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BULLETIN OF THE SCANDINAVIAN SOCIETY FOR PARASITOLGY

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 Níðhoggr is ceaselessly gnawing at the roots of the ash. The second spring - Mímisbrunnr - is the source of wisdom and is guarded by Mímir. The third spring - Urðarbrunnr - is guarded by three women, the Norns, which mete out man's thread of life.

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PROCEEDINGS

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22-24 May, 1997

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WELCOME BY THE PRESIDENT OF THE SCANDINAVIAN SOCIETY FOR PARASITOLOGY

By INGER LJUNGSTRÖM

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 18th Symposium of our Society.

We are a Society with members from the Nordic countries, which is clearly reflected in the participation in this Symposium. But the Society also have members from many other countries, which we are very proud of. 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 microbiology and many others.

However, one thing brings us together - the parasites. The subjects we will discuss during these three days are parasites from different points of view, which reflect the scientific interest of the members, although this symposium will concentrate on tropical medicine and veterinarian parasites. I hope the various presentations will give you all inspirations for your future research.

A special welcome is directed to the invited speakers. During this Symposium we will listen to "Neuro-immune integration to host defences" and "Genetic and immunological influences on host-parasite interactions", which will be of great interest. More specific subjects such as "Lymphatic filariosis" and "Distribution and diagnosis of gyrodactylids in salmonids" will also be presented during these days. Two subjects far away from each other but of equal importance. Finally, we will be brought up-to-date about "Drug resistance and novel drug development in malaria".

Dear friends, without you as dedicated scientists, who are going to present exiting results, this symposium would not have been possible. I am really looking forward to fruitful discussions and that you all will bring new ideas back home.

Finally, I will thank the local organising committee headed by Kurt Buchmann, the scientific committee chaired by Birgitte Jyding Vennervald, and Elaine Svenningsen, who has elegantly handled the "practical work". These organisers have all performed a formidable task in arranging this symposium on this beautiful island, Bornholm. 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.

INVITED PAPERS

GENETIC AND IMMUNOLOGICAL INFLUENCES ON HOST-PARASITE INTERACTIONS

By D. WAKELIN

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Variation in intensity of infection is a universal phenomenon among naturally parasitized hosts and reflects the summation of many different influences. Laboratory studies have shown that genetic influences on natural and acquired resistance can play a major role in determining levels of infection and there is evidence that similar influences can affect parasitic infections in the field. Analysis of experimental models has revealed both the genetic basis of these influences and the ways in which they are expressed. It is now clear that all components of resistance, from induction to effector mechanisms, are under genetic control, are variable within populations and can significantly affect the degree to which parasites cause disease. Recent data from laboratory models (including Leishmania and intestinal nematodes) and from field studies (including malaria and schistosomiasis in humans and gastrointestinal nematodes in sheep) will be reviewed, and their consequences for understanding aspects of parasitology as diverse as epidemiology, pathology, chemotherapy and vaccination will be discussed.

DISTRIBUTION AND DIAGNOSIS OF GYRODACTYLIDS IN SALMONIDS

By C.O. CUNNINGHAM[1] & K. MacKENZIE[2]

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The number of gyrodactylid species reported from salmonid fish depends firstly on which fish classification scheme is accepted. Some classifications include thymallid coregonid, osmerid and plecoglossid fish under the collective title of "salmonids". In this review we include gyrodactylid species reported from thymallid and coregonid fish only. On this definition, over 20 species of *Gyrodactylus* and one of *Gyrodactyloides* are known from salmonid fish. *Gyrodactyloides bychowskii* is the only marine species amongst these. The number of true species of *Gyrodactylus* is uncertain because identifications have until recently been based on minor morphological differences which

show considerable variation within a single species. Five species have been described from North American salmonids: *G. avalonia, G. brevis, G. colemanensis, G. nerkae* and *G. salmonis.* One species, *G. masu,* has been described from Japanese salmonids. None of these has been found in Europe. Four species have been reported from both Asia and Europe: *G. birmani, G. derjavini, G. lavareti* and *G. thymalli.* A possible eight further species have been described only from Europe: *G. bohemicius, G. caledonensis, G. salaris, G. truttae* (including possibly two species), *G.* sp. of Ergens (1983) and *G.* spp. (possibly) of Shinn *et al.* (1995). A further five species have been described only from Asia: *G. asiaticus, G. brachymystacis, G. magnus* and *G. taimeni.* The pathogenic species *G. salaris* appears to be most closely related to three species which occur on fish with East Asian distributions. This may be an indication of the origin of *G. salaris.* This paper gives the known distributions of all these species and describes the spread through Europe of *G. salaris* from reports of its occurrence on wild Atlantic salmon in Norwegian rivers in the mid-1970s to its present known distribution.

Identification of *Gyrodactylus* species has traditionally relied on morphological features, particularly those of the opisthaptor. Recently, the study of *Gyrodactylus* DNA has led to the development of alternative methods of identifying species commonly found on salmonids in Europe. Molecular studies have concentrated on the ribosomal RNA gene array which occurs in multiple copies in the genome and contains regions of variable sequence which have been used to discriminate other congeneric platyhelminth species.

A PCR method was designed to amplify variable region V4 of the small sub-unit ribosomal RNA gene from single *Gyrodactylus* specimens. The nucleotide sequences of this region from *G. salaris, G. derjavini* and *G. truttae* were determined and compared. Sixteen nucleotide positions were found to vary between species. From these sequences, species-specific restriction fragment length polymorphisms (RFLPs) were predicted and oligonucleotide probes designed. Amplification of the V4 and digestion with enzymes *HaeIII* and *DdeI* produces fragment patterns which can be used to discriminate these three species. However, in the presence of unincorporated nucleotides and primers from the PCR, the restriction fragments can be difficult to discriminate. Hybridisation of species-specific probes to amplified V4 is a longer procedure but produces clear results.

The internal transcribed spacer (ITS) region of *Gyrodactylus* ribosomal RNA genes has also been studied. A PCR method to amplify the ITS1 - 5.8S - ITS2 region was developed. Digestion of this PCR product with a variety of restriction enzymes revealed RFLPs between species. Digestion with *Sau3AI* produces a particularly clear RFLP which allows the identification of *G. salaris, G. derjavini* and *G. truttae.* Amplification of the ITS followed by *Sau2AI* digestion of the PCR product provides a rapid and clear means of differentiating these species. The ITS from *G. salaris* and *G. thymalli* have been completely sequenced. These sequences were found to be identical, as are the small sub-unit gene V4 region sequences from these species.

Random amplified polymorphic DNA (RAPD) can also reveal genetic variation in *Gyrodactylus*. Although not particularly suitable for routine diagnosis of species, this method is sufficiently sensitive to detect differences in the genetic material of different populations within a single species.

DEVELOPMENT OF A VACCINE FOR THEILERIA INFECTION IN MOROCCO

By M. KACHANI, M. El HASNAOUI & H. OUHELLI

Department of Parasitology, Hassan II Institute of Agronomy and Veterinary Medicine, Rabat, Morocco

Objective: The purpose of this work was to develop a vaccine against tropical theileriosis.

Materials and methods: A cellular vaccine has been prepared and various experiments were designed to assess its safety and efficacy in susceptible animals. These made it possible to determine the relevant dose and passage number to be used in the field. Long term epidemiological studies were carried out in a pilot area and revealed a high prevalence of the disease in imported breeds of cattle. The vaccine was used to immunise thousands of animals exposed to natural infections in an endemic area.

Results: The vaccine proved to be efficient against both experimental and natural challenges. From 99-100% of the vaccinated animals were protected against natural infection.

Conclusion: The described vaccine against tropical theileriosis in Morocco is now ready for commercialisation. A sero-epidemiological survey was carried out and the prevalence of theileriosis in various cattle areas in Morocco was determined prior to large-scale vaccination.

EVALUATION OF EDUCATIONAL AIDS ON CYSTIC ECHINOCOCCOSIS IN MOROCCO

By M. KACHANI, H. OUHELLI, M. El HASNAOUI & M. BOUSLIKHANE

Department of Parasitology, Hassan II Institute of Agronomy and Veterinary Medicine, Rabat, Morocco

Objective: To determine the prevalence of hydatid cysts in intermediate hosts and *Echinococcus granulosus* infection in dogs in various regions in Morocco and

demonstrate the role of health education in a small village in the Middle Atlas, where the causative agent is highly prevalent both in man and domestic animals.

Materials and methods: A study using two types of aids for education was conducted at three elementary schools. These covered important aspects of transmission and preventive measures.

Results: The causative agent is highly prevalent in both man and domestic animals. Major factors which likely contribute to the relatively high levels of hydatidosis in Morocco include a lack of general knowledge in the rural populace about the disease and its routes of transmission and the low level of hygiene in rural villages.

Conclusion: The effectiveness of specifically designed educational material on children was evaluated in order to make recommendations on the potential use of such material in cystic echinococcosis control efforts in rural Morocco.

DRUG RESISTANCE AND NOVEL DRUG DEVELOPMENT IN MALARIA

By A. KHARAZMI

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Malaria is one of the most serious health problems in many parts of the world, particularly in Africa, Asia and Latin America. The global prevalence of malaria is 400 million clinical cases annually with over two million deaths each year. More than 80% of the world's malaria cases are found in Africa, where approximately two million children die from the disease every year. The situation is further complicated by the spread of drug resistant parasites in many parts of the world where *Plasmodium falciparum* is endemic. Today, in some areas of the world such as Thailand, multiple drug resistance is so prevalent that there is little to choose from for the prophylaxis or treatment of malaria. The recent discovery of chloroquine resistance in some *P. vivax* parasites adds to the problem. Therefore there is a great need for the development of effective and safe drugs for the prophylaxis and treatment of malaria. A number of the available antimalarial drugs have originated from plant sources and the potential of plants for the production of new antiprotozoal agents has recently been emphasised. The discovery of the antimalarial property of artemisinin and its derivatives is such an example.

Using a bioassay-guided fractionation our group has identified an oxygenated chalcone, licochalcone A, with potent antimalarial and antileishmanial activity *in vitro* and *in vivo* in several animal models. Licochalcone A and a large number of related structures inhibited the *in vitro* growth of chloroquine sensitive and chloroquine resistant strains of the human parasite *Plasmodium falciparum.* Oral administration of these compounds to mice protected the animals from lethal infections of *P. yoelii* and *P.*

berghei. Preliminary toxicological studies in rats showed that these compounds up to a 20-fold *in vivo* protective dose did not exhibit any toxicity. The absorption studies showed that these compounds are absorbed through the gastrointestinal tract, an important advantage for the development of a new antiprotozoal drug. Studies on the site of action of licochalcone A and related structures revealed that the target organelle was the parasite mitochondria. The mechanism of action of these compounds appears to be inhibition of the activity of some of the mitochondrial enzymes such as dehydrogenases thereby interfering with the parasite energy metabolism. Structure-activity studies indicated the importance of oxygenation pattern on the selectivity of antiprotozoal activity of these compounds.

In summary, oxygenated chalcones exhibit potent antimalarial activity and can provide the basis for the development of a new generation of antiparasitic drugs.

TOWARDS THE CONTROL/ELIMINATION OF LYMPHATIC FILARIASIS

By E.A. OTTESEN

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The disease and its outlook: Lymphatic filarial parasites infect 120 million people in 73 countries of the tropics and subtropics, especially in Africa and the Indian sub-continent. Elephantiasis, lymphoedema, genital damage (particularly hydrocoele and scrotal pathology in men), and lung disease occur in 44 million men, women and children; another 76 million have hidden internal damage to their lymphatic and renal systems.

In the past, tools and strategies available for control were inadequate, but during the last decade there have been dramatic new developments, including: 1) new techniques for defining pathology (lymphoscintigraphy and ultrasound); 2) new tools (based on antigen and DNA detection) for diagnosing infection and monitoring control efforts; 3) new recognition of the social and economic consequences of disease and 4) most importantly, new treatment tools and control strategies.

The new strategy to control/eliminate lymphatic filariasis focuses principally on treating the human population through community-wide (mass) treatment programmes that should completely replace older techniques. One-day, once-yearly co-administration of 2-drugs (ivermectin + either diethylcarbamazine (DEC) or albendazole) reduces blood microfilariae by 99% for a full year, with early field studies demonstrating that transmission can be interrupted. Equally effective is treatment using DEC-fortified salt daily as a substitute for regular table/cooking salt.

"*This disease may be eradicable.....*". Of 94 infectious diseases evaluated by the International Task Force for Disease Eradication (1993) lymphatic filariasis was

identified as one of only six considered "eradicable" or "potentially eradicable", principally because humans are essentially the only reservoir host, the available treatment tools are excellent and programmes to eradicate filariasis from some countries have already demonstrated success.

Current progress: The general approach to global control/elimination of lymphatic filariasis taken by the Division of the Control of Tropical Diseases (CTD) at WHO is to develop national control strategies with each endemic country (initially 1-2 countries per region per year and preferably integrated with other health programmes encompassing multiple-disease control activities), to develop appropriate training materials and workshops, to assist in securing funds, and to assist in coordinating, monitoring and certifying programme effectiveness.

These efforts benefit from a valuable, unique "window of opportunity" provided by the willing generosity of ivermectin's manufacturer (Merck & Co., Inc.) to donate its drug to countries collaborating with CTD to demonstrate the feasibility of eliminating lymphatic filariasis by the new control strategies. It is hoped that similar arrangements can be made with the manufacturers of albendazole and DEC as well.

Of the 12 countries approached thus far, Revised National Strategies for Filariasis Control/Elimination have already been drafted and approved (or are being approved) in Egypt, India, Fiji and Samoa, with the latter two having already commenced their yearly, 2-drug mass treatment programmes. Eight other countries are actively revising strategies that should be in effect in 1997.

Recognising the importance of enhancing the visibility of global filariasis control/elimination efforts and of sustaining the momentum now established because of the new tools and opportunities available to control this formerly hopeless disease, the Executive Board of the World Health Assembly in January 1997 adopted a Resolution to be recommended to the full Assembly in May 1997 calling for the global elimination of lymphatic filariasis as a public health problem.

INTEGRATION OF NEURO-ENDOCRINE-IMMUNE RESPONSES IN HOST DEFENCES AND PATHOGENESIS

By D. BEFUS

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In an effort to understand the scientific bases of the beliefs held by philosophers and healers of ancient and modern cultures about the mind-body connection, there has been a growing interest in the interfaces of neuroscience, endocrinology and immunology. The nature of the connection as it relates to disease susceptibility, remission and relapse has intrigued humankind for centuries and remains a prime target of several alternate therapies practised today. In the past neuro-endocrine-immunology has often been

viewed as a "soft" science because of our limited ability to define and control the mechanisms involved in complex phenomena such as stress, circadian rhythms and Pavlovian conditions. However, in recent years major advances have been made in our understanding of the cellular and molecular mechanisms involved. Reproducible experimental systems have been established and increasingly exciting and powerful technologies provide a solid basis for future progress.

Given the complexity of the biological pathways involved in mind-body connections, it is not surprising that reductionist approaches have been utilised to begin to dissect the underlying mechanisms. Several research themes have evolved. One focus has been on the influence of the nervous system on immune and inflammatory responses. Definition of the sympathetic, parasympathetic and non-adrenergic, non-cholinergic innervation of the lymphoid system has provided some understanding of the communication lines and chemical signalling involved. In the gastrointestinal tract, the complexity of the enteric nervous system in the innervation of the mucosal immune system has begun to be mapped. Detailed investigations of the influences of specific neuropeptides and other neurotransmitters on cells and pathways in the immune system have provided insights into the nature of the immune functions under neuronal controls. In turn, it has become recognised that cells of the immune system make several neuropeptides and neurotransmitters, opening up the possibility that communication within the immune system itself employs pathways previously thought to be restricted to neuronal involvement. A corollary is that the immune system communicates to the nervous system using chemical signals previously thought to be restricted in their origin to the latter.

Holistic influences of the nervous system on the immune system that appear to be highly relevant in host responses to infectious diseases include classical Pavlovian conditioning of immune responses, stress effects on immunity and the circadian effects on host responses and immunopathogenesis. Numerous examples of conditioning of specific immune responses have been defined and more recently, the effects of stress and circadian rhythms on host defences have been studied.

Another central theme has been the effects of the immune system on the nervous system. The immune system produces factors that activate or modulate functions of the nervous system and in addition to utilising neuropeptides or neurotransmitters made by cells of the immune system, it has been recognised that cytokines produced by cells in immune responses are also chemical messengers in the nervous system. Indeed, cytokine receptors have been mapped in both central and peripheral sites in the nervous system and neurons and structural cells in the nervous system produce many cytokines themselves. This sharing of signalling molecules and complementary receptors makes the boundaries we have defined that separate the systems seem increasingly, and inappropriately, arbitrary.

Immune and inflammatory responses to infectious agents can also lead to significant damage to the nervous system. Chagas disease has been one example that has been studied, but more recently, it has been established that damage and repair to the nervous

system can occur during responses to other infections. Such studies have demonstrated the plasticity of the nervous system and its ability to modify tissue innervation in response to inflammatory infiltration, damage and remodelling.

Perhaps the most challenging, but exciting research has been in the integration of neuro-endocrine-immunologic responses and elucidation of the relevance of such communication in host defences and immunopathogenesis. Complex phenomena such as neurogenic inflammation and pathways including the hypothalamic-pituitary-adrenal axis and the cervical sympathetic trunk-submandibular gland axis have received considerable study. Examples such as mast cell - nerve interactions, particularly in intestinal helminth infections, or the neuropeptide pathways involved in schistosome granuloma have uncovered intriguing information and we can begin to develop conceptual models of the neuroimmunology of host-parasite relationships.

Clearly, neuro-endocrine-immunology has advanced remarkably in recent years, from "soft" science to an exciting science with a rigorous experimental base. There are many challenges before us, but hidden jewels are being uncovered regularly and there is much promise for increasingly greater understanding of complex homeostatic mechanisms in mind-body connections and host-parasite symbiology.

SUBMITTED PAPERS - ORAL PRESENTATION

MINI SYMPOSIUM - HUMAN AND VETERINARY TROPICAL PARASITOLOGY

THE EFFECT OF PERMETHRIN IMPREGNATED BEDNETS ON THE RESTING AND FEEDING BEHAVIOUR OF LYMPHATIC FILARIASIS VECTORS ON THE KENYAN COAST

By C. BØGH[1], E.M. PEDERSEN[1], J.H. MUKOKO[2] & J.H. OUMA[2]

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The impact of permethrin impregnated bednets on the resting and feeding behaviour of vectors of lymphatic filariasis was studied in six pairs of villages before and after intervention. Mosquitoes were collected in each village once a week both indoors using pyrethrum spray catches and outdoors using pit traps, during the months just after the long rainy seasons in 1994 and 1995. Before intervention, 33.6% of the vector mosquitoes were *Anopheles gambiae sensu lato*, 30.0% *A. funestus* and 36.4% *Culex quinquefasciatus*. PCR analysis of the *A. gambiae* complex species collected in 1995 demonstrated that 98.5% were *A. gambiae sensu strictu*, 1.0% *A. arabiensis* and 0.5% *A. merus*. Introduction of impregnated bednets reduced the number of indoor resting *A. gambiae s.l.* by 95.6% and *A. funestus* by 98.1%, but there was no significant change in the number of *C. quinquefasciatus* collected indoors. There was no change in the number of outdoor resting *A. funestus* or *C. quinquefasciatus*, but there was a significant decrease in the number of outdoor resting *A. gambiae s.l.* ELISA analysis of mosquito blood meals showed a shift from human to animal feeding after the introduction of treated nets, most markedly for indoor resting *C. quinquefasciatus* where the human blood index was reduced from 93.1% to 14.4%. These results indicate that permethrin impregnated bednets are likely to be effective in reducing lymphatic filariasis transmitted by *A. gambiae, A. funestus* and *C. quinquefasciatus*.

THE *in vitro* AND THE *in vivo* ANTIMALARIAL PROPERTIES OF A NOVEL OXYGENATED CHALCONE, 2,4-DIMETHOXY-4'BUTOXYCHALCONE

By M. CHEN[1,2], S.B. CHRISTENSEN[5], L. ZHAI[1], M.H. RASMUSSEN[5], T.G. THEANDER[3,4], S. FRØKJÆR[6], J. DAVIDSEN[6] & A. KHARAZMI[1]

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Our previous studies have shown that licochalcone A, an oxygenated chalcone, has antileishmanial and antimalarial activities. We have now synthesised a number of analogs of licochalcone A and have also tested their biological activity. The present study was designed to examine the antimalarial activity of one of these analogs, 2,4-dimethoxy-4'butoxychalcone (2,4m4'bc). 2,4m4'bc inhibited the *in vitro* growth of both a chloroquine sensitive (3D7) and chloroquine resistant (Dd2) *Plasmodium falciparum* in a ^{3}H-hypoxanthine uptake assay. The *in vivo* activity of 2,4m4'bc was tested in a mouse model infected with *P. yoelii* or *P. berghei* and was also tested in a rat model infected with *P. berghei.* 2,4m4'bc administered either intraperitoneally, subcutaneously or orally for five days protected the mice from the otherwise lethal infection. 2,4m4'bc administered orally for five days reduced parasitemia in the rats infected with *P. berghei* infection. 2,4m4'bc at concentrations up to 20 times antiparasitic dose did not inhibit human lymphocyte proliferation and neutrophil oxidative burst. These results demonstrate that 2,4m4'bc exhibits potent antimalarial activity and might be developed into a new antimalarial drug.

ELECTROPHORETIC DIFFERENCES BETWEEN *Schistosoma japonicum* FROM DIFFERENT PROVINCES IN MAINLAND CHINA

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Human schistosomiasis japonicum has long been recognised to be of major public health importance in Asian countries. This disease is caused by the blood fluke, *Schistosoma japonicum*, which is assumed to consist of distinct geographical "strains" in different areas of Asia. Until recently, *S. japonicum* in China was considered to

represent a single "strain". We therefore conducted an allozyme electrophoretic study to examine the extent of genetic variation in *S. japonicum* from mainland China. The allelic profiles of *S. japonicum* from seven provinces were established at 16 enzyme loci, seven (43%) of which were polymorphic. Of significance was the magnitude of the fixed genetic differences detected between *S. japonicum* from different provinces. Those from Sichuan Province had four (25%) fixed differences from those from Anhui, Jianzi, Hunan and Hubei Provinces, suggesting that *S. japonicum* may represent a species complex. Also of significance was the detection of genetic variation among individuals within populations that had been maintained in the laboratory for at least 20 years. Thus, this study is the first to report electrophoretic variation within a population of *S. japonicum.* Furthermore, the establishment of genetic markers for different populations and/or cryptic species of *S. japonicum* has significant implications for studying the biology of these organisms in human and animal hosts, and for the control of human schistosomiasis japonicum.

USE OF RAPD TO DETECT GENETIC VARIATION IN THE HUMAN BLOOD FLUKE *Schistosoma japonicum*, FROM MAINLAND CHINA

By R.B. GASSER[1,2], B.-Z. QIAN[3], P. NANSEN[1], M.V. JOHANSEN[4] & H.O. BØGH[1]

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A random amplified polymorphic DNA (RAPD) technique using 16 decamer oligonucleotide primers was employed to characterise isolates of *Schistosoma japonicum* from seven geographical locations (Sj1: Zheijiang; Sj2: Anhui; Sj3: Jiangxi; Sj4: Hunan; Sj5: Hubei; Sj6: Sichuan; Sj7: Yunnan) of the People's Republic of China. Distinct differences between some isolates were reproducibly detected in RAPD patterns produced using five of the primers. The analyses showed that both Sj6 and Sj7 were quite distinct genetically from Sj1-5 based on the presence/absence of particular bands (A10-200 bp, A9-220 bp, B17-520 bp, P205-680 bp and P235-930 bp). These findings are in line with previous reports of the biological, biochemical, immunological and chemotherapeutic differences of *S. japonicum* from Sichuan and Yunnan compared with other geographical regions. The present study showed, based on RAPD profiles, that genetic differences exist within *S. japonicum* from mainland China. This finding may have important implications for studying the population biology, epidemiology and clinical forms of the disease in China, well as for developing vaccines and diagnostic test systems.

WORM ESTABLISHMENT AND FECUNDITY OF *Schistosoma japonicum* IN PIGS INFECTED BY THE PERORAL ROUTE OF INFECTION

By H. GIVER[1,2], M.V. JOHANSEN[1], N.Ø. CHRISTENSEN[1] & P. NANSEN[2]

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The aim of the study is to compare the effect of a peroral with an intramuscular route of infection in pigs experimentally infected with *Schistosoma japonicum.*

Fifteen pathogen-free Danish/Yorkshire/Duroc crossbred male and female pigs were divided into three groups. Two groups, each of six pigs, were in the middle of February 1997 infected with 1000 *S. japonicum* cercariae, either by the peroral or by the intramuscular route of infection. One group of three pigs was kept as uninfected controls. All infected pigs were killed by an overdose of pentobarbital 10 weeks post infection and worms were recovered by perfusion. Egg counts from faeces and specific antibody titers from blood, as well as worm recovery, will be presented later when results are available.

