens*.

109 (2.1%) of the 5076 animals tested by a CFT showed positive titres against *A. marginale*, 188 (3.8%) reacted anticomplementary.

The results obtained showed that infections with *B. divergens* and *A. marginale* occur in different areas of Austria. Last year unusual cases of simultaneous infections with *Anaplasma* and *Babesia* could be diagnosed.

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**MORPHOLOGY OF THE CUTICLE OF OESOPHAGOSTOMUM DENTATUM (STRONGYLOIDEA, NEMATODA):  
ULTRASTRUCTURAL OBSERVATIONS, DEVELOPMENT, AND  
PHYLOGENETIC IMPLICATIONS**

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The ultrastructure and morphogenesis of the body cuticle of adult and juvenile Oesophagostomum dentatum (Rudolphi, 1803) (Strongyloidea, Nematoda) was investigated by SEM and TEM. The cuticle of the first three juvenile stages consists of a trilaminar epicuticle, an amorphous layer and a radially striated basal layer. In the last juvenile stage and the adult worm the radially striated layer is replaced by a fibrous layer with 3 layers of giant fibres and a basal amorphous layer. The new forming cuticle of the third juvenile stage exhibits the epicuticle and the radially striated layer; later amorphous material appears between these layers. Functional aspects of the radially striated layer during the formation of the cuticle are discussed in comparison with previous observations on other nematodes.

When screening the literature for a phylogenetic comparison of the cuticular morphology we realized that a radially striated layer occurs in almost all major taxa of the Nematoda, at least during the postembryonic development. Such a layer seems to be missing primarily only in the Spirurida and most Ascaridida. A cuticle consisting of a trilaminar epicuticle, an amorphous layer, a radially striated layer, and possibly an amorphous basal layer may already belong to the ground pattern of the Nematoda.

Our finding of a structural change in the cuticular morphology between the third and fourth juvenile stage harmonizes with earlier reports about the Strongylida. Such a change seems to be missing in other nematodes.

POPULATION DYNAMICS OF THE ENDOPARASITIC FAUNA OF  
*POMATOSCHISTUS MINUTUS* (GOBIIDAE) OF THE GREVELINGEN  
VOORDELTA AREA (SW-NETHERLANDS)

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Gobies of the genus *Pomatoschistus* are a major faunal component of the shallow coastal Grevelingen Voordelta area. Gobies are important food items for several commercial and non commercial fish species. Therefore, their role as possible transmitters of parasites to higher levels in the food chain is of great interest. In this study the temporal changes in endoparasitic fauna of *P. minutus*, the most abundant gobyspecies, was investigated.

Monthly samples were taken in the Grevelingen Voordelta area from September '92 till July '94, using a 2.7 m beam trawl with a 5\*5 mm mesh size in the cod end. The gobies were anaesthetised in a ethylamino-4-benzoate solution in seawater and preserved in a 7% formaldehyde solution. The gobies were checked on parasites using a dissecting microscope.

The highest densities of *P. minutus* were found in September '93 (690 individuals/1000 m<sup>2</sup>). The mean density over the 20 months sampling period was 247 individuals/1000 m<sup>2</sup>.

The most common parasite species of *P. minutus* were the trematodes *Asymphylogora demeli* and *Derogenes varicus*, the cestode *Bothriocephalus scorpii* and the nematodes *Hysterothylacium aduncum* and *Cucullanus sp.* A clear temporal shift in parasite fauna was observed. *A. demeli* showed the highest prevalence (prev.=57.5 %) and mean intensity (m.i.=81.1) during late summer (September '93), while *D. varicus* was found to be more abundant in spring (prev.=61.9 %, m.i.=2.7 in March '94) as well as *B. scorpii* (prev.=14.3, m.i.=3.4). The nematodes *H. aduncum* and *Cucullanus sp.* are more abundant during winter (prev.=11.7% and 5.8%, m.i.=1 for both species in January '94). The densities of parasites carried by the population of *P. minutus* calculated for an area of 1000 m<sup>2</sup>, gives a global idea of the relative importance of *P. minutus* as a host. *A. demeli* reached densities as high as 12278 individuals/1000 m<sup>2</sup>, *D. varicus* 150 individuals/1000 m<sup>2</sup>, *B. scorpii* 72 individuals/1000 m<sup>2</sup>, *H. aduncum* 24 individuals/1000 m<sup>2</sup> and *Cucullanus sp.* 12 individuals/1000 m<sup>2</sup>.



THE EFFECT OF WATER POLLUTION ON THE OCCURENCE  
AND DISTRIBUTION OF METAZOAN PARASITES  
OF CHUBB (*LEUCISCUS CEPHALUS* L.)

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During 1993-1994, 129 specimens of chubb (*Leuciscus cephalus* L.) from two localities (polluted and unpolluted) of the Morava river were examined for metazoan parasites. Altogether 30 parasite species including helminths, parasitic crustacea, leeches and glochidia belonging to 18 genera as follows: Monogenea - 16 species - *Dactylogyrus crucifer*, *D. fallax*, *D. folkmanovae*, *D. micracanthus*, *D. namus*, *D. nanoides*, *D. naviculoides*, *D. prostrae*, *D. vistulae*, *D. vranoviensis*, *Gyrodactylus carassii*, *G.gracilihamatus*, *G. laevis*, *Paradiplozoon ergensi*, *P. megan* and *P. homoion*, Cestoda - 2 species - *Caryophyllaeus brachycollis*, *Caryophyllaeides fennica*, Digenea - 5 species - *Asymphylogora imitans*, *Diplostomum* sp., *Posthodiplostomum cuticola*, *Rossicotrema donicum* and *Sphaerostomum brauae*, Nematoda - 2 species - *Philometra abdominalis* and *Pseudocapillaria tomentosa*, Acanthocephala - 2 species - *Pomporhynchus laevis* and *Acanthocephalus anguilla*, Hirudinea - 1 species - *Piscicola geometra*, Crustacea - 1 species - *Ergasilus sieboldi* and finally Mollusca (glochidia sp.- larv.) have been found. On the unpolluted locality, 28 of parasite species have been recorded, however, on the contrary only 17 species have been found on the polluted one, it means 11 species less. Moreover, significant differences have been also observed in the course of seasonal occurrence of core parasite species. Comparison were made also on the matter of the effect of the host fish length and weight, its sex and frequency distribution of core parasite species within the population of the host fish originating from the mentioned localities. To compare the effect of water pollution on parasite micro-habitat selection, the spatial distribution of oviparous monogeneans parasitic the gill apparatus of invaded fish have been investigated.

## MORTALITY OF REARED SEA TROUT AND BROWN TROUT CAUSED BY CESTODE LARVAE, *DIPHYLLOBOTHRIUM DENDRITICUM* (NITZSCH, 1824)

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The plerocercoid larvae of *Diphyllbothrium dendriticum* (Nitzsch, 1824) has been found to infect the heart of the sea trout *Salmo trutta* m. *trutta* (L.) and brown trout *Salmo trutta* m. *lacustris* (L.) in fresh water at the Muonio State Fish Farm in northern Finland. The infection caused a varying degree of mortality among one to three-year-old trout during the summer months of 1991-1992. The mortality began when the water temperature reached 12 °C. One to six *D. dendriticum* larvae have been found in the atrium and in some cases the ventricle in 61 to 86 % of the dead 1+ and 2+ sea trout and brown trout in July 1991 and from June to September, 1992. Histologically, a massive, chronic myocarditis was evident around larvae in the atrial wall. The one-year-old trout received the first worms in early spring, probably via *Eudiaptomus graciloides* (Lilleborg) and/or *Cyclops scutifer* (Sars). *D. dendriticum* larvae were also found in the heart of live two-year-old trout in the winter in 1993. A few encapsulated *D. dendriticum* as well as *D. ditremum* (Creplin, 1825) larvae were found within the visceral cavity in just 10 % of the fish studied.

THE EFFECTS OF *LERNAEOCERA BRANCHIALIS* ON THE GROWTH RATES OF CAGED ATLANTIC COD.

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In this study we describe how *Lernaeocera branchialis* affects growth rates of Atlantic cod (*Gadus morhua* L.) caged in a fjord in Northern Norway (70 °N). The number of *L. branchialis* was recorded every third month over a period of 18 months.

The effects of both physiological variables in the host and the presence of other parasites on the level of *L. branchialis* infection are discussed. Furthermore, we evaluate if previous infection by *L. branchialis* affects the probability of present infections.

## **GYRODACTYLUS SALARIS ON SALMON ON THE SWEDISH WEST COAST**

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Five rivers with a natural salmon reproduction were investigated: Örekilsälven, Sävleån, Högvadsån, Fylleån and Stensån. *G. salaris* was found in three of the rivers. River Sävleån: the infection on parr was larger in 1993 and 1994 than in 1989 and 1990, but in August 1994 no *G. salaris* could be established; the infection was smaller after "warm" period than after or during "cold period" (seasonal variations), and was larger on smolt than on parr (hormonal host influence).

Ätran river system: River Högvadsån: the most infected river in Sweden (1991-1993). At the beginning of 1994, the infection on parr was diminished, in August 1994 no *G. salaris* were found, during the following "cold period" (1994-95), a moderately increasing infection was established. Smolt and mature precocious males (1991, 1994) were more infected than parr specimens. Tributaries of Högvadsån: in Fageredsån, (1991, 1992, 1994) and in Lillån, (1992) the infection was larger than in Högvadsån; in Lillån an abundance of 790 was established. In Hiärtaredsån, (1994), a moderate infection was found on two out of four precocious males. In River Ätran: (September and November 1994) no *G. salaris* were found.

River Fylleån: in 1989, no *G. salaris* were found; in June and October 1994, a moderate infection, also on yearlings, was established.

**Conclusions:** The tributaries seems to represented a source of reinfection of the River Högvadsån. Presumably, the salmon in the investigated rivers has a sufficiently good *G. salaris* resistance: the salmon was free from pathogenic gyrodactylosis; in rivers with large infections, no dead parr specimens were found. Basically, different resistance to *G. salaris* infection, the age of an infection, seasonal variations of *G. salaris* and macroenvironmental differences between rivers may explain the established infection differences. The dry and hot summer 1994, causing a long-range, high water temperature and a low amount of water in the rivers may have had a negative influence on the *G. salaris* reproduction, in turn causing a distinct diminishing of the *G. salaris* infections.

## INVESTIGATION OF SOME PARASITES OF THREE-SPINED STICKLEBACK *GASTEROSTEUS ACULEATUS* L. FROM WESTEN NORWAY WITH COMMENTS ON ITS FUNCTIONS IN THE PARASITE COMMUNITY.

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There has been very few studies of the fish parasite fauna of western Norway. Due to the zoogeographical isolation of this region the freshwater fish fauna is limited to a few species. *G. aculeatus* is a common small fish in the system and may take on different functions in the parasite community.

### Objectives

The primary aim was to identify parasites of *G. aculeatus* and secondarily to discuss *G. aculeatus* as host for different parasite species in western Norway.

### Methods

Unbaited clear Plexiglas traps were used to catch *G. aculeatus* from 9 localities, both fresh- and brackishwater, in western Norway. Fresh, frozen and formalinixed specimens (at least 30 in each sample) were investigated for parasites.

### Results

Two flagellates, one microsporidean, one coccidean, two myxosporideans, three ciliates, two monogeneans, four trematodes, five cestodes, three nematodes and one copepod species were identified. The following species are previously not reported from *G. aculeatus* in western Norway, *Ichthyobodo necator* (brackishwater), *Cryptobia* sp., *Sphaerophora elegans*, *Myxobilatus gasterostei*, *Eimeria* sp., *Gyrodactylus branchicus*, *Podocotyle atomon*, *Diplostomum* spp., *Cryptocotyle concavum*, *Brachyphallus crenatus*, *Eubothrium* sp., *Proteocephalus* sp., *Hysterothylacium aduncum* (Brackishwater) and *Eustrongylides* sp. larva.

### Conclusion

Twenty-four parasites are identified which indicates a great species diversity in western Norway. *G. aculeatus* is possibly the main host for *I. necator* in western Norway. *G. aculeatus* can also function as host for non-host specific parasites like *P. atomon*. Occasional findings of *Eustrongylides* sp. in *G. aculeatus* may indicate an intermediate host function, but further investigations are necessary.

ECOLOGICAL STUDY OF THE *GASTEROSTEUS ACULEATUS*-*TRIAENOPHORUS NODULOSUS* HOST-PARASITE RELATIONSHIP IN THE FRESHWATERS OF DERBYSHIRE, UK. G. Hunjan, J. Thomas & R. Siddall. The University of Derby, Derby, U.K.

The host-parasite relationship between *Gasterosteus aculeatus* and the plerocercoids of *Triaenophorus nodulosus* has previously not been studied in British waters. The host-parasite relationship is discussed in terms of seasonal, sexual and age related variations in infection prevalence and abundance.

A three year study based on a protocol of monthly sampling from freshwater in Derbyshire, U.K. has shown a clear seasonal pattern of infection. In any one generation of the host (18 months maximum life-span) two periods of potential infection are noted, the first occurring between the ages of 1-6 months and the second between the ages of 10-17 months. Logistic regression models have shown both the length and temperature are important factors in the prediction of an infection. Further, the seasonal feeding behaviour of *G. aculeatus* on the first intermediate hosts of *T. nodulosus*, a freshwater copepod and the plerocercoid induced *G. aculeatus* mortality are implicated in determining this seasonal pattern of infection.

THE EFFECT OF *OESOPHAGOSTOMUM DENTATUM* POPULATION DENSITY CHANGES ON THE DEVELOPMENT OF ADULT WORMS.

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This experiment was designed to examine the consequences of reducing the size of an *Oesophagostomum dentatum* worm population by transplanting a known, small number of worms from a high density population into helminth naïve recipient pigs. In a previous experiment it was demonstrated that following a single inoculation with 200,000 infective *O. dentatum* larvae (L<sub>3</sub>) adult worms were stunted in their growth, and the female worms displayed a significantly reduced fecundity than worms from smaller inoculations (2000 L<sub>3</sub>). In the present experiment, seven pigs were infected with 200,000 L<sub>3</sub> (Group A). Four weeks post infection (p.i.) two of the pigs were sacrificed, and the worms were recovered from the large intestinal contents by an agar-gel technique. Within 3 hours, aliquots of 1,500 worms (30% adults, 70% L<sub>4</sub>) had been surgically implanted into the caecum of each of five helminth naïve recipient pigs (Group B). Four weeks later, the experiment was terminated and the worm burdens determined. Faecal egg counts (EPG) were determined starting at week 3 p.i.. By one week after transplantation, the EPG of group B had exceeded that of group A, and this continued throughout the experiment. Similarly, fecundity of the worms was markedly increased in the transplanted worms. In the recipient pigs, the worms (approx. 70% establishment) had all developed to the adult stage and were significantly larger than worms recovered from the group A pigs, which remained stunted in their growth and development. This experiment was a preliminary step in addressing the question of whether *O. dentatum* worm populations are regulated via the host immune response or via worm-worm interactions.

## THE EFFECT OF *ASCARIS SUUM* TRICKLE INFECTIONS ON *OESOPHAGOSTOMUM DENTATUM* CHALLENGE INFECTION IN PIGS.

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The objective of the present study was to investigate the possible interaction between *Ascaris suum* and *Oesophagostomum dentatum* when coexisting in pigs. Polyparasitism is a very common phenomenon, but until now it has not received much attention.

The experiment comprised 24 pigs, which were divided into two groups according to litter, sex and weight. Group A was trickle infected with approx. 500 infective *A. suum* eggs twice a week for eight weeks and Group B was a control group. Both groups were infected with 5000 L<sub>3</sub> *O. dentatum* at week 8 p.i. and were slaughtered 28 days after. The large intestine was divided into five sections: caecum and 0-20%, 20-40%, 40-60%, 60-100% of the colon and the *O. dentatum* worms were recovered from the contents of each section by an agar-gel technique.

The results demonstrated that the total *O. dentatum* recovery in group A was lower than group B, though it was not statistical significant. Neither the male/female ratio nor the mean fecundity differed for *O. dentatum* between the two groups. There was no correlation between the number of *A. suum* worms and the number of *O. dentatum* worms recovered. The distribution of *O. dentatum* through the sections was significantly different in the two groups. Thus, the worms in group A were located in a more proximal region of the large intestine than group B, thereby indicating a certain degree of interaction.

This experiment is one of several designed to examining whether or not *A. suum* interferes with other helminths when they coexist.



## IMPACT OF DIET ON THE ESTABLISHMENT AND LOCATION OF *ASCARIS SUUM* AND *OESOPHAGOSTOMUM DENTATUM* IN EXPERIMENTALLY INFECTED PIGS

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**Objectives:** The impact of diet on the establishment and location of *Ascaris suum* and *Oesophagostomum dentatum* in swine was investigated.

**Methods:** Fifty worm-free pigs, from a specific pathogen-free (SPF) farm were used. The animals were randomly divided into five groups, and infected with a mixed culture of 600 infective *A.suum* eggs and 6000 infective larvae of *O.dentatum* per pig. The groups received traditional ground barley plus protein supplement feed (A), commercial full-constituent pelleted feed (B), barley flour plus protein (C), barley flour plus rafterline plus sugar beet fibre plus protein (D), and barley flour plus wheat bran and protein (E), respectively. All experimental pigs were slaughtered at 8 weeks post infection. Worm burdens, worm location and female worm fecundity were determined.

**Results:** In all groups *A.suum* worm burdens were low and comparable. However, *O. dentatum* worm burdens were significantly lower in groups B, C and D than in groups A and E.

**Conclusions:** Low content of diet fibre and increased amount of fermentable carbohydrates may have led to a more distal location of *O.dentatum* and reduced fecundity of female worms.

## Stage specific features and growth of the fourth-stage larvae of *Ascaris* sp. from the pig.

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In order to relate the stage specific structures of the L4 larva of *Ascaris* sp. from the pig to earlier and later stages of the parasite in the experimentally infected host, an analysis of early and late stage L4-worms were made by LM and SEM. Besides assertion of known features some new interesting observations were made. The L4 stage is typified by an extremely fast and divergent length growth of individual worms compared with the initial relatively uniform size of the new L4 at day 10. p.i. This is one indication of the fact that only few worms, if any, will, due to largely unknown reasons, ultimately be established as adults in the pig. The short duration of this stage (about 16 days) is a common feature in the Ascaridoidea. We observed that when the L4 cuticula is formed, the transverse annuli of the L3 constitutes a mould, the number of annuli in the L4 being strictly two times that present in the L3. In the newly emerged adult the new annuli are at least four times as narrow as in the cast L4 cuticula. The extension of the lateral alae (lacking in the adult) is known to be specific for the stage analysed. A pair of prominent lateral mid-body sensors were present in the larvae analysed. These have previously been named centrids when observed in the Ascaridoidea (*Contracaecum*, and subsequently in *Toxocara* and *Porrocaecum*). Apparently homologous structures were reported as Mittelkörperpapillen in Cucullanidae (Seuratoidea) by Törnquist (1931) and as postdeirids by Sulston et al. (1980) in *Caenorhabditis*. The centrids are present just dorsally to the lateral line. They were found, as in *Cucullamus*, to be asymmetrically oriented. The left side centrid was present in the mid-body region of the worm. On the other hand, the right side centrid was always present much more anteriorly. The centrid in the L4 was found to have a globular appearance. It is more than 60 % larger (diam. 12-13 µm) than the deirids. We tentatively suggest that the function of this sensor would be to transmit information of a site-specific position in relation to other parts of the body and the environment, but could also be involved in the propagation or modulation of the sinusoid movements of the worm. The growth in the L4 worm is related to the development of other stages of *Ascaris*.

## EXAMINATION OF STRONGYLES POPULATION IN LITHUANIAN CATTLE FARMS

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**Objective.** The role of strongyles in parasite-associated bovine diarrhea has been increased in recent years and significant production losses due to strongyles infection is out of any doubt. Purpose of our study was to detect the infection level of strongylatoses, to examine population and distribution of those nematodes in Lithuania.

