BULLETIN
OF THE SCANDINAVIAN SOCIETY FOR PARASITOLOGY

PROCEEDINGS OF THE XVI SYMPOSIUM OF THE SCANDINAVIAN SOCIETY FOR PARASITOLOGY, NORWAY, 30 SEPT. - 2 OCT. 1993

Vol. 3 No. 2 1993

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

16th Symposium of the Scandinavian Society for Parasitology

Oslo * Vettre * Norway

September 30 - October 2, 1993

Editor: Bjørn Gjerde

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Vettre Hotel, Asker, Norway

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The generous financial support from the following sponsors is gratefully acknowledged:

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Norges veterinærhøgskole
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Orvar og Gertrud Nybelins fond
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SmithKline Beecham Animal Health

Introduction by the editor

The present Proceedings comprise the Welcome speech of the President of the Scandinavian Society for Parasitology, the abstracts/manuscripts of seven invited lectures, and the abstracts of 43 oral presentations and 25 poster presentations at the 16th Symposium of the Scandinavian Society for Parasitology. On behalf of the Organizing Committee I would like to thank all contributors for their interest in the symposium.

The abstracts have generally been treated as ordinary contributions to the *Bulletin of the Scandinavian Society for Parasitology*, and the majority of them have been subjected to some editorial changes. All edited abstracts were returned to the presenting authors for proofreading and a final check of the contents. However, not all presenting authors have responded to the edited version of their abstract, and consequently errors due to the editorial changes might occur in some abstracts. Since it is the policy of this journal to use the suffix -osis rather than -iasis to denominate parasitic diseases and infections, the former suffix has been used throughout the proceedings.

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WELCOME BY THE PRESIDENT OF THE SCANDINAVIAN SOCIETY FOR PARASITOLOGY

Hans-Peter Fagerholm
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It is a fact that it is very difficult these days to keep abreast of the latest advances, even in one's own field: this is true of the biological sciences (which are represented by the tree of Yggdrasill on the front page of our Bulletin), and particularly so in the case of our discipline, parasitology, where so many facets of science combine to make what must be the jewel in the biological crown. Although the new and highly efficient tools which we use today to obtain, store, retrieve, and distribute information often make our tasks much easier, we still need to learn to utilise such facilities more efficiently.

Parasitology is usually more difficult than one thinks. It may be difficult enough for a scientist in the field of genetics, for instance, to tell the sequence of nucleotide bases of the actin gene, but then think of the parasitologist who is asked to eradicate malaria by combining information from all fields of science. However, because parasites are such fascinating animals, the parasitologist is still willing to learn and develop methods, and sometimes even to pay for the work himself, in order to find the solution to a problem. It is evident that the importance of our symposia increases as the amount of new information accumulates.

Today, on behalf of the Scandinavian Society for Parasitology, I have the privilege to welcome all of you to our 16th symposium. It is my hope that we will become even better acquainted with parasites and parasitology by listening to our invited guest lecturers, by hearing the numerous short communications and by analyzing the posters, but also by contributing to discussions both during and between the sessions. Furthermore, on the social side, it is not totally irrelevant if Professor Helle and his co-workers are fit and are able to run as usual, or if Karin has bought a new horse, or if Tor has finished his dictionary, as some of these persons are responsible for the arrangements of this symposium.

At the present symposium, we are discussing the consequences of the implementation of new legislation, and the possible increased risks of the spread of zoonotic and economically important parasitic diseases, when new countries join the European Community. However, we must not forget the formidable parasitic problems found in some tropical regions outside Europe. The bodies in the Scandinavian countries responsible for foreign aid, with the exception of Denmark and to some extent Sweden, have, contrary to the situation in numerous other European countries, made only limited use of their own countries' expertise in different aspects of parasitology associated with health care, food production, education and research in developing countries. It is important that we make better use of this expertise and potential, as they do it in Denmark via the new programmes of the Danish Bilharziasis Laboratory. We must inform our authorities of the situation and urge them to analyze ways in which our expertise in parasitology can be more efficiently used in such work.

The Scandinavian Society for Parasitology tries to promote the field of parasitology by supporting universities and research institutes when they are planning courses, seminars and similar activities, although the funding has to come through other

channels. Since our Bulletin can now be used to spread information of general interest efficiently, we should not forget to use it for this purpose. In order to reduce the production costs, commercial advertisements are also welcomed. We have been discussing the possibility of arranging some of our regular symposia outside our region. However, it is suggested that while extraordinary meetings, usually based on special themes, may be arranged, the regular meetings will still be held bi-annually in alternate Nordic countries.

The organising committee headed by Professor Oddvar Helle (Karin Andersen, Björn Gjerde, Svein Gunnar Gundersen, Reidar Mehl, Tor Atle Mo, Gudbrand Stuve, Jorun Tharaldsen) have performed a formidable task in arranging this symposium, and it is an honour and privilege to be able to thank them on your behalf for their successful efforts. We are also indebted to numerous sponsors which have made the symposium possible. It is a pleasure to come to Oslo and to get acquainted with a city that has changed so much during the last decade. I hope, among other things, to find the time at least to pay a visit to the famous Munch Museum.

CONSEQUENCES OF MORE OPEN BORDERS IN WESTERN EUROPE CONCERNING TRADE IN ANIMALS AND ANIMAL PRODUCTS

Live L. Nesse
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Establishment of the European Community internal market

The foundation stone in the building of the European Community was laid on 18 April 1951 when six countries (Belgium, Germany, France, Italy, Luxembourg, the Netherlands) signed a Treaty establishing the European Coal and Steel Community (ECSC). Further integration was achieved with the two new Treaties establishing the European Atomic Community (Euratom) and The European Economic Community (EEC) signed in Rome in 1957. Today the European Community comprises twelve countries (Britain, Denmark, Greece, Ireland, Portugal and Spain in addition to the first six).

The focal point of economic integration within the EEC is the common market, in which the Member States have combined to create a unified economic territory undivided by customs or trade barriers. This common market rests on the pillars of four fundamental freedoms: the free movement of goods, persons and capital, and freedom to provide services.

To create this large European internal market (almost 320 million people) the Community countries have had to dismantle all manner of trade barriers, harmonize legislation, administrative practices and tax structures, and extend their cooperation on monetary policy.

Regulation of trade in live animals and animal products within the European Community

One goal of the EEC is to obtain a high health standard throughout the Community both for animals and for humans. Free movement of live animals and animal products is not compatible with this goal today. Therefore a harmonized EEC legislation regulates both surveillance and control of animal disease, as well as trade in animals and animal products. In fact, several hundred EEC directives and decisions are concerned with these matters.

When a live animal is intended for trade, both the animal itself and the holding of origin, must fulfil certain requirements concerning animal health. This must be certified by the competent veterinary authority at the place of origin. By this, basic guaranties concerning certain specified diseases are always given. In addition, a region which has been declared free from a certain disease, may be granted the right to require additional guarantees concerning this disease before an animal may enter the region.

When an animal product is intended for trade, health requirements concerning the holding of origin, the animal and the production plant must be fulfilled and certified by the competent veterinary authorities at the place of origin. There are also Community rules for processing and placing on the market of animal waste, as well as feedstuffs for animals, to prevent the spread of pathogens from such sources.

In addition, there is a safeguard clause which allows Member States on serious public or animal health grounds, to take interim protective measures according to their own procedures with regard to the introduction into their territory of animals or animal products.

Most of the above mentioned EEC legislation is concerned with infectious diseases, whereas parasite infestations are less focused. Bovine semen must originate from animals free from *Tritrichomonas foetus*, and meat must be examined for cysticercosis (swine) and trichinosis (swine and horses). Community rules have been laid down to prevent and reduce the appearance of zoonoses which pose a threat to human health through food of animal origin, e.g. trichinosis, echinococcosis and toxoplasmosis.

Regulation of trade in animals and animal products under the EEA Agreement

The European Free Trade Association (EFTA) was founded in 1959, comprising Britain, Norway, Sweden, Denmark, Austria, Portugal, Iceland and Switzerland, with Finland as an associate member. Later, Britain, Denmark and Portugal left EFTA to join the EEC. The objectives of EFTA were purely economic, unlike those of the EEC which were also political. Within EFTA, the Member States apply national legislation on trade in animals and animal products in order to protect animal and human health.

In 1989, negotiations for an expanded cooperation between EEC and EFTA were started, resulting in the proposed EEA Agreement. Under this agreement, the EFTA countries will be obliged to follow most of the EEC legislation on trade in animals and animal products. However, the EFTA countries will sustain their border control, and there will also be border control of animals and products entering the EEC from EFTA countries.

Consequences of the more open borders

To predict the consequences of the more open borders, one must bear in mind the large differences in animal health standards in the countries concerned. It is also necessary to differentiate between the diseases covered by legislation and those which are not. It is reasonable to believe that the unified Community efforts to raise animal health standards will have a positive effect in those areas were the standard today is low. The areas with a high standard should be reasonably well protected against those diseases that are regulated by common legislation. However, in these areas an increased trade may lead to a higher risk of the diseases which are not regulated. Unfortunately, most diseases resulting from parasites belong to the latter category.

In this context, it is important to be aware that all the above mentioned regulations are confined to official requirements, i.e. stating which guarantees the official authorities of the Member States can demand. In addition, the private buyer can demand any guarantee he or she wants in order to protect against disease. This means that the responsibility to protect the health standard when trading with animals and their products, is shared between the official authorities and the private community. In several of the countries concerned, this is a new situation. Earlier, setting of standards has mostly been left with official authorities, and the private community has traditionally felt less obliged to assume responsibility in these matters. Therefore, appropriate information is crucial to succeed in obtaining and maintaining a high animal health standard when the borders in Western Europe become more open.

CONSEQUENCES OF MORE OPEN BORDERS IN WESTERN EUROPE FROM A PARASITOLOGICAL POINT OF VIEW - THE DANISH SITUATION

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Denmark is characterized by having a large animal industry and an export that covers the demand of animal proteins of more than 18 million people. Hitherto, there has been a very limited import of animal products, and a negligible import of live animals. Imported animals have been subject to control and quarantine regulations according to the Danish veterinary legislation. This control has mainly focused on certain exotic, epidemic diseases caused by viruses and bacteria, and only to a lesser extent on parasitic diseases. Nevertheless, the overall restrictions have presumably ensured that the import of parasites has been limited (e.g. an imported case of *Echinococcus granulosus*, a case of *Rhipicephalus sanguineus*, and a few cases of warble flies over the last decades). The import of various exotic helminths, such as gastrointestinal nematodes of livestock, may have passed unnoticed, since infections with these parasites are usually not associated with overt, clinical disease and since a complete checklist of helminths in Denmark does not exist.