Pilot studies on mice infected perorally resulted in worm establishment and excretion of eggs from all individuals. Compared to mice infected by the percutane route of infection, worm recovery was significantly lower for the mice in the perorally infected group ($p<0,05$), except for the immature worms. No difference in number of eggs excreted in faeces, expressed as eggs/g faeces/female was seen between the two groups of mice.

Hopefully the present study on pigs will elucidate the role of the peroral route of infection.

Schistosoma japonicum AND *Trichuris suis* INFECTIONS IN PIGS FED DIETS WITH HIGH AND LOW PROTEIN

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Objective: The aim of the study was to measure the impact of *Schistosoma japonicum* and *Trichuris suis* infections in young growing pigs fed a low or high protein diet.

Material and methods: Thirty pigs, 6-10 weeks old, were randomly allocated into two groups receiving either a high or a low protein diet. After 11 weeks half of the pigs from each of the two groups were infected with 1500 *S. japonicum* cercariae and 4000 *T. suis* eggs. The weight of the pigs was measured every three weeks and blood and faecal samples were collected biweekly from the time of infection.

Results: At infection time, the pigs on a low protein diet had significantly lower mean body weights and haemoglobin levels compared to those on a high protein diet and that pattern continued throughout the study ($p<0.05$). Significantly more *S. japonicum* worms as well as faecal and tissue eggs were found in the low protein diet pigs compared to the high protein diet pigs ($p<0.01$). No differences between the two diet groups were observed in *T. suis* establishment rates or fecundity.

Conclusion: The low protein diet increased the establishment rates of *S. japonicum*, favoured larger depositions of eggs in the liver and in faeces as well as reduced weight gains and caused anaemia and hypoalbuminaemia in young growing pigs as compared with the high protein diet.

THE ROLE OF *Bulinus truncatus* IN THE TRANSMISSION OF *Schistosoma haematobium* AND *Paramphistomum microbothrium* IN TESSAOUT-AMONT IRRIGATION SCHEME, MOROCCO

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A cross-sectional snail study has been carried out in Tessaout-amont irrigation scheme to identify the parasite fauna and describe the spatial distribution of the snail intermediate hosts. *Bulinus trunatus* has been found to carry infection with either *Schistosoma haematobium* or *Paramphistomum microbothrium* and no snail with double infection was encountered. During the last three years, the prevalence of schistosomiasis was reduced drastically and no *B. truncatus* naturally infected with *S. haematobium* were collected in the area. However, snails infected with *P. microbothrium* were found although the infection rate was low. The infection reached a maximum of 3.49% with an average around 1% throughout the study period. The pattern of snail infection was irregular and appeared to be linked to the seasonal slaughtering of small ruminants near the canals. The disease has never been described in the area before and likely has been introduced after the implementation of the irrigation scheme. Water in the siphon boxes in the tertiary canals is used for watering livestock and cleaning the viscera of animals. Environmental measures implemented in

the siphon boxes to control schistosomiasis haematobium are expected to have a positive effect on the control of paramphistosomiasis. A longitudinal study is ongoing to evaluate the effects on the transmission of both diseases through the reduction of the snail population. Histological examination of the snail organs showed that the ovotestis and the digestive gland are the elective sites colonised by the sporocysts and rediae stages.

HOST-PARASITE RELATIONSHIPS IN *Schistosoma bovis* INFECTION OF GOATS

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Objective: Experimental *S. bovis* infection in goats features a suppression of faecal egg excretion after early patency and a high degree of resistance to homologous challenge exposure. This study aimed at elucidating events at parasite and tissue level behind this course of infection.

Materials and methods: 44 West African dwarf goats were divided into four groups (A: primary infection with *S. bovis* week 0; B: as A and homologous challenge infection week 16; C: challenge control infection with *S. bovis* week 16; D: unexposed). The dose per exposure was 1,000 cercariae. The course of infection was monitored with faecal egg counts, clinical observations and haematology. Post mortem studies, including recovery of worms by vascular perfusion, determination of tissue egg counts (TECs) and histopathological analysis of the intestines and liver were performed on subgroups of goats at weeks 16, 22 and 32.

Results: The course of infection was concordant with that previously observed, *i.e.* showing a suppression of faecal egg output after an early peak and only a minor rise post challenge, neither of which resulted in increased TECs. There were no signs of attrition of the worm population from primary infection during the course of patency. The establishment of worms from challenge was similar to that from primary infection, though with a delay in maturation. In early patency of primary infection when egg excretion started to rise (group C week 22), tissue reactivity to eggs in the intestine was minor. Necrosis of the liver from egg deposition was prominent only at that stage. Later in primary infection and after challenge, the histopathology of the intestine was characterised by an anti-oval granulomatous response and was consistent with a slow-down of egg transfer into the gut wall.

Conclusion: The study indicates that resistance phenomena in caprine *S. bovis* infection involve a depression of worm fecundity and that the peak in egg output in early patency is dependent upon a low degree of host reactivity to eggs at that phase of infection.

MASS DEC CHEMOTHERAPY FOR CONTROL OF BANCROFTIAN FILARIASIS: A 4-YEAR FOLLOW-UP STUDY ON THE EFFECT OF FOUR DIFFERENT STRATEGIES

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Mass chemotherapy with diethylcarbamazine (DEC) is an effective measure for the control of bancroftian filariasis. However, only a few studies have assessed the effect of long-term treatment. The present study evaluated and compared four mass DEC control strategies in four endemic communities of Tanzania. Each community received treatment according to a different strategy. Strategy I: Standard DEC treatment (6 mg/kg body weight given daily for 12 consecutive days); Strategy II: Semi-annual single dose treatment (6 mg/kg body weight given once every six months for one year); Strategy III: Low monthly dose treatment (50 mg to children and 100 mg to adults for one year); Strategy IV: DEC-medicated cooking salt (given for a period of one year).

Results from the first two years of the study have already been published. This presentation will analyse the changes taking place from two years to four years after start of treatment. Although a slight increase in microfilaraemia (mf) prevalence was observed in this period, the mean mf intensities remained very low. The majority of individuals who developed microfilaraemia from the 2-year to the 4-year follow-up examination were also microfilaraemic before the start of treatment. Thus, the rate of re-development of microfilaraemia was much higher among those who were mf-positive at pre-treatment than among those who were mf-negative. The long-lasting effect of treatment adds a promising potential to the use of mass DEC chemotherapy for the control of bancroftian filariasis. A decision on the most appropriate DEC strategy and the frequency of intervention will be discussed.

ASSESSMENT OF PERMETHRIN IMPREGNATED BEDNETS ON THE TRANSMISSION OF LYMPHATIC FILARIASIS IN KWALE DISTRICT IN THE COASTAL REGION OF KENYA: PRE-INVENTION DATA

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Studies on the effect of permethrin impregnated bednets on the transmission of lymphatic filariasis in Kwale District Kenya have been going on since 1994. The study design and pre-intervention data are discussed here. Twelve study villages endemic for lymphatic filariasis were selected in Kwale District. People in the twelve villages were registered in household units. Parasitological and clinical manifestations were conducted to establish the infection rates with *Wurchereria bancrofti* and the prevalence of clinical manifestation of the disease in the study population. In entomological studies, mosquitoes were caught by light traps every fortnight in each of the twelve villages for a year. The vector species of the mosquitoes caught were dissected to record infection and infectivity with *W. bancrofti.* After one year's pre-intervention studies, the twelve villages were paired. One of each pair was selected randomly to give six intervention and six control villages. In the intervention villages enough permethrin impregnated bednets were distributed to cover all villagers, while the control villages remained without bednets. Both entomological and parasitological studies continued as in the pre-intervention period. Results from the parasitological examination in 9,056 people registered in the study indicate microfilaraemia rates ranging from 8% to 27%. Entomology results show that three vector species, *i.e. Anopheles gambiae s.l., A. funestus* and *Culex quinquefasciatus* are present. Moderate infection rates in the mosquitoes were observed, with widely varying annual transmission potentials in the twelve villages.

THE PROPORTION OF HELMINTH INFECTIONS REACHED BY SCHOOL-BASED HEALTH PROGRAMMES IN WESTERN KENYA

By A. OLSEN

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School-based health programmes are often the best possible way of controlling helminth infections, but the impact of such programmes depends among other things on school enrolment and the age-distribution of the helminth in question. This study used

data from an epidemiological survey of 752 persons in three villages in western Kenya to examine the proportion of infected persons as well as helminth eggs which will be reached in a school-based programme. Overall prevalences of hookworm, *Ascaris lumbricoides, Trichuris trichiura* and *Schistosoma mansoni* infections were 63%, 16%, 24% and 24% respectively, and intensities were low for all infections. Of the school-aged children, only 79% were enrolled. For all four infections, a school-based programme will thus reach between 76% and 87% of the number of infected school-aged children, as well as the number of eggs in this age group. Of the total population, school programmes will reach between 31% and 50% of the infected persons and 24%, 54%, 30% and 40% of the total number of hookworm, *Ascaris, Trichuris* and *S. mansoni* eggs excreted. Assuming that attendance rates are high in the study area, the school-based programmes seem to be efficient in improving the helminth infection status of school-aged children. On the other hand, adults, non-enrolled and pre-school children not reached by school-based programmes represented more than half of the helminth infected persons and they harboured between half and three quarters of the total burden of helminth eggs in the area. It is expected that school-based programmes implemented in the study area will have little impact on the contamination level of the environment and hence on the transmission of helminth infections.

A 16 YEAR FOLLOW-UP STUDY ON BANCROFTIAN FILARIASIS IN TANZANIA: IMMUNOLOGICAL ASPECTS

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Many aspects of the natural history of bancroftian filariasis, including those related to host susceptibility, the longevity of infection and the relationship between infection and disease, remain poorly understood. To help elucidate some of these aspects, a 16-year follow-up study was carried out on bancroftian filariasis in three communities in north-eastern Tanzania. All individuals were examined for microfilariae and clinical manifestations in 1975 and again in 1991, and as many as possible of the individuals examined in 1975 were re-identified in 1991. Detailed analyses of the parasitological and clinical data have been documented previously.

Individuals were selected from the same communities in 1991 for further immunological studies. Two studies will be presented which analyse the antibody responses in relation to the long-term observations on microfilaraemia. The first study examines antibody responses in individuals in relation to their microfilarial status in 1975 and 1991. It includes individuals who were mf positive at both surveys, individuals who were mf positive in 1975 but mf negative in 1991 and individuals who were mf negative at both surveys. The second study examines the antibody responses

in young people (aged 10-20 years in 1991) in relation to the mf status of their mothers in 1975 and 1991. In both studies, sera from the study individuals were examined for the following: filarial specific circulating antigens (by ELISA), filarial specific IgG1, IgG2, IgG3, IgG4 and IgE (by ELISA), and specific IgG2 to the sheath of *Wuchereria bancrofti* microfilariae (by IFAT). The results are presented and discussed.

ANTHELMINTIC TREATMENT IMPROVES PHYSICAL FITNESS, GROWTH AND APPETITE OF KENYAN SCHOOL BOYS WITH HOOKWORM, *Trichuris trichiura* AND *Ascaris lumbricoides* INFECTIONS

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Objective: We studied physical fitness with the Harvard Step Test (HST), growth and appetite in primary school boys with hookworm (96% baseline prevalence), *Trichuris trichiura* (98% prevalence) and *Ascaris lumbricoides* (41% prevalence) who received a single 600 mg dose of albendazole or an identical placebo.

Materials and methods: Boys were examined, allocated at random by decreasing hookworm egg count to placebo (PL, n-26) or albendazole (A, n-27) groups, treated and re-examined four months later.

Results: Four months after treatment, the A group showed highly significant improvements in fitness score, resting heart rate and heart rates at 1,2,3 and 4 minutes after the HST, while the PL group had not changed significantly. The A group also exhibited significantly more rapid growth judged by weight gain (1.0 kg greater than PL, $p<0.0002$), height increment (0.6 cm more, $p<0.003$), arm circumference (0.3 cm more, $p<0.0002$) and triceps and subscapular skin folds (1.0 mm more, $p<0.0002$), and showed improved appetite with objective and subjective measures.

Conclusion: We conclude that single dose treatment with albendazole can allow improved physical fitness, growth and appetite in areas where these helminths and poor growth are highly prevalent.

THE INFLUENCE OF AGE ON THE RESISTANCE OF PIGS TO A PRIMARY INFECTION WITH *Schistosoma japonicum*

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The current study examined if any pattern exists between age and resistance to infections with *Schistosoma japonicum* in pigs. Seventeen Landrace/Yorkshire crossbred specific pathogen-free pigs in three different age groups (groups A-C), aged 7, 24 and 37 weeks, were infected by intramuscular injections of 1,000, 1,500 or 2,400 cercariae, respectively. Faecal egg counts were obtained twice a week from seven to ten weeks post infection (w.p.i.), and the pigs were euthanised 11 w.p.i. The number of worms established in the mesenteric veins were counted and sexed subsequent to perfusion of the portal system. Tissue egg counts were estimated on subsamples from the liver. The results indicated that no age related increase, or decrease, in resistance to infection of *S. japonicum* occurs in pigs. The worm recoveries for the three groups were 3.2%, 8.1% and 3.8% for groups A-C, respectively. The fecundity parameters, *i.e.* faecal egg counts per mature female and liver egg counts per mature female, showed no significant difference among the three age groups. No differences were observed between the male/female ration of the three groups. These results indicate that no age related increase in resistance to infection with *S. japonicum* exists in the pig.

FISH PARASITOLOGY

METAZOAN PARASITES OF WILD-CAUGHT ATLANTIC HALIBUT FROM SOUTH-EAST NORWAY

By C. APPLEBY & T.A. MO

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Objective: The objective was to study the occurrence of metazoan parasites from wild-caught Atlantic halibut (*Hippoglossus hipoglossus*), and to evaluate their potential as possible pathogens for artificial rearing of halibut in the future.

Materials & methods: Adult halibut between 2.3 and 16.5 kilos were caught in gill nets and trawl. Some were kept alive until examination, while others had to be killed

and shipped on ice to the laboratory. All external and internal organs were examined by visual inspection and with a dissecting microscope.

Results: So far we have found nine metazoan parasite species: five Digenea, one Nematoda, one Acanthocephala and two Copepoda, the most common species being *Derogenes varicus* (Digenea), *Anisakis simplex* (Nematoda) and *Hatschekia hippoglossi* (Copepoda).

Conclusion: Based on our preliminary results, it is difficult to predict which species might cause problems in future halibut aquaculture, but based on general knowledge, parasites with one-host life-cycles, *e.g. Entobdella hippoglossi* (Monogenea) and *Lepeophtheirus hippoglossi* (Copepoda) will possibly be of greatest importance. However, this also depends on what technical solutions are chosen for the rearing of halibut to market size. Our studies show that metacercariae of *Cryptocotyle lingua* and other digeneans might negatively affect the flesh quality of small halibut caught in shallow waters.

PARASITES OF MARINE ATLANTIC SALMON (*Salmo salar* L.) IN WINTER

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Objective: The normal parasite fauna of wild marine Atlantic salmon is poorly known and is practically unknown during the winter. Here we report the results of a study on parasites of winter-caught salmon in the Norwegian Sea.

Materials and methods: One of us, J.A. Jacobsen, obtained more than one hundred salmon caught in the winters 1993/94 and 1994/95 at sea north of the Faroe Islands and froze them at sea in order to search them for salmon lice, *Lepeophtheirus salmonis*. A sub-sample of 82 frozen salmon, 53 wild and 29 escaped farmed salmon, from the 94/95 winter, were received in Bergen for B. Berland to study their other parasites.

Results: No parasites were found on gills or in eyes. *Myxobolus* sp. was found in spinal cord squashes of nine wild salmon, *Chloromyxum* in kidney in some fish. Trematodes of the genera *Derogenes, Brachyphallus* and *Hemiuris* were found in stomach, but no trematodes in intestine. The cestode *Eubothrium* sp. was present in intestine, with prevalence higher in wild fish, but intensity higher in escapees (prevalence - mean intensity respectively: 59% - 7; 24% - 24). The nematode *Hysterothylacium aduncum* was present in both wild and escaped fish, almost exclusively as small third, fourth and young fifth stage specimens. No fully matured specimens were seen; they do not reproduce during the winter. *Anisakis simplex* larvae in viscera had a higher prevalence in wild than in escaped fish, while the intensity was

higher in escaped farmed fish (prevalence - mean intensity respectively: 81% - 6; 69% - 21).

Conclusion: The marine salmon's general parasite fauna is poorer in winter than in summer. The differences in infection with *Eubothrium* and *Anisakis* in wild and escaped salmon may possibly be explained by assuming that some escapees feed in fjords and near coasts before they disperse on the high seas.

INFRASOUND SENSITIVITY IN A PARASITIC COPEPOD

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Objective: The parasitic copepod *Lepeophtheirus salmonis*, the salmon louse, is one of the major pests in salmon farming. By feeding on mucus, skin and blood of salmonids, it causes skin damage and secondary infections. The cues that trigger infestation by parasitic copepods are largely unknown. We wanted to determine if simulations of fish-generated water movements could trigger responses that may bring the salmon louse in contact with the host.

Materials and methods: The responses of salmon louse copepodids to uniform, linear accelerations at 1, 3, 5 and 10 Hz frequency was videotaped and analysed. The animals were stimulated in a completely water-filled, clear perspex chamber, which was suspended like a swing in four wires from a steel frame. The chamber was moved by a vibrator which was fed amplified sine curves from a function generator.

Results: On stimulation, about 40% of the copepodids responded by executing swimming bursts of 1.3 seconds duration. There was no apparent preferred swimming direction. Sensitivity, as measured by the behavioural response, was highest at 3 Hz, with a threshold value of 5.10^{-3} ms^{-2}(rms). However, at 1 and 5 Hz, thresholds were less than 6 dB higher.

Conclusions: The results indicate that the copepodids may react to near-field accelerations produced within centimetres of a swimming fish. Acceleration sensitivity could therefore be the cue that triggers high-speed swimming and subsequent infestation of the host.

PARASITES OF PARR OF ATLANTIC SALMON (*Salmo salar* L.) FROM THE ALTA RIVER, NORWAY - A PRELIMINARY REPORT

By T. LARSEN & F.R. LUND

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Objectives: The purpose of this study is to describe the parasite fauna and the health status of salmon parr from the Alta river.

Material and methods: Atlantic salmon (2+) were examined by Dogiel complete technique supplemented by bacteriological sampling, differential counting of blood cells, histology and measurements of hematocrite and osmolarity.

Results: Ten parasitic species, one viral disease (papillomatosis) and one unknown strain of non-pathogen bacteria are found. The parasites included five protozoan, two digenean, one monogenean and two nematode species. *Tetraonchus alaskensis* (Monogenea) and *Ichtyobronema hamulatum* (Nematoda larva) are not previously reported from parr of Atlantic salmon. There were no signs of serious diseases and no correlation between any physiological/haematological parameters and the intensity of the various parasites.

Conclusion: A preliminary list of parasites of parr from the Alta river is presented. The preliminary results indicate no signs of parasitic diseases.

Tetrahymena sp. (CILIOPHORA, HYMENOSTOMATIDA) CAUSING HEAVY MORTALITIES IN CULTURED NORWEGIAN JUVENILE TURBOT (*Psetta maxima*)

By A. LEVSEN & G.A. BRISTOW

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Objectives: The aim of the present study was to identify the causative agent of heavy mortalities among reared juvenile turbot in south Norway during autumn 1996.

Materials and methods: Formalin-fixed samples of moribund juvenile turbot were sent to us and examined for parasites. All measurements and observations so far were done as a) stained histological sections and b) squash preparations and thin smears of various organs temporarily mounted in glycerol and examined using interference-contrast microscopy. Numerous smears were air dried for silver impregnation and samples were taken for further studies using SEM.

Results: The examination revealed heavy infection with a ciliophoran parasite in all organs and body parts. Ciliated trophozoites showed highest infection level in visceral organs including swim bladder, and in the eyes. Non-ciliated reproductive cysts (early

stage) containing at least eight tomites were found mostly in the CNS and connective tissue close to the gill cartilage. All measurements and observations are in accordance with the original description of the genus *Tetrahymena* Furgason, 1940. Preliminary histopathological examination of fins and gills revealed severe tissue damage, especially haemorrhages, hyperplasia and hypertrophy in the gills. Little or no host reaction due to cysts was observed in the CNS.

Conclusion: *Tetrahymena* sp. was identified as the causative agent of heavy mortalities among reared juvenile turbot in Norway. This finding represents a) a first report of a parasitic *Tetrahymena* from Norway and b) apparently a first case of *Tetrahymena* parasitizing a marine fish. Further examination using SEM and silver impregnation will be done for additional elucidation of its taxonomy.

HEAVY MORTALITIES IN CULTURED NORWEGIAN JUVENILE TURBOT (*Scophthalmus maximus*) CAUSED BY A CILIATE

By A. LEVSEN[1], T.A. MO[2] & G.A. BRISTOW[1]

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[2]*National Veterinary Institute, PB 8156 Dep., 0033 Oslo 1, Norway*

Objectives: The aim of the present study was to identify the causative agent of heavy mortalities among reared juvenile turbot in south Norway during autumn 1995 and 1996.

Materials and methods: Live and formalin-fixed samples of moribund juvenile turbot were examined. Observations and measurements were made on live and fixed parasite specimens in smears from various organs. Fixed parasites were temporarily mounted in glycerol and studied in an interference-contrast microscope. A few turbots were fixed in glutaraldehyde and studied in SEM. Pathology was studied in a large number of HE stained histological sections from several turbots.

Results: The histological examination revealed heavy infections with a ciliophoran parasite in all organs and body parts. In the most heavily infected turbots, most internal organs, but especially skeletal muscle and CNS, had been "replaced" by parasites. Little or no host reaction due to cysts was observed in the CNS. Examination of the fins and gills revealed severe tissue damage, especially haemorrhages, hyperplasia and hypertrophy, especially in the gills. Ciliated trophozoites showed highest infection level in visceral organs including swim bladder, and in the eyes. Reproductive cysts (early stage) containing at least eight tomites were found mostly in the CNS and connective tissue close to the gill cartilage. Several measurements and observations on the parasites are in accordance with characters of the Hymenostomatida while others indicate a Scuticociliatida.

Conclusion: This is the first report of a ciliate causing high mortality among juvenile farmed turbot in Norway. The taxonomic position of the parasite is still uncertain and further studies of parasites in SEM and silver impregnated smears are in progress.

DOES HOST FINDING BEHAVIOUR OF THE *Cryptocotyle lingua* CERCARIAE AFFECT THE TRANSMISSION IN NATURAL ENVIRONMENTS?

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Atlantic cod, *Gadus morhua*, were caged at increasing distance from a rocky shore where natural infections of the digenean *C. lingua* occurred in the snail *L. littorea.* In each cage the fish were kept at two different depths in the sea. During a six month period the fish living close to the surface accumulated significantly more cysts than those at deeper waters. The distance from the shore, however, did not affect the number of metacercariae in the cod. We conclude that host finding behaviour of cercariae seems to influence the infection pattern under natural conditions, including both transmission from snail to fish and probably also transmission to fish-eating birds as final hosts. The results also show that within the scale studied here, cercarial infectivity does not decrease with the distance from the shore.

HOST SEARCHING BY *Argulus foliaceus* L. (CRUSTACEA: BRANCHIURA): THE ROLE OF VISION AND SELECTIVITY

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In laboratory experiments, the swimming behaviour of the ectoparasitic crustacean *Argulus foliaceus* and its infection rates on juvenile perch (*Perca fluviatilis*) and roach (*Rutilis rutilis*) were examined. The highest infection rate and preference for perch juveniles were obtained in the dark, while the lowest infection rate and lack of preference were found in the light when aquaria with glass walls (high reflectivity) were used. In the light, when aquaria were lined with black plastic inside (low reflectivity) intermediate levels of infection for perch and the highest for roach were recorded. Under such conditions roach were significantly more heavily infected that perch; in fact

the attack rate was four times higher for the brighter (more reflective) roach juveniles than for perch. Within the aquaria with a low reflective interior, parasites swam at only a quarter of their speed in highly effective aquaria and were observed predominantly in the central area rather than close to the walls. The primary role of visual stimuli in the host search behaviour of *A. foliaceus* in the light is suggested. Parasites can effectively use visual stimuli only in low reflective surroundings. Highly reflective glass aquaria walls produce numerous secondary local light sources, which cause fast and erratic parasite movements and tend to prevent the efficient location of potential hosts.

PROTOZOAN AND MYXOSPORIDIAN PARASITES OF WILD-CAUGHT ATLANTIC HALIBUT FROM SOUTH-EAST NORWAY

By T.A. MO & C. APPLEBY

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Objective: The objective was to study the occurrence of protozoan and myxosporidian parasites from wild-caught Atlantic halibut (*Hippoglossus hippoglossus*), and to evaluate their potential as possible pathogens for artificial rearing of halibut in the future.