**Methods.** Following research project, 97 faecal samples in 16 districts were collected and examined for the presence of strongyles. 76 samples were taken from big dairy (>100 milking cows) farms and 21 samples from small (<10 milking cows) farms. Average age of tested cattle from big farms was 1,5 years and in small ones varied from 9 months to 6 years.

The modified McMaster method were used to detect the number of eggs per gram of faeces (EPG). To identify the strongyles, copro-cultures were examined: the faeces were mixed with sawdust and incubated in moist conditions at 24°C for 7 days. Larvae isolated by Baermann method.

**Results.** 85,5% of coprologically tested cattle were infected with strongyles and 84,3% of them by mixed infection. Following results were obtained: 44,7% of tested cattle from big farms were infected with *Bunostomum* spp.; 32,8% with *Cooperia oncophora*; 32,8% with *Ostertagia* spp.; 30,2% with *Haemonchus* spp.; 21% with *Strongyloides papillosus*; 17,1% with *Trichostrongylus* spp.; 15,7% with *Nematodirus* spp. and 6,5% with *Oesophagostomum venulosum*. In the small farms there were 76,1% of tested cattle infected with *Bunostomum* spp.; 42,8% with *S. papillosus*; 42,8% with *Nematodirus* spp; 42,8% with *Ostertagia* spp.; 19% with *C. oncophora*; 19% with *Trichostrongylus* spp. and 9,5% with *Haemonchus* spp. The average of eggs per gram of faeces EPG = 103 in big farms and EPG = 46 in small farms in winter period; 52,1% of tests EPG = 0.

## COMPARISON OF THE PARASITES SPREADING IN LARGE SCALE AND SMALL FARMS

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Objective: The forming of large scale animal breeding farms have been injuring evolutionary approved quantitative proportions in ecosystems, and have been becoming a reason of parasitological problems.

The purpose of this study was to compare spreading parasites in large scale farms and small ones.

Methods: We have examined feces by methods of McMaster and Berman of 263 sheep from large scale farms (50-200) and of 78 sheep from small ones.

The studies included 82 cattle from large (200-400) farms and 15 cattle from small (2-4) ones. We also have investigated by method of bacteriological examination 980 milking cows from 6 large scale farms and 50 cows from 12 small farms to reveal an incidence of mastitis.

Results: According to the data of our research 100% of the sheep and cattle from large scale and small farms have been infected with strongylus nematodes. The large scale farms' sheep and cattle were infected with 3 species of parasitic strongylus nematodes. There were 1 species of sheep and 2 species of cattle nematodes in the private farms.

The cows udders from large farms have been infected with 7 species microorganisms in 430 cases (44% ) and ones from small farms -- with 1 species in 3 cases (6%)

Conclusion: The parasitic diseases that have been caused by various species of strongylus helminths and microbial parasites predominate in large scale farms.

THE POTENTIAL OF AN INTESTINAL NEMATODE TO CONTROL ITS  
FLUCTUATING HOST POPULATION

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The nematode *Heligmosomum mixtum* (Heligmosomidae) occurs commonly in the bank vole *Clethrionomys glareolus* (Arvicolinae) throughout the host's distribution range. We have studied the role of this nematode as a potential regulator of its host population by collecting field data for 17 years at Pallasjärvi, Finnish Lapland, and by accomplishing laboratory and field experiments at the same locality. According to the long-term data, the autumnal prevalence of *H. mixtum* is positively associated with host density (one year time-lag) and with precipitation during the preceding summer. Laboratory experiments showed that *H. mixtum* is able to cause direct host mortality if the transmission of the parasite is efficient and the protein content of the hosts' diet is low (3%). On the other hand, the experimental reduction of parasite's infection level in field did not affect the population density and survival of the host. We conclude that *H. mixtum* may occasionally have a limited impact on the host population, but it does not affect the overall pattern of density variation in bank vole.

## THE PARASITOFAUNA OF SEALS IN ESTONIAN WATERS

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In the parasitological study of seals in Estonian waters two main objectives were pursued: to evaluate seals as the final hosts for some of fish parasites and to obtain supplementary data on the range of seals migrations using parasites as biological tags. 11 specimen of gray seal and 1 of harbor seal were examined by total autopsy in 1994. The main parasites were Contracaecum osculatum imagos and larvae in the stomach, Corynosoma semerme and C.strumosum imagos in the intestine and Trichinella sp.l. in muscles. 3 of those species were found at the prevalence of 100% but Trichinella sp. - in one specimen only. Beside these some larval nematods of the family Anisakidae also, mainly Raphidascaris acus and Hysterothilacium auctum (both specific for fish) were found in the stomach in all specimen analysed. As the content of parasitofauna and the ratio of sexes of helminthes were very stabile we consider the seals analysed to be considerable habitual locality in Estonian waters. The possibility of connection between the infestation by nematods and local lesions in the stomach of some specimen are discussed.

## DISPERSION AND STRUCTURE OF MONOGENEAN INFRACOMMUNITIES ON GILLS OF ROACH FRY

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The material was collected from a brown water stream Rutajoki in Middle-Finland. 291 roach were studied from River Rutajoki by taking 20 roach weekly with a net between 25.6 and 6.10.1995. Parasites were studied from the gills of the left side of freshly killed fish. All adult monogeneans were identified to species, and postlarvae were counted. Identifications were based on the chitinized parts of the parasites.

The length of roach fry examined varied between 9-47 mm. Four *Dactylogyrus* species were found, of which the first were *Dactylogyrus* larva on the gills, when fry were 12 mm long. The occurrence of parasites is considered to be connected with the physiological development of the gills. *D. nanus* was the first species that established and it was the most common parasite on the gills of roach fry. *D. nanus* prevalence was 65.6 % and abundance 1.31 in the pooled material. *D. crucifer* occurred in the river only from the end of July, prevalence being 6.9 % and abundance 0.08 in the pooled material. Surprisingly, *Gyrodactylus* sp. occurred in high prevalence on the gills (maximum prevalence 73 % and abundance 1.62 on 15th June) until the beginning of August, when they disappeared. *Gyrodactylus* usually occurs on fins and skin and migrate to gills only to protect themselves against unfavourable macroenvironmental conditions. The reason for *Gyrodactylus* sp. on the gills of roach fry is discussed. There was a slight tendency for *Dactylogyrus* larvae and *Gyrodactylus* sp. to prefer the first two gill-arches at the beginning of an infection. The other parasites occurred more or less evenly on all gill-arches during the study period. The development of the community structure is, however, seen clearly because abundances increased evenly till the end of August and decreased when water temperature fell. The absence of competition between the parasite species is seen e.g. between *D. nanus* and *D. crucifer*, The latter emerge to the gills when the abundance of *D. nanus* is at highest and even to the same sites.

## ENVIRONMENT POLLUTION, VECTOR PATHOLOGY AND LYME DISEASE PATHOGEN TRANSMISSION ABILITY

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Changes in vectors of diseases as biota components suffered due to the environment pollution were studied. Furthering our investigation of the environment pollution impact upon the tick morphology and physiology we have scrutinized the correlation between the pollution level, tick exoskeleton transformation frequency and *Borrelia burgdorferi* sensu lato prevalence. *Ixodes persulcatus* ticks with exoskeleton pathologies proved to be more abundant on more polluted territories. Prevalence of *Borrelia* infected ticks was greater among the transformed specimens than among the normal ones, both males and females. Prevalence of the transformed ticks peaked at the beginning and at the end of the tick activity period during the season and displayed no dependence on the specimens' physiological age. In St.Petersburg Region the peak of the infected tick prevalence occurred in the middle of *I.persulcatus* activity period (end of May, beginning of June). The said period was also characterized by the ultimate divergence between *Borrelia* prevalence curve for transformed and normal ticks, the first curve peaking the highest. With the ticks having small anomalies in their exoskeleton *Borrelia* infection was more prevalent than with the ticks having significant pathologies. The moving activity of main *Lyme borreliosis* (LB) vector *I.persulcatus* females with pathologies was the same or even a bit greater than that of the normal ones. All the above strongly suggests that the growing number of ticks with pathologically transformed exoskeleton will increase the danger of their becoming LB vectors to promote LB distribution.



## PARASITES AND METAPOPOPULATION THEORY

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The persistence of a parasite population can be modelled using the metapopulation concept. Here a host individual is considered as one patch, harbouring a local parasite population. Several metapopulation models predict a bimodal distribution of the fraction of patches occupied, i.e., prevalence. We have used data on prevalence of mammalian nematodes from the literature to test whether prevalence is bimodally distributed as predicted by these models.

## THE ROLE OF DISEASES IN MICROTINE CYCLES IN NORTHERN FENNOSCANDIA

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Pronounced fluctuations (cycles) of microtine rodents (voles and lemmings) are a prominent feature of the vertebrate community in the northern Fennoscandia. Several features of the cycles as well as experimental work suggest that these cycles are caused by extrinsic factors. The role of food, and more recently predation have been in the focus. It has been known that diseases (in a wide sense) occur in microtine populations, but so far there are surprisingly few data on the dynamics of disease agents in the rodents and on their ecological consequences. We have studied diseases and parasites in small mammal fluctuation in Finland over several cycles. We have monitored field material as well as brought voles into lab in the peak - decline phase to see whether latent infection could be common. The occurrence of parasites (helminths and coccidia) depends on the host population structure. Thus they may affect primarily a specific subgroup of the host population (e.g. old or very young animals), and are obviously not decisive at the population level. The prevalence of *Pneumocystis carinii* increases in the peak - decline phase, but we have not seen such a physical deterioration in voles as found in lab rats. Bacterial epidemics can be common in voles, e.g. *Microtus agrestis* in decline phase. Our most common finding is *Bordetella bronchiseptica* which can also kill voles. Opportunistic infections like *Listeria monocytogenes* also seem to be common. Enteritis with unknown (viral?) origin has also been observed. On the other hand, there is a pronounced geographic and host specific variation in the occurrence of diseases. Diseases can contribute to the population declines at a host species level but their role at the rodent community level does not seem as obvious. The research on additive, compensatory and interactive impacts of diseases, nutrition and predation on the microtine population crashes remains a challenge.

## PLANT NEMATOLOGY IN ESTONIA

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A historical review about the investigations in the field of Plant Nematology since 1955 is given. Most of the work in our Institute has been done on Taxonomy and Ecology of pathogenic species as well as on Physiology of host plants. Host-parasite relationships were studied in greenhouse experiments with clovers a. o. legumineous plants using the clover cyst nematode, Heterodera trifolii Goff. and with potato using the golden potato cyst nematode, Globodera rostochiensis (Woll.). Field and greenhouse experiments with cereals using the oat cyst nematode, Heterodera, (Bidera) avenae (Woll.) revealed several hundred resistant clones among oat and barley received from the Jõgeva Plant Breeding Institute.

Estonian nematologists have collected material also from Karelia/Karjala, Central Asia and Siberia. Of several hundred publications and research notes, 3 books are available also in English.

INFECTIVITY DIFFERENCES IN SELECTED CLONES OF NEMATODE  
HETERODERA TRIFOLII GOFFART, 1932

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Three clones of white clovers sort Jegeva-4 and two clones of nematode H. trifolii, singled out in my early work, were used for experiments. Growing temperature and inoculum density was controlled.

One test: one clover leaf with roots was innoculated with one clone nematode larvaes; after one and a half month the mature females was counted. Nematodes infectivity depending on initial larvaes dose and mature females was evaluated. The values of nematode infectivity did not correspond to normal distribution, therefore for comparison of infectivity the Kolmogorov-Smirnov method was used. In my early experiments nematodes infectivity in various tests for one nematode clone was highly different, that was the reason to test the homogeneity of the H. trifolii clones.

An experiment. On one clover clone three generations of the one nematode clone were cultivated. Larvaes from the third nematode generation was transfered to other clover clone. Significance level of the differences in nematodes infectivity in various white clover clones was counted.

All clover clones resistance against nematodes was statistically different. It shows that sort Jegeva-4 is heterogenetic in resistance to nematode H. trifolii. Significance level differences of infectivity between tests with various nematode generations was evaluated for each of clover clones.

There was not found difference between nematodes infectivity in various nematode generations. Selection of nematodes on the one clover clone have not influence on the ability of nematode outspring to grow on the other clover clones. Nematode infectivity was very different in various tests for each of Jegeva-4 clover clones, but average nematode clone infectivity is stable through some nematode generations and do not depend on nematode selection.

## EFFECT OF THE POTATO CYST NEMATODE AND FUNGUS RHIZOCTONIA UPON THE RESISTANCE OF POTATO VARIETIES GROWN IN KARELIA

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Globoderoze and rhizoctonioze are widespread and harmful diseases decreasing the potato harvest under combined infection. In this connection a question of interactions between two pathogens arises. The aim is to study potato variety resistance to given pathogens. An experiment was carried out on growing potato varieties resistant and sensitive to potato cyst nematode (PCN) under the conditions of separated and combined soil infestation with fungus mycelium Rhizoctonia solani, Kuhn and PCN Globodera rostochiensis (Woll.) Behrens. The following plant resistance indicators were used: terms of phenophases, number of leaves, growth increment, the number of nematode cysts, estimation of plant organ infestation by fungus.

Fungus and nematode invasions both separately and in combination caused inhibition of plant development: the phenophases were delayed, growth intensity and number of plant leaves decreased. Lowering of plant productivity was observed (2-4 times). Nematode infestation intensity increased 17-40 times on sensitive potato roots. Nematode invasion level accounted for 1/2-1/4 of the initial nematode quantity in variants with resistant potato, but single females still developed. Thus, resistant potato varieties under reproduction preserved nematode traits of resistance sufficiently well. Planting of resistant sorts of potato on plots infested with nematodes decreases the soil invasion level considerably (70%). To eliminate the harmful effect of fungus seed-potatoes can be treated with disinfective chemical substances (according to our data: formalin and chloramin).

## A HELMINTHOLOGICAL SURVEY OF FOXES FROM THE COPENHAGEN METROPOLITAN AREA

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### Objective

The density of urban foxes in Denmark is increasing with their presence in city parks and private gardens becoming very common. The last published comprehensive survey of helminths infecting Danish foxes was conducted over 20 years ago (1). The aim of the presented study was to survey helminths infecting wild foxes from the greater Copenhagen metropolitan area with special attention paid to those parasites transmissible to domestic animals as well as those of public health concern.

### Methods

During March to September, 1993, 68 wild red foxes (*V. vulpes* L.), killed or found dead in the metropolitan Copenhagen area, were examined for intestinal helminths by routine faecal flotation. Faeces from 39 of these foxes were examined for lungworm larvae using the Baermann method. Stomach and intestinal contents from 1/4 of the foxes were examined and intestinal helminths collected. A sample of the diaphragm from each fox was examined for *Trichinella spiralis*.

### Results

Faecal flotation indicated that 24% were positive for ascarids, 75% for hookworms and 37% for trichurids, with 13% negative for helminth eggs. Of the specimens submitted for Baermann, 36% contained *Angiostrongylus vasorum* larvae, 28% *Crenosoma vulpis* and 15% *Filaroides* spp. Eight of the foxes had multiple lungworm infections. No larvae of *T. spiralis* were found in the muscle samples.

### Conclusions

The results indicate that intestinal and cardiopulmonary helminths are prevalent in the urban fox population of Copenhagen. The finding of *A. vasorum* larvae in the faeces of foxes recovered in areas other than the north of Copenhagen signifies that the endemic area for this parasite may be greater than previously thought (2). Further epidemiological studies are warranted concerning the possible transmission of these parasites from foxes to domestic pets and, in the case of ascarids and hookworms, to man.

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## PARASITES OF PERCH (*PERCA FLUVIATILIS* L.) IN A EUTROPHIC LAKE AND TWO MAN-MADE RESERVOIRS

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About 300 perch from one eutrophic lake and two man-made reservoirs located in Western Finland were studied for parasites during 1988 and 1989. The pH-level of the eutrophic lake is about 6.6 for most of the year. The water of the reservoirs is polyhumous and quite acid; in Kyrkösjärvi Reservoir the pH-level is 5.6-5.9 and in Liikapuro Reservoir 5.1-5.3.

A total of 18 parasite species were recorded, 17 of which were present in the lake, 12 in Kyrkösjärvi and only 6 species in Liikapuro. In the lake the most common species were the larvae trematodes *Diplostomum* sp. and *Tylodelphis clavata* in the vitreous humour of the eye. In Kyrkösjärvi and Liikapuro these parasites were not found due to the absence of their first intermediate hosts, lymnaeid snails, which can not tolerate water of low pH.

In the reservoirs the acanthocephalan *Acanthocephalus lucii* had the highest prevalences (94-100 %) due to the availability of *Asellus aquaticus*, its intermediate host, in the bottom fauna and diet of the perch. The protozoan *Trichophrya piscium* also prefers water of low pH-levels, because it was significantly more common in the reservoirs than in the lake.

**SUSCEPTIBILITY OF THE HOUSE FLY, *MUSCA DOMESTICA*  
(DIPTERA: MUSCIDAE) TO A SWEDISH STRAIN OF THE  
ENTOMOPATHOGENIC NEMATODE *STEINERNEMA*  
*CARPOCAPSAE* (RHABDITIDA: STEINERNEMATIDAE)**

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The infectivity of the nematode, *Steinernema carpocapsae*, of first-, second- and third-instar larvae of the house fly was investigated. This strain of *S. carpocapsae* (Nemalogic™) is pathogenic to sciarid larvae. Here, larvae of *Musca domestica* were exposed to infective nematodes in standard house fly rearing media.

First-instars of *M. domestica* was the most susceptible stage to *S. carpocapsae* infection as 89.5% of nematode-exposed fly larvae became infected. The proportion of nematode-infected second- and third-instars were 66.7% and 21.7%, respectively. Nematode infections occurred within 24-60 hours in the first-instars, while the main proportion of the other larvae's nematode infections was detected at 84 hours. All these late infections were superinfections. A small proportion (5%) of the nematode-infected second-instars reached pupation. Nematode-infected larvae were dead or in poor vigour.

These data indicate that first-instar larvae and larvae just about to pupate are the most susceptible larval periods of *M. domestica* to *S. carpocapsae*.



## RECOVERY OF *ASCARIS SUUM* LARVAE FROM LIVER AND LUNGS OF PIGS BY AN AGAR-GEL METHOD.

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**Objective.** The present study was designed to compare two different methods for recovering *A. suum* larvae from liver and lungs of pigs.

**Methods.** Pigs inoculated orally with infective *A. suum* eggs were killed on post inoculation day 3 and 7. Liver and lungs were removed and separately cut in a kitchen blender until the size of tissue specimens were 3-5 mm. Half of each tissue sample was Baermannized overnight at 38°C in isotonic saline, and larvae were subsequently recovered and enumerated. The other half was examined by a newly developed agar-gel method. In brief, the cut tissue specimens were embedded in 2 per cent agar, which was allowed to set on a paper cloth. The agar gel on the cloth was placed overnight at 38°C in a vertical position in a jar with isotonic saline, and the sedimented larvae were then recovered and enumerated.

**Results.** The mean number of *A. suum* larvae recovered from the liver by the two methods was similar, but counting of larvae was greatly facilitated by the agar-gel method. A clean suspension of larvae was obtained, in contrast to the large amount of cellular debris, that troubled the larval counting by the Baermann method. Lung larval counts, in contrast, were higher following the Baermann method than the agar-gel method.

**Conclusion.** The agar-gel method appears to be an attractive time saving alternative to the traditional Baermann method for recovering the early migratory stages of *A. suum* from the liver of pigs.