The establishment of the Inner Market in the CEC countries has implied that the previous control and quarantine at the national borders have been repealed, and replaced by control of foods and live animals at the production site. However, these control regulations do not cover a range of parasitic infections and have not yet proven to be fully operative.

Due to the new control principles and the increased overall trade between countries, the risk of parasites being 'imported' is obvious. The majority of these parasites may already be present in Denmark, but some, especially those from Southern Europe, may be introduced and cause unexpected disease problems in livestock and man. One should not only be aware of the possible introduction of new species, but also of the possible import of new strains of indigenous species, e.g. anthelmintic resistant strains.

CONSEQUENCES OF MORE OPEN BORDERS IN WESTERN EUROPE FROM A PARASITOLOGICAL POINT OF VIEW. PARASITES CAUSING DISEASE IN AQUATIC ANIMALS.

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Introduction

As we all know, outbreaks of transmissible diseases in aquatic animals in most cases can be traced back to movement of live aquatic animals and their introduction into new areas. History is full of such "successful" transfers and many of you know the history of the introduction and spread of the swim bladder nematode *Anguillicola crassus* in Europe.

Thus, in order to achieve disease control, it is necessary to have an established legal framework which can be used as a tool to avoid the introduction and spread of serious fish pathogens, including serious parasites. Once a disease agent has been established in a free living or a farmed stock, the possibility of subsequent spread to other areas or farms via water or through the transfer or trade of aquatic animals, poses a permanent threat to the fish farming industry, as well as to wild populations.

Although the borders in Western Europe - that is in the EEC countries - have "disappeared" due to the introduction of the single market, there are still rules and regulations which have to be strictly followed both for trade within the EEC and for importation from countries outside the EEC. The situation is not necessarily as open as one might initially think.

Principles for disease control

Before going into details on the possible consequences of more open borders, I would like to focus on the basics of disease control, as legislation and regulatory measures are still of utmost importance in order to minimize disease introduction into a country, in spite of the establishment of the single market.

Both outside and within the EEC, a wide range of steps must be taken to control the spread of diseases. Control in its broadest sense may be summarized as follows:

1. Clearly defined procedures for inspection and health control.
2. Import regulations.
3. Quarantine measures.
4. Regulations concerning the introduction of new species into new areas.
5. Transport regulations.
6. Restrictions on movement of live aquatic animals.
7. Disinfection procedures.
8. Defined procedures for dealing with outbreaks of certain notifiable diseases of socioeconomic importance, including eradication procedures (i.e. "stamping out"), sanitary slaughtering, etc.

Disease Control in the EEC and Norway

In this section I shall highlight the basis for disease control in the EEC countries and Norway.

Although border control within the EEC has been lifted after the introduction of the single market from 1st January 1993, the risk of disease spread is not necessarily any

greater than before, as this risk depends on the previous national legislatory framework and the demands for health certification of certain notifiable diseases of parasitic origin. In most EEC countries, the only notifiable diseases of aquatic organisms have been socioeconomically important diseases of bacterial or viral origin, while the parasitic diseases, except for those caused by *Gyrodactylus salaris* and *Myxobolus cerebralis*, have seldom been recognized as being serious enough to necessitate the documentation of their absence. Unless a country imposes restrictions with regard to any parasitic disease in connection with importations, the introduction of new parasites could occur just as easily now as before 1st January 1993. The incidence rate of dissemination would thus depend upon the volume of trade in live aquatic animals and their eggs. If the opening of borders leads to increased trade in aquatic animals and their products, the risk would be greater of new parasites spreading to new regions, especially if the presence of these parasites is not notifiable. In Norway, there are indications that a *Diplectanum* spp. has been associated with importation, the eggs of *Diplectanum* have been attached to the surface of sea bass eggs.

Current EEC regulations based upon directives and decrees do not have direct legal validity in member countries, but it is a presupposition that member countries establish or adjust their national legislation in such a way that it is in agreement with the intentions of the Directives given.

The most important EEC regulations are the following:

- Council Directive 91/67/EEC of 28th January 1991, concerning the animal health conditions governing the placing on the market of aquacultural animals and products.
- Commission Decision 92/532 of 19th November 1992, laying down the sampling plans and diagnostic methods for the detection and confirmation of certain fish diseases.
- Proposal 92/204 for a Council Directive introducing Community Measures for the Control of certain fish diseases.
- Council Directive 89/662/EEC of 11th December 1989, concerning veterinary checks in intra-community trade with a view to the completion of the internal market.
- Council Directive 90/425/EEC of 26th June 1990, concerning veterinary and zootechnical checks applicable in intra-community trade in certain live animals and products.
- Council Directive 90/675/EEC of 10th December 1990, laying down the principles governing the organization of veterinary checks on products entering the Community from third countries.
- Council Directive 93/54/EEC of 24th June 1993, amending Council Directive 91/67/EEC, concerning the animal health conditions governing the placing on the market of aquacultural animals and products.
- Council Directive 93/53/EEC of 24th June 1993 introducing minimum Community measures for the control of certain fish diseases.

Council Directive 91/67 contains definitions of aquacultural animals, aquacultural products, aquaculture establishments, approved zones, approved farms, etc., and it also applies to transfer of live feral fish from one river to another river, i.e. "wild to wild".

This directive also gives a definition of List I and List II diseases which are

recognized as the most socioeconomically important diseases needing special countermeasures. The restrictions imposed on affected farms only refer to susceptible species for the disease(s) in question. In addition to List I and II diseases, the Directive also has a List III of additional diseases, which member countries may ask for recognition of freedom from if they have drawn up a voluntary or compulsory programme to combat these diseases. Table I shows the listed diseases/pathogens of fish, molluscs and crustaceans in Directive 91/67 according to the suggested amendments given in Council Directive 92/458/EEC.

As can be seen from the table, very few of the diseases to be considered are of parasitic nature, and thus any parasitic condition other than those mentioned may easily be transferred from one country to another. However, a possible control may be achieved on the basis of Directive 91/67 with regard to approval of zones into which no live fish should be introduced from infected zones or infected farms. Control thus might be achieved when/if the official services have subjected fish from the lower catchment area to a health inspection twice a year for consecutive years.

Article 3 in Directive 91/67 gives certain general requirements regarding the marketing of aquaculture animals: they should not show clinical signs of disease on the day of loading; they should not be intended for destruction or slaughter for eradication purposes; and they should not be from farms subjected to prohibition.

The Directive also lays down principles for transportation and health certification, as well as rules governing imports from third countries.

All countries outside the EEC are regarded as third countries, and before the EEA treaty is signed, this will also apply to EFTA countries. However, even after the EEA treaty has been signed Finland, Iceland and Norway will have status as third countries regarding importation of live fish, but as member countries regarding dead fish. Sweden, on the other hand, will have membership status for both live and dead fish, and can thus no longer apply its own regulations. This enables better protection of the aquaculture industry in Iceland, Finland and Norway from hazardous importations, as one may lay down the principles of national disease regulations for the importation of live material.

In Norway there is a general ban on importation, and in principle the open borders in Western Europe will thus not have any effect on the present situation.

However, if Norway becomes a member of the EEC, the regulations given in the Directive 91/67 will have to be followed and the previous restrictive importation policy will only apply for importation from countries outside EEC. Thus any parasite may be transferred within the EEC if not listed. Both live and dead fish may be possible sources of parasite dissemination, live fish being the product most at risk.

Table I. Proposed list of diseases/pathogens of fish, molluscs and crustacea, against which special countermeasures are needed/required (* May be reclassified into List I).

Disease/Pathogen	Susceptible species
LIST I	
Fish	
Infectious salmon anaemia (ISA)	Atlantic salmon (*Salmo salar*)
LIST II	
Fish	
Viral haemorrhagic septicaemia (VHS) virus	Salmonid species, including grayling (*Thymallus thymallus*) and whitefish (*Coregonus* sp.), pike (*Esox lucius*), sea bass (*Dicentrarchus labrax*), turbot (*Scophthalmus maximus*)
Infectious haematopoietic necrosis (IHN) virus*	Salmonid species
Molluscan shellfish	
Bonamia ostreae	Flat oyster (*Ostrea edulis*), Chilean oyster (*Ostrea chilensis*), New Zealand oyster (*Tiostrea lutaria*)
Marteilia refringens	Flat oyster (*Ostrea edulis*), mussel (*Mytilus edulis, M. galloprovincialis*)
Haplosporidium amoricana	Chilean oyster (*Ostrea chilensis*)
Perkinsus atlanticus	Manilla clam (*Ruditapes phillipinarum*), carpet shell clam (*Ruditapes decussatus*)
Iridovirus gill disease	Portuguese oyster (*Crassostrea angulata*), Pacific oyster (*Crassostrea gigas*)
LIST III	
Fish	
Infectious pancreatic necrosis (IPN)	To be specified in the programme referred to in Article 12.
Spring viraemia of carp (SVC) virus	
Bacterial kidney disease (BKD) (*Renibacterium salmoninarum*)	
Furunculosis (*Aeromonas salmonicida*)	
Enteric redmouth disease (ERM) (*Yersinia ruckeri*)	
Gyrodactylus salaris	
Crustaceans	
Crayfish plaque (*Aphanomyces astaci*)	
Gaffkemia of lobsters (*Aerococcus viridans*)	

PARASITISM AND PARASITOLOGY: ANACHRONISTIC FLAGS

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Parasitism and parasitology are anachronistic terms when viewed from the new data of molecular biology and ultrastructure research. Comparable to prejudicial terms like "slave" or "prisoner", "parasite" implies the restricted movement of the members of one species in relation to another (the "master", "host" or "warden". " Autopoiesis" refers to the self-maintaining metabolic activities of living systems. Respecting A. DeBary's 19th century intention, "symbiosis" simply means the protracted physical association between members of different species without regard to outcome for either partner. David Lewis, Sheffield University, UK, in the early 70's tried to alleviate the damage wreaked by the inaccuracy and political aspects of terms like "parasite" and "pathogen". He distinguished nutritional from other relationships by developing appropriate terminology such as symbiotrophy (living together where the basis of the relationship is nutrient flow between symbionts), etc. Pathogenesis becomes the extreme version of necrotrophy, as noted in the Table that accompanies this paper, where an attempt is made, following Lewis, to replace obsolete and politicized terminology.