Materials and methods: Adult halibut between 2.3 and 16.5 kilos were caught in gill nets and trawl. Some were kept alive until examination, while others had to be killed and shipped on ice to the laboratory. All external and internal organs were examined by visual inspection and with a dissecting microscope.

Results: So far we have found two (possibly three) myxosporidian species but no protozoans. All myxosporidians were found in the gall bladder. *Ceratomyxa drepanopsetta* has been found in every fish examined, but in some fish only pseudoplasmodium stages have been observed. *Myxidium gadi*, or possibly a similar species, was found in most fish examined. A third "species" with three polar capsules has been found. However, this could have been deformed *C. drepanopsetta* spores.

Conclusion: Based on recent knowledge that myxosporidians need an intermediate host, production of halibut in closed cages or tanks should exclude these parasites as potential pathogens except if the inlet water is taken from an area where the intermediate hosts occur.

THE OUTPUT OF POLISH ICHTHYOPARASITOLOGY WITHIN THE PAST 23 YEARS

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The basic published aid for Polish fish parasitologists has been for years the catalogue compiled by J. Grabda "Parasites of cyclostomates and fishes". This catalogue appeared in 1971 and covered the records of parasites of Polish fish up to 1970. It listed 225 different parasite species found in 74 fish and one cyclostomate. Prompted by the need for updating the records, the present authors attempted to gather the relevant information on Polish fish parasites published within the period from 1970-1993. They collected nearly 200 publications and figured out that parasitic surveys on fish in Poland hitherto have been performed on 83 fish species. Out of this number, 67 were studied within the past 23 years, while 23 only before 1970. Nine fish were studied only in the past two decades. A total of 432 parasites has been recorded from Polish fish. Out of this 360 occurred in the publications from the last 23 years and 72 were found before 1970. The recent period of 23 years yielded a total of 203 parasite species new to Polish fauna. In this number 16 were new to science. In 141 cases parasites were found to infect hosts which have not been attacked by them in Poland. A total of 51 new host records has also been reported. Updating the years 1994-1996 is now in progress.

THE IMPACT OF WATER TEMPERATURE ON THE MORTALITY OF TROUT (*Salmo trutta* m. *lacustris* (L.)) CAUSED BY *Diphyllobothrium dendriticum* PLEROCERCOIDS

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Objective: A cestode, *Diphyllobothrium dendriticum,* is one of the very few helminth parasite species which occurs commonly and has been demonstrated to kill salmonid fish both in the wild and at fish farms. The method of running the entire life cycle in the laboratory is known, which makes *D. dendriticum* a valuable tool for studying a poorly known area: the influence of parasites on fish populations. The aim of the present experiment was to study the impact of water temperature on the mortality of the fish intermediate host caused by *D. dendriticum* plerocercoids.

Materials and methods: Brown trout (*Salmo trutta* m. *lacustris*) aged 1+ were used as intermediate hosts. A dose of 15 copepods, representing 7-10 procercoids, was intubated into the stomach of each fish in a drop of 0.3% (w/v) pepsin in physiological saline (0.9%), pH 2. The experimental design included four treatment aquaria with heated water and four with non-heated water. In addition, two heated and two non-heated aquaria served as control aquaria to monitor mortality other than *D. dendriticum* induced. Control fish received only a drop of 0.3% pepsin solution. Each aquarium contained 17 fish: altogether 136 infected and 68 control fish. Water temperature was 11.5°C in all aquaria on the day of infection. The following day, the temperature was increased to approximately 15°C in every second aquarium. The experiment was terminated after three months.

Results and conclusions: Results and conclusions on the impact of temperature on mortality induced by *D. dendriticum* will be presented.

MAIN HELMINTH ASSOCIATIONS IN FISH IN SOME SALMONID SPAWNING RIVERS

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Objective: The purpose of this study was to acquire a concept of the compositional stability of infestation by helminths important for the stock of salmonids in Estonian spawning rivers discharging into the Gulf of Finland.

Materials and methods: About 4,100 specimens of 14 species of fish (mainly sea trout, pike, perch, bleak, stickleback (nine- and three-spined) and bullhead from 12 spawning rivers located in the southern Gulf of Finland basin were analysed by method of total parasitological autopsy in 1985-96. The majority of those specimens of fish were also analysed trophologically. Additionally, a number of samples of forage invertebrates from those rivers (copepods, amphipods, other crustaceans, water insects, molluscs, etc.) were analysed parasitologically yearly in the same period. The main part of the material was obtained in stationary stations on rapids by electrofishing in May-June and August-September. In all 12 rivers under surveillance, sea trout spawn regularly and six of those rivers are the only ones in Estonia where salmon spawn.

Results: Fish in rivers investigated are parasitized by 28 species of parasites important for O+ - 1+ salmonids. The main ones are the species belonging to the F. *Urceolaridae (Ciliophora)* and helminths of the g.g. *Cyatocephalus Diphyllobothrium, Proteocephalus (Cestoda), Bunodera, Crepidostomum, Phyllodistomum (Trematoda), Rhabdochona, Cystidicoloides, Capillaria (Nematoda)* and *Acanthocephalus, Metechinorhynchus (Acanthocephala).* The composition of associations of helminths has been very stable in 1985-96 in turbot (acanthocephalans

and trematodes in the intestine; *Phyllodistomum* sp. sp. in urinary-bladder), bullhead (*Bunodera*, acanthocephalans), sea trout (1+ : either *Bunodera* and nematodes or trematodes and acanthocephalans). Helminth associations of labile composition were observed in bleak, pike and stickleback. The two latter species have been the main supplementary and paratenic (pike) hosts for diphyllobothrids in the area. The helminth associations in perch have been comparatively stable in composition only in typical small salmonid spawning rivers (trematodes, nematodes, acanthocephalans). In all rivers investigated, the prevalence of proteocephalans and *Cyathocephalus* infestations, comparatively high in the 1980s, decreased considerably in the early 1990s. The helminth associations in 1+ sea trout are very particular for certain rivers: *Bunodera luciopercae* and *Cystidicoloides tenuissima* develop obviously two generations *per annum* (in spring and in autumn). Cestodes highly pathogenic for salmonids (*Diphyllobothrium, Cyathocephalus*) occur mainly in stickleback in spring and in pike in autumn. The young salmon are practically free from helminths in most spawning rivers.

Conclusion: The helminth associations in the Estonian salmonid rivers of the Gulf of Finland basin are very well established and have been compositionally quite stable from 1985-95. The most stable are helminth associations in sea trout.

THREE MAJOR CHANGES IN THE PARASITOFAUNA OF FISH IN THE COASTAL WATERS OF ESTONIA IN THE EARLY 1990s

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Objective: The aim of this study was to emphasise three trends of radical changes in the parasitofauna of cyprinids and percids in 1990-96. These trends have developed simultaneously in the coastal waters of Estonia, but never occurred in the same fish in the inland water bodies.

Materials and methods: Eight species of cyprinids (mainly roach, ide, bleak and silver bream) and three species of percids (mainly perch), altogether about 7,400 specimens from the coastal waters and additionally about 3,200 specimens of the same fish from inland water bodies of Estonia were analysed in 1978-96 by total parasitological autopsy (*in sensu* V.A. Dogiel). The main materials were obtained twice a year from spawning migrant schools in spring and August-October periods.

Results: In 1978-86 the parasitofauna of cyprinids and percids have been comparatively stable in the composition of species (about 230 species of parasites from 13 classes of invertebrates) as well as in the rates of the parameters of infestation.

From 1986 on, a number of trends occurred in changes in the associations of parasites of those fish of which the following are by now well established in the coastal waters:
1) A considerable decrease in the number of species and the rate of prevalence of myxosporidians parasitising in the gall or urinary bladder (g.g. *Chloromyxum, Zschokkella, Myxidium*) and in the same time a very obvious increase in the rates of infestation by muscle, gill and connective tissue myxosporidian parasites (mainly g.g. *Myxobolus* and *Henneguya*).
2) Rather drastic decline in the fauna of Monogeneans: of 29 species which have been quite common in the early 1980s, 22 species (mainly g. *Dactylogyrus*) became eventually rare from 1986 and 12 species have not occurred in analyses at all in the 1990s.
3) The position of the dominant species in the infestation by trematode metacercariae has shifted from g.g *Diplostomum* and *Thylodelphys* to g. *Posthodiplostomum* (*P. cuticula m.* in cyprinids and *P. brevicaudatum m.* in percids). In the 1980s the prevalence of the latter did not exceed 1.5%, but in 1993-96 its rating was between 23% and 56%. No such changes have occurred in fish in the inland waters.

Conclusion: In our opinion the simultaneous changes in the infestation by so different parasite species as described above could be caused mainly by the factors connected with the state of populations and the behaviour of hosts - in this case fish.

VETERINARY PARASITOLOGY

SUPPRESSION OF SHEDDING OF *Toxoplasma gondii* OOCYSTS BY TOLTRAZURIL TREATMENT OF CATS

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Three trials were conducted to evaluate the efficacy of toltrazuril to suppress excretion of oocysts of *Toxoplasma gondii* by cats. The drug was applied daily via conventional cat food from day 1 post infection onwards in a dose of 5 mg per kg body weight (b.w.). The cats were housed in individual steel cages throughout the experimental period. During the experimental period the complete individual faeces were collected daily and examined for the presence of oocysts of *T. gondii*.

Trial 1: Twelve cats were infected by feeding them infected mice (10 g of cyst-harbouring mice/kg b.w.). Six cats were treated subsequently with toltrazuril, the other

six cats served as non-treated controls. All cats seroconverted within four weeks post infection. The non-treated cats excreted 4.33×10^5 to 3.40×10^7 oocysts after a prepatent period of three to eight days. Patency lasted for four to nine days. In contrast, none of the treated cats excreted any oocysts within the experimental period of four weeks.

Trial 2: Eleven cats that were (6 cats) or were not (5 cats) treated with toltrazuril after a primary infection with cysts of *T. gondii* were subjected to a secondary infection 10 weeks later by feeding them cyst-harbouring mice. Toltrazuril was not given after the secondary infection. During the investigation period of four weeks no oocysts were found in the faeces of any of the re-infected cats.

Trial 3: Twenty-four cats were infected by oral inoculation of 2.5×10^5 sporulated oocysts of *T. gondii.* Twelve cats were treated with toltrazuril during the whole experimental period of eight weeks, the other 12 cats served as non-treated controls. All non-treated cats seroconverted while seven out of 12 treated cats remained serologically negative. Four non-treated cats excreted 2.57×10^5 to 1.98×10^7 oocysts after a prepatent period of 20 to 43 days. Patency lasted for six to nine days in these cats. All treated cats remained coproscopically negative. Thus it can be concluded that treatment with toltrazuril via medicated feed is suited to suppressing oocyst excretion by cats irrespective of the mode of infection. The development of immunity against consecutive infections with cysts is not impaired by toltrazuril treatment.

THE EFFECT ON FEMALE *Oesophagostomum dentatum* FECUNDITY OF THREE DISTINCT SEX RATIOS AT LOW AND HIGH WORM POPULATION DENSITIES

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To study details of the population biology of *Oesophagostomum dentatum* in pigs a new non-surgical worm transplantation technique was developed. The pig is sedated and a set of pvc tubes is inserted into the rectum. The worms are then pushed with air through the tubes into the distal colon from where they migrate to their predeliction site. To examine the effect of different sex rations on worm fecundity, initially helminth-free "donor" pigs were inoculated with infective third stage larvae of *O. dentatum.* Five weeks later the donor pigs were killed to recover the adult worms. To six groups of helminth-free "recipient" pigs were then transplanted different female to male ratios (FMR) of *O. dentatum* (1:9, 5:5, 9:1) at either high (300 worms) or low (30 worms) doses. Faecal egg excretions (eggs per gram faeces - EPG) were measured once weekly following transplantation until the pigs were killed four weeks post transplantation to

assess the worm burdens. At the low dose level, equal sex rations gave rise to significantly higher EPG than at the 1:9 FMR. Due to high variability in egg counts there were no significant differences between the other groups. In the low dose 9:1 FMR group, the female worm fecundity (last EPG/number of female worms) was significantly lower than in the equal ratio group. This study suggests an upper limit for the FMR at very low worm densities, above which *O. dentatum* females produce fewer eggs.

DIFFERENTIAL EFFICACY OF LOW DOSE FENBENDAZOLE (0.35 mg/kg) AGAINST *Hyostrongylus rubidus, Ascaris suum* AND *Oesophagostomum* spp. IN THE PIG

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The objective of this study was to examine the effect of low dose fenbendazole (0.35 mg/kg) against *Hyostrongylus rubidus, Ascaris suum* and *Oesophagostomum* spp. in the pig. Two experiments (experiment A and experiment B) were carried out. In experiment A pigs were inoculated with a mixture of *H. rubidus* and *Oesophagostomum* spp. third stage larvae. Using a modified McMaster technique excretion of helminth eggs in faeces was determined regularly until termination of the experiment. Larval cultures of faeces were set up of each faecal sample to differentiate between *H. rubidus* and *Oesophagostomum* spp. and larvae were harvested by a Baerman technique after two weeks of culture at room temperature. On day 27 post inoculation (PI) pigs were allocated into two comparable groups on the basis of eggs per gram (EPG) in faeces. One group of pigs was treated orally with 0.35 mg/kg of fenbendazole and the other group was left non-treated. On day 34 PI all pigs were slaughtered and the gastrointestinal tract was removed for worm recovery. Experiment B had a similar design, sampling and laboratory procedures as in experiment A, except that the pigs were inoculated with infective eggs of *Ascaris suum* and infective third stage larvae of *Oesophagostomum* spp. In experiment A pre-treatment samples were composed of 18% *H. rubidus* and 82% *Oesophagostomum* spp., whereas post-treatment samples from the day of slaughter contained >99% *H. rubidus*. Non-treatment control pigs harboured a mean of 821 *H. rubidus* and 660 *Oesophagostomum* spp. and in the treated group a mean of 681 *H. rubidus* was detected and no *Oesophagostomum* spp. worms were recovered. In experiment B *Oesophagostomum* spp. eggs disappeared after treatment and excretion of *Ascaris suum* eggs was not

affected. In the non-treated group *Ascaris suum* and *Oesophagostomum* spp. worms were recovered, whereas in the treated group only *Ascaris suum* worms were found.

THE EFFECT OF THE NEMATODE-TRAPPING FUNGUS *Duddingtonia flagrans* ON FREE-LIVING STAGES OF HORSE STRONGYLES - AN EXPERIMENTAL PILOT STUDY

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Objective: The aim of this study was to determine the ability of the nematode-trapping fungus *Duddingtonia flagrans* to reduce the transmission of infective larvae of horse strongyles from experimentally deposited dung onto surrounding herbage.

Material and methods: At three different times (June, July, August) during the grazing season 1995, three groups of horses naturally infected with strongyles were fed three different doses of *D. flagrans* spores, while a fourth group remained as non-fungal fed control. Faeces from all four groups were deposited as artificial dung pats on a parasite-free plot. During two months, every second week after deposition, grass from specified areas surrounding each dung pat was cut, and the number of infective larvae on the herbage was determined. Additionally, faecal cultures from all groups of horses were set up to evaluate the larval reduction capacity of the fungus *in vitro.*

Results: After a dry period that apparently diminished the larvae transmission, the number of infective larvae escaping from dung pats to herbage was significantly reduced from mid August onwards. Faecal cultures showed that *D. flagrans* survived the passage through the animals and was able to significantly reduce the number of infective larvae developed *in vitro.*

Conclusion: This work has demonstrated the ability of *D. flagrans* to act as biological control agent against nematodes, reinforcing the idea that the use of nematode-trapping fungi is a useful tool in an integrated strategy to control nematode parasites.

THE ANALYSIS OF CERCARIAL DERMATITIS IN CENTRAL EUROPE

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Larval stages of bird schistosomes are the causative agent of cercarial dermititis in Europe. It seems that the number of clinical cases increased during the last few years. The aim of the study was to analyse some factors influencing the occurrence of cercarial dermatitis in Central Europe with special attention to the Czech Republic such as the density fluctuation in populations of schistosome intermediate hosts and the prevalence of infection with larval and adult parasites in intermediate and definitive host respectively. The influence of environmental conditions on the occurrence of human infection is discussed. An examination of 53,464 snails (12 species belonging to the families Lymnaeidae, Planorbidae, Physidae and Hydrobiidae) resulted in findings of *Trichobilharzia szidati, T. franki* and *Bilharziella polonica* larval stages during the period of 1988-1996. An examination of 2,595 birds (14 species belonging to the orders Anseriformes, Podicipediformes, Ralliformes and Lariformes) resulted in detection of adult schistosomes of *B. polonica, Dendritobilharzia pulverulenta, Gigantobilharzia acotylea* and *Ornithobilharzia canaliculata* during the period of 1960-1995.

During the period of 1985-1996 a total of 139 cases of cercarial dermatitis were diagnosed either by serological reaction or by clinical symptoms of patients with a swimming history in the water bodies where larval stages of schistosomes were found.

EFFECTS OF STRATEGIC TREATMENTS WITH IVERMECTIN ON SET-STOCKED CALVES NATURALLY EXPOSED TO TRICHOSTRONGYLID IN LITHUANIA

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Objective: The study was carried out to examine the effects of strategic treatments with ivermectin of first season grazing calves exposed to trichostrongylid nematodes on naturally contaminated pasture.

Methods: Seventeen first season calves were divided into two groups according to live weight and on May 22 each group was transferred to a 1 ha pasture plot. Group A was treated with ivermectin at weeks 3-8-13 after the start of the grazing season, while group B served as untreated controls. Heifers were weighed, clinically inspected and faecal, blood and grass samples were collected every two weeks.

Results: Following the second treatment the egg counts from the treated calves dropped to zero while controls continuously excreted trichostrongyle eggs. Pasture larval counts in the plot grazed group B rose gradually in the first part of the summer, followed by a steep rise towards the end of July. In contrast, the numbers of larvae recovered from the group A plot remained low throughout the season. Both groups showed comparable weight gains from late May up to the middle of July. Subsequently, the gains of group B diminished while those of group A increased markedly. Most of the untreated calves showed mild clinical signs of parasitic gastroenteritis from the end of July onwards.

Conclusions: The results showed that the treatments significantly reduced the animals' trichostrongylid loads towards the end of the season. The egg counts and herbage larval counts clearly demonstrated that this had been accomplished through suppression of contamination and hence pasture infectivity.

Ascaridia galli POPULATIONS IN CHICKENS FOLLOWING SINGLE INFECTIONS WITH DIFFERENT DOSE LEVELS

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Objective: The purpose of this study was to examine the influence of different levels of experimental *Ascaridia galli* infections on population size, individual worm size, sex ratio and fecundity of female worms.

Materials and methods: Three groups each consisting of 20 day-old Lohman Brown chickens were orally infected with doses of 100, 500 or 2500 embryonated *A. galli* eggs, respectively. Eight weeks later counts (eggs/gm faeces (EPG)) were determined for all animals prior to slaughter. The gastrointestinal tracts were examined for the presence of adult and immature stages of *A. galli*.

Results: All groups had roughly similar worm burdens, hence significantly different establishment rates, *i.e.* 14.2%, 2.9% and 0.5%, respectively. A significantly lower mean female worm burden was seen in the high dose group (p=0.02) which also showed a significant lower egg excretion (p=0.01). However, fecundity (EPG per female) was not significantly different between the groups (p=0.55). The mean lengths of adult worms as well as the weight of the mean worm burdens were significantly smaller in the high dose group.

Conclusion: This study demonstrated that single infections with varying doses of *A. galli* eggs influenced the establishment rate, sex ratio, egg excretion, worm size and weight, but not the fecundity.

THE IMPACT OF FASTING ON *Ascaris suum* AND *Oesophagostomum* spp. IN PIGS

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Objective: This investigation was conducted to study the possible influence of fasting on *Ascaris suum* and *Oesophagostomum* spp. infection in growing pigs.

Materials and methods: Young crossbred pigs naturally infected with *A. suum* and *Oesophagostomum* spp. were used. In one experiment 10 pigs were fasted and offered water *ad libitum* for six days. In another experiment, the pigs were fasted for 10 days. These pigs, together with non-fasted control pigs were slaughtered and worm numbers, worm location, sex, developmental stage and female worm fecundity were determined.

Results: Pigs fasted for 10 days had decreased numbers of *A. suum* and *Oesophagostomum* spp. at slaughter vs. controls and worms were found in more distal locations in the gastrointestinal tract. Fasting for both six and 10 days significantly lowered the fecundity of both worm species.

Conclusion: The present study indicates that fasting for six and 10 days leads to unfavourable conditions for *A. suum* and *O. dentatum* in the gut of the pig.

LEVAMISOLE RECEPTORS FROM SENSITIVE AND RESISTANT *Oesophagostomum dentatum*: A PATCH STUDY

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Objective: To characterise changes in single-channel properties of levamisole receptors associated with resistance.

Materials and methods: Adult female *O. dentatum* were recovered from pigs at slaughter following experimental infection with either levamisole sensitive or levamisole resistant strains in Denmark and shipped overnight to Scotland for electrophysiological examination. Muscle membrane vesicles were prepared and single-channel recordings were made using the cell-attached patch configuration with levamisole in the patch pipette.

Results: In the sensitive strain, single-channel currents conductances in the range 24.6-57.7 pS were seen. Mean open-times were in the range 0.98-4.43 ms. There were two main channel substances: one type *G35* had a mean conductance of 35.2 pS and open-time of 1.6 ms; another subtype, *G45*, had a mean conductance of 44.6 pS and open-time of 2.7 ms. A model for the heterogeneous population of levamisole receptors is proposed. Levamisole activated channel currents were more difficult to record from levamisole-resistant strains. Preliminary analysis suggests that they occurred less frequently in patch recordings and had mean open-time less than the *G45* subtype.

Conclusion: The patch-clamp can be used to study changes in the properties of levamisole receptors associated with resistance.

TRANSMISSION DYNAMICS OF *Ascaris suum* AND *Trichuris suis* IN OUTDOOR PIGS

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Objective: In Denmark, alternative outdoor production systems for pigs are becoming more frequent and information on the transmission of *Ascaris suum* and *Trichuris suis* under continuously grazed pasture conditions is needed.

Materials and methods: A group of pigs was turned out on a pasture in May 1993 (Year One of the study), inoculated with 200 eggs of *A. suum* and 1000 eggs of *T. suis*, and followed parasitologically. A non-experimentally infected group of pigs was similarly turned out on the same pasture the following year (Year Two) and again followed parasitologically. Pasture infectivity was measured using helminth naïve tracer pigs.

Results: During the summer of year 1, *A. suum* eggs became infective within 4-6 weeks on the pasture. However, transmission was moderate until August of Year Two, when a pronounced increase in transmission occurred. After two months on the pasture, the continuously exposed pigs in summer seasons of both Years One and Two harboured small overdispersed populations of adult *A. suum*, moderate numbers of liver white spots and high specific IgG responses. These parasitological measures on chronically exposed pigs did not, however, correlate well with pasture infectivity or with each other. In contrast, the liver inflammation and specific IgG responses (but not the intestinal *A. suum* burdens) of the tracer pigs were highly correlated (p=0.0001) and appeared to better reflect pasture infectivity. The inoculated pigs excreted *T. suis* eggs by late summer of Year One, but no transmission took place before August of Year

Two. Thus, the *T. suis* population of infected eggs built up very slowly. The results indicate that *T. suis* eggs may survive for considerable time, however.

Conclusions: The study results revealed that *A. suum* and *T. suis* eggs are much more resistant to environmental factors than free-living infective larvae of pig parasites such as *Oesophagostomum dentatum* and *Hyostrongylus rubidus.* Control of these parasites in outdoor systems will present more difficult challenges than that for parasites transmitted by free-living larvae.

TRANSMISSION DYNAMICS OF *Oesophagostomum dentatum* AND *Hyostrongylus rubidus* IN OUTDOOR PIGS

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Objective: An increase in alternative outdoor pig production systems is occurring in Denmark and this study was designed to elucidate the transmission patterns of *Oesophagostomum dentatum* and *Hyostrongylus rubidus* in pigs allowed to graze continuously on a pasture.

Materials and methods: A group of pigs were turned out in May 1993 (Year One of the study) and subsequently inoculated with low numbers of both helminths. These pigs were followed parasitologically until October by serial necropsy and sampling of faeces, grass and soil. A non-inoculated group of pigs was similarly followed on the same pasture in Year Two (1994). Pasture infectivity was measured using helminth naïve tracer pigs during all seasons.

Results: The pasture vegetation was rapidly destroyed by the pigs, resulting in a dirt lot by the autumn of Year Two. The area was soon contaminated with eggs, resulting in heavy pasture infectivity and increasing worm burdens in late summer, whereafter the numbers of larvae declined markedly. In May of Year Two, newly exposed pigs became only lightly infected (mostly *O. dentatum*), and no transmission was observed in July-August of Year Two, probably due to an unusually dry summer and a lack of protecting vegetation. The results indicate that both *O. dentatum* and *H. rubidus* are very sensitive to environmental factors, because significant transmission occurred only under the most favourable conditions (summer combined with protecting vegetation as in Year One). Transmission was severely reduced during the low temperatures experienced during the winter between Years One and Two and during the summer of Year Two, when vegetation was lacking.

