Bulletin

of the

SCANDINAVIAN SOCIETY FOR PARASITOLOGY

WITH PROCEEDINGS OF THE BALTIC-SCANDINAVIAN SYMPOSIUM on PARASITIC ZOONOSES AND THE ECOLOGY OF PARASITES, VILNIUS, LITHUANIA 7 - 8 SEPT. 1994

Vol. 5 No. 1 1995

BULLETIN OF THE SCANDINAVIAN SOCIETY FOR PARASITOLGY

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

Subscriptions are available to non-members upon request from the Publisher. The subscription rate is SEK 300 per year (two issues annually).

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.

Printed in Norway by HS-Trykk A/S ISSN 0803-4907

PROCEEDINGS

of the

BALTIC-SCANDINAVIAN SYMPOSIUM

on

PARASITIC ZOONOSES AND THE ECOLOGY OF PARASITES

7-8 September 1994, Vilnius, Lithuania

Editor of Proceedings:
Peter Nansen, with technical assistance from Jane Røken, Fritz Østergaard and Helle Kürstein

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THE SCANDINAVIAN SOCIETY FOR PARASITOLOGY
THE LITHUANIAN ACADEMY OF SCIENCES
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PREFACE

On 7 and 8 September 1994 the Baltic-Scandinavian symposium on Parasitic Zoonoses and Ecology of Parasites was held in Vilnius, Lithuania. It was the first parasitological meeting jointly organized by the Baltic and the Scandinavian societies for parasitology. All societies involved - i.e. Baltic Society for Parasitology, Lithuanian Academy of Sciences, Lithuanian Institute of Ecology, Scandinavian Society for Parasitology, Danish Society for Parasitology, Danish Centre for Experimental Parasitology and Danish Centre for Parasitic Zoonoses - had strongly supported the idea of bringing together the many parasitologists in this region to promote exchange of information and to encourage collaborative arrangements. The symposium attracted more than 100 persons, mainly from Baltic and Scandinavian countries, but fortunately also from neighbouring countries, and even from overseas, as will appear from the present proceedings. The main themes of the symposium, i.e. parasitic zoonoses and ecology of parasites, were chosen because of the relevance for human and animal health problems and because of their basic role in the understanding of parasitism and parasite transmission. At the same time, these rather broad themes provided an opportunity for interdisciplinary discussions with contributions from parasitologists having biological, medical and veterinary backgrounds. Additional themes allowed parasitologists with other interests to present their results and to exchange information and opinions.

The organizing board and committees express their deepest thanks to all participants, not least the invited speakers, for their valuable contributions to the success of the symposium. We are not only thinking of the scientific achievements, but also the open and warm social atmosphere which prevailed despite the language barriers that exist. We also owe deep gratitude to the active and helpful individuals involved in the practical planning and carrying through of the symposium.

The symposium in Vilnius was altogether a great success, and a first step towards future Baltic-Scandinavian meetings with similar or other themes, hopefully rotating between the countries.

The present proceedings are based on the various contributions, in the form of invited lectures, oral presentations or poster presentations. Through an editorial process some language corrections have been made, but the individual authors are responsible for the presented figures, interpretations, terminology etc.

December 1994

V. Kontrimavičius P. Nansen

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INVITED PAPERS

FOODBORNE HELMINTH ZOONOSES

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Introduction

Foodborne diseases are major causes of morbidity and mortality throughout the world, including the so-called developed countries. A recent report of the Food and Agricultural Organization and the International Atomic Energy Agency on food safety concluded that "illness due to contaminated food is perhaps the most widespread health problem in the contemporary world and an important cause of reduced economic productivity" (WHO 1984). Diarrhoeal diseases, 70% of which result from contaminated food, account for about 25% of all deaths in developing countries (WHO 1992). In western countries such as the United States, annually up to 14% of the population may contract a foodborne infection, with more than 9,000 associated fatalities. The costs of these diseases, for both the United States and Canada, are estimated at $ 9,700 million. Of the numerous causative agents of foodborne diseases, parasites represent an important, but difficult to quantify, burden.

Comprehensive and reliable statistics on the incidence and impact of foodborne parsitic zoonoses are generally not available for all but a few countries. However, the data available from the United States and Canada, which have relatively high levels of food safety and quality, are provocative. Studies on the incidence and impact of major foodborne diseases such as toxoplasmosis, trichinellosis, and cysticercosis reveal that the public health burden imposed by these diseases are substantial although not well recognized.

The rising public anxiety over food safety, and the concomitant distrust of safeguards has caused a re-examination of both government policy and industry practices. The perception of industry's ability to deliver safe food and its basis in fact is also a concern for the scientific community. The public trust in our food production systems will only be gained from the development of effective safeguards which in turn will depend upon a greater understanding of the nature and epidemiology of these zoonoses. Because of public concern, the opportunity to achieve this is greater now than at any time in the recent past.

This review will present the current understanding of the biology and epidemiology of the major foodborne helminth zoonoses and the issues they present to authorities responsible for establishing a comprehensive system for safeguarding public food and water supplies.

Trichinellosis

Although all five species of *Trichinella* are potentially zoonotic, *T. spiralis* is

responsible for most human infections. It has a 'domestic' epidemiology and can be transmitted from livestock (pig, horse and cattle (?)) and wild game. The distribution of human trichinellosis is worldwide with recent outbreaks in Europe, North and South America, Africa, the Middle East, China, and Thailand. Interestingly, the predominant sources in Western Europe are infected horse meat and wild boar, while outbreaks in Eastern Europe are attributed to domestic pigs and game. The economic costs of human trichinellosis are both direct and indirect. In the United States, health costs for an average of 52 cases per year are nearly US$ 800,-000, but the costs for prevention (regulatory oversight of meat processing) is nearly US$ 1,000 million/year. Domestic pig infections are strongly associated with poor management and extensive rearing facilities. Under these conditions, transmission via rodents, cannibalism, and feed containing pork scraps can occur. Frequently, wild animals associated with such farms become infected, thereby serving as a reservoir for both pig and human infections.

Effective control includes both stringent husbandry practices and meat inspection (including game meat). Meat irradiation is also under serious consideration in regions where the above control stategies have either not been enforced or have not been completely effective.

Cysticercosis/taeniosis

The important features of this particular zoonosis are that the cestode larvae are meat-borne (beef or pork), and the adult stages develop only in the intestines of humans (obligate host). There are two species, *Taenia saginata* ('beef tapeworm'), and *T. solium* ('pork tapeworm'). The latter species, *T. solium*, is of greatest clinical importance because, unlike *T. saginata*, man may also serve as the host for the larval (cysticercus) stage if the adult worm's eggs are accidentally ingested. Neurocysticercosis is a major public health problem affecting especially Latin America, Asia, and Africa. In Mexico, *T. solium* cysticercosis accounts for 1% of all deaths in general hospitals and 25% of all intracranial tumours. It is estimated that the costs to Mexico in 1992 for medical treatment of neurocysticercosis and lost wages totalled US$ 195 million. The World Health Organization (WHO) estimates that these infections affect about 50 million people and that 50,000 deaths occur worldwide each year due to neurocysticercosis (WHO 1983). Animal infections are also costly. In Mexico, the equivalent of 58% of the country's total investment in pig production was lost due to cysticercosis condemnations in 1980. Bovine cysticercosis is estimated to cost Africa up to US$ 2,000 million/year. Control currently relies on detection of infection at slaughter. However, high-risk animal husbandry practices should be part of any control stategy, and these aspects will be discussed. Irradiation of pork is also being evaluated in Latin America.

Fishborne parasites

There are a number of zoonotic fishborne parasites that, although generally not widely distributed, may be of significance in certain regions or countries, and

their potential impact should be understood by those with responsibilities for food safety. It has been estimated by WHO that there are 750 million people at risk to fish and invertebrate-borne trematode parasites found in 50 countries; the number infected may exceed 40 million. Chief among these zoonotic diseases are anisakiosis, clonorchosis/ opisthorchosis, diphyllobothriosis and heterophylosis. The epidemiology and control of diseases will be discussed.

TOXOPLASMOSIS: THE ANIMAL RESERVOIR

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Toxoplasma gondii, a protozoan parasite, is found in a great variety of mammals and birds. Also humans are vulnerable to infection. Definitive hosts are certain feline species, among them the domestic cat. Oocysts are shed with the cat faeces and the parasite is transmitted to the intermediate host by ingestion of the viable, sporulated oocyst. A second important transmission route is the consumption of raw or insufficiently cooked meat of *T. gondii* infected animals. *T. gondii* is considered to be widespread among farm animals. Although infection in healthy adult animals seldom leads to clinical symptoms, it may cause severe damage in young animals. Also, in pregnant animals the infection may lead to abortion, miscarriage and stillbirth.

Based on the presence of sporulated oocysts in the environment, which are very resistant to climate conditions, the transmision to livestock and wild-life animals is very frequent.

The prevalence of toxoplasma infection in various species is merely based on serzological surveys. The seroprevalence may vary from country to country, between animal species and even varies between investigators. The laboratory methods of choice are likely due to the latter variations. There seems to be a general pattern which may be recognized:

- the seroprevalence in animals (and man) increases with age.
- the seroprevalence within Europe decreases with the geographical latitude.

In horses, the seroprevalence is between 5 and 10%, in pigs up to 65% may be positive, and in the toxoplasma-sensitive sheep the percentages are between 75 and 100%. Cattle are relatively often seropositive; however, they are the only animal species which reckon with toxoplasma, i.e. after some 1-2 years they become negative again. So far, it has been very difficult to demonstrate toxoplasma tissue cysts in various tissues, particularly in meat from chronically infected cattle.

The majority of cats which have access to free wandering around are seropositive within their first year of life.

Generally, whenever cats are infected for the first time, oocysts are excreted for only a limited period of time. After a second infection, no more than 20% of the

cats may again shed oocysts for 1 to 2 weeks, but thereafter immunity of the cats prevents reshedding, even when they become infected again. The contamination of the environment (pastures, gardens, parks) leads to infection of small animals, birds, livestock and man. So far, no reliable methods exist for the study of the degree of contamination in the environment.

TRICHINELLA SYSTEMATICS AND ITS PRACTICAL IMPORTANCE IN EPIDEMIOLOGY AND PATHOLOGY

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The parasite *Trichinella spiralis* was described in 1835 by Owen; and for a long time, domestic pigs and synanthropic rats were considered as main reservoirs, and the presence of this nematode in other mammals was considered circumstantial. Only one century later, there was evidence of the presence of *Trichinella* in other mammals, especially carnivores and omnivores.

Since 1943, some authors evidenced biological differences among *Trichinella* strains collected from different regions and hosts. In particular in 1961, Nelson et al. evidenced biological and clinical differences between African isolates and European pig isolates. Other differences were observed by Rausch et al. between isolates from arctic carnivores and pig isolates. These authors considered these strains as geographical variants of the cosmopolitan species *T. spiralis.*

In 1972, Britov and Boev described two new sibling species within the genus *Trichinella: Trichinella nativa* and *Trichinella nelsoni,* and in the same year Garkavi described *Trichinella pseudospiralis.*

Since then, controversy has reigned over the taxonomy of the genus *Trichinella,* primarily over the criteria to be employed for the diagnosis of species.

In the last eight years, more than 350 isolates were studied using biochemical (27 allozymes), molecular (Dot and Southern blots with specific DNA probes, PCR with specific primers, RAPD) and biological methods, including environmental data at the *Trichinella* Reference Centre. These studies were intercongruent and showed the presence of eight differentiated clusters within the genus *Trichinella,* five of which, *T. spiralis, T. nativa, T. britovi, T. pseudospiralis* and T. *nelsoni* at species level, and three *Trichinella* T5, T6 and T8 at uncertain taxonomic level. All isolates examined belong to one or other of these clusters (Pozio et al., J Parasitol 1992; 78: 654-659). The main biological parameters utilized in differential diagnosis are: i) newborn larvae production; ii) infectivity to birds, rats and pigs; iii) resistance to freezing; iv) nurse cell development; v) intestinal phase. The environmental parameters utilized in differential diagnosis are the isotherms in January and July in the place of isolate origin.

Most experimental studies on this genus were carried out on laboratory mice and/or rats. Rodents, in particular

rats, are good hosts for *T. spiralis* and to a lesser degree for *T. pseudospiralis.* In contrast, only a few individuals of the 'sylvatic' group are able to reproduce in the guts of rodents, and the majority of newborn larvae are rapidly destroyed. Usually, no living larvae are detectable in mice and rats 12-15 and 3 months after infection, respectively. In pigs, 'sylvatic' *Trichinella* species behave similarly as in rats. Circulating specific IgGs rapidly disappear in rats and pigs infected with 'sylvatic' trichinellas, while they persist in *T. spiralis* infected rats (or pigs) for a longer period of time.

These biological characteristics explain why 'sylvatic' trichinellas are seldom present in the traditional domestic hosts (i.e. rats and pigs). When these parasites infect domestic pigs, the number of infective larvae established in these hosts muscles is very low and their number drops rapidly. Consequently, there are no reports of domestic ↔ domestic or domestic →synanthropic transmission of 'sylvatic' parasites; i.e. 'sylvatic' trichinellas cannot be maintained by a domestic cycle involving rats and pigs. A similar picture is observed among sylvatic rats and wild boars where the infection with 'sylvatic' trichinellas is only circumstantial (for example, in Italy the prevalence of *T. britovi* infection is less than 0.1% in wild boars in areas where the prevalence of infection in foxes is 20-25%).

The main reservoirs of 'sylvatic' trichinellas are carnivores belonging to the families of Canidae, Ursidae, Hyaenidae and Felidae. In those hosts, the 'sylvatic' trichinellas have established a good parasite/host relationship characterized by: i) production of a low number of newborn larvae per female; ii) survival of muscle larvae in host tissue for a long time (years); iii) minimum or absent immunological protective reaction of the host due to the low number of parasites; iv) presence of a low number of muscle larvae/g. Only in deprived (i.e. immunodepressed) animals the host/parasite balance is altered.

The host range of *Trichinella* species have implications on their world-wide distribution. The distribution of 'sylvatic' trichinellas is correlated with the distribution of sylvatic carnivores. The transmission areas of these parasites are reduced by progressive changes of the natural habitat of these hosts that are banished in protected areas and parks or in the mountains. Unlike 'sylvatic' trichinellas, *T. spiralis* seems to infect the majority of mammals. This wide host spectrum and the global distribution of domestic pigs and synanthropic rats account for the cosmopolitan distribution of this species among domestic, synanthropic and sylvatic animals. These data bear strong evidence that the *Trichinella* biomass is greater in sylvatic than in domestic animals.

A rigorous comparison of clinical and pathological differences in human infections due to different *Trichinella* parasites is impossible, because the number of living larvae ingested by patients is generally unknown. However, there is strong evidence of clinical and biological differences observed in humans infected by different species of *Trichinella.* The percentage of patients with intestinal

symptomatology appears to differ among species. In *T. spiralis* human infections eosinophilia persists over six months, while in *T. britovi* infections the duration of eosinophilia is less than three months. Similar differences were observed in specific IgG. The Western blot analysis shows some differences in protein profiles among *T. spiralis*, *T. britovi* and *T. pseudospiralis*.

This new classification of *Trichinella* parasites shows a very important and practical spin-off in the epidemiology (identifying different cycles and reservoirs, evaluating the human risk and carrying out epidemiological surveys) in the diagnosis of human and animal infection (identifying the probable source of infection and its geographical origin, interpreting laboratory features, and evaluating the time of development of muscle larvae, the clinical course of infection and the need of treatment) and in control programmes (establishing appropriate strategies and methods for the diagnosis of infection in animals, identifying the target reservoirs, and evaluating the resistance to freezing).

AGENTS OF DISEASES AND VECTORS OF ZOOANTHROPOZOONOSES AS A SYSTEM WITH NEW FEATURES

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The notion of the helminth/host couple as a system is now generally accepted. The purpose of this communication is to prove that vector-borne disease agents and their specific vectors are also systems with new features and feedbacks which increase the probability of the successful circulation of the pathogen in the environment. Among these are analyzed not only such simple pathogen actions as suppression of apyrase production in malaria-infected *Anopheles* salivary glands (Ribeiro et al., J Insect Physiol, 1985; 31: 689-692) or trypanosoma-infected tse-tse mechanoreceptors blocking (Jenni et al., Nature 1980; 283: 383-385), but such complicated features as the deep physiological changes which influence the blood digestion, water balance, reactions to environmental stimuli, moulting periods, etc.

Flea / plague couple. According to our investigation, the bactericidal flea gut factor (Alekseev et al., Parazitologia 1969; 3: 228-235) decreased microbe quantity in the sucked blood and triggered the selection process, as a result of which newborne coccal forms of *Yersinia pestis* block the flea proventriculus. Specific plague plasmids not only strengthen block formation, but depress blood digestion and, feedback-acting, stimulate the host to try to feed more frequently.

Tick-borne encephalitis virus (TBEV) / ixodid tick couple. For the first time in the world science it was demonstrated, according to our investigations (Alekseev, Tick-tick-borne pathogen system and its emergent qualities, ed. Daiter AB, St. Petersburg, Zoological Institute 1993), that TBEV is reproduced in the most active and viable tick-vector specimens and is changing their moving activity and reac-

tions to the host and plant odours (result: on the host (man), infected *Ixodes persulcatus* are found 7 times more often than on the vegetation); and that TBEV suppresses water losses in the infected specimens which are able to move higher on the vegetation and to hunt longer than naive ones. The specificity of TBEV reproduction in the specific *Ixodes* tick host salivary glands permits virus to be transmitted by the transsalival and distant routes during naive and infected tick cofeeding on the nonviraemic hosts and by the omovampiric route of transmission by the bite of males with infected saliva during copulation with females in the nature. Feedback-physiological peculiarities of *Ixodes* salivary gland cells determine the TBEV strains qualities along their area (e.g. DS + strains from *I. persulcatus*) and distant virus exchange ability. Our investigations and analyzed literature permit us to suppose that similar systemic features do exist among other pathogen/vector couples.

THE ROLE OF FAO IN THE CONTROL OF ZOONOTIC HELMINTH PARASITES AND OTHER PARASITIC DISEASES

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The Food and Agriculture Organization (FAO) is one of the specialized agencies of the United Nations, similar to the World Health Organization (WHO), UNESCO, UNICEF and others. The major objective of FAO is to promote the improvement of production and productivity in all areas of agriculture, fishery and forestry. This takes place at several levels, but the main activities of the Organization are in the areas of:

- collecting, analyzing and disseminating information; and through FAO, the member countries have access to some of the world's largest data bases covering all areas of interest to the Organization;
- advising the governments on development policies and the formulation, planning and implementation of these;
- the FAO Field Programme including the formulation and implementation of development projects and programmes for adaptation and transfer of appropriate technology to the developing countries.

FAO cannot initiate any activities in the the two latter areas without receiving a request from member countries.

Development of activities, projects and programmes related to the above- mentioned fields as they relate to livestock production and health, is the responsibility of the Animal Production and Health Division, which comprises three services, the Animal Production Service, the Meat and Milk Service and the Animal Health Service. The latter is subdivided into: 1) The Veterinay Services Group, which is primarily responsible for the field of epidemiology, assisting in the development of veterinary services, veterinary education, and the Animal Health Yearbook. 2) The Infectious Diseases Group, which is in charge of viral and bacterial diseases, emergency disease prevention, and vaccine production. 3)

The Parasitic Diseases Group, which is responsible for all aspects of control of tsetse and trypanosomosis, ticks and tick-borne diseases, and helminth infections.

While the world in general has experienced a rather astonishing increase in crop production during the last 20 years, livestock production in many areas of the world, particularly in Africa, has stagnated or even declined. This lack of progress is somewhat surprising in view of the fact that the great effort in controlling the major infectious and parasitic diseases (rinderpest, contagious bovine pleuropneumonia, tsetse and trypanosomosis, ticks and tick-borne diseases) has to a large extent been successful.

The reasons for this lack of progress are many and varied, and include a partial or complete neglect of the control of a number of important production-related diseases (helminth infections, reproductive disorders, nutritional factors and other non-infectious conditions). This is, however, not the sole reason, but is interrelated with a complex set of circumstances, such as lack of government incentives, erroneous price policies, and a failure to understand the socio-economic background of many of the livestock producers.

FAO has of course been aware of the importance of helminth infections and non-infectious diseases for years, but has only recently decided to focus more on the production diseases and has to that effect created positions for two Animal Health Officers in helminthology and non-infectious diseases. Since the establishment of the positions four years ago, the main task has been to establish a FAO programme for the control of helminth infections and non-infectious diseases.

With only one helminthologist in the Animal Health Service, input from colleagues around the world with regard to the importance of helminths in various countries, the need for basic research on epidemiology and control programmes, the latest developments within the field of diagnostics, and many other aspects related to animal health and production, is extremely important. It has, therefore, been one of the priorities to establish a network of individuals and laboratories through which information can be received and the awareness of the economic importance of helminth infections be promoted.

A first important step in implementing this strategy was the organizing of an FAO Expert Consultation on Helminth Infections of Livestock in the Developing Countries, which was held in Rome in September 1991. At this meeting, 20 experts on helminthology, representing various geographical regions and fields of interest, formulated global guidelines and recommendations for FAO activities in all areas of this subject in short, medium and long-term plans. The report from this meeting is available upon request to the Animal Health Service.

Another key component is the establishment of links between FAO and well-known laboratories with activities in the fields of epidemiology, diagnosis and control of helminths. The excellence of these laboratories is monitored regularly, and the network can be expanded according to the need for specific expertise. At present, the following laboratories have

been designated FAO Collaborative Centres for Helminth Infections:

- Commonwealth Agricultural Bureau, St. Albans, UK. Areas of expertise: taxonomy, information, training.
- USDA, Parasitology Laboratory, Beltsville, USA. Areas of expertise: immunology, diagnostics, trichinellosis, development of vaccines.
- CSIRO, Division of Animal Health, Australia (several sites). Areas of expertise: epidemiology, anthelmintic, resistance, control, genetic resistance, modelling.
- Danish Centre for Experimental Parasitology, Royal Veterinary and Agricultural University, Copenhagen, Denmark. Areas of expertise: epidemiology, control, biological control, diagnostics.

It has also been part of the strategy for obtaining information on the current status of helminthology in various regions of the world to use consultants, who at the end of their visit will prepare detailed reports including recommendations for follow-up activities. South-East Asia and West Africa have been targets for such consultancies which have, together with the experience of the Animal Health Officer in charge of helminthology, resulted in the creation of a substantial data base comprising information about the priorities and the needs of the Veterinary Services in the countries visited with regard to the control of helminth diseases.

A number of publications covering various fields and aspects of helminth infections have been or will be prepared. An inventory of activities in this field in developing countries, with the title "Distribution and Impact of Helminth Diseases of Livestock in Developing Countries", was published early in 1992. This book lists more than 1,000 references (1975-1990) of published research and can be used as a guide to past and ongoing activities and may possibly stimulate collaboration between neighbouring countries. The Animal Health Service has been updating (1990-1993) this reference publication, and it is now available on disk with abstracts, enabling scientists to perform their own literature search.

The second edition of the manual "Epidemiology, Diagnosis and Control of Gastro-Intestinal Parasites of Ruminants in Africa" has been published and is now available. This new edition has been expanded to cover all helminths and geographically comprise all developing countries. These changes naturally required a modification of the title, and it is now called "Epidemiology, Diagnosis and Control of Helminth Parasites of Ruminants". The manual will be available in French later this year.

A contract for the preparation of a similar manual for helminth infections of pigs has been signed with the FAO Collaborative Centre on Helminth Infections, Copenhagen, Denmark, and Professor Nansen and his colleagues have already started the preparatory work on this much-needed handbook.

A series of extensively illustrated publications regarding fluke diseases of livestock have been prepared for different target groups and is in the process of being translated into French and Spanish and printed, and will be available around December/January. The pamphlets pre-

pared for extension personnel and farmers are suitable for translation into local (tribal) languages.

The rapidly increasing problem of anthelmintic resistance in sheep parasites is of great concern to FAO, and funds have been allocated to activities which attempt to map the extent of the problem in the developing countries. A consultant from CSIRO was hired for an evaluation of the situation in the southern part of Latin America, and a similar consultancy covered selected countries in Africa last autumn. As a follow-up to the consultancy to Argentina, Brazil and Uruguay, a regional project comprising the three countries and Paraguay was designed and funding was secured for the start of the project in October 1993.

Of other ongoing or planned projects specifically related to helminth infections and control, a small project in Tanzania where different control stategies for parasites in small ruminants are being tested at village level, should be mentioned. A project which has been designed to establish the epidemiology of helminth infections and test various control strategies for measuring of production benefits has recently been started in Mozambique. This, together with another project designed to measure the economic impact of helminth infections on ruminant productivity in villages in Vietnam, will reveal much needed data on the economic importance of these infections.