Genetic, ultrastructural and molecular biological data underlie the perspective of symbiosis as a major mechanism of taxa origin. These sciences lead us to distinguish taxa of minimal reproducing autopoietic entities (e.g., bacteria) from replicating nonautopoietic entities such as viruses, plasmids, or nucleic acids) and recognize their recombination and integration in the formation of more complex autopoietic entities at higher levels of individuality. That is, all protoctists including apicomplexa, kinetoplastids, algae, as well as other more obvious organisms such as lichens, bovines, termites, etc. are holobionts.) The replacement of anthropocentric, zoological language with more microbially-oriented and adequate descriptives is a prerequisite to the development of an analytical biological science. The need for accurate description of organismic properties and relationships is especially acute in traditional ecological "fields", such as parasitology.

The number of extant species of organisms is estimated to be from fewer than 3 to more than 30×10^6. Molecular biology and genetics coupled with ultrastructural studies provide new insights into evolutionary relationships between these species, including increasing detail concerning the role of symbiogenesis in the origins of species and other higher taxa. Accumulation of random mutations and large macromolecular sequence change in all organisms since the Proterozoic eon has been importantly supplemented by acquisition of inherited, especially microbial, genomes ("symbiogenesis" or "symbionticism" in the early 20th century literature). Karyotypic alterations (polyploidization and karyotypic fissioning) have been added to these other mechanisms of species origin in plants and animals during the Phanerozoic eon. Such new concepts of evolution, coupled with current rapid rates of species extinction and ignorance of the extent of biodiversity, prompt my insistence that human and veterinary necrotrophies be analyzed as extreme cases of symbiotic association. I even suggest you change the name of this program to the "Scandinavian Symposium on Necrotrophy"!

Incorporation of bacterial, protoctist and probably even fungal (e.g., yeast) and animal (e.g., nematode, pentastome) species into pre-existing others in the emergence

of new life forms has occurred frequently throughout evolutionary history. Many necrotrophic associations tend toward the symbiotrophic over time: well-integrated symbioses lead to symbiogenesis. Systematic and logical reorganization of biology requires a new look at the placement of extant species, all of which are probably heterogenomic, into higher taxa. The following provisional scheme is presented for open discussion.

Two superkingdoms (=Domains: **Prokaryotae** and **Eukaryotae**) and five kingdoms (**Monera**=Procaryotae or Bacteria; **Protoctista**: algae, amoebae, ciliates, foraminifera, oomycetes, slime molds, etc.; **Mychota**: "true" fungi; **Plantae**: one phylum (=division) of bryophytes and nine phyla of tracheophytes; and **Animalia**) are recognized. Two subkingdoms comprise the monera: the great diverse lineages are Archaebacteria and Eubacteria. Logically consistent, technical definitions for each group can be given with their time of appearance as inferred from the fossil record. Viruses and other non-autopoietic entities are omitted from this five kingdom scheme since they do not meet the minimal criteria for living systems. Adoption of this type of classification scheme, which most closely reflects the evolutionary history, molecular biology, genetics and ultrastructure of extant life, requires changes in social organization of life scientists who, as botanists and zoologists (e.g., "protozoologists", "parasitologists"), still behave as if there were only two kingdoms (plant and animal). The parasitologists, especially the Scandinavians with their optimal relations with both eastern and western scientists, have an opportunity to lead the global community of biologists and related researchers in the difficult task of putting our systematic and evolutionary house in order.

Conclusion

"Parasitism" and "parasitology" are ecological descriptions of ontogenetic outcomes between members of at least two species. They are anachronistic and invalid terms from the molecular biological perspective that recognizes the importance of environmental conditions on the establishment and maintenance of associations. Such anthropocentric and restrictive labels as "parasite" and "pathogen", which impede the recognition of the extent to which symbiosis is a major mechanism of taxa origin, require replacement by "autopoietic entity", "symbiotroph", "necrotroph", "holons that form holarchies" and other non-prejudicial descriptors of organismic characteristics and relationships.

GENE TECHNOLOGY AND RIBOSOMAL RNA AS DIAGNOSTIC AND TAXONOMIC TOOLS IN MICROBIOLOGY

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Introduction

Techniques used in molecular biology, often referred to as gene technology, have revolutionized life sciences. These techniques are today used not only in basic research, but also in applied research and in routine laboratories for diagnosis of genetic diseases, tumour related diseases, and infectious diseases. The hybridization reaction, for instance, by which two single stranded and complementary nucleic acid molecules can form a duplex molecule with very high specificity, has been known and used in research laboratories for more than 30 years. DNA probes have during the last 10 years also been utilized in hybridization experiments in diagnostic laboratories. Restriction enzyme analysis is another technique which has been used for many years in research, and recently also in diagnosis. Restriction enzymes are endonucleases which cleave double stranded DNA at specific sites. The recognition site of a restriction enzyme is usually a palindromic sequence of 6 nucleotides, but other types of recognition sites also exist. The high specificity of a restriction enzyme can be utilized in restriction enzyme analysis for diagnostic or epidemiologic purposes. *In vitro* amplification by the polymerase chain reaction (PCR) is a method by which a segment of a DNA molecule can be enzymatically amplified with a thermostable DNA polymerase. The specificity of the system can be very high and, the PCR may therefore be utilized in diagnostic procedures. The specificity is determined by the PCR primers, which are short oligonucleotides complementary to the flanking regions of the segment to be amplified. The PCR reaction is sensitive to mismatched base pairs in the 3'-end of the PCR primer. PCR is today an indispensable tool not only at research institutes, but also in many diagnostic laboratories.

Diagnostic systems based on sequences of ribosomal RNA (rRNA) or rRNA genes (rDNA) have properties which are particularly useful for infectious diseases caused by microorganisms (except viruses).

Ribosomes and ribosomal RNA

The ribosomes constitute the protein synthesizing machinery of the cell and are composed of proteins and rRNA. The ribosome is universal in all life forms (except viruses) and is composed of two subunits, the small and the large subunit, which in eucaryotes are also referred to as the 40S and 60S subunits. The small subunit contains 33 proteins and one rRNA species (18S), and the large subunit contains about 45 proteins and three rRNA species (5S, 5.8S and 28S). The length of the 18S rRNA is about 2000 nucleotides. The 18S and the 16S rRNA in eucaryotes and procaryotes, respectively, are collectively referred to as the small subunit rRNA (srRNA). The 18S, 5.8S and 28S rRNAs are transcribed from an rRNA operon into a 45S rRNA precursor. The precursor rRNA is then processed to the three mature rRNAs. The 5S rRNA molecule is in eucaryotes transcribed from another operon. The number of rRNA operons varies in different organisms.

Ribosomal RNA as a phylogenetic tool

Ribosomal RNA is perhaps the most versatile molecule for phylogenetic studies (1, 2). There are several reasons for this. The ribosomes are ubiquitous and have the same important function in all cells. Certain components of the ribosome, for instance the srRNA molecules, are accordingly under the same evolutionary pressure in all organisms, which is essential for drawing correct phylogenetic inferences. The sequences of the rRNAs are composed of regions of different evolutionary variability, which makes it possible to study a large span in the evolution. There are highly conserved regions (Universal regions) with very few nucleotide substitutions in different species. There are also highly variable regions (Variable regions) where many nucleotide substitutions have occurred also between closely related species (or even subspecies). Finally, there are regions of intermediate variability (Semi-conserved regions), which are essentially the same for closely related species within a family or a genus. Eucaryotic cells have 8 variable regions (V1-V9). The variable region corresponding to V6 in procaryotic srRNA is lacking in eucaryotes (3). Region V4 is in eucaryotes the most variable region, and the length of it varies between 230 and 500 nucleotides and there are even eucaryotes which lack V4 (4).

So far, it is the srRNA molecule which has been mostly used for phylogenetic inferences, because it contains much more sequence information than the smaller rRNA molecules, and it is still possible to determine the complete sequence of it without too much effort. Due to the great interest in using rRNA sequences in phylogenetic studies, a lot of sequences have been deposited in the data bases for nucleotide sequences, and a special database for rRNA sequences has been created (5).

Ribosomal RNA as a diagnostic tool

Probes complementary to rRNA, so-called rDNA probes, have proved very useful for detection and identification of microorganisms (6). There are 10^4-10^5 ribosomes in a rapidly dividing cell, and rRNA is also present in the corresponding high copy number. A diagnostic system based on rDNA probes will therefore have a high sensitivity. A diagnostic system based on rRNA sequences is very stable and can most probably be used for detection of all "variants" of the species that it was designed for, because the primary structure of rRNA is comparatively conserved. The sensitivity of a diagnostic system based on radiolabelled oligonucleotide probes complementary to rRNA in direct filter hybridization experiments is about 10^3-10^4 organisms (at least for bacteria) applied onto the filter. This sensitivity is sufficient for certain clinical applications, but is too low for many purposes, and one possibility of increasing the sensitivity is to use PCR for in vitro amplification of the target molecule. Conventional PCR can be used to amplify a segment of the rRNA gene, but it is also possible to amplify directly a segment of rRNA by PCR with reverse transcriptase in the first cycle of the process. This will further increase the sensitivity of the system.

Determination of the primary structure of ribosomal RNA

Sequence information is essential for the design of a diagnostic system based on oligonucleotide probes or PCR. Furthermore, it is important to have sequence information, not only for the species to be detected, but also for closely related species which are likely to be found in the same ecological niche, to avoid cross-hybridization or cross-amplification of these species. This is particularly important for rRNA based systems, because of the conserved nature of the molecule. Sequence information of

rRNA can be obtained fairly easily, since PCR primers or sequencing primers can be designed for the universal regions in the proximity of the variable region(s) to be sequenced.

Direct rRNA sequencing: The sequence of rRNA can be determined by the dideoxynucleotide chain termination method with reverse transcriptase. This method is comparatively simple and rapid, but the sequence information is often incomplete due to the secondary structure of the rRNA molecule.