Conclusions: Continuous grazing actually reduced transmission of *O. dentatum* and *H. rubidus* because of the reduction in vegetation. This, however, is not a desirable

alternative farming system, because of its adverse environmental effects. This environmental impact may be mitigated by employment of a pasture rotation system in place of continuous grazing.

EFFECTS OF STRATEGICAL TREATMENTS WITH IVERMECTIN ON CALVES NATURALLY EXPOSED TO TRICHOSTRONGYLIDS IN INTENSIVE CALF-REARING IN LITHUANIA

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Objective: This study was carried out to examine the effects of strategic treatments with ivermectin on calves naturally exposed to trichostrongylid infection in small outdoor pens in intensive calf-rearing systems. These are characterised by very small outdoor plots, less than 0.1 ha per calf and a hut in which the calves are fed an almost full diet comprising concentrate, fresh cut grass and hay.

Materials and methods: Twenty heifer calves (5-8 months of age) were divided into two groups according to the live weight. Calves in group A were treated with ivermectin at weeks 3-8-13 after start of the outdoor season (1 June) while group B served as untreated controls. With regular intervals, blood, faeces and herbage samples were collected for laboratory analyses. The larval infectivity in the grass of the small pens was monitored using parasite naïve tracer calves for two weeks starting on 30 September.

Results: Following the first treatment the egg counts from the treated calves dropped to zero while controls continuously excreted trichostrongylid eggs. Herbage larval counts in all the pens were comparable till the end of July. Subsequently, there was a steep rise in August and onwards in "untreated" pens. The numbers of larvae recovered from group A pens remained low throughout the season. Both groups showed comparable weight gains during June and July. However, the gains in group A were higher from August till the end of the study.

Conclusions: Higher weight gains, presumably explained by reduced herbage larval counts due to ivermectin treatments, suggest that the intensive calf-rearing systems in Lithuania may be confronted with severe trichostronglyid problems.

FIPRONIL, A NEW INSECTICIDE

By M. van MAANEN

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Fipronil is a new active of a new family of insecticides called phenyl pyrazoles. Rhône Poulenc scientists working at the Rhône Poulenc research station at Ongar in England discovered Fipronil and identified its insecticidal properties in 1987.

Fipronil, used at low doses, is a highly effective insecticide against a broad range of insect pests in agriculture, animal health and public health.

Fipronil has a unique mode of action, different from other insecticides on the market. It is a potential blocker of the GABA (gamma-aminobutiric acid) regulated chloride channel, interfering with the central nervous system (CNS). Because of its unique mode of action, Fipronil is effective in controlling insects resistant to commonly used insecticides.

Since 1987 the efficacy of Fipronil has been established by laboratory and field experiments and by commercial users. It is a versatile insecticide for use in many different markets at low dose rates whilst providing high level efficacy against target insect pests.

Fipronil is effective against a broad range of insect pests of various crops including rice, cotton, sugarcane, sugar beet, sunflower, potatoes and corn, as a foliar spray, soil application or wood treatment.

Fipronil is also very effective for the control of various non-crop pests. At this time Fipronil is either already commercialised or under development for the control of fleas and ticks on cats and dogs, flies and ticks on cattle, in animal health, as for locusts, mosquitoes, flies, fleas, bedbugs, in public health and against household insects like cockroaches, ants and termites in PCO or OTC.

Schistosoma japonicum: A REVIEW OF ITS AGRICULTURAL IMPORTANCE

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Schistosoma japonicum, a zoonotic digenean trematode, is considered to be one of the most important helminth infecting humans in Southeast Asia, particularly in the People's Republic of China and the Philippines. In these areas domestic animals, including cattle, water buffaloes, dogs and pigs, also serve as definitive hosts of the parasite and play an important role in the transmission of *S. japonicum* to other animals

and humans. However, little attention has been directed at agricultural aspects of schistosomiasis japonicum in endemic regions in spite of the role that livestock play in transmission of the parasite and the effects of infection on their health and productivity.

The main route of definitive mammalian host infection with schistosomes is generally considered to occur by dermal penetration when the potential host enters cercarial infested bodies of water. Research reported in eastern literature has indicated that animals and humans may also become percutaneously infected with *S. japonicum* by walking through wet grass along river banks where amphibious *Oncomelania* snails, the intermediate host, are present. In addition, peroral infection from drinking water containing cercariae as well as transplancental congenital infections in livestock and humans have been reported.

Studies have shown that cattle, water buffaloes and pigs not only harbour *S. japonicum* but also manifest clinical signs of the disease and may die from infection especially during the acute phase of infection. This is especially true in areas where it is necessary to graze animals near lakes and canals. Abortions and stillbirths of livestock have also been associated with *S. japonicum* infection, further reducing their productivity.

DIAGNOSTICS

IDENTIFYING PARASITES BY PCR-SSCP OF rDNA

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Recent research has demonstrated that internal transcribed spacer rDNA (ITS) provides genetic markers for a range of parasites for diagnostic purposes and establishing systematic relationships. DNA sequencing, RFLP and cloning techniques are usually used to define genetic markers in spacer rDNA but they do not reliably detect sequence variation. Advances in DNA screening procedures have included the development and improvement of denaturing gradient gel electrophoresis (DGGE) and single-stranded conformational polymorphism (SSCP) techniques. In spite of their usefulness, such methods have not been exploited in molecular parasitology. In this study, we evaluated the use of PCR-SSCP to identify parasitic helminths to the species level. PCR-SSCP allowed a fingerprint of a species to be generated based on the existence of consistent differences in the ITS sequence between species ("interspecific heterogeneity") and low levels of variation within a species ("intraspecific homogeneity").

DIFFERENTIATION OF SPECIES AND STRAINS OF PORCINE *Oesophagostomum* spp. BY RAPD-PCR

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Although *Oesophagostomum* spp. are among the most common nematodes parasitizing pigs, virtually nothing is known about their genetic makeup. The purpose of this study was to create genetic fingerprints of different *Oesophagostomum* isolates by random amplified polymorphic DNA (RAPD) PCR and to create specific DNA markers for the differentiation of species and strains.

Total genomic DNA from ten laboratory strains and ten field isolates of porcine *Oesophagostomum* spp. (14 pure *O. dentatum*, one pure *O. quadrispinulatum* and five of mixed composition) were subjected to RAPD-PCR using 33 different random primers. The products were analysed by agarose-gel electrophoresis followed by ethidium bromide staining and photography.

Nineteen primers could amplify reproducible fingerprints for all the samples tested. Nine primers amplified specific polymorphic DNA markers: five *O. dentatum* markers and eight *O. quadrispinulatum* markers. Four primers produced markers specific for strains or groups of strains. These markers could be used to identify samples with mixed species composition and to delineate defined anthelmintic resistant and susceptible strains of *O. dentatum.*

It was shown that RAPD-PCR was able to reproducibly amplify fingerprints from different strains and isolates of porcine nodular worms. Band patterns were shown to be clearly distinct between species with all primers use. Intraspecific differences could be demonstrated with some of the primers, indicating that there is a certain degree of variation within *O. dentatum.* Specific polymorphic bands were considered as genetic markers for the delineation of species and strains.

A MODEL FOR THE TRANSMISSION PRESSURE OF TOXOPLASMOSIS TO DANISH CATS USING AGE-RELATED ELISA SEROPOSITIVITY

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As an alternative to faecal oocyst detection, the usefulness of serological data for estimation of the toxoplasmosis transmission pressure to populations of cats was investigated.

Sera obtained from 543 Danish cats of known age were tested in an indirect ELISA for antibodies to *Toxoplasma gondii*. Visual inspection of the distribution of OD-values for all age groups indicated an optimal separation between seropositive and seronegative individuals at OD = 0.55, which was close to the cut-off value of 0.58 determined by calculation of differential positivity rate (DPR) for the ELISA vs. Dye Test positivity obtained on a smaller serum panel. There was a marginal difference between the percentage seropositivity for cats of designated race and other cats (31 vs. 39%), but this was not reflected as a significant difference in the age-related increase of acquired infection. Modelling transmission pressure was performed assuming an equal infection risk for the known categories of cats and assuming a constant infection pressure over the last two decades.

Because the ln-transformed seronegative proportion of cats conformed reasonably to a linearly decreasing function of age, maximum likelihood estimation was performed for transmission pressure (λ) in the simple model for proportion seropositives (p) as a function of age (a):

$$p = 1 - \exp(-\lambda a)$$

The estimated value of λ was 0.0109/month with 95% confidence limits 0.0094 to 0.0126.

Further investigation of subpopulations of cats with more clearly defined restraints in hunting activity will be necessary in order to establish models for acquisition of infection and oocyst excretion in the Danish cat population.

Dictyocaulus viviparus LARVAE: MORPHOLOGY AND *in vitro* CULTIVATION

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Objective: The aim of this study was to provide further information about the optimal conditions for *in vitro* cultivation of *D. viviparus* larvae. Furthermore, characteristic features of the morphology of different larval stages of the bovine lung worm should be described.

Materials and methods: First stage larvae (L1) were collected from the faeces of experimentally infected calves and incubated in tap water at room temperature for at least seven days until development to third stage larvae (L3) was completed. L3 were exsheathed by incubation in 0.1625% NaOCl at 40°C for 5 or 10 minutes followed by three washing steps. Exsheathed larvae were *in vitro* cultivated in RPMI-1640 (GIBCO-BRL, pH 7,2) including 20% New Born Calf serum, 200 U/ml Moronal and 200 U/ml Penicillin/Streptomycin. Incubation was performed at 39.5°C at 5, 10, 20% or without CO_2 incubation. Development was measured by occurrence of the third sheath (3M) or development of the primordial vulva of the female larvae. The rate of *in vitro* cultivation was documented daily. *In vitro* cultured larvae were measured by image analysis system Q500MC (LEICA) and photomicrographed. Fifth stage larvae (L5) were collected by perfusion of the lungs of an experimentally infected calf at 16. d.p.i. and also photomicrographed. Inhibited L5 were collected after incubation of L3 for six weeks at 4°C and infection of a calf with those larvae followed by perfusion of the lungs at 15 d.p.i.

Results: Average exsheathment rates after incubation in 0.1625% NaOCl for five min were 83%, after 10 min incubation more than 93%. No further development was observed without CO_2 incubation. Average development rates at 5% CO_2 incubation were 8.33% (SD±7.76), 22.52% (SD±13.09) at 10% CO_2 and 38.01% (SD±15.63) at 20% CO_2. These differences were statistically significant. Uninhibited L5 were measured to be about ten times bigger than inhibited L5.

Conclusion: The influence of the CO_2 concentration for the development rates of *in vitro* cultured lung worm larvae is demonstrated. Morphological differences of inhibited and uninhibited L5 are documented for the first time and corresponding photomicrographs are presented.

EPIDEMIOLOGY

SOME PECULIARITIES OF BORRELIOSES FOCI FUNCTIONING IN THE BALTIC REGIONS OF RUSSIA: DISEASE NOSOFORMS, PATHOGEN GENOSPECIES AND VECTORS' BEHAVIOUR

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Studies of the borrelioses foci functioning in the regions of St. Petersburg and Kaliningrad Baltic beaches were carried out from 1992-1996. Erythema migrans (EM) with or without neurological syndrome was seen among patients from the Kurish Spit Region and from the recreation zone of the northern part of the beach of St. Petersburg on the Gulf of Finland. During the season of vector activity, the behaviour of *Ixodes ricinus* and *I. persulcatus* ticks collected by flagging and the level of infection by the different borreliae genospecies were compared with the occurrence of clinical symptoms. Only *Borrelia afzelii* were detected by PCR among all investigated adults and among 12 *I. ricinus* nymphs from the Kurish Spit. In 11 PCR-negative nymphs spirochetes were detected by dark field microscopy. It is thought that these spirochetes originated from birds. *B. afzelii* and other unidentified spirochete infected adult *I. ricinus* had the same or even higher moving activity as the control ones. The same picture was demonstrated in the St. Petersburg region for *I. persulcatus* infected by *B. afzelii* and *B. garinii*. The activity of ticks which had a dual *B. garinii* and *B. afzelii* infection was suppressed by these pathogens. Such kind of suppression might be the reason why only a limited number of patients (13%) had an EM accompanied by neurological symptoms, whereas dual infected *I. persulcatus* ticks in the St. Petersburg region were abundant (52%). Ticks with a mixed infection and patients with EM and neurological symptoms were seen in the middle and the last half of the *I. persulcatus* tick activity season (June-August).

HOST DENSITIES AS DETERMINANTS OF ABUNDANCE IN PARASITE COMMUNITIES

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Objective: Epidemiological theory predicts a positive correlation between host population density and parasite abundance across parasite communities harboured by different host species. The purpose of this study was to test this prediction.

Materials and methods: We used literature data on (i) abundance of gastrointestinal strongylid nematodes in mammals and (ii) population densities of the mammalian hosts, and controlled for confounding factors using data on relevant variables (*e.g.* host body weight) and using information on mammalian phylogeny.

Results: Host population density and average parasite abundance within host species were strongly positively correlated within mammalian taxa, and across all host species when the effects of host body weight were controlled for. Controlling for other variables did not change this. Positive relationships were seen also for single parasite species: when a parasite species is found in two closely related host species, its abundance is likely to be higher in the host species living at the higher density.

Conclusion: These patterns suggest that transmission rates are positively affected by host population density, which has implications for how we understand the processes structuring parasite communities and the role of parasites in more broadly defined natural communities.

EXPERIMENTAL DESIGN, POWER AND PROBLEMS IN FISH PARASITOLOGY

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Objective: This paper is to introduce the concepts of modern experimental design and power analyses with concrete examples from the literature as well as with examples of how the methods should be applied under varying conditions.

Materials and methods: Current publications in fish parasitology (and parasitology in general) indicate that fish parasitologists have not yet put in place the techniques that

modern experimental design and statistical analyses have made available to us. These techniques are particularly pertinent when trying to determine what is an adequate sample size to describe given host or parasite population, prior to beginning experimental work. They are also critical when trying to come to grips with β or type II error. The latter (Power analyses), when properly applied, can give direct estimates of the sample size needed to test hypotheses both within and between populations prior to beginning experiments, and tell us after the experimental work is complete how great a chance we had of finding significant differences with the design and sample size which we have used. This is particularly necessary when the results of one's experiments (natural or laboratory) do not allow one to reject the null hypothesis. These methods are applicable to both parametric and non-parametric statistical methodology and specific power analyses have been worked out for specific testing methods.

Conclusions: The application of modern experimental techniques coupled with power analyses is critical to the designing of experiments and testing of hypotheses in fish parasitology. The methods need to be adopted by all of us in the planning and testing of our ideas.

GEOPHAGY AS A RISK FACTOR FOR GEOHELMINTH INFECTIONS: A LONGITUDINAL STUDY AMONG KENYA PRIMARY SCHOOLCHILDREN

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A study on geophagy among primary schoolchildren and its impact on geohelminth infection was conducted in Nyanza Province, Kenya. Two hundred and four children in Standard 5 and 6 aged 10-18 years, 112 girls (54.9%) and 92 boys (45.1%), were interviewed about their soil eating habits and examined parasitologically for helminth infections at baseline. The children that were found initially uninfected or cured after treatment were followed up after 4, 8 and 11 months for reinfections. The 8 month follow-up included a survey on geophagy to provide data on the soil eating habits during the study period. Soil samples were collected with the geophagous children and examined for helminth eggs.

At baseline, a 77% prevalence of geophaagy was found. The children ate soil daily, most of them from the surface of termitaria. 48% of all samples were contaminated with eggs of *Ascaris lumbricoides*. The median intensity of contamination for the

positive samples waas three *Ascaris* eggs per 2 g soil (range 1-15). Few other helminth eggs were found. Twenty-nine (14.2%) oc the children were found infected with *A. lumbricoides*, 87 (42%) with *Trichuris trichiura*, 128 (62.7%) with hookworm and 162 (79.4%) with *S. mansoni*.

Significant associations between geophagy and infection with *A. lumbricoides* and *T. trichiura*, but not *S. mansoni* and hookworm were found at baseline. *A. lumbricoides* intensities above 1000 epg were only seen among the geophagous children (12.0 % *vs.* 0%; p=0.014). The reinfection study showed that geophagy significantly increased the risk of acquiring an infection with *A. lumbricoides*. The reinfection rate was twice as high among geophagous children as compared to non-geophagous children (28.8% *vs.* 14.3%; p=0.035). Furthermore, the median intensity of reinfections was much higher among geophagous children (642 epg *vs.* 4 epg; p=0.024). No significant differences in *T. trichiura, S. mansoni* or hookworm reinfection rate or intensity were seen.

It was concluded that geophagy is a major source of infection with *Ascaris* and possibly other geohelminths among primary schoolchildren. The cultural context of geophagy as well as nutritional causes and consequences should be investigated further in different societies, in order to provide a solid basis for improved health education on the prevention of geohelminth infection.

Pneumocystis carinii INFECTION IN APPARENTLY HEALTHY COMMON SHREWS (*Sorex araneus*)

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Objective: *Pneumocystis carinii* is an eukaryotic organism previously presumed to be a protozoan but recently recognised as an organism more closely related to fungi than to protozoans. The presence of *Pneumocystis carinii*, more than almost any other organism, is associated with immunodeficiency, and this pulmonary pathogen is capable of causing life-threatening pneumonia (PCP) in immunocompromised hosts. *P. carinii* has been demonstrated from many animal species but this elusive organism has been shown to have an exceptionally high prevalence in apparently healthy shrews. The purpose of the present study is to characterise the nature of *P. carinii* infection in this widespread, insectivorous host species.

Material and methods: *Sorex araneus* (n>500) were live-trapped during all seasons in various locations in Finland. Standard histological lung sections were stained with Grocott's modification of Gomori's Methenamine Silver (GMS) stain. This histologic scoring system was used as a standard for comparison with other methods (cyst

concentration, histopathological analysis, commercial immunofluorescence kits, electron microscopy) used for further characterisation of the infection.

Results and conclusions: The prevalence of *P. carinii* was high in every sex and age group, in all study areas, which represented different habitat types, and both in low and high host density populations. The infection was common during all seasons with a peak prevalence in late fall. The intensity of infection was low, and no histopathological changes typical for PCP were found. *P. carinii* in *S. araneus* is both genetically and phenotypically discrete from organisms originating from laboratory rats. The high metabolic rate of *S. araneus* may contribute to the high prevalence of *P. carinii* infection in this host species.

GEOPHAGY AND ITS INFLUENCE ON GEOHELMINTH INFECTION IN RURAL SOUTH AFRICAN SCHOOLCHILDREN

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Objectives: The aim of this study was to investigate the prevalence of soil eating and its possible influence on *Ascaris lumbricoides* and *Trichuris trichiura* infection in a population of schoolchildren.

Materials and methods: Single stool specimens were collected from 137 children from grades one to four of a rural combined primary school on the KwaZulu-Natal south coast. Of these specimens two sub-samples of approximately one gram were examined for parasite eggs by use of the MIF technique. Soil eating habits of these and 180 more children (grades one to seven) were assessed by structured interviews.

Results: The overall prevalence of soil eating as determined by interviews was 68.9% (mean amount eaten: 6.8 ml/day) with no larger difference between girls and boys. In the eldest age group (13-18 years) however, this habit was less common in boys (52.8%) than in girls (75.0%). The prevalence of ascariasis and trichuriasis was 82% and 100%, respectively, with higher egg counts for both parasites in soil eaters than in non soil eaters. There was a significant difference between these two groups in prevalence of *A. lumbricoides* infections >500 epg (p=0,043) whereas the difference in *T. trichiura* egg numbers was less marked.

Conclusion: The results indicate that soil eating is a more common habit among schoolchildren than previously recognised and a risk factor for ascariasis and trichuriasis. This supports the findings of another study conducted in Kenya.

THE PARASITOLOGICAL ASPECT OF SOME TROPHIC CHAINS IN THE COASTAL WATERS OF ESTONIA

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Objective: This study was dedicated to the investigation of possible ways of formation of the helminth association in main final hosts: seals, sea birds and predatory fish in Estonian coastal waters.

Materials and methods: Twenty-three specimens of seals and 76 specimens of birds (mainly *Larus*) were analysed by the method of total parasitological autopsy in Estonian coastal waters in 1988-96. The data from parasitological monitoring on 24 species of fish in the same area (over 12,000 specimens of clupeids, smelt, gasterosteids, sea-sculpins, flounder, etc.) were used along with the trophological data on the same material. Additionally, a number of samplers of forage invertebrates (zooplankton, nectobenthos, zoobenthos) were analysed parasitologically.

Results: The main helminth associations in grey seal were *Contracaecum osculatum* in stomach and *Corynosoma strumosum* and *C. semerme* in intestine, and in common seal both *Corynosoma* sp. and (optionally?) *C. osculatum* are formed via baltic herring, flounder, smelt, eel pout and sea-sculpins as supplementary, and amphipods and mysids as intermediate hosts. The helminth associations in herring gull were a composition of *Diplostomatidae* g.g. sp. sp. and *Cryptocotyle concava*, mainly developed via clupeids, sticklebacks, smelt, flounder, bleak and roach (in the Western Estonia waters also perch) as supplementary, and above mentioned crustaceans and molluscs of g.g. *Bithynia* and *Hydrobia* (in very shallow waters *Lymnaea* and *Radix* as well) as intermediate hosts. The parasitofauna of great and lesser black backed gulls are of great variability in composition so we could not comment on the ways of their formation yet. The complex trematode and cestode associations in black headed gull allude to its feeding mainly on invertebrates, also sticklebacks and bleak. The helminth associations in predatory fish such as salmonids, pike, pike-perch, turbot and cod, quite multifarious as they are, nevertheless permit the suggestion that there are three main groups of forage fish used as supplementary hosts by main nematodes (*Anisakidae* in the first place) and cestodes (*Bothriocephalus, Eubothrium, Proteocephalus*, etc.): a) herring and smelt for salmonids as final hosts; b) sticklebacks for pike-perch, salmonids, turbot, etc.; c) small demersal fish such as young eel pout, young flounder, bullhead, for cod, turbot, etc. and d) cyprinids (roach, dace) for pike. In the 1990s the importance of sticklebacks as supplementary hosts for anisakids and cestodes has been steadily on the increase.

Conclusion: There are comparatively few channels for the formation of the main helminth associations in seals, gulls and predatory fish in the coastal waters of Estonia.

All those channels depend on mass-species of forage fish such as herring, smelt, sticklebacks and flounder, mainly, as the supplementary hosts.

FIRST DATA ON INFECTION OF CHIRONOMIDS WITH MERMITHIDS IN CHUKOTKA

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The investigations were carried out in the Chaun Lowland (North West Chukotka, Russia, 68.5° n.l.). Sixty water bodies were examined and more than 50 thousand chironomid larvae were dissected. Free-living forms of mermithids (Nemathoda: Mermithidae) have been registered in all the examined water bodies. Ten species of mermithids belonging to seven genera have been discovered as parasites of chironomids (Diptera: Chironomidae). Prevalence of infection in different tundra lakes varied from 0 to 100 per cent. The most common species was *Hydromermis macrospiculatus* Mulvey et Nicle, 1978. The five species of chironomid larvae registered as being infected were *Chironomus anthracinus* Zett., *Chironomus salinarius* Kief., *Chironomus* sp., *Cryptochironomus gr. Defectus* Kief. and *Stictochironomus crassiforceps* Kief. The investigation on the dynamics of *H. macrospiculatus* infection in *Ch. anthracinus* was carried out in one lake during a three year period. The highest prevalence of infection was registered after the basin thawed (16.0, 13.6 and 12.7 per cent in different years) and before it froze (9.0, 9.7 and 8.0 per cent). The results can be explained by the seasonal course of life cycle of the parasites, *i.e.* (i) by the appearance of a lot of mature mermithids in the environment in spring and (ii) establishment of a new infection in summer. General distribution of mermithids in the host population satisfactorily corresponds to the negative binomial distribution. However, the distribution of female mermithids corresponds to Poison's distribution and that of males corresponds to the negative binomial distribution.