Historically, FAO has had a commitment to assist member countries in the control of zoonotic diseases. This has naturally been in collaboration with the World Health Organization (WHO), particularly the Veterinary Public Health Unit. With regard to parasitic zoonoses, the focus was previously only on echinococcosis/hydatidosis and taenia/cysticercosis. However, during the last four years FAO has expanded the activities into the area of trichinellosis, fasciolosis and other food-borne trematode infections.

Among some of the projects and activities that FAO has been and is currently involved in, is a hydatidosis control project in Uruguay with a potential expansion into a regional programme. The Organization is also in the process of preparing a project proposal for a regional control programme for echinococcosis/hydatidosis in the North West African countries. In collaboration with WHO and O.I.E., FAO is participating in the revision of the guidelines for hydatidosis control. An FAO representative recently participated in a WHO established working group on food-borne trematode infections. At present the Organization is assisting the World Trichinellosis Reference Centre in identifying funding possibilities, and FAO is planning to officially recognize the Centre.

Training is obviously an important part of FAO activities, and all projects usually contain a considerable training component. This can be as individual scholarships and fellowships, or in the form of workshops and seminars. A regional workshop on epidemiology, diagnosis and control of helminth infections was recently held in Sri Lanka for participants from South-East Asian coun-

tries. A similar workshop will be held in East Africa in December 1994.

While these activities will be consolidated and expanded in the future, others, including support to research on genetic resistance and modelling, will be initiated.

This short review is intentionally focusing on the activities related to helminth infections, but it should be kept in mind that these diseases are only one component of animal health and production issues and should not be viewed in isolation. It is important that scientists work together with the national Veterinary Services in establishing priorities and subsequently develop appropriate disease control programmes.

SUBMITTED PAPERS - ORAL PRESENTATIONS

Parasitic Zoonoses

PROBLEM OF NATURAL NIDUS OF OPISTHORCHOSIS IN LITHUANIA

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The first to have described human opisthorchosis in Lithuania was Askanazy (1900, 1906), who had observed patients in the Königsberg clinic. They had come from the coastal regions of the Curonian Lagoon, Kintai, Rusnė, Šilutė and from other settlements. Rindfleisch (1910) claimed that Braun had also observed opisthorchosis among Lithuanian inhabitants in the western settlements (near the Curonian Lagoon, coastal regions as well as in the lower reaches of the river Nemunas) at the beginning of the 20th century. Braun and Seifert (1925-1926), Vogel (1929, 1937), Ischucke, Szidat and Wigand (1932), Steiner (1933), Erhardt (1934), Blumberg (1938), Erhardt, Germer, Horning (1962) and many others mention the cases of human opisthorchosis in Lithuania's western settlements.

The evidence of the existence of the pathogen of opisthorchosis (near the Curonian Lagoon and the lower reaches of the Nemunas) can be found in the works of Vogel (1934), Šulman (1949), Gecevičiūtė (1954), Šulman and Krotas (1959), who claimed to have found opisthorchan larvae in the fish of the Nemunas and of the Curonian Lagoon. Having investigated 29 species of fish from the Curonian Lagoon, Gecevičiūtė stated that she had found cercariae of this helminth in five species. After autopsies of some of the fishermen's cats in those parts, she found that their livers harboured the larvae of this parasite.

After helminthological examination of more than 6,000 inhabitants in the coastal region of the Lagoon, of which 283 inhabitants were complaining of pains in the liver area, as well as 40 human corpses, opisthorchas and their elements were not detected.

In order to follow the incidence of opisthorchosis among the domestic and wild fauna in the coastal regions of the Curonian Lagoon and the Baltic Sea, helminthological investigations were made: 228 cats, 5 pigs, 3 foxes and 2 wild boars were autopsied: 180 cats, foxes and wild boars of the Curonian coastal regions and the lower reaches of the Nemunas, and 42 cats from the settlements of the Baltic coastal regions. 70.7% of the examined cats, 2 foxes, 1 wild boar and 2 pigs from the Curonian coastal settlements, harboured opisthorchas in their livers. The intensity varied from animal

to animal. This parasite was not found in the cats of the Baltic coastal homesteads.

The investigations carried out bear evidence of the long-standing existence of the natural nidus of opisthorchosis in the coastal region of the Curonian Lagoon and the lower reaches of the river Nemunas.

(List of references may be obtained from the author on request. - Ed.)

TOXOPLASMOSIS IN VARIOUS PROFESSIONAL GROUPS OF HUMAN BEINGS

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Toxoplasma gondii is an obligate intracellular protozoan parasite widespread in all mammals, including human beings. The parasite causes severe disease - toxoplasmosis - especially in immunocompromised people, like those with malignancies, transplanted organs, AIDS, etc. - by reactivation of latent toxoplasma infection. Manifestations of reactivated toxoplasmosis as an opportunistic infection can be encephalitis, myocarditis, pneumonia, with lethal outcome. This shows the great importance of early diagnosis of toxoplasmosis, in its latent as well as acute forms.

The aim of this work was to examine various human professional groups for toxoplasmosis and to determine high-risk personnel in Lithuania.

2,366 persons of various professions were examined serologically for toxoplasmosis by Indirect Immunofluorescent Test (IFT) and were also examined by specialists.

The lowest percentage of infected individuals was in donors: 10.6±2.3% (control group) and among pupils and students: 14.8±3.9%. The significantly higher percentage of toxoplasma infection was found in the professional groups who handled animals and raw meat; it was within the limit of 57.8±4.2 - 58.9±4.5%. What is noteworthy here is the high infection percentage which was observed in medical personnel: 72.5±4.3%, and in those who were faced with many stress situations in their daily work, such as teachers, lecturers, economists, accountants; the latter were infected within the limits of 67.0±4.7 - 84.6±7.1%.

Thus, the conclusion can be drawn that high-risk personnel groups for toxoplasmosis in Lithuania are those persons who handle animals and raw meat, as well as those who are confronted with many stress situations in their daily work. Thus, first of all, we must suspect toxoplasmosis in these groups in all unclear cases of disease.

CRYPTOSPORIDIUM SP. IN DANISH RODENTS

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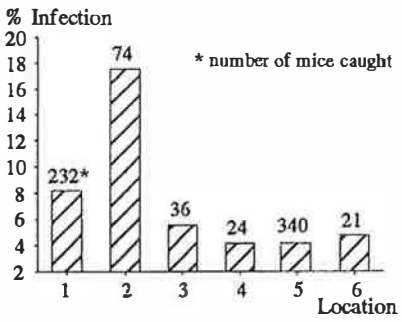
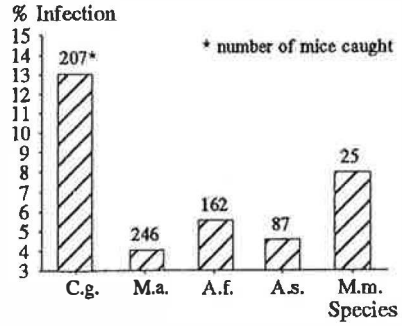
Until now, investigations of cryptosporidiosis in Denmark have mainly focused on the epidemology in humans and livestock (e.g. Holten-Andersen et al., J Infect 1984, 9: 277-282; Henriksen and Krogh, Nord Vet-Med 1985, 37: 34-41). In the present investigation, the prevalence of *Cryptosporidium* sp. in rodents was investigated in Denmark for the first time.

Rodents, totally 727 (*Clethrionomys glareolus* (C.g.), *Microtus agrestis* (M.a.), *Apodemus flavicollis* (A.f.), *A. sylvaticus* (A.s.) and *Micromys minutus* (M.m.)), were caught from 6 locations in Denmark during an 18-month period. Faecal smears were taken and fixed in methanol/1% HCL and thereafter stained by a modified Ziehl-Neelsen technique (Henriksen and Polenz, Acta Vet Scand 1981; 22: 594-596).

The overall prevalence of *Cryptosporidium* sp. was 7.2%. At all the locations *C. glareolus* was significantly more often infected than all the other species except *M. minutus*. Location 2 had the highest infection rate, not only because *C. glareolus* had a high infection, but also the other species were more often infected at this location.

This study showed that 7.2% of the investigated rodents harboured *Cryptosporidium* sp., and there were some differences between different species of mice and different locations. However, the investigation cannot conclude whether the species found in the rodents also act as a reservoir for infections in humans and livestock.





DYNAMICS OF ANTIBODY TITERS IN SPONTANEOUS TOXOPLASMOSIS IN CHILDREN

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The aim of our work was to reveal the fluctuations of antitoxoplasmic antibody titers in blood serum of children with spontaneous toxoplasmosis (without any effect of specific medicine).

233 children with ocular pathology were examined according to the intradermal test (IDT), complement fixation test (CFT), and indirect fluorescent antibody test (IFAT). The afflicted children were from 8 to 17 years old. 50 (21.4±2.6%) children were found to be seropositive. 111 healthy children were examined for control. 6 (5.4±2.1%) of these were found to be seropositive. Thus, compared with healthy children, children with ocular pathology responded to toxoplasmin more frequently ($P<0.05$).

Dynamics of antitoxoplasmic antibody titers was tested in blood serum of 33 invaded children. Serological studies were carried out in May, September, October and November. During our studies, CFT and IFAT antibody titers were at the same level or changed insignificantly (within the range of one dilution) in blood serum of 13 (5.6±1.5%) children. In 7 (3.0±1.1%) children, antitoxoplasmic antibody titers increased gradually, while in 9 (3.8±1.2%) children they decreased gradually. In 2 (0.8±0.5%) children, CFT antibody titers decreased considerably and low IFAT titers increased at the end of the study. In the blood of 2 children, CFT and IFAT high antitoxoplasmic antibody titers decreased gradually and increased again at the end of the study.

In all cases, IFAT antibody titers were higher by 2-5 dilutions as compared to CFT.

After the examinations were completed, children with high or increased antitoxoplasmic antibody titers underwent a treatment with specific preparations.

Our studies indicated that in cases of spontaneous toxoplasmosis, antibody titers in blood serum in children fluctuate; thus, the process of toxoplasmosis can grow acute or become chronic.

SEROPREVALENCE STUDIES OF TOXOPLASMOSIS IN DANISH PIG POPULATIONS

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Toxoplasmosis may be transmitted to man from domestic animals such as pigs and sheep, by way of unfrozen and undercooked meat. Serology is an alternative to direct demonstration of viable *Toxoplasma gondii* by e.g. mouse inoculation, which involves time-consuming and expensive procedures. The present study investigated the usefulness of monitoring *T. gondii* seroprevalence in pigs by means of herd and slaughterhouse samples.

An indirect ELISA, measuring pig IgG antibodies to *T. gondii* tachyzoite lysate was established. A cut-off OD value of 0.36 for positive sero-reaction was determined on the basis of 69 sera from 4 herds, investigated by Dye-Test (serum dilution 1:10) and ELISA. The chosen cut-off gave optimum combined sensitivity and specificity of 0.94 and 0.92, re-

spectively, using the Dye-Test as a standard. In experimental infections with different doses of cyst-forming strains, 40/42 pigs, positive by mouse inoculation, were seropositive in ELISA. Sera from a total of 87 pigs, experimentally infected with bacteria of the genera *Salmonella, Yersinia* or *Actinobacillus* and with the parasites *Isospora suis, Trichinella spiralis* or *Ascaris suum,* did in no case produce cross-reactions in the ELISA. However, 3/9 pigs inoculated with 50,000 sporocysts of *Sarcosystis miescheriana* gave maximum OD readings of 0.40-0.45 during the 13-15 weeks observation period.

The seroprevalence in 30 Danish sow herds was 11.9% (N=807) with a significant age-related increase from gilts (5.9%, N=443) to sows with parity ≥5 (23.5%, N=102). Between herds, seropositivity varied from 0 (14 herds) up to 46%. When 16 of these herds were re-examined two years later, 9/10 herds remained seronegative and 5/6 herds remained seropositive. Seropostivity persisted in 15/21 individual sows on retest.

The seroprevalence in 4 quarterly samples of serum from 4,016 slaughter pigs, taken at random as a pre-fixed percentage of slaughterings from all Danish abattoirs during a 2-week period each quarter, was 3.1%. This figure agrees well with a recent finding of 3.3% seroprevalence in 1,398 Danish pigs.

Meat juice, drained from the frozen and rethawed hearts and tongues of slaughtered pigs, was investigated for the content of anti-*Toxoplama* antibodies. When tested by ELISA at a 10 times lower dilution step, meat juice samples produced excellent correlations with corresponding serum samples (r=0.97, slope=1.11, y-intercept=0.06, N=38).

The cost/efficiency ratio of integrating toxoplasmosis testing into a quality control programme for Danish slaughter pigs will depend i.a. on whether the same degree of clustering of seroreactors exists in slaughterhouse herds as in breeding herds, and whether feasible control measures can be established on individual farms for reducing the transmission of *T. gondii* from cats to pigs.

ECHINOCOCCOSIS IN LITHUANIA: EPIDEMIOLOGICAL AND SURGICAL ASPECTS

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In Lithuania, immunological reactions have been carried out since 1979: latex agglutination reaction (1979-1988), ELISA (1989-1992), nondirect haemagglutination reaction (from 1993). Echinococcosis caused increased morbidity. This disease was confirmed in 1979 in 4 patients, 1980 - in 3, 1981 - 4, 1982 - 4, 1983 - 2, 1984 - 2, 1985 - 0, 1986 - 1, 1988 - 12, 1989 - 1, 1990 - 9, 1991 - 8, 1992 - 19 (one died), 1993 - 22 (one died).

The liver is the organ most commonly involved, but the lungs and the brain may be affected. In rare cases,

hydatidosis is localized in the muscular or skeletal system, ovaries, or spleen. Surgical treatment is performed to avoid cyst complications and anaphylactic reactions. Operations are carried out as soon as possible. The indications for operation are absolute in complicated cases.

From 1993 to June 1994, 2 patients with hepatic echinococcosis underwent operations at the 1st abdominal surgery department of the Vilnius University Emergency Hospital. One of them, a 22-year old man, was operated for a traumatic rupture of a hepatic echinococcal cyst in the abdominal cavity. After trauma, diffusive peritonitis was diagnosed. During laparotomy, rupture of the cyst (5-6 cm in diameter) of the left hepatic lobe near the anterior border was found. A marginal resection was performed. The epidemiological anamnesis was as follows: the man served in the SU Army in 1989-1990 in Uzbekistan. During night service in the mountains he slept together with the dogs to keep warm. On another patient (a 65- year old woman) an elective operation for a gigantic hepatic cyst was carried out in May 1994: cystectomy. She died after the operation.

Echinococcosis has become a problem in Lithuania. People can contract the disease by migration, not only in places that are considered to be endemic centres, but also in our republic. Care should be taken, not only by the medical personnel, but by the entire society. This means that this particular area of surgical work will be increased. In cases of echinococcosis, any complications are possible, including those described above.

INFANT HYMENOLEPIOSIS

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Invasion by *Hymenolepis diminuta* is very rare in Lithuania. Only individual cases of this invasion are described in Lithuanian medical literature (Lenkauskaitė, 1990), but until now it has not been diagnosed in infants. We are presenting the case report of an infant with the diagnosis of *H. diminuta* invasion.

The girl G.E., at the age of 11 months, was not of normal weight for her age and had sporadic episodes of diarrhoea. When she was 13 months old, worm ova were found during an examination of faeces at the polyclinic laboratory. That was why the patient was hospitalized in the Children's Clinic of Infectious Diseases. She arrived in a satisfactory state, without diarrhoea, bodyweight being 8,200 g (birth weight 4,050 g). On the 3rd day in clinic, diarrhoea repeated and persisted for four days. The temperature of the patient was normal. In the peripheral blood test, Hb 111 g/l, leu $8.4*10^9$/l, eo 4, neutr 13, lymph 79, mon 4, ESR 9 mm/h. Urine tests without abnormalities. In coproscopy tests, *H. diminuta* ova were found. EIEC 0151 was also found in two faecal bacteriology tests.

Epidemiological anamnesis: The infant was brought up in a village with poor sanitary conditions, where rodents were observed. The infant had direct contact

with flour and crops. After the diagnosis was confirmed, the patient was treated with 1 g of phenasalum once per day. After 40 days of treatment, the same *H. diminuta* ova were found. The patient had no complaints. After that the girl was treated with 150 mg biltricide per day three times a day. Repeated dehelmintization was effective.

Conclusion: *H. diminuta* invasion at the age of one year is very rare. The episodes of diarrhoea and abnormal age weight may be one of the reasons for this invasion. Biltricide proved to be effective in the treatment of the *H. diminuta* invasion.

DIAGNOSIS AND TREATMENT OF TOXOPLASMOSIS

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The diagnosis of toxoplasmosis is easy in the grown-up patient with an acute, acquired infection where high IgG antibodies can easily be demonstrated and IgM antibodies are present. However, the diagnosis of toxoplasmosis poses special problems in pregnant women and adults with late onset congenital toxoplasmosis.

During the last few years, several new tools have been introduced into the routine diagnosis of acquired and congenital toxoplasmosis, especially sensitive assays for toxoplasma-specific IgM and IgA antibodies and the toxoplasma IgG avidity assay.

Infections in pregnancy present special problems in cases where it is important to know whether the infection has passed from the mother to the child. With the development of the polymerase chain reactor (PCR), it is now possible to determine within a few days whether a sample of amniotic fluid contains *Toxoplasma gondii*, whereas previously it took at least six weeks to perform an *in vivo* culture in mice. At the same time it is becoming evident that transmission rates in the beginning of the pregnancy are lower than previously expected, and it now hardly seems indicated any longer to perform induced termination of a pregnancy, based on the presence of specific IgM antibodies, without proving that the foetus is infected.

Treatment of toxoplasmosis has for many years relied on the traditional drug combination of sulfadiazin and pyrimethamin. This drug combination is used in pregnancy and congenitally infected children, when the infection is certain, and spiramycin is used as a suppressive treatment in the same category of patients in cases of uncertainty whether infection has actually taken place.

Patients after the neonatal period will only very rarely need treatment, but occasional severe, prolonged infections and especially myocarditis and encephalitis will need treatment.

New drugs on the horizon are azithromycin and atovaquone, which are at present undergoing clinical trials in HIV-

infected patients, and will hopefully also be of use in pregnancy and neonatals.

SPREADING OF *ECHINOCOCCUS GRANULOSUS* IN LITHUANIA; ITS CAUSES

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It is an old tradition in Lithuania to slaughter domestic animals, especially pigs and sheep, at private farmsteads possibly meeting sanitary requirements.

Sometimes farmers do not pay attention to *Echinococcus* larvae vesicles in the liver, being ignorant of their parasitic origin. Such liver is readily accessible to dogs. When the infected liver is eaten, the cycle of *Echinococcus* is repeated. Pigs in Lithuania are usually infected with *Echinococcus oncospherus* in spring and autumn.

Pigs are the main intermediate hosts of the cestode *E. granulosus* in Lithuania. The larval stage of this helminth can occur in sheep, but the epizootological role of sheep is rather negligible. In 1993, 143242 pigs were slaughtered at the Lithuanian slaughterhouses. Approximately 0.4% of the pigs were infected with *Echinococcus*. In comparison with data from the preceding 10 years, the number of cases with these helminths have been reduced by 2-3 times.

Dogs in Lithuania are dewormed according to annual plans developed by the state veterinary service, which are not always fulfilled. Often the owners of dogs ask veterinary services to examine their dogs for intestinal helminths and deworm for nematodes and cestodes.

The opinion is held that the number of domestic animals infected with *Echinococcus* should be reduced in the future.

TAENIOSIS AND CYSTICERCOSIS IN A RURAL VILLAGE IN HONDURAS

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Taenia solium infection in man is acquired by ingesting the larval stage (the cysticercus) in raw or undercooked meat from pigs that acquire the infection by ingesting *T. solium* eggs in faeces from a human tapeworm carrier. Accidentally, man can be infected by eggs, and the larval stage develops, which can lead to neurocysticercosis, a life-threatening disease.

The aim of this study was to assess the prevalence of taeniosis and cysticercosis and to determine risk factors associ-

ated with their transmission in a rural community of Honduras where 60.5% of the population live in rural areas. The selected village had a population of 1,384 inhabitants in 217 households. Of these households, 110 were randomly selected to be included in the study.

ELISA was used to determine antibodies against *T. solium* cystercercus vesicular fluid antigen. Sera were obtained from 526 humans and 179 pigs. Three stool samples were collected from each person at 3-day intervals. The samples were examined for taeniosis and other intestinal parasites, using formol-ether concentration technique. Pigs were examined for lingual cysticerci. *Taenia* spp. positive persons were given niclosamide. Expelled worms were identified using carmine staining.

Sensitivity, specificity, and predictive values for the ELISA were calculated. The association between seropositivity and various background characteristics was expressed as odds ratio with 95% exact confidence interval. Data analyses were performed by means of Epi info software package, version 5, 1990.

Ninety-six per cent of the human population was parasitized by one or more of 17 identified parasites. Of 11 persons (2%, 9 females, 2 males) found to be infected with *Taenia* spp., nine were treated and four expelled *Taenia* segments were identified as *T. solium*. In humans, the seroprevalence of anti-cysticercus antibodies was 38%, in which a statistically significant association between seroprevalence and age was found. Risk factors associated with seropositivity were: having earthen floor, overcrowding, previous taeniosis, and living in the same household as a person with previous taeniosis. In pigs, the seroprevalence of antibodies was 43%, whereas tongue examination revealed 7% infected animals. These findings suggest that a few tapeworm carriers are enough to spread the infection to humans and pigs. Furthermore, the spread of the infection is strongly associated with socio-economic and sanitary conditions.

Problems on Trichinellosis

ANIMAL TRICHINOSIS IN LITHUANIA

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No data existed about trichinosis among the wild and synantropic fauna in Lithuania until 1957. Kazlauskas and Prūsaitė (1958) were the first to investigate trichinosis in wild animals. Having investigated 21 homeless cats, 2 foxes and 36 wolves that had been caught in various regions, they found that one cat, one fox and 12 wolves were trichinized. Most of the animals infected with trichinous larvae were from south-east Lithuania. According to data collected by Musteikaitė and Prūsaitė (1959), 33.4% of wolves, 10.7% of foxes and 21% of wild dogs were trichinized. Trichinosis among wild boars was registered only in 4 regions of Lithuania until 1968 (Biziulevičius et al., 1969). Fur-bearing animals from 20 farms were investigated, but trichinae were not detected (Dubnickis, 1967; Stankevičius et al., 1973). Trichinoscopy was performed on 8,827 domestic, synantropial and wild animals (29 species in all) in the course of 1969-1974 by Stankevičius, who found that 9 of these species harboured trichinae.

The Institute of Zoology and Parasitology carried out investigations in various regions of Lithuania in 1970-1982; of 29,491 investigated animals, comprising 28 species, 20 species were found to be infested with trichinae.

The first data about trichinosis among pigs in Lithuania were published in 1926-1932; six pig carcasses were registered as harbouring trichinae. After World War II, pig trichinosis was detected only in the south-east regions. In 1971-1975, more than 8 million pigs were investigated at meat-processing plants, and trichina larvae were found in 23 slaughtered pigs. Over 9 million pig carcasses were investigated in 1976-1980, 23 of them were found trichinized.

In the course of 1952-1967, 5 wild boars were found to harbour trichinae. 34,648 hunted wild boars underwent a veterinary examination in 1965-1973, and the meat of 67 wild boars was trichinous. An obligatory trichinoscopy of wild boar meat was resorted to in 1973. It has become widely known that not only pig meat may be trichinous, but also that of wild boars. Wild boar trichinosis is evenly spread in the Republic, while that of pigs is found mostly in south-east and eastern regions.

The incidence of trichinosis among humans having become a more frequent phenomenon, active measures have been taken in the campaign against trichinosis in Lithuania.

OBSERVATIONS ON THE PREDILECTION SITES OF *TRICHINELLA NATIVA* MUSCLE LARVAE IN ARCTIC FOXES *(ALOPEX LAGOPUS)* CAUGHT WILD

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It is known that the predilection sites of muscle larvae of *Trichinella* depend on the host species. However, few reports exist on the the predilection sites of *Trichinella* larvae in carnivores.

For the diagnosis of a *Trichinella* infection, it is of major practical importance, in particular in field situations, to know the predilection sites of the parasite, since this will make the diagnosis more sensitive.

Of 270 arctic foxes *(Alopex lagopus)*, all caught wild in Greenland, 16 were positive for *Trichinella*. The foxes were stored frozen for several weeks before examination. 18 selected muscles/muscle groups, freed from all tendons and fasciae, were examined using a HCL/Pepsin digestion in a Stomacher blender.

No differences were found when comparing the average weight between infected and uninfected animals. The level of infection varied considerably between foxes, where average numbers of larvae per gram (l/g) showed values from 1 to 148. The number of muscle larvae did not exceed 460 l/g in any of the samples examined. The highest relative larval densities were found in the following muscle/muscles groups: The muscles of the eye *(m. rectus dorsalis/medialis/lateralis/ventralis + m. obliquus dorsalis/ventralis)*, the lower part of the hind leg *(m. gastrocnemius)*, the upper part of the front leg *(m. biceps brachii/m. triceps brachii)*, the sublumbar muscles *(m. psoas minor)*, and the upper part of the hind leg *(m. rectus femoris)*.