Cloning and rDNA sequencing: This method is more cumbersome since cloning is involved, but the sequence information obtained is more complete and often very accurate. Only one of the operons is usually sequenced by this procedure, and it is important to keep in mind that microheterogeneity might exist between the rRNA operons.

In vitro amplification and rDNA sequencing: The fastest method to generate rRNA sequences is to sequence the PCR-product after in vitro amplification of rRNA genes. Solid phase sequencing can be utilized if one of the PCR primers is biotinylated (7). This procedure can be further automated with robotic work stations and by automated laser fluorescence sequencing for very efficient generation of sequence data. This technique has been applied to 16S rDNA sequencing of bacteria (8).

Applications in parasitology

The number of articles referring to the use of gene technology in parasitology is rapidly increasing, and only a few representative examples can be mentioned here. DNA probes have been constructed for several parasites, for instance *Plasmodium* sp., *Leishmania* sp. and *Trypanosoma cruzi*; for a review, see (9). One of the first parasites for which rRNA was utilized as a target molecule for identification probes, was *Plasmodium* sp., and different species specific rDNA probes have been designed (10). *In vitro* amplification by PCR has also been utilized in parasitology, and for a review see (11). *Giardia muris* is an example of a protozoan parasite from which the complete rRNA operon has been sequenced, and the sequence data have been used for phylogenetic discussions (12).

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EPIDEMIOLOGY AND CONTROL OF HUMAN HELMINTHOSIS

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Intestinal helminthoses are amongst the most common of chronic infections in children and in the poorest societies. Such high prevalence does not necessarily imply that these infections are of importance to public health or justify a need for control. Instead, it raises two questions: why are the morbid consequences of infection not more commonly recognised, and is control on such a large scale affordable and sustainable by developing societies?

In addressing the first of these questions the presentation will focus on the insidious nature of helminthic disease, its developmental rather than clinical implications, its consequences for intellectual as well as physical health, and its resulting under-recognition by public health services. In addressing the second question, emphasis will be given to the careful targeting of interventions, to the need for a multiple-species approach, and to the results of cost effectiveness analyses. The implications will be discussed with respect to the Partnership for Child Development, an international programme which seeks to improve the health and educational achievement of children through school based services.

IMMUNOLOGY OF HUMAN PARASITIC DISEASES: EXPERIENCE GAINED FROM HIV/AIDS-PATIENTS

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The immunological abnormalities that develop in HIV-infected individuals are qualitatively and quantitatively so severe that they render the individual susceptible to a wide range of parasitic infections. However, the HIV-infected patients are not susceptible to all parasites, since only parasites that can exploit the immune defects produced by the HIV-virus, will be able to flourish. Thus, epidemiological, clinical and immunological studies of AIDS-patients have given valuable information on the immunology of parasitic diseases in man, and have also served as a stimulus for experimental research.

DIAGNOSIS OF SCHISTOSOMOSIS: OPTIMISATION OF MAGNETIC BEAD ANTIGEN CAPTURE ENZYME IMMUNO-ASSAY (MBAC-EIA) FOR USE IN ZIMBABWE AND A COMPARISON WITH DIAGNOSIS BY EGG COUNTING

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Objectives

The main aim of the study was to establish the MBAC-EIA in Zimbabwe and assess its potential use as a field test for diagnosis of schistosomosis.

Methods

The MBAC-EIA was developed in Oslo as a serodiagnostic test for the circulating anodic antigen (CAA), a schistosome antigen. Monoclonal antibodies (Mab) against CAA (produced in Leiden) were bound to magnetic beads. CAAs in serum samples bind to these bound antibodies, and are detected by using the same Mab labelled with alkaline phosphatase. When substrate is added, a colour is produced, the intensity of which is proportional to the amount of CAA present in the serum sample.

Using the MBAC-EIA, the effects of temperature, incubation time and assay design were determined. Conditions were optimised to allow the assay to be carried out within 2-3 hr at room temperature, using 25 µl of serum or whole blood. In order to compare the MBAC-EIA with diagnosis by egg counting, serum samples from individuals with known egg counts were assayed by the MBAC-EIA. Samples were obtained from both *Schistosoma haematobium* and *S. mansoni* endemic areas. Controls included sera from non-endemic areas within and outside Africa.

Results

A high specificity (close to 100%) was found for the MBAC-EIA, and a significant correlation between egg count and antigen concentration ($r=0.61061$ $p<0.001$) was noted.

Conclusion

The MBAC-EIA appears to be a possible alternative to egg counting for diagnosis of schistosomosis. Further comparisons between the methods are necessary; the MBAC-EIA also needs to be tested under field conditions.

THE ROLE OF BASOPHILS IN *SCHISTOSOMA MANSONI* INFECTION. DIFFERENCES BETWEEN RESISTANT AND SUSCEPTIBLE INDIVIDUALS WITH RESPECT TO THEIR IGE AND IGG_4 RESPONSE

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Objectives

The objectives of this study were: 1) to examine the role played by IgE and IgG_4 with regard to resistance and susceptibility to reinfection with *Schistosoma mansoni*; 2) to examine the role played by histamine release in relation to intensity of infection and antibody response; 3) to study the possible effect of praziquantel treatment of *Schistosoma mansoni* infected individuals on the above parameters; and 4) to develop a histamine micromethod which may be of importance in pathology assessment and diagnosis of the disease.

Methods

Occupationally hyper-exposed Sudanese canal cleaners were examined. A longitudinal study was carried out to investigate the role of humoral immunity in these workers. According to the parasitological examination and follow-up, two sub-groups were identified: A resistant group, which remained negative for up to one year after the infection had been cleared with praziquantel treatment (n=16), and a susceptible group, which acquired a new infection after treatment (n=14).

Blood samples were collected before and 3 months after praziquantel treatment. Total and specific antibodies were determined by the ELISA technique. Another function of IgE and IgG_4 was examined through their ability to sensitize umbilical cord blood basophils and the subsequent histamine release after stimulation with schistosomosis antigens. Direct histamine release experiments were also performed on whole blood from schistosomosis patients.

Results

Our results suggest that IgE plays an important role in protection against schistosomosis, as also shown recently by Hagan *et al.* (1991) and Butterworth *et al.* (1992). Thus, in our study there was a statistically significant negative correlation between IgE and infection intensity ($r=-0.82$, $P<0.01$). The significant increase in histamine release after praziquantel treatment, together with the significant decrease in IgG4 in the resistant group, demonstrated the effectiveness of treatment in regulating the immune response. The direct histamine release experiments demonstrated the sensitivity of the histamine micromethod, and the possibility of using this method for a better understanding of the role of IgE in parasitic infections. A better understanding of the modulation of immediate hyper-sensitivity responsiveness in parasitic infections may also bear significantly on prospects for improving the immunological control of atopic diseases.

THE MOLECULAR MECHANISM OF RESISTANCE TO THE SCHISTOSOMICIDES OXAMNIQUINE AND HYCANTHONE

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Objectives

Hycanthone (HC) is now an obsolete drug for the treatment of schistosomosis, but oxamniquine (OXA), which has the same mechanism of action, is still one of the two compounds in use for *Schistosoma mansoni* infections. Unfortunately, OXA is inactive against other schistosome species. In addition, OXA-resistant schistosomes have been isolated. A better knowledge of drug mechanisms should permit the design of analogs capable of overcoming the above limitations.

Methods

In addition to standard parasitological techniques, specific procedures have been described in our previous publications and references can be found in a recent review (Parasitol Today 1993; 9: 162-6).

Results and discussion

HC and OXA cause an irreversible inhibition of nucleic acid synthesis in sensitive schistosomes, while the inhibition was only transient in resistant worms. A short contact with the drug *in vitro* followed by repeated washes led to a delayed parasite death 10-15 days later. This suggests that the drug establishes a covalent bond with some vital parasite component, presumably a nucleic acid. When drug-resistant *S. mansoni* were crossed with drug-sensitive parasites, the progeny were invariably sensitive. Results of the F_2 generation and of backcrosses confirmed that resistance was a recessive trait, thus indicating that drug activity cannot be exerted in the absence of some critical parasite factor.

We propose that HC and OXA are enzymatically transformed by sensitive (not by resistant) schistosomes into a reactive ester which, upon dissociation, yields an electrophilic moiety capable of alkylating parasite DNA. Several pieces of evidence support this hypothesis. An artificial HC ester was synthesized and was shown to be active against sensitive and resistant worms. Tritiated HC and tritiated OXA were shown to form covalent bonds with deoxyguanosines in the DNA of sensitive worms, but not in resistant parasites. An enzymatic activity effecting the covalent binding of tritiated drugs to DNA was detected in extracts of sensitive schistosomes and was absent in resistant worms. Preliminary results indicate that this enzyme is a sulfotransferase. The molecular cloning of the enzyme is under way.

Conclusion

A detailed study of the activating enzyme structure should permit the design of drugs recognized by the enzyme variants present in resistant and in naturally insensitive schistosomes.

MODIFIED ELISA FOR HYDATID SERODIAGNOSIS: THE POTENTIAL OF PERIODATE TREATMENT AND PHOSPHORYLCHOLINE INHIBITION

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Objective

The presence of carbohydrate moieties and phosphorylcholine in the antigen may be involved in immunological cross-reactions. In an attempt to improve the sensitivity and specificity of an ELISA designed to diagnose hydatid disease, the carbohydrate moieties of the antigen were destroyed by sodium periodate treatment and/or antibodies with specificity for phosphorylcholine were blocked by addition of free phosphorylcholine.

Materials and methods

The sera examined were from patients with either confirmed hydatidosis, non-hydatid tapeworm infections, or other disorders, and from healthy donors. The sera were collected in Uruguay (n=73) and in Sweden (n=112). ELISA was performed with lyophilised fertile bovine hydatid cyst fluid as antigen.

Results

When ELISA was performed with sodium periodate or free phosphorylcholine, the sensitivity increased, but the specificity decreased. Western blot analysis showed reduced recognition of a 38 kD antigen following both treatments, and also of a 15.5 kD antigen after periodate treatment. The use of free phosphorylcholine in the assay resulted in an apparently increased recognition of two antigens, 26 and 50 kD, which may represent cross-reacting epitopes. The cross-reaction obtained in sera from patients infected with non-hydatid tapeworms was not eliminated by the various modifications of the ELISA.