## A METHODOLOGICAL STUDY ON TISSUE EGG COUNTS IN PIGS INFECTED WITH *SCHISTOSOMA JAPONICUM*

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Trematode infections with *Schistosoma japonicum* are of public health and economic importance in many regions of South-East Asia. Humans and a wide range of vertebrates, including pigs, are potential final hosts. In many experimental situations it is important to estimate the number of eggs in the tissues of the host. Therefore, the present study examined the possibility of using a tissue subsample, taken directly from the host tissue, to give the total number of *S.japonicum* eggs in a specific organ. Sixteen male Landrace/Yorkshire crossbred pigs were infected with 500 *S.japonicum* cercariae and killed 12 weeks post infection (wpi). Five gram samples were taken from specific areas of the liver, colon, caecum and rectum and the tissue egg counts were determined by standard digestion of tissue in 3% KOH for 18 hours. Five 1 ml samples were counted for each sample the mean of which was used to determine eggs per gram. For the liver, counts from 5g samples taken from the left medial and left lateral lobes were not significantly different from counts for the whole liver, but counts from 5g samples from the right lateral, right medial and central lobes did differ significantly from whole liver counts. The results suggest that the processing time of liver tissue egg counting from large scale pig experiments can be greatly diminished since digestion of a 5g sample from certain lobes of the pig livers is sufficient to give a reliable estimate of the TEC of the whole organ. Variable results were obtained when studying 5g tissue samples taken at 6 specific points in the colon compared to the whole colon digests. This latter result corresponds well to observed pathological findings.

HEXAMITA SALMONIS FROM RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) IN-  
TESTINE: IN VIVO AND IN VITRO STUDIES

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Rainbow trout fry from a Danish hatchery were examined for infections with *Hexamita salmonis* during a one year cycle. Studies on microhabitat selection of the flagellates indicated crowding mechanisms in this parasite-host system. Heavily infected fish were parasitized along the entire length of the intestine whereas weakly infected hosts primarily were infected in the pyloric caeca and the anterior intestine. Infected fish showed a significantly lower body weight and length compared to uninfected hosts. In addition, infected hosts had reduced food content and yellow mucus in the intestine showing signs of enteritis.

Two different media were used for *in vitro* cultivation. MEM (Minimum Essential Medium) was superior to Keister's medium and was used for studies on temperature dependent growth and life cycle observations. Optimal cultivation temperature was 10 °C and cysts were readily produced *in vitro*. Continuous maintenance of the *Hexamita salmonis* population *in vitro* was conducted for five months at 5 °C without addition of fresh medium. Two division forms were identified. The formerly described binary fission was found in addition to a multiple fission form containing at least three daughter cells.

## KARYOTYPIC DIFFERENTIATION AND CHROMOSOMAL VARIABILITY IN PSEUDOPHYLLIDEA (CESTODA)

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Due to the presence of high intraspecific variability and the scarcity of taxonomically useful morphological characters, karyotype morphology may give useful indications both for species differentiation and for establishing of phylogenetic relations in Cestoda. Unfortunately, knowledge of Cestoda karyology is still rather scanty. So far chromosome numbers are known only for 15 species of pseudophyllideans, belonging to the families Bothriocephalidae, Triaenophoridae, Amphicotylidae, Diphyllbothriidae and Ligulidae.

I have therefore studied the karyotypes of 8 species. Chromosome materials were prepared by using an air-dried method of cell suspension. A fairly wide range of chromosome numbers, from  $2n=12$  to  $2n=26$ , has been found; the species with  $2n=18$  predominate. Chromosomes of most pseudophyllideans are of middle size, up to 8.5  $\mu$ m long. General majority of species show fairly symmetric complements, with biarmed chromosomes prevailing.

The representatives of different families generally form karyologically distinct groups, and no obvious homology could be detected in their chromosome sets structure. But very close karyological affinity was established between studied species of Diphyllbothriidae and Ligulidae. Apparently, order Pseudophyllidea is a taxon of moderate karyotypic dynamism, so that the existence of generalized karyotypes does not exclude the identification of species by karyotypic characters. It seems that systematics could be essentially improved by the study of chromosomal characters. To recognize the ancestral karyotype of a group is not an easy task when only a very small sample of the extant species have been worked out, but a wide distribution of the karyotype with  $2n=18$  in different groups of cestodes lead to the assumption that this number could be a plesiomorphic condition for pseudophyllideans.

**GYMNOPHALLUS SOMATERIAE FROM THE CLAM MACOMA BALTHICA: DEVELOPMENT FROM METACERCARIA TO ADULT**

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**OBJECTIVES.** Although it is known that *Gymnophallus somateriae* (Levinsen, 1881) (syn. *Gymnophallus gibberosus* Loos-Frank, 1971) occurs in the clam *Macoma balthica* (L.) both in the Baltic Sea and the northeastern Pacific Ocean (Pekkarinen & Ching 1994) and the cercaria--metacercaria development of this species (with the name *G. gibberosus*) in Baltic clams has been described by Pekkarinen (1987), further development and characterization of the adult require further investigation and description.

**METHODS.** Ovigerous Baltic adults of *G. somateriae* were obtained by incubating metacercariae on the chorioallantoic membrane or in the allantoic sac of chick embryos. The vitelline glands of the worms were stained with Fast red B. Adults of *G. somateriae* as described by Ching (1973) from the Vancouver region were used as a comparison.

**RESULTS.** In adults, the vitelline glands had grown in size and acquired phenolic substances. The glands were somewhat irregular in contour, and they were united by a full vitelline reservoir. The proximal part of the bipartite seminal vesicle could nearly reach a size similar to that of the ventral sucker. Moreover, there is a prominent pars prostatica, situated ventrally. The uterus is situated between the Mehlis' gland and the mouth. Individuals incubated for four days contained up to 55 eggs. The egg sizes of Baltic and Pacific worms partially overlapped (slightly greater in Baltic worms, 24--30  $\mu\text{m}$  by 16--21  $\mu\text{m}$ , than in Pacific worms, 21--28  $\mu\text{m}$  by 12--20  $\mu\text{m}$ ). The difference might result from including the dimensions of preserved eggs from the Pacific region.

**CONCLUSIONS.** There are no significant differences between the Pacific specimens of *G. somateriae* and the Baltic worms (previously described as *G. gibberosus*). The development of *G. somateriae* from the cercaria to adult stages is now described.

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THE DIFFERENTIATION OF PARASITES OF GRAYLING *THYMALLUS THYMALLUS* L. FROM THE RIVERS IN NORTH OF POLAND.

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In the years 1992-1994, 127 grayling were examined from the rivers: Łeba, Reda and Radunia (the north of Poland). Eleven species of parasites were noticed and one of them was determined to the kind. They are: *Echinorhynchus truttae* (Shrank, 1788); *Crepidostomum metoecus* (Braun, 1900); *Echinorhynchus borealis* (Linst); *Diplostomum spathaceum* (Rud), metacercaria; *Sterliadochona tenuissima* (Sinker, 1788); *Cyathocephalus truncatus* (Pallas, 1781); *Plagioporus stefański* (Ślusarski, 1958); *Cucullanus truta* (Fabricius, 1794); *Trichodina* sp.; *Pomphorhynchus laevis* (Müller, 1779); *Ichtipothirius multifillis* (Fouquet, 1876).

The populations of grayling from the rivers: Łeba, Reda and Radunia indicate their parasitological differentiation. From among the 11 species only 2 are common to the all three rivers, when 5 are characteristic for individual populations. This fact is caused by the differences among the water invertebrates which are eaten by the fish in particular rivers.

## PARASITE INFECTIONS IN DANISH TROUT FARMS

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Monthly samples from five Danish freshwater trout farms rearing rainbow trout (Oncorhynchus mykiss) were examined for parasite infections from October 1993 until November 1994 and recorded parasites are listed. In addition, results from an examination of a mariculture net cage system are presented as well. A total of 10 metazoan and 9 protozoan parasites were recorded. The metazoans included Gyrodactylus derjavini, Gyrodactylus salaris, Eubothrium crassum, Triaenophorus nodulosus, Proteocephalus sp., Diplostomum spathaceum, Tylodelphus clavata and Argulus foliaceus from the freshwater farms. The protozoans Hexamita salmonis, Ichthyobodo necator, Ichthyophthirius multifiliis, Apiosoma sp., Epistylis sp., Trichodina nigra, T. mutabilis, T. fultoni and an Ichthyophonus like intestinal parasite were also detected in the freshwater trout farms. Based on lectin binding studies few fish were found positive for the myxosporean parasite PKX. In the mariculture system Lepeophtheirus salmonis and Caligus elongatus were found.

# Helminths Of Fish: Reliable Indicators Of Heavy Metal Pollution In Aquatic Ecosystems?

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## Objectives and Methods:

In recent years there has been an increasing interest in the interrelation between pollution and parasitology. The acanthocephalans *Pomphorhynchus laevis*, *Paratenuisentis ambiguus* and *Acanthocephalus lucii*, the nematode *Anguillicola crassus* and the cestode *Monobothrium wageneri* were analysed in field studies for their lead and cadmium concentrations. Additionally, the heavy metal contents of different organs (muscle, liver, intestine) of their final fish hosts were also determined by electrothermal atomic absorption spectrometry. Furthermore, the metal burden of *A. lucii* was compared between larval and adult stages in its intermediate and final hosts (*Asellus aquaticus* and *Perca fluviatilis*, respectively).

## Results:

It is clear that mainly acanthocephalans and cestodes accumulate very high amounts of lead and cadmium whereby the lead content of *A. crassus* was significantly lower compared to its final host. Adults of *A. lucii* contained up to 400 times more lead and up to 100 times more cadmium compared to the muscle of perch. The concentration of lead and cadmium of the adult *A. lucii* was  $2.2 \times 10^3$  and  $2.2 \times 10^4$  times higher than the heavy metal concentration of the aquatic environment of its fish host. Compared to the adults of this acanthocephalan the larvae showed a lower degree of contamination. The adult worms contained 30 times more lead and 180 times more cadmium than the larvae.

## Conclusions:

Comparing the heavy metal concentrations in adults and larvae of *A. lucii* one may assume that the toxic metals lead and cadmium were predominantly accumulated by the adult acanthocephalans and not by their larvae. Because of the enormous heavy metal accumulation capacity of some helminths (especially acanthocephalans) these organisms may serve as sensitive indicators of heavy metal pollution in aquatic ecosystems.



## **PARASITES OF MATURE SALMO SALAR L. RETURNING TO WESTERN NORWAY: WITH EMPHASIS ON THE SALMON LICE, *LEPEOPHTHEIRUS SALMONIS*, AND, *CALIGUS ELONGATUS*.**

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### **OBJECTIVES**

1. To provide base line information on the parasites of mature salmon, *Salmo salar* L., returning to the western coast of Norway prior to spawning. 2. To examine the validity of current techniques for parasitological sampling.

### **METHODS**

For two years salmon returning to the coast of Norway prior to the spawning run have been monitored for parasites. This investigation was the first complete parasitological study of salmon returning to Norway. Fish were taken from weirs anchored off the seaward side of small coastal islets. The Dogiel complete technique, modified to include blood sampling, was used to examine the fish for parasites.

### **RESULTS**

4 protozoan, 2 monogenean, 3 cestode, 4 trematode, 2 nematode, 1 acanthocephalan, and, 2 copepod species were found. 2 of the protozoans and the monogenean were previously unknown from western Norway. 2 of the protozoan species are previously undescribed. 2 copepod species and 1 cestode are known to cause problems in marine salmonid aquaculture, the monogenean has recently been reported associate with such problems, and, at least one of the protozoans is suspect.

The population dynamics of the copepods *Lepeophtheirus salmonis* and *Caligus elongatus* described here lead to serious questions in experimental design of earlier works and the validity of the techniques in many modern field studies of these commercially important parasites. Sample size and design criteria are discussed with regard to the present report and other studies.

### **CONCLUSIONS**

Our present knowledge of parasites of anadromous salmonids in their marine phases is inadequate. Current techniques used to examine *Lepeophtheirus salmonis* and *Caligus elongatus* are, generally, ineffective. Techniques exist to improve sample size estimates before sampling is started, but these are inadequately used.

ON THE ADAPTATION OF *DERMOCYSTIDIUM* IN ESTONIA

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The classification of genus *Dermocystidium* is still not clear and data on the lifecycle of the parasite is scarce (Dykova, Lom, 1992).

Objectives of the study include investigation of morphological and ecological adaptations in the framework of general data gathering on dermocystidiosis in Estonia.

Material and methods. Number of invaded fish, number of cysts per fish, mortality caused by dermocystidiosis, water temperature and other parasites of *Cyprinus carpio* L. from two fishfarms from 1982 were registred. 20-100 fish were inspected every month. Histological and electronmicroscopical methods have used for identification the species and investigation of its micromorphaology from 1992. Digital database on the disease containing above mentioend features is created at present.

Results. Cystes were found only on fingerlings from January till April, most of them in March. These were morphologically identified as *Dermocystidium cyprini* Cervinca et Lom, 1974. With less than 30% of fish invaded only cystes were found and no mortality was observed. Since 1990 some cases with remarcable mortality where registred when all inspected specimens were invaded and high diversity of life-forms of the parasite was observed. Although the fish from ponds of invaded fish have been sold to other fishfarms there is no data on spread of the disease. There was statistically significant correlation ( $p < 0.001$ ) between average water temperature in winter and number of invaded fish. No correlation with infection of other parasites have been observed.

Conclusions and perspectives. Big number of parasites and higher mortality coincided with diversity of life stages of parasite. These invasions have happened during last winters. Whether it is a sign of adaptation of the parasite to climate change remains to be investigated but given the correlation with temperature it seems possible. It also appears that adaptation of the fish to moderate invasion of the parasite fails them during more intence epizootic. Morhological species identity should be further checked using molecular genetics.

ON THE PARASITOLOGICAL FAUNA OF SALMON PARR (*SALMO SALAR* L.)  
FROM THE RIVER TENO AND TRIBUTARIES (NORTH FINLAND).

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**ABSTRACT:** In the investigation of salmon parr from 20 places has been found 22 parasites. Most of the species, the Flagellate *Trypanosoma* sp., monogeneans *Tetraonchus alaskensis* and other, were previously unknown in this area due to the lack of research. The general ecology of the parasites noted is discussed and a detailed discussion of parasitological situation and epidemiology is presented.

**RESULTS.** Parr from 20 places were sampled and studied during September and October during the year 1993 and 1994 respectively. Fishes were collected from different biotops by electrofishing. In the year 1993 it has been studied 50 salmon parr and 250 in the year 1994. Collection, fixation and laboratory processing of the material was carried out according to Bykhovskaya-Pavlovskaya (1985), modified to include blood samples.

It has been found 22 parasite species of 6 systematic groups (Flagellate, Myxosporidia, Infusoria, Monogenea, Trematoda, Nematoda) were found. The parasite fauna of the investigated rivers consists exclusively of freshwater species, and 11 have direct life cycles.

The parasite fauna of the individual rivers varied from 6-13 species. Only *Chloromyxum truttae*, *Apiosoma* sp., *Crepidostomum farionis*, *Diplostomum* sp., *Ichthyocotylurus erraticus*, *Raphidascarisacus* may be considers common. The research carried out in the year 1994 made it possible to describe the parasite fauna of young salmon in main tributes of the River Teno. The level of the infection of most parasites was not high. The exceptions were *Chloromyxum truttae*, *Capriniana piscium*, *Apiosoma* sp., *Crepidostomum farionis*, *Ichthyocotylurus erraticus*, *Raphidascarisacus* in the Rivers Iskurasjoki, Iesjoki lower part, and Karasjoki. The infection of salmon parr markedly higher in tributaries compared to the River Teno. The infection of parr by *Crepidostomum farionis*, *Raphidascarisacus* (l), *Capillaria salvelini*, *Cystidicoloides tenuissimum* (developing with the change of host) takes place mainly while feeding on larvae of various aquatic insects and oligochaetes. High prevalence and intensity of their infection indicates that these invertebrates have an important part in the food of fish.

It is well known that the balance between the parasite and host in natural condition usually does not lead to catastrophic increase in the quantity of parasites and to the mortality of the infected fish. Under normal conditions only the least resilient hosts die under the effect of parasites. In this connection, the existence of *Trypanosoma* sp. (blood), *Chloromyxum truttae* (gall bladder), *Diplostomum* sp (eyes), *Ichthyophthirius multifiliis* (skin, gill) is dangerous in for salmon parr in the River Teno. With the increasing of salmon farming, and other human activities parasite prevalence and intensity may reach epidemic levels.

## PARASITIC PERITRICHA OF FISH FROM KARELIAN ACID LAKES

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Acidification of Northern lakes associated with mobilisation of trace and heavy metals have a negative effect on fish and their parasites. There are 13 lakes from 2 regions of Karelia (Suoyarvi and Kondopoga water systems) had been investigated. Two of them were circumneutral (pH 7.0 and 7.9), others were acidic (pH 4.5-5.8) humic or clear-water. These lakes were mainly with perch *Perca fluviatilis*. SEM-investigation had discovered 2 species of parasitic Peritricha infected the perch gills: *Apiosoma campanulatum* and *A. minimicronucleatum*. There is correlation between species and level of gill infestation with these parasites and pH and colourness of Karelian lakes water. The gills of perch from acid clear-water lakes are infected with *A. campanulatum* and the level of infestation is sufficiently lower than in humic ones. In humic lakes (pH 4.9-5.8) the perch gills are infected with both species of Peritricha, but *A. campanulatum* is prevalent. Number of *A. minimicronucleatum* is increased in parallel with decreasing of water pH. The most intensive infestation of perch gills with this parasite takes place in the most acidic humic lakes (pH 4.5)

BIOLOGY AND ULTRASTRUCTURE OF *DIPLOCOTYLE OLRIKII*  
(CESTODA, SPATHEBOTHRIIDEA) FROM WHITE SEA

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Peculiarities of life cycle and morphology *Diplocotyle olrikii* from Kandalaksha Bay have been investigated. From 4342 studied littoral amphipods was found to be infested only *Gammarus oceanicus* (prevalence - 6%, abundance - 1,5, range - 1-4 specimen). Examined fishes - ordinary definite hosts of these cestodes: *Gadus morpha marisalbi* - 39, *Elegans navaga* - 25, *Pleuronectes flesus* - 22, *Myxocephalus scorpius* - 26, *M. quadricornis* - 20 specimen were uninfested. Experimental infestation of flounders with *D. olrikii* was not successful. We found in gammarids progenetic procercooids of similar morphology with adult worms. They were gravid worms (50 mm in length) with fully developed scolex and reproductive systems. Up to 70 reproductive complexes were there; uterus was filled with eggs, parts of which contained oncospheres. SEM- and TEM-investigaitons shown that worm body was surrounded by cyst. Tegumental microtrichiae were of tubular type. Unlike intestinal cestodes they are undifferentiated on the scolex and body. In the scolex there was found grandular apparatus, bundles of musculature and ciliarless receptors.

Thus, the progenetic development of *D. olrikii* in amphipods from White Sea shore was observed. Probably, cycle of these cestodes in North region is limited with gammarids without participants of their definite host - sea fishes.

## ANTIBODY RESPONSE OF ROACH (*RUTILUS RUTILUS*) TO INFECTION OF *RHIPIDOCOTYLE FENNICA*

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Digenean trematode *Rhipidocotyle fennica* uses *Anodonta anatina* as the first and *Rutilus rutilus* as the second intermediate host. The final host of *R. fennica* is *Esox lucius*. Antibody response to *R. fennica* cercariae infection and the role of antibody in the protection against the parasite were studied. Anti-*R. fennica* antibodies in roach serum were determined with ELISA-method.

Antibodies against *R.fennica* in the serum and the number of parasites in the tail of fish from different lakes were studied. The roach in lake Peurunka were free from *R.fennica* because there is no *A. anatina* in the lake. The fish from other lakes with *A. anatina* were infected by the parasite and specific antibodies against *R.fennica* were found in their sera. The number of parasites and the level of antibodies increased during the summer.

Fish from lake Peurunka were used for immunizing studies in aquaria. The roach were treated in two ways: group A was immunized with *R.fennica* cercariae shedding from *A.anatina* and group B were given a single injection (i.p.) of homogenized *R. fennica* cercaria. After four weeks the fish in group A had developed high levels of antibody but group B responded only weekly.