DIFFERENTIATION OF *Oesophagostomum bifurcum* FROM *Necator americanus* BY PCR USING GENETIC MARKERS IN rDNA

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Oesophagostomiasis in humans due to infection with *Oesophagostomum bifurcum* (nodular worm) is of major human health significance in northern Togo and Ghana where *Necator americanus* (human hookworm) also exists at high prevalence. Yet very little is known about the transmission patterns of *O. bifurcum,* which is in part due to the difficulty in differentiating *O. bifurcum* from *N. americanus* at some life-cycle stages using morphological features. To overcome this limitation, a molecular approach utilising genetic markers in the second internal transcribed spacer (ITS-2) of ribosomal (r) DNA was developed. The ITS-2 sequence of each species was determined, and specific oligonucleotide primers were designed to regions of greatest sequence difference between the species. Utilising these primers, rapid PCR assays were developed for the specific amplification of DNA of *O. bifurcum* or *N. americanus*, which now have the potential to confirm the identity of eggs from faeces and larvae from the intestine or environment. The application of species-specific PCR has important implications for studying the epidemiology and population biology of *O. bifurcum.*

HEXAMITID PARASITES IN NORWEGIAN FRESHWATER FISH

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Objective: Hexamitid flagellates believed to be *Hexamita salmonis* have occasionally been encountered in farmed fish in fresh water in Norway since 1973. A closer study of these flagellates was started after serious outbreaks of systematic hexamitosis in sea farmed Atlantic salmon.

Materials and methods: Wild fish of different species and farmed Atlantic salmon, *Salmo salar,* have been examined for hexamitid flagellates. Three isolates have been axenically cultivated and their ultrastructure studied by scanning and transmission electron microscopy.

Results: Flagellates from Atlantic salmon, grayling *Thymallus thymallus* and arctic char *Salvelinus alpinus* are found to be the same species. This is not *Hexamita salmonis* but a new species belonging to the genus *Spironucleus*. Hexamitid flagellates have also been found in trout *Salmo trutta*, whitefish *Coregonus lavaretus* and burbot *Lota lota*. These isolates have not been identified.

Conclusions: There seems to be a tendency in diagnostic laboratories, not only in Norway, that whenever hexamitid flagellates are encountered in salmonids, they are automatically called *H. salmonis*. Flagellates fitting the description of *Hexamita salmonis* have never actually been found in Norway and previous reports of this species should be regarded as misidentifications. All isolates of hexamitid flagellates identified in Norway are shown to be the same *Spironucleus* sp. Electron microscopy is at the moment the only reliable way to discriminate between genera and species of hexamitid flagellates and should always be performed before species are identified.

In vitro CHARACTERISATION OF ANTHELMINTIC SUSCEPTIBILITY OF THE NODULAR WORM *Oesophagostomum dentatum* AND THE RED STOMACH WORM *Hyostrongylus rubidus* OF PIGS

By M. VARADY[1,2], H. BJØRN[1,3] & P. NANSEN[1]

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Objective: The objective of the present study was to determine and compare *in vitro* effects on the development of two different nematode species of pigs.

Materials and methods: A field isolate of *O. dentatum* that had no prior exposure to anthelmintics and a sensitive *Hyostrongylus rubidus* obtained from a farm were used. Both isolates have been routinely maintained by passages through worm-free two to three month old Danish Landrace/Duroc/Yorkshire crossbred pigs. Pigs were infected orally with 6,000 - 8,000 L_3 of both isolates. Faecal samples were collected 25-40 days after experimental infection and subsequently a larval development assay was carried out to estimate the concentration of drug (ivermectin, levamisole, pyrantel, morantel and thiabendazole) required to inhibit the development of 50% larvae (LD_{50}).

Results: Comparison of LD_{50} values of *Oesophagostomum dentatum* and *Hyostrongylus rubidus* demonstrated significant differences in response to levamisole, pyrantel and thiabendazole (ovicidal effect) anthelmintics. Non-significant differences were recorded for ivermectin, morantel and thiabendazole (larval development effect).

Conclusions: The results from this study show that two different nematode species of pigs produce significantly different responses to anthelmintics *in vitro*, which has also been documented *in vivo* in other studies.

HOST PARASITE INTERACTIONS

CONCOMITANT IMMUNITY IN ADULT TAPEWORM INFECTIONS

By J. ANDREASSEN

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Concomitant immunity, which was first described in *Schistosoma mansoni* infections in Rhesus monkeys, means that an existing infection of adult worms cannot be killed by the host, while it is immune against new infections. The same phenomenon has been described in larval tapeworm infections in mammals and the following experiments with the bile duct tapeworm *Hymenolepsis microstoma* show that it also exists in adult tapeworm infections in mice.

NMRI mice were infected with two cysticercoids (primary infection) and after 2, 3, 4, 7, 14 and 21 days superimposed infected with five cysticercoids. All mice were autopsied two weeks after the superimposed infection.

Two days between the primary and superimposed infections resulted in 93% of 11 mice being infected with superimposed worms of which 66% were recovered. Seven days between primary and superimposed infections resulted in only 27% of 11 mice being infected and only with 9% of the superimposed worms. Three weeks between the primary and superimposed infections resulted in none of the 11 mice being infected. The mean total dry weight of superimposed worms per mouse was 18.1 mg with two days interval, but only 0.5 mg with seven days interval between primary and superimposed infections.

These findings could be interpreted as a result of an increasing biomass of the two primary worms causing a debilitating effect on the five superimposed worms. However, nude thymus-deficient mice were also infected with two primary worms and seven days later superimposed with five cysticercoids and autopsied two weeks later together with normal mice. All nude mice were infected with superimposed worms compared to 27% of normal mice and 88% of the superimposed cysticercoids were recovered as adult worms and in total weighing 64.9 mg dry weight per mouse, while only 9% of the superimposed worms in normal mice were found and weighing only 0.5 mg per mouse.

Since the mean total dry weight of the two primary worms was 75% higher in the nude mice (69.2 mg) than in the normal mice (39.5 mg), it is concluded that the deleterious effect on the superimposed worms in the normal mice was caused by immune reactions and not simply by crowding.

ESTABLISHMENT OF *Ascaris suum* IN NATURALLY INFECTED PIGS

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Objective: The objective of this study was to investigate if a predisposition exists in pigs to infection with *Ascaris suum*, by studying parasite distribution and host response in naturally infected pigs.

Materials and methods: Fifty helminth-naïve pigs were turned out on a pasture contaminated with *A. suum* eggs. The pigs received anthelmintic treatment after 10 weeks when egg counts indicated patent infections. All pigs were housed individually in cages and their faeces were collected for three consecutive days. The expelled worms from each pig were counted, after which the pigs were reintroduced to the infected pasture. After a subsequent 10-week period, all pigs were slaughtered and their worm burdens recorded. Blood samples were taken regularly and analysed by means of an ELISA test. The degree of aggregation of *A. suum* was measured using the negative binomial parameter, k.

Results: Heavy over-dispersion was observed at treatment (k=0.77). The number of pigs that harboured worms increased from 34% at treatment to 84% at time of slaughter. Mean worm burdens did not differ significantly (10.44 versus 9.35), but a significant correlation was found between worm burden at treatment and at slaughter (r=0.39, p<0.01). Chi-square analysis showed that pigs harbouring worms at treatment were more likely to harbour worms again after re-infection (X^2=4.73, p<0.05).

Conclusion: The results of this study suggest that a predisposition exists in pigs to establishment of adult *A. suum*. Degree and length of exposure and their influence on the immune response, including the ELISA results, will be discussed.

EXPERIMENTAL *Oesophagostomum dentatum* INFECTIONS IN THE PIG: WORM POPULATIONS RECOVERED AT REGULAR INTERVALS DURING TRICKLE INFECTIONS WITH THREE DOSE LEVELS OF LARVAE

By C.M. CHRISTENSEN, E.H. BARNES & P. NANSEN

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A trickle infection experiment was undertaken to study in detail the population dynamics of *Oesophagostomum dentatum* in pigs. Three groups of 32 pigs were inoculated with *O. dentatum* via the feed twice weekly with 100 L_3 (Group A), 1,000 L_3 (Group B) or 10,000 L_3 (Group C). Five pigs from each group were killed 2, 4, 8, 12, 16 and 20 weeks after the first inoculation (weeks p.i.) to determine their worm burdens. Faecal egg counts were determined at weekly intervals from day 17 p.i.. At slaughter, worms were counted, differentiated according to sex and developmental stage, and their length measured. Faecal egg counts were proportional to dose rate until week 15, but later were more variable. The worm establishment was proportional to the dose rate, but in group C decreased over time, whereas in groups A and B there was no consistent pattern over time. Adult worms were located more proximally in the large intestine in group A than in group C, and in all groups worms were more proximal at later slaughter dates. Worm fecundities (epg per female worm) in groups A and B were similar to each other and higher than in group C. The lengths of the female worms increased over time, whereas the lengths of the male worms remained approximately constant from week 8 p.i. The study suggests a reduced establishment of incoming larvae and lower fecundity of the female worms at the high dose levels.

PREPATENT PERIODS IN *Oesophagostomum* spp. IN PIGS FOLLOWING PRIMARY AND MULTIPLE INFECTIONS

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There is variable and conflicting information in the literature concerning the length of the prepatent period of *Oesophagostomum* spp. in pigs, reported in textbooks to be 5-7 weeks. Therefore, the prepatent period of several nodular worm isolates were studied following single or multiple infections with *Oesophagostomum dentatum* or *O. quadrispinulatum.* Twenty-four helminth free pigs were pairwise given a single infection with 2000 L_3 larvae from either of 10 *Oesophagostomum* spp. field isolates or

two laboratory isolates. Daily faecal egg counts were determined from day 15 p.i., and the worm species composition was ascertained at slaughter 8 weeks p.i. To assess a possible resistance to re-infection, 3 groups of 10 helminth free pigs were trickle infected twice weekly for 8 weeks with 1000 L_3 of *O. dentatum* or *O. quadrispinulatum*, or no larvae, respectively. All pigs were anthelmintically treated 10 days before challenge inoculation with 5000 L_3 of the homologous or the heterologous species. Faecal egg counts were determined from day 16 p.i., and worms were recovered from the pigs six weeks after challenge inoculation. Following the single infection all pigs commenced egg excretion on days 18-24 p.i. with a mean prepatent period of 20.2(±1.4) days and no differences between species or isolates. No delays in onset of egg excretion were found in the trickle infected groups, which commenced egg excretion on days 17-23 after challenge infection. Based on these observations there is a need for revision of the textbook information concerning prepatent periods of *Oesophagostomum* spp. in pigs.

SUBMITTED PAPERS - POSTER PRESENTATION

POPULATION STRUCTURE OF *Pseudoterranova decipiens* IN THE COMMON SCULPIN (*Myxocephalus scorpius*)

By K. ANDERSEN[1] & S. des CLERS[2]

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During the period 1992 to 1996, sculpins were caught at Hvaler in the outer Oslofjord close to some skerries which are regular haul-sites for a colony of common seals (*Phoca vitulina*). Sculpins from this area were found to be heavily infected with seal worm larvae (*P. decipiens*), with a maximum infection of 424 larvae in a 30 cm long fish. Age, length, weight and infection were registered for all fish studied (212).

The purpose of the study was to detect patterns in the infection, if possible, in relation to host size and age and size within age groups. The infection varied from year to year.

Sculpins less than three years of age were hardly infected. The infection first really developed at the age of three and increased between three and four years. In older fish the infection varied more and the standard deviation was greater than the mean. The infection seemed to decrease again in the oldest fish caught (9-10 years of age). Larger fish within a given age group had more worms than smaller fish of the same age. This

applied to all age groups from one to seven, while in the oldest it was the other way around.

COMPARATIVE CHARACTERISTICS OF HELMINTH FAUNA OF EUROPEAN MINK *Mustela luterola* AND PINE MARTEN *Martes martes* IN BELARUS

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A comparative study of the helminth fauna of two native mustelid species, European mink *Mustela lutreola* and Pine Martin *Martes martes* was carried out from 1990-1996. These two species have different population structures and our goal was to test whether it affects the formation of their helminthocenoses. Seventeen helminth species were found in European mink and 92.6% of the animals studied were infected. Helminth fauna of Pine Marten appeared to be poorer with 11 helminth species being recorded for it. Total infestation of Pine Martin was lower than in European mink - 65.7% ($\chi^2 = 6.2$, $p = 0.01$). The apparent reason for higher infestation of European mink seems to be the characteristic localisation of the whole invasion process in this species (definite host concentration in line home ranges along water-bodies, localisation of helminth eggs and infestation during feeding in shoreline ecotones). On the contrary, spatial dispersion over the large areas are characteristic both for Pine Marten and its prey animals carrying infestation stages of the helminths.

MALACOLOGICAL LENGTH PROFILE OF IRRIGATION CANALS IN MOROCCO, EMPHASIZING *Bulinus truncatus*, INTERMEDIATE HOST OF URINARY SCHISTOSOMIASIS

By E. BOELEE, H. LAAMRANI, K. KHALLAAYOUNE & H. MADSEN

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In a modern irrigation system in Morocco, a technical and malacological length profile of a secondary canal and some of its tertiaries has been made. The main objective was to obtain more information on the ecological requirements of *B. truncatus*.

Several different hydraulic structures such as drop structures, weirs, angle structures and siphons have distinct ecological characteristics that can be explained by water management and technical features. *B. truncatus* was frequent at the tail end of

the secondary canal and in all tertiaries. It is mainly confined to tertiary siphon boxes, where a significant difference between densities of *B. truncatus* in upstream and downstream boxes may be explained by hydraulic differences in water flow.

Trypanosoma cruzi TISSUE AFFINITY *in vitro*

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T. cruzi parasites isolated from both humans and insect vectors differ in their tissue affinity *in vivo*. The mechanism of tissue tropism is not well understood.

Objective: The aim was to study *in vitro* tissue affinity, adherence, cell-invasion and the development of intracellular stages using *T. cruzi* strains with different properties *in vivo*.

Materials and methods: *In vitro* raised trypomastigotes of cardiomyotropic (strain NICI) and rheticulotropic (strain Tulahuen) parasites were incubated with normal mouse tissue sections obtained from different organs and with monolayers of two types of tissue cultures. The number of attached and transformed parasites was evaluated by IFL using human chagasic serum anti human Ig FITC-conjugate.

Results: *In vitro* tissue affinity assay comparing affinity for heart and spleen shows that cardiomyotropic parasites expressed 6,3 x stronger affinity to heart tissue sections while rheticulotropic parasites adhered 8,4 x more to spleen tissue.

Conclusion: Results obtained in the *in vitro* assay are compatible with *in vivo* studies on mice.

Gyrodactylus derjavini IN FOUR SALMONIDS: COMPARATIVE HOST SUSCEPTIBILITY AND SITE SELECTION OF PARASITES

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Objective: The purpose of the study was to determine the susceptibility of four salmonids, rainbow trout, brown trout, Scottish salmon (Conon) and Finnish salmon (Iijoki) to infection with the monogenean *Gyrodactylus derjavini*. In addition, the microhabitat selection of the parasites on the hosts was studied.

Materials and methods: Parasites were obtained from a Danish rainbow trout farm. In the laboratory salmonid fry (3-4 months post hatching) were experimentally infected by exposing fish to detached parasites in small aquaria. The infection was followed in fish tanks (volume 200 l) containing 40 rainbow trout and 40 fish of the species to be tested (brown trout, Scottish salmon or Finnish salmon). The infection was followed for six weeks. All experiments were conducted at 12-13°C. The lysozyme activity and density of mucus cells in the fins were recorded in the four salmonids.

Results: Rainbow trout were most susceptible reaching high infection levels within 3-4 weeks. Brown trout were less susceptible compared to rainbow trout but reached relatively high infection levels. Both Scottish and Finnish salmon were relatively resistant to infection experiencing only few parasites per fish despite a heavy infection pressure. The fins and especially the tail fin was the preferred microhabitat in all four hosts. However, in later stages of infection the corneal surface became an increasingly important site. The lysozyme activity was not correlated to susceptibility but the most resistant salmonids showed the highest density of mucus cells in the tail fins.

Conclusion: The introduced salmonid, rainbow trout, showed the highest susceptibility to infection with this gyrodactylid with an original area of distribution in Europe. This can be explained on an evolutionary basis. Although several factors are involved in host resistance to infection, the immunologically active compounds in mucus are liable to play a role in this context.

CERCARIOSIS PROBLEM IN MINSK AND MINSK ENVIRONS

By E. BYCHKOVA

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The civilisation process causes formations of enlarged aggregations of wild birds (*e.g.* mallards, mute swans, etc.) near big cities and health resort areas. A high number of mallard ducks in Minsk (120-500 couples) in combination with a high population density of pulmonate snails creates favourable conditions for the rise of cercariosis infestation niduses. A similar situation was observed in the resort area of Naroch Lake in 1994 (Anisimova & Bychova 1995).

In 1996 30 mallards from the Minsk environs were dissected and examined for trematodes and 176 pulmonate snails were examined for cercariae using the compressor method.

Examinations showed the following findings: trematodes *Bilharziella polonica* (Kowalewsky, 1895) Looss, 1899 and *Trichobilharzia ocellata* (La Valette, 1854) Brumpt, 1931 were found in 25 mallards; from three species of molluscs examined, one, *Limnaea stagnalis*, was found infected by cercariae. General infection rate in this case was 23.0%.

Great numbers of mallards and the high rate of their infection along with the high cercariae infection of intermediate hosts, pose a threat of schistosomiasis propagation in Minsk and environs of the city.

THE TEST OF AVERSECTIN "EQUISECT" PASTE FOR CONTROL OF HORSE NEMATODOSES AND PERSPECTIVES OF INTRODUCTION OF BIOLOGICAL CONTROL OF PARASITES IN HORSE FARMS IN RUSSIA

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Introduction of modern techniques and effective drugs for parasite control are important preconditions for the revival of horse breeding in Russia.

The original paste "Equisect" was elaborated by the firm "PharmBioMed" for antiparasitic treatment of pedigree horses. The active substance of it is avermectin C, and the natural avermectin complex was received from soil fungus *Streptomyces avermitilis* by means of microbiological synthesis.

"Equisect", which contains 1% of active substance, in dose 20 mg/kg in peroral treatment shows 100% efficacy against adult Strongylidae, *Strongyloides, Parascaris, Oxyuris, Parafilaria* and larvae of *Gastrophilus* spp. The paste is also highly effective against cyathostomes, which are resistant to benzimidazoles.

The programme of using "Equisect" has been worked out according to season, age of hosts, geographical and climatic peculiarities. In many cases the state horse farms of Russia and Ukraine have a large number of horses and spacious pastures, so the perspectives of introducing modern biological techniques are wide enough and are discussed at present.

A METHOD OF USING BIRDS FOR DETECTION OF NATURAL FOCI DISEASES

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Objective: An investigation of the role of birds in the feeding Ixodinae ticks in Belarus was undertaken.

Materials and methods: Three species were registered in the birds: *Ixodes arboricola* Schulze et Schlottke, *Ixodes lividus* Koch. - a bird's nest-burrow parasite and *Ixodes ricinus* (L.) - a parasite of pasture type.

Results: It was established that the birds do not play the main role in the feeding *Ixodes ricinus* in the forests of Belarus, but the amount of ticks feeding on birds seems enough to support the tick population in periods of quantitative depression of the rodents. Two strains of TBE virus were isolated from *Ixodes lividus* larvae, the specific parasites of the bank swallow, collected from the nests before the spring arrival of the birds. Earlier the strain of TBE virus from *Gamasina* was isolated in this colony. Furthermore, 5% of blood serum of inspected *Riparia riparia* L. contained antihemagglutinins to TBE virus and 3.3% to West Nale virus.

Conclusion: The results indicate that the bank swallow - the feeder of *Ixodes lividus* Koch. - is the host of TBE virus. The isolation of TBE virus from *Gamasina* ticks in summer and seven years later from *Ixodes lividus* Koch., which was collected from the nests of *Riparia riparia* L., indicated the stability of the natural foci of TBE, localised in the breeding colony of bank swallows. The discovery of TBE virus in the spring before the arrival of the birds showed that the infection reserved in the diapaused Ixodinae ticks.

A STUDY ON INTESTINAL PARASITES IN THE FAECES OF DOGS IN ICELAND USING THE FORMALIN-ETHYLACETATE CONCENTRATION METHOD

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Objective: To study the occurrence of intestinal protozoans and helminths in the native dog population in Iceland.

Materials and methods: In 1996 in the Reykjavik urban area in SW Iceland faecal samples were collected from 25 dogs ranging in age from 2-12 months, 39 dogs over

one year old and 8 bitches and their 5-6 week old puppies. In a rural area in E Iceland samples were collected from 4 dogs, 2-12 months in age and 8 dogs over one year old. The formalin-ethylacetate concentration method was used in order to detect protozoan cysts and helminth eggs. A modified Ziehl-Neelsen staining method was used on the concentrates of the samples from all dogs under one year of age for the specific detection of *Cryptosporidium* sp.

Results: No protozoan cysts, trematode eggs or cestode eggs were detected. Eggs of *Toxocara canis* were found in puppies from two bitches and in one 3 month old puppy from the Reykjavik area.

Discussion: In the past most of the common parasites of dogs have probably reached Iceland and in recent years several protozoan and nematode species have been found in imported dogs in quarantine. This is the first comprehensive search for protozoans in native dogs in Iceland and none were found. Trematodes have never been found in dogs in Iceland. Cestodes were very common in dogs in Iceland in the last century and six species have been reported. Because of the fight against echinococcosis their prevalence has diminished drastically and some of the species have probably been eradicated. However, the method used in this study is not very reliable for the detection of several of the cestode species. *Toxocara canis* is the only nematode species which has been found in native dogs in Iceland and the finding of *T. canis* in two out of eight litters indicates that this parasite is common in Iceland.

IMMUNOCHEMICAL CHARACTERISATION OF RECOMBINANT *Ancylostoma caninum* ANTIGENS

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Objective: The purpose of the immunochemical characterisation of *Ancylostoma caninum* antigens is the production and evaluation of recombinant antigens concerning their diagnostic potential for serological tests and their protective potential as vaccine candidates.

Material and methods: Adult worms of *A. caninum* were dissected and manually separated into cephalic glands, cervical glands, intestine, esophagus and cuticula. These fractions as well as third stage larvae were separated with Triton X-114 into water soluble (hydrophilic), Triton soluble (hydrophobic) and insoluble proteins. These fractions were characterised by immunoblotting with serum from rabbits immunised either with a pool of cervical, cephalic glands and intestine, or the esophagus fraction as well as with sera from percutaneously infected dogs and rabbits. Lambda-ZAP II cDNA libraries of male and female adult worms were screened with the sera mentioned

above and reacting clones were isolated and characterised by sibling analysis. Some of the resulting clones were subcloned into the QIAexpress™ system and the expressed proteins characterised with the hyperimmune sera of different organs.

Results: Three clones (341, 342 and 350) and their correspondent fusion proteins (35kDa, 38kDa, 38kDa) could be allocated to esophagus and intestine. The DNA sequence of clone 341 shows homologies to the sequence of a Paramyosin gene of *C. elegans*, other clone homologies with enzymes of *C. elegans* or *Onchocerca volvulus*.

Conclusion: Immunodominant antigens were found that reacted with dog or rabbit post infection sera and could be useful as antigens in serodiagnostic tests. Antigens were found in fractions from esophagus and intestine that might have protective potential as hidden antigens and that will be used in immunisation studies.

THE PREVALENCE OF *Balantidium coli* AND OTHER ZOONOTIC PARASITES IN ICELANDIC PIGS

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Objective: When amoebic cysts were detected in faeces from imported Norwegian pigs kept in a quarantine station in Iceland in 1994, it was decided to examine the occurrence of gastrointestinal parasites in the native pig population.

Material and methods: Ten pig farms from various parts of Iceland were selected for the survey. In 1995-1996 faecal samples from five slaughter pigs from each farm were collected in slaughterhouses and examined for the presence of protozoan cysts and helminth eggs by the formalin-ethyl concentration method.

Results: The following protozoan cysts were found: *Balantidium coli, Entamoeba polecki* and *Idoamoeba bütschlii*. The only helminth eggs recovered were eggs of *Ascaris suum*. *B. coli* and *E. polecki* were found at all farms, *I. Bütschlii* at nine (90%) and *A. suum* at two (20%) farms. The overall prevalence was as follows (n=50): *B. coli* 86%; *E. polecki* 98%; *I. bütschlii* 40% and *A. suum* 10%.

Conclusion and discussion: *B. coli, E. polecki* and *I. bütschlii* are reported for the first time in the native swine population in Iceland. In addition to *A. suum* the following parasites have been reported previously in Icelandic pigs: *Cryptosporidium parvum, Eimeria* sp. and *Isospora suis*. All the parasites found in this survey and *C. parvum* are considered to be zoonotic species. However, only *B. coli, C. parvum* and *A. suum* are regarded as pathogenic to humans.

THE ADULT STAGE OF THE ASCARIDOID NEMATODE *Ascaris suum:* GROWTH AND STRUCTURAL FEATURES

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Objective, materials and methods: For studying the morphogenesis of the adult stage of *Ascaris suum*, worms were obtained from experimentally infected domestic pigs. Structural and morphometric features of *A. suum* were analysed by light microscopy and scanning electron microscopy, and the observations were related to earlier studies.

Results: It is shown that the length increase of the worm from day 35 to 56 is based on a region specific lengthening of individual transverse annuli in the cuticle. A pair of lateral midbody papillae, centrids, are here shown to be prominent sensory organs in the adult male and female worm. The centrids have an asymmetric placement, the right centrid being more anterior on the worm than the left centrid. An analysis of the variability of certain features of diagnostic significance in the male tail is made.