Conclusion

To obtain an increased sensitivity and to avoid a decreased specificity a combination of basic and modified ELISA has to be used.

SERODIAGNOSIS OF HYDATID DISEASE: DYNAMICS OF CIRCULATING ANTIGENS AND ANTIBODIES IN THE SERA OF MICE EXPERIMENTALLY INFECTED WITH *ECHINOCOCCUS GRANULOSUS*

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Objective

Mice were used as an experimental model to imitate re-infection occurring in humans after surgical leakage of protoscoleces from the hydatid of *Echinococcus granulosus*. The anti-hydatid antibody production and the presence of circulating antigens were examined after infection with dead or alive protoscoleces.

Materials and methods

Balb/c mice in Group I were each inoculated intraperitoneally with 2000 living protoscoleces, of which 0.25-10% had developed into cysts 16 months post infection (p.i). Mice in Group II were each inoculated with 2000 dead protoscoleces. The specific antibody concentration was measured by ELISA, using fertile bovine hydatid cyst fluid as antigen. The antibody response against non-carbohydrate antigens was determined after sodium periodate treatment of the antigen. The antibody response to carbohydrate antigens was obtained by subtracting the response to periodate-treated antigen from the response to untreated antigen. In order to measure the circulating antigens a sandwich-ELISA was developed.

Results

In Group I, the antibody response to carbohydrate antigens showed 3 peaks: at 19-25, 29-41 and 43-54 weeks p.i., respectively. The highest antibody concentration was measured in the second peak. As to the response to non-carbohydrate antigens in Group I, only one peak could be recognized, at 39-50 weeks p.i. In Group II, only one peak, at 19-26 weeks p.i, was observed against carbohydrate antigens, whereas no response against non-carbohydrate antigens was seen. Circulating antigens were observed in Group I mice, but not in Group II mice. The highest amount of antigens was measured from 1-21 weeks p.i, but even one year after infection some circulating antigens were detected.

Conclusion

Detection of circulating antigens may be an early marker of post surgical re-infection. By examining sera for both circulating antigens and antibodies the possibility for immunodiagnosis of hydatidosis seems to be increased.

GASTEROSTEUS ACULEATUS AS A RESERVOIR FOR *ICHTHYOBODO NECATOR* IN WESTERN NORWAY

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Ichthyobodo (Costia) necator continues to plague smolt production in Norwegian salmon *(Salmo salar* L.) aquaculture and this protozoan, or a sibling species, is occasionally responsible for mass mortalities on the marine side. Although much has been learned about how the parasite interacts with its host and the host pathology, little progress has been made in understanding the natural ecology, epidemiology, or life cycle of this parasite. As to therapy or prophylaxis, formalin is still being used, even though the resistance to this treatment is increasing.

I. necator is apparently euryxenous, and is generally noted only when it reaches epizootic proportions in aquaculture. Although it is generally accepted that *I. necator* survives best at temperatures around 20° C, it is clear from Norwegian studies that this parasite may be epizootic even at temperatures just above the freezing point. Even though *I. necator* is a major cause of mortalities in smolt in western Norway, it has not been recorded from wild salmonids in the same area (2, 3). This is important, because in this area there are commonly only 5 fish species present: *Salmo salar*, *S. trutta*, *Salvelinus alpinus*, *Anguilla anguilla* and *Gasterosteus aculeatus.*

Objectives

The primary objective of this study was to elucidate the role of *G. aculeatus* as a reservoir of *I. necator* in watercourses in western Norway. Secondary objectives were to obtain details on the natural ecology and epidemiology of *I. necator* as a basis for a better understanding of its interaction with aquacultured salmon.

Methods

Specimens of *G. aculeatus* were taken by unbaited, clear plastic traps. At least thirty specimens from each sample were placed in 4 % formalin for later microscopic investigation.

Results

I. necator was present on *G. aculeatus*, primarily on the skin and fins, throughout the sampling period. This corresponded to the period when "costiosis" occurred in a nearby smolt production facility. *I. necator* was not found on other fish in the watercourse.

Conclusions

G. aculeatus appears to be the primary reservoir for *I. necator* in watercourses in western Norway, and thus the primary source for outbreaks of costiosis in aquacultured salmonids in this area. Ongoing work with this host/parasite system may lead to a clarification of the parasite's life cycle and natural ecology.

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GYRODACTYLUS SPP. ON THE FINS AND SKIN OF WOLFFISH (*ANARHICHAS SPP.*) IN NORTHERN NORWAY

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During the spring of 1992, wild Atlantic wolffish, *Anarhichas lupus*, were caught off the coast of northern Norway. An examination of the skin and fins of the fish revealed hundreds of gyrodactylids. For taxonomical studies, specimens of these parasites were prepared using the ammonium picrate-glycerine method.

A closer examination revealed two different species of *Gyrodactylus*. Both species belonged to the *Gyrodactylus perlucidus*-group Malmberg, 1970. Based on haptoral sclerite morphology, one species was very similar to *G. errabundus* Malmberg, 1970, while the other species differed markedly from both *G. perlucidus* Bychowsky et Polyanskii, 1953 and *G. errabundus*. A detailed description of the latter species will be presented.

In October 1992, gyrodactylosis was observed on the skin and fins of spotted wolffish (*A. minor*) and Atlantic wolffish in a fish farm in northern Norway. All the examined specimens seemed to be identical to the *Gyrodactylus errabundus*-like species that we had found earlier on wild Atlantic wolffish.

EXPERIMENTAL STUDY ON THE INFLUENCE OF PULP MILL EFFLUENT ON THE METAZOAN ECTOPARASITE COMMUNITIES OF ROACH (*RUTILUS RUTILUS*)

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Materials and methods

Eighty roaches were caught on 23rd May, 1992 in the oligotrophic, clean Lake Peurunka. All fish were kept in cages in this lake for two weeks, and then 35 of them were transferred to cages in the nearby Lake Vatia, which is influenced by pulp mill effluents. Before moving the fish, gill parasites were examined from 10 fish. Five *Dactylogyrus* species, a *Gyrodactylus* sp. and *Paradiplozoon homoion* were found, the main components of the infracommunities being dactylogyrids. Thereafter, five fish were studied weekly from both lakes between 1st July and 17th August. Metozoan parasites were recorded from gill arches, which were divided into four sectors along the dorso-ventral axis.

Results

Dactylogyrus crucifer, D. nanus, D. micracanthus and *D. suecicus* occurred in both lakes throughout the study; *D. caballeroi* was found only at the beginning of the experiment in the oligotrophic lake, and *D. vistulae* was only found in the polluted lake during the course of the study. *Gyrodactylus* sp., *Ergasilus briani* and *P. homoion* occurred in low numbers in both lakes.

Differences between the lakes were seen in the prevalence of *D. micracanthus*, which was higher in Lake Vatia, and in increased numbers of *D. crucifer* and *D. micracanthus* in Lake Vatia. Abundances of other *Dactylogyrus* species remained, on average, at the same level in both lakes. Surprisingly, no change was recorded during the experiment in the location of the two most common species, *D. crucifer* and *D. nanus*, on the host gills in either of the lakes, both species favouring the 2nd and 3rd gill arches, especially the inner parts of the gills.

The apparent lack of competition between the two most common *Dactylogyrus* species on the gills of roach indicates that no limitation of resources on roach gills occurred for dactylogyrids, even when the intensity of infection was high.

PARAMETERS OF INFECTION OF THE SALMON LOUSE *LEPEOPHTHEIRUS SALMONIS* ON SALMONIDS, PARTICULARLY WILD SEA TROUT, *SALMO TRUTTA* L., IN SCOTTISH WATERS

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Sea trout are of considerable economic value in Scotland, both as game fish, and as commercial catch. Catches of sea trout have been in decline for several decades and in some areas, this has become more severe in recent years. However, in Scotland, unlike the west of Ireland, there have been no substantiated reports of the premature return to fresh water of young sea trout, carrying high levels of *Lepeophtheirus salmonis* chalimus stages.

Sampling of Scottish sea trout for bacterial and viral pathogens, particularly those associated with intensive aquaculture, has not revealed a likely causative agent responsible for catch declines. In both Scotland and Ireland the regions most heavily involved in salmonid aquaculture are also those worst affected by a decline in sea trout catches. Popular opinion has created a link between the decline in sea trout catches and the presumed rise in availability of the infective stages of *Lepeophtheirus salmonis* due to increasing salmonid aquaculture.

For the last three fishing seasons we have recorded the infestation intensity of *L. salmonis* on Scottish sea trout. Meristic measurements on gravid female *L. salmonis* have revealed considerable variation between fishing sites, and also within the lice population on fish returning to the same site. We have shown differences in lice populations on the east and west coasts of Scotland using this method. In addition, preliminary studies indicate that the variation in both carapace length and egg length is greater for lice obtained from wild sea trout compared to those from cultured salmon.

During the 1993 fishing season we have obtained lice from wild sea trout and salmon, and also cultured salmon, from the same site. Lice from tank reared sea trout were also available. We will consider the possible effect of host species and culture method on the size of female *L. salmonis*, and attempt to evaluate measurement techniques as a simple method of identifying the source of lice populations on wild fish.

ECOLOGICAL AND PHYSIOLOGICAL EFFECTS OF SALMON LICE ON SALMONIDS IN NORWEGIAN FJORD SYSTEMS

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Salmon lice (*Lepeophtheirus salmonis*) cause serious problems for fish farming, leading to economic losses each year. During the last years severe attacks on wild fish in Norwegian fjord systems have also been reported.

The first part of the investigation was initiated in the summer of 1992. We recorded salmon lice on salmonids caught in fish traps in the rivers Imsa (Rogaland county) and Halselva (Finnmark county) and on salmonids caught along the Norwegian coast using floating nets and bag nets. High numbers of salmon lice on the fish were common.

In the second part of the investigation, beginning in the summer of 1993, we will examine the physiological effects of salmon lice infestations on salmonids. We will also try to quantify the effect of salmon lice on the survival of smolts of Arctic char (*Salvelinus alpinus*), sea trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*) in areas with and without fish farms. In addition, physiological investigations will be carried out in the laboratory in order to investigate the effect of salmon lice on the osmoregulatory and immunological capacity of salmonids. Results from this part of the investigation will be presented.