The role of antibodies in protection against *R.fennica* was studied 4 weeks after the immunization described above. The fish in both groups (A and B) and untreated control fish (group C) were given a challenge infection by cercariae shedding from *A.anatina*. The number of the parasites in the tail and the level of the antibodies in the serum were studied on days 0 and 14 after the challenge. There were no difference in the number of metacercariae between control fish (C) and fish injected with *R. fennica* homogenate (A). In the group of fish infected by cercariae (B) the number of parasites did not increase during the 14 days post challenge but instead a decrease in the number of strongly infected fish was found.

Taken together the results show that roach naturally infected by *R. fennica* develop specific antibodies in the serum. High levels of antibody may be protective against the parasite, but induction also of other components of resistance cannot be ruled out on the basis of these results.

## **Population aspect of morphologically variability of fish parasites**

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The high inraspecific variability which in the most important adaptive mechanism of the species to the concrete environmental conditions lies on the basis of the population phenotypical diversity. Due to variability there are always some individuals in the population which retain their adaptivity to the fluctuating environmental conditions. Change in signs leads to the change in functional activity, reconstruction of the organisms at the individual level and realizes at the population level.

The use of population-morphological approach in the parasitology allows to represent the species as a set of morphologically different populations, to assess their condition, adaptivity to the environmental conditions and interrelations in the system "parasite-host". It is well known that the variability of the freeliving species fluctuates from 4 to 10 %, most often the coefficient of variation is 5-6% (Giller,1988). The study of the parasite morphology has shown that they have more wide limits of the parameter variation. The materials obtained indicate that high degree of variability is natural for the parasitic species. It is directed to the more complete assimilation of their ecological niche that is necessary for the successful passing of the life cycles. However, in spite of the fact that every population is adapted to the concrete environmental conditions and is unique on its morphological parameters, as a rule there are no differences in the character of separate signs variations. Distinct differences are revealed only in the geographically distant populations, territorially separated during long period of time (Roitman, Kazakov, 1977; Pugatchev, 1988). These facts show that parasitic populations have large reserve of genetic variability due to the high morphological variability. High variability is the base of their existence stability and some extent inhabits their microevolution.

## CERCARIOSES PROBLEM IN HEALTH-RESORT ZONE OF THE NARCOCH-LAKE (BELARUS)

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More than 300 observations of allergodermatites caused by cercaria of trematodes from the family *Schistosomatidae* (Looss, 1899) were recorded in peoples resting in Narcoch Lake health resort area (NW Belarus). This problem is connected with the population increase of water pulmonate snails and birds and high number of resting places in the area.

Complex eco-parasitological research was carried out in the Narcoch Lake area in 1994. Full parasitological necropsy was performed on 16 Mallards and 5 Mute Swoms, and the presence of trematode larvae was studied in 334 pulmonate snails by means of compressor method.

Both males and females of *Bilharziella polonica* (Kowalewsky, 1895) Looss, 1899 and *Trichobilharzia ocelata* (La Valette, 1854) Brumpt, 1931 were found in 6 (38%) Mallards, Mute Swoms were free of trematodes. Six species of transition hosts (*Limnaea stagnalis*, *L.ovata*, *L.auricularia*, *L.peregra*, *L.palustris*, *Planorbis corneus*) were infested by cercaria. Highest density (up to 100-150 ind./m<sup>2</sup>) was recorded for *L.palustris* and *L.stagnalis* populations, highest cercaria infestation was recorded in the same species (26% and 57% respectively). Mean proportion of infested snails (all studied species) was 19%, and estimated number of cercaria in 1 m<sup>3</sup> of water reached 35000 specimens. Our results indicate high risk of cercaria infestation in humans in the study area.



INFLUENCE OF DIPLOSTOMUM SP. ON COLOUR CHANGE IN SALMO SALAR  
AND SALMO TRUTTA

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**Objectives.** Heavy infection with Diplostomum sp. was found in salmon produced for stocking at one fish farm in 1993. Some of the fish had white or opaque lenses (max=28 metacercariae/lens). Our purpose was to examine whether Diplostomum sp. has any effect on the behaviour of the fish (colour change) and whether there are any differences between salmon and sea trout in this respect.

**Methods.** One-year-old salmon and sea trout were infected with approximately 1500 cercariae/litre at one fish farm at the end of August 1994, the cercariae having been obtained from isolated snails (Lymnaea palustris) under warm light conditions. The water temperature at the time of the infection was 15.2 °C and that at the end of the experiment (in the middle of October) was 6.4 °C. The time which a fish need to change its colour was measured after transfer from total darkness to a white basin placed in a bright light.

**Results.** The average times for the colour change were 204 seconds for the salmon and 258 seconds for the sea trout. No significant difference was observed between the control and infected fish in either species. An average of 21.6 metacercariae was found per salmon and 12.4 per sea trout (Student t-test, df=49,  $p < 0.001$ ).

**Conclusions.** Salmon seem to be more susceptible to Diplostomum sp. than are sea trout. The reason why there were no differences between the infected and control groups in the colour change experiment may be that the metacercariae were not yet infective, but more examinations would be required to prove this.

## NEMATODE COMMUNITIES IN STOMACHS OF FOUR SEAL SPECIES FROM THE VARANGERFJORD, NORTH NORWAY

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We compare the community structures of nematodes (Anisakis, Contracaecum, Pseudoterranova and Phocascaris) in the stomach of seals (grey seals (Halichoerus caryus), harp seals (Phoca groenlandica), ringed seals (P. hispida) and common seals (P. vitulina)) caught in the same area in North Norway. During the three winter-spring seasons 1989-1991 a total of 32 grey seals, 34 harp seals, 14 ringed seals and 18 common seals were sampled and used for this purpose. Subsamples of the nematodes were identified to species, stages and maturity. The data was analysed by multivariate methods (Bray Curtis similarity index and MDS-ordination). Friedman's test was used in the comparison between the nematode composition in the individual stomachs. Essentially three different communities were identified in the seals. These were dominated by respectively Pseudoterranova, Anisakis and Contracaecum. In harp seals most of the communities were dominated by Contracaecum, while grey seals exhibited the largest varieties of communities. Anisakis and Contracaecum seem to have in an inverse relationship to each other. Our data indicate that the nematode community is dependent on both the host species and the diet.

THE DANISH SCIENTIST OTTO FREDERIK MÜLLER (1730-1784) AND HIS IMPORTANCE TO FISH PARASITOLOGY.

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The great Danish scientist Otto Frederik Müller created in the 18th century outstanding works in the fields of Botany and Zoology. However, especially fish parasitologists are familiar with the author name O.F. Müller. He described a huge number of invertebrates including parasite species and should be regarded as one the fathers of fish parasitology. The present paper provides information about his life and scientific production.

O. F. Müller was born in Copenhagen in 1730 where he lived for the first 12 years of his life. From 1742 until 1748 at a latin school in Jutland (western part of Denmark) he obtained his basic education in history and music whereafter he started a theology study in Copenhagen. He later shifted to the study of jurisprudence and got his degree in 1760.

As a privat teacher for the son of prime minister Schulin O. F. Müller had to teach natural sciences as well. Probably it is from here he got his interest in biology. From 1758 he implemented collections and studies in botany and zoology. These were first performed at Frederiksdal (near lake Furesø) but after his marriage in 1773 with Anna Catharina Paludan he obtained possibilities to conduct studies in Oslo Fjord (formerly a part of Denmark). Later also other parts of the Danish waters were examined by Müller.

Among a number of publications *Zoologia Danica* was the greatest. Müller endeavoured to describe all the animal species of Denmark and Norway, a task which was never accomplished. However, the tremendous work *Zoologiae Danicae Prodrromus* from 1776 containing more than 3000 species descriptions was a preliminary work to be followed by further publications. Of these he personally published two volumes with illustrations. Later further two volumes with a similar number of drawings were published posthumously. Another great Danish fish parasitologist, the scientist P. C. Abildgaard (the founder of the Veterinary School in Copenhagen) was involved in the publication of these volumes (1789, 1806).

Müller suffered seriously from rheumatism and died in 1784 but before he passed away he wrote to the new generations of fish parasitologists (*Zoologia Danica* 1781): "Further findings it would give to examine the cod, flounder and herring nets of the fishermen from Bergen when they are drawn from the bottom of the sea. But for this my lost health is needed. However, with joy I throw my eyes back on what has happened and with thanks to God I am happy to conduct the resolute on a path, which will satisfy their inquiring minds and provide them with lasting pleasure".

## ACAROFAUNA OF HOUSE DUST FROM BASEMENT FLAT

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The aim of this study was to investigate the species composition of house dust mites during the year. Material was collected monthly (1 sample per 1 month) from basement flat (Poznań, Poland) during 26 months (Nov. 1984 - Dec. 1986). House dust samples were sieved by means of laboratory strainer (mesh - about 2mm) and analysed microscopically. Mites were isolated from dust, prepared, specified, counted and dust infestation was stated (number of mites per 1g of dust).

All (26) samples were settled with mites. Average infestation of them amounted 188,9 mites per sample (9,4 mites/1g dust). Among total number 4911 mites found, over 15 species were specified. The most frequent (almost 100% infested samples) and numerous of them were allergogenic mites: Dermatophagoides pteronyssinus (average number 2,7 per 1g dust), Glycyphagus domesticus (2,4/1g) and Euroglyphus maynei (2,3/1g). Cheyletus eruditus, Lepidoglyphus destructor and species belonging to Tydeidae and Tarsonemidae groups were fairly frequent but not very numerous (0,2-0,3 specimens per 1g dust), however they occurred over the months of whole investigations. Other mite species (mostly house dust and stored products mites) were found rarely and usually in small numbers.

MYSTERY OF *SCHIZOCARPUS* (LISTROPHOROIDEA,  
CHIRODISCIDAE) EXCHANGE BETWEEN TWO BEAVER SPECIES

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Genus of *Schizocarpus* is known to include of 51 species: 32 species are specific for *Castor fiber*, 19 species are specific for *Castor canadensis*.

Even though both beaver species live on the same territories *C.canadensis* having once been introduced in Finland, Karelia and Far East, ectoparasite interchange between Canadian and European beavers is practically impossible due to their ecological isolation. *C.canadensis* ectoparasite investigation in Karelia has detected only two species of *Schizocarpus* (*S.paramingaudi* and *S.centralis*). But in beaver farms on which both species are bred ectoparasite interchange was found: 6 species of *C.fiber* were collected on *C.canadensis*. Same breeding tools and farm hands facilitate the process of ectoparasite interchange.

Only the most biologically plastic mites of the *Schizocarpus* genus proved to be able to change the animal host. When mites change their host their localization on a new host tends to be different. In our case *S.numerosus* when found on the *C.fiber* is a whole body parasite, whereas when found on *C.canadensis* in 47% cases is on its head.

## **METACERCARIAE OF *BUCEPHALOIDES GRACILESCENS* (DIGENEA) IN COD AND HADDOCK FROM ICELANDIC WATERS**

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The anglerfish *Lophius piscatorius* is the only known final host of *Bucephaloides gracilescens* and the bivalve *Abra alba* is the only known 1st intermediate host. Various gadoid fish serve as 2nd intermediate hosts. *B. gracilescens* has been recorded from the Mediterranean to the Faroe Islands and Norway. The known geographical distribution exceeds that of the bivalve host *A. alba* which does not occur in Icelandic waters.

### **Objective**

The objective of this study was to investigate whether *B. gracilescens* metacercariae occur in gadoids caught off the coast of Iceland.

### **Methods**

A total number of 237 Atlantic cod (*Gadus morhua*) and 10 haddock (*Melanogrammus aeglefinus*) caught in 1993-1994 were examined for the presence of *B. gracilescens* metacercariae. In all cases the cranial cavity was examined macroscopically and in addition brain, eyes, kidneys, skin and muscles from most fish were prepared for histological examination.

### **Results**

In cod *B. gracilescens* metacercariae were found in the cranial cavity of three fish. Histological examination demonstrated metacercariae in seven additional cod, i.e. submeningeally, in cranial nerve bundles, in nerve bundles of muscle and kidney, in the dermal layer of skin and in the eye close to the optic nerve. In haddock metacercariae were found in kidney nerve bundles in five out of ten fish. All metacercariae detected were encysted and evoked a host response (encapsulation) to a varying degree. All infected fish were caught off the south and southwest coast of Iceland.

### **Conclusions**

These findings extend the known geographical distribution of the digenean *B. gracilescens*. This also supports the view that molluscan species other than *A. alba* can act as 1st intermediate hosts for this parasite. A distinct host response to infection was observed.

THE ECOLOGY OF VIVIPAROUS MONOGENEANS OF THE GENUS  
GYRODACTYLUS NORDMANN, 1832 IN THE LIGHT  
OF RECENT KNOWLEDGE

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Monogenean parasites of the genus *Gyrodactylus* Nordmann, 1832 are widespread and important parasites of fishes, both in freshwater and marine ecosystems. According to current approach to study of the total relation of an animal species to its abiotic and biotic environments, ecology is a complex science and must deal explicitly with three levels of biological hierarchy, the organism (species) under investigation, population of these species and communities of species. With this in mind, we can compare and review the present status of our knowledge of gyrodactylids ecology. Till now, only a few gyrodactylid species have been investigated from the point of view of the effect of the ecological factors on the course of its life cycles. On the other hand, very much results have been obtained dealing with seasonal aspects of gyrodactylids occurrence and population dynamics. Several papers on the effect of host fish size, age, sex and physiological status of host fish organism including their immunological response and stress have been published. During recent years, many interesting papers concerned with the influence of a host fish susceptibility and resistance and the effect of genetic origin of host fish on the course of gyrodactylids life-cycles have been published. However, also the factors of water environment (e.g. water temperature, light regimen, concentration of oxygen dissolved in water, water salinity, its pH and water pollution) play an important role and affect the reproduction and transmission of these parasites. The study on the level of a parasite community and co-occurrence of gyrodactylids with other fish parasites were practically neglected and there are no informations concentrated on this approach.

EFFECTS OF HOST AGE AND SEX ON *EIMERIA* (PROTOZOA:  
COCCIDIA) INFECTIONS OF THE ROOT VOLE *MICROTUS*  
*OECONOMUS*

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### Methods

Two root vole populations were live-trapped at Pallasjärvi in western Finnish Lapland during the summer 1992. *Eimeria*-infections of individually marked root voles of known age and sex were studied from faeces. The observed *Eimeria* were classified in two size groups: *Eimeria* A (about 30×40 µm) and B (about 40×60 µm).

### Results

Practically all root voles were infected with *Eimeria* A. Age of root vole had a significant effect on the intensity of infection. Overwintered voles had the mildest and immature voles had the strongest infections. The prevalence of *Eimeria* B was low in all age groups. It was never found from overwintered root voles and in mature summer-born voles it was met only occasionally. Immature summer-born voles had heavier infections than mature summer-born voles. There was no difference between infections of male and female root voles.

### Conclusions

Induced immunity may cause the observed lower prevalence and intensity of infections in old root voles. *Eimeria* A probably does not induce effective immunity. The results suggest that immunity to *Eimeria* B is stronger and it also develops faster than immunity to *Eimeria* A. This may indicate differential pathogenicity of these coccidian 'species'.



## HELMINTHS INVASION OF ROE DEER UNDER DIFFERENT ECOLOGICAL CONDITIONS

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The environmental conditions, where the host settles in, should be taken into consideration to clarify complicated relations between helminth and host.

**Objective.** To find out the influences of some ecological conditions on the helminths invasion of roe deers.

**Methods.** Helminthological investigations of 128 roe deers from 29 different parts of Estonia were carried out. On the basis of the population density (Pd) the animals were divided into 3 groups: 1) 6 - 30, 2) 31 - 50 and 3) 51 - 119 roe deers per 1000 hectares. On the ground of biotope woodiness (Bw) the locations of roe deer were also divided into 3 groups: 1) 25 - 40%, 2) 41 - 60% and 3) 61 - 88%. A comparison between the invasion with helminths in roe deers on mainland and on the islands was made (population isolation - Pi). The number of helminth species (Hsp.) and the total number of helminths (Ht) in one roe deer were calculated.

**Results.** In total 28 helminth species were found in roe deers, 99% of animals investigated were infected. The most widespread species were as follows: *Ostertagia leptospicularis*, *Chabertia ovina*, *Bunostomum trigonocephalum* and *Nematodirus filicollis*. *N.filicollis*, *O.leptospicularis* and *Ch.ovina* were dominant in the intensity of invasion.

Pd: Hsp. and Ht were smaller in the I group (3.9 and 134) in comparison with these in the II (5.7 and 543) and III group (5.8 and 394).

Bw: Hsp. and Ht were greatest in the area with woodiness of 41 - 60%, where the bushes formed the majority of the biotope.

Pi: Hsp. and Ht were much bigger on the mainland, accordingly 5.3 sp. and 379 helminths in comparison with 2.3 sp. and 24 helminths on the islands.

**Conclusion.** The population density, biotope woodiness and isolation of population have an effect on the helminths invasion of roe deer in essential way. These circumstances must be taken into consideration in planning helminthoses control of games.

## THE SPATIAL DISTRIBUTION AND MICROHABITAT SPECIFICITY OF GILL MONOGENEANS OF BARBEL (*BARBUS BARBUS* L.)

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During the investigation of 64 barbels (*Barbus barbus* L.) originating from the river Danube near Vienna, Austria, three monogenean species of the genus *Dactylogyrus* Diesing, 1850 (*D. carpaticus*, *D. dyki* and *D. malleus*) have been recorded. Distribution of the ectoparasites on the gill apparatus of the host fish was examined by means of the number of specimens of each dactylogyrid species on each of the 72 sectors distinguished. To analyse the occurrence, spatial distribution and microhabitat specificity of two core ectoparasite species (*D.carpaticus* and *D. malleus*) non-parametric Chi-square test has been used. There was no significant difference between left and right side of the gill apparatus. The third gill arch has represented a place where the highest number of both parasite species have been occurred. Moreover, there were differences in preference in occupying the individual segments of gill arches. In the case of *D. carpaticus* there was a strong preference for the dorsal segment, whereas for *D. malleus* the preference not only for dorsal but also for medial segment have been found. During the whole period of investigation *D.carpaticus* exhibited a significant preference for the central area of gill filaments where 72% of all specimens were attached. In the case of *D. malleus* we can state, that this parasite has preferred the proximal area where 54% of all parasites have been distributed. Finally, it was found that the biggest proportion of both core parasite species have been attached on the inner surfaces of the hemibranchs. Only very small number of 8% (*D.carpaticus*) and of 9% (*D.malleus*) have been recorded on so called outer surface of the gill apparatus.

## BLOOD PARASITES AND REPRODUCTION BY FEMALE REINDEER

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The contemporary evolutionary theory includes a prediction that, in polygynous mammals, prospective mothers in poor physiological condition will produce more daughters than sons. While blood parasites (Trypanosoma sp) were most common amongst non-pregnant females in reindeer (Rangifer tarandus), females carrying a female foetus had more blood parasites (Trypanosoma sp.) than those having a male foetus. Differences in blood parasite intensity were probably related to female vigor. One possible proximate explanation for our findings is a connection between female social rank and their position in the summer herd. Subordinate individuals are forced to locate at the periphery of the herd where the attack rates of blood-sucking Diptera that act as parasite vectors, are highest.

## LONG TERM STUDY ON FISH PARASITES IN LAKES WITH DIFFERING WATER QUALITY

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Occurrence of parasites of roach and perch was studied from two lakes polluted by effluents from a paper and pulp mill and from a lake which is in a natural state. A total of 335 roach and 299 perch were studied during the year 1986. In 1994 326 roach and 345 perch were examined from the same lakes. During these eight years between these studies the water quality of the two polluted lakes had changed significantly due to changes in the processes of the pulp mill. In 1986 all parasites were studied from both fish species, while in 1994 only those species were selected which were known to show differences in prevalence or abundance between the lakes. The most significant change in the occurrence of parasites between those two periods of study was seen in prevalences and abundances of those parasite species which have molluscs as their first intermediate hosts, for example *Diplostomum* spp., *Tyloodelphys* spp. and *Rhipidocotyle* spp.. Higher prevalences and abundances of these species in 1994 in the most polluted lake refer to better condition of the bottom fauna of this lake when compared with the situation in 1986. This was seen also as increase of glochidia-larvae on the gills and fins of both of the studied fish species.