In the present study, the larval distribution shared the same characteristics as those found in other studies on closely related wild-living carnivores and on experimentally infected arctic fox *(A. lagopus)*, but differed from experimentally infected herbivorous species. This observation supports the assumption that the relative density of *T. spiralis* muscle larvae will depend on the functional importance to which the muscles are predisposed in that particular genus/species of host, whether the muscles are being frequently used or not.

FOCUS OF TRICHINELLOSIS AND FACTORS DETERMINING ITS MILD CLINICAL COURSE

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An outbreak of trichinellosis near Poznan provided the opportunity to describe the clinical course of the disease.

Out of 81 reported persons, 68 patients were studied, all of whom were subjected to a set of clinical and laboratory tests. Serological tests aimed at trichinellosis included the determination of total, IgG, IgM and IgA class antibodies by ELISA techniques. Three groups were distinguished: the patients of group I (10 patients) had consumed raw pork. In this group, trichinellosis had a moderate course in 1 patient, abortive course in 7 patients, and was asymptomatic in 1 patient. IgG class antibody titers were low in 8 patients (1:10 to 1:80) and only in 2 patients, IgG total titers were 1:640 and 1:320, associated with IgM ab titers of 1:80, and 1:320. All the patients were treated with mebendazole (vermox) at 200 mg twice daily with good effects. In group II (13 patients) who also had consumed raw or semi-raw pork, no symptoms were noted and eosinophil levels in blood were normal. Only in 1 case IgM ab titer was 1:80, in 2 other patients IgG an IgM titers were at the borderline of significance. All the patients were prophylactically treated with mebendazole. In group III (45 persons), who had consumed cooked pork only or pork products, neither symptoms of the disease nor eosinophilia were detected, and the group was excluded from further observation. Parasitological examination of the consumed pork detected only 5 *T. spiralis* larvae/g meat. Trichinoscopy showed that most of the larvae were uncapsulated, digestion of meat samples showed that all *T. spiralis* larvae were uncoiled and immobile, probably dead. Biological test (xenodiagnosis) aimed at detecting reproductive capacity of the larvae yielded a negative result: 3 months after infection with 60 larvae, parasitological examination of rat muscles demonstrated no larvae of *T. spiralis*.

In the focus trichinellosis was diagnosed in only 16.1% of the cases (out of 68 persons examined) and its clinical course was mild. This was explained by low intensity of invasion in the consumed pork and low invasiveness of *T. spiralis* larvae most of which were uncapsulated and unable to reproduce in a subsequent host. This was probably due to the early stage of invasion in the animals from which the pork originated. Devitalization of larvae after digestion of the meat sample points to their injury, possibly due to the meat processing.

The parasitological characters of the *Trichinella* invasion determined its mild clinical course in the epidemical focus.

ON THE OCCURRENCE AND DIAGNOSIS OF TRICHINELLOSIS IN WILD ANIMALS IN ESTONIA

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The objective of this study was to establish the extent of *Trichinella* invasion in Estonian wild animals, in order to determine the most intensively invaded muscles and to identifity *Trichinella* spp.

Under examination were raccoon dogs *(Nyctereutes procyonoides)* foxes *(Vulpes*

vulpes), badgers *(Meles meles)*, wild boars *(Sus scrofa)*, pine martens *(Martes martes)*, squirrels *(Sciurus vulgaris)*, lynxes *(Felis lynx)*, wolves *(Canis lupus)*, bears *(Ursus arctos)*, black rats *(Rattus rattus)*, polecats *(Mustela putorius)*, minks *(Mustela lutreola)*, dogs and cats. Muscle samples from 13 muscles and muscle groups were examined by an artificial digestion method. *Trichinella* spp. were identified by the RAPD method at the Trichinella Reference Centre.

Trichinellosis was diagnosed in boars, raccoon dogs, foxes, lynxes, pine martens, wolves, and cats in 14 rural districts. Raccoon dogs and cats were most invaded. M. masseter, m. temporalis, corpus and radix linguae, m. extensor digitorum communis, m. extensor digitorum longus, m. flexor digitorum lateralis, and m. flexor digitorum profundus were most infected. Raccoon dogs were infected with *T. nativa* and *T. britovi*, foxes with *T. britovi* and wild boars with *T. nativa*. One raccoon dog was infected with *T. nativa* and *T. britovi*.

TRICHINELLA SPIRALIS AND DENSITY OF HOST POPULATIONS IN LITHUANIA

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The extensive spreading of *Trichinella spiralis* among wild and domestic pigs has been registered in Lithuania in the past years. Of the 7,876 wild and 2.1 million domestic pigs investigated in 1993, 119 wild and 692 domestic pigs were found to be infected.

A great number of infected domestic pigs was from large-scale collective farms. Domestic pigs were infected mainly from rodents.

Only 30 domestic pigs were found infected with *T. spiralis*, of 1.95 million pigs investigated during the period of 1933-1937.

The *T. spiralis* infection rates in wild and domestic pigs have increased correspondingly with the increasing densities of susceptible wildlife, rodents, and domestic pig populations in Lithuania.

The densities of wild and domestic pig populations increased 2.5-10 times from 1933 to 1990. The densities of rodent populations in fields also increased during that time.

The forming of large-scale collective farms has disrupted the balance of ecosystems, and has caused serious problems with the *T. spiralis* parasite in Lithuania.

DATA ON THE SEROEPIDEMIOLOGY OF HUMAN TRICHINELLOSIS IN LITHUANIA

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Until recently, the distribution of human trichinellosis in Lithuania was determined according to evident cases (i.e. foci and number of patients). However, such a method does not completely

reveal the distribution of all cases of trichinellosis, due to the fact that most of them, subclinical cases especially, remain unknown. Our task was to estimate the immunological aspect of human trichinellosis in Lithuania.

Blood serum samples were taken all over Lithuania from 562 persons who were not suspected by their physicians to be suffering from trichinellosis. The sera were tested by ELISA using excretion/secretion antigen and DAKO reagents.

The results obtained are given in the table below.

Distribution of tested persons according to age:

Age (years)	Total	Sero-positive suspected	Percentage sero-positive
0-1	15	0	0.0
1-5	31	7	22.6
6-10	65	23	35.4
11-15	64	26	40.6
16-20	53	19	35.9
21-30	76	20	26.3
31-40	111	33	29.7
41-50	79	32	40.5
51-60	43	13	30.2
60 and more	25	12	48.0
TOTAL	562	185	32.9

According to our tests, trichinellosis antibodies are found in the blood of 1/3 of the Lithuanian population. However, such antibodies were negative in the blood of babies under 1 year of age. The titer of the most common antibodies was 1:10 for 68.1% of seropositive persons; it was 1:20 for 22.7% of such persons, 1:40 for 7.57% and 1:80 for 1.62%. The blood of 95.65% of seropositive healthy donors manifested IgM antibodies, and 4.35% of these had IgG.

INVESTIGATION ON THE IMMUNE ACTIVITY OF *TRICHINELLA* ISOLATES

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The objective of this paper is to examine the immune activity of the trichina isolates, both rat-adapted and extraced from a pig, a wild boar, and a cat.

The investigation was carried out dynamically in the course of the first and the second trichina passages. The humoral antibodies were determined by applying double diffusion to agar gel and immuno-electrophoresis methods with a standard antigen. The antibody titers were determined from a complement linkage reaction. Subsequent to infection of white rats with adapted isolate, the precipitates were formed already after the 7th-14th day and remained until the 60th day of invasion. We obtained a positive reaction on the 14th-28th day of invasion, the antibody titers being 1:5 and 1:20. Formation of expressed precipitation patterns were observed in the area of globulins.

Upon infection with a pig isolate, the antibodies were determined in 1:5 titer in

the period between 21 and 60 days of the first passage and between 14 and 30 days of the second passage. In agar gel the antibodies were expressed on the 30th day and remained until the 60th day of invasion. The precipitation was particulary expressed on the 45th day. The precipitation remained expressed until the 60th day during the second passage.

Upon infection of rats with a wild boar isolate, immune precipitates were determined by the two above-mentioned methods until the 60th day of invasion during the first and the second passages; however, they were more expressed in the latter case.

Upon infection with an isolate extracted from cat muscles, precipitating antibodies in agar gel were determined on the 30th day with antibody titers being 1:5. A particularly expressed reaction was observed on the 60th day of invasion.

Our investigations have shown that the trichina isolates, though not being adapted to a new host, are able to induce significant antibody responses.

COMPARATIVE TRICHINOSCOPIC EXAMINATION OF STAINED AND UNSTAINED FROZEN MEAT

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In order to evaluate the efficacy of a compressor slide technique in detecting trichina in stained and unstained frozen meat, samples of wild boar with *Trichinella spiralis* larvae were used for examination. 140 samples were prepared and placed in 5 compressors of 28 samples each. At first unstained frozen meat samples were examined microscopically and with a portable projective trichinoscope, then the same samples were stained for 5 min. with methylene blue (1:500) and washed with 5% acetic acid and distilled water. After this procedure, the samples were again examined microscopically and with a portable projective trichinoscope. Cuts of unstained meat from 140 samples were studied: 24 of them contained 27 larvae. The examination of these samples took 59 min. The examination of the same samples with a portable projective trichinoscope revealed 29 larvae of *T. spiralis* in 25 samples. The examination took 60 min. The microscopical examination of dyed meat cuts took only 47 min., and 45 min. with a portable projective trichinoscope. Microscopical investigation of dyed samples revealed 43 larvae of *T. spiralis* in 38 samples. Investigations with a portable projective trichinoscope showed 46 larvae of *T. spiralis* in 39 samples.

Therefore, the examination of dyed frozen meat samples allowed us to detect 1.6 times more invaded samples and 1.6-1.7 times more larvae of *T. spiralis*. The investigations described revealed that the larvae of *T. spiralis* are more distinct in dyed samples. This method is less tiresome to the eyes of the analyst and makes the examination faster. Taking into consideration the possibility of missing invaded carcasses in the examination of

unstained meat samples, we think the staining of meat samples should be compulsory.

Ecology, Morphology and Taxonomy of Parasites

MUSSELS, MITES AND MIDGES

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A unique association occurs between freshwater mussels (*Anodonta* spp.), mites (*Unionicola* spp.) and midges (*Chironominae*). The larval mites are parasitic on adult midges, whereas the nymphal and adult stages of some Unionicolids live on and between the gills of freshwater bivalves. The water mite life cycle can now be successfully completed in the laboratory. Infected adult Chironomids are produced by maintaining mud, containing larval midges and one species of mussel, in a tank covered with muslin. The mud, covered to a depth of a few cm with water, is aerated and maintained at constant temperature (13°C) and a 16-hour daylight regime. Emerging adult midges are collected by aspiration using a pooter.

Mite larvae released from *Anodonta cygnea*, occur mainly in the intersegmental region between the thorax and abdomen (54%) or on the first and second abdominal segments (25%) of midges [Infestation rate is 17% and average mite load 2.4]. Those in the intersegmental area are *Unionicola ypsilophora* and those on the anterior abdominal segments are *U. aculeata*. Unionicolid larvae released from *A. anatina* are found mainly on the first and second abdominal segments (83%) [Infestation rate is 37% and mite load 2.5] and are *U. aculeata*. Although *U. intermedia* also occurs in *A. anatina*, the larval stage has not been recorded from Chironomids, leading to speculation that this species does not require a midge host.

NEW DATA ON THE LIFE CYCLE AND TAXONOMY OF *SARCOCYSTIS*

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Species of *Sarcocystis* Lankester, 1882, are distinguished by their obligate-heteroxenous life cycles. An opinion exists that an intermediate host can become infected with parasites only after having ingested sporocysts and oocysts excreted into the environment by definitive hosts. There are scarce data about the role of such ecological factors as cannibalism and predation among the intermediate hosts in the spreading of sarcosporidians.

Our work was devoted to the investigation of sarcosporidians of rodents in Lithuania. It is known that cannibalism is common among rodents. We have proved that *Sarcocystis rodentifelis* from the bank vole (*Clethrionomys glareolus*) and the Norway rat (*Rattus norvegicus*) can circu-

late among rats and mice without the participation of the definitive host (cat). Transmission of this parasite from an intermediate host to another occurs by an alimentary route through the sarcocystic stages. This characteristic feature of a parasite survived after four passages. Typical sarcocysts are developing in muscles of an intermediate host infected by this route. In addition, our research revaled that *S. rodentifelis* can be transmitted to laboratory rat (*Rattus norvegicus var. alba*) young by transplacental transmission after infection of female rats with sarcocysts.

The analysis of our original data, as well as data from literature, enables us to expand the diagnosis of the genus *Sarcocystis* (Grikienienė, Arnastauskienė, Kutkienė, Ekologija 1993; 1: 16-24). We suggest dividing this genus into 3 subgenera: 1) *Sarcocystis*, 2) *Levitia*, 3) *Matuschkia*. The first subgenus should include those species of *Sarcocystis* which are not notable by cannibalistic and predatory relations among the intermediate hosts. The newly described *S. rodentifelis* species is included in the *Levitia* subgenus. The representatives of this subgenus can circulate among intermediate hosts of the same or related species through the cyst stages, by cannibalism or predatory routes. Asexual and sexual development of the parasites of the *Matuschkia* subgenus takes place in a single host or in different individuals of the same host species (dihomoxenous life cycle). Cannibalism and autotomy are the main routes of transmission of these sarcosporidians.

We are convinced that to understand the life cycle of parasites, features of specific ecological hosts should be taken into account.

NEW SIGNS IN THE TAXONOMY OF HAEMOPROTEIDS

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The identification of species of haemoproteids (Haemosporidia: Haemoproteidae) is based on very limited data of gametocytes morphology and specificity of the parasites. The main aim of the investigation is to determine new signs which can be used in the taxonomy of haemoproteids.

Observations were made through examination of thin blood films which were prepared at appropriate intervals from citrated fresh blood of infected birds. The comparative investigation of gametogenesis, zygote and ookinete formation of *Haemoproteus tartakovskyi*, *H. belopolskyi*, *H. fringillae*, *H. pallidus*, *H. balmorali*, *H. dolniki*, and *H. majoris* under a ligth microscope was performed for the first time. The most informative signs which can be used for haemoproteid systematics, are microgamete length, zygote structure, morphological peculiarities of developing ookinete as well as the rate of ookinete formation.

H. pallidus microgametes are almost half the length of the microgametes of other species. A large clear vacuole is formed in the zygote of *H. balmorali* and *H. fringillae* only. Three types of initial

stages in ookinete formation were distinguished. The first type is characteristic of *H. tartakovskyi*, *H. belopolsyi*, *H. balmorali*, *H. dolniki*, and *H. majoris*. Ookinete formation in these species starts with the appearance of thin long digital growth. The second type is characteristic of *H. fringillae*. A short blunt growth appears in the middle part of the parasite. The growth expands, and the forming ookinete attains the shape of a pear. The third type is observed in *H. pallidus*. The ookinete of this species acquires a triangle form. One side of the 'triangle' accumulates some pigment and the opposite angle becomes prolonged, thus giving the beginning of the ookinete.

The peculiarities of gametogenesis, zygote and ookinete formation under stable conditions in vitro can be useful additional signs for the determination of haemoproteid species.

SOME ECOLOGICAL ASPECTS OF DIPLOSTOMOSIS IN LITHUANIA

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In a study on the distribution of cercariae of *Diplostomum* in Lithuanian water bodies (Curonian lagoon, Nemunas delta, water reservoirs and lakes included in the cooling system of the electric power station, various water bodies of east region of Lithuanian), 5,233 limnaeid snails were examined. Six *Diplostomum* species were found: *D. pseudospathaceum*, *D. paracaudum*, *D. spathaceum*, *D. mergi*, *D. parviventosum*, and *D. baeri*, in five species of Limnaea snails. Higher degrees of infection of snails and foci of diplostomosis were obtained in the littoral of the Curonian lagoon (24.0%). The limnaeid snails were concentrated in small bays sometimes drying-up. These were resting places for migrating birds as well as resting and feeding places for wintering *Mergus merganser*. The foci of diplostomosis were obtained in the Nemunas delta in the river of Ulmas (16.9%) and in the water reservoirs of Elektrénai. The influence of heated water created favourable conditions for the development of fish diplostomosis.

ON THE POPULATION DYNAMICS OF HELMINTHS IN A BANK VOLE POPULATION

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In the population biology of parasites, factors regulating population density are of major importance. Generally it is accepted that both density-dependent and density-independent factors are involved in the regulation of helminth populations, but their relative importance is still being discussed and differs in various host-parasite systems. Here I provide data about population dynamics of three helminth species in bank vole *(Clethrionomys glareolus)*, and discuss factors deter-

mining the dynamics.

The material was collected in South-West Lithuania (Šakiai district). The biotope is the wood-cutting areas of mixed coniferous-deciduous forest. Rodents were caught in seasonal assays in 1983-1986 and in the winter of 1990. In 1983-1986, total helminthological autopsies of 340 bank voles were carried out. For the 58 voles caught in the winter of 1990, only the large intestine, including the caecum, was examined.

The maximum density of the bank vole population was found in the autumn of 1983 (56.0 voles per 100 trap-days). In the winter the density decreased and in the spring of 1984 it was 1.1. Thereafter the population density slowly increased (autumn 1984: 10.7; autumn 1985: 18.9; autumn 1986: 20.5).

Seventeen helminth species were found in the investigated population of bank voles. The population dynamics of *Notocotylus noyeri* (Trematoda), *Taenia mustelae* (Cestoda) and *Heligmosomum mixtum* (Nematoda) was studied.

N. noyeri: No evident seasonal dynamics was detected. The rodents become infected with this fluke only when the environmental conditions for the transmission of infection are favourable.

T. mustelae: The maximum levels of infection parameters were found in 1984 (spring: prevalence of infection E=76.2%, abundance M=1.6 parasite per host; summer: E=17.9%, M=2.4), i.e. in the period of low vole population density. Later the values of the parameters were lower. The dynamics of the *T. mustelae* population is influenced by processes of interaction of its hosts: voles and mustelids.

H. mixtum: The levels of the *H. mixtum* infection parameters in the autumn of 1983 were not high: E=17.1%, M=0.2). In 1984 the values increased considerably and were at a maximum in the autumn of 1984 (E=52.6%, M=1.28) and in the winter of 1985 (E=61.5 %, M=1.31). Later the values decreased, but were higher than in the autumn of 1983.

It should be noted that the population dynamics patterns and regulation factors differ in the examined host-parasite systems. The high parasite burdens when the host population density was low can be explained by the delay in density-dependent regulation.

ORIGIN AND DIFFERENTIATION OF THE ONCOSPHERAL TEGUMENT IN THE CESTODE *ECHINOCOCCUS MULTILOCULARIS* (CYCLOPHYLLIDEA, TAENIIDAE)

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Rybicka's description (Rybicka, Parasitology 1973; 66: 9-18) of the oncospheral tegument in *Hymenolepis diminuta* exhibiting a distal cytoplasm with an internal cyton makes an attractive theory, suggesting homologies between the teguments of the oncosphere, the metacestode, and the adult worm. The oncospheral tegument

plays an important role at the stage of infective, activated oncosphere (Swiderski, La Cellule 1972; 69: 207-237); its distal cytoplasm, after hatching and rupture of the hook region membrane, spreads around the hexacanth. The aim of the present study is to describe the origin, differentiation and ultrastructure of the oncospheral tegument in the taeniid cestode *Echinococcus multilocularis*.

Tissue samples from mature and gravid proglottids of *E. multilocularis* were fixed for 7 h in 4% glutaraldehyde buffered to pH 7.4 with cacodylate buffer and post-fixed for 2 h in 1% osmium tetroxide. The samples were then routinely processed for TEM and embedded in Spurr's resin. Thin sections were double-stained with lead citrate and uranyl acetate and examined with Zeiss EM-10 electron microscope.

The oncospheral tegument of *E. multilocularis* originates from a syncytial binucleate complex, which appears at the early stage of morphogenesis and organogenesis of the hexacanth. At this stage, the binucleate complex primordium appears as a syncytial cup or "calotte" situated beneath the inner envelope at one pole of the developing embryo. During oncosphere differentiation, the cyton of the syncytium migrates progressively into the central part of the embryo, but remains always connected with the distal cytoplasm by a tendrillar cytoplasmic connection. Following cyton migration, numerous cytoplasmic vesicles appear in the distal cytoplasm. The vesicles join, finally forming by a fusion a single large cavity or lacuna, the walls of each becoming the walls of two delaminated layers: the oncospheral tegument distal cytoplasm and the so-called "hook-region membrane". This delamination of the initially compact layer of binucleate complex cytoplasm into two layers seems to be closely associated with differentiation of oncospheral hooks, the elongating blades of which protrude progressively into a newly formed cavity. The pression of hook blades on the hook region membrane, appears to facilitate its further separation from the distal cytoplasm. In the mature oncosphere the surface of the distal cytoplasm forms a regular brush border of cytoplasmic processes or microvilli. The distal cytoplasm accumulates numerous membrane-bound, dense granules, mitochondria and vesicles, and represents the true body covering of the hexacanth. The cytoplasmic connection between the distal cytoplasm and the cyton, or so-called "medullary binucleate cell", appears on the longitudinal and cross-sections as a narrow cytoplasmic strand, the plasma membrane of which is reinforced by numerous microtubules.

The above results indicate that the origin and differentiation of the oncospheral tegument in *E. multilocularis* is essentially similar to that described by Rybicka (Rybicka, Parasitology 1973; 66: 9-18) in *H. diminuta*, but differs from that reported by Gabrion (Gabrion, Z. Parasitenk. 1981; 65: 191-205) and Kashin (Kashin, Parazitologija 1990; 24: 23-27) in other cyclophyllidean species.

INFLUENCE OF HAEMOSPORIDIANS ON WILD BIRDS

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Haemosporidians (Sporozoa: Haemosporidia) are common parasites of wild birds in the Holarctic region. Data on the relationship between these parasites and free-living birds are very scanty. A notion of the harmlessness of haemosporidians to wild birds dominates in the literature nowadays.

The long-term populational investigation of the Curonian Spit in the Baltic Sea provides several pieces of evidence that the haematozoa harm their free-living avian hosts.

(1) At the peak of parasitaemia by *Haemoproteus fringillae* for a 3-day period, young chaffinches (*Fringilla coelebs*) become weakly mobile and secretive. They are usually not trapped by net catching, but may be shot. The limited mobility of the birds leads to an increased probability of the elimination of infected individuals.

(2) The high parasitaemia by *Haemoproteus* sp. influences negatively the accumulation of migratory fat in small passerine birds during spring migration. The migratory fat is a main energy material for migratory flight. So, heavy parasitaemia cannot be neutral for migrants.

(3) The prevalence of *Leucocytozoon* sp. in chaffinches increases significantly at the late stages of the autumnal migration. This can be regarded as a peculiar parasitic filter delaying the infected birds in their migration.

(4) The rarity of *Haemoproteus* sp. in very old (4-11 years) chaffinches in comparison with younger ones may indicate a reduced longevity of infected birds.

Most probably, the notion of the harmlessness of the haemosporidian parasites to wild free-living birds is not correct. Data on the non-pathogenicity of some species of haemosporidian parasites for wild birds, and probably also of other parasites, obtained under experimental conditions, cannot be directly applied to wild animals under field conditions. Special ecological investigations may reveal pathogenic effects of parasites which are known to be non-dangerous to their hosts in captivity.

Ecology, Morphology and Karyology of Parasites

KARYOTYPE CONSERVATISM AND OTHER CHROMOSOME SET PECULIARITIES OF TREMATODES

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Up till now, karyological studies have not been applied to the orders *Heronimiformes*, *Haploporiformes*, and *Lepocreadiformes*. Digeneans of the orders *Hemiuriformes*, *Opisthorchiiformes* and *Plagiorchiiformes* appear to be insufficiently studied. I have had the opportunity to study the

chromosome sets of 95 trematode species.

The overwhelming majority of the species was studied during the parthenogenetic stage of the development, i.e. chromosome sets were described in embryonic cells of rediae or sporocysts. Molluscs infected with the parthogenetic generation of trematodes were collected in different water bodies in Lithuania, Poland, Bulgaria, Kazakhstan, Azerbaijan and Chukotka, and in the White Sea. Air-dried slides were examined under a Jena Med cytology microscope.

The rate of karyotype evolution was relatively low. The diploid number of chromosomes varied from 12 to 28. Chromosome sets with 22 and 20 elements predominated (52.7% of the species). There was an extraordinary left asymmetry in the distribution of trematode chromosome classes (2n = 18, 16, or 12 included 40.4% of the species; while 2n = 24, 26, 28, or 3n included only 6.9% of the species). There was additional evidence of the chromosome junction in the evolutionary processes of trematodes. Besides, there were no gaps and disruptive patterns in the distribution of trematode chromosome classes. The minimal interpopulation karyotype differences showed also a high conservatism level of trematodes. Pericentric inversions and accumulation of DNA repetitive sequences are the driving forces at the early stages of karyotype evolution. The next stages of evolution are marked by Robertsonian translocations, reduction in chromosome numbers and the symmetrization of karyotypes. There are at least four main phyletic lineages of trematodes: *Echinostomatoidea*, *Strigeoidea*, *Opisthorchoidea*, and *Plagiorchoidea*. Their ancient forms probably contained 22, 20, 18 and 24 one-armed chromosomes, respectively. The formation of giant chromosomes is marked in highly specialized trematodes and in those being nearer to the stem of the phylogenetic tree.