The results obtained so far show that salmon lice may be a threat to wild stocks of salmonids, and that a further study of the effects of this parasite is necessary in the years to come. Through physiological experiments we hope to identify which numbers of the different stages of salmon lice may lead to mortality in smolts and adult salmonids. Such knowledge will enable us to better assess the importance of salmon lice under field conditions.

INFESTATIONS WITH SALMON LICE (*LEPEOPHTHEIRUS SALMONIS*) CAUSE PREMATURE RETURN OF SEA TROUT POST SMOLTS (*SALMO TRUTTA*) TO RIVERS

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Objective

The aim of the present study was to examine if heavy salmon lice infestations may lead to premature return of sea trout post smolts to rivers.

Methods

The study was performed in River Lønningdal, in western Norway. Four hundred sea-trout smolts were caught in a fish trap situated at the river outlet. The fish were marked individually and divided into two groups of 200 smolts each. One group was supplied with filtered sea water to prevent salmon lice infestations. The other group was supplied with unfiltered sea water and thereby exposed to infective salmon lice larvae. After twelve days, all the fish were released in the sea, 200 meters from the river outlet. By that time, all the fish that had been supplied with unfiltered water were heavily infected with salmon lice, and about 25% of them were moribund.

Results

A higher proportion of post smolts infected with salmon lice as compared to uninfected fish returned to fresh water shortly after they had been released. Thirty minutes after the fish had been released, 94 of them were swimming towards the river outlet in a school. Only seven of these fish were from the uninfected control group.

Conclusion

The present study indicates that infestations with salmon lice are the main cause of premature return of sea trout post smolts to rivers.

PARASITES OF WILD ATLANTIC SALMON (*SALMO SALAR* L.) RETURNING TO THE WEST COAST OF NORWAY: WITH COMMENTS ON THEIR INTERACTIONS WITH AQUACULTURED SALMON

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Although recent emphasis on interactions between wild, native salmon and their aquacultured sibs is commendable, too little is known of wild salmon parasites to adequately address parasitological interactions. This is especially true for freshwater salmonids in Norway, and for marine salmonids in general. This report on parasites found in 45 fish returning for the spawning run of 1992 off Telavåg, Sotra, in western Norway is especially relevant in this connection, as this location is one of the centres of the Norwegian aquaculture industry.

Objectives

The objectives were: 1) to identify parasites of wild Norwegian marine salmon 2) to elucidate their epidemiology; and, 3) to determine the extent and/or potential of interactions of wild salmon parasites with aquacultured fish.

Methods

Fish were caught by weir to minimize the loss of skin and gill parasites which happens with the more common gillnetting. Each fish was examined after a modified version of the Dogiel complete technique, which included routine blood samples.

Results

Three protozoan species, 7 trematode species, 2 nematode species, 3 cestode species, 1 monogenean species, and 2 copepod species were found. Of these species, the myxosporidian *Myxobolus arcticus*, the ciliate *Trichodina* sp., and the monogenean *Gyrodactyloides bychowskii* have not previously been reported from wild salmon in western Norway (3). The *Trichodina* sp. appears to be previously undescribed. Four of the parasites described here, the cestode *Eubothrium* sp., the monogenean *G. bychowskii*, and the copepods *Lepeophtheirus salmonis* and *Caligus elongatus*, are known for their negative interactions with aquaculture salmon. A trichodinid, possibly the *Trichodina* sp. here, has also had a negative effect on *S. salar*. Some of the other species found in our study may also have a negative effect on their hosts.

Conclusions

Eighteen parasite species are reported from wild marine salmon on the west coast of Norway. Four of these species are known from Norwegian aquaculture. Three species are reported for the first time from wild salmon from this area, and 1 species may represent a new species. Sample size and sites need to be increased to gain a comprehensive picture of the epidemiology and interactions of salmonid parasites on the Norwegian coast.

THE EFFECT OF LIVER COCCIDIOSIS IN SOME MARINE FISH FROM SCOTTISH WATERS

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Objective

To investigate the effect of the liver coccidian *Goussia clupearum* in five of its fish hosts and establish the relationships between prevalence and intensity of coccidiosis with specific host and environmental factors.

Materials and methods

Samples of mackerel, *Scomber scombrus*, herring, *Clupea harengus*, Norway pout, *Trisopterus esmarkii*, poorcod, *T. minutus* and sprat, *Sprattus sprattus*, were collected from October 1988 to January 1991 from the North Sea and West coast of Scotland. Fish length, weight, age, sex, sampling place, date and area were recorded. Squash preparations of livers were examined and prevalence calculated as P(%)=number of infected fish/number of examined fish x 100. Seasonal and regional variations and variations of prevalence with age were studied whenever possible. Condition factors were calculated as K=body weight x 100/length^3, and correlated when possible with sex and intensity of infection. Standard statistical analyses were used to test the effect of intensity of coccidiosis and sex of the fish host upon condition factor, and the relationship between prevalence and both age of the host and sampling month.

Results and conclusions

Prevalence of *G. clupearum* was shown to have seasonal variations, with two peaks, one in late spring and another in autumn. These fluctuations of prevalence are thought to be related to the feeding activity of the fish through the year. The lower prevalence of *G. clupearum* infections in poorcod, and the absence of the coccidian in sprat might be due to the different feeding habits of these two fish species, compared with those of herring, mackerel and Norway pout, which have many food items in common. The influence of season on prevalence was statistically significant. Prevalence of liver coccidiosis varied with age. It increased from juveniles to adults. Between adult age groups it is uncertain how the prevalence evolves, though an initial rise was observed. However, due to the impossibility of collecting samples on a monthly basis for all the fish species, it is not known whether prevalence of liver coccidiosis rises continuously or decreases at a certain age.

Although uninfected fish as a rule had higher mean condition factors than infected fish, and higher intensities of infection with *G. clupearum* were associated with lower mean condition factor, no definitive conclusion could be drawn. Statistical analysis of condition factors related to the different levels of infection (uninfected, low, moderate and heavy infections) did not show a consistently significant effect of the infection level upon condition factor. Therefore it is considered that environmental and fish specific factors other than infection with the coccidian alone, or an interaction of those factors are involved.

EPIDEMIOLOGY OF *CRYPTOCOTYLE* SPP. ON CAGED ATLANTIC COD (*GADUS MORHUA* L.)

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Methods

Atlantic cod were caged about 70 meters from the shore in a fjord in Finnmark county (northern Norway). Every third month 50 fish were randomly sampled, killed immediately, and examined for metacercariae of *Cryptocotyle* spp. in the laboratory.

Results

The abundance of *Cryptocotyle* spp. metacercariae in the caged fish increased significantly during the 18 months of the study, and was significantly higher than in a comparative free-living cod population sampled at the end of the study. Overdispersion of metacercariae among the caged cods increased over the first nine months, but thereafter showed a pronounced decline. The duration of exposure to cercariae, i.e. time in cage, explained 30% of the variation in the number of metacercariae. Differences in susceptibility between individual cod probably influenced both abundance and distribution of metacercariae.

INFLUENCE OF WATER QUALITY ON INTERMEDIATE HOST SURVIVAL AND PARASITE TRANSMISSION

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Extremely low levels of infection with digenean eyeflukes, *Diplostomum* spp., have been recorded in roach, *Rutilus rutilus*, from a freshwater lake in central Finland receiving waste water from a pulp and paper mill. The sensitivity of the snail intermediate hosts, *Lymnaea* spp., to the effluent has been suggested as an explanation for this condition.

By laboratory experiments we have examined the influence of mill effluent and parasites, both independently and in combination, on the survival of naturally infected *Lymnaea stagnalis*. The viability of *Diplostomum spathaceum* cercariae exposed to the effluent was also investigated.

The effects of pollution on *Diplostomum* transmission to the fish host and the potential role of using these effects in monitoring temporal changes in water quality will be discussed in the light of these studies.

TRIAENOPHORUS CRASSUS MASS INFECTION: ARE CHANGES IN THE BEHAVIOUR OF THE FIRST INTERMEDIATE HOST INVOLVED?

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The occurrence of *Triaenophorus crassus* in whitefish, *Coregonus lavaretus*, from Lake Saimaa, SE Finland, was studied throughout the years 1991 and 1992; the prevalence and abundance being as high as 98% and 7 worms/fish, respectively. The mass-infection is suggested to be related to changes in the relative abundance of copepod species (the first intermediate host of *T. crassus*). These changes might have been caused by changes in the abundance of coregonid species, either by pollution or by regulation of the lake.

The extremely high transmission rate of *T. crassus* from copepods to whitefish in Lake Saimaa indicates that infected copepods are not included in the diet of whitefish only by chance. Our hypothesis was that the high prevalence is due to selective predation by whitefish on infected copepods. The aim was to examine whether *T. crassus* procercoids are able to affect copepod behaviour so that infected copepods become more vulnerable to fish predation.

TRANSMISSION OF METAZOAN PARASITES: A PISCIVOROUS FISH AND ITS PREY AS AN EXAMPLE

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Materials

Metazoan parasites in the body cavity and intestine and on the outer surface of the three-spined stickleback (*Gasterosteus aculeatus*) and burbot (*Lota lota*) from the Bothnian Bay, Baltic Sea, were studied after sampling throughout 1978. The three-spined stickleback is known to feed on a great variety of invertebrates. The burbot studied were piscivorous due to their large size. Stickleback is among the most frequently consumed food items of burbot in the study area.

Results

The numbers of parasite species found in three-spined stickleback and burbot were 14 and 22, respectively, of which 10 species occurred in both fish species. A transmission of 9 of these species from stickleback to burbot was apparent. Thus, higher prevalences in burbot as compared to the three-spined stickleback were recorded for *Triaenophorus nodulosus* larvae (57.8% versus 8.4%), *Echinorhynchus borealis* (4.8% versus 0.2%), *Raphidascaris acus* (62.7% versus 11.6%) and *Eustrongyrus* larvae (39.8% versus 1.2%). In contrast to this, the prevalence of *Diphyllobothrium dendriticum* was at the same level in both fish species, and most *D. ditremum* were not able to transmit to the burbot (prevalence of 4.8% versus 63.8%).

Sixty per cent of the stickleback specimens had only one or two parasite species, the maximum being five, and the most common combination was the two allopatric cestodes *D. dendriticum* and *Schistocephalus solidus*. Eighteen per cent of the sticklebacks were uninfected. All the burbots were infected, and half of them harboured 6-8 parasite species, with a maximum of 12 species.