PARASITE COMMUNITIES IN TWO SYMPATRIC MORPHS OF ARCTIC CHARR  
*Salvelinus alpinus* (L.) IN LAKE FJELLFRØSVATN, NORTHERN NORWAY.

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**Objectives:** Lake Fjellfrøsvatn has two morphs of Arctic charr; an autumn spawning 'normal' charr utilising all major lake habitats, and a winter spawning 'dwarf' charr exclusively found in the deep profundal. Our hypothesis is that separate life strategies should give differences also in parasite infection between the two morphs.

**Methods:** 'Normal' and 'dwarf' charr were sampled by gillnets in the period from June 1992 to May 1993, and examined for macroparasites while fresh.

**Results:** The mean number of parasite species present in each fish was 5.2 for the 'normal' and 1.7 for the 'dwarf' charr. Obligate *Gammarus* transmitted parasites were exclusively found in the 'normal' charr. All copepod transmitted species also had a higher occurrence in this morph, except *Proteocephalus* sp. which was frequently found in the 'dwarf' charr (see Fig. 1).

**Conclusions:** The broader niche of the 'normal' charr results in a higher risk of acquiring several different parasites at a higher rate than the 'dwarf' charr. Thus, the parasite community was clearly more diverse in the 'normal' charr. The 'dwarf' charr seem to feed exclusively upon profundal prey throughout life, and the high occurrence of *Proteocephalus* sp. in these fish suggests that a benthic copepod (probably *Acanthocyclops gigas*) serves as intermediate host.

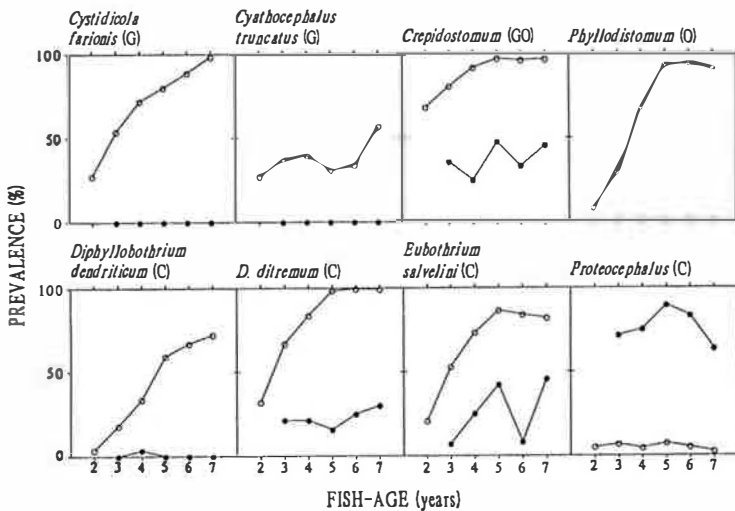


FIGURE 1: Age-specific prevalence of macroparasites in 'normal' charr (o) and 'dwarf' charr (●) from Lake Fjellfrøsvatn. (G) = transmitted by *Gammarus lacustris*, (O) = transmitted by other benthic invertebrates, (C) = transmitted by copepods. (n=1460)

COCCIDIA IN PEAK DENSITY *MICROTUS AGRISTIS*  
POPULATIONS IN FINLAND

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### Objective

Coccidia are known to be important cause of death of young animals of many mammalian species (e.g. hares). Our goal was to investigate the possible role of eimerians in the cyclic population crashes of voles by monitoring the seasonal changes in the occurrence of coccidia (*Eimeria* spp.) in peak density and declining field vole, *Microtus agrestis*, populations.

### Methods

Voies were live-trapped at Luhanka (n=185), central Finland, from September to March in 1991-1992, and at Evo (n=149), southern Finland, from September to January in 1992-1993. Faecal samples originating from the rectums of the voles were stored in 2,5% aqueous potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>). Oocysts were allowed to sporulate at room temperature, after which 1 ml of the suspension was centrifuged. The pellet was resuspended in saturated magnesium-sulphate (MgSO<sub>4</sub>) solution and the oocyst number was counted with McMaster counting chamber.

### Results and conclusions

In both areas the prevalence and the number of oocysts per gram of rectal content (OPG) were highest in September (sexes pooled). The prevalence of oocysts was higher (but not significantly) in subadult (57%) voles than in adult (50%) ones both at Luhanka and at Evo (46% and 36%, respectively) in September. The mean number of oocysts was much higher in subadult voles than in adult voles both at Luhanka (subadults OPG 107 989; adults OPG 17 159), and at Evo (OPG 44 846 and OPG 3620, respectively) in September. The mean weight of the infected subadult voles (22.8g) did not differ significantly from that of the non-infected ones (23.7g). Since the prevalence and the intensity of eimerian infections decreased before the densities of the host reached the peak levels, it seems that eimerians do not have any significant effect to the decline of the field vole populations. The reduction in the parasite burden is probably consequence of the decreasing number of young, susceptible voles in the population after September.

ECOLOGICAL STUDIES ON *DIPOSTOMUM SPATHACEUM*  
IN A DANISH FRESHWATER TROUT FARM

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Eye fluke infection in rainbow trout in a Danish trout farm was monitored during a one year cycle. Intermediate host snails (*Lymnaea stagnalis* and *L.pereger*) were collected in the farm ponds and infected snails were used in laboratory studies. In addition to few specimens of *Tyloodelphus clavata* in the vitreous humor the eyeflukes recorded were located in the lens exclusively and identified as *Diplostomum spathaceum*.

The prevalence of infection was 100 % during most of the year. In the cold season a slightly lower prevalence and intensity of infection was found. Prevalence of infected snails (snails shedding *D.spathaceum* cercariae ) was 3.3 %. Cercarial shedding from host snails was temperature dependent and the number of cercariae increased significantly when temperature was raised from 10 °C (100-1.000 cercariae/day) to 20 °C (5.000-60.000 cercariae/day). Shedding of cercariae was light independent and the cercariae showed positive phototaxis. Experimental infection of rainbow trout at 7 °C with cercariae shedded at both low and high temperatures was performed. However, migration of the diplostomulum larvae inside the trout was partly inhibited at 7 °C.

INFESTATIONS WITH THE SKIN PARASITE *TRICHODINA JADRANICA* RAABE, 1958 (CILIOPHORA: TRICHODINIDAE) IN DANISH EEL FARMS.

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The Danish annual production of European eels (*Anguilla anguilla*) in eel farms using heated recirculated water amounts app. 1000 metric tonnes. The number of eel farms has decreased markedly during the latest 10 years but in contrast the production per eel farm has increased significantly. The production suffered previously from infections with gill monogeneans. However, anthelmintic treatments (with mebendazole) control this helminthosis satisfactorily. Unfortunately, during the latest few years infestations with the skin trichodinid *Trichodina jadratica* have caused problems in several eel farms. In order to evaluate different control methods eels were collected from an eel farm (24 °C, pH 7, 6 ppt NaCl) and exposed to various treatments in controlled laboratory experiments.

Bath treatments using formaldehyde (50 ppm), formic acid (pH 5 and pH 6), freshwater and different salt concentrations were tested. The best depression of ciliate population increase was achieved with formaldehyde exposure but also freshwater depressed the population increase markedly. In formic acid and 15 ppt NaCl population increase was inhibited compared to the rapid multiplication of trichodinids observed in 3 ppt NaCl.



## DIGITAL PHOTOGRAPHY FOR SEM AND IMAGE ANALYSIS

G. Malmberg, Department of Zoology, Stockholm University, Stockholm, Sweden

The poster presents results from experiments with "digital photography" for the SEM and the image analysis equipments at the Department of Zoology, Stockholm University. The experiments started in August, 1994.

For the scanning electron microscope (Novascan 30), an "ImageSlave" system is used, transferring the SEM images to a PC computer. The image analysis system (Q500MC, Leica) receives its images via black-white and color video cameras and transfer them to another PC computer. The SEM and the Q500 have each a laser writer (LaserJet 4) with a printer controller ("LazarPrint") for grey scale images. Within **about 10 seconds**, the "LazarPrint" produces an image with a resolution of **3.400 or 4.800 dpi**, printed out on A4 size paper (see the poster images).

For storing images, each system (SEM and Q500) also has a "Bernoulli Transportable Drive" for "IOMEGA SCI" disks, **230 mb**. Thus disk stored SEM images can easily be brought from the SEM to the image analysis room for measurements and other kinds of analyses.

The SEM is calibrated for giving the highest resolution at a working distance (W) of 5 mm. For the record mode of the SEM, contrast (C) 9 and brightnes (B) 5 is used. Brightness and contrast of stored images on a desk in the Bernoulli driver, in turn, can be influenced by changing a curve in the computer menue.

The image analysis system is equipped with a stereomicroscope (Wild M3Z), a mikroskope (Leitz DMRBE) for brightfield, phase contrast, interference contrast and polarized light, a video cassette recorder (Panasonic AG 73330) and an illuminated desk.

## AGE- AND SEX-SPECIFICITY, SEASONALITY OF PARASITE SIZE AND LONG-TERM CHANGES IN PREVALENCE OF *PHILONEMA SIBIRICA* IN A VENDACE POPULATION

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Members of the genus *Philonema* (Nematoda) are parasites in the body cavity of salmonid fishes in the northern part of North America and Russia. Their intermediate host is a copepod crustacean. *Philonema sibirica* occurs in the vendace (*Coregonus albula* (L.)) populations of Eastern Karelian lakes (Russia) close to Finland but has rarely been reported from Finnish lakes. However, we found a population of *P. sibirica* parasitising the vendace of Lake Puulavesi, central Finland. The objectives of this study were to describe: 1) the occurrence of *P. sibirica* in relation to the characteristics of its host fish 2) the distribution of *P. sibirica* in the lake and 3) the seasonality of the life cycle and 4) annual differences in prevalence of the parasite.

Prevalence of *P. sibirica* was estimated from random seine catch samples of vendace during 1985-1994. The samples were frozen and examined in a laboratory. The length and weight of the fishes were measured and age determined from the scales. Occurrence of the worms was checked by visual examination of the coelom. During 1985 and 1986 prevalence was estimated in summer, autumn and winter. The spatial distribution of *P. sibirica* was studied from the autumn samples of 1985 and 1986. During 1988-1991 and 1993-1994 prevalence was estimated mostly from the winter samples. In 1986 the number of worms per fish was counted and the length of the worms was measured.

*P. sibirica* was never found in the fishes less than 1 year old. The prevalence in the age group 1+ was lower than that of older fish ( $p < 0.001$ ) being 2 % in 1+ and 12 % in older fishes. Prevalence was also sex-dependent ( $p < 0.001$ ) being 9.5 % in females but only in 1.5 % in males over 1-year-old. Neither size nor fecundity of the parasitised fishes differed statistically significantly from the unparasitised fishes. The worms occurred in the whole study area, the prevalence varying from 1 to 15 % between the sites in over 1-year-old fishes. No spatial gradient in the prevalence was observed. The worms performed an one year life cycle. The average length ( $\pm$ s.e.) of the worms was 17 ( $\pm 0.5$ ) mm in June-August, 30 ( $\pm 3.0$ ) mm in October and 51 ( $\pm 5.1$ ) mm in March-April. The adult worms left their host approximately at the time of ice break around early May. The prevalence of the worms seemed to fluctuate from year to year and had a declining trend towards the end of the study period.

***Trichinella spiralis* IN THE RACCOON DOG (*Nyctereutes procyonoides*) IN FINLAND**

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The raccoon dog (*Nyctereutes procyonoides*) is a medium-size carnivore originating from eastern Asia. The raccoon dog colonised Finland rapidly from western Russia. Since the raccoon dog was the main reservoir of rabies virus during the recent outbreak in Finland, we started to research the role of the raccoon dog as a carrier of another significant zoonose, the *Trichinella spiralis*. We studied the occurrence of the *Trichinella* with respect to the sex, age and condition of the host animal. Diaphragm muscle samples (n=134) were analysed by Stomacher digestion method. The *Trichinella* was found to be a common parasite of the raccoon dogs in southern Finland (50% of the raccoon dogs were infected). The number of larvae per gram in the infected hosts (n=66) varied between 0.2-766.8 (mean 95.5). The occurrence of the *Trichinella* was significantly related to the age and sex of the host animal, adult males (72%) showing higher prevalence than adult females (53%) and young males (43%). The physical condition of the animals (fat index) did not differ between infected and non-infected females.

## **RHIPIDOCOTYLE CAMPANULA IN FISHES FROM LAKE KUIVASJÄRVI IN NORTHERN FINLAND**

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Fins and gills of roach and intestines of perch and pike were examined monthly from April to October in 1989 for the occurrence of *Rhipidocotyle campanula* in Lake Kuivasjärvi in northern Finland. The prevalence in the region of the gill-arches was close to 100% throughout the study period, but there was a statistically significant difference in abundance between the period of April-June (before the cercarial emergence) and the period of July-October (after the beginning of the cercarial emergence). In the fins the prevalence was low from April to June (2.2%) and high (74.0%) from July onwards. The abundance in the fins was very low (1.5) compared with the gill-arches (92.2). The most frequently infected fins were the pectoral fins, tail and the abdominal fins. Only in October metacercariae were harbouring in all fins. The abundance in the fins was very low (1.5) compared to the gill-arches (92.2). The overall prevalence of infection in perch intestine was 20.9% and the abundance 6.0. Both the prevalence and the abundance were high in spring in perch and decreased until August when no worms were found. Gravid specimens were encountered in September and October when the prevalence was 10%. Mature worms were obtained in May and June so it seems obvious that *R. campanula* produce miracidia twice a year: in midsummer and in autumn and their origin are of different year infected roach. Of the pike 13% were infected with *R. campanula* and no mature adults were found.

## **HUMORAL IMMUNE RESPONSE OF THE EUROPEAN EEL (*ANGUILLA ANGUILLA*) AGAINST ANTIGENS FROM DIFFERENT ORGANS AND SECRETIONS OF *ANGUILLICOLA CRASSUS* (NEMATODA)**

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Different organs and secretions/excretions of the swimbladder parasite, *Anguillicola crassus* (Nematoda), were tested for the presence of antigens to the humoral immune response previously seen in European eel, *Anguilla anguilla*. The swimbladder nematodes were collected from naturally infected eels and kept in a nematode ringer for 48 h before dissection into cuticula, gonads, gut and content of gut. Furthermore, nematode protein in the ringer was precipitated with acetonitrile. Proteins from the different fractions of *Anguillicola crassus* were separated using SDS-PAGE (sodium-dodecyl-sulphate polyacrylamide-gel electrophoresis) under reducing conditions and electroblotted onto nitrocellulose membranes. The nitrocellulose membranes were incubated in diluted serum from either infected or non-infected eels. Secondary and tertiary antibodies were rabbit anti-eel Ig serum and phosphatase-conjugated swine anti-rabbit Ig antibodies, respectively. Infected eels showed a specific antibody response to a 43 KDa antigen in the cuticula. Furthermore, two antigens around 28 KDa antigen was found, both in the protein precipitated from the ringer and in the cuticula. There were weak antibody responses to a 43 Kda and a 34 Kda antigen in the gonads and no response to the intestinal wall.

THE INTESTINAL HELMINTH COMMUNITIES OF SHREWS  
(INSECTIVORA: SORICIDAE) IN CENTRAL SIBERIA

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Seven *Sorex* species and *Neomys fodiens* were studied for helminths in Central Siberia (the total number of examined shrews was 350). Recorded 14 Cestode, 4 Trematode and 8 Nematode species. Being the most generalists in the given shrew community, *S. araneus* and *S. caecutiens* had the heaviest parasite burden both in prevalence and number of helminth species. Cestodes *Urocystis prolifer*, *Neoskrjabinolepis singularis*, *Ditestolipis diaphana* and *Molluscotaenia crassiscolex* were the dominant parasite species. All of the helminths tended to occupy a specific site in a host gastrointestinal tract. There was no a significant difference of parasite infection levels in shrews between the three years of study despite a significant change in the 4-year cycle abundance dynamic of shrews community in the studied area.

*PLEISTOPHORA MIRANDELLAE* (PROTOZOA: MICROSPORA)  
 FOUND IN ROACH (*RUTILUS RUTILUS*) FROM FINNISH COASTAL  
 WATERS

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**OBJECTIVES.** To confirm the identity and assess the prevalence of a parasite which caused oocytes in the ovary of the roach, *Rutilus rutilus* (L.), to turn whitish (first recorded in 1988 in fish from the Åland Archipelago) the parasites were studied among roaches in the Gulf of Finland off Helsinki in 1994 (Pekkarinen 1995).

**METHODS.** Ovaries and an ovotestis were examined under a binocular microscope, and the parasites found were examined under a normal light microscope as stained smears and paraffin sections. For measurements, fresh spores were photographed in 0.9% saline. TEM and SEM were also used. The morphological characteristics of the parasite found were compared with descriptions of *P. mirandellae* Vaney & Conte, 1901 in the literature (described under several different names).

**RESULTS.** About 50% of roaches examined had spores of *P. mirandellae* in their larger oocytes. Oocytes occupied by spores were not numerous but merogonial stages of the parasite occurred frequently in smaller, basophilic oocytes. The merogonial stages, with total diameters of 10--40  $\mu\text{m}$ , had thick capsules (3--9  $\mu\text{m}$ ). The merogonial vesicles contained up to 20 or more meronts or merozoites. Young sporophorous vesicles (with plasmodia or sporoblasts) had walls composed of a membranous labyrinth (about 0.4  $\mu\text{m}$  high). In older sporophorous vesicles, the wall became thinner and acquired some secreted matter to the inside. The numbers of spores in sporophorous vesicles varied greatly. The size of normal spores was also very variable: length from 7.1 to 14.1  $\mu\text{m}$  and breadth from 3.5 to 7.2  $\mu\text{m}$ . Polar tubes, with lengths of 462 to 693  $\mu\text{m}$ , made 28--77 turns within the spores. Sporophorous vesicles with vicarious microspores (with spore sizes 3.4--4.3  $\mu\text{m}$  by 1.9--2.2  $\mu\text{m}$ ) were very rare. The "microspores", with a poorly defined exospore, occurred in common exosporous matrix and had no more than about ten turns of polar tube.

**CONCLUSIONS.** *P. mirandellae*, which elsewhere has been reported to occur in fish living in freshwater, is now described in roach in brackish water. The labyrinth-like wall of the sporophorous vesicle becomes thinner with age. Distinct macrospores were not found.

**REFERENCE.** Pekkarinen, M. 1995: *Pleistophora mirandellae* Vaney & Conte, 1901 (Protozoa: Microspora) infection in the ovary of the roach, *Rutilus rutilus* (L.), from Finnish coastal waters. - Memoranda Soc. Fauna Flora Fennica, in press.

THE DISTRIBUTION OF *TRIAENOPHORUS CRASSUS* CYSTS IN THE MUSCLES OF WHITEFISH (*COREGONUS LAVARETUS* S.L.) IN LAKE SAIMAA

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1067 whitefish were examined from three sites in Lake Saimaa, SW Finland, during 1991-1993 for *Triaenophorus crassus* infections. Each fish was divided in four parts along the median and lateral lines. The location of each individual parasite in these parts was recorded. The establishment of parasite cysts in these parts was described by means of a linear regression. Judging from the works of Newton (1932) and Petersson (1971), cysts were expected to be concentrated in the dorsal muscles and on the right side of the fish.

When the total number of cysts in each fish increased, cysts were situated more often in the dorsal parts of the fish than ventrally. The parasites were also situated in the dorsal region when previously uninfected fish first acquired the parasites. There were slightly more cysts in the dorsal region on the right side than on the left. The right and left sides of the ventral region didn't differ from each other. These results are in accordance with the results of Newton (1932) and Petersson (1971). They also show that the dorsal region of the fish harbours more parasite cysts not only because of the greater amount of muscle but also because the parasites appear to prefer the dorsal muscles when first entering the host.