Conclusion: The results, which are of diagnostic and systematic significance, point to the need of a redescription of *Ascaris suum* and *Ascaris lumbricoides*, preferably combined with a genetic analysis.

MINK EIMERIOSES AND ISOSPOROSES ON FUR FARMS OF BELARUS

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Objective: The purpose of the study was to develop and introduce a scientifically grounded system of measures against mink eimerioses and isosporoses on the basis of agents study, epizootology, pathogenesis, symptoms and efficacy of a series of preparations to be used for given diseases in the Republic of Belarus.

Materials and methods: To achieve the goal, parasitological, clinical and biochemical methods of investigation have been applied involving 6178 mink, eimeria and isospora oocysts as well as medico-prophylactic preparations.

Results: On 17 fur farms of the Republic of Belarus four species of parasites have been found in mink: *E. vison, E. furonis, I. laidlawi* and *I. eversmanni.* 100% of the farms have been reported to be liable to eimerioses and 94.1% to isosporoses. The most intensive rate of infection has been stated in young mink aged 2-4 months (50.47%). Maximal EI for summer period is reported to be 24.77%, minimal EI for winter is 9.4%. Pathogenic effect of *E. vison* on mink organisms has been manifested in a lowering of haemoglobin level, erythropenia, leukocytosis in reduction of total protein amount, in decreasing of lyzocymic activity against a raise in activity of aminotransference in blood serum, loss of appetite and diarrhoea with a touch of blood and mucus. Baycox, biovit-120, chimcoccide, olachindox, salinomicin, peat oxydate and fir needles are reported to be effective means for treatment and prophylaxis.

EFFECT OF LOW PROTEIN DIET ON FECUNDITY AND RELATIVE DISTRIBUTION OF *Schistosoma japonicum* EGGS IN PIGS

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The purpose of the study was to estimate the fecundity and relative distribution of *Schistosoma japonicum* eggs in pigs fed low or high protein diets and infected with *S. japonicum* and *Trichuris suis.*

Sixteen pathogen-free Danish Landrace/Yorkshire Duroc crossbred female pigs were divided into two groups of eight pigs and fed on either a high or low protein diet. After 11 weeks all 16 pigs were infected with 1500 *S. japonicum* cercariae and 4000 infective *T. suis* larvae. All pigs were killed week 12 post infection and worms were recovered by perfusion. Faecal and tissue egg counts, including counts from liver and mucosa scraped from the large intestine, were done.

Except for the immature worms, worm recovery was significantly higher for the pigs in the low protein diet group compared to the pigs fed on high protein diet ($p<0,01$). The number of eggs excreted in faeces, expressed as eggs/g faeces/female was significantly higher for the pigs in the low protein diet group ($p<0,05$) compared to the pigs in the high protein diet group, but no difference in number of eggs deposited in tissue, expressed as eggs/g tissue/female worm, was seen between the two groups of pigs.

In the low protein group, more than three quarters of the mean total eggs excreted were found in the caecum and only about one tenth in the rectum, whereas in the high protein group only about one third of the mean total eggs excreted were found in the caecum but more than half were found in the rectum.

In conclusion, the low protein diet gave rise to increased worm recovery, seemed to change the localisation of adult worms but only marginally increased the fecundity.

SUSCEPTIBILITY OF *Gyrodactylus salaris* TO HOST COMPLEMENT

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Gyrodactylus salaris is an important pathogen of Norwegian Atlantic salmon, *Salmo salar*. The parasite is extremely susceptible to host complement, lysing within five minutes in a 1:50 dilution of serum in fresh water, and within two hours in a 1:200 dilution. Specific lysis due to complement cannot be detected at a 1:1000 dilution. Mucus is 10-100 times less effective than serum at lysing *G. salaris*, but nevertheless complement activity is clearly detectable. Lysis is abolished by heat treatment, and shows divalent cation sensitivity characteristic of the alternative complement pathway. Lysis cannot be enhanced by pre-incubation in heat-treated serum of salmon with long-standing infections, suggesting that the classical antibody mediated, pathway is not involved. Lysis is not restricted to particular host-parasite combinations, and a cyprinid parasite, *Gyrodactylus decorus*, can be lysed by salmon serum as effectively as *G. salaris*. During long-standing (up to six weeks) infections, there is no evidence of a specific anti-*Gyrodactylus* antibody response, although there is a slight increase in total antibody titre in mucus, probably because of leakage of serum as a result of inflammation. Complement in serum does not show clear trends during infections of *G. salaris* on salmon, and after six weeks some fishe have high (up to 400% of controls) complement activity in serum, while in others this is barely detectable. Complement in mucus on the skin of the fish has clear potential to damage *G. salaris in vivo*, and it is paradoxical that the parasite appears able to survive on the skin despite this anti-parasite activity.

CORTISOL INDUCED IMMUNOSUPPRESSION RENDERS BROOK TROUT (*Salvelinus fontinalis*) SUSCEPTIBLE TO *Gyrodactylus salaris* INFECTION

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Gyrodactylus salaris infects many salmonids experimentally, although it is only pathogenic to Norwegian strains of Atlantic salmon. Previous work has shown that *G. salaris* transiently infects American brook trout, *Salvelinus fontinalis*, populations of parasites growing for ca. 30 days before declining to extinction after ca. 50 days. This pattern of growth is suggestive of a host immune response. It was therefore reasoned that treatment with cortisol, an immunosuppressant, would render brook trout more susceptible to this parasite. Brook trout (50 grouped individuals) were given intraperitoneal implants of hydrocortisone acetate (20 mg ml) in cocoa butter. Controls included similar groups injected with cocoa butter alone, and uninjected fishes. The immunosuppressant cyclophosphamide (200 $\mu g\ g^{-1}$ body weight) was also injected into another group of brook trout. All fishes were maintained in groups in freely circulating dechlorinated tap water at 10° C, and initially infected with ca. 25 parasites per fish.

Over the subsequent 35 days, infections declined to ca. nine parasites per fish on all except the cortisol treated fishes. In this group, infections increased up to 44.2 parasites per fish. This strain of brook trout appears relatively resistant to infection, and parasite populations failed to grow on healthy fish. Immunosuppression with cortisol reversed this trend, allowing *G. salaris* to infect brook trout for extended periods. Interestingly, cyclophosphamide had no effect on *G. salaris* population growth. The reasons for this are not clear.

DISSEMINATION OF PARASITES THROUGH INTRODUCTION OF FRESHWATER FISH SPECIES: INTRODUCTION OF MINNOW (*Phoxinus phoxinus*) AND ITS PARASITES TO HIGH ALTITUDE LAKES WITH BROWN TROUT (*Salmo trutta*)

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The purpose of the study was to describe and quantify the occurrence of minnow (*Phoxinus phoxinus*) parasites in a set of lakes which differed with regard to time since the introduction of minnows and to examine the overlap in parasite fauna between

minnow and brown trout (*Salmo trutta*) within the same lakes. Minnow and brown trout were collected by gill nets in four high altitude lakes in southern Norway. Minnows were introduced to the area about 70 years ago, and time since introduction varies between a few years and up to 70 years. After collection the fish were deep-frozen and later examined for parasites in all internal organs including brain, eyes and gills. It was predicted beforehand that parasites with a simple life cycle and direct transmission would be the most numerous, as they do not require the presence of intermediate hosts. It was also predicted that parasites with fish-eating birds as final hosts would be more common than parasites with fish as the final host and it was expected to find a low degree of similarity among parasite assemblages in minnow due to the influence of unpredictable events. The results showed that minnows introduced new parasite species. These parasites did not infect brown trout, but they infected other organisms in the lakes (snails, mussels, insects and fish-eating birds). It was difficult to relate the occurrence of parasites to life cycle or transmission route, except that parasites with fish as the intermediate host were the most numerous. The results also showed that there was a core of common parasites and only a few rare parasites. The results will be discussed in relation to theory about the introduction of freshwater fish parasites and parasite assemblage formation.

WHY ARE THERE SO FEW PARASITES IN FENNOSCANDIAN LEMMINGS?

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Objective: There are two species of lemmings in Fennoscandia, the endemic Norwegian lemming, *Lemmus lemmus*, and the wood lemming, *Myopus schisticolor*, which is distributed from Fennoscandia to easternmost Siberia. From a parasitological point of view, the most interesting feature of Fennoscandian lemmings is the low number (Norwegian lemming: 1 species) or total absence (wood lemming) of gastrointestinal helminth species. This pattern is supported by the absence of certain microparasites (*Pneumocystis carinii, Cryptosporidium*) in Fennoscandian lemmings. Our objective is to seek a plausible explanation for this phenomenon by analysing interspecific and geographical patterns of helminth species diversity in arvicoline rodents (voles and lemmings) from Fennoscandia and Siberia.

Materials: All data on helminths and microparasites from Fennoscandia and Siberia come from our own studies, except for the data on helminths of Siberian wood lemmings.

Results and conclusions: The rarity of helminths in Fennoscandian lemmings seems to be due to the following patterns of helminth diversity: i) lemmings have fewer species of helminths (0-5) than voles (4-10); ii) in lemmings, helminth diversity decreases from east to west. However, the specialised food consumption of *Lemmus* and *Myopus* (high proportion of moss in diet) and the erratic fluctuations of lemming populations may also have contributed to the rarity of helminths in Fennoscandian lemmings.

DIFFERENCES IN THE RIBOSOMAL SECOND INTERNAL TRANSCRIBED SPACER DNA BETWEEN SIX SPECIES OF GASTROINTESTINAL NEMATODES FROM RUMINANTS

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Infections of domestic ruminants are of major economic significance because of their impact on animal health. An accurate diagnosis is important for their effective control. It is not possible (except for *Nematodirus*) to distinguish reliably between the eggs of different trichostrongyle genera. Ribosomal DNA and their spacer regions proved to be useful for species differentiation.

The second internal transcribed spaces (ITS-2) of the ribosomal DNA of *Ostertagia ostertagi, Cooperia oncophora, C. punctata, Trichostrongylus axei, Haemonchus contortus* and *Nematodirus helvetianus* were amplified by PCR using ITS-2 specific primers. After agarose gel electrophoresis bands were isolated, DNA extracted, cloned into the plasmid vector pCR II and sequenced.

ITS-2 sequences of the six species ranged between 230 and 241 bp in length. *C. oncophora* and *C. punctata* showed 100% sequence homology, whereas homologies between the different genera varied between 63 and 79%. The differences allow the design of genus-specific primers for the differentiation of eggs and larvae.

The work was supported by a grant from the German Research Council (DFG grant No SCHN 267/8-1 u. 2).

POSSIBLE EFFECTS OF A PRIMARY *Schistosoma japonicum* INFECTION ON A SUPERIMPOSED *Ascaris suum* INFECTION IN PIGS

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The study was designed to examine the impact of a primary *Schistosoma japonicum* infection on the establishment and location of a superimposed *Ascaris suum* infection. A *S. japonicum* infection causes hepatic lesions as a result of the host's immune reaction to trapped eggs. Therefore, migrating *A. suum* larvae might be arrested in their development during the liver passage in *S. japonicum* infected pigs.

The study comprised two experiments each containing two groups. Within each experiment one group was primary infected with 1,000 *S. japonicum* cercariae and challenge infected with 1,000 *A. suum* eggs only. The *A. suum* challenge infections took place 11 or 16 weeks after the primary *S. japonicum* infection in experiments one and two, respectively. All pigs were slaughtered 10 days after the *A. suum* challenge infections. The recovery of *A. suum* worms in the liver, lungs and small intestine which was divided into four equal sections was estimated. Furthermore, the mean (±std) number of *S. japonicum* eggs per gram (epg) liver tissue was obtained in order to check for successful primary infection.

In the first experiment the mean (±std) *S. japonicum* epg in the livers was 100 (±100) and moderate fibrotic lesions were observed. The mean recovery of *A. suum* worms was 604 (±205.5) for the *S. japonicum* infected group and 577 (±211.4) for the control group. In the second experiment the mean *S. japonicum* epg in the livers was 600 (±482.0) and severe fibrotic changes were observed. The recovery of *A. suum* worms was 449 (±110.1) and 402 (±125.7) for the *S. japonicum* infected and control groups, respectively. No significant difference in the recovery of *A. suum* worms was obtained between the groups in each experiment. The relative location of *A. suum* worms in the liver, lungs and sections of the small intestine was similar in the two groups in each experiment. Therefore, we conclude that a primary *S. japonicum* infection does not have an affect on the establishment and distribution of a challenge infection with *A. suum.*

CERCARIA-SCHISTOSOMULUM TRANSFORMATION OF *Trichobilharzia szidati* AND ITS IMMUNOLOGICAL CONSEQUENCES

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Schistosome cercariae of the genus *Trichobilharzia* are the causative agents of swimmers' itch. In order to characterise the changes of parasites during and after the penetration of the host skin, the *in vitro* and *in vivo* (*Anas platyrhynchos, Mus musculus*) transformations of *T. szidati* cercariae into schistosomula have been performed. Ultrastructural observation revealed that cercariae possess a simple outer tegumental membrane with a thick glycocalyx. The latter structure disappears during the transformation. More types of vesicles are formed in the cytons and then they are transported towards the tegumental surface. As in human schistosomes, vesicles with packed membranes fuse with the surface in order to create double membrane with putative protective function.

Biochemical and immunological observations showed that the carbohydrate-rich glycocalyx of cercariae serves as a good target of commercial lectins. The early stage of cercarial transformation can be detected by enhanced reactivity of two lectin probes (PNA, ConA) with the surface. However, the advanced schistosomula appear to have no surface ligands for 12 lectin probes being tested. Similarly, the cercarial surface is recognised by sera from immunised mice and people with cercarial dermatitis. On the other hand, the schistosomula fail to react with antibodies. The non-reactivity as a part of parasite immune evasion within the host is discussed.

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PARASITES AND COMMENSALS OF THE RED KING CRAB, *Paralithodes camtschaticus* (Thilesius), IN THE BARENTS SEA

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The red king crab, *Paralithodes camtschaticus,* is native to the North Pacific. During the 1960s and 1970s it was introduced to the Barents Sea by Russian scientists, where it has thrived to the extent that a commercial fishery is now a viable proposition.

The aims of this survey were to list the main parasites and commensals infesting these crabs, to distinguish species native to the Barents Sea from species introduced to the area from the North Pacific, and to identify potentially serious pathogens.

Crabs (weight range 1080 - 4300 gm) were caught in baited pots in Varangerfjord (April 1996) and transported to Hammerfest, where they were held in indoor tanks of running sea water. A sample of 15 crabs (9 males and 6 females) was examined within 14 days after catchment. The crabs were killed and the carapace removed to expose internal organs. All major organs of the crabs were thoroughly examined by standard methods.

Twelve species of parasites and commensals were found. Of these, only two, one cilate and one acanthocephalan *Coronysoma* sp. can be regarded as true parasites of the crabs. The different species found, site of infestation, prevalence and abundance will be presented.

The results will be discussed in the perspective of possible effects of introducing species with respect to infectious organisms.

OCCURRENCE OF *Borrelia burgdorferi* INFECTED *Ixodes ricinus* IN DENMARK

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Objective: The purpose of this study was to investigate the variation in number of ticks and number of *B. burgdorferi* infected ticks, consequently leading to a risk map for Lyme's disease in Denmark.

Materials and methods: Ticks were collected from two different forest habitats at 21 different locations in Denmark (at nine locations only one habitat). The locations represented a variation in the presence of roe deer from 0 to 135 per 1000 ha. Differentiation of the soil quality in each location based on texture analysis led to six different soil classes, giving information on soil water capacity and capillary conductivity. The ticks were collected by flagging 80 minutes at each site. Detection of *B. burgdorferi* was performed by IFA using polyclonal antibodies.

Results: The average infection rates in *I. ricinus* females and males were 8.3% and 4.0%, respectively, and in nymphs 5.9%. The number of ticks increases drastically with increasing numbers of roe deer and increasing water capacity of the soil. Maximum occurrence was 5.7 nymphs/min.and minimum occurrence 0.15 nymphs/min. The infection rate of the nymphs ranged between 0 and 12% and was positively correlated with the adult/nymph ratio. All variation could be explained by variation in adult/nymph ratio, which reflects the diurnal activity pattern by uninfected nymphs. Following the corrections for differences in diurnlal activity leading to differences in the

infection rate, the average rate of infection for nine different combinations of soil type and presence of roe deer was found to be 4.9% (STD 1.1).

Conclusion: *I. ricinus* is widespread in Denmark and therefore *B. burgdorferi* infected ticks may be found in all forests. Since all areas have the same rate of infection in nymphs the potential risk may be determined by the abundance of ticks, which means that the eastern part of Denmark with high presence of roe deer and soil with high water capacity is a high risk area, while the western part is a low risk area.

DYNAMICS OF *Coccidia* FAUNA FROM *Eimeria* AND *Isospora* GENERA IN RODENTS IN THE CHERNOBYL ACCIDENT ZONE

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The goal of the present work is to estimate changes in the *Coccidia* fauna in rodents over the radionuclide contaminated area.

The paper contains material collected in the period 1987-1995 in the evacuation zone at the Chernobyl Nuclear Plant. Rodents caught with the standard zoological methods were treated with Darling's method.

We found 34 *Eimeria* and 4 *Isospora* species in 11 rodent species. During the entire period of the study *Sporozoea* fauna was the richest in the bank vole (8 species) and the yellow-necked (8) and field (6) mice. Moist biocenoses such as oak and alder forests and flood plain meadows are most preferred by *Coccidia.* The maximum number of Eimeriidae species was found in the initial period after the accident: 34 species (including 4 *Isospora* species). Subsequently, the species diversity decreased and now it varies at the level of 14-16 species (2-3 *Isospora*). It is found that the protozoan fauna tends to decrease in the background hosts to 1-2 parasite species that are the most typical of a particular host. This trend is accompanied by increased extent of invasion of rodents by each of them. *Eimeria rysavyensis* Arnastauskiene, 1982 in the bank vole, *E. arvalis* (Ivanoff-Gobsem, 1932) Vejsov, 1963 in *Microtus* voles and *E. polessica* Kireenko, 1990 in the field mouse can be an example. In particular years infestation of the hosts with these species reaches 70%, which is twice as high as the average figure for Belarus. After 1991 species of *Isospora* genera are extremely scarce.

Thus, in the Chernobyl accident zone the *Coccidia* fauna is characterised by the trend of a general decrease in the species composition which is accompanied by an increase in the frequency of occurrence of dominant species of the *Eimeria* genera.

CLINICAL EVALUATION OF FAMILIAR TOXOPLASMOSIS IN POZNAN REGION (WEST POLAND)

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A total of 102 families were examined, in which index cases manifested a fully symptomatic form of lymphonodular toxoplasmosis. The latter was thought to reflect acquired *Toxoplasma gondii* invasion and to represent a dependable index of the invasion source in other members of the patient's family.

Material and methods: A total of 388 persons from urban or rural areas of Poznan region were examined, performing in each of them a set of clinical tests. *T. gondii* invasion was confirmed by positive ELISA test (Vidas, BioMerieux), which permitted determination of antibodies of IgG and IgM classes.

Results: Two groups of patients were distinguished. Group I included 102 patients (index cases) with fully symptomatic form of lymphonodular toxoplasmosis: lymphadenopathy, subfebrile status, weakness, sweating, positive ELISA-IgG (94.1% cases) and with IgM class antibodies (68.7% cases). The group included 42.1% patients aged 1 to 14 years. Group II included 286 patients (168 children). In 28 children lymphonodular taxoplasmosis was ascertained; seven children manifested CNS lesions. Eight adults with lymphonodular toxoplasmosis included three pregnant women. In the group IgG class antibodies were found in 158 patients (55.2%) and IgM class antibodies in 35 patients (12.2%) which indicated active toxoplasmatic process and need for treatment. Children with neurological signs were subjected to a more thorough clinical analysis.

Conclusions: Clinical and serological examination demonstrated that index cases with lymphonodular form of toxoplasmosis are useful markers for the detection of acquired or congenital *T. gondii* invasion in families.

A GENE PROBE FOR MOLECULAR EPIDEMIOLOGICAL STUDIES ON *Fasciola hepatica*

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A repetitive gene fragment of *Fasciola hepatica* was evaluated for its use in molecular epidemiological studies.

Two primers were synthesized according to the original sequence. Using these primers, PCR amplifications of genomic DNA from *F. hepatica* from Northern Germany generated a ladder of multiple copies of a 114 bp fragment. To determine the intraspecies variations, five individual adult worms from four countries in four different continents were investigated (Australia, China, Germany, USA). The repetitive fragments were sequenced and homologies compared. To determine specificity and interspecies variations, genomic DNA from other trematodes, *F. gigantica* (China), *Dicrocoelium dendriticum* (Germany), *Paramphistomum* spp. (China, Germany) as well as genomic DNA from intermediate host snails *Lymnaea truncatula* (Germany) and *Lymnaea pervia* (China) were investigated.

Intraspecies variations ranged from 1 to 6% without showing significant differences between isolates from different countries. Sequence homologies with *F. gigantica* were between 95 and 99%, whereas no amplificates were generated with DNA from trematodes belonging to other genera or from intermediate hosts.

The gene fragment will be used as a specific probe in epidemiological studies on the occurrence of developmental stages of *Fasciola* in snails in Germany and China. (This work is supported by the EU, INCO-DC grant No. ERB 3514PL950001.)

PARASITES OF MINNOW (*Phoxinus phoxinus*) FROM A HIGH MOUNTAIN WATER SYSTEM OF SOUTHERN NORWAY

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Objective: The aims of this study were to do a parasitological survey on minnows recently spread to a high mountain area, to estimate pathological effects on the host and to detect population regulating factors.

Material and methods: Minnows of different age classes were sampled and fixed separately in formalin during the summer months of 1996. Fresh material was also examined. Measurements and observations were done by means of squash preparations of the fish larvae and dissection of bigger fish. Countings and studies of some parasites were based on histological sections.

Results: Parasite species found included the ciliates *Paratrichodina incissa, Apiosoma* sp. and *Capriniana* sp., the myxosporidian *Myxobolus mülleri,* the monogeneans *Gyrodactylus* spp. and *Dactylogyrus borealis,* the digeneans *Diplostomum phoxini* and *Allocreadium isoporum,* the acanthocephala *Neoechinorhynchus rutilii* and the cestode *Ligula intestinalis.* Some adult nematodes yet to be identified have also been observed. One metazoan parasite species occurred in considerable amounts. Metacercariae of *Diplostomum phoxini* in the brain counted

close to 4000 individuals in one fish. Minnows acquired this parasite at an early developmental stage. Accumulated infections seemed frequent. Trichodinids and diplostomatid metacercariae were the most pronounced parasites on the fish larvae.

Conclusions: Minnows newly spread to this high mountain water system carry a wide variety of parasites. The digenean *Diplostomum phoxini* is found in large numbers, also infecting fish larvae, and may have an impact on population regulation of *Phoxinus phoxinus*. Further estimation of the pathological effect will be done by experimental infection of fish larvae and by quantifying the amount of glucose assimilated by this metacercaria.

BIOMETRICS OF THE PIKE PERCH, *Stizostedion lucioperca* (L.) IN THE COURSE OF INFECTION WITH ACANTHOCEPHALANS IN THE DAM RESERVOIR "PRZECZYCE" (SOUTHERN POLAND)

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Materials and methods: In the dam reservoir "Przeczyce" (the Silesian Upland) 423 pike perch were collected with mean body weight 776.4 g and length (*longitudo corporis*) 52.1 cm, at the age 1-9 years. The material was collected with hand and barrage nets.

Results: In examined populations of the pike perch, infection with three species of acanthocephalans was ascertained (*Acanthocephalus lucii, A. clavula, A. anguillae*), at mean extent of infection 37%. Intensity of infection was 3-46 acanthocephalans. A significant decrease in body weight of infected fish in comparison to non-infected individuals, was observed when the intensity of the infection was more than 15 parasites. Sub-populations of fish infected by more than 25 acanthocephalans showed in addition to a decrease in body weight a reduction in the increase in body length (mean about 12%) in comparison to healthy fish. Extent of the infection in the examined pike perch population was varied in particular age classes. The lowest amount of all infected fish (9%) was ascertained among one year old individuals, the highest of all (46%) in the six year old class.

Conclusion: Under conditions of relatively high intensity of infection, reductions related to increases in body weight and body length were observed in sub-populations of the pike perch which were infected with acanthocephalans, as well as a significant decrease in the condition factor, in comparison to healthy individuals in the dam reservoir "Przeczyce".

MAPPING THE DENSITY OF *Ixodoidea* IN BELARUS

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The goal of this work was to map the distribution and density of the most abundant *Ixodidae* species, namely *Ixodes ricinus* L. and *Dermacentor pictus* Herm, which are the main carriers of epidemic diseases in Belarus.