The relationships between the parasite species were also studied and will be discussed in order to interpret the mechanisms of transmission.

SPREADING OF PARASITES IN RELATION TO DENSITY OF LIVESTOCK POPULATIONS

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Objective

The objective was to investigate the spreading of parasites on large scale collective farms (more than 200 head of cattle, more than 2000 pigs) and on small private farms (less than 10 head of cattle, less than 20 pigs).

Methods

We have carried out helminthological investigations of faeces and have used data from the State Veterinary Department.

Results

93% of the pigs and 37% of the cattle from large scale farms and 80% of the pigs and 47% of the cattle from small farms were infected with enterohelminths. Diseases of the digestive and respiratory tracts of younger animals, mastitis and metritis of adults treated with antibiotics or other antiparasitic drugs comprised 91.3-92% of the total disease rate. The disease rates of pigs and cattle were 3.6 and 2.9 times higher, respectively, on large scale farms than on small ones. Although 95-99% of all antibiotics, anthelmintics, and disinfectants applied were used on large scale farms, the animal husbandry production on these farms accounted for only about 50% of the total production. 59.9% and 7.7% of the cows were infected with the bovine leucosis virus on large and small scale farms, respectively.

Conclusion

The high density of livestock populations have been injuring evolutionary approved quantitative proportions in ecosystems and have caused parasitological problems.

EFFECT OF GASTROINTESTINAL NEMATODES ON APPETITE IN REINDEER *(RANGIFER TARANDUS TARANDUS)*

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Objective

Our objective was to assess the effect of naturally acquired gastrointestinal nematode infections on the appetite of female reindeer calves.

Methods

Eighteen six-month-old female reindeer with naturally acquired parasitic infections were divided into two groups of nine. Blocked randomization was used to provide comparable between-group distributions of live weight and parasite transmission stages per gram faeces. In November, one group (I) was injected subcutaneously with one ml of Ivermectin (Ivomec 1%; Merck, Sharp & Dome) and the other group (C) received one ml of an isotonic saline solution. The animals were fed a high protein reindeer diet (RF-80) ad lib, and food intake was recorded daily from 20 days before to 194 days after treatment. Live weights, serum concentrations of pepsinogen, rectal temperatures and estimated number of parasite transmission stages per gram faeces were recorded throughout the experiment. At slaughter (day 194), the intensities of gastrointestinal nematodes and other parasites were estimated.

Results

Food intake was significantly higher in group I from day 113 to the end of the experiment (t-test and repeated measures ANOVA). In the control group, there was a significant negative correlation between the food intake during days 160-194 and the intensity of gastrointestinal nematodes. However, there was no evidence of such a relationship between food intake and the other parasites found in the control animals.

Conclusions

Our data suggest that natural infections of reindeer with gastrointestinal nematodes may depress the appetite of the animals. A different and less conclusive result has been reported previously on the basis of data from the same animals during the earlier stages of this experiment (up to 63 days after treatment).

ECOLOGICAL INTERACTIONS BETWEEN GASTROPODS, MUSHROOMS AND THE NEMATODE *ELAPHOSTRONGYLUS RANGIFERI*

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The parasite *Elaphostrongylus rangiferi* use terrestrial snails and slugs as intermediate hosts. Reindeer, the final host, do not feed on gastropods, but get the infection by accidentally ingesting infected gastropods with their food. Here we examine the hypothesis that one important route of transmission is by infected slugs feeding on mushrooms.

Objectives

The aim of the study was to examine which gastropod species was most commonly associated with mushrooms, to describe activity patterns of slugs in relation to mushrooms, and to look for differences in behaviour patterns in infected and uninfected slugs.

Results

Arion subfuscus was the most commonly observed gastropod species associated with mushrooms. This species was most frequently found on mushrooms during the night. Experimentally infected *A. subfuscus* showed no significant difference in activity pattern or affinity to mushrooms compared to uninfected controls. The importance of these observations in relation to the transmission ecology of *E. rangiferi* will be discussed.

THE EFFECT OF INTESTINAL HELMINTHS ON THE BODY CONDITION OF EIDERS, *SOMATERIA MOLLISSIMA*, BEFORE INCUBATION

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Introduction

Female eiders do not feed during incubation, and reproductive success is therefore assumed to be directly related to stored energy reserves. Individual birds may, however, show large variations in body condition before incubation. Since this variation is closely associated with individual fitness, it is important to identify factors that affect body condition in the prelaying phase. We have examined the effect of intestinal helminths on female energy reserves.

Methods

Fifty-one eiders were shot before the incubation period. Helminths were removed from the alimentary tract and counted. Body fat of eiders was measured using an electromagnetic scanner after each bird had been minced in a food processor.

Results and conclusion

The abundance of digeneans and nematodes was inversely and significantly correlated with percentage body fat of eiders. The correlation between acanthocephalans and body fat was not significant. Digeneans and nematodes explained 25 % of the variation in body fat of eiders. It is concluded that intestinal helminths may be an important factor affecting female reproductive success.

DENSITY-DEPENDENT SEX RATIO IN *ECHINOMERMELLA MATSI* (NEMATODA), A PARASITE OF THE GREEN SEA URCHIN (*STRONGYLOCENTROTUS DROEBACHIENSIS*)

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Close to the Vega island on the coast of mid-Norway sea urchins of the species *Strongylocentrotus droebachiensis* were collected and examined for the nematode *Echinomermella matsi*. *E. matsi* show a sexual dimorphism in that females often become more than 50 cm in length, while the males never exceed 7 cm. Female nematodes were classified according to the degree of gonad development, males were recognised when they had developed spicules. Among 119 randomly selected infrapopulations of *E. matsi*, 70 infrapopulations had nematodes that could be sexed. The intensity of female nematodes was found to be low (<13) in all infrapopulations independent of the intensity of males and the total intensity of infection including juveniles, which could reach several hundreds. Moreover, the recruitment of females was low in infrapopulations already inhabited by more developed female nematodes. The results suggest that the intensity of females of *E. matsi* is regulated to a low level. The observed patterns indicate that the sex of *E. matsi* is determined by density, resulting in males when females already are present in the infrapopulation.

PREVALENCE OF *ANOPLOCEPHALA PERFOLIATA* IN HORSES IN CENTRAL SWEDEN - PRELIMINARY RESULTS

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Objectives

The tapeworm *Anoplocephala perfoliata* is localized to the ileo-caecal junction and the caecal mucosa of equines. Many reports have emphasized the potential pathogenicity of this parasite, but its prevalence in Swedish horses has not been known. The aims of the present work were to study the prevalence of *A. perfoliata* in horses submitted to the abattoir of Linköping in Central Sweden during a one-year period and to study possible pathological lesions associated with the presence of the parasite.

Materials and methods

From October 1992 until March 1993, the distal ileum, caecum and proximal colon from 222 horses of different ages (mean 12.8 yrs), breeds and sexes were examined. The numbers of tapeworms, their sizes, localization and associated lesions, as well as previous anthelmintic treatments, were recorded. Faeces from the horses were examined for *A. perfoliata* eggs by a modified McMaster technique, using 30 g of faeces.

Results

A. perfoliata worms were found in 64% of the horses examined, and there were only small differences in relative prevalence over time. The numbers of tapeworms ranged from 1 - 648, with a mean of 88. *A. perfoliata* eggs were found in 33% of worm positive horses. A large proportion of the horses with *A. perfoliata* showed a thickening and hyperaemia of the mucosa of the distal ileum, and necroses of the caecal mucosa were prevalent in association with attached tapeworms.

Conclusions

A. perfoliata is a common parasite of Swedish horses. Coprological investigation using standard techniques is insufficient for establishing the diagnosis. The pathological lesions observed in connection with the tapeworms suggest that further attention should be paid to this parasite.

INFLUENCE OF FLUCTUATING TEMPERATURES ON THE GROWTH AND PREDACIOUS CAPACITY OF SELECTED NEMATODE-TRAPPING FUNGI AGAINST THE FREE-LIVING LARVAL STAGES OF *OSTERTAGIA OSTERTAGI*

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Objectives

Recent research in Denmark has demonstrated the potential of nematode-trapping microfungi as an alternative or supplement to anthelmintic treatment of ruminants. A criterium in the selection of fungi as candidates for biological control is their ability to grow and capture nematode larvae in the dung environment, in particular during the early grazing season. The aims of this experiment were 1) to examine how simulation of temperature fluctuations, expected to prevail on pasture in Denmark, would affect the growth of selected nematode-trapping fungi; and 2) to test if such temperature fluctuations affected the ability of five of these fungi to reduce the number of third stage larvae (L_3) of *Ostertagia ostertagi*.

Materials and methods

Seven isolates of *Duddingtonia flagrans*, two isolates of *Arthrobotrys superba* and one isolate of *A. oligospora* were used. Growth on 1:10 CMA plates was recorded daily. Daily and weekly fluctuating temperature regimes simulated periods with increasing (spring), constant (summer) and decreasing (fall) temperatures in Denmark. Three *D. flagrans* isolates, one *A. superba* and one *A. oligospora* isolate were tested for nematode-trapping capacity in a dung pat bioassay. The cultivation of the fungi and the set-up of the bioassay were according to Larsen *et al.* (1). The number of eggs of *O. ostertagi* in the faeces used were adjusted to approximately 400 EPG. Five test dung pats for each fungal isolate and five control pats without fungal material were incubated for three weeks. Larvae were harvested by a modified Baermann technique.

Results

Both *Arthrobotrys* spp. had a higher growth rate than the *D. flagrans* isolates, and there was a clear species difference in growth. There was a tendency of isolate differences between the seven *D. flagrans* isolates. The reduction in L_3 after spring simulation was significant for only one *D. flagrans* isolate and one *A. oligospora* isolate. By the summer simulation, all five fungal isolates caused a significant reduction in L_3. The fall simulation gave numbers of L_3 equal to or higher than the numbers found in the control pats.

Conclusions

Although *Arthrobotrys* spp. showed a higher growth rate when tested on CMA, the dung pat assay showed that the use of selected *D. flagrans* isolates will be more appropriate in future field experiments. Thus, the choice of the right isolates of fungal species will be of importance in future studies on biological control of nematodes.