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## TREMATODE PARASITISM OF PISIDIUM AMNICUM MÜLL. IN SIILAISENPURO, EASTERN FINLAND

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Digenean parasitism of P. amnicum Müll. was studied during 1992 - 1993 in Siilaisenvuono River in eastern Finland. 790 clams were measured by length, weighed and dissected. Size, parasitic situation and gravidity of the clam were then checked. Seasonal variation of different trematode species was also studied. Parasitism in relation to host size was studied by comparing the prevalences in different host size classes. The dominant species was Bunodera luciopercae, which seemed to not have any regular seasonal fluctuation. Two other species, Palaeorchis sp. and Phyllodistomum elongatum, disappeared almost completely in winter. Palaeorchis sp. had its infection peak from May to June and P. elongatum had its highest prevalence in August. Prevalences of B. luciopercae were higher in big clams, while the other species preferred middle-sized clams. Joint infections were significantly lower than random. Only 1.2 % of the clams had joint infections. All parasites castrated their hosts. Any clam containing both parasites and embryos was not found.

**TIMING OF EXISTENCE OF TWO BLOODPARASITE SPECIES IN  
MIGRATING WILLOW WARBLER (PHYLLOSCOPUS  
TROCHILUS)**

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We studied bloodparasites during autumn migration in common migrating passerine species, willow warbler, in Tauvo birdstation on the Gulf of Bothnia, Baltic Sea, Finland. Parasite studies made in birds have so far concentrated mainly on how they effect on sexual selection process. In our data two parasite species (*Haemoproteus beloposkyi* and *Leucosytozoonia phylloscopus*) were most common (85 % of all infections). *H. beloposkyi* was most common of these two species (70 %). Total of 12 % of willow warblers were parasited by some parasite species. Timing of existence in these species differs remarkably: *H. beloposkyi* was common at early migrating season while *L. phylloscopus* was later in autumn. This difference was not affected for example by age or sex, because almost all samples were taken from juvenile males. We assumed that this difference in existence between parasite species is most convincingly explained by vectors. In early autumn, Ceratopogonidae-insects are those that transmit *H. beloposkyi*. Similarly, later in autumn Simulidae-insects transmit *L. phylloscopus*.

## ARE COSTIASIS AND WHITE SPOT DISEASE ALWAYS PRESENT WHEN NATURAL RIVER WATER IS USED AT A FISH FARM

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**Objectives.** The occurrence of protozoan parasites was examined at Montta fish farm over the ten-year period 1984-1993, and the factors influencing infection with *Ichthyobodo necator* (costiasis) and *Ichthyophthirius multifiliis* (white spot disease) were traced.

**Methods.** A total of 10 726 salmon (*Salmo salar*), sea trout (*S. trutta* m. *trutta*) and brown trout (*S. trutta* m. *lacustris*) were studied at the Montta farm, which produces two-year-old smolt for stocking. Random samples of freshly killed fish of two age groups were examined, and also fish with disease symptoms.

**Results.** Both parasites were found to occur on salmon and the two trout forms in each year studied. Costiasis occurred mainly on fingerlings, especially on the sea trout, the prevalence of its infection in the pooled material being 27 %. Only 6 % of the sea trout yearlings had the disease over the ten years. A total of 46 % of the salmon yearlings were infected with *I. multifiliis* in pooled material from earth ponds in June-September in the ten years, but only 26 % of each of the trout forms. Higher numbers were counted on the skin, gill and fin samples from the infected salmon than on the two trout forms (mean intensity 16 in salmon versus 6 in trout).

**Conclusions.** Costiasis is the problem of first feeding fish and white spot disease of yearlings reared in earth ponds in summertime. Mortality has been kept at a low level in most cases at the Montta farm by means of formalin and malachite green-formalin baths, respectively.

*SCHISTOCEPHALUS* LARVAE IN SCULPIN (*COTTUS GOBIO* )- A NEW HOST- PARASITE- RELATIONSHIP FOUND IN AN ARCTIC RIVER IN NORTHERN FINLAND

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**Introduction:** Cestodes of the family *Schistocephalus* are known to be highly host-specific at the plerocercoid stage. *S. solidus* lives in the body cavity of *Gasterosteus aculeatus* and *S. pungitii* in *Pungitius pungitius*. Plerocercoids can be identified on the basis of the number of proglottids. No ecological research on *Schistocephalus* living in sculpin (*Cottus gobio*) has been done.

The aims of this work are to study the occurrence of *Schistocephalus* plerocercoids in six populations in a river flowing into the Arctic Ocean and to compare their proglottid numbers in sculpin and *G. aculeatus* and to study the possible origin of infection in sculpin.

**Methods:** Sculpins were electrofished in 1991 and *G. aculeatus* in 1994 from the river Utsjoki. The first sampling site was situated 14 km from the river mouth and the last about 15 km upstream. When counting proglottid numbers only worms from fish infected with a single plerocercoid were used.

**Results:** In most cases plerocercoids in sculpin had 130–160 segments (mean = 146, n = 57) and in *G. aculeatus* 80–95 segments (mean = 85, n =9) (Mann-Whitney- U=570,0 p<0,001).

The plerocercoids in sculpin were also significantly longer than in *G. aculeatus* (51,2 mm and 37,7 mm , T=3,90, p<0,001, df=65).

Prevalence of *Schistocephalus* infection in sculpin was 23 % and it varied between 18 – 43 % among the sampling sites ( $X^2= 27,25$ , p< 0,001, df=5).

Prevalence was highest in medium sized sculpins although worms accumulated in larger fish.

**Conclusions:** Although only a few infected *G. aculeatus* were studied from the river Utsjoki, the drastical difference in proglottid numbers of *Schistocephalus* in sculpin and *G. aculeatus* may indicate that the origin of infection in sculpin is not from *G. aculeatus*, but it may involve a different *Schistocephalus* species.

However, well established *Schistocephalus* populations in all six sites studied indicates that sculpin is not a new introduction to the area as suggested.

Further studies are needed to confirm the status of sculpin in this Arctic river.

## IMMUNOLOGICAL DIVERGENCE OF THE *DIPHYLLOBOTHRIMUM* TAPEWORMS IN KARELIA

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The possibilities of the quantitative immunological analysis for the resolving of the taxonomical, ecological and evolutionary problems were evaluated during the researches in Karelia on the cestodes belonging to the genus *Diphyllobothrium* Cobbold, 1858.

The immunological distance index (I.d. as in Mainardi, 1957) was used to differentiate between the species, including closely related ones, which were hardly differentiated by conventional taxonomical characters.

Through the use of I.d., the quantitative level of specific differences was determined. The reconstruction of evolutionary relationships as also some factors of speciations inside the group were performed on the base of immunological analysis.

The prominent correlation was demonstrated between the immunological divergence between the species studied and such their ecological features as intermediate and definitive hosts.

## NEUROTRANSMITTERS (BIOGENIC AMINES) IN ADULTS AND LARVAE OF *LIGULA INTESTINALIS* (CESTODA)

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Neurotransmitter serotonin (5-Hydroxytryptamine, 5-HT) has been identified in adults and plerocercoides of *Ligula intestinalis* by spectrofluorimetrically. The concentration of compound was lower in larvae from body cavity of fishes (*Abramis brama*, *Rutilus rutilus*) than those in adults from intestine of gulls (or experimental infected chickens).

It has been demonstrated that 5-HT level in intestine of gulls (and chickens) is fairly high. Probably, adults of *L.intestinalis* are capable take up 5-HT from intestine of hosts.

Preliminary results showed the presence of the key enzyme of 5-HT biosynthesis - tryptophan hydroxylase - in plerocercoids of *L.intestinalis*. The activity of enzyme was relatively low as compared with those in some trematodes.

The investigation of 5-HT inactivation indicated on the low rate of 5-HT catabolism in worms.

Dopamine has been detected in adults of *L.intestinalis*. Probably this compound has nontransmitter function in this parasite.

## HELMINTHES OF LEPORIDAE FAMILY IN BURJATIA

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Rodents of Leporidae-family belonging to two species are explored: Lepus tolai Pallas - 3 specimen, Lepus timidus L - 22 specimen.

High invasion extensity is registrated /92,0%/, intensity from 1 to 276 specimen of helminthes. 8 species of helminthes discovered; Trematodes - 1, Cestodes - 2, Nematodes - 5: Dicrocoelium orientalis Sudarikov et Ryiikov, 1951, Mosgovaja pectinata/Goeze, Taenia pisiformis, Protostrongylus kamenskyi Schulz, 1930, P. terminalis/Passerini, 1884, Nematodirus aspinosus Schulz, 1931, Hepaticola hepatica/Bancroft, 1893, Trichocephalus leporis Froelich, 1789.

Trematodes D.orientalis and nematodes P.terminalis first registrated for hare of Saxt Siberia. Mostly spread in hare of Buriatya is lung nematodes - P.kamenskyi. Intestines helminthes N.aspinosus and M.pectinata have the second place as soon as frequency is concerned. For hare of Jakutya these parasites are also most popular, which is connected with similar places of occupation and forage circumstances. Invasion of hare by helminthes differs depending on the place of occupation. Maximal invasion is discovered in forest - steppe zones and sea-buckthorn bushes areas. These places have best forage circumstances and characterized by high density of hare.

Hare of Buriatya territory have no trematodes typical for other regions of UIS / foreexample, D.lanceatum /. It applies equally to helminthofauna of hare of Jakutia.

## HELMINTHES OF DOMESTIC DEER IN BURYATIA

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Domestic deer are spread and play a significant economic role in two regions of republic: North-Baical and Bauntovsky. Examination of helminthofauna of domestic deer has been undertaken in North-Baical region at a deer - farm of sovhoz "Severny", situated at Okun mountain foot. Necessary to emphasize close contact to domestic and wild deer. Invasion exchanges between them are possible due to this fact. 7 species of helminthes are registrated for domestic and wild deer in South Zabaikalie, belonging to 7 genus and 7 families: Trematodes - 2, Cestodes - 2, Nematodes - 3: Fischoederius elongatus, Liorchis scotiae, Moniezia benedeni, Avitellina arctica, Nematodirella longissimespiculata, Setaria tundra, Skrijabinema tarandi. L. scotiae is found in 34,6% of domestic deer with invasion intensity up to 2133 specimen. This species is registrated for both domestic and wild deer. Intermediate hosts of these helminthes are mollusc Anisus/Gyraulus/stroemi. These parasites larva are discovered in it in the first half of August.

F. elongatus/Gastrothilactidae family/ is registrated in UES for the first time.



## PARASITES OF FERAL MINKS (*MUSTELA VISON*) IN ICELAND

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In Iceland farmed minks escaped and a feral population gradually colonized all suitable habitats from the early thirties until the seventies.

Various studies have been carried out on the parasites of native North-American mink populations as well as on farmed and feral minks on other continents. A provisional review of the literature on mink parasites revealed not less than 70 species; 7 protozoans, 20 trematodes, 4 cestodes, 22 nematodes, 6 acanthocephalans and 11 various ectoparasites.

### Objective

The objective of this study was to search for and identify for the first time parasites of feral minks in Iceland. Previously, however, antibodies of the protozoan *Encephalitozoon cuniculi* had been detected in the feral population.

### Methods

Carcasses of 26 adult minks (12 females, 14 males), killed by local hunters in 1991 and 1992 (6 animals from coastal areas, 20 from inland habitats) were examined for ecto- and endoparasites. Likely sites of parasites (skin, fur and ears, nasal sinuses, respiratory and digestive tract, gallbladder, kidneys and diaphragm) were systematically investigated and faecal samples were examined for protozoan cysts and helminth eggs by using the formalin-ethylacetate concentration method. In addition the digestive tracts of 47 adult animals killed in 1986 and 1987 (25 females, 22 males; 38 from coastal areas, 9 from inland habitats) were examined for intestinal parasites.

### Results and conclusions

No parasites were found in minks from inland habitats but four acanthocephalans, *Corynosoma strumosum*, *C. semerme*, *C. magdaleni* and *C. wegneri* (syn. *hadweni*) were found in 54,5% of the coastal minks.

The *Corynosoma* species are probably all endemic in the Icelandic environment and normally they are found in seals. *C. strumosum* and *C. semerme* have previously been recorded from Icelandic seals but *C. magdaleni*, not previously recorded from Iceland, was originally described from North-American seal species but has not been found previously in mink. *C. wegneri* (syn. *hadweni*) has previously been found in Icelandic arctic foxes *Alopex lagopus*.

Icelandic coastal minks probably acquired the infections by eating infected fish.

Interestingly Icelandic feral mink does neither seem to share any parasites with the native North-American mink population nor the farmed Icelandic population which recently was found to harbour three coccidians and the flea *Monopsyllus sciurorum*.

## INFLUENCE OF TREMATODE INFECTION ON POPULATION STRUCTURE OF INTERMEDIATE SNAIL HOSTS *LITTORINA SAXATILIS* AND *L. OBTUSATA* IN THE WHITE SEA

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Trematode infection is recognized as an important factor influencing host populations. We have performed a complex long-term study of populations of the intertidal snails *Littorina saxatilis* and *L. obtusata* with heavy trematode infection in the White Sea. The objectives of this study was to analyze distribution and temporal changes of infection within snails populations over long periods, and to reveal influence of trematodes on host population structure.

Since 1982 annually we have surveyed coexisting populations of *L. saxatilis* and *L. obtusata* from the West Spit of Ryazhkov Island (Kandalaksha Bay of the White Sea). Snails were quantitatively sampled from different intertidal horizons and various substrates.

List of trematodes comprised of 10 species and was identical in *L. saxatilis* and *L. obtusata*. Because of irreversible parasitic castration of the winkles, trematode infection significantly effected host reproduction and demography. Infection prevalence by most trematodes was low (5 to 7%) and rather constant over 13 years period. In contrast, infection by *Microphallus piriformes* was high and showed great year-to-year variability (20 to more than 50%). Significant age-related, sex- and genotype-dependent differences in infection prevalence by *M. piriformes* were found. Spatial heterogeneity in prevalence of *M. piriformes* between intertidal horizons and substrates was observed. Experiments suggest the parasite-induced modifications of host behaviour as a probable reason for this heterogeneity. Some patterns of trematode distribution in snails populations were highly repeatable in different years, whereas others varied depending on the overall infection prevalence.

Despite strong impact of trematodes on host populations, our study showed that infected populations can persist for a long period (at least for decades), that suggests effective compensatory mechanisms supporting recruitment of host populations and stability of host-parasite interactions.

THE GAMASID MITES OF BATS FROM RUSSIA  
AND ITS ADJACENT TERRITORIES  
(GAMASINA: SPINTURNICIDAE, CRONYSSIDAE)

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The important and interesting group of gamasid mites from bats was totally neglected in the former USSR for decades. The results of processing our own material and also the collection material on the gamasids of Russia and adjacent countries, as well as the analysis of literature data, have led us to conclusion that the fauna of 34 species of bats of the investigated region includes 41 species of 7 genera from two gamasid families. Were added 5-100% of the new species of gamasid mites of bats from any ex-Soviet republics to the species that have been formerly known (for example, 26 species of gamasids became new for Russia). Also, 2 entirely new species have been described, while some unknown stages (males and protonymphs) for another 3 species have been described. For the very first time one species have been found in the Palaearctic, while five species have registered in the embraced territory. The gamasid mites of bats have been divided into two groups by their parasitic links with their hosts, and into three groups (monoxenic, oligoxenic, polyxenic) according to their specificity.

## THE DYNAMICS IN THE PARASITE FAUNA OF FISHES IN THE COASTAL WATERS OF WESTERN ESTONIA

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A parasitofaunistic study was maintained in 1978-94 in the area between western Estonian Archipelago and the mainland on 34 fish species. In toto 173 species of parasites were determined, of these 103 helminthes mainly Trematoda (27) and Monogenea (29), and 51 protozoans, mainly Myxosporidia (32). For oecological monitoring the parasite faunas of herring, perch, roach, ide, dace, breamflat, bleak, eelpout and flounder were chosen and analysed by standard methods of total autopsy and cameral treatment for 6-10 times per annum including mass-analyses from spawning migrant schools. Certain trends of shifting in the seasonal development of infestations as well as in the dominance of species in parasite faunas were observed. Some fluctuations in the multiparasitical associations in connection with the rate of hostality of parasites and the oecology of hosts are discussed. The eliminative and restructive influences of predation, fishery and eutrophication on the parasite fauna are pointed out.

The associations of parasites of fishes in Western Estonian coastal waters are more multifarious and stabile than in the coastal waters of the Southern Gulf of Finland.

## **CONGENITAL HYDROCEPHALUS CAUSED BY TOXOPLASMA GONDII IN A LITTER OF NEWFOUNDLAND PUPPIES**

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Hydrocephalus is a common canine congenital anomaly but often a riddle by etiology.

A hydrocephalus was diagnosed with magnetic resonance imaging (MRI) in a litter of Newfoundland puppies showing severe neurological signs.

Diagnosis was confirmed by postmortem examination, and histopathology revealed periventricular calcifications, which are considered suggestive of prenatal toxoplasmosis in children (Best & Fintayson). However, no signs of *Toxoplasma* organisms could be detected by histological methods.

By allowing the detection of very low amounts of DNA the polymerase chain reaction (PCR) has revolutionized diagnostics of toxoplasmosis. Also latent infections, as in our case, can be found with PCR. We targeted the B1 gene of *T. gondii*, which is shown to be highly specific and well conserved among nearly 20 strains already tested (Burg et al.).

We studied nine samples from two puppies, four of them being positive. We conclude that even though patho-anatomical findings led our suspicion to toxoplasmosis, the specific etiology could not have been verified without PCR. Thus we think that congenital toxoplasmosis may be an underdiagnosed disorder in veterinary medicine.

### References:

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## EFFICACY OF SOME ACARICIDES PRODUCED IN ESTONIA

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Objectives. Sarcoptic mange in breeding swine is recognised as a major contributor to losses in swine productivity. To control swine sarcoptosis, experimental work with different compounds prepared at the Estonian Institute of Chemistry of the Academy of science was carried out.

Methods. 12 preparations which have reported to have antiparasitic effects in some insect species were applied against Sarcoptes suis. Our investigations were made to measure the effect of 5 juvenoids (efoksen, efotrin, efoksen P, 3434 E and T-634) and 7 chemotherapeutic preparations (bensilate, bensoksen, sulfoksen, saroksen, saroksen-0, S-10, saroksen-B and saroksen-E). About 250 animals showing clinical signs of mange were used in our trials. The basic dosage used contained 1-1,5g of active product per 100g water. Clinical evaluations were done up to 45 days after treatment.

Results. Our results indicate the suitability of several home-produced preparations in animal treatments. No adverse toxicological effects were found with any of the preparations in swine. The most active compounds were sulfoksen and saroksen-E due to synergistic effects of their components efoksen, sulfur and benzyl benzoate. Our investigation demonstrated that pigs recovered fully and live mites were eliminated completely.

Conclusions. To control mange mites, the most effective preparations were the mixtures containing as well poisonous substances as juvenomimetic compounds. According to our suggestions the components with insecticidal effects may play major role in these mixtures.

## PIG NEMATODE INFECTIONS IN ESTONIA AND THEIR CONTROL BY COMBINED METHODS

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Objectives. Our previous studies on pig experimental infections refer to increased nutritional requirements in the presence of internal parasites. The main purpose of this study was to investigate the distribution dynamics of nematodes on our pig farms. The second purpose was to work out nematode infection control methods in the conditions of the modern housing systems and minimal anthelmintic treatment.

Methods. Observations were carried out in 10 successive years on 5 large-scale pig farms where no-dehelminthized swine groups of different ages were kept in separate sections. Only the pregnant sows were dehelminthized when needed. Faeces samples were taken once a season and 3-10% of pigs have been coprologically investigated every year. Several pig farms with different keeping systems belonged to the comparable investigation.