The material was collected according to standard zoological methods used for accounts of the ticks. All administrative districts of Belarus were examined. The work was financed by the Soros International Science Foundation.

As a result of the studies, *I. ricinus* was recorded over the whole territory of Belarus, except for two districts in the Mogilev region, while *D. pictus* waas found to be absent almost in the whole Vitebsk region and in some districts of the Mogilev region. The highest density of both species was found in Gomel and Brest regions. The maximal density of *I. ricinus* was found at Belovezhskaya Pushcha and the density of *D. pictus* was the highest in the evacuation zone of the Chernobyl Nuclear Plant (< 50 at a maximum of 2800 ind./km^2). Similar densities were found in local foci in a few districts of Mogilev and Grodno regions. The lowest density of the ticks was recorded in Vitebsk and Minsk regions (0-1 to 1-10 ind./km^2). Foliaceous (oak and alder) forests and forest margins at the boundaries with flood plains and settlements are preferred biocenoses for *Ixodidae.*

Thus, the southern part of Belarus, primarily the Pripyat basin and the territory of Belovezhskaya Pushcha, are potentially the most dangerous as regards natural foci diseases carried by *D. pictus* and *I. ricinus*, respectively.

Schistosoma haematobium INDUCED LESIONS IN THE FEMALE GENITAL TRACT IN A VILLAGE IN MADAGASCAR

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To assess the morbidity related to female genital schistosomiasis, FGS, in a *Schistosoma haematobium* endemic area in Madagascar, a study was carried out

among women in the childbearing age as part of an overall community based morbidity survey including parasitological and ultrasonographical examination. A medical history was taken and particular attention was paid to symptoms and signs related to the female reproductive tract. Of 175 women interviewed, 103 were included for a gynecological examination and visible lesions of vagina and cervix were biopsied in order to determine the origin of the lesion. Furthermore, all the women were screened with PAP smears from vagina and exo/endocervix.

Fifteen women in all were detected with schistosome ova from the vagina and/or cervix (median age 24 years and range 15-36). Of 36 women biopsied for cervical abnormalities, 12 were detected with eggs in the histological sectioning (33%) of which two women additionally presented vaginal induration and both were found egg positive in the vaginal biopsy. Six women were found positive in the PAP smears of which three were also among those 12 women with egg positive biopsy. The overall prevalence of positive *S. haematobium* egg excretion in the urine was 69% and the geometric mean egg count of positive individuals was 51 eggs/10 ml of urine. Five of the 15 women with confirmed FGS had <1 egg/10 ml urine. Bladder lesions and congestive changes in the kidneys shown by ultrasonographic examination were observed in 33% and 9% of the 103 women, respectively. None of the 15 women with confirmed FGS had renal congestion. Our study demonstrates that FGS is a common manifestation of an infection with *S. haematobium.*

EVIDENCE OF OBLIGATORY PARASITISM OF *Dermocystidium cyprini*

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Objective: High numbers of cysts of *Dermocystidium cyprini* have caused remarkable mortality of fingerlings of common carp but a low number of cysts has minimal influence on fish health. There is a lack of information about the mechanism of pathogenesis of this parasite. The damages caused by mechanical irritation can be the reason for pathological changes in fish gills in the case of high numbers of cysts. The gills may be used only to hitch in purpose of spreading. The aim of the work was to reveal the possibility of the maturation of *D. cyprini* cysts under *in vitro* conditions.

Material and methods: The cysts of *D. cyprini* in three different developmental stages: plasmodium (N=17, removed from fish in February), cleavage of plasmodium (N=21, removed in March) and beginning of sporogenesis (N=20, removed in April) were collected from fingerlings of common carp. The cysts were kept in fresh water at 4°C in the absence of antibiotics until mature spores were formed. In culture where maturation did not occur the experiment was finished after 60 days.

Results: The maturation did not occur in stages of plasmodium where the diameter of cysts was 0,1-0,3 mm. The cysts which were in stages of beginning of sporogenesis matured normally in two weeks. Three of 21 cysts which were in the beginning of the experiment on plasmodium cleavage stage matured after three weeks, others did not develop.

Conclusions: The identification of dependence of the host in all developmental stages of *D. cyprini* was not possible in this work as the full life cycle is not known at present. The few number of cysts found could not give us a large statistical material. However, the experiment shows the obligatory parasitism in plasmodium stage and the possibility of sporogenesis out of the host organism.

PREVALENCE OF *Anisakis simplex* IN HERRING *(Clupea harengus)* FROM NORWEGIAN WATERS

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Objective: The objective of this investigation was to examine the prevalence of nematodes of the genus *Anisakis* in herring (*Clupea harengus*) from Norwegian waters.

Material and methods: During March and April 1996 a total of 250 samples of herring were collected at the major landing sites. These samples were examined visually for nematodes in the viscera and muscle.

Results: In this material we were able to detect nematodes in 94% of the examined samples. These nematodes were mainly found in the mesenteric and plyoric region, in numbers ranging from 1 to 42. 36% of the fish had nematode numbers higher than 10. In 10% of the examined herring, nematodes could be found in the muscle. The number of visible nematodes in muscle was in all cases low, and when detected they occurred in numbers of one to two in each fish. The nematodes found belong to the species *Anisakis simplex*, as shown by morphology and ability to fluoresce under UV-light.

Conclusion: The prevalences of *Anisakis simplex* found in this investigation seem to be in line with those reported in previous studies in these waters. The herring examined had been frozen, and none of the observed nematodes were found viable.

POST-TREATMENT RE-INVASION DYNAMICS OF SOME ANIMAL PARASITES IN ESTONIA

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Objective: The purpose of the study was to investigate post-treatment re-invasion dynamics of pig and sheep parasites (*Ascaris suum, Oesophagostomum dentatum, Sarcoptes suis, Strongylata* spp., *Moniezia* spp.) and to compare the effect of different treatments in Estonian climatic and maintenance conditions.

Methods: Every year from 1989-1996 several trials were carried out in all districts of Estonia. Our observations were carried out during 3-10 months after treatment. The extent of invasion was studied comparatively on small as well as on large-scale farms.

Results and conclusions: Our data show that re-invasions of sarcoptosis suis were formed 3-6 months after treatment. The most effective drug against sarcoptic mange mites was ivomec and this preparation prolonged the period before re-invasion up to 8-10 months. In pig ascaridosis and oesophagostomosis the re-invasions took place generally 50-70 days after the treatment (11-15% of the pigs). In application of piperazin the re-invasions took place more quickly compared with other anthelmintics (imidazole preparations and ivomec). Where treated sheep were grazing on pastures contaminated with *Strongylata* spp. larvae, the extent of invasion was 2±2%, 4±4%, 22±6%, 34±7% and 53±7% after 10, 20, 30, 60 and 90 days, respectively. According to our experience, the main principles in prophylaxis of animal parasites are 1) to avoid the re-invasions with mange mites, repeated treatments of pigs are needed after six months; 2) in pig helminthoses sows are the main source of invasion for piglets. Sows should be treated at the end of pregnancy about 10 days before farrowing and 3) where sheep graze on contaminated pastures the dehelminthizations against digestive strongylatoses and monieziosis are needed once a month.

Demodex folliculorum AND *Demodex brevis* (ACARI: DEMODICIDAE) IN A POPULATION SAMPLE OF OJOBI, GHANA

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Objective: Reported prevalences of hair follicle mites in humans from different countries vary from a few percent to 100 percent. One study only reports observations

from an arctic area and three studies report observations from tropical areas. More observations from areas with extreme climatic conditions are needed to evaluate whether the climate influences the prevalence. This report presents observations from an equatorial West African country.

Materials and methods: Eyelashes were obtained by epilation from 100 Africans, 56 men and 44 women, aged 0-100 years old. All donating persons were chosen at random among the inhabitants of Ojobi near Accra in Ghana. From 8 to 20 eyelashes (average 9.4) were obtained from the upper eyelid of both eyes of each person. The eyelashes were fastened to object glasses with scotch tape and mites were identified by ordinary light microscopy at 100 and 400 times enlargement according to described criteria. A Demodex Index (DI) was calculated as the number of mites per eyelash examined.

Results: Evidence of hair follicle mites was obtained in 26 of the examined persons, 9 women and 17 men. The difference between women and men was not significant (χ^2=1.256, p>0.2). A low prevalence was found in young persons and the highest prevalence among the oldest persons. Eggs were found only in one person, larvae in two persons and ghosts (degenerated mites) only in two persons. Mites identified as *Demodex folliculorum* were found in 18 and *D. brevis* in 3 of 21 persons, giving a *D. folliculorum/D. brevis* prevalence ratio of 6,1. The DI was 2,4. As a DI of 8,9 was found earlier in a Danish population sample and a DI of 23 found in a population sample from Greenland the data seem to support the hypothesis that climatic factors might influence the intensity of the infections but not the prevalence of mites.

PROBLEMS ENCOUNTERED WITH TECHNOLOGY TRANSFER TO THE RESOURCE LIMITED FARMER IN SOUTH AFRICA

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Objective: Problems encountered with technology transfer to the resource limited farmer include illiteracy, language barriers, little contact with areas outside their rural towns and a lack of understanding of internal parasites, to name but a few.

Material and methods: Animal health technicians and extension officers are an excellent means of contact between farmers and the Onderstepoort Veterinary Institute (OVI). OVI trains these officers in veterinary parasitology and they relay the message to the farmer. OVI receives feedback on problems encountered with farming from the officers. Field trips and information days are invaluable to the farming communities. Illiterate adults are often taught by their school-going children. Demonstration kits, laminated life cycles, cartoons, pamphlets and videos are materials used successfully by the OVI to demonstrate and educate these farmers. It is essential to learn an African language.

Results: Extension service for veterinary parasitology is a new concept, but excellent work is being started in spreading knowledge and information about the many services that are available. Pamphlets, cartoons and videos are produced and improved for training. Field trips motivate and persuade farmers to adopt new ideas, to improve their skills and create opportunities for interaction between resource limited farmers, extension officers and OVI.

Conclusion: It is our responsibility to promote the dissemination of information on animal health and veterinary services and to educate the many new and upcoming farmers.

UNUSUAL LOCATION OF *Ergasilus sieboldi* ON PIKEPERCH AND VOLGA PIKEPERCH OF LAKE BALTON, HUNGARY

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Ergasilus sieboldi infection of different fish species living in natural waters is well known in Europe. Numerous data are available on the occurrence of that parasite in Lake Balaton, too. As a parasitic copepod, *E. sieboldi* spends the larval stage of its life in the plankton, and only its imago stages establish themselves on fish. *Ergasilus* specimens have been detected on the gills of the majority of Lake Balaton fish species examined. The parasites adhered to the gill filaments, resulting in epithelial proliferation. In contrast with data reported by German and Polish authors, we did not find intensive infection in cyprinids, and the majority of Lake Balaton fish were colonised by as few as 3-30 specimens. The only exception was the pikeperch in which occasionally an infection of very high intensity and prevalence could be recorded. In pikeperch, a characteristic feature of *Ergasilus* infection was that, besides the gills, the copepods established themselves also on the external surface of the operculum, in the folds located there, as well as on the base of the fins. A similar unusual location has been reported by Russian and Polish authors; however, in their cases the copepods appeared on the base of fins and in the anal region only if extremely intensive gill infection was present. In our cases, the number of copepods colonising the folds of the gill cover and the fins always exceeded that present on the gills. The number of copepods clinging to the operculum was 3 to 10 times higher than that of specimens found on the gill filaments. Infection by as many as 800 copepods was also observed. In general, it can be established that in pikeperch specimens with a body length exceeding 30 cm, infection is common and its prevalence reaches 100%. At the same time, in pikeperch smaller than 20 cm, the prevalence of infection is only 20-25% and its intensity is also low.

The Volga pikeperch was unique in that it had only gill infection and even that was of low intensity and prevalence.

The pathological importance of copepods present on the body surface seems to be lower than that of specimens occurring on the gills. Although on the base of the fins inflammatory processes and small ulcers were macroscopically visible in some cases, in the folds of the operculum degenerative and proliferative changes could be detected only in histological sections.

A TRIAL OF SINGLE DOSES OF PRAZIQUANTEL WITH ALBENDAZOLE OR MEBENDAZOLE IN CONTROL OF SCHISTOSOMIASIS AND HOOKWORM

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Objective: A randomised single-blind control trial was undertaken among schoolchildren of Sangabuye in Mwanza, Tanzania, to compare egg negative conversion rates (NCR) and egg reduction rates (ERR) for *Schistosoma mansoni* and hookworm of a single oral dose of praziquantel (pzq) 40 mg/kg body weight (BWT) followed after five days by either albendazole (alb) 400 mg or mebendazole (med) 500 mh.

Materials and methods: Side effects were documented by teachers in a questionnaire 24 hours after treatment, whereas NCR and ERR were determined six months later.

Results: Praziquantel therapy had relatively higher incidence of side effects than albendazole or mebendazole. However, the side effects were mild and transient. At six months the NCR for pzq/alb and pzq/meb groups were 79.7% and 93.5% and ERR were 68.5% and 81.4& for *S. mansoni* and hookworm respectively and they were not statistically different among the interventional groups.

Conclusion: On the basis of few side effects and relatively high NCR and ERR at six months (low reinfections), praziquantel 40 mg/kg BWT and albendazole 400 mg or mebendazole 500 mg separated by weekly intervals may be recommended for mass chemotherapy in areas where schistosomiasis mansoni and hookworm are highly prevalent.

THE ORIGIN AND FORMATION OF PARASITOFAUNA OF FISH OF THE FAMILY GOBIIDAE FROM THE BLACK AND ASOV SEAS

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Objective: Analysis of the parasites of gobiid fish.

Materials: In the Black and Asov Seas the endemic species of family Gobiidae, relicts of the ancient basis, are the most numerous. They dwell mainly in oligo- and mesohaline zones of the seas. The settling of the seas by Gobiidae Mediterranean tenants (immigrants) took place in the contemporary epoch, after "the break" of Dardanell.

Results: Nineteen species, belonging to eight genera of Gobiidae were investigated and 141 species of parasites were found in them.

Host	Number of species parasites (endemic species)							
	a	b	1		2		3	
			a	b	a	b	a	b
	119 (26)		43 (6)		23 (8)		53 (12)	
		46 (2)		5 (2)		11 (3)		30 (2)
Immig.	68 (11)		5 (2)		16 (4)		47 (5)	
Total	141 (30)		43 (6)		28 (9)		70(15)	

1 - freshwater parasites; 2 - brackish parasites; 3 - marine parasites;
a - number of species for host; b - common number of species

Conclusion: The specific structure of the parasitofauna of Gobiidae and the intensity of their infection by separate parasites is defined with two categories of factors: 1) phylogenetic factors - systematic position of a host and parasite, their last joint evoluation in the process of which the definite speciality of a host is worked and 2) ecological factors - the mode of life, nutrition, food structure etc. The parasitofauna of Gobiidae reflects the tight correlation with the environment and illustrates excellently the life history of a host.

METAZOAN PARASITES IN LONG ROUGH DAB (*Hippoglossoides platessoides limandoides* Bloch, 1787) IN ICELANDIC WATERS

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Objective: The aim of the study was to add information to the limited knowledge of parasite infections in marine fish in Icelandic waters.

Materials and methods: Metazoan parasites were collected from fish flesh of 562 and intestines, gills and organs of 358 long rough dabs. The fish were caught in February and March from five locations around Iceland.

Results: Thirty parasite species were observed in long rough dab in the study: *D. varicus, B. crenatus, L. gibbosus, S. ovacutum, S. furciger, S. vetustum, P. atomon, Z. viviparus, P. subaquephorous, Aporocotyle simplex, S. baccatum, Otodistomum* sp., *Grillotia* sp., *Diphyllobotrium* sp., Tetraphyllidean larvae, *Anisakis simplex, P. decipiens, Pseudoterranove* sp., *H. aduncum, H. rigidum, C. osculatum, Contracaecum* sp., *C. heterochrous, S. oviflagellis, Ascarophis* sp., Acuarioid larvae, *E. gadi, Corynosoma* spp., *L. branchialis* and *A. cornuta.* Some geographical difference in infections was found in the study area.

Conclusion: The parasite fauna in long rough dab in Icelandic waters seems similar to what has been observed in other areas in the North Atlantic Ocean. Distribution of necessary hosts in life cycles of the parasites is probably the most important factor in causing geographic differences in the parasite distribution. Differences in diet and migration of long rough dab or its prey may also affect the distribution of the parasites.

CORRELATION BETWEEN CLINICAL MANIFESTATIONS AND LABORATORY DATA, PECULIARITIES OF TREATMENT OF CHILDREN WITH TRICHINOSIS

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Objective: The purpose of the study was to find out the changes in clinic and laboratory data during the period of 1992-1995 in comparison to the classic form of trichinosis.

Materials and methods: Two hundred and forty-one patients from two to 15 years old, were examined and treated. The diagnosis was based on epidemiological anamnesis, clinical manifestations, eosinophilia and ELISA method (69.9% of

patients). The disease has spread through the use of smoked pork products that have not been veterinary checked.

Results: The average duration of incubation period was 12 days. The dyspeptic symptoms were present in 95 cases (39.4%). Fever was present in 52 cases (21.6%) and 45 patients (18.6%) had subfebrile temperatures. Rash was present in 52 cases (21.6%) and went together with marked eosinophilia in more than 20%. Conjunctivitis and periorbital edema were observed in 95 older patients (39.4%), and simultaneously with rash in 102 cases (42,3%). Mebendazole (in 222 cases) and thiabendazole (in 19 cases) were used for treatment. Clinical symptoms lasted longer when thiabendazole was used. Since 1994 all patients have been treated for 5-7 days with mebendazole (10-15 mg/kg/24h for children under five years old and 200-300 mg/kg/24h for older children). The average hospitalisation time decreased from 13.8 to 9.3 days in the mentioned period.

Conclusion: The course of trichinosis in children was uncomplicated, light and moderate. The therapy of mebendazole for 5-7 days 10-15mg/kg/24h is optimal for children.

TYPING OF *Toxoplasma gondii* ISOLATES BY AUTOMATIC SEQUENCING OF THE C-TERMINAL PART OF THE IMMUNODOMINANT SURFACE ANTIGEN 1, SAG1 (P3O)

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Isoenzyme analysis has shown that *Toxoplasma gondii* can be divided into different zymodemes and analysis of DNA has shown that Restriction Fragment Length Polymorphism (RFLP) patterns can distinguish *T. gondii* with different virulence in the mouse model. However, both techniques are laborious and time consuming, and the isoenzyme analysis requires large amounts of parasite material. Previous studies have found nucleotide differences between the mouse virulent RH-isolate and the avirulent C and P-isolates.

Objective: The aim of the study was to develop automatic sequencing of the C-terminal part of the SAG1 gene as a rapid tool for typing *Toxoplasma gondii* isolates.

Material and methods: *Toxoplasma gondii* isolates from livestock and wildlife in Denmark and well characterised isolates from abroad were collected. Genomic DNA was amplified with the Polymerase Chain Reacion, PCR, and the reaction product sequenced in an ABI-3773 automatic sequencing machine using standard protocols.

Results: The published sequences from the RH and C/P isolates were used as references and compared to the sequence of the RH and SSI-119 isolates kept at Statens

Seruminstitut for many years. The published RH and RH_{SSI} were identical. The RH were identical to the BK isolate. The mouse avirulent isolates showed nucleotide substitutions at positions 751 (C to G), 788 (G to T), 809 (G to C), 891 (A to C) and 930 (G to T). All nucleotide changes were non-silent resulting in amino acid substitutions. All isolates from Danish livestock animals fell in the mouse avirulent group.

Conclusion: Automatic sequencing of PCR amplified DNA is an easy and rapid method for typing *Toxoplasma gondii* isolates in mouse virulent (RH type) and avirulent (C/P type) isolates.

COMPARATIVE PARASITOLOGICAL STUDIES OF SOME REPRESENTATIVES OF FAMILY RANIDAE IN WESTERN POMERANIA

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Objective: In the course of the research, the examination of parasitic fauna of the family *Ranidae* inhabiting the western Pomerania region in Poland was undertaken. The impact of parasites on host organisms was investigated.

Materials and methods: As a whole, 239 specimens of three species, *Rana esculenta* (L.), *Rana temporaria* (L.) and *Rana arvalis* (Nilson, 1842) from the environs of Szczecin, Jaroslawiec, Insko and Wolin were examained parasitologically, anathomopathologically and hematologically.

Results: Parasites from types of *Sarcomastigophora, Microsporidia, Trematoda, Nematoda* and *Acanthocephala* were found. From general analysis it appears that there is variability in number of species, intensity and extent of infection. Some centres of epizootic were found in the environs of Zaroslawiec. Species of microsporidia *Glugea danilewskyi* (not reported in Poland up to now) were found. The mass occurrence of metacercariae *Codonocephalus* sp. in the muscles, peritoneum, liver, kidney and gonads was stated. The analysis of the collected material showed that the most scanty parasitic fauna of frogs occurred in the environs of Szczecin.

Conclusion: Collected material was preliminarily diagnosed. At present cytodiagnostic and histochemical examination of conserved material is conducted.

FOOD PREFERENCES OF THE PIKE PERCH, *Stizostedion lucioperca* (L.) IN THE COURSE OF INFECTION WITH ACANTHOCEPHALANS IN THE DAM RESERVOIR "PRZECZYCE" (SOUTHERN POLAND)

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Materials and methods: In the dam reservoir "Przeczyce" (the Silesian Upland) 423 pike perch were collected with mean body weight 776.4 g and length (*longitudo corporis*) 52.1 cm, at the age 1-9 years. The material was collected with hand and barrage nets.

Results: In examined populations of the pike perch, infection with three species of acanthocephalans was ascertained (*Acanthocephalus lucii, A. clavula, A. anguillae*), at mean extent of infection 37%. Intensity of infection was 3-46 acanthocephalans. There were no observed differences in food preferences in non-infected and infected one year old pike perch. The food of these fish was dominated by crustaceans of the genera *Daphnia* and *Eudiaptomus*. The fundamental foods of non-infected older pike perch were: bream (*Abramis brama*), which amounted to 29-53% during the year period, roach (*Rutilus rutilus*) 21-46%, rudd (*Scardinius erythrophthalmus*) 11-26%, perch (*Perca fluviatilis*) 119-22% and sunbleak (*Leucaspius delineatus*). The last species was dominant in the food of juvenile pike perch from II and III age classes. The food of adult pike perch infected with acanthocephalans was dominated by roach (35-58%), perch (29-46%), bream (19-26% and rudd (16-19%). Fish caught by infected pike perch were 12-19% shorter in comparison to species found in the digestive tract of non-infected individuals.

Conclusion: The authors conclude that food preferences of pike perch infected with acanthocephalans have changed, probably caused by the altered condition of the infected fish.

THE OCCURRENCE OF *Myxosporea* IN SKELETAL SYSTEM OF SALMONIDAE AND ONCORHYNCHIDAE BREEDING AND WILD FISH

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One of the most pathogenic species of *Myxosporea* is the protozoan *Myxobolus cerebralis*. Its disease is distributed in Europe, North America, Africa and Asia and attacks mainly rainbow trout, *Oncorhynchus mykiss*, which is a common breeding fish.

The parasite attacks cartilage of the fry tissue and locates mainly in the cranium and candel vertebras. It is a source of big movement of fish and is one of the main diseases of salmonidae fish. It was found in 136 examined fish belonging to four species: *Oncorhynchus mykiss* (114 fish), *Salmo trutta m. trutta* (9 fish), *Salmo trutta m. fario* (7 fish) and *Coregonus lavaretus* (6 fish). The material was taken from 10 fish farms, from the river Gizdepka and the mouth of the Vistula river. *Myxobolus cerebralis* were found in the cranium of four fish among 29 specimens of the examined rainbow trout, which were taken from the fish farm on the river Leba near the village of Tluczewo. Most of two tons of the fish from these ponds indicated wrong coordination of movement. All fish were killed.

SCREENING OF DRUGS FOR THEIR INHIBITION OF *Plasmodium falciparum* PROLIFERATION USING THE ^{3}H-HYPOXANTHINE INCORPORATION METHOD

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Objective: We have adapted a ^{3}H-hypoxanthine incorporation assay, developed by DesJardin *et al.* for large-scale screening of antimalarial drugs. One person can now easily analyse up to 300 compounds a week.

Materials and methods: Asyncronous *Plasmodium falciparum* parasites in culture are diluted to a parasitemia of 0.2% and a hematocrit of 2% and are added in 50 µl aliquots to microtiterplates. The compounds to be tested are diluted in RPMI-hepes to one or several concentrations and are added to the wells. After incubation for 24 hours, to allow inhibition of parasite growth, the ^{3}H-hypoxanthine is added and the culture is continued for another 18 hours (total 42 hours). The cells are harvested and the incorporated radioactivity is estimated in a microbeta counter. The uptake of ^{3}H-hypoxanthine is directly proportional to the number of parasitized erythrocytes and thereby also to the inhibition of the proliferation achieved by the drug. Hypoxanthine is a purine, used as a nucleotid precursor. The malaria parasite is incapable of hypoxanthine synthesis, so the uptake of the radioisotope ^{3}H-hypoxanthine is directly proportional to the growth of the parasite.