IS *HEXAMITA SALMONIS* (FLAGELLATA) CAUSING WIDESPREAD SYSTEMIC DISEASE IN FISH?

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Recent reports by several authors describing systemic infections with the protozoan flagellate *Hexamita salmonis* in farmed salmonid fish, as well as the involvement of this parasite in the development of the 'hole-in-the-head'-disease and its hypothetical involvement in the development of the 'head and lateral line erosion syndrome' (HLLE), raise the question of how common systemic hexamitosis might be.

An analysis of the occurence of fish parasites, including microscopical examinations of intestinal contents, gall bladder contents, fresh smears of liver tissue and of fresh and fixed Giemsa-stained blood smears, was carried out throughout Finland in fishes from fresh and brackish water sites (both wild and farmed fish). A total of 1,700 specimens of fish, including

37 different species, was analyzed.

The study revealed *Hexamita salmonis* to be common only in a few salmonid and gadoid fishes, the parasites occuring free in the intestinal lumen, in the gall bladder, and rarely in liver tissue. Only *Onchorhynchus mykiss, Thymallus thymallus, Gadus morhua* and *Lota lota* were found infected. In turbot, analyzed for seasonal occurrence of infection, the parasite was present throughout the year. *Hexamita* was never observed in the blood samples, although flagellates of the genera *Trypanosoma* and *Cryptobia* were frequently encountered.

Conclusion: The present study indicates that in the region studied, *Hexamita* is common only in a few fish groups. Systemic infections were not observed. However, further studies are needed to evaluate any role of *Hexamita* in the development of the HLLE syndrome in cod, a disease recorded also in south-western Finland.

CERCARIAE OF THE GENUS *ECHINOCHASMUS* IN POLAND AND LITHUANIA

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Trematodes of the genus *Echinochasmus* Dietz, 1909 occur mainly in piscivorous birds, Podicipedidae and Ardeidae. Their intermediate hosts are prosobranch snails of the genus *Bithynia*, and metacercariae encyst in the gills and mouth cavity of various cyprinid fish. Of about 10 species recorded in Central Europe, the life cycles have been recognized in 4 species (Karmanova, Trudy Gelm Lab, 1973; 23: 71-76; Helminthologia, Bratislava, 1974; 15: 669-678; Trudy Gelm Lab, 1974; 24: 45-52), and chaetotaxy in 3 (Grabda-Kazubska et al., Ch Ann Parasitol Hum Comp, 1991; 66: 263-268; Dymitrov and Kanev, Helminthology, Sofia, 1992; 32: 19-23).

The cercariae show great similarity in body structure and dimensions, except the tail, which may be of various sizes and structures. Short tail (shorter than the body) was observed in *E. (Echinochasmus) beleocephalus* and *E. (E.) coaxatus*, long tail (1.5-2 times longer than the body) in *E. (Monilifer) spinosus*, and in *Echinochasmus* sp. - probably the larva of *E. (M.) spinulosus* and large (much longer than the body) in *E. (Episthmium) shigini*. The length of the tail seems to be in accordance with the subgeneric qualification of the species, except the cercaria of *E. (Episthmium) bursicola* which, according to Karmanova (Trudy Gelm Lab, 1973; 23: 71-76), has a short tail.

Chaetotaxy in both representatives of the subgenus *Echinochasmus* is very similar; only small differences may be noted in group C IV_5 and in ventral and lateral rows on the body. The tails of both cercariae bear the same number of sensillae: 4 pairs on the dorsal and 1 pair on the ventral side, near the end. *Echinochasmus* sp. cercariae have a greater number of sensillae in C II and C IV rings, in lateral rows and on the tail. The tail bears

5 pairs on the dorsal and 5 pairs on the ventral side plus 2 lateral pairs on its proximal part. Despite the above-mentioned differences, the pattern of chaetotaxy is the same and seems to be characteristic of the whole family Echinochasmidae. It is much more similar to the chaetotaxy recognized in representatives of *Psilotrema* and *Sphaeridiotrema* than to Echinostomatidae.

NEW DATA ON THE KARYOTYPES OF PSEUDOPHYLLIDEA (CESTODA)

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Karyological studies in the mitotic complements of pseudophyllidean cestodes were carried out in order to extend the very scanty knowledge existing on karyology of this group and to contribute to the elucidation of a number of questions on the systematics and phylogeny of cestodes. So far, karyotypes of 15 species of pseudophyllideans, including literature and present new data, have been described. In the present work the preliminary data on karyotypes of 3 species are presented for the first time.

Analysis of Giemsa-stained metaphase plates of plerocercoids of *Schistocephalus solidus* Müller, 1776 (from the body cavity of *Gasterosteus aculeatus*) revealed the mode diploid number of chromosomes 2n=18. Chromosome pairs 1, 5, 6, 8 and 9 are metacentric; pairs 2, 4 and 7 are subtelocentric, and pair 3 is subtelocentric to acrocentric. The karyotype is characterized by mean absolute chromosome length between 1.9 and 5.9 µm.

A diploid set of *Bothriocephalus gowkongensis* Yen, 1955 (from *Cyprinus carpio*) consists of 14 comparatively small biarmed elements. A diploid chromosome set of *Eubothrium* sp. (the parasite of *Clupes harengus membras*) contains 16 biarmed elements with the first two pairs of large metacentric chromosomes.

The range of diploid chromosome numbers among species of *Pseudophyllidea* spans from 12 to 26; most of the species have 18 chromosomes. The congeneric species tend to have the same number and the similar chromosome morphology. The wide distribution of the karyotypes with 2n=18, not only between pseudophyllideans, but also between other groups of cestodes - caryophyllideans, proteocephalideans, and cyclophyllideans, leads to the consideration that 2n=18 could be an initial number for cestodes.

THE KARYOTYPES OF *BRACHYLAIMA MESOSTOMA* AND *RHIPIDOCOTYLE ILLENSE* (TREMATODA: BRACHYLAEMOIDAE)

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Karyotype analysis of two species, *Brachylaima mesostoma* Rudolphi, 1803 (fam. Brachylaemidae) and *Rhipidocotyle illense* Ziegler, 1883 (fam. Bucephalidae), of the superfamily Brachylaemoidae

Joyeux and Foley, 1930, was performed in order to increase our karyological knowledge of the order Strigeiformes. The material was collected from naturally infected molluscs of the genus *Unio* from the river Neris (Lithuania) and from experimentally infected molluscs *(Xeropicta krynickii)* from the Crimean peninsula. Chromosomes of somatic cells of the parthenites were examined after air-drying of the cells.

The chromosome complement of *B. mesostoma* was found to consist of 9 pairs of uniarmed chromosomes which decreased gradually in length (from 3.87 to 1.43 μm). Chromosome pairs 1-4 were acrocentric, pairs 5-8 were acrocentric to subtelocentric, and pair 9 was subtelocentric. 68% of the cells of the parthenites obtained from the experimentally infected snails (infective eggs were obtained from one marita) contained B-chromosomes. The B-chromosomes were small elements with submedian to subterminal centromeres.

The chromosome complement of *R. illense* consisted of 7 pairs. All chromosomes were biarmed. They had median (pairs 1, 4, 6, 7) and submedian (pairs 3, 5) centromeres, except the 2nd pair, which was submetacentric to subtelocentric. The total length of the chromosomes ranged from from 5.78 to 1.55 μm.

The high degree of symmetrization of *R. illense* karyotypes indicates that bucephalids are not primitive trematodes. The karyotypic differences that were found between the species investigated show that their phylogenetic relationships may be close. Trematodes of the families Bucephalidae and Brachylaemidae form a karyologically distinct group within the order Strigeiformes.

EXCHANGE OF METAZOAN PARASITES BETWEEN THREE WHITEFISH *(COREGONUS LAVARETUS,* L.s.l.) STOCKS IN CENTRAL FINLAND

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Component communities of parasites of freshwater fishes are dependent on both evolutionary (specificity and immunological responses, for example) and ecological factors (as migrations and feeding behaviour).

The exchange of parasites has been documented to be more common between relative species and mainly generalists are exchanged between unrelated fish species. The significance of the differences between fish stocks and their taxonomical status are problematic, especially in coregonids.

Our purpose is to examine whether parasites can be used in understanding more thoroughly the ecological relationships between three species of whitefish *(Coregonus lavaretus* L.s.l.) stocks *(C.l. wartmanni, C.l. nilssoni* and *C.l. pallasi)* in an oligotrophic large lake, Lake Konnevesi, in Central Finland.

Another aspect is to see how stable the system is in relation to the establishment

of each fish species over time. Whitefish were studied in the early 1970s and again in the early 1990s.

The present results indicated that the composition and abundance of the 11 metozoan parasite species in the whitefish stocks were similar in both study periods. However, some surprising features were found, including the dominance of *Ergasilus sieboldi* (96-100%) and *Ichthyocotylurus erraticus* (96-100%), and the low level of cestode infections.

Some parasite species, such as the copepod *Salmincola coregonorum* and the acanthocephalan *Acanthocephalus lucii*, were found more rarely in the early 1990s than in the 1970s.

These results give support to the idea that only one *Coregonus* species occurs in Lake Konnevesi. However, to separate different stocks which differ, for example in gillraker numbers and breeding behaviour, is essential when the maintenance of whitefishes in the area is to be considered.

Parasite Populations

ECOLOGY AND MODELLING OF THE FREE-LIVING STAGES OF *TRICHOSTRONGYLUS COLUBRIFORMIS*

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A long-term field study was carried out at Badgery's Creek, NSW, Australia, to investigate the ecology of the free-living stages of *Trichostrongylus colubriformis*, a nematode parasite of sheep. Plots were set up each month for three years. Faeces containing known numbers of *T. colubriformis* eggs were deposited once on each plot, then samples of herbage were collected weekly to determine the availability of infective larvae on pasture. Daily weather data were also recorded.

Faeces deposited in autumn and winter yielded more larvae that persisted for longer on pasture, than faeces deposited in spring and summer. Moisture availability in the first week after eggs were deposited was an important factor for the development and survival of larvae.

The results of the study were used to develop a mathematical model describing the survival and development of eggs and infective larvae and migration of larvae to the herbage. The number of larvae on pasture is predicted from standard meteorological measurements - maximum and minimum air temperatures, rainfall and evaporation. The model was fitted to data from the artificially contaminated plots, then adjusted to predict larvae on pastures contaminated by grazing sheep.

The model has been incorporated into a model of the complete life-cycle of *T. colubriformis* in sheep, including farm management and the evolution of anthelmintic resistance. This model is

used to simulate traditional worm control program and new worm control technologies, and their impact on worm populations and the development of anthelmintic resistance.

COMPARISON OF THREE METHODS OF EXPERIMENTAL TRANSFER OF ADULT *OESOPHAGOSTOMUM DENTATUM* FROM DONOR TO RECIPIENT PIGS

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Objective: Experimental establishment of adult parasitic worms in hosts is usually achieved by artificial infection with the infective stages. However, some experimental designs in helminthological research may require establishment of adult worms which have not undergone the normal development from the infective to adult stage, and where it is a prerequisite that the established worm population has a known composition of history, size and sex. The purpose of this study was to examine 3 methods of transfer of *Oesophagostomum dentatum*, a common large intestinal nematode of pigs, from donor to helminth naïve recipient pigs. Two non-surgical and one surgical procedure were examined.

Materials and methods: Each of the following methods was tested in 5 pigs: A. Transfer of worms by stomach tube to the ventricle of pigs treated with 20 mg omeprazol to increase gastrointestinal peristalsis; B. Transfer by stomach tube to the ventricle of pigs pre-treated with 20 mg omeprazol and cisaprid (0.5 mg/kg), a drug blocking hydrochloric acid secretion; C. Surgical transfer of worms to caecum of pigs.

Results: Procedures A and B resulted in establishment rates corresponding to only 0.5% and 7.6% of the transferred worms. In contrast, surgical transfer allowed 74.2% of the transplanted worms to be established. In all groups the transplanted worms migrated to the normal predilection site, i.e. the middle part of the large intestine. More female than male worms established in all groups.

Discussion: Surgical transfer allowed the highest establishment rate of worms, and the variation in establishment was reasonably low, indicating that this was a reliable and reproducible procedure for establishment of transplanted worms.

STUDIES OF THE POPULATION BIOLOGY OF EXPERIMENTAL *OESOPHAGOSTOMUM DENTATUM* INFECTIONS IN PIGS

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The development of *Oesophagostomum* in pigs is known only to a limited extent. Hence, the present work was undertaken

to describe the effect of different dose levels of infection on the growth of the parasite.

Three groups of 40 nine-week old, helminth naïve pigs were infected once with either 2,000 (group A), and 20,000 (group B), or 200,000 (group C) infective third-stage larvae of *O. dentatum*. The pigs were fed *ad libitum* throughout the study, and their weight gain measured at regular intervals. Subgroups of 5 pigs from each group were slaughtered at frequent intervals after the infection in order to examine the composition of the worm burdens as a function of the dose level. The last slaughtering took place on day 47 post infection (p.i.). Faecal samples were collected at regular intervals after day 13 p.i., and faecal egg counts were determined.

There were no overt clinical signs of gastrointestinal helminthosis in the course of the experiment. Faecal egg counts became positive in groups A and B at around day 19 p.i., whereas the high-dose group (C) did not have positive egg counts until day 27-33 p.i. Correspondingly, significant differences in the size of the worms were observed, with adult worms from the low-dose group (A) being significantly larger than those from group C. Moreover, towards the end of the experiment the worm populations in group C consisted mainly of immature larval stages, while those of group A and B were predominantly adult stages.

These results indicate a stunting of the worms at high dose levels, and may be applied to future studies on the phenomenon of arrested development.

TRANSPLACENTAL TRANSMISSION AS ONE OF THE WAYS OF CIRCULATION OF *SARCOCYSTIS*

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Transplacental transmission of sarcosporidians as a phenomenon has not yet been studied throughly.

For elucidation of the possibility of transplacental transmission of sarcosporidians, 5 experiments on 381 non-pedigree rats and Norway rats (*Rattus norvegicus var. alba*) of the line "Wistar" were carried out, 13 females in the first and 9 in the last week of pregnancy were infected by alimentary with cysts of *Sarcocystis rodentifelis* from the Norway rat. 88 young were born from the females infected in the first week of pregnancy. In 45 (51.1%) of the individuals, cysts of *S. rodentifelis* were determined by sarcoscopy 78-210 days after birth. From the females infected in the last week of pregnancy, 64 young were born. Sarcocysts were found in 58 (90.6%) of the individuals. This parasite was found in the young even of those females which were infected 2.5 days before giving birth to their young. It was shown that the parasite has been transmitted to the foetus directly after the infection of the females. Infected rats were found in the broods of all 22 females. The index of extensity and intensity of infection in different broods varied markedly. Sarcocysts were not found in 145 control ratlings born from 20 females.

The data obtained allow us to confirm that *S. rodentifelis* can be exposed to trans-

placental transmission to foetuses of laboratory rats after sarcocyst infection of females during the first and last weeks of pregnancy.

This route of transmission of some *Sarcocystis* species is, most probably, of epizootological significance.

POTENTIAL LARVAL REDUCTION CAPACITY OF THE NEMATODE-TRAPPING FUNGUS *DUDDINGTONIA FLAGRANS* IN DUNG CULTURES FOLLOWING PASSAGE THROUGH THE GI-TRACT OF HORSES

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In recent years the role of small strongyles (cyathostomes) in parasite-associated equine diarrhoea has been highlighted. At the same time surveys document that anthelmintic resistance in cyathostomes against benzimidazoles is a growing problem. Biological control of free-living stages of horse strongyles by using nematophagous microfungi could be a way to lessen the present intensive use of anthelmintics.

This study was undertaken to examine the potential of the nematophagous microfungus *Duddingtonia flagrans* to survive passage through the gastro-intestinal tract of horses and subsequently destroy free-living stages of cyathostomes in faecal cultures.

Fungal material was cultivated on autoclaved barley grains for 4-6 weeks, washed off and filtrated through fine gauze. Three different oral dose levels (10^5, 10^6 and 10^7 fungal units/kg bodyweight) were tested, two horses being used for each level. Doses were given orally as single doses. Faeces were collected twice daily, and the numbers of strongyle eggs per gram of faeces were determined. The numbers of infective third-stage larvae which developed in faecal cultures were determined after the cultures had been incubated for two weeks at 24°C.

The fungus was found in the cultures the following days after dosing, and the numbers of positive cultures were positively correlated with increasing dose level. After dosing with both 10^6 and 10^7 units/kg, a significant reduction in the number of larvae in the faecal cultures was observed.

This experiment clearly demonstrated that *D. flagrans* chlamydospores can survive passage through the GI-tract of the horse and significantly reduce the number of larvae in faecal cultures. These positive results form the basis for further evaluation of the biological control potential of this fungus against free-living larvae of horse strongyles in a field experiment.

BIOLOGICAL CONTROL OF FREE-LIVING LARVAE OF PARASITIC TRICHOSTRONGYLES IN CALVES ON PASTURE

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Evidence to the potential of nematophagous fungi as biological control agents of nematode parasites of livestock has increased significantly over the last five years. *In vitro* and *in vivo* experiments have shown that particularly isolates of the species *Duddingtonia flagrans* exhibit very promising properties. The results of three field experiments conducted in two grazing seasons are presented here. The aim was to test the ability of an isolate of *D. flagrans* to reduce the number of infective third-stage larvae of parasitic nematodes on herbage and subsequently reduce parasitism in grazing calves.

Each of the three experiments had two equally sized groups (based on bodyweight) of young first-year grazing calves set to graze two plots of equal size. At turnout in spring all animals were given a primary infection dose of 5,000 infective larvae of a Danish *Ostertagia ostertagi* strain to ensure a uniform initial infection level. Fungal material was produced by growing *D. flagrans* on barley grains (Grønvold et al., J Helminthol 1993; 63: 115-126). In 1992 and one of the 1993 experiments, fungal material was given daily to the fungus- treated group of calves for two months from turnout. In the second 1993 experiment, treatment with fungal material lasted three months. Throughout the grazing season, the following data were collected fortnightly: faeces, blood and bodyweight of the animals, plus grass from the plots of the fungus-treated and control animals. Faecal samples were used for faecal egg counts (EPG) and faecal cultures to determine the genera of nematodes present. The potential reducing effect of the fungus was evaluated by comparing the number of larvae resulting from the faecal cultures of fungus-treated animals with those of cultures from control animals. In the 1992 experiment, one-half of the animals in the two groups were slaughtered in August and the rest in October to determine the worm burdens.

In 1992, where the fungus feeding period coincided with an unusually dry summer, the fungus treatment had a significant effect on EPG larvae on herbage, animal worm burdens, and serum pepsinogen levels. In 1993, the climatic situation again was somewhat unusual, with a very wet summer. The control animals in both the two and three months fungus trial developed clinical trichostrongylosis, while the treated animals were unaffected. In the two months fungus-feeding trial, herbage infectivity, number of infective larvae developed in faecal cultures, and pepsinogen levels

were lower in the fungus-treated group. For the three months fungus feeding trial, there was a significant difference in weight gain and herbage infectivity by the end of August. Also, a significantly lower number of larvae developed in faecal cultures of the fungus-treated group than in those of the control group during the feeding regime.

The trial demonstrates that treatment with fungal material in the early grazing season is able to prevent subclinical as well as clinical trichostrongylosis.

EFFECTS OF DIET ON THE DEVELOPMENT AND LOCATION OF EXPERIMENTAL *ASCARIS SUUM* AND *OESOPHAGOSTOMUM DENTATUM* INFECTION IN PIGS

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The influence of nutritional diet on the development and location of *Ascaris suum* and *Oesophagostomum dentatum* in pigs was investigated. Forty-eight initially worm-free pigs, originating from a specific pathogen-free (SPF) farm, were used. The animals were randomly divided into two groups which were infected with a mixed dose of 600 infective *A. suum* eggs and 6,000 infective larvae of *O. dentatum* per pig. One group received traditional ground barley plus protein supplement feed, while the other group was fed commercial, full-constituent, pelleted feed. One-half of the pigs in each group were slaughtered at 3 weeks post infection and the other half at 12 weeks p.i.

At 3 weeks, *A. suum* larvae were rather numerous in pigs fed ground barley plus protein supplement, but were absent in pigs fed a commercial full-constituent pelleted fodder. At 12 weeks this marked difference had diminished, and average adult worm burdens were low and comparable. *O. dentatum* worm burdens were significantly lower in the group receiving commercial feed than in the other group at both 3 and 12 weeks, showing that the commercial diet exerted a strong population regulatory influence. In addition, this diet led to a more distal location of the parasite and a reduced fecundity of the female worms.

OBSERVATIONS ON EARLY LARVAL POPULATIONS OF *ASCARIS SUUM* IN PIGS

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The present study was carried out to investigate the effect of a low-level primary infection by the large roundworm, *Ascaris suum*, on the establishment and migration of a secondary challenge infection with a moderate number of infective *A. suum* eggs.

Forty Landrace x Yorkshire x Duroc outbred ♀ pigs from a helminth-free pig

herd were allocated into 10 groups of 4 pigs each according to weight, and all pigs were housed in contamination-free pens during the whole experiment. Twenty-four pigs (6 groups) were each inoculated with 50 infective *A. suum* eggs by stomach tube at week 0. Two of these groups were slaughtered after 4 weeks and 10 weeks, respectively, in order to evaluate this primary infection. At week 10 post infection, the pigs of the remaining 4 groups, together with the 4 groups of previously non-infected pigs, were challenged with 50 infective eggs per kg body weight. Thereafter, one primarily and one secondarily infected group were slaughtered on day 4, 7, 10, and 14. At slaughter, the numbers of white spots were enumerated, the total numbers of migrating larvae in the livers and the lungs were estimated by macro--baermannization of the chopped tissues, and subsamples of 20% of the small intestinal contents were examined.

At 4 weeks after the primary infection, a few intestinal immatures were found in one pig, while later on only one mature *A. suum* was found in the remaining 20 primarily infected pigs, and all faecal samples were negative throughout. The short-term primary infection did not seem to influence the establishment rate and migration of the challenge infection, as the size and distribution of the challenge larval populations were quite similar in helminth-naïve and in previously infected pigs. On days 4, 7, and 10 post challenge, the numbers of larvae recovered in livers, lungs, and small intestines were 23 (~0.6% recovery), 1,016 (~26.0% recovery), and 1,083 (~31.3% recovery), respectively. On day 14 post challenge, the intestinal worm burdens seemed to have moved distally in the small intestines, and in some pigs almost all larvae seemed to have been expelled.

Conclusion: Experimental infections with low doses of *A. suum* eggs may result in liver and lung migration and few intestinal immatures, but may not result in patent infections or acquired resistance against challenge. The larval populations resulting from moderate doses of *A. suum* eggs may easily be followed during the migration and in the small intestine, where rather high burdens of tiny worms may be found shortly after completed migration, but these large intestinal worm burdens seemed to be expelled rather quickly.

PECULIARITIES OF THE DEVELOPMENT OF *FASCIOLA HEPATICA* IN LAMBS

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While investigating the peculiarities of *F. hepatica* development in the organism of 34 lambs, we carried out two experiments. In the first experiment we studied the sexual maturity of fasciolas under different invasion intensities in cases of single and repeated experimental infection. Our investigations revealed that in the case of intensive invasion, fasciolas develop at slower rates, i.e. are smaller in

size, weigh less, and reach the stage of sexual maturity much later. During the autopsy of experimental lambs with 127-164 day old intensive invasion of fasciolas in their organisms, we found immature forms in all cases. The livers contained from 301 to 435 fasciolas, of which less than 1% were immatures. It should be noted that 4.4% of the fasciolas were only 4-6 mm long.

In the second experiment we investigated the development of fasciolas, their sexual maturity and distribution in the cases of initial invasion and re-invasion. The lambs from group I were infected during eight successive days (4,500 cercariae in all). 100 days after first day of infection, the lambs were dehelminthized with raphoxanide (10 mg/kg body weight). Dehelmintized lambs were infected repeatedly after 6 weeks, 200 cercariae at a time. The lambs from group II were infected only once, 200 cercariae each. It was observed that in the case of re-invasion and very strong degeneration of the liver resulting from the intensive fasciola invasion, fasciolas in the liver were smaller in size and weight after a repeated invasion. The number of immature fasciolas re-invaded in lamb liver was 2.5 times greater, compared with the initial invasion. In the case of initial invasion most fasciolas were located in the left half of the liver, whereas in the case of re-invasion, they were in the right half.