Results. The nematodes of economically important invasions are as follows: Ascaris suum, Oesophagostomum spp., Strongyloides ransomi, Trichocephalus suis and Metastrongylus spp. Occurrence of Strongyloides ransomi, Trichocephalus suis and Metastrongylus spp. is diminished essentially during the last decade. According to our results, on 5 investigated farms only Ascaris suum and Oesophagostomum spp. invasions were found. If the pregnant sows were not prophylactically dehelminthized in 1-2 years, the extensivity of invasion increased up to 50% in some pig groups. Ascariidosis was observed more often in the first pregnancy than in older sows.

Conclusions. In order to control nematode infections on large-scale pig farms is needed: 1) application of isolated farrow principles, 2) to keep different age groups in separate sections and to complete the sections on the principle "all out and all in", 3) regular investigations of all swine groups (3-10% of pigs), 4) anthelmintic treatment of pregnant sows (10 days before farrowing) only in case of invasion occurrence.

## COCCIDIOSIS OF PIGS IN LATVIA: SPECIES, OCCURRENCE, PROPHYLAXIS

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Coccidiosis of pigs and the occurrence of the species of coccidia have been investigated in the Republic of Latvia. Studies were carried out according to the age and sex of the animal, keeping technology and season, as well as the effectiveness of trihopol and himkokoid-7 was determined. Coprological tests were performed by the standardized Fileborn method. The species of coccidia were determined according to their morphological indices and time of sporulation.

Pigs were affected in all districts of Latvia. In some farms the extensity of infection was within the range of 0.5 to 92 per cent. The highest degree of infection was in fattening pigs at the age of 4-9 months. The infection was lower in piglets aged to 1 month and in boars. In large well equipped farms the extensity of coccidiosis infection of pigs was lower than that in the small ones.

In most cases (61.2%) there were several species of coccidia in the intestines of pigs at the same time, as well as balantidia (*Balantidium coli*) and helminthes (*Oesophagostomum dentatum*, *Ascaris suum*, *Trichocephalus suis* or *Strongyloides ransomi*). There were 8 species of coccidia of pigs determined: *Eimeria deblickei*, *E.scabra*, *E.perminuta*, *E.polita*, *E.spinosa*, *E.scrofae*, *E.suis* and *Isospora suis*.

The most widespread species of coccidiosis in pigs of all ages in Latvia is *E.deblickei*. *E.scrofae* and *I.suis* were found mostly in piglets until the age of 4 months. The richest coccidiofauna was found in veaned piglets in late summer period. Rarer were *E.spinosa* and *E.scrofae*. Treatment with trihopol was rather effective (EE-69.5%, IE-88.2%) in the doses of 15 mg/kg body weight two days in succession and himkokoid-7 (EE-56%, IE-73.0%) 5 mg/kg seven days in succession.



## HELMINTHOSES OF SHEEP (GOATS) IN LATVIA AND INVESTIGATIONS OF THE EFFECTIVENESS OF THEIR TREATMENT

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The epizootic situation of helminthosis in sheep and goats has been analyzed according to the results of helminthocoprological and postmortem examinations. The effectiveness of some antihelminthic drugs has also been studied.

It was found out that sheep and goats were infected mostly with strongylatoses of gastro-intestinal system (*Ostertagia*, *Chabertia*, *Haemonchus*, *Oesophagostomum*, *Nematodirus*, *Trichostrongylus*, *Cooperia*, *Bunostomum*).

Extensity of infection (EI) varied within the range of 27.7-47.5 per cent for some years. Protostrongylidoses (*Protostrongylus* and *Muellerius*) was diagnosed in sheep (EI 1.6-18.3%) and in goats (EI to 46.9%). Besides those, in some farms fasciolosis (*Fasciola hepatica*), paramphistomidosis (*Paramphistomum cervi*), dicrocaeliosis (*Dicrocoelium lanceatum*), monieziosis (*Moniezia expansa*, *M. benedeni*), trichocephalosis (*Trichocephalus ovis*), strongyloidosis (*Strongyloides papillosus*), dictyocaulosis (*Dictyocaulus filaria*) and cysticercosis (*Cysticercus tenuicollis*) were also diagnosed. Cenurosis and echinococcosis were found only in some animals.

Treatment of the associative infection of strongylatoses, muelleriosis and strongyloidosis appeared to be highly effective (91-100%) with 2.4 per cent rinal premix (Febantel) given orally 5 mg/kg of the body weight 7 days in a row, ivomec (Ivermectin)- 1 ml/50 kg subcutaneously, nilverm 50m/kg orally. Panakur (Fenbendazol) appeared to be highly effective for strongylatosis, but less effective (83.4%, 87,8%) for muelleriosis in goats.

## BOVINE SARCOCYSTOSIS IN ESTONIA

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The aim of the present study was to determine the extent and causative species of bovine sarcocystosis spread in Estonia, the localisation of sarcocysts in bovine muscular tissue and their shape and size in different muscles, to establish the best sites for sample taking and thus improving the research methods in common use. Histological examination of the parasite and the tissue reaction of the invaded bovine myocardium was made. The role of ecological conditions in the sarcocystosis spread in different parts of Estonia was studied.

The spread of bovine sarcocystosis in Estonia was studied in 1982 - 1990. The total number of cattle examined is 2,892, among them 1,418 milking cows and 1,474 heifers on 194 large agricultural farms in 13 districts of Estonia.

The muscle samples taken from the line between the right atrium and ventricle and the apex of the heart were collected at the 7 Meat Processing Plants. Muscle samples were studied by the microscopical compression method. The choice was made with the statistical simplex method on computer ЭBMEC-1035 of Russian make. For histological examination the muscle slices were embedded in parafin and stained after van Gieson method and with PAS haematoxylin. To establish the kinds of sarcocysts an experiment was carried out on laboratory animals. Bovine sarcocystosis was diagnosed in the animals of all the 13 districts. Of 2,892 animals examined sarcocysts were found in 1,537 with the invasion extent (IE) of 53.1%. The average invasion intensity (II) was 64.2 sarcocysts per one gram of muscle tissue (1g m.t.). Of 1,418 cows examined 818 were infected with sarcocysts (IE=57.7%), II=66.3 sarcocysts per 1g m.t. Of 1,474 heifers examined 719 were infected with sarcocysts (IE=49%), II=61.7 sarcocysts per 1g m.t. Seasonal changes did not have a marked effect on the IE of sarcocystosis.

Sarcocysts were found in all parts of the muscle, lying along the tissue fibre. The greatest IE of sarcocysts invasion was at the cardiac apex (83.3 %). Sarcocysts of oval, ovoid, sacciform, fusiform, ribbon-like and atypical shape were detected. The shape and size of sarcocysts depends on the sarcocysts localisation in the muscle. Sarcocysts of 0.010-0.500\*0.01-0.100 µm were detected. Pathological changes manifest themselves slower in animals of good nutritional condition.

The apex of the heart and the upper part of the *m. extensor carpi radialis* are the best sites for sample taking to diagnose sarcocystosis by the simplex method. The highest sarcocystosis spread was found to be in East Estonia and in the central and western parts of South Estonia. The muscular tissue of the animals examined were invaded by the cysts of *Sarcocystis cruzi* (*S. bovicanis*).

## TRICHINELLOSIS IN ESTONIA

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Trichinellosis, spread among wild animals is one of the most hazardous anthroozoonosis. It is frequent that trichinellosis spreads from its nidi among wild animals to synanthropic animals and people.

In Estonia trichinellosis among wild animals was first diagnosed in 1966: 1 wolf - *Canis lupus*, 3 raccoon dogs - *Nyctereutes procyonoides* and 3 badgers - *Meles meles* (Peebsen, 1967). Human invasion cases were registered in 1969 and 1985 caused by the victims having consumed wild boar (*Sus scrofa ferus*) meat, which had not undergone any veterinary check. In 1993 there were serious outbreaks of human trichinellosis of unknown origin. Wild animal trichinellosis has been thoroughly studied in Estonia since the 1970'ies. In 1994, the first cases of trichinellosis in domestic pigs and in the animals of farm fur-bearing animal were diagnosed.

All in all 1384 wild animals by 15 species have been examined for trichinellosis. Besides, 51 cats, 12 dogs, 4 domestic pigs, 18 blue foxes (*Alopex lagopus*) and 9 minks (*Mustela lutreola*) have been investigated. Muscle samples from 18 muscles/muscle groups were studied by the artificial digestion method and the microscopical compression method. *Trichinella spp.* were identified by RAPD method in the Trichinella Reference Centre.

Trichinellosis has been diagnosed in 8 species of wild animals (invasion extensity 5.4 - 6.9 % in different years), 2 cats, 4 domestic pigs and 8 blue foxes in 14 rural districts and on the islands of Saaremaa and Hiiumaa in Estonia.

Raccoon dogs, rats (*Rattus norvegicus*) and cats were the species most seriously invaded. Wild boars and raccoon dogs were infected with *Trichinella britovi* and *T. nativa*. One raccoon dog was infected with *T. nativa* and *T. britovi*. A domestic pig was infected with *T. britovi*, a fox (*Vulpes vulpes*) with *T. britovi*, blue foxes and a brown rat with *T. spiralis*.

Trichinellosis is of wide spread among several species of wild animals in Estonia. The most important source of *Trichinella* invasion is raccoon dogs. *T. nativa* and *T. britovi* were identified in wild animals, *T. britovi* and *T. spiralis* in a domestic pig, a blue foxes and a brown rat.

Of interesting findings the first case of double infection in a naturally infected host was detected in 1993 and the first *T. britovi* infection was diagnosed in a domestic pig in 1994.

## A survey of red poultry mite, *Dermanyssus gallinae*, in different types of production systems for egg-layers in Sweden.

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**Objective.** The study was undertaken to investigate the prevalence of poultry associated haematophagous mites in Sweden. A particular aim was to establish the relationship between occurrence of mites and different types of production systems for egg-layers.

**Methods.** A postal questionnaire study was performed in May 1994 and some months later it was followed up by inspections, sampling and interviews on site of poultry farms.

**Results.** The only haematophagous mite species found was the red poultry mite, *Dermanyssus gallinae*. About 4% of the cage systems were affected while 33% of the deep-litter flocks and as much as 65% of the backyard flocks housed mites. Most farmers were combatting the mites by cleaning the empty houses and thereby using chemicals such as organophosphorous compounds, carbamates and synthetic pyrethroids. In several instances the problems were still not solved despite preventive or curative measures.

**Conclusion.** Although *D. gallinae* was found to be present both in cage batteries, deep litter systems and in backyard flocks, it was less prevalent among birds housed in cages. However, it seems as the magnitude of the mite problem is not solely determined by the kind of housing system. A control programme for *D. gallinae* is suggested by regular screening and certification of ectoparasite status of birds before their transfer to production sites.

## **TOXOPLASMA GONDII IN FINNISH CATS: SEROLOGICAL AND COPROLOGICAL STUDY**

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### **Objectives**

The purpose of this study was to get an overview of *Toxoplasma*-infection prevalence in Finnish cats. We also wanted to evaluate the rate of oocyst-spread in these cats.

### **Methods**

Cat sera and feces were collected from two lost-animal houses in Helsinki and a few veterinary clinics in Finland. Total amount of sera and feces were 141 and 46 respectively (some of these coprological samples were "common samples" of 2-10 cats). The sera were analysed with direct agglutination test (Toxo-Screen DA) to screen IgG-antibodies. The titers used were 40 and 4000. Feces were analysed using the sedimentation-flotation method to find *Toxoplasma*-oocysts.

### **Results**

*Toxoplasma*-specific IgG-antibody seroprevalence was 44,7 % in the whole material. Old and male cats were more often positive than young and female cats. The difference between sexes was not statistically significant. One *Toxoplasma*-like oocyst -positive sample was found in the coprological study.

### **Conclusions**

*Toxoplasma*-specific IgG-antibodies were quite common in the cat population of this study, which should be remembered when human health is concerned. Fortunately, the oocyst spreading time is short, which was also recognized in this study: oocysts were found only in one sample.

## Prevalence of strongyles of Swedish horses

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### Objective

A survey was carried out to determine the prevalence of strongyle parasites of Swedish horses and to establish possible regional differences.

### Methods

Eighty horse farms in north Sweden and forty in south Sweden were selected for faecal sampling. The criteria for a farm to be included in the survey was that there had to be at least five horses on the farm and that no treatment with anthelmintics had been undertaken during the last eight weeks before sampling. Faecal samples were collected by the horse owners and sent to laboratory for analysis during January to March 1995. All samples were examined by McMaster technique for strongyle eggs. Larval cultures were performed to differentiate small strongyles (*Cyathostominae*) and large strongyles (*Strongylidae*). The main purpose of larval cultivation was to establish if the farms were infected with *Strongylus vulgaris*. In connection with sampling a questionnaire on anthelmintic usage, frequency of treatments and pasture management was filled in.

### Results

All participating horse farms were infected with strongyle parasites. In the northern region of Sweden 69% of the horses sampled had a faecal output of strongyle eggs and in the southern region corresponding percentage was 90%. The mean output of eggs of horses in north Sweden was 354 per gram faeces and in the south 656. *S. vulgaris* was found at 10% of the northern farms and at 20% of the southern farms.

### Conclusion

This survey has shown that horses in the southern part of Sweden were infected with strongyle parasites to a higher extent than horses in the northern part. Despite frequent usage of anthelmintics, *S. vulgaris* seems to be a parasite that still deserves attention in Sweden.

## ZOONOTIC ECTOPARASITES FOUND FROM DOGS IN FINLAND

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### Objectives

"Of all the animals associated with man, the dog is probably the most important source of parasitic disease" (Beaver 1954). The purpose of this study is to get overview of those canine ectoparasites found in Finland, that are capable to cause clinical signs also in human beings.

### Material and Methods

This report is based on the identification requests sent to the Parasitological Laboratory of the College of the Veterinary Medicine in Helsinki during the years 1980 - 1994. The material was evaluated by routine light microscopy, histology, scanning electron microscopy and when needed also by transmission electron microscopy.

### Results and Conclusions

The following zoonotic ectoparasites seem to be common among the dogs in Finland: *Cheyletiella yasguri*, the fleas (*Arhaeopsylla erinacei*, *Monopsylla sciurorum*, *Ceratophyllus spp*; recently also the cat flea, *Ctenocephalides felis*), *Otodectes cynotis*, *Sarcoptes scabiei* and *Ixodes ricinus*. Brown dog tick, *Rhipicephalus sanguineus* has also become more common especially in the southern part of Finland. *Rhabditis strongyloides* -dermatitis is only occasionally found from dogs in Finland. Recently the sporadic cases of canine leishmaniosis has been also diagnosed in imported dogs.

## THE PROPORTION OF TROPHOZOITES INCREASES DURING RECOVERY FROM *PNEUMOCYSTIS CARINII* PNEUMONIA IN RATS

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### Objectives

*Pneumocystis carinii* is an extracellular opportunistic pathogen of the lung with an uncertain taxonomic status; either protozoal or fungal classification has been suggested. It causes pneumonia (PCP) in immunocompromised hosts and is best known as a pathogen of AIDS patients and immunosuppressed transplant and cancer patients.

*Pneumocystis carinii* has two developmental stages, the trophozoite and the cyst, but no information is available on whether the trophozoite or cyst is more sensitive to the host response and on whether the relative proportions of different developmental stages are constant or changes over time during recovery from PCP

### Methods

Four groups of Wistar rats (seven male in each group) were immunosuppressed with dexamethasone (1 mg/l) in drinking water for 12 weeks. After immunosuppression one group was sacrificed immediately and the other groups one, two and four weeks after discontinuation of immunosuppression. The differential specificity of commercial diagnostic immunohistochemistry kits, SMoAb (cysts only; Detect if *Pneumocystis carinii* kit, Shield Diagnostics Ltd, Dundee, UK) and CMOAbs (cysts and trophozoites; Pneumo-cel i.f. kit, Cellabs PTY Ltd, Brookval, NSW, Australia) was utilized to analyze the numbers of trophozoites and cysts during recovery from PCP. The number of trophozoites was calculated by subtracting the cyst number (SMoAb-reactive) from the total number of organisms (CMoAbs-reactive). Analyses were carried out on lung homogenates. The lung sample was digested in 10 ml of 0.2% collagenase in 0.9% NaCl at +40°C with continuous stirring; a 10 µl sample of each homogenate and dilutions 1:10 and 1:100 were used for the preparation of the cytocentrifuge (Cytospin 3 Shandon, Shandon Scientific Ltd, Cheshire, UK) specimens, which were air dried and fixed in +4°C acetone. Stainings with both kits were carried out in accordance with manufacturers' recommendations.

### Results

In tissue homogenates the cysts decreased from week 0 to week 4 ( $4.10 \pm 1.57 \cdot 10^6/g$  vs  $0.069 \pm 0.021 \cdot 10^6/g$ ,  $p < 0.001$ ), whereas the trophozoites decreased much less ( $99.75 \pm 15.08 \cdot 10^6/g$  vs  $59.57 \pm 19.10 \cdot 10^6/g$ ,  $p > 0.05$ ) leading to a change in trophozoite-to-cyst ratio from week 0 and 1 ( $72 \pm 33$  and  $49 \pm 14$ , respectively) to week 2 and 4 ( $1752 \pm 652$  and  $1163 \pm 482$ ,  $p < 0.001$ ).

### Conclusion

Four weeks recovery time was not sufficient to all *P. carinii* organisms to be cleared out from the lungs. Trophozoites and cysts showed outstanding difference in clearance speed. During the four weeks recovery period the cyst number had diminished to 60-fold, but the trophozoites number had diminished to only about half the number observed in immunosuppressed PCP rats. This either shows different sensitivity to host defense or/and its due that trophozoites continued to multiply asexually, but stop developing to cyst stage. Therefore, a relatively bigger number of cysts than trophozoites is cleared away.



## INTERNAL PARASITES OF DOGS IN TARTU DISTRICT

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Objectives. Dog-keeping is very popular in Estonia. Private kennels have appeared, dog-shows are organized but since homeless dogs are roaming along the streets. And not each dog-owner is regularly deworming his or her pet. It was therefore necessary to clear out the present parasitological status of dogs in Tartu region.

Methods. The previous study was carried out during several years in our parasitological laboratory. Dogs from different locations and with different health statuses (routinely dewormed and not dewormed and also homeless dogs) were studied. All age groups were involved. The total number of studied faeces samples was 1087. The routine Fuelleborn method was used for faeces examination as well as macroscopical determination of cestodes segments.

Results. 24,9% of investigated dogs were excreting parasite eggs, oocysts or segments. The nematode infections dominated, 190 (17,5%) dogs excreted Toxocara canis eggs, only few dogs (0,9...0,2%) possessed Uncinaria stenocephala, Trichocephalus vulpis and Toxascaris leonina infections. From cestodes Dipylidium caninum, Diphyllobothrium latum, Echinococcus granulosus and Taenia sp. were determined. Dipylidium caninum infection was dominating (3,4%). From coccides Cystoisospora (1,8%) and Sarcocystis spp. (0,09%) oocysts were found.

Conclusions. In spite of less or more regular deworming many dogs excrete eggs and oocysts of internal parasites with their faeces. The most wide-spread internal parasite of dogs in Tartu district is Toxocara canis.

## A TECHNIQUE FOR QUANTITATIVE ASSESSMENT OF *SCHISTOSOMA JAPONICUM* EGGS IN PIG FAECES

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### Objective

The objective of this study was to develop a convenient method for assessment of *Schistosoma japonicum* faecal egg excretion which could 1) detect low egg numbers and 2) differentiate *S. japonicum* eggs from similar-sized *Balantidium coli* cysts which are frequently present in pig faeces.

### Methods

A technique was devised which incorporated portions of a filtration method (1) and a sedimentation/centrifugation method (2) of egg counting. A mixture of 5 g faeces in 1.8% saline was washed through a series of 3 sieves of 400  $\mu\text{m}$ , 100  $\mu\text{m}$  and 50  $\mu\text{m}$ . The residue left in the 50  $\mu\text{m}$  sieve was washed and sedimented twice for 20 and 15 minutes, respectively. The final sediment was washed into a conical tube and centrifuged for 1 minute at 1500 RPM after which the supernatant was removed. Saline was added to the sediment to make a 2.25 ml solution of which 0.15 ml was transferred onto a microscope slide, covered with a glass coverslip and microscopically examined for eggs. Three counts were conducted for each sample, the sum of which was equivalent to eggs/g faeces.