Results: Within six months we have tested about 3700 compounds of which 1% showed a significant inhibition of the proliferation in concentrations below 0.5 µg/ml.

Conclusions: Compared to manual counting of malaria parasites in giemsa stained films this method is faster, easier and more sensitive.

In vitro MAINTENANCE AND SURGICAL TRANSFER OF *Schistosoma japonicum* WORMS FROM DONOR TO NAÏVE RECIPIENT PIGS

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The purposes of the first part of this study were to find a culture medium and a temperature range suitable for *in vitro* maintenance of adult *Schistosoma japonicum* worms just prior to surgical transplantation. The objective of the second part of the study was to establish a method for transplanting adult *S. japonicum* worms from experimentally infected donor pigs to naïve recipient pigs. Adult *S. japonicum* worms were cultivated in four different media (NCTC 135, NCTC 109, RPMI 1640 and physiological saline) at three different temperatures (4°C, room temperature (22-25°C) and 37°C). Based on survival and evaluation of morphology, incubation in NCTC 135 at room temperature was judged as the best combination. Six Landrace/Yorkshire crossbred pigs were used as donors and two as recipients. Worms for transplantations were obtained by perfusion of the mesenteric veins of the donor pigs. A total of 148 and 150 were surgically transplanted into a caecum vein of the two recipient pigs. Six weeks after the transplantation 12% and 36% of the transferred worms were recovered by perfusion of the two recipient pigs. The results suggest that the transplantation technique can be useful as part of future studies on population genetics, dynamics and regulation in the pig/*S. japonicum* model.

HELMINTHS MUSTELINE (CARNIVORA: MUSTELIDAE) OF BELORUSSIAN POLESSIE IMPORTANT FOR MEDICINE

By V. SHIMALOV

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Objective: One of the trends of our work is an investigation of helminths spreading among the wild vertebrate animals (Vertebrata) of southern Belarus (Polessie). These helminths are important and one needs to elucidate their role in the epidemiology of human helminthosis. The substance of this report is the result of investigations of animals of the Mustelidae family (1986-1995).

Materials and methods: Two hundred and thirty-five carcasses of eight kinds of animals (*Mustela nivalis* L., *M. erminea* L., *M. putorius* L., *M. vison* Brisson, *Martes martes* L., *M. foina* Erxleben, *Meles meles* L., *Lutra lutra* L.) were subjected to helminthological revelation according to Skrjabin.

Results: The total infection of musteline by helminths is 79.2±2.7%. 42.6±3.2% exmined animals possess medically important helminths. We found nine kinds of such helminths: *Fasciola hepatica* L., 1758 - host: *L. lutra; Opisthorchis felineus* (Rivolta, 1884) - hosts: all kinds of musteline besides *M. martes, M. foina; Pseudamphistomum truncatum* (Rudolphi, 1819) - hosts: *M. nivalis, M. erminea, M. putorius, M. vison, L. lutra; Metorchis albidus* (Braun, 1893) - hosts: *M. putorius, M. vison, L. lutra; Spirometra erinacei* Rudolphi, 1819) larvae - all kinds of musteline; *Mesocestoides lineatus* (Goeze, 1782) - all kinds of musteline besides *M. vison; Thominx aerophilus* (Creplin, 1839) - all kinds of musteline besides *M. vison; Trichinella* sp. larvae - all kinds of musteline; *Spirocerca lupi* (Rudophli, 1809) larvae - hosts: *M. nivalis, M. erminea, M. putorius, M. martes, M. foina.* Most frequently encountered helminths belong to the Opisthorchidae family. 11.1±2.1% examined animals are infected by them.

Conclusion: Musteline take part in preservation and spreading in natural Belorussian Polessie biocenoses of nine kinds of medically important helminths. These animals promote the support of natural helminthosis centers which in turn may be dangerous to human health.

PINWORM INFECTIONS OF CHILDREN IN PLAY SCHOOLS IN ICELAND

By K. SKÍRNISSON & B. JÓNSSON

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Objective: The aim of this study was to examine the prevalence of pinworm (*Enterobius vermicularis*) infection of children in play schools in the Reykjavik area, Iceland.

Materials and methods: In November and December 1992 cellulose tape samples were taken from the anal region of 184 of the 526 two to five year old children at nine play schools in Reykjavik and Kópavogur. Furthermore, the teachers and parents were questioned about known pinworm cases in the children in the previous six months.

Results: Eleven of the 184 children examined (6%) were infected with pinworms. Infection was found mainly in children in fifth (13.2%, n=53) and sixth (7.1%, n=42) year. No infections were found in three year old children (n=44), but one two year old child had pinworms (2.2%, n=45). The prevalence of infection within the play schools varied between 5.9 and 9.1%.

Pinworm eggs were found in six of the nine play schools and children in at least one additional play school had a recent history of pinworm infection (78%). Fourteen of the children not examined (4.1%, n=342) had a history of pinworm infection in the previous six months.

Conclusions: The results indicate that pinworm infections are rare in two and three year old children but every tenth of the four or five year old children had pinworms. In most cases neither the staff nor the parents had suspected the infection. Disadvantages of the cellulose tape method are that a recent or light pinworm infection can not be detected and that eggs can be removed from the perianal area prior to the sampling by washing or cleaning. Some pinworm infections could therefore have been missed in the present study indicating that the actual infection prevalence could be higher than reported.

DISPERSAL OF *Gyrodactylus salaris* BY THE SEA-RUNNING STAGES OF ATLANTIC SALMON (*Salmo salar*)

By A. SOLENG[1], T.A. BAKKE[1] & L.P. HANSEN[2]

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[2]*Norwegian Institute for Nature Research, Tungasletta 2, 7004 Trondheim, Norway*

Objective: We wanted to elucidate the importance of sea-running smolt and adult Atlantic salmon (*Salmo salar*) in the dispersal of *Gyrodactylus salaris*.

Materials and methods: The susceptibility of smolts to *G. solaris* was examined in laboratory experiments. The infection levels on smolts caught in the River Lierelva and in brackish water in the Drammensfjord in spring were assessed. The infection level on adult wild salmon caught as prespawners, spawners and postspawners (kelts) in the River Drammenselva was also assessed.

Results: Population growth of *G. solaris* increased exponentially on salmon smolts in laboratory experiments at 12.0°C. The prevalence and intensity of infection of *G. solaris* on wild salmon smolts caught approximately 30 km from the river outlet in the Drammensfjord (surface salinity 2.0-3.5%), were comparable to the parasite burden on wild smolt caught in the neighbouring River Lierelva. Adult wild salmon caught as prespawners, spawners and postspawners (kelts) in the River Drammenselva were infected with *G. solaris*. The prevalence and abundance increased from autumn to spring.

Conclusions: The present results give further support to the hypothesis of brackish water dispersal of *G. solaris* with infected salmon migrating between rivers in estuaries and fjords. The results also demonstrate the possible importance of adult salmon in the population dynamics of *G. solaris*.

HEXAMITID PARASITES IN NORWEGIAN FRESHWATER FISH

By E. STERUD

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Objective: Hexamitid flagellates believed to be *Hexamita salmonis* have occasionally been encountered in farmed fish in fresh water in Norway since 1973. A closer study of these flagellates was started after serious outbreaks of systematic hexamitosis in sea farmed Atlantic salmon.

Materials and methods: Wild fish of different species and farmed Atlantic salmon, *Salmo salar,* have been examined for hexamitid flagellates. Three isolates have been axenically cultivated and their ultrastructure studied by scanning and transmission electron microscopy.

Results: Flagellates from Atlantic salmon, grayling *Thymallus thymallus* and arctic char *Salvelinus alpinus* are found to be the same species. This is not *Hexamita salmonis* but a new species belonging to the genus *Spironucleus.* Hexamitid flagellates have also been found in trout *Salmo trutta,* whitefish *Coregonus lavaretus* and burbot *Lota lota.* These isolates have not been identified.

Conclusions: There seems to be a tendency in diagnostic laboratories, not only in Norway, that whenever hexamitid flagellates are encountered in salmonids, they are automatically called *H. salmonis.* Flagellates fitting the description of *Hexamita salmonis* have never actually been found in Norway and previous reports of this species should be regarded as misidentification. All isolates of hexamitid flagellates identified in Norway are shown to be the same *Spironucleus* sp. Electron microscopy is at the moment the only reliable way to discriminate between genera and species of hexamitid flagellates and should always be performed before species are identified.

PRELIMINARY RESULTS OF A SURVEY ON THE PARASITE FAUNA OF THE KIS-BALATON WATER RESERVOIR, HUNGARY

By Cs. SZÉKELY & K. MOLNÁR

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In recent years, members of our research team have conducted a comprehensive parasitological survey involving the most important fish species of Lake Balaton. The results have been reported in the form of presentations and scientific papers in several places. However, there was still a great need for a detailed parasitological study of fish species living in Kis-Balaton, regarded as the "filter" of Lake Balaton, as well as for a comparison of relevant results obtained in the two water systems during the survey

encompassing several years. We carried out a detailed parasitological examination of a total of 98 fish specimens belonging to 13 species, collected from storage-lake I of Kis-Balaton at five different sampling times in the year 1996, with the help of Balaton Fisheries Co. Ltd., using electrofishery equipment. Although originally we planned to examine samples of identical species composition and containing the same number of fish, the number and species composition of the fish examined depended on the catching results.

The gibel carp population has repeatedly experienced massive mortality in recent years. According to expectations, the parasite fauna of that population is extremely scanty: in addition to some protozoans (*Goussia carpelli, Hoferellus caarassii,* in the spring *Goussia leucisci, Trypanosoma* sp.) found in the majority of cases, only three helminth species belonging to the genera *Diplostomum, Philometroides* and *Dactylogyrus* have been detected.

Kis-Balaton has a valuable and consistent wild carp population, in which 7 protozoan and 12 metazoan parasite species have been detected. The occurrence of a previously undescribed *Skrjabillanus* nematode species, detected from the scales, was an interesting finding.

The bream population of Kis-Balaton has much better body condition than that of Lake Balaton. The study of that population has yielded a much lower number of parasite species than expected, comprising a total of 3 protozoan and 4 metazoan species. Despite the presence of a large avian population, Kis-Balaton lacks many helminth species that can be considered common in Lake Balaton, *e.g. Paradilepis scolecina.*

Ot the other cyprinid fish, some specimens of tench, rudd, roach, bleak and white bream were examined. In these fish species a total of 15 protozoan and 16 metazoan parasites were detected.

Of the predatory fish species, small numbers of pikeperch, perch, ruffe, pike and sheatfish could be examined, in which we detected 4 protozoan and 11 metazoan parasite species. An interesting finding was the detection of *Lucionema* nematode larvae in *Argulus foliaceus,* parasitic on pikeperch, as the adult stages of that nematode have so far been detected only once in Lake Balaton. This nematode is not only a new species but represents a new genus, too.

Summing up the "exploratory" results obtained in the first year of the four-year project, it can be stated that the parasite fauna of Kis-Balaton fish have proved to be much scantier than expected. This may be due to the absence of snails and lower crustaceans serving as intermediate hosts in the parasites' life cycle, as well as to the excellent general condition of the fish. From 1997, we intend to draw into the survey the fish population of storage-lake II located closer to Lake Balaton, as it has fish species which are absent from the fauna of storage-lake I.

DISTRIBUTION OF INSECT VECTORS (DIPTERA, CULICIDAE, SIMULIIDAE, TABANIDAE) IN THE BIOCENOSES OF THE CHERNOBYL ACCIDENT ZONE

By N. TERESHKINA, M. TRUKHAN & V. KAPLITCH

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One of the purposes of investigation which was begun in 1987 in connection with probable epizootic danger at the Chernobyl accident zone was to find the areas with the most stressed parasitological situation.

The study was carried out in the pine, oak and black alder forests and at the flood plain meadows within a 30 km distance around the reactor. Insects were collected with an insect net and traps modified from prototypes used in Canada (Manitoba horse fly trap).

The greatest species variety and abundance of haematophagous dipterians were established in oak forests situated at flood lands and in swampy alder thickets. In the oak forests 31 species belonging to *Culicidae* (12 species), *Simuliidae* (7) and *Tabanidae* (12) have been found. The average abundance of insects was 67 specimens per sample. There were habitats of 12 species of *Cucilidae* larvae and three species of *Tabanidae* larvae in these biotopes. In the alder thickets 29 species were collected, among which there were 11 species from *Cucilidae,* seven from *Simuliidae* and 11 from *Tabanidae.* Their average abundance was 75 specimens per sample. The least number of species and the abundance of haematophagous dipterians were recorded in unwooded areas at flood land of the Pripyat river.

Thus, the most stressed parasitological situations in respect of insect vectors are formed at the damp oak and alder forests, where there are conditions for all stages of insect development, from egg to imago.

ANTIBODIES IN SERUM FROM PATIENTS WITH SWIMMERS ITCH DETECT CROSS REACTING ANTIGENS OF *Trichobilarzia* AND *Schistosoma*

By C. THORS[1], G. BYLUND[2], E. NIEMELÄ[3] & E. LINDER[1]

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Objective: To study the antibody response in swimmers itch (SI).

Material and methods: Serum antibodies from patients with SI react with *Schistosoma mansoni* larval and adult stages. Serum was obtained from eight patients

with an anamnesis of cercarial dermatitis from Säkylä at the Finnish southwestern coastal region. The immunofluorescence reactivity of these sera was tested on sections of *Trichobilharzia* infected *Lymnaea* snails and on sections of adult *S. mansoni* worms and larval stages present in *Biomphalaria glabrata* snails.

Results: The sera reacted with the cercarial surface and secretions identified at the surface of adult schistosomes and subtegumental cells in contact with surface knobs. Reactivity was also seen with ducts of the genital tracts of both male and female worms but no reactivity was observed with the gut of either male or female schistosomes. The reaction with the *S. mansoni* cercariae was indistinguishable from the reactivity with *Trichobilharzia* cercariae seen in an infected *Lymnaea stagnalis* snail.

Conclusion: Our results suggest that the inflammatory skin reaction in SI is associated with an antibody response against cercarial products common to *Trichobilharzia* and *Schistosoma.*

ELIMINATION OF *Toxocara canis* HELMINTHS UNDER THE INFLUENCE OF OXIBENDAZOLE AND PYRANTEL PAMOATE

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Objective: The purpose of our investigations is to set up the differences in elimination of *Toxocara canis* helminths from the organism of dogs using anthelmintics belonging to different chemical groups.

Materials and methods: We used seven puppies invaded with *Toxocara canis* helminths. During the first experiment three puppies were given Oxibendazole (20 mg/kg/b/w). During the second experiment four puppies were given Pyrantel pamoate (14.4mg/kg/b/w). After the introduction of the preparations the dynamics of the elimination of *Toxocara canis* helminths and eggs were investigated.

Results: Affected by Oxibendazole the first *Toxocara canis* helminths were eliminated from the organism of dogs 23-23 hours after the introduction of the preparation. The most intensive elimination was registered 24-96 hours after the introduction of the preparation. It was observed that at the beginning of elimination the females dominated but from the third day the majority of eliminated helminths were males. Elimination of helminths lasted for 5-7 days. After dehelmintization by Oxibendazole a continual decrease in the number of *Toxocara canis* eggs was observed and on the seventh day they were absent. A total of 51 *Toxocara canis* (25 males and 26 females) was eliminated. The puppies dehelmintized by Pyrantel pamaote started eliminating helminths 8 hours after the introduction of the preparation. During the first 24 hours as much as 87.5% of all helminths were eliminated. *Toxocara* males dominated at the beginning of elimination.

Conclusion: It was detected that *Toxocara canis* helminths affected by Pyrantel pamoate are eliminated from the organism of dogs much quicker than *Toxocara canis* helminths affected by Oxibendazole.

PROTOZOANS PARASITIC ON GILLS OF THE BLUE BREAM, *Abramis ballerus* (L.) FROM DABIE LAKE (POLAND)

By J. WIERZBICKA & E. SOBECKA

Division of Fish Diseases, University of Agriculture, Szczecin, Poland

The present survey covered detailed post-mortem examination of gills of 295 blue bream collected from Dabie Lake, linked to the Odra River estuary. The above-mentioned material, representing all four year seasons, yielded a total of five protozoan species: *Myxobolus* sp., *Chilodonella piscicola* (Zacharias, 1894), *Capriniana piscium* (Bütschli, 1889), *Ichthyophthirius multifiliis* (Fouquet, 1876) and *Trichodina kupermani* Arthur & Lom, 1984. Out of the parasites listed above, the most common turned out to be *T. kupermani.* The intensity of this parasite ranged from single to mass-occurring. They were characterised by having 21-28 denticles (n=90) and 8-10 radial pins per denticle (n=20). Their morphometric features, except for the length of the denticle, fitted as well in the ranges specified in the original description. None of the species recovered has been recorded previously for this particular host in Poland.

PARASITE FAUNA OF THE TENCH, *Tinca tinca* (L.) FROM SELECTED LAKES OF WESTERN POMERANIA (POLAND)

By J. WIERZBICKA, E. SOBECKA & D. GRONET

Division of Fish Diseases, University of Agriculture, Szczecin, Poland

During the summer of 1996 a detailed parasitic survey was conducted on a total of 32 tench. The fish were collected from three lakes of the Western Pomerania region: Insko Lake, Mielno Lake and Woswin Lake. A total of 20 parasite species was recovered representing the following systematic groups: Protozoa (6), Monogenea (1), Cestoda (1), Trematoda (4), Nematoda (3), Hirudinea (1), Acanthocephala (2) and Crustacea (2). Prevalence of individual parasite infection ranged from 3.12 to 65.62%. Intensity of infection was also variable, starting from single encounters, ending on mass occurrence. The most prevalent species in the material studied turned out to be *Asymphylodora tincae* (Modeer, 1790), *Ergasilus sieboldi* Nordmann, 1832, *Ichthyophthirius multifiliis* (Fouquet, 1876) and *Dactylogyrus* sp. The monogeneans

found on the gills significantly differed from those hitherto described and they constitute a new species.

INTERFERENCE OF THE FUNCTION OF PARASITE MITOCHONDRIA BY OXYGENATED CHALCONES

By L. ZHAI[1], J. BLOM[3], M. CHEN[1,3], T.G. THEANDER[2], S.B. CHRISTENSEN[4] & A. KHARAZMI[1]

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[2]*Institute for Medical Microbiology and Immunology, Copenhagen University, Copenhagen, Denmark*
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Our previous studies have shown that licochalcone A, an oxygenated chalcone, has antileishmanial and antimalarial activities. The present study was designed to examine the mechanism of action of the antileishmanial activity of licochalcone A. Electron microscopic studies illustrated that licochalcone A in a concentration dependent manner altered the ultrastructure of the mitochondria of *L. major* promastigote and amastigote, without damaging other cell organelles and the function of human macrophages. Studies on the function of the parasite mitochondria showed that in a concentration dependent manner licochalcone A inhibited the respiration of the parasite as shown by inhibition of O_2 consumption and CO_2 production by the parasites. Moreover, licochalcone A inhibited the function of the parasite mitochondrial dehydrogenase and fumarate reductase in a concentration dependent and time dependent manner. The inhibition of the mitochondrial function of the parasite correlated well with the changes observed in the ultrastructure of the mitochondria as shown by electron microscopy. Similar findings were observed with other oxygenated chalcones.

ON TRANSMITTING MALARIA IN LITHUANIA DURING THE PERIOD 1992-1996

By M. ZYGUTIENÉ

State Public Health Centre, Vilnius, Lithuania

Objective: To investigate the prevalence of *Anopheles* genus mosquito in Lithuania as well as the dominating species of them to analyse the possibility of sporogonia and its duration in Lithuania.

Materials and methods: Ninety-six adult mosquitoes and 300 larvae of the IV growing stage of *Anopheles* were used and the species determined using binocular microscope. The adult mosquitoes were collected at cattle-sheds and in natural surroundings. The larvae were collected in closed water bodies. The sporogonia was calculated on the basis of methods of Organov-Rejevskij and Mashkovskij using the average day temperatures according to the data of the Hydrometeorlogical Centre.

Results: All the adult mosquitoes and the larvae were of *Anopheles maculipennis* species. Each year the possibility of transmitting malaria was observed and duration of sporogonia was calculated. Seasonal observations on the transmission of malaria are presented in the Table (see following page). In 1992 the season of malaria continued from July 1 to October 11, the duration of sporogonia was 42 days and there were 95 warm days within the year. In 1993 there were no favourable conditions for transmitting malaria and in 1994 the spring was cool. Only on June 26 did the average day temperature rise to 16°C. On July 19 the season of transmitting of malaria started. It is important that the first generation of *An. maculipennis* mosquitoes had developed into mosquitoes before the beginning of the effective infection. The season came to an end on September 10. An exceptional situation took place in 1995 with the duration of sporogonia being 21 days and transmission starting on June 14 and lasting for 114 days. Cool weather in 1996 accounted for the late season of transmitting malaria and the duration was only 41 days.

Conclusion: The possibility of transmitting malaria in Lithuania is ever increasing with the spreading of *Anopheles maculipennis* mosquitoes with a gradual warming up of the climate and influx of persons from potentially dangerous countries.

Year	Malaria transmission season	Duration of sporogonia Days	Number of days, average temperature more than 15°C
1992	01.07 - 11.10	42	95
1993	-	-	56
1994	19.07 - 10.09	24	59
1995	14.06 - 03.10	21	99
1996	22.08 - 02.10	41	76

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NEWS

Members are encouraged to submit items of news, information on forthcoming meetings, personnel etc. for publication in the News section. Letters and points of view are also welcome.

NEWS Baltic Section

Award to young scientist from Lithuania

In November 1996, Mindaugas Šarkūnas, a 25 year old veterinary parasitologist from the Veterinary Academy in Kaunas, Lithuania, received a prestigious award from Prince Joachim and Princess Alexandra's Foundation for a training and research stay at Danish Centre for Experimental Parasitology, Copenhagen, Denmark.

ERRATUM TO

"PARASITES OF COMMON ASP (*ASPIUS ASPIUS*), BREAM (*ABRAMIS BRAMA*) AND ZANDER (*STIZOSTEDION LUCIOPERCA*) FROM THE RIVER NITELVA, SOUTH-EASTERN NORWAY"

[Bull Scand Soc Parasitol 1996; 6 (2): 134-138]

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[2]Central Veterinary Laboratory, P. O. Box 8156 Dep., N-0033 Oslo, Norway,

There are errors in the published version of this manuscript. Please substitute the section **Materials and methods** with the following:

Introduction

The asp *Aspius aspius* (L.) is one of the rarest Norwegian cyprinids, being restricted to the south-eastern parts of the river Glomma water system. Its parasite fauna has never been studied in Norway. The bream *Abramis brama* (L.) is common throughout south-eastern Norway, and its helminth fauna has been studied by Halvorsen (1971) and Hartvigsen (1995). The zander, *Stizostedion lucioperca* (L.), is only slightly wider distributed than the asp, but much more commonly caught. The zander has been introduced to lakes outside its natural distribution as a part of top-down control projects for improvement of water quality. No reports are available on its parasite fauna. The poor knowledge of the parasite fauna of asp, bream and zander prompted the present study of both protozoan and metazoan parasites of these species.

Materials and methods

The fish were caught May 28 and June 12, 1996 by fishing with rod in the river Nitelva, close to its inlet into the lake Øyeren (Skedsmo municipality in Akershus county). All the asp and zander except one individual of each species, died shortly after capture or during transportation to the laboratory. The fish were kept in river water, placed in a refrigerator and examined ca. twelve hours later. All bream were kept alive in river water until examination. Fish were were killed by a sharp blow to the head, and all external and internal organs were examined for parasites using a dissecting microscope and a light microscope equipped with phase contrast. Klein's dry silver impregnation method (see Lom & Dykova, 1992) was used to prepare trichodinids before identification.

Some parasites were found only on fish that were alive until examination. Furthermore, two bream were examined only for monogeneans. Numbers infected in Table 1 may therefore not reflect the parasite prevalences in the examined fish populations. If possible, the number of each parasite species was counted and presented as intensity range in Table 1. The literature generally used for species identification included: Bykhovskaya-Pavlovskaya *et al.* (1962) (protozoans, cestodes, trematodes, monogeneans, acanthocephalans), Malmberg (1970) (*Gyrodactylus*), Lom & Dykova (1992) (protozoans), Moravec (1994) (nematodes). Other references used for species identification are mentioned below.

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 inch disk in MS-DOS or MS-DOS compatible format. The text should be written in Words or WordPerfect or other word processing programs convertible to these. **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.

The articles/communications should normally 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.

Line drawings should be provided as good quality hard copies suitable for reproduction as submitted.

Photographs must be provided as glossy prints, and be of sufficiently high quality to allow reproduction on standard (not glossy) paper. Colour plates will not be printed.

References in the text shold 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: 401-5. 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

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