Chemotherapy and Control

EFFICIENCY OF RINTAL AGAINST GASTROINTESTINAL NEMATODES IN PIGS

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A lot of investigators look for new antihelmintic preparations in the benzimidazole group. Numerous effective preparations in this group have been investigated. The purpose of our work was to evaluate the therapeutical efficiency of Rintal treatment of pigs infected with intestinal nematodes.

We carried out our investigations on Rintal efficiency with pigs naturally infected with *Oesophagostomum dentatum* and *Ascaris suum*. We administered the preparation individually and by group method with forage once a day in doses of 40, 50, 70, and 80 mg per kg body weight. Rintal contains the active ingredient of 10% febantel. An experiment was conducted by giving Rintal individually to 20 pigs which were divided into 3 experimental groups and one control group. Rintal by group method with forage was given to 30 pigs divided into 3 groups, 10 pigs each.

During the helminthological dissections it was determined that in the case of applying 40 mg/kg Rintal by individual method, the IE (intensity efficiency) was 76.6%, EE (extensity efficiency) 60.0% for *Oesophagostomum*, and IE 80.0%, EE 60.0% for *Ascaris*; in the case of 50 mg/kg dose

IE was 80.6%, EE 80.0% for *Oesophagostomum*, and IE 89.6%, EE 60.0% for *Ascaris*. The efficacy of 70 mg/kg of Rintal dose was 80.0% for *Oesophagostomum* and 100% for *Ascaris*.

The analysis of the results in the field of trial indicates that when giving this preparation with forage by group method at a dose of 70 mg per kg of body weight, the following efficiency was achieved: IE 91.5%, EE 80.0% against *Oesophagostomum*, and IE 95.0%, EE 80.0% against *Ascaris*. Giving Rintal at a dose of 80 mg/kg, the efficiency was 100% against *Oesophagostomum* and IE 98.6%, EE 90.0% against *Ascaris*.

After dehelminthization, *Oesophagostomum* is removed from the pigs' organisms with faeces in 12-36 hours, and *Ascaris* in 12-48 hours.

VACCINOPROPHYLAXIS AGAINST COCCIDIOSIS IN CHICKENS

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Every floor-reared commercial flock of chickens in Estonia will be exposed to coccidia during their growing period. Coccidiosis is frequently found in combination with other diseases. The poultry farmer can avoid coccidiosis by the use of a coccidiostat or planned immunization.

Coccidiosis vaccine of the Estonian Agrobiocentre is a measured suspension of the sporulated oocysts, designed to introduce into chickens at a controlled number of commercially important species of coccidia to stimulate an immune response.

The data demonstrate that vaccinated birds had a significant resistance to coccidiosis.

ON THE DIAGNOSIS AND CONTROL OF NEMATODES IN HORSES IN ESTONIA

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Horses in Estonia frequently have nematode parasites. Data on the helminthofauna in horses have become obsolete. The purpose of this study was to investigate the occurrence of helminths in horses at two farms and to test some anthelmintics.

Faecal samples from 82 horses were investigated using a flotation method. Subsequently, one group was treated with Strongid-P at a dose rate of 12.5 mg/kg p.o. by syringe; one with Nematox vet. 15 mg/kg p.o. with concentrate; one with Ivomec 0.2 mg/kg s.c. by injection; while the fourth group was an untreated control. The results of the treatments were estimated 2 weeks later.

Seventy-eight % of the horses examined were invaded. 4 species of helminths: *Parascaris equorum* (prevalence (P) 8%), *Strongyloides westeri* (P 8%), *Oxyuris equi* (P 2%), *Trichostrongylus axei* and other representatives of strongylides (P 100%) were diagnosed. The horses of the

corporation "Lennu" were much more invaded with strongylids than the horses from the Ihaste Riding Centre, due to insufficient veterinary service and stabling conditions. The most effective drugs were Strongid-P and Ivomec.

Parasites, Miscellaneous

SCHISTOSOMA BOVIS IN GOATS: CIRCULATING ANTIGEN AND ANTIBODY RESPONSES TO EGG AND ADULT WORM ANTIGENS

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The circulating antigen and antibody responses in *Schistosoma bovis* infected West African dwarf goats were evaluated during patency and following treatment with praziquantel. 30 goats were infected with 2,000 cercariae each, and 15 of them received praziquantel treatment (60 mg/kg) 13 weeks post infection. One day, one week and four weeks post treatment, representative goats from each group were euthanized and perfused for worm recovery. For comparison, parasite-free control animals were monitored, some of which received treatment with praziquantel. Every two weeks during the study, blood and faecal samples were collected.

Two gut-associated antigens, circulating cathodic (CCA) and circulating anodic antigen (CAA), were measured using monoclonal antibody- based ELISA technique. For specific antibody detection, crude *S. bovis* adult worm and egg homogenates were prepared. Total Ig, IgG, and IgM were measured using an ELISA technique. The level of CCA in the infected groups rose significantly ($p<0.05$) from the time of onset of ovideposition (six weeks post infection). In the group receiving treatment, the CCA titers dropped to control levels one week post treatment, whereas the infected non-treated group remained at a significantly elevated level ($p<0.001$) throughout the study period. On the individual level, strong correlations were found between CCA and faecal egg counts from eight weeks post infection ($p<0.01$) and between CCA and worm counts in the infected non-treated group ($p<0.01$). CAA failed to show similar correlations. The total specific antibody response (Ig) and the specific IgG and IgM responses for both worm and egg antigen followed nearly identical patterns during the study period. Specific Ig, IgG and IgM against worm antigen were significantly elevated ($p<0.05$) four weeks post infection, two weeks before the onset of egg excretion, and the titers gradually increased during the study. An additionally significant ($p<0.01$) increase in antibody titers (IgG and IgM) was observed one week after chemotherapy in the treated group, but two weeks later no

differences were observed between the two infected groups, both being significantly elevated as compared to the control groups ($p<0.05$). The antibody responses to egg antigen in the infected non-treated group rose significantly ($p<0.001$) from eight weeks post infection and throughout the study period, whereas in the treated group the titers dropped significantly ($p<0.05$) three weeks after chemotherapy. Correlations of antibody titers to worm counts and faecal egg counts were very poor, except for a reasonable correlation found three weeks after treatment between antibody titers and faecal egg counts ($p<0.05$).

HYPERSENSITIVITY REACTIONS DURING PRIMARY AND RE-INFECTIONS WITH *SCHISTOSOMA MANSONI* IN MICE

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The objective of the present study was to examine hypersensitivity responses during primary and re-infection with *Schistosoma mansoni* in mice. Histamine release from peritoneal mast cells from mice infected with *S. mansoni* was measured at different times post infection using a modified Histamine Release Micro Test. Mast cells were stimulated with soluble *S. mansoni* worm antigen preparation (SWAP), anti-IgE or anti-IgG1. Total IgE and IgG1, and *S. mansoni* specific IgE and IgG1 responses were measured by ELISA. Significant differences in mast cell sensitivity was observed between the *S. mansoni*-infected mice and the non-infected mice. Mast cell sensitivity to anti-IgG1 were observed in the prepatent infection (ns) followed by an increase in mast cell sensitivity to anti-IgE in the patent infection. A re-infection increased mast cell sensitivity to anti-IgG1, and anti-IgE and SWAP increased to a maximum level throughout the study period. *S. mansoni* specific IgG1 and IgE responses increased 2-4-fold from week 3 p.i. At patency, the total IgE response increased 13-fold and the total IgG1 response increased two-fold in the primary infection. The total IgE response in the re-infected group was 20-fold of the control level. The specific IgG1 response increased 4-6-fold in the re-infected group. The results of the present study could indicate that IgG1 plays a role in hypersensitivity reactions in *S. mansoni* infections in mice. The modified Histamine Release Micro method is a reproducible and sensitive method for measuring biologically active IgE in relevant animal or human models.

LONG-TERM INVESTIGATIONS OF *TYLODELPHYS CLAVATA* (TREMATODA) METACERCARIAE IN THE PERCH *(PERCA FLUVIATILIS)* POPULATION

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Investigations were carried out in Drūkšiai Lake (the basin of the river Daugava), which in 1984 became the cooling reservoir of the Ignalina Nuclear Power Plant. From 1984 to 1986, during the operation of one power block and a thermal loading (ThL) of 60 W/m^2, the defined area of water of 12 sq.km was subjected to heat, while in 1987, during the performance of two power-blocks and a ThL of 120 W/m^2, the area amounted to 42 sq.km.

Within ambient and thermally altered locations of Drūkšiai Lake, the prevalence and mean density of *T. clavata* metacercariae were monitored in perches for 50 months from March 1985 to April 1989. At the ambient site, the prevalence of *T. clavata* ranged from 96% in November 1985 to 4% in October-November 1988. During the study period, peak seasonal infection percentages were erratic. In the heated area, seasonal changes in prevalence were also erratic over the duration of the study, although it tended to be highest in spring and to decline in late summer and early autumn.

Mean densites at the ambient area fluctuated over time. There was, however, no consistent pattern according to season. Moreover, after reaching a maximum of 7.1 metacercariae/host in May 1987, there was a sharp decline in density, and then the numbers were persistently low. In the heated area, the mean density fluctuated over time. Seasonal patterns, although apparent, were not sharp and distinct. In general, the highest seasonal densities were observed in spring, followed by a decline in late summer and early autumn.

During 26 out of the 50 months, the prevalence was significantly higher at one of the two thermal sites. For 18 of these 26 months, the prevalence was significantly higher in the heated area, and for 8 months it was highest at the ambient site. During 29 of the 50 months, mean densities were significantly higher at one of the two sites: for 20 months in the heated area, and for 9 months at the ambient site. During the last 24 months of investigations, when the power of the Ignalina NPP increased, the metacercariae prevalence and mean density were significantly higher in the heated area: prevalence for 12 months and mean density for 11 months. At the ambient site the increase was insignificant.

During the second and the third years of operation of the Ignalina NPP, a marked increase in the variability of *T. clavata* metacercariae invasion parameters was recorded at both thermal sites. When the second power-block was launched in 1987, the prevalence and mean densiy in the thermally altered site became significantly higher. However, in the autumn of 1988, the autumnal invasion number of *T. clavata* metacercariae, which was recorded every year, did not increase. The reason

can be found with the changes in the populations of the first intermediate hosts-molluscs - because of the increased thermal pollution.

SOME QUESTIONS CONCERNING THE OCCURRENCE OF TRICHODINE PROTOZOANS ON FISH IN POND CULTURE

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Four fish species were examined during three years: *Cyprinus carpio* (common carp) and three introduced species, *Ctenopharyngodon idella* (grass carp), *Hypophthalmichthys molitrix* (silver carp) and *Aristichthys nobilis* (big-head carp). Seven species of trichodines were recorded, but only three of them (*Trichodina mutabilis*, *Trichodinella subtilis*, *Chilodonella hexasticha*) occurred commonly on all fish under study. All trichodines represented autochthonous species. In spite of this, they colonized more often the introduced fish than the common carp. Four species (*Trichodina pediculus*, *Trichodinella subtilis*, *Chilodonella hexasticha*, *C. piscicola*) occurred mainly on these fish, whereas 2 species (*Trichodina domerguei*, *T. nigra*) were noted very rarely on all fish species. Only *T. mutabilis* occurred more often on common carp than on introduced fish.

All trichodines showed a distinct preference for young fish. They appeared on fish very early (they were present on the first fishes sampled, about 1 month old), and all were maintained through the first year of fish life. Three species (*Trichodina mutabilis*, *Trichodinella subtilis*, *Chilodonella hexasticha*) occurred also on fish older than one year, but their prevalence decreased with fish age.

There were also some relationships between the level of trichodine prevalence and seasons of the year. Spring appeared to be the most favourable season for their occurrence, summer being the worst period. Out of 7 species, 5 (*Trichodina domerguei*, *T. nigra*, *T. pediculus*, *Chilodonella hexasticha*, *C. piscicola*) were absent in summer samples, and only *Trichodina domerguei* was more abundant in autumn than in spring. *Trichodinella subtilis* and *Trichodina mutabilis* were the only species found also in summer, but the peak of their occurrence was also in spring.

BOVINE EIMERIOSIS IN ESTONIA

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Bovine eimeriosis is an enzootic disease of calves in Estonia. Five *Eimeria* species have been found in a research project of bovine eimeriosis etiology and epizootology. These are *E. zuernii*, *E. bovis*, *E. auburnensis*, *E. ellipsoidalis* and *E. subspherica*. The first two of the above-mentioned species were heavily pathogenic in the large intestine, the next two were midly pathogenic in the small intestine, and the last one was of insigni-

ficant pathogenicity.

The average *Eimeria* prevalence in the cattle was 78%, with calves of 3-4 months and heifers of 12-18 months being invaded at an equal extent of 87% and cows of 27%. The *Eimeria* species most frequently found were *E. bovis* (44%) and *E. ellipsoidalis* (34%). Oocysts of *E. zuernii* made up 12%, *E. auburnensis* 9%, and *E. subspherica* only 1% of the total number of oocysts found.

INVESTIGATION OF IMMUNOLOGIC AND BIOCHEMICAL MARKERS RELATIVE TO RESISTANCE AND SUSCEPTIBILITY TO PARASITIC DISEASES

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The relationship between a parasite and a host is stipulated by many factors. Currently, a number of immunity investigators have determined that this relationship is subject to genetic control. To solve some aspects of the parasite-to-host relationship, we have infected goslings and ducklings with adolescaria of two trematode species of the *Notocotylus* genus, *N. attenuatus* and *N. ephemera*, obtained from spontaneously infected intermediate hosts, *Lymnaea stagnalis (Lymnaeidae)* and *Planorbarius corneus (Bulinidae)*. Five goslings and five ducklings were individually infected with adolescaria produced by a single mollusc. The marita gained from the birds infected in this way constituted one genetic clone.

The antigenic analysis was carried out by applying rabbit antisera in immunoelectrophoresis and double diffusion agar gel methods. The rabbit antisera were obtained after immunization of a rabbit by using serum proteins of final hosts (different species of the goose order).

The obtained test data indicated antigenic similarities and differences between two investigated species of notocotylids and their final hosts, as well as different virulence of genetic adolescaria clones within the investigated species. Virulence was determined on the basis of adaptation ability of notocotylids in a final host. Genetic homogeneity of individual clones was proved on the basis of enzymatic systems investigated by an electrophoresis method in polyacrylamid gel. It has been reliably determined that the difference in morphological features of the investigated notocotylids depends on the host species.

INFLUENCE OF INOCULUM DENSITY ON GENOTYPE INTERACTIONS OF THE PHYTONEMATODE *HETERODERA TRIFOLII* GOFFART, 1932 AND ITS HOST *TRIFOLIUM REPENS* L.

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Different authors have described different effects of phytonematode density on phytonematode/plant interactions. Phytonematode populations and sorts or populations of plants were used in these

experiments.

The present paper deals with the differences in interactions between clones of parasite and host using six initial inoculum densities (from 20 to 455 juveniles and eggs). Three clones of clover (sort Jegava-4) and two clones of *H. trifolii* were used, and the survival of phytonematodes was evaluated. The original equipment and methods of experiments and cultivation of phytonematodes were used. Distribution of the data on survival of *H. trifolii* on *T. repens* was not normal, thus the method of Kolmogorov- Smirnov was applied for the evaluation of statistically significant differences in survival.

The analysis of the results points to the existence of two variants of survival of phytonematodes: 1) no differences between groups, 2) the second inoculation group (51-80 juveniles and eggs) differs. These differences occur in both nematode clones, and depend on the pairs of phytonematode and plant clones used.

The used clones of plants showed genetical differences. Also the clones of phytonematodes are genetically different. On the background of these data, it can be concluded that some sorts of plants cannot be used as purely genetic objects in such experiments.

MOLECULAR CHARACTERIZATION, LOCALIZATION, AND VACCINE POTENTIAL OF A π-CLASS GLUTATHIONE-TRANSFERASE FROM *ONCHOCERCA VOLVULUS*

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A full-length cDNA clone (Ov24) coding for a 24 kD pie-class glutathione S-transferase of *O. volvulus* has been isolated.

Immunohistochemistry performed with antisera raised against Ov24 suggest differential regulation of GST gene expression in the gametocytes and in the different stages of the life cycle of *O. volvulus*.

Anti-Ov24 serum also recognizes a similar sized antigen in the infective L3 larvae of *Litomosoides sigmodontis* which is a rodent filarial parasite currently being used as a model to study the immunology of filarial infections.

Tetanus toxoid has been used as an adjuvant for chemically coupled antigens. We have expressed two Ov24 constructs from pTECH, a modified form of the *nir*B-TetC plasmid, pTET*nir* 15. pTECH-Ov24C was designed to express a fusion protein of TetC and the terminal portion of Ov24 (12kD). pTECH-Ov24F was designed to express a fusion protein of TetC and the full-length GST (24kD). We have obtained stable expression of these

constructs *in vivo*, and fusion proteins were recognized by Western Blot by both rabbit polyclonal sera raised against TetC and against GST. There was a comparable level of expression of fusion proteins from *E. coli* TG2 and *Salmonella typhimurium* SL32261. Groups of 12 mice were given a single i.v. dose of approximately 1 x 106 live recombinant *S. typhimurium* SL32261 expressing TetC, TetC-Ov24C and TetC-Ov24F. There was stable *in vivo* persistance of constructs in the *Salmonella*, persisting up to 9 days post inoculation. Circulating antibodies (total IgG) to both TetC and GST were detected by ELISA.

Vaccination with TetC-Ov24F resulted in a 30% reduction in the worm burden of mice challenged with L3 of *L. sigmodontis.*

RUMINANT HELMINTH RESEARCH PROJECT - RESEARCH COLLABORATION IN VETERINARY HELMINTHOLOGY BETWEEN EAST AFRICAN COUNTRIES AND DENMARK

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Strengthening of research activities and recruitment of researchers are of utmost importance for sustainable veterinary services and relevant veterinary education in developing countries. Four years ago, the Danish International Development Agency (DANIDA) launched a programme called ENRECA (ENhancement of REsearch CApacity); and our Ruminant Helminth Research Project was one of the first projects to be established within the programme.

The major aim of the project is long-term research assistance in the field of tropical helminth diseases of cattle, sheep and goats of primarily small-scale farmers. Apart from a few specific infections, these infections are often concurrent, subclinical, and chronic, and will inevitably result in sub-optimal production levels, unthriftiness, and increased susceptibility to other diseases. This area has been grossly neglected due to the main focus internationally on 'big killer diseases' like Rinderpest and the haemoparasitic diseases. So far, researchers from the veterinary faculties of Kenya, Tanzania, and Denmark have participated, but the project is extended to cover also Zambia and Zimbabwe from 1994 onwards. The project comprises postgraduate training involving local supervisors and Danish co-supervisors, provision of sufficient funding for research and for participation in international conferences, establishment of a local network for information in this field and, to a lesser degree, transfer of new technology. Linking up with research in animal science and research of local or international agricultural institutions, e.g. FAO, is also important.

Hallmarks of our project are: local enrolment of M.Sc. and Ph.D. students, extensive communication between participating countries including a bi-annual

Newsletter, a 4 days annual workshop hosted by participating African countries, and ex-change of researchers, technical staff, and knowledge between laboratories involved.

It is the aim of our current research project to demonstrate the pathogenicity and influence of subclinical infections on productivity, to provide prevalence and basic epidemiological data on these infections to be able to define sustainable ways of control in the future, and not least, to look into the problems of poor efficacy of drugs due to either resistant parasites or poor quality of pharmaceutical products. At present, one Ph.D. student and one M.Sc. have finished, and 3 Ph.D. and 3 M.Sc. studies are ongoing. Research is carried out on nematode infections (e.g. *Haemonchus spp.)* in cattle, sheep and goats, *Schistosoma bovis* in cattle, *Taenia saginata* in cattle, *Fasciola gigantica* in different breeds of sheep, nematode- predacious fungi, and anthelmintic resistance. Research on paramphistomes and interactions with nutrition is in the planning phase.

Research collaboration creates international scientific understanding and communication, and helps in breaking down barriers between North and South and not least between African countries.

DETECTION OF ANTIBODIES AGAINST *ENCEPHALITOZOON CUNICULI* IN THE BLUE FOX *(ALOPEX LAGOPUS)* BY ELISA

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Heavy losses among pups of domesticated blue fox *(Alopex lagopus)* have periodically been caused by *Encephalitozoon cuniculi* in Norway since the first cases were diagnosed in 1968 (Nordstoga, Nord Vet-Med 1972; 24: 21-24). During the period 1975-1982, research was performed to establish the routes of transmission, pathology and pathogenesis, and measures to control spreading of the parasite (Mohn, Thesis, National Veterinary Institute, Oslo, Norway, 1983). Diagnosis in live foxes has until now been based on detection of antibodies using the CIA (Carbon Immuno Assay) (Waller, Lab Anim 1977; 11:93-97; Mohn, Acta Vet Scand 1982; 23: 99-106), which is, however, time-consuming and somewhat difficult to interpret. An ELISA was therefore developed to increase the diagnostic capacity of our laboratory, and hopefully also to avoid some of the occasional problems seen with the CIA. So far, the ELISA has produced results which seem to meet our requirements, and a comparison between these and those obtained with the CIA will be presented. Further work to improve the method will continue at our laboratory, together with epidemiological studies.

NEMATODE INFECTIONS IN ORGANIC DAIRY CATTLE HERDS IN DENMARK

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Organic farming in Denmark is characterized by the following rules laid down by the Ministry of Agriculture, 1991: 1) Summmer grazing at least 150 days for all cattle. 2) Preventive use of medicine is prohibited. 3) Withdrawal time following medical treatment is increased 3 times the statutory withdrawal time. 4) Herd size must not influence health negatively. 5) If required, anthelmintics must only be given orally.

Control of nematode infections in traditionally reared dairy heifers in Denmark is based largely on preventive or curative use of anthelmintics. In the organic farming system it is, on the contrary, necessary to develop preventive strategies not involving anthelmintics, including certain grazing strategies and supplementary feeding. However, due to limitations in use of drugs, heifers in organic herds were nevertheless expected to be more likely affected by endoparasites during the grazing season.

A study was performed to investigate levels of nematode infections and grazing management strategies applied in 11 Danish organic dairy herds. 1st- season grazing calves were examined at housing in 1991, and 1st and 2nd- season grazing animals at turnout (May) and housing (October-December) in 1992. Five farms were also sampled in August. All animals were blood sampled, weighed and body condition scored. Clinical observations were made monthly. Serum was analyzed for antibodies against *Ostertagia ostertagi*, *Cooperia oncophora*, and *Dictyocaulus viviparus* using an indirect ELISA test, and selected samples for pepsinogen. Faeces were sampled from 10-15 animals per age group per farm and analyzed for trichostrongyle eggs (EPG).

Strategies used were: moving to clean pasture at mid-summer or later (1 and 5 herds, respectively), late turnout (after June 1st) (4 herds), supplementary feeding of 1st-seasongrazers during dry periods (9 herds), no introduction of new stock into established flocks (6 herds), and strict separation of 1st and 2nd-season grazers (3 herds).

In 3 herds, faecal soiling of hind legs indicating diarrhoea was observed (7-32% of both 1st and 2nd-season grazers). In 5 herds, coughing was observed (5-18%), and *D. viviparus* was detected in 2 of these. One herd was found infected but without clinical signs. Two herds infected with *D. viviparus* were treated with anthelmintic in August, and 1st-season grazers were housed. No other treatments were given.

Positive faecal egg counts were observed in many 2nd-season grazers at turnout (41% of samples, max. 240 EPG) and during the season mean counts were low or nil. A similar pattern was observed in serum pepsinogen. 1st-season grazers had egg counts of 20-380 EPG in

August and 0-340 EPG at housing. Serum pepsinogen was generally increasing during the season, and at housing up to 25% of the animals per herd had values in excess of 1.0 i.u./l. The levels of antibodies corresponded relatively well with the pattern described, but titres against different nematode species were strongly correlated, probably indicating some cross-reaction.

Conclusion: Grazing management routines in organic herds were not carried out as meticulously as expected. However, apart from cases of lungworm disease, little or no clinical illness was recorded, and judged by parasitological parameters, only low or moderate levels of gastrointestinal nematode infections were encountered in 1992.

OBSERVATIONS ON THE EPIDEMIOLOGY OF LYME BORRELIOSIS IN DENMARK

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It is estimated that at least 2,000 human cases of the tick-borne zoonosis Lyme borreliosis occur each year in Denmark. The disease is caused by the spirochaete *Borrelia burgdorferi*, transmitted by the hard tick *Ixodes ricinus*.

In 1990 a research project was conducted, aimed at determining the infection level in ticks and the prevalence of antibodies in the probable spirochaete reservoir animals from different localities in Denmark.

Ticks were collected from vegetation and their mid-guts were examined for the presence of spirochaetes by indirect immunofluorescence. A total of 1,087 mice (6 species) were caught in traps, and blood samples from 156 deer (3 species) were taken at hunts. The prevalence of antibodies against *Borrelia burgdorferi* was determined by indirect immunofluorescence.