### Results

We were able to detect eggs in the faeces of pigs with low intensities of infection using this technique. The samples could be kept several days in refrigeration. Differences between *S. japonicum* eggs and *B. coli* cysts were usually evident but when in doubt could be resolved by using a higher magnification. This also permitted observation of active miracidial flame cells. We were able to conduct a hatching test on the residual sample following egg counting giving us information on egg viability.

### Conclusions

This technique is convenient and simple requiring minimal equipment and supplies and avoids the use of toxic chemicals. Direct viewing of the eggs allows assessment of egg viability and easy differentiation from *B. coli* cysts. The additional feature of conducting a hatching test on the same faecal sample makes the technique very useful.

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## Identification of Lectin-Reactive Structures in *Fasciola hepatica* by Immunofluorescence and Lectin Blotting

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We have used lectins as histological markers and lectin blotting for the identification of *Fasciola hepatica* glycoconjugates. In order to determine the carbohydrate distribution in the liver fluke, a battery of fluorochrome-lectin conjugates with wide sugar specificities were used on both paraffin sections and frozen sections of adult *F. hepatica* worms. In addition, parasite extracts were electrophoresed and electrotransferred to a polyvinylidene difluoride membranes for lectin blotting using peroxidase-labeled lectins.

FITC-labelled lectins used were *Bauhinia purpurea* (BPA), *Canavalia ensiformis* (Con A), *Griffonia simplicifolia* (GS I or GS II), *Dolichos biflorus* (DBA), *Triticum vulgare* (WGA), *Ulex europaeus* (UEA I), *Glycine max* (SBA), *Arachis hypogaea* (PNA), *Maclura pomifera* (MPA) and *Limulus polyphemus* (LPA).

The lectin-binding results are summarized in Table:

Lectin	Sugar specificity.	Surface	BM zone	Tegu-ment	Paren	Testes	Vitelline Glands	Gut surface
Con A	Man, Glc	+	-	+	+++	+	+	++
PNA	Gal, GalNAc	+	-	++	cells	-	++	-
GS I	"	-	-	-	++	-	-	-
MPA	"	-	-	++	+++ cells	-	+/-	+
DBA	GalNAc, Gal	-	+	-	-	-	+	-
SBA	"	+	+	+	cells	+	++	-
BPA	"	+		+		+	+	-
LPA	sialic acid	+	-	-	-	-	+	-
WGA	(GlcNAc) <sub>1-3</sub>	-	-	++	cells	+++	++	+/-
GS II	GlcNAc	-	-	-	-	-	-	-
UEA I	Fuc	-	-	-	-	-	-	-

Several lectin reactive components were identified by lectin blotting in detergent extracts of adult flukes and in excretory secretory products.

## ANTHELMINTIC USAGE OF EXTRACTS OF *EMBELIA SCHIMPERI* FROM TANZANIA

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*Embelia schimperi* Vatke, belonging to the family Myrsinaceae, is used among the traditional Masai people of Tanzania and Kenya since it is believed to eliminate adult *Taenia saginata*, the beef tapeworm. In the present study, the anthelmintic effect of an extract of the dried fruits of *E. schimperi* was studied in experimental parasite/rodent models. In two experiments, rats with 10 adult tapeworms of *Hymenolepis diminuta* were treated with a diammonium salt of embelin (DE), isolated from the fruits. Significant lower numbers and total worm biomass of *H. diminuta* were observed in rats treated with 100 mg DE/kg. Furthermore, clear indications of the occurrence of destrobilation was observed in faeces after treatment and in 16% of the worms found at autopsy. The killing effect shown in vivo was corroborated by in vitro studies, which showed that all adult *H. diminuta* were killed when incubated in a culture medium containing as little as 0.08 mg DE/ml. No significant effect of DE was observed against *H. microstoma*, the trematode *Echinostoma caproni* and the nematode *Heligmosomoides polygyrus* in mice, although the worms could be killed in vitro. These results indicate that the crushed seeds of *E. schimperi* taken orally by the Masai people probably has an anthelmintic effect against human intestinal tapeworms.

## CHAGAS' DISEASE IN NICARAGUA

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Chagas' disease is a considerable problem in Central and South America. Prevalence values for Nicaragua have not been published despite a reported presence of *Trypanosoma cruzi* in *Rhodnius prolixus* and *Triatoma dimidiata* in 1967. In this study from the villages Somoto and Masaya 180 dwellings were examined using "sensor boxes" to trap the triatomine vectors, one third was successful, showing presence of *Triatoma dimidiata*. Fifty per cent of the bugs were shown to be positive.

The inhabitants of Somoto, Masaya, León, Matagalpa and Quilali, were examined seroepidemiologically and for presence of infected triatomine vectors. By immunofluorescence (IFL) and passive haemagglutination using the epimastigote stage as antigen, 77/1600 serum samples were found to be positive (4.8%).

Furthermore, the serological method for detection of *T. cruzi* infection was evaluated using mammalian stages of the parasite life cycle and a locally isolated strain (NIC 1) as antigen. The results showed an increased sensitivity of detection and significantly higher titers using mammalian stages of NIC 1 when compared to epimastigote stage of the reference strain Corpus Christi as antigen in an IFL assay. Thirtyone out of 52 of positive samples (59.5%) showed higher titres in epimastigote-IF assay using the local strain. Two out of 16 negative (12%) and 34 out of 52 of positive samples (65%) tested in higher titre and/or showed more intense reaction with mammalian stages of the local strain of *T. cruzi* used as an antigen.

## Urinary Schistosomiasis in Bissaque-Njalla, a Village in Guinea Bissau.

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A study at the National health laboratory (LNSP), Guinea Bissau aiming at schistosomiasis control was supported by the Swedish International Development Authority, SIDA. Field work was started in 1992 in the village Bissaque-Njalla close to the capital of Bissau. Adequate laboratory diagnostic procedures including demonstration of *S. haematobium* infection by identification of excreted parasite eggs and urine examination for haematuria were set up at LNSP.

The specific aims were to establish the prevalence of urinary schistosomiasis in Bissaque-Njalla and to evaluate the effects of treatment with Praziquantel.

One third (333) of 1001 children examined had schistosomiasis. Upon follow-up about a year later we found 58 out of 63 to be negative, suggesting a cure rate of above 90%. Five of the 63 (8%) were positive a year after treatment, either because of reinfection or treatment failure. Interestingly 6 out of 76 (8%) originally uninfected were positive a year later.

The data seem to support the concept of mass chemotherapy followed by yearly identification of infected cases and repeated targeted treatment for schistosomiasis control in an endemic area.

## CD59 Immunoreactivity in Parasitic Protozoa

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CD59 is an important inhibitor of complement mediated cell membrane lysis in several mammalian tissues and cells. Available data suggest that an analogous component is present in some parasites.

The purpose of the present study was to look for CD 59 immunoreactivity in a number of pathogenic protozoa, [*Trypanosoma. cruzi*, (6 strains tested: *Nic.I*, *Nic.II*, *CR4*, *CAI*, *Corpus Christi*, *Tulahuen*) *T. rangeli*, *T. brucei*, *Leishmania tropica*, *L. donovani*, *L. 527/94 isolate (L.infantum?)*, *L. 3152/94 isolate (L. tropica?)* *Toxoplasma gondii*, *E. histolytica*, *Acanthamoeba castellanii* and *A. rhysodes*] by immunofluorescence using three different anti-CD 59 antibodies, two monoclonal and one polyclonal antibody.

Parasites were maintained *in vitro* in axenic conditions except for monoxenic *Acanthamoeba spp.* culture with *E. coli* K12. Intracellular forms of *T. cruzi* were generated in a glioma cell-line. Blood-trypomastigotes of *T. cruzi* and *T. brucei* and *P. falciparum* were obtained from experimentally infected mice.

The most marked finding was anti-CD 59 reactivity in flagellae and basal bodies of culture-derived *T. cruzi* trypomastigotes and epimastigotes of all strains tested. No reactivity found in the other trypanosomes or *Leishmania spp.* *E. histolytica* trophozoites presented a granular staining of the cytoplasm and weak cytoplasmic staining was seen in *Acanthamoebae*. Strong background staining of erythrocytes interfered with *P. falciparum* staining.

Flagellar staining of American trypanosome species suggests that CD59 may be a parasite-protective molecule.

## **Detection of Microsporidiosis by Microscopy and Serology in African Patients with Aids.**

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Diagnostics of microsporidiosis is difficult due to the small size of the parasite and the lack of standardized serology. In the present study we looked for intestinal parasites in 28 HIV infected individuals with chronic diarrhoea in Guinea Bissau. Using a modified trichrome staining procedure of standard formalin fixed stool samples, we observed two positive cases, both with HIV-2 infection. No microsporidian spores were seen in 10 HIV-negative cases with chronic diarrhoea.

To evaluate serology using smears of *Encephalitozoon cuniculi* as antigen in an immunofluorescence assay we examined sera from the above-mentioned stool-positive cases and 6 additional sera from the same group of HIV-positive diarrhoeal cases. The two microsporidiosis cases were positive and so were two additional sera. One out of 14 Swedish Aids cases but none of 9 randomly selected hospital cases were positive. Interestingly 7/11 african sera from patients with ongoing or suspected schistosomiasis showed antibodies against *E. cuniculi*.

The results suggest that microsporidiosis is a significant cause of diarrhoea in HIV-2 infection and that serology for microsporidial infections should be further developed.



## **Antibodies Against $\beta$ -Tubulin: A Cause of False Positive Reactions in Pneumocystis Serology.**

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Immunisation with pneumocystis (PC) organisms isolated from infected human lung tissue for the generation of monoclonal antiPC antibodies yielded antibodies against  $\beta$ -tubulin in addition to parasite-specific antibodies. One such antibody, 26G8 was used to identify cross reacting antigenic epitopes in mammalian tissues and protozoa. These studies showed that the ventral flagellae of *Giardia lamblia* express the  $\beta$ -tubulin cross reacting epitope.

This enabled screening of patient sera for presence of antibodies against  $\beta$ -tubulin using *Giardia lamblia* as antigen in an immunofluorescence (IFL) test. We observed that sera with  $\beta$ -tubulin reactivity gave a positive reaction when tested in our IFL assay for antiPC reactivity. We could also show that about 10% of sera tested positive in the antiPC assay had antibodies against  $\beta$ -tubulin. Absorption experiments suggested that anti $\beta$ -tubulin antibodies may cause false positive antiPC serology.

## ANTIGENEMIA AS A INDEX OF TOXOPLASMOSE INFECTIONAL PROCESS ACTIVITI

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The search for clinical and laboratorial diagnostic criteria of the transfer of *Toxoplasma gondii* from latent stadia to the acute infectious processes against the immunodeficient background is very actual. For this purpose there was the investigation of some serological indexes in the modeling toxoplasmosis on the rabbits.

The animals were infected with 10000 cells of *T.gondii* RH-strain. Antibodies to *T.gondii* were discovered by counter current immunoelectrophoresis (CCIEP), indirect immunofluorescence (IIF) and ELISA-test. The detection of antigenemia was carried out with the help of ELISA-test.

It was found that the persistence of antibodies in response to infection was shown in all 10 rabbits. The positive results of ELISA-test were registered from 1-st to 6-th week. In the 6 months after infecting the antibodies and soluble antigens of *T.gondii* were not indicated in the animals' blood. In this period the rabbits were irradiated by X-Ray in 5 Gy.

It was found that the antigenemia was present in any experimental animals from 2-nd to 6-th week after irradiation. The results of antigens revealing in ELISA-test were negative in 8 weeks. On the 5-th week after irradiation the positive antibodies seroconversions in ELISA-test were registered first, and their maximum was shown on the 6-th week. In this period the positive results in CCIEP were found. In the control group (10 x-rayed rabbits without infection) the results of serological reactions and ELISA-test were negative all period of observation.

The analysis of the results allows to consider the antigenemia as an index of toxoplasmosis infectious processes activity.

## **Airborn transmission of pneumocystis: Detection of *Pneumocystis carinii*-DNA by filtration of air**

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*Pneumocystis carinii* is considered as the most important parasite in non-tropical geographical regions (even if it can be regarded as a fungus), mostly due to the high prevalence of pneumonia caused by this organism in immunosuppressed patients. The mode of *P. carinii* transmission is thought to be air, but the transmissive stage has not been identified or isolated.

We attempted to capture *P. carinii* by filtration of air in the vicinity of cages containing *P. carinii* infected Wistar rats. On such filters we were able to demonstrate *P. carinii* DNA by nested PCR amplification of the thymidylate synthase gene of rat *P. carinii* in 10 out of 10 experiments. The volume of air filtered was about 3 000 litres in each experiment (900 l. was later shown to be sufficient). No *P. carinii* DNA was seen in control experiments, filtration of air in a cell culture laboratory.

The result strongly supports the hypothetical airborne transmission route for *P. carinii* and suggests experiments aiming at further investigations of this stage of the parasite.

## PRESENT STATUS OF EPIDEMIOLOGY OF LYME DISEASE IN LITHUANIA

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Geografically Lithuania occupies the territory of the European continent where Lyme disease plays an important role in pathology of communicable diseases. Preliminary data are presented here on epidemiology of Lyme disease during 1992 - 1994.

There were 1451 case of Lyme disease diagnosed and reported in Lithuania in 1992 - 1994. The average annual incidence rate of Lyme disease for 100.000 population was 3,47 in 1992, 17,82 in 1993 and 17,58 in 1994. People of different age groups were infected. Number of Lyme disease cases was the highest for elderly persons. People in both sexes fall ill with Lyme disease - female more often (60,14 %) then male (39,86 %). Their occupation varies. The data show that most of them were office employer (44,06 %) the rest were workers (17,51 %), retired and unemployed persons (13,5 % of each category), students and school children (11,8 %). The retired persons seem to get infected most often.

Most patients usually get bitten by ticks in July, August and September. The first case of tick-borne disease occurred on 6 April 1994. The last occurrences were noticed on November 8. The period of tick activity is rather long in Lithuania: it lasts for 7 - 8 months. In nature Ixodes Ticks are natural carriers of Borrelia - the agent of Lyme disease. They belong to the variety of Ixodes ricinus and are found all over Lithuania. In the period between 1992 and 1994 there were 6,8 - 7,2 ticks found per one kilometer. According to the data of tests that were made in 1992 - 1994 - 5,0 - 8,62 % of I. ricinus ticks are infected with Borrelia.

## **Antibodies against paramyosin (PM) in metazoan infections and in sera with antismooth muscle antibodies (SMA)**

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Paramyosin (PM) is a 96kDa muscle component absent from mammalian tissues. It has been shown to be immunogenic in human schistosomiasis and some other metazoan infections.

AntiPM antibody reactivity could be demonstrated by immunoblotting (Western transfer) and by immunofluorescence. In the present study we also established a semiquantitative "dot blot" assay for antiPM antibodies and evaluated the significance of this serological assay for the detection of antiPM antibodies.

Sera from patients suffering from worm infections such as schistosomiasis, filariasis, trichinosis and fasciolasis were positive for antiPM antibodies, whereas control sera from patients with some protozoan infections were negative. Surprisingly, some sera with antismooth muscle antibodies (SMA) used as controls, also were positive. The latter sera also reacted with paramyosin from *Schistosoma mansoni*.

The results show that antiPM antibodies are associated with worm infections but the reason for antiPM reactivity in SMA sera is not obvious. Two possibilities will be considered, presence of cross reacting antimyosin autoantibodies or coincidental presence of worm infection.

## GUIDELINES FOR CONTRIBUTORS

All contributions should be submitted as word-processed manuscripts on floppy disk, accompanied by two exactly matching print-outs of good reading-quality. The preferred storage medium is a 3½ or 5¼ inch disk in MS-DOS or MS-DOS compatible format. The text should be written in WordPerfect or other word processing programs convertible to WordPerfect. **With a Macintosh computer, save the file in the MS-DOS compatible option.** Please indicate the word processor (and version) used to generate the file, the type of computer, the operating system, and the formatted capacity of the diskette.

Short articles/communications should not exceed 4 printed pages, including tables, figures, and references, and may contain a maximum of 2000 words if there are no figures or tables. The first page should show the title of the article, and the name(s) of the author(s). The authors' addresses should be given, and the complete correspondence address with telephone and telefax number (if available). The text should follow, without subheadings, but a short summary, maximum 100 words, may be included.

The text should be typed unjustified (unaligned right margins), without hyphenation (except for compound words), and at 1 ½ line spacing. Do not type page numbers. Label the hard copies by hand at the bottom of the page. Please ensure that the digit 1 and the letter 'l' have been used properly, likewise with the digit 0 and the letter 'O'. Do not use decorative formatting, such as boldface and centred headings, or underlining of titles or subheads.

Authors are obliged to follow the rules governing biological nomenclatures, as laid down in e.g. the *International Code of Zoological Nomenclature*. Disease names should follow the principles of *Standardized Nomenclature of Parasitic Diseases* (SNOPAD).

Figure legends must be included on the diskette, but the **figures will be handled conventionally**. They should be marked on the back with the title of the article and name of the (first) author.

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References in the text should be stated by giving in brackets the name of the author and the year of publication, e.g. (Thornhill, 1987) or (Austin & Austin, 1987). If there are more than two authors, only the first name plus *et al.* is given (Lund-Larsen *et al.*, 1977). The reference list should be in alphabetical order, and follow the style set forth in *Uniform Requirements to Manuscripts Submitted to Biomedical Journals*, Br Med J 1988; 296: 401-05. References to journals should contain names and initials

of the authors, article title, the abbreviated name of the journal, year of publication, volume, and first and last page numbers of the paper. Journals should be abbreviated according to the "List of journals indexed in *Index Medicus*". Authors without access to this list may type the full name of the journal, and the Editor will take care of the abbreviations. If there are more than six authors, list only the first three and add 'et al'. Personal communications and unpublished data should not be used as references, but may be inserted in the text (within parenthesis marks).

Examples of correct forms of references are given below:

*Standard journal article:*

Anonymous. Some facts on small animal practice. *Vet Rec* 1987; 120: 73

Horsberg TE, Berge GN, Høy T et al. Diklorvos som avlusningsmiddel for fisk: klinisk utprøving og toksisitetstesting. *Nor Vet Tidsskr* 1987; 99: 611-15

Lund-Larsen TR, Sundby A, Kruse V, Velle W. Relation between growth rate, serum somatomedin and plasma testosterone in young bulls. *J Anim Sci* 1977; 44: 189-94

*Books and other monographs:*

Austin B, Austin DA. Bacterial fish pathogens: disease in farmed and wild fish. Chichester: Ellis Horwood, 1987

McFerran JB, McNulty MS, eds. Acute virus infections of poultry: a seminar in the CEC programme, Brussels 1985. Dordrecht: Martinus Nijhoff, 1986. (Current topics in veterinary medicine and animal science 37)

Sosialdepartementet. Tsjernobyl-ulykken: Rapport fra Helsedirektoratets rådgivende faggruppe. Oslo: Universitetsforlaget, 1987 (Norges offentlige utredninger NOU 1987: 1)

Thornhill JA. Renal endocrinology. In: Drazner FH, ed. Small animal endocrinology. New York: Churchill Livingstone, 1987: 315-39

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## ERRATUM

The authors of the article "Occurrence of *Gyrodactylus* (Monogenea) on salmon and rainbow trout in fish farms in northern Finland", Bull SSP 1995 vol 5 no 1, would like add a sentence to the last paragraph of page 81 to clarify the results presented, the addition typed with bold letters):

Both uninfected and infected farms were found within the same water system. In the River Kemijoki, *G. salaris* was found in an upstream farm but not at two other downstream farms (Table 1, **results of Rintamäki (1993) combined with our material**):

- farms B21 and B22, ca. 110 km apart. The farms were monitored for half a year.
- farms B21 and B25, ca. 100 km apart. The farms were monitored for one year.



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