The infection rate of *Ixodes ricinus* nymphs was 18%. 25% of the mice and 44% of the deer had elevated antibody titers. Differences in antibody prevalence between species and localities were shown for both mice and deer.

Conclusion: *Borrelia burgdorferi* is present and widely distributed in the wildlife fauna in Denmark.

SUBMITTED PAPERS - POSTER PRESENTATIONS

FORMATION OF HELMINTHOCENOSES IN SMALL MAMMALS IN THE CHERNOBYL ACCIDENT ZONE

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The effect of radioactive contamination on parasites is not yet enough researched.

The anthropogenic influence on ecosystems of strongly radioactively contaminated areas had changed, and the succession from anthropogenically transformed ecosystems to natural types of ecosystems was observed. Under these conditions, the biodiversity of zoocenosis undergoes considerable changes. This is especially significant for parasites.

Investigation of this problem was carried out at the Polessky Radiation Ecological Reserve. The same parasitocenosis in an unpolluted area was researched at the Pripyat Landscape and Hydrology Reserve. The relevant biocenosis typical of the southern part of Belorussia was examined.

The unstable state of helminthocenosis of the micromammalian community was observed in the radionuclide-contaminated area. In 1991, micromammalian infestation was 2 times higher than in the previous year. Then some stabilization of the process took place, with the infestation levels becoming lower. The same trend was observed in biocenoses infestation of rodents inhabiting forests. The alder forests constitute the base for the formation of the helminthocenosis of the micromammalian community in forest ecosystems. The main parasite host here is the bank vole (*Clethrionomys glareolus* Schreb). Helminthic infestation of rodents in open areas was lower, but during the research period it increased more than 10 times. One of the reasons is the increasing density of the root vole (*Microtus oeconomus* Pall). The study of helminth species variety in micromammals of both reserves showed that the helminthic complex is being transformed, i.e. a decrease of general species is recorded. The factor of fauna similarity lowered from 28.6% within the research period. The general ecological situation in the zone favoured the greater diversity of species as compared to the situation in the Pripyat Reserve. This is indicated by Shinnon-Wiver's index, amounting to 2.43 and 1.84, respectively.

ECOLOGY OF PARASITES OF SOME WILD ANIMALS IN LITHUANIA

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The results of the long-standing parasitological investigation into Li-

thuanian wild animals are briefly reviewed. During a 20-year period, thousands of aurochs (*Bison bonasus*), elks (*Alces alces*), red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), wild boars (*Sus scrofa*) and hares (*Lepus europaeus*) from different regions of Lithuania were examined.

Endoparasites were collected from the inner organs of dead animals. Samples of excrements were collected around the mangers at the feeding-places, where the largest gatherings of the wild animals were noticed, and examined. 56 species of helminths and 28 species of coccidia were diagnosed. The greatest variety was found in the helminths of roe-deer (28 species), and the highest extensity of invasion was found among the hares (up to 83.1%). 7 species of coccidia were isolated from the roe-deer and also from the hares, and 9 from the wild boars. These coccidia are parasites of domestic pigs as well. The highest level of infection with coccidia was observed also among hares (up to 83.0%). A high percentage of the wild boars was affected by the *Sarcocystis* sp. (49.4%), and recently the trichinellosis had spread widely.

The level of the infection is determined by the density of its hosts (intermediate and definitive) in a particular area, the changes in the structure of the host's population, the character of the biotope, the culture of the hunting economy, and the economic activity of the population. The influence of the climatic conditions was of no importance.

Seven species of the wild animals' parasites that are dangerous to the human population have been discerned: *Toxoplasma gondii, Sarcocystis suihominis, Fasciola hepatica, Dicrocoelum lanceatum, Trichostrongylus colubriformis, Trichinella spiralis,* and *Echinoccocus granulosus.*

OUTBREAK OF TRICHINELLOSIS IN KAUNAS DURING THE YEARS 1993-1994

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The major outbreak of trichinellosis in Kaunas from December 1993 to January 1994 was caused by *Trichinella*-contaminated pork. 246 patients were suffering from trichinellosis (189 adults and 57 children), and 579 exposed individuals underwent preventive treatment (51% males and 49% females), the majority of them (58%) being under the age of 40. Patients were hospitalized as follows:

- 61% during the first 5 days,
- 19% - 6-10 days,
- 18% - 11-20 days,
- 3% over 21 days.

The main complaints were of dyspeptic character. Oedema of the face was present in 86%, conjunctivitis in 34%. At the peak of the disease, 16% of the patients had normal body temperature: 37-38°C in 67%, 38.1-39°C in 12%, and above 39.1°C in 5% of the patients.

The number of eosinophils was 5 in 13%, 6-10 in 15%, 10-20 in 36%, 21-40 in 34%, 41-60 in 4% of the patients. Serological tests were positive in 30% of the pa-

tients under study. The patients were given Vermox and symptomatic drugs. Of the patients observed, 62% had a mild form of the disease. 32% moderate to severe, and 27% had a severe form. In 4% of the patients, the course was not apparent.

STRUCTURAL DISTURBANCES OF HELMINTH TISSUES UNDER THE EFFECT OF DIFFERENT ANTHELMINTICS

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All the investigations of the histological and electron microscopic studies that we carried out were associated with studies on the specificity of structural disturbances in the tissues of *Opisthorchis* after the application of anthelmintics: Albendazol, Mebendazol, Triclabendazol, and Bitricid 'Bayer AG' (Proziquantel). The anthelmintics have an effect on the tissues of helminths. It was determined that the greatest structural changes in all *Opisthorchis* were induced by the Biltricid from 'Bayer AG'. Under its effect in the course of 6 hours, a loosening of the external part of tegument takes place. The interior part of the tegument becomes vacualized. The organs of the reproductive system are under destruction. Pathological changes (however, to a lesser extent) take place in tissues of *Opisthorchis* following 12 hours of the introduction of Triclabendazol. Destruction of *Opisthorchis* tissues under the effect of Albendazol and Mebendazol was less expressed and was less noticeable after a longer period.

Thus, it should be assumed that the anthelmintic Biltricid from 'Bayer AG' is considered at present to be the most effective preparation in opisthorchosis. Opisthorchosis is considered to be the most dangerous and widespread helminthiasis in Siberia. The main control method against opisthorchosis is chemotherapy. On the basis of the experimental investigations it was determined that the most effective anthelmintic preparations were of great importance in the histological and electron microscopic studies of tissues and helminth systems.

OLIGOCHAETES IN THE LIFE CYCLES OF HELMINTHS OF THE WOODCOCK *(SCOLOPAX RUSTICOLA L.)*

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The woodcock (*S. rusticola*) is one in a few *Charadriiformes* birds inhabiting leaf-bearing and mixed forests. Earthworms (Lumbricidae) prevail in the feed of the birds. According to literary data, the helminth fauna of the woodcock include 58 species (Trematoda 16, Cestoda 32, Nematoda 9, Acanthocephala 1). Some of these are probably doubtful or sporadic in the woodcock. There is, with a certain degree of assumption, information about

25 species of helminths which use the oligochaetes as intermediary hosts. The cestode fauna was represented by species of the families *Dilepididae* (17) and *Hymenolepididae* (15). 7 species of *Dilepididae* develop on oligochaetes.

During 1983-1987 I was specifically focusing on cestodes of the genus *Aploparaksis* Clerc, 1903 of woodcocks from different parts of Russia and Lithuania and their life history. The results of studying the life cycles of 7 from 8 species of this genus are listed below.

Experimental intermediate hosts of cestodes of the genus *Aploparaksis* from woodcock: *A. filum (Goeze, 1782) Clerc, 1903* (Intermediate host (IM): *Eisenia tetraedra, Octolasium lacteum*); *A. australis Johnston, 1911* (IM: *E. foetida, E. tetraedra*); *A. pseudofilum (Clerc, 1902), syn. A. sanjuanensis sensu Demschin, 1985* (IM: *Bryodrilus arctica, Henlia diverticula, (Enchaetraeidae:) O. lacteum, Nicodrilus roseus, E. foetida, E. nordenskioldi, E. tetraedra, Dendrobaena octaedra*); *A. scolopacis Yamaguti, 1935* (IM: *E. nordenskioldi, D. octaedra*); *A. sinensis Tseng Shen, 1933, syn. A. sachaliensis sensu Demschin, 1985* (IM: *O. lacteum, N. roseus, E. foetida, E. tetraedra, D. octaedra*); *Aploparaksis sp. nov. 1* (IM: *B. arctica, E. foetida, E. nordenskioldi, D. octaedra*); *Aploparaksis sp. nov. 2* (IM: *D. octaedra*).

Distinct dependence on the dietary habits of the woodcock was clearly demonstrable. Among the oligochaeta, prevalent species are represented by amphibiotic forms (*B. arctica, H. diverticulata, E. tetraedra*), litter dwellers (*D. octaedra*) and by top soil surface (*E. foetida, E. nordenskioldi*). Among the soil-dwelling forms are the following: *O. lacteum, N. roseus.*

FORMATION OF HELMINTHOCENOSES OF WILD BIRDS UNDER CONDITIONS OF ANTHROPOGENIC IMPACT ON THE ECOSYSTEMS

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The broad-scale transformation of ecosystems resulting from drainage amelioration and radioactive contamination of the Belorussian territory induces violation of stability in the existing parasite-host systems and in some cases causes formation of new systems.

Most vividly this can be seen in birds' helminths, since at different stages of ontogenesis they change their vital forms which adapt to the varying ecological background. The running processes reveal either adaptability or vulnerability of helminth species to sudden ecological changes.

The problem was studied at research stations in the south of Belarus by investigating the biocenosis series passing from natural-life sites (Pripyat Landscape and Hydrological Reserve) to those affected by drainage and radioactivity (Polessky Radiation Ecological Reserve). 978 specimens of wild birds belonging to 10 characteristic species were processed using full dissection.

It has been found that helminthocenoses of birds in forest ecosystems are most resistant to all types of anthropogenic influence. The helminthic community includes stenoxenic and euryxenic species. Drainage amelioration of innated grasslands causes the vanishing of stenoxenic species and a two-fold decrease in the total number of species. Dominating hosts acquire new helminth complexes. Radioactivity causes practically no change in helminthocenoses of birds. The wide specific variety is retained, and helminthic community preserves both stenoxenic and euryxenic species. As a result of the removal of anthropogenic pressure on drained grasslands after the Chernobyl accident, the species variety is being restored, and general helminth infestation of characteristic hosts is noted to be growing.

MITES (ACARINA) OF BUMBLE-BEES *(BOMBUS LATR.)*

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A very important field of activity of modern apiculture is the pollination service of entomophilous plant cultures. Bumble-bees, along with honey bees and solitary bees, are very useful pollinators, especially in greenhouses (tomato cultures). Therefore, colonies of bumble-bees are being mass produced. However, such artificial insect monocultures are often infested with pests.

Results of acarological analyses of bumble-bees and their nests in nature as well as in culture, confirm the hypothesis that these insects are usually accompanied by mites. The most frequent and numerous of these are the following:

Parasitellus fucorum (phoresy, transport parasitism, commensalism, coprophagy, predatism). Other mesostigmatic mites (*Laelaptidae, Parasitidae*, e.g. *Parasitus bombophilus*) are also quite commom in these habitats.

Scutacarus acarorum (phoresy, commensalism; fungivorous pseudoparasite).

Kuzinia laevis, Cerophagus granulatus (phoresy, transport parasitism, commensalism, nest parasitism, noxiousness: pests of bee bread).

Artificial monocultures of bumble-bees feeding on bee-collected pollen are also infested with beehive acarids (*Acaridae, Carpoglyphidae, Glycyphagidae*) which are harmful to their brood.

A typical endoparasite attacking the tracheal system of bumble-bees is *Locustacarus buchneri*, not yet registered in Poland.

The estimated total number of mite species associated with Polish bumble-bees in nature and in colonies under artificial conditions covers more than 20 species.

ACAROSES OF HONEY BEES

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Many years of Polish as well as foreign investigations and observations of honey bee colonies, individual bees, and hive detritus, show that the species composition of the acarofauna associated with bees is very rich and varied (more than 50 species). Some of these species are parasites of insects. Bees are affected by external as well as internal mites.

In Poland and in other European countries, the following species are widely known:

Varroa jacobsoni - a world-wide common and harmful ectoparasite infesting adult bees and brood.

Acarapis woodi - a cosmopolitan endoparasitic tracheal mite causing so-called 'acarine disease' ('Isle of Wight disease') in adult bees; the other two allied species (*A. dorsalis, A. externus*) are rarer and not so harmful to honey bees.

Pyemotes ventricosus - a common species, mostly ectoparasitic on various insects - pests of bee nests, sometimes occurring on bee brood.

There are other ectoparasites of bees, such as Asiatic mites (e.g. *Euvarroa sinhai, Euvarroa wongsirii, Varroa underwoodi, Tropilaelaps clareae, Tropilaelaps koenigerum*) and some controversial pseudoparasitic species (e.g. *Mellitiphis alvearius, Neocypholaelaps indica, Pseudoacarapis indoapis*).

Although they are rather exotic and have not so far been found on honey bees in Baltic-Scandinavian region, it is worthwile being familiar with them, because they are likely to be a potential European and world-wide problem in the future, maybe beyond 2000.

HOUSE DUST MITES IN THE HOMES OF ALLERGIC PATIENTS IN LITHUANIA

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The house dust acarofauna in Lithuania consists of 23 species with a great prevalence of *Dermatophagoides pteronyssinus*.

House dust samples from the homes of 110 asthmatic patients and 50 healthy persons were studied. The house dust mites were detected 4 times more often in asthmatic homes than in a control group ($P<0.01$). In patients' houses, the mite *D. pteronyssinus* was found in 82.6% of cases, in great amounts - up to 4,600 mites per gram, on the average 399.2±170.2. This kind of mite was 15 times more abundant in beds than in other sites, on the average 551±237.3 as compared to 35.4±10.45 mites/gram ($P<0.05$). The second is the genus *Glycyphagidae* - 9.8% with a prevalence of *Glycyphagus destructor*, 6.4%, 185±155.1 mites/gram. *Thyrophagus putrescentia* from the Acarides family comprized 1.05% of all the mites, on the average 38±13.8 mites/gram. Species

from the family Cheyletidae were 6.6%, mainly *Cheyletus eruditus*, on the average 56.35±24.9 mites/gram. The other species were not significant.

The sensitivity to mite allergens was examined by prick tests which were performed on patients as well as healthy persons. The sensitivity to *D. pteronyssinus* was established in 96.4% of asthmatics and in 12% of the healthy population ($P<0.001$).

The present investigation indicates the great role of mites in the allergy problem in Lithuania.

ANTIBODIES AND CIRCULATING ANTIGENS IN HUMAN TRICHINELLOSIS

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The object of the presented surveys was the evalution of reciprocal relationships among antibodies IgM (IgM-Ab), IgG (IgG-Ab) detected using somatic and metabolic larval antigens, as well as circulating antigens (CAs), to determine these by ELISA. The subject of examination were serum samples from patients included in "family outbreaks", either hospitalized or receiving out-patient treatment in the Clinical Hospital of Infectious Diseases in Bialystok.

235 serum samples obtained from 103 patients were examined, including 43 samples from 26 asymptomatic patients. In the first testing after admission to hospital, 12 samples from asymptomatic patients were negative, likewise 19 samples from symptomatic patients. However, in the sera from 48 patients, IgM-Ab and IgG-Ab were detected upon the first examination. Antibodies of both classes were found simultaneously in 39 serum samples. IgG-Ab only was present in 22 samples.

Serum samples in which IgM-Ab and IgG-Ab were present simultaneously constituted 71% of the samples and derived from the period between the 10th and 20th day of illness. Until the 10th day of trichinellosis, the antibodies detected belonged mainly to one class. The specific antibody was never found before the 2nd day of illness.

CAs were detected in 42% of the serum samples examined, not before the 3rd day of illness, and not later than 12 months after the appearance of the first symptoms. In the first testing, CAs were detected in 32 serum samples, of which 10 were from asymptomatic patients. Between day 0 and 10 of illness, CAs were registered in 36% of the sera examined.

DISTRIBUTION OF BRACONIDAE (HYMENOPTERA) IN THE LANDSCAPES OF LITHUANIA

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Reactions of the world's insects to anthropogenic impact have not been much studied. Parasitic insects are considered to be, on the one hand, regulators of the numbers of other Arthropoda, and, on the other hand, components of animate nature reacting sensitively to any environmental changes. Therefore, from a scientific point of view, it is interesting to draw attention to the study of insects in natural reserves and changed landscapes.

Using parasitic insects, Hymenoptera, Braconidae, as a sample, an analysis was made of the results of investigations on the occurrence and distribution of these insects in agrobiocenoses and natural communities, their relations with hosts, and the effect of anthropogenic activity on them.

Analysis of the distribution of the entomofauna has shown that definite species and their groups (even of the same biotope) are not considered to be constantly the same. The density of these insects usually depends on the character and combination of arboreal species and grass cover. The most abundant and diverse fauna is found in forest and meadow biocenoses, the more scanty in agrocenoses. The fauna of sandy and boggy biocenoses has shown a conditional specificity.

Landscape management has an unfavourable effect on numbers of parasitic species. However, according to data of many years, not a single aspect of this question was revealed: in agrobiocenoses under certain conditions, the chains between natural and cultivated biotopes were not entirely broken. For example, the orchard fauna consists of species from surrounding remote and near-region cenoses. The study of places and the distribution of adult stages of Braconidae according to grass covering and bushes, as well as the determination of habitat of their preimaginal phases through the definitive and intermediate hosts has shown the existence of regulating mechanisms and stabilizing natural systems. It is even possible to utilize this purposefully. In addition to intercenotic relations in agrocenoses, parallel, rather stable secondary chains were revealed which, according to their properties, are close to natural cenoses of a primitive character. However, the connections in agrocenoses are very vulnerable and sensitive to any impact.

MINK COCCIDIOSIS IN BELORUSSIA

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Coccidiosis is one of the common parasite diseases of minks which are kept in cages. We found 4 species of coccidiae on fur farms in Belorussia: *Eimeria vison*

(Kingscote, 1934), 83.5%; *E. furonis* (Hoare, 1927), 2.3%; *Isospora laidlawi* (Hoare, 1927), 14.1%; *I. eversmanni* (Svanbaev, 1956), 0.08%.

The prevalence in 3-month old pups was 57.63%; in adults 12.16% in spring, in summer 9.46%, in autumn 3.04%, and in winter 5.95%.

The disease manifests itself in depression, loss of appetite, thirst, and diarrhoea with admixture of mucus and blood.

The most effective drugs were 0.3% Bycox water solution for drinking instead of water, chimcoccidae 30 mg/kg body weight by 2 courses of 5 days' treatment at 4-day intervals.

For prophylaxis we used MKK-2 probione, 25 mg/kg body weight for 14 days; oxidate peat 0.5 ml/kg body weight for 10-14 days; and crude pine-needle biomass 1-2 g per animal for 7 days.

SCHISTOSOMA BOVIS IN GOATS: EVALUATION OF SEROLOGICAL DISEASE MARKERS DURING INFECTION AND FOLLOWING TREATMENT WITH PRAZIQUANTEL

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Potential serological disease markers for *Schistosoma bovis* infection in West African dwarf goats were evaluated before and after treatment with praziquantel.

30 goats were infected with 2,000 cercariae each, and 15 of them received teatment with praziquantel (60 mg/kg) 13 weeks post infection. One day, one week and four weeks post treatment, representative goats from each group were euthanized. For comparison, parasite-free control animals were monitored, some of which received treatment with praziquantel. Every two weeks during the study, the goats were weighed, and blood and faecal samples were collected. The serological parameters included: haemoglobin, eosinophils, albumin, lactate dehydrogenase (LDH), gamma glutamyl transferase (GGT), amino terminal propeptide of type III procollagen (PIIINP), and antipyrine clearance (APC).

The levels of haemoglobin, albumin, LDH and PIIINP were significantly reduced ($p<0.05$) in the infected goats from the time of onset of ovideposition. In the infected non-treated animals, the levels remained significantly reduced throughout the study period ($p<0.05$), whereas the levels in the infected treated goats returned to control levels three weeks post treatment. Eosinophilia was observed in the infected animals during prepatency and again at the time of maximum ovideposition, 8 and 10 weeks post infection, being significantly elevated as compared to the controls ($p<0.05$). An additional eosinophilia peak was observed in the treated goats one week after treatment ($p<0.05$), but two weeks later the level resembled that of the controls. No statistical differences in the GGT

levels were found between the groups during the study period. The antipyrine clearance test was performed on a cohort of 17 goats one day, 4 days and 24 days after treatment. However, the test did not show any significant differences between infected and non-infected goats, nor between treated and non-treated goats.

None of the evaluated parameters indicate any side effect of praziquantel treatment except for a temporary rise in the level of eosinophils one week after treatment.

DERMOCYSTIDIOSIS ON POND FISHES IN ESTONIA

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The genus of *Dermocystidium* is of uncertain classification at present and has been assigned by investigators either to the lower fungi or to Protozoans.

This is the first report on *Dermocystidium* infection in gills of common carp and rainbow trout in Estonia. First cysts of *Dermocystidium* on carp were found in March 1983. Cysts were found only on fingerlings from January to April. Neither visible pathological changes of the gills nor mortality of the fish were observed. Micropathological changes included local haemorrhage, epithelial hypertrophy and hyperplasia, as well as local inflammations. Cysts and developing spores were investigated by electronmicroscopy. By morphology of matured spores the parasite was identified as *Dermocystidium cyprini* Červinka et Lom. 1974. During 11 years, from 0.5% to 33.5% of the fish were invaded. The difference between numbers of invaded fish during the years, and positive correlation with warmer winters and more active invasion, were both statistically significant ($p<0.001$).

Dermacystidiosis of rainbow trout caused the death of a large number of spawners in March and April 1991. From some tens to several hundreds of *Dermocystidium* sp. cysts were found on the gills of the fish.

INVESTIGATION OF THE PARASITE FAUNA IN FISHES OF RUSSIAN WATERS OF THE BALTIC SEA

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An investigation of the parasite fauna in commercially important fishes of the waters of the Baltic sea and the Curonian gulf has been carried out since May 1992. The study covered 2,175 specimens of 5 species in the Baltic sea (*Clupea harengus, Sprattus sprattus, Gadus morhua, Scopthalmus maximus, Platichthys flesus*) and 256 specimens of 6 species (*Abramis brama, Rutilus rutilus, Carassius carassius, Perca fluviatilis, Stizostedion lucioperca*) in the Curonian gulf. During researches of the specific parasites and diseases by incomplete analysis, 5,628 specimens were in-

vestigated. In the Baltic sea, 27 parasite species were discovered, none of which were limiting fisheries. However, in February and March 1993 and in February 1994 in Clupea near Baltyisk, the nematode *Anisakis simplex* was found. The invasion extensivity was 0.03% with an intensivity of 1-4 species. A sharp decrease in the abundance of the marine parasites was found, which probably was related to the noticeable desalination of the Baltic in recent years. A deterioration of the ecological situation in the sea leads to the occurrence of several diseases in the Russian waters (ulcus syndrome and skeleton deformation in cod, lymphocystis in flatfish, Clupea and others). In the Curonian gulf we found 36 parasite species. Among these, the cestodes *Ligula*, occurring only in bream (fish size 17-25 cm) with an invasion intensivity of 1-12 species, can influence the fish quality negatively. The metacercariae of the trematodes *Postodiplostomum cuticula* were discovered in juveniles of roach and bream (25-50%, depending on time and place of the fish sampling), where they induced black-spotted disease. The definitive hosts of these trematodes are herons, a colony of which exists in the area of our investigations. In recent years, local swellings of the epithelial tissues (papillomas) were found in roach and bream on the surface of the body, fins and opercle, in severe cases papillomatoses. The number of affected fish makes up some 0.5-5% of the catch, depending on the season and fish age. An increased content of heavy metals was found in these.

PATHOMORPHOLOGICAL FINDINGS IN BIRD INTESTINES INVADED BY NEMATODES OF THE FAMILY CAPILLARIIDAE NEVEU-LEMAIRE, 1936

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The purpose of this work was to study the developmental dynamics of a host organism reaction in the case of experimental capillarosis. The dynamics of micromorphological changes in the small intestine of experimental fowl (chickens and turkey-poults) infected with *Aonchotheca caudinflata* (Molin, 1858) Moravec, 1982 and *Baruscapillaria obsignata* (Madsen, 1945) Moravec, 1982, at various stages of the postembryonic development of helminths was investigated. The first species was biohelminth, the second was geohelminth. It was found that the reaction of a host organism and its dynamics depended on the capillaria species. Different species induced pathomorphological changes which are typical of the individual species. Significant changes in small intestine mucosa induced by *A. caudinflata* were observed. From the 15th (4th developmental stage) to the 35th day (adult), these helminths penetrate deeply into intestinal mucosa and provoke there an inflammatory process which manifests itself by infiltration, haemorrhage, and cellular swelling. Adult helminths were eliminated from their locations, leaving cavities covered by connective tissue, and in many places of intestine mucosa large

accumulations of lymphoid tissue were observed. *B. obsignata* capillariae induced less pronounced changes in the fowl intestines.

TRICHINELLOSIS AT THE KAUNAS CLINICAL INFECTION HOSPITAL DURING THE PERIOD 1974-1993

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Since 1974 the incidence of trichinellosis has been increasing, and before 1994 we had 998 cases. Until 1971 the main source of infection was contaminated pork, and in 1971-1984 it was boar meat. Since 1984 the occurrence of infection from contaminated pork has increased again and remained the same until recent years. During the first decade, only sporadic cases were present, but since 1984 trichinellosis started to occur in outbreaks, and in the recent years the outbreaks were rather severe (171 patients were hospitalized in 1992, 182 patients in 1993). Changes of the clinical picture were inconsiderable, symptoms being typical. Cases of the first decade were characterized by the fever syndrome (61% of patients had a temperature for up to 10 days) but in separate years of the second decade, from 16.2% to 12.3% of the individuals had normal body temperature. In 1974-1983 leucocytosis above 8.10 g/l was found in 70%-90% of the patients, but in the recent decade it decreased. In 1993 the number of leucocytes was below 8.10 g/l in 53% of patients. In the first decade eosinophilia was more marked than in the recent one. In the first decade, 8.2%-8.5% were severe forms of the disease, and in 1981-1983 severe forms reached 18.3%. In the second decade severe forms started to decrease, and in 1993 they constituted only about 2%. The serological reaction was positive in one-third of the patients.

CLINICAL PECULIARITIES OF GIARDIOSIS IN CHILDREN

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Giardia lamblia is a microorganism which can cause disease, according to data of a scientific group of WHO (World Health Organization). 4,759 patients with enteric dysfunctions were treated in children's departments of the Kaunas clinical hospital of infectious diseases in 1991-1993. 288 (6.1%) of the patients had an initial form of giardiosis. 24 of these were infants, 201 were pre-school children, and 63 were in the school age. Giardiosis was accompanied by acute viral respiratory infection with complications (6 cases in infants) or without complications (10 cases in pre-school children). Hypotrophy was observed in 4, anaemia in 4, and rachitis in 2 infants.

Enteric diarrhoea, anorexia, nausea, flatulence and abdominal pains in older children continued for 5 days in 264 cases. The duration of these symptoms

was from 5 to 10 days in 20 patients and more than 10 days in 4 patients. Protracted diarrhoea can be explained by late diagnosis and late antiprotozoic treatment. Other invasive and enteric diseases were excluded in these patients.

Mild forms of disease were predominant in 284 patients, and 4 infants had accompanying diseases (hypotrophy and complicated acute viral respiratory infection). Changes in peripheral blood were not common. 226 patients were treated with Metronidazole or Tinidazole and 12 with Furazolidone. The duration of the treatment was 5 days, doses were common. The treatment with Metronidazole or Tinidazole was effective for 100%. Three stools examinations were made after the treatment, and *Giardia lamblia* were not found.

It may be concluded that *Giardia lamblia* is a conditional protozoan which can cause disturbances of the digestive system.

SEROEPIDEMIOLOGICAL DATA OF HUMAN TOXOPLASMOSIS IN LITHUANIA

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Data on the distribution of toxoplasmosis among the healthy population of Lithuania are scanty. Our task was to clarify the immunological situation during an incidence of toxoplasmosis. 750 persons were examined; 285 donors, 405 pregnant women, and 60 sailors. Blood sera were taken in various areas of Lithuania and were tested by the IF method using the Odessa-made lyophilised antigen and DAKO conjugates.

The results of our tests were as follows:

400 persons out of 750 tested, i.e. 53.3±2.5%, were found seropositive. They had IgG and IgM antibodies of 51.4±2.5% and 9.86±3.5%, respectively.

Thus, preliminary immunological tests manifest that about one-half of the examined persons in Lithuania may be carriers of antibodies against *Toxoplasma* and are primarily IgG antibody carriers.

HUMAN CRYPTOSPORIDIAL DIARRHOEA IN VILNIUS

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Organisms of the genus *Cryptosporidium* are small coccidian parasites that infect the microvillous region of epithelial cells lining the digestive and respiratory organs of vertebrates. *Cryptosporidium* is an important, widespread cause of diarrhoeal disease in humans and some domestic animals. In immunocompetent persons, *Cryptosporidium parvum* may cause a short-term (3-20 days) diarrhoeal illness that resolves spontaneously. However, in immunocompromised patients, cryptosporidiosis usually presents as a life-threatening, prolonged, cholera-like disease. At the time of this

writing, no effective therapy for cryptoporidiosis has been identified.

The first case of human cryptosporidiosis in Lithuania was reported in 1986. The specific routine diagnosis of cryptosporidiosis was applied in a medical practice in Vilnius in 1989.

The purpose of the study was to determine the presence of *Cryptosporidium* infection among acute and chronic diarrhoeic patiens.

In the period of 1988-1991, all samples of stools of 1,492 acute diarrhoeic and of 246 chronic diarrhoeic patients were examined for the presence of cryptosporidial oocysts. All samples were also tested for Salmonella, Shigella, Yersinia, Escherichia, Lamblia, Rotavirus, etc.

By modified Ziehl-Neelsen staining, cryptosporidial oocysts were found in stools from 3.2% of the acute patients and in 1.3% of the chronic patients.

The results showed that acute cryptosporidiosis is more frequent among children under 5 years of age. *Cryptosporidium* spp. ranks with Salmonella and Shigella as a leading cause of non-viral diarrhoea in Vilnius. Transmission of cryptosporidium infection has been noticed as occurring directly from animal reservoirs (calves), via a waterborne route, and person-to-person via the faecal-oral route, especially among children. Diarrhoea, vomiting, abdominal cramps and fever were the most common clinical syptoms, which were observed for 1-2 weeks. The prevalence of infection was highest in March, April and June. Most of the patients with clinical acute cryptosporidiosis were treated symptomatically, a few of them with antibiotics.

Other results showed that chronic cryptosporidiosis is more frequent among adults under 60 years of age with symptoms of persistent diarrhoea and with premorbidity, such as immunosuppression after chemotherapy and frequent use of antibiotics, and with chronic symptoms of gastroduodenitis. Persistent diarrhoea and weight loss were the most common clinical symptoms. No data have been presented to suggest a carrier stage among tested patients and control groups.

Thus, these results indicate the significance of *Cryptosporidium* infection among diarrhoeic patients in Lithuania.

HIERARCHIC CLASSIFICATION OF HOSTS BASED ON THEIR ROLE IN THE PARASITE LIFE CYCLE

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Proposed for discussion is the classification of hosts (H) based on their actual role in the realization of the life cycles (LC) of parasites, with endoparasitic helminths (P) taken as an example. Three levels of H in LC of P were revealed:

1. Primary structure of H. Genetically strictly determined circles of obligate hosts, where preparatory morpho-physio-

logical processes and achievement of the adult stage occurs in P.

1.1 Intermediate (1st, 2nd and following levels of H) and

1.2. definitive H.

2. *Secondary structure of H* (intermediate only). There is no morphogenesis of P inside them. Ecologically determined H promote the realization of LC of P and sometimes determine its possibility. As to the role in the enclosement of LC there are:

2.1 reservoir H, where P larvae accumulation and transfer to the next H occur.

2.2. Accessory H: transfer of P larvae without accumulation.

3. *Tertiary structure of H.* Ecologically determined definitive H. P may be found; they do not participate in the realization of LC. As to the longevity of the life there are:

3.1 Deadlock H: P can live for a long time, but the possibility of invasion of the next H is close to zero.

3.2 H-killers: P inevitably die soon after invasion.

H of the 1st and 2nd levels are cyclogenic, of 3rd, acyclogenic. The formation of secondary and tertiary structures in LC of P and their complicacy is a function of the complicacy of the trophic channels used by parasites that integrate a community. One of the main directions of the evolution of LC of P is a closer adaption of P to H and transition of H in the range: 3 - 2.1 - 2.2 - 1 in different variants or, more rarely, 2 or 1.1 - 1.2 (neoteinia). Application of this approach will be fruitful only when parasitological investigations are used in the general context of a synecological study of the concrete communities. This can further the understanding of the actual structures of LC of H and the peculiarities of their evolution.

PECULIARITIES OF THE FISH PARASITE FAUNA FROM THE WEST ANTARCTIC AND EPIZOOTOLOGICAL CONDITION OF THE REGION

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A special position of the West Antarctic fauna in the World Ocean - its high endemism, the original history of the formation, and also a keen interest in separate objects of the fishing industry, are the causes of a profound detailed study of the parasite fauna and peculiarities of the trophoparasitic relations of the region.

Examination of the fishes was carried out in the region of South Georgia Island, South-Shetland Islands and the Antarctic Peninsula. A total of 997 fishes belonging to 38 species of 12 families was studied. 53 species of parasites were found.

The influence of the specific variety of the hydrofauna and the hydrological conditions of the local region on the distribution of the parasites by their hosts in every region and all the area studied was determined. The dependence of the fish parasite fauna peculiarities of the trophic relationhips in the Antarctic was

shown. For example, the fish from more southern Antarctic localities showed an increased number of species and growing quantitative indices of infection caused by helminths having euphausiids in their life cycles. Euphausiids are the main food components of the Antarctic fishes.

The epizootological significance of the fish parasites of the region was elicited. 11 of the parasite species were dangerous to the health of human beings, and 3 species were not too dangerous but affecting the trade qualities of the raw fish or fish products.

PECULIARITIES OF THE HELMINTH-FAUNA OF DIFFERENT ECOLOGICAL GROUPS OF SQUIDS FROM THE OPEN SEA OF SOUTH AMERICA (SOUTH-EAST PACIFIC AND SOUTH-WEST ATLANTIC)

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During the years 1985-1992, 13 species of squids of 6 ecological groups in the area of South-east Pacific and South-west Atlantic were investigated. These groups are: oceanic squids (*Sthenoteuthis pteropus*, *Ommastrephes bartrami* and *Eucleoteuthis luminosa*), neritic-oceanic (*Todarodes angolensis* and *Dosidicus gigas*), bottom-oceanic (*Illex argentinus*, *Martialia hyadesi*, *Moroteuthis ingens* and *Pholidoteuthis boschmai*), bathypelagic (*Lycoteuthis diadema*), pelagic (*Architeuthis dux* and *Tetronychoteuthis dussumiery*), and inhabitants of the open shelf (*Loligo patagonica*).

In general, the helminth-fauna of squids has some common traits, such as high-percentage invasion of a didymozoid metacercariae, the presence of plerocercoids from the fam. Phyllobothriidae or Trypanorhynchidae, and larval nematodes from the fam. Anisakidae. However, these common traits have a kind of specific realization due to the difference in areas and ecological groups of squids. The helminth-fauna of the oceanic, neritic-oceanic and bottom-oceanic squids is roughly similar. Among the others, pelagic squids of the fam. Lepidoteuthidae seemed to be closer to the above-mentioned. The helminth-fauna of oceanic squids presented by the most widespread species includes more than 80% of the parasites recorded for the neritic-oceanic, bottom-oceanic and pelagic squids. The poorest fauna (a few species only) was recorded for bathypelagic and neritic squids.

ECOLOGY OF THE TABANIDAE (DIPTERA) FROM URBAN TERRITORIES

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Increasing of urban areas involves alteration of faunistic complexes of parasitic organisms, including bloodsucking

dipterian insects, and causes new medical problems in these territories.

Investigations were undertaken in 1992-1993 in three parts of a big industrial city (Minsk): (1) in a town garden situated in the centre of the city, (2) in the park zone of outskirts (both without water reservoir), and (3) in the park zone around Komsomolskoe-lake. At the same time, a study took place in the identical forest areas of the Berezina National Reserve. Flies were collected with an insect net and traps modified from prototypes used in Canada (Manitoba horse fly trap).

Collections of Tabanidae in urban territories yielded 14 species - 3 of *Chrysops Mg.*, 3 of *Tabanus L.*, 1 of *Atylotus O.S.*, 4 of *Hybomitra End.*, 1 of *Heptatoma Mg.*, and 2 of *Haematopota Mg.* In the reserve areas, Tabanidae were represented by 18 species, of which the genera *Atylotus* and *Heptatoma* were absent. Eight species were common for both areas. In urban territories the greatest number of species - 14 - was collected in the park zone of outskirts; 7 species were taken in the park zone around the lake, and 4 in the town garden. The average relative abundance was the same in all parts of the city (about 18 specimens for a test). Abundance of Tabanidae in the reserve areas was 1.6 times larger than in urban territories. *T. bromius L.*, the dominant species in urban territories, and *A. fulvus Mg.* and *H. pellucens F.*, the common species, were absent in the collection from the reserve areas. This suggests a synanthropic tendency of their ecology.

HAEMORRHAGIC FEVER WITH RENAL SYNDROME IN ESTONIA IN 1990-1993

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The examination by enzyme-linked immunosorbent assay (ELISA) of samples from 3,501 small mammals of 12 species, trapped in the territory of 14 municipal districts of Estonia, revealed the presence of Hantaan virus antigen in five species of the small mammals investigated. It is known that the bank vole (*Clethrionomys glareolus*) is the main source of HFRS infection in European foci. In Estonia, the species most frequently captured was *Clethrionomys glareolus* (1,191), followed by *Microtus arvalis* (837) and *Apodemus agrarius* (789). Also Hantaan virus antigen was most frequently found in *Clethrionomys glareolus*, 14.4% (51/1,191); in *Apodemus agrarius*, 2.4% (19/789); in *Microtus arvalis*, 1.7% (15/837); and in *Apodemus flavicollis*, 1.2% (4/319). It should be pointed out that Hantaan virus antigen has been found in the specimens of *Clethrionomys glareolus* in all municipal districts of Estonia. These data demonstrate that practically each landscape zone has natural foci of Hantaan virus, which provided the ground for the study of the incidence of HFRS, as not a single clinical case of HFRS has been registered until 1991. In 1992, in order to recognize cases of HFRS, we carried out a study of the serological

and clinical signs in 296 adult patients with various diseases, and also in 15 sick children. According to the results of the detection of antibodies to Puumala virus and clinical symptoms, the diagnosis of HFRS was confirmed in 4 patients in 1990-1991, in 11 patients in 1992, and in 15 patients in 1993. As there have been only 30 cases of NE, we have only preliminary data on clinical manifestations, epidemiological characteristics as well as some other problems of HFRS in Estonia. All cases occurred during autumn and winter in five towns of Estonia. Hantaan and Seoul virus infection have not yet been diagnosed in Estonia. That needs further studies.

SEROLOGICAL INVESTIGATIONS OF LYME DISEASE

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The number of Lyme disease cases per 100,000 inhabitants has increased 5.2 times between 1992 and 1993. Serological diagnosis has become important in Lithuania at present. The number of persons subjected to serological examinations has also increased, from 825 persons in 1992 to 2,075 in 1993. The number of examinations has increased threefold. An increase in serologically confirmed diagnoses is also observed.

An indirect immunofluorescence (IF) method was employed when investigating the various categories of persons. The following results were obtained:

Categories of persons	Persons examined	Sero-positive	Sero-positive (%)
1. Pregnant women	277	92	33.2
2. Foresters	57	7	12.3
3. Patients with erythema	149	39	26.2
4. Patients with dermatitis	30	7	23.3
5. Patients with syphilis	12	10	83.3

In the first group, pregnant women represented the highest seropositive percentage (33.2%) of those having antibodies.

Among foresters, seropositives amounted to 12.3%; among patients with dermatitis, 23.3%; among patients with erythema whose titers vary from 1:10 to 1:160, 26.17%.

We also investigated a group of syphilitic patients, since *Borrelia burgdorferi*, the agent of the Lyme disease, belongs to the Spirochaetales series. These cross-reacted, emphasizing that differential diagnosis is necessary.

PARASITES OF FLOUNDER (*PLATICHTHYS FLESUS*) IN THE EASTERN PART OF THE BALTIC SEA

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During the last 13 years, 22 species of flounder parasites have been found in the Eastern Baltic. They belong to the following systematic groups: Virales - Lymphocystis disease, Protozoa - 4 species, Monogenea - 1, Trematoda - 3, Cestoda - 3, Nematoda - 6, and Acanthocephala - 4 species.

The most frequent parasites in flounder populations are *Trichodina raabei* Lom, *Diplostomum* sp., *Cucullanus heterochrous* Rudolphi, *Dichelyne minutus* (Rudolphi), *Hysterothylacium aduncum* (Rudolphi), *Raphidascaris acus* (Bloch), *Echinorhynchus gadi* (Müller), *Corynosoma semerme* (Frossell), and *Pomphorhynchus laevis* (Müller). More rarely flounders were infected by *Trichodina jadranica* (Raabe), *Gyrodactylus flexibiliradix* (Malmberg), *Cryptocotyle concavum* (Creplin), *Bothriocephalus scorpii* (Müller), and *Scolex pleuronectis* (Müller). The rarest parasites of flounders among those established are *Myxobilatus platessae* (Hagenmuller), *Posthodiplostomum brevicaudatum* (Nordmann), *Eubothrium* sp., *Ascarophis* sp., *Porrocoecum* sp., and *Corynosoma strumosum* (Rudolphi). The occurrence of lymphocystosis was established more rarely in the last years. Probably this is due to changes of the host fish population. The extensity and intensity of infection by fish parasites among flounders depends on their age, sex, season and habitats - the Gulf of Riga, coastal zone, or open sea.

SOME DATA ON TICK INFECTIOUSNESS WITH *BORRELIA SPIROCHAETA*

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Republican Centre of Hygiene,
Vilnius, Lithuania

Ixodes ricinus and *Dermocentor pictus* are the prevailing species of ticks in Lithuania. *I. ricinus* is found in all the administrative regions of the country.

Observations carried out in recent years show an increase in the number of ticks. *I. ricinus* are found not only in the woods, but also in city parks and recreational zones.

In the Republican Centre of Hygiene, ticks are examined annually for the presence of the tick encephalitis virus, the Lyme disease and the carrying of *Pasteurella tularensis*.

During the two-year period of 1992-93, a total of 835 *I. ricinus* ticks, 768 adults and 67 nymphs, were collected from different localities in 14 regions and 2 towns in Lithuania. All ticks were examined for the presence of *Borrelia spirochaeta* by means of fixed stained smears.

388 females (5.26% infected), 380 males (2.63% infected), and 67 nymphs (0.71% infected) were examined. Overall infectiousness of the ticks examined was 8.62%.

Infectiousness of ticks varies from forest to forest within the limits of 0-15.7%.

With the increasing number of ticks and the possibilities of their contact with human beings getting stronger, an increase in the number of Lyme disease cases may be expected.

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FINAL ANNOUNCEMENT

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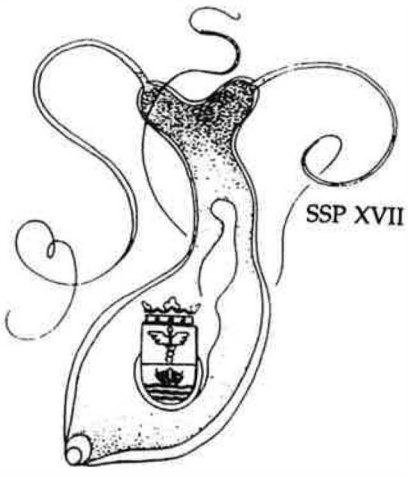
17th SCANDINAVIAN SYMPOSIUM OF PARASITOLOGY



UNIVERSITY OF JYVÄSKYLÄ, FINLAND 15-17 JUNE 1995

has now been sent to those who responded to the first announcement.

For further information, Please contact:

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Bull Scand Soc Parasitol 1995; 5: 76-88

OCCURRENCE OF *GYRODACTYLUS* (MONOGENEA) ON SALMON AND RAINBOW TROUT IN FISH FARMS IN NORTHERN FINLAND

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Abstract

35 salmonid farms within Northern Finland (between latitudes 64° and 69° north) were investigated for *Gyrodactylus* during the years 1990, 1991 and 1992. The investigations were performed during the cold water period (September-June, water temperature ≤+10°C) and generally included the pectoral fins of 60 specimens per fish species, farm and year. A total of 3976 fish were examined.

G. salaris was found on salmon, *Salmo salar*, and rainbow trout, *Oncorhynchus mykiss*, in a total of 13 out of 33 farms, including 9 out of 27 salmon or rainbow trout farms investigated within the Baltic area (both fresh and brackish water), 1 out of 3 farms in the Arctic Ocean area and 3 rainbow trout farms in the White Sea area. *G. lavareti* was found only in freshwater farms and only on rainbow trout, being present in 11 out of 27 rainbow trout farms investigated, including 10 out of 21 in the Baltic area and 1 out of 3 in the Arctic Ocean area, but none in the White Sea area. Clinical gyrodactylosis was not observed in any of the farms. No *Gyrodactylus* specimens were found on the Saimaa salmon, a landlocked form of *Salmo salar*, the brown trout, *S. trutta*, or the arctic char, *Salvelinus alpinus*.

The results indicate that *G. salaris*, and even *G. lavareti*, reproduce on rainbow trout and are more likely to spread between farms in Finland via the frequent interchanges of live salmonids than via the outflow water and rivers. Thus transport of live salmonids between farms is of special interest to fish health screening programs for preventing a further spread of *G. salaris* to wild populations of the Atlantic group of *S. salar*.

Introduction

Gyrodactylus salaris Malmberg, 1957 has been highly detrimental to wild populations of the Atlantic salmon, *Salmo salar* L. in Norway, where it has been found since 1975 (Johnsen & Jensen, 1991). Before that it was reported in the Baltic drainage areas of Sweden (Malmberg & Malmberg, 1993) and the former Soviet Union (Ergens, 1983). *G. salaris* was identified on salmon parr specimens in a Russian White Sea water system for the first time in 1992, a heavy infection in

the river Keret (Kierettijoki) (Ieshko, 1993, unpublished). So far it has not been found in the water systems of the Kola Peninsula, however (Mitenev, 1989; Ieshko, 1993, personal communication).

The first report of *G. salaris* in Finland dates back to 1984 and concerns findings on 1+ year-old parr specimens of *S. salar* in a fish farm on the River Kemijoki, which drains into the Baltic in Northern Finland (Rintamäki, 1989). Later on, Rimaila-Pärnänen & Wiklund (1987) and Keränen *et al.* (1992) reported the parasite in a number of salmonid fish farms in different water systems likewise draining into the Baltic Sea. In 1987, it was found in a salmon farm in the brackish water of the Baltic Sea itself (Malmberg and Malmberg, 1991a).

Gyrodactylus lavareti Malmberg, 1957 was originally described on whitefish, *Coregonus lavaretus* (L.), captured in a Swedish lake, and was later reported on coregonids in natural waters in Karelia and Kamchatka, the former Soviet Union (Ergens, 1983). At low magnification , the parasite species can be easily mistaken for *G. salaris*, however, which may cause irrelevant measures. Thus increased information on this species is important for health screening programmes in connection with farming of the rainbow trout, *Oncorhynchus mykiss* (Walbaum).

The present paper deals with findings of *G. salaris* and *G. lavareti* in salmonid fish farms in Northern Finland in 1990-92. The paper also deals with the host-parasite relationship between *G. salaris* and both the rainbow trout and the salmon.

Material and methods

The investigations were performed during the cold water period (September-June; water temperature not exceeding +10°C) in the years 1990-92 with the exception of October and December. The material was obtained from 35 salmonid farms in three drainage areas in Northern Finland, between latitudes 64° and 69° north (Table 1): I. 28 farms in six basins draining into the Bothnian Bay, northern tip of the Baltic Sea, together with one farm (net cages) in the Bothnian Bay itself (brackish water, salinity ca. 0.2 %); II. 3 farms in one basin draining into the Arctic Ocean; III. 3 farms in one basin draining into the White Sea. The map of the drainage areas (Fig. 1) is based on the 1980 Hydrological Yearbook of the National Board of Waters (1983).

A total of 3976 live salmonid specimens were taken from the fish farms by the owners and sent to the Oulu Regional Laboratory of the National Veterinary and Food Research Institute. The majority of the consignments consisted of rainbow trout, 2416 specimens and Baltic salmon (the Baltic group of *Salmo salar* L.), 1019 specimens, and the remainer of other salmonids: Saimaa salmon, a land-locked form of *Salmo salar*, 300 specimens, arctic char, *Salvelinus alpinus* (L.) 181 specimens, and of brown trout, *Salmo trutta* L., 60 specimens (Table 1). Generally at least 60 specimens per fish species, farm and year were investigated. A negative result was not included in the calculations of the frequency of infection if the number of fish examined was less than 60.

Table 1: Findings of *Gyrodactylus salaris* and *G. lavareti* in fish farms in Northern Finland

Drainage areas		Year 1990 Fish[1]			Gyr.[2]		Year 1991 Fish[1]			Gyr.[2]		Year 1992 Fish[1]			Gyr.[2]		1990-92 Gyr.[2]	
	Fish farm	Species	Age	No. examined	*G. salaris*	*G. lavareti*	Species	Age	No. examined	*G. salaris*	*G. lavareti*	Species	Age	No. examined	*G. salaris*	*G. lavareti*	*G. salaris*	*G. lavareti*
BALTIC SEA AREA (B)																		
Bothnian Bay	B 1	RT	2	30	II	-[3]											X	-[3]
Oulujoki basin	B 2	BS	1	60	-	-											-	-
	B 3											RT	0-2	140	-	-	-	-
												AC	0+	60	-	-		
	B 4	RT	1	60	III	-											X	-
	B 5						RT	2	60	-	I						-	X
	B 6	RT	1	30	-[3]	II											-[3]	X
	B 7	RT	1	60	-	II	RT	0	60	-	-	RT	0-2	60	I	-	X	X
		BS	1	60	-	-	BS	1	60	-	-							
	B 8	RT	0	60	-	-	RT	1	60	-	-						-	-
	B 9	RT	2	30	-[3]	I	RT	1	60	-	I						-	X
Kiiminkijoki basin	B 10						RT	1	60	-	I						-	X
Iijoki basin	B 11	RT	2	30	-[3]	III											-[3]	X
	B 12	BS	1-2	60	-	-	BS	2	60	-	-						-	-
	B 13	BS	1	60	-	-	BS	1	60	-	-						-	-
							RT	1	60	-	-							
	B 14						RT	0-1	120	-	-						-	-
	B 15	RT	1	60	-	I											-	X
	B 16	RT	1	30	-[3]	II	RT	1	60	-	II						-	X
	B 17	RT	2	30	I	-[3]											X	-[3]
	B 18	RT	1	60	-	II											-	X
Simojoki basin	B 19	BS	1+	60	-	-	BS	1	59	-	-	BS	1	60	-	-	-	-

Kemijoki basin	B 20	BS	2	60	III	-	BS	2	60	I	-	BS	1+-2	120	I	-	X	-
												BT	2	60	-	-		
	B 21											BS	0+-1+	60	-	-	-	-
	B 22	RT	1	45	-[3]	III						RT	1	60	II	-	X	X
	B 23	RT	0+-3	150	I	-											X	-
	B 24	RT	0-1	112	I	-											X	-
	B 25						RT	1	60	I	-	RT	2	60	I	-	X	-
	B 26						BS	2	60	-	-						-	-
	B 27						RT	0	60	-	-						-	-
Tornionjoki basin	B 28											BS	1-2	60	-	-	-	-
	B 29						LS	1	60	-	-	LS	2	60	-	-	-	-
ARCTIC OCEAN AREA (A)																		
Paatsjoki basin	A 1											AC	1-1+	121	-	-	-	-
	A 2	RT	0-1	151	I	I						RT	0+	188	I	-	X	X
	A 3	LS	1	60	-	-	LS	1	60	-	-	LS	1	60	-	-	-	-
WHITE SEA AREA (W)																		
Koutajoki basin	W 1	RT	1	60	III	-											X	-
	W 2											RT	0+-1	70	II	-	X	-
	W 3	RT	1	60	III	-	RT	1	60	III	-	RT	1	60	III	-	X	-
TOTAL				1478					1199					1299				

Fish[1]: Species: RT = rainbow trout (tot. 2416 specimens); BS = Baltic salmon (tot. 1019 specimens); LS = land-locked salmon (tot. 300 specimens); BT = brown trout (tot. 60 specimens); AC = arctic char (tot. 181 specimens).
Age: 0 =fry, 0+ = one summer old, 1=one year old, 1+ = one year and one summer old, a.s.o.

Gyr[2]: Amounts of *Gyrodactylus*:
-: no *Gyrodactylus*
I = < 4 specimens per one pectoral fin; II = 4-10 specimens ; III = > 10 specimens per pectoral fin.
X: *Gyrodactylus* found

-[3]: Negative result not included in the calculation of prevalence.

Each fish specimen was decapitated or euthanised with benzocain Ph. Eur. (Tamro, Finland) in the laboratory. Its pectoral fins were then cut off, placed in the water of the fish farm and examined for *Gyrodactylus* under a dissecting microscope (Olympus VMT-4F-W) against a black background. The examinatory results (Table 1) were ranked as follows:

- \- no *Gyrodactylus* specimens
- I less than 4 specimens per pectoral fin
- II 4-10 specimens per pectoral fin
- III more than 10 specimens per pectoral fin

For subsequent species identification, 1-3 *Gyrodactylus* specimens from each infected fish species per fish farm sample were fixed in ammonium picrate-glycerin between a slide and a cover glass (Malmberg, 1970). Totally, 87 *Gyrodactylus* specimens were prepared. With one exception, the species determinations were made by the second author. The statistical tests were performed according to Ranta *et al.* (1989) by the first author.

Results

The investigation revealed two *Gyrodactylus* species, *G. salaris* and *G. lavareti*,

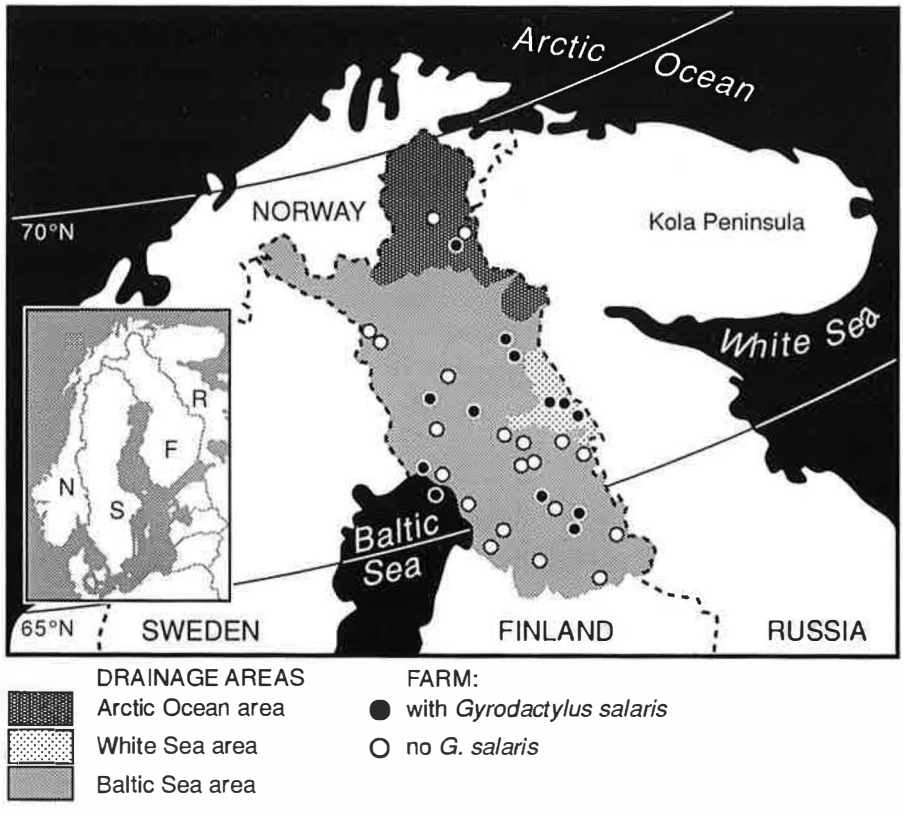

Fig. 1. Findings of *Gyrodactylus salaris* at salmonid fish farms in three drainage areas in Northern Finland (Baltic Sea, Arctic Ocean and White Sea) in 1990-92.

in the salmonid fish farms in Northern Finland. No *Gyrodactylus* specimens were found on the pectoral fins of the land-locked Saimaa salmon, the brown trout or the arctic char (Table 1).

G. salaris:

The species was identified in 13 of the 35 fish farms (ca. 39 % of the farms included in the calculation of prevalence, Table 1). *G. salaris* was found in both brackish water (Bothnian Bay, salinity ca. 0,2%; one infected farm) and freshwater farms in areas draining into the Baltic Sea (8 infected farms), the Arctic Ocean (one infected farm) and the White Sea (3 infected farms). The distribution of the infected fish farms is shown in Fig. 1.

Both rainbow trout and salmon specimens could be infected by *G. salaris*, but no infection seemed to cause any disease. The rainbow trout had a higher intensity of infection than the salmon (Fig. 2), and the prevalence of infection was also found to be higher in the populations of rainbow trout examined (ca. 49% of samples) than in the Baltic salmon (ca. 16% of samples, Table 1; chi-square test result X^2=ca. 5.78, $p<0.05$).

The records of the fish health monitoring programme undertaken by the National Veterinary and Food Research Institute contain nine farms which have a history of farming only rainbow trout (B 1, 4, 6, 14, 18, 27, A 2, W 1 and 2; Table 1), and *G. salaris* was found at five of these. The parasite was also present in one of the seven salmon farms without a history of raising rainbow trout (B 12, 19-21, 28-29 and A 3; Table 1).

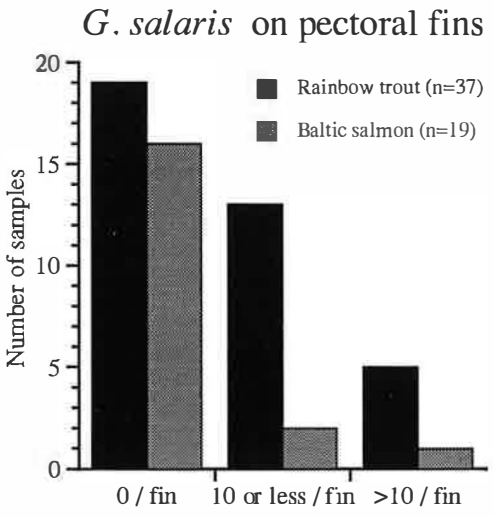

Fig. 2. Findings of *Gyrodactylus salaris* on the pectoral fins of rainbow trout and Baltic salmon specimens in samples from salmonid farms in Northern Finland (1990-92). The G-test result (G^2= ca. 6.29) shows a significant association between fish species and intensity of infection ($p<0.05$).

Comparison of the fish specimens in their first and second winter showed no significant association between either the amount or the prevalence of the infection and the age of the fish. Seven samples of fry (Age 0 in Table 1) were investigated, but no specimens of *Gyrodactylus* were found. No specimens of *G. salaris* were found in November, January and February (Fig. 3).

Both uninfected and infected farms were found within the same water system. In the River Kemijoki, *G. salaris* was found in an upstream farm but not in two other downstream farms (Table 1):

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

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

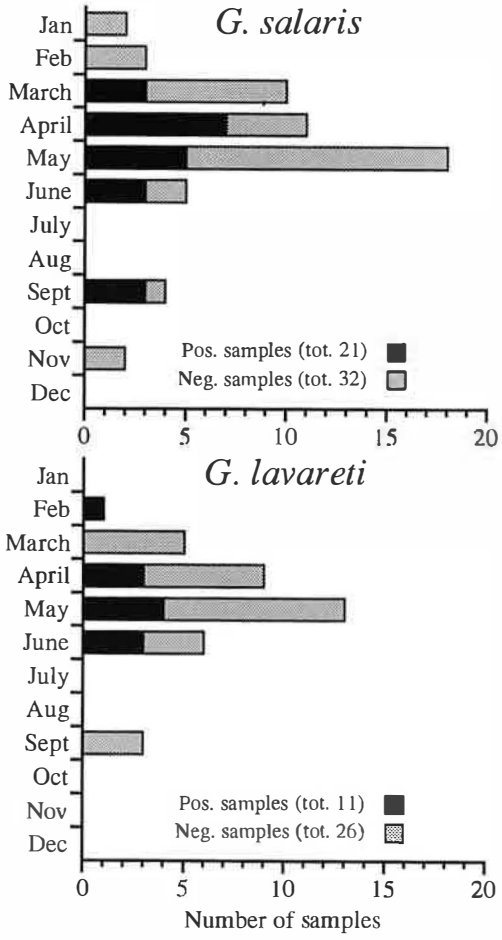

Fig. 3. Samples collected at farms in Northern Finland in various months over three years (1990-92): findings of Gyrodactylus salaris on pectoral fins of rainbow trout and Baltic salmon and of *Gyrodactylus lavareti* on pectoral fins of rainbow trout.

G. lavareti:

The species was found only on rainbow trout, in 10 "Baltic" and one "Arctic" farm (totally ca. 33% of the farms investigated), but it was not found in the three farms within the drainage area of the White Sea (Table 1). No specimens of *G. lavareti* were found in September or March (Fig. 3). *G. lavareti* seemed not to be pathogenic for the rainbow trout.

Discussion

Material and methods

An earlier investigation on *G. salaris* in Northern Finland (Keränen *et al.*, 1992) concerned 16 farms where the sampling was performed by the authors themselves. In order to complete the present investigations within the stipulated time, the sampling had to be performed by a large number of persons, which raises the question of whether or not the number of samplers could have influenced the result. The farmers were supposed to use the same methods for sampling and transport as Keränen *et al.* (1992), however, and a comparison of the findings of *Gyrodactylus* shows that similar results were obtained in the two investigations.

When possible, 60 fish specimens per fish species, farm and year were examined. On the assumption that the prevalence of infection is 5%, such a sample size will give a 95% probability of finding at least one infected fish per sample (Ossiander and Wedemeyer, 1973). Some of the 35 farms (Table 1) were not monitored for three years, however, or less than 60 fish specimens from some of them were examined, which reduced the chances of finding *Gyrodactylus* in such a farm. Even our results based on lower numbers of fish (30 or 45) may reflect the degree of *Gyrodactylus* infection in a farm at the time of sampling, however.

In accordance with earlier Finnish investigations (Rintamäki, 1989; Keränen *et al.*, 1992), the microscopic analysis was limited to the pectoral fins. This is based on observations that *G. salaris* prefers the fins rather than the body of its "true" host, *S. salar*, generally achieving its highest infection intensity on the dorsal and pectoral fins (Malmberg and Malmberg, 1991b; Jensen and Johnsen, 1992; Mo, 1992). Although the dorsal fin of salmon parr is habitually more infected, the prevalence of *G. salaris* on the dorsal and pectoral fins of salmon seems to be fairly similar (Jensen and Johnsen, 1992; Malmberg, unpublished). Furthermore, in a Finnish investigation of farmed Baltic salmon, more *G. salaris* specimens were found on the pectoral fins than on the dorsal one (P. Rintamäki, personal communication). However, the examination of the two pectoral fins can be expected to be more revealing as to the presence of *G.salaris* than an analysis limited to the dorsal fin only.

G. lavareti was found exclusively on rainbow trout, and thus only farms rearing rainbow trout could be infected by both *G. salaris* and *G. lavareti*. In fact, the present investigations revealed a concurrent infection in only one rainbow trout farm. This may be an account of the small number of *Gyrodactylus* specimens available for identification.

The occurence of Gyrodactylus salaris in fish farms

G. salaris was found here in 13 of 33 farms (ca. 39%, Table 1), whereas in an investigation two years earlier of salmonid farms in Northern Finland, likewise involving 60 specimens per fish species (Keränen *et al.* 1992), *G. salaris* was found in only 3 of the 16 farms investigated (ca. 19%). This proportionally low number of infected farms may be connected with the fact that the farms were investigated on only one occasion, while in the present study, a farm could have been examined on three occasions. At a cursory glance, the present results may be conceived as rather similar to those of Malmberg and Malmberg (1993) in salmonid farms in Sweden, where *G. salaris* was found in 8 of 18 farms (ca. 44%). The two investigations were performed differently, however, and consequently the results are not fully comparable. In the Swedish study all the fins and the head, and in certain cases also the body, were investigated, which increased the opportunity of finding solitary parasites, and yielding higher prevalence figures. On the other hand, the smaller sample size in the Swedish investigation (generally 10 specimens per fish species and sample) will have reduced the possibility of an infected fish being included in a sample at a low prevalence of infection. Furthermore, the majority of Swedish farms were investigated between 1986 and 1990, but certain investigations date back to the 1970's (in one case to the 1950's). Moreover, most of the Swedish farms had been investigated on only one occasion, and certain farms that had been reinvestigated were not infected on all occasions.

Seasonality of Gyrodactylus in fish farms

The present investigations were performed during the cold water period in Northern Finland, from September to June, when the water temperature was ≤+10°C. This limitation was based on the observation that *G. salaris* in salmonid farms in Finland (Rintamäki, 1993) and Sweden (Malmberg and Malmberg, 1993) seems to favour cold water. *G. salaris* was found from March to June and in September and *G. lavareti* from February to June, but the small number of investigations carried out at seasons other than spring makes proper analysis of the annual fluctuation impossible (Fig. 3). The present results are nevertheless consistent with the usual seasonal pattern of *G. salaris* infection in three salmon farms in Northern Finland where monitoring continued all the year round. Rintamäki (1993) found *G. salaris* to be present on the pectoral fins of Baltic salmon mainly after the water temperature had fallen below +10°C. Her material of 15709 fish showed prevalence and the intensity of the infection to increase during the winter, leading to more heavily infected fish in the spring than during the previous summer. The present results are also in accordance with observations in salmon farms in Northern Sweden, where the treatment of salmon parr for *G. salaris* may have to be performed during the period March-May (Malmberg and Malmberg, 1991a).

The seasonal occurrence of *G. lavareti* in natural water is still unknown, but the present results may indicate that in Northern Finland the species is found more frequently in a period around May than during other times of the year.

Presence of G. salaris in Northern Finland:

The present paper is the first report of *G. salaris* in Finnish waterways draining into the Arctic Ocean and the White Sea (Table 1). The salmon in both areas belong to the Atlantic group of salmon, which is less resistant to *G. salaris* than the Baltic group (see Bakke *et al.*, 1990). *G. salaris* can live on rainbow trout, and the exchanging of live fish between farms can cause the parasite to spread. In order to eradicate the infection from the farm in the Arctic Ocean area (farm A 2, Table 1) and in the hope of preventing it from spreading to the Paatsjoki drainage basin, all the fish in the farm were killed and a programme was started for controlling the *G. salaris* situation of the salmonid farms and wild fish in the Paatsjoki basin (Koski, 1993, unpubl.).

There are no wild populations of salmon, however, in either the River Paatsjoki in the Arctic Ocean area or the Finnish parts of the River Koutajoki in the White Sea area (Koli, 1990). *G. salaris* can spread directly upon contact between two host specimens or via the substratum, but it cannot swim and is short-lived without a host (Malmberg, 1993). This implies that live *G. salaris* specimens in the outlet water from a salmonid farm have little opportunity to infect wild fish stocks, especially when a river system lacks suitable host specimens (salmon or rainbow trout). Within the Baltic area of Finland (Fig. 1; Table 1) it is only the River Tornionjoki and the River Simojoki

that have wild populations of salmon (Koli, 1990) and the stocking of rivers with rainbow trout was uncommon at the time of the present work. Thus, even within this area *G. salaris* has limited opportunities for spreading further in natural waters. This may explain why *G. salaris* could be present in the upstream salmon farms B22 and B25 but not in the downstream farm B21 on the River Kemijoki. The absence of suitable host specimens in the river may have prevented any spread downstream.

Rainbow trout farming and G. salaris in Northern Finland:

The rainbow trout can harbour several *Gyrodactylus* species under farmed conditions in fresh water (e.g. *G. salaris* and *G. lavareti*; see Malmberg and Malmberg, 1987), and *G. salaris* can even reproduce on the rainbow trout under such conditions (Bakke, 1991). The present findings confirm this relationship, however, as *G. salaris* was in fact found on rainbow trout in 11 out of 23 farms investigated (Table 1), five of which had never reared salmon and to our knowledge had no salmon in their respective rivers.

Furthermore, more specimens of *G. salaris* were found on rainbow trout specimens than on salmon parr (Fig. 2), the opposite result of the expected, since the salmon is regarded as the "true" host of *G. salaris*. The present findings may be explained, however, by the different methods of rearing salmon and rainbow trout in Finland. Salmon farms, for example, keep the year classes in separate plastic or glass fibre tanks with a parallel flow of water and rearing almost exclusively takes place on the "all in - all out" principle, whereas rainbow trout farms often keep different year classes in successive earth ponds with water passing through them. Thus the rearing of rainbow trout facilitates a constant presence of *G. salaris* in a farm, and may also enable an accumulation of parasite specimens on individual fish.

G. lavareti on rainbow trout:

G. lavareti was found in 11 out of 23 rainbow trout farms (Table 1), whereas investigations in Sweden revealed the parasite on the same temporary host in 2 out of 4 farms (Malmberg and Malmberg, 1987; 1991a; Malmberg, 1993). Its presence in rainbow trout farms must arise from an original infection from wild or farmed infected specimens of its true host *Coregonus lavaretus*. Extensive farming of coregonid fingerlings is very common in Finland, and according to Statistics Finland (1993) there were 9113 hectares of natural food rearing ponds mainly for coregonids in 1992 . One might assume that this farming would provide a continuous potential source of infection, allthough it is true that Finnish fish farmers very seldom keep coregonids and rainbow trout in the same farm. The newly hatched coregonid fry are usually moved to temporary, isolated natural food ponds and the young coregonids are transferred to natural waters in autumn at an age of 0+. In fact, parasitological analyses of coregonid stocks in such ponds revealed no speci-

mens of *Gyrodactylus* (see Mannermaa-Keränen, 1991). Thus the Finnish coregonid farms seem not to be directly involved in the spread of *G. lavareti.*

Spread of G. salaris and G. lavareti between Finnish rainbow trout farms:

Within a rainbow trout farm the water supply may act as a vehicle for the spread of *G. salaris* and *G. lavareti* (above). On the assumption that the farms are situated within the same waterway system and suitable transport hosts are present, spreading via the outlet water (passive spreading) may be possible. Both *Gyrodactylus* species were found in farms within different areas or drainage basins, however, which indicates that they had probably spread in connection with the exchange of live rainbow trout between the farms. Such movements of live rainbow trout have been taking place in Scandinavia since the end of the nineteenth century (Malmberg, 1993), which may imply that "farmed" populations of *G. salaris* and *G. lavareti* exist that originate from specimens of old parasite populations adapted to the European rainbow trout farming.

Conclusions

The present results indicate that *G. salaris* is introduced to rainbow trout farms in Northern Finland mainly in connection with exchanges of infected live fish between them. Passive spreading via the outlet water released from the farms into natural waters is improbable in waterway systems without suitable host specimens, mainly salmon.

The populations of *G. salaris* and *G. lavareti* on rainbow trout specimens in farms may not be a recent introductions from wild *Salmo salar* and *Coregonus lavaretus,* respectively, but may be of much earlier origin.

Aknowledgements

We thank all the people at the fish farms who helped us to obtain the material for this investigation. Dr. Tor Atle Mo of Oslo, Norway, kindly identified the *Gyrodactylus salaris* material from the farm on the River Paatsjoki. We are also grateful to Mrs. Bibbi Mayrhofer for making the figures by means of computer graphics and Mr. Malcolm Hicks for revising the language.

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NOTES FROM THE EDITOR

This year there will be three issues of the Bulletin, instead of the normal two. The next issue will contain the proceedings of the SSP XVII in Jyväskylä, Finland, 15-17 June 1995, and will be ready for this symposium.

The next ordinary issue will be printed in October.

Proceedings of the SSP-Paraqua-94, Heimaey, Iceland, 2 - 6 July, 1994:

These were printed in the previous issue of the Bull SSP (Vol. 4, No. 2, October 1994). We have extra copies for sale (Kr. 150 per copy), please contact Hans Peter Fagerholm. Present address:

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ANNOUNCEMENTS

FISH PARASITOLOGY - GRADUATE COURSE

Date: August 12-23, 1995

Location: Marine Biological Laboratory (University of Copenhagen), 3000 Helsingør, Denmark

Language: English

Number of Participants: 16

The course will cover accomodation expenses, but the participants must cover their own travel expences and food, kitchen facilities are available at the laboratory.

The course is primarely aimed at Danish Ph.D. students. To promote international cooperation, participants from other countries, primarely from the Scandinavian countries, may also participate.

Deadline for application: May 1, 1995. For further information, please contact: **Dr. Marianne Køie, Marine Biological Laboratory, DK-3000 Heslingør, Denmark**

SPRING SYMPOSIUM - March 4, 1995

Theme: *LEISHMANIA/TRYPANOSOMA*

Organizers: Danish Society for Parasitology &
Danish Society for Tropical Medicine

Time: Saturday March 4, 1995; 8.30 a.m. - 4.45 p.m.

Venue: August Krogh Instituttet, Universitetsparken 13, 2100, Copenhagen Ø

Registration fee: DKK 150.-/75.- (with/without lunch)

TOXOPLASMA MEETING

The Swedish Institute for Disease Control is planning a meeting on *Toxoplasma* on Monday 24 April 1995.
For further information, please contact **Dr. Inger Ljungström, Parasitology Laboratory, Swedish Institute for Infectious Disease Control, S-105 21 Stockholm, Sweden**
tel: +46 8 735 1429, fax: +46 8 735 1162, e-mail: inger.ljungstrom@ki.se

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

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

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

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

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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:

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

Books and other monographs:

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

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

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

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BULLETIN OF THE SCANDINAVIAN
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VOL. 5 No. 1 CONTENTS FEBRUARY 1995