References

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A FIELD EXPERIMENT ON THE EPIDEMIOLOGY OF *OESOPHAGOSTOMUM DENTATUM* AND *HYOSTRONGYLUS RUBIDUS* INFECTIONS IN A HERD OF OUTDOOR-REARED PIGS IN DENMARK

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Objective

This study was performed to acquire basic information on some aspects of the epidemiology of *Oesophagostomum dentatum* and *Hyostrongylus rubidus* in outdoor-reared pigs in Denmark.

Methods

The study comprised three groups, each of six worm-free piglets. At 10 weeks of age, the piglets in group 2 were each experimentally infected with approximately 10.800 infective third stage larvae (L_3) of *O. dentatum*, whereas the piglets in group 3 each received about 8700 L_3 of *H. rubidus*. The piglets in group 1 were kept as non-infected controls. All pigs were reared together on an initially parasite-naive pasture, and were monitored for strongyle egg excretion, serum pepsinogen levels and bodyweight gain. The herbage infectivity was also monitored during the experiment, which lasted for 148 days. At the end of the experiment, the pigs were slaughtered and the stomach and large intestine of each pig were collected for worm recovery. In the data analysis, the groups were statistically compared using one-way analysis of variance.

Results

In all pigs, the egg excretion of *O. dentatum* gradually increased during the experiment, while that of *H. rubidus* remained at the same level after the initial increase. The herbage infectivity of both species showed a similar pattern, i.e. increased progressively during the experiment. The geometric mean worm burden of *O. dentatum* adults was significantly higher in the experimentally infected group 2 ($F=12.28$, $P=0.0007$) than in both the other groups. There were no significant differences ($p>0.05$) between the groups with respect to geometric mean worm burdens of *H. rubidus*, bodyweight gains and serum pepsinogen levels.

Conclusions

The pattern of herbage infectivity of *O. dentatum* and *H. rubidus* was similar to what is seen when cattle and sheep infected with trichostrongyles are reared on pasture. The pigs that were experimentally infected with *O. dentatum* had a higher worm burden of this parasite than the groups acquiring only a natural infection on pasture, possibly due to a persistence of the initial, experimental infection. Detailed and lengthy studies are required to cater the future parasitological needs on outdoor-reared pigs in Denmark.

THE POSSIBLE EFFECT OF DIET ON THE ESTABLISHMENT AND/OR SURVIVAL OF *ASCARIS SUUM* AND *OESOPHAGOSTOMUM* SPP. IN THE PIG

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Objective

In different studies on experimental infections of pigs with *Oesophagostomum* spp. and *Ascaris suum* it has been noted that pigs fed ground barley and protein supplement often acquired higher worm burdens and shed more eggs in their faeces than pigs fed commercial full constituent pellet fodder. The purpose of this experiment was to examine the possible effect of two types of common diets for pigs on faecal egg excretion and worm burdens after controlled infection with *A. suum* and *Oesophagostomum* spp.

Methods

Forty Landrace-Yorkshire crossbred piglets were weaned at 26 kg live weight and allocated, on the basis of weight and sex, to one of four equally sized pens with 10 pigs in each pen. All pigs were treated with fenbendazole at 5 mg/kg daily for two consecutive days before being moved to the pens. One group of 20 pigs (2 pens) was fed ground barley and protein supplement, while the other group (20 pigs in 2 pens) was fed full constituent pellet fodder. Both groups were fed according to a standard feeding regimen. After two weeks of adjustment to the fodder, the pigs in both groups were each infected with 6.000 *Oesophagostomum* spp. L_3 and 600 *A. suum* eggs through the fodder. Faecal samples were taken at weaning, 5 days pre-infection and every two weeks after infection until termination of the experiment. The pigs were weighed at monthly intervals. The pigs were slaughtered at day 84 and 91 after infection. The small and large intestines were removed and the numbers of *A. suum* and *Oesophagostomum* spp. worms were determined, using standard parasitological procedures.

Results

The infection became established in both groups. There was a marked and statistically significant difference in egg excretion between the groups. In the group fed ground barley and protein supplement, the excretion of *A. suum* and *Oesophagostomum* spp. eggs was 6-34 and 2-6 times higher, respectively, than the excretion of the group fed full constituent pellets. These observations were substantiated by post mortem worm counts, showing that pigs fed barley and protein supplement harboured a geometric mean of 5.1 *A. suum* and 1995 *Oesophagostomum* spp. worms, in contrast to 1.8 *A. suum* and 1331 *Oesophagostomum* spp. worms in the group fed full constituent pellets. There was no difference in weight gain between the groups.

Conclusion

In pigs fed commercial full constituent pellets, there was a significantly lower establishment and/or survival of both *A. suum* and *Oesophagostomum* spp. compared to pigs fed ground barley and protein supplement. The factors responsible for these observations remain to be substantiated.

PREDILECTION SITES OF *TRICHINELLA SPIRALIS* MUSCLE LARVAE IN EXPERIMENTALLY INFECTED FOXES (*ALOPEX LAGOPUS, VULPES VULPES*)

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Objective

It is well known that the predilection sites of muscle larvae of *Trichinella spiralis* depend upon the host species. However, there are few reports on the predilection sites of *T. spiralis* larvae in carnivores. For the diagnosis of a *T. spiralis* 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.

Methods

Eight male, arctic foxes (*Alopex lagopus*), and three male, silver foxes (*Vulpes vulpes*), all raised in cages, were inoculated with larvae of *T. spiralis* by a stomach tube. Four arctic foxes and one silver fox were inoculated with 500 larvae each, while the remaining animals were inoculated with 5000 larvae each. Six weeks after the inoculation the foxes were killed and tissue samples from 18 selected muscles/muscle groups, freed from all tendons and fasciae, were examined using a combined digestion and Baermann technique.

Results

No clinical symptoms were observed following the inoculation with *T.spiralis* larvae. The digestion techniques yielded positive results for nearly all the muscle groups examined (97%). The level of infection varied considerably between the foxes, where average numbers of larvae per gramme (l/g) showed values from 2 to 291. The number of muscle larvae did not exceed 660 l/g in any of the samples examined. The highest relative larval densities were found in the following muscles/muscle groups: The muscles of the eye (*M. rectus dorsalis/medialis/lateralis/ventralis & M. obliquus dorsalis/ventralis*), the lower part of the front leg (*M. flexor carpi ulnaris/M. extensor carpi radialis*), the hind leg (*M. gastrocnemius*), the tongue (*M. lingualis proprius*), and the diaphragm (*M. pars lumbalis/pars costalis*).

No significant difference could be demonstrated with regard to the predilection sites between the two fox species *V. vulpes* and *A. lagopus* (two-factor analysis of variance, $P>0.05$). Even though the higher inoculation dose (5,000 larvae) gave the higher average number of l/g, this tendency was not significant (two-factor analysis of variance, $P>0.05$).

Conclusion

The larval distribution in the present study was comparable with that of other studies on closely related wild-living carnivores, but different from experimentally infected herbivorous species. This observation supports the assumption that the relative density of *T. spiralis* larvae in particular muscles will depend on the functional importance of those muscles in that particular genus/species of host, i.e. whether the muscles are being frequently used or not.

THE CHEMOTHERAPY OF *GIARDIA* INFECTIONS: A CLINICAL TRIAL WITH BACITRACIN AND ITS ZINC SALT

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Objective

The awareness of drug resistance in cases of giardiosis is increasing, as are calls for the development of alternative medication to replace the present day drugs. Laboratory studies have shown zinc bacitracin to be ten-fold more active than the dodecapeptide antibiotic bacitracin, which has previously found service as an amoebacide. The present clinical trial was designed to determine the efficacy of both bacitracin and zinc bacitracin in the treatment of *Giardia* infections. In addition, neomycin has been shown to be synergistic with bacitracin in the treatment of amoebiosis. We therefore also included treatment groups given neomycin alone or a combination of neomycin and zinc bacitracin.

Methods

The study was performed according to EEC GCP guidelines as a prospective, randomised, open, Phase II clinical trial of a 10 day treatment with two daily doses of 120,000 U (USP) zinc bacitracin; 120,000 U (USP) bacitracin; 120,000 U (USP) neomycin sulphate; or 60,000 U (USP) zinc bacitracin and 60,000 U (USP) neomycin sulphate.

The drugs were administered orally as tablets. Stool samples were examined daily, if possible, during the treatment phase to determine when the drugs were active. All stool samples were read blind. Cure was determined on the basis of diagnostic results obtained for 2-5 stool samples collected in the 2-week period following completion of treatment, in accordance with the specific EEC guidelines (1993) for testing reagents on *Giardia* infections. Eighty-two persons, with or without symptoms of giardiosis, participated in the trial.

Results

Final cure rates were 87.5% for bacitracin, 94.7% for zinc bacitracin, 86.4% for neomycin and 87.5% for zinc bacitracin and neomycin. The treatment failure in the zinc bacitracin group was due to a relapse occurring with a known non-compliant patient.

Conclusions

All four treatment regimens provided acceptable levels of cure with side effects limited to nausea in a small number of subjects. No synergistic effect was noted for neomycin and zinc bacitracin. Zinc bacitracin appears to be a suitable alternative medication for the treatment of giardiosis.

TOXOPLASMA GONDII INFECTION AMONG NORWEGIAN AIDS PATIENTS

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Cerebral *Toxoplasma gondii* infection is a life-threatening complication to AIDS. Effective prophylaxis is available but the indications for this has not yet been established.

Objectives

In order to clarify the indication for prophylaxis we wanted to determine the incidence of cerebral *T. gondii* infection in a Norwegian AIDS population and to find out at what level of immunodeficiency this complication occurs.

Methods

This was a retrospective study of 598 HIV positive patients at Ullevål Hospital during the period from 1983 until April 1993 (50% of all HIV positive patients in Norway). The diagnosis was based on clinical and computer tomography manifestations, response to treatment and sometimes neuroautopsy. The patients were identified by a diagnostic computerbase.

As a measure of immunodeficiency T4 (CD4) cell levels were used. This marker is closely correlated to clinical signs of immunodeficiency in HIV infection. T4 levels are measured in all patients regularly (analysis by Dep. of Immunology); reference range: 0.3-1.5 x 10^9 cells/l. The T4 level closest in time to the diagnosis of cerebral *T. gondii* infection was used.

Results

176 patients had developed AIDS. Among these, 14 patients with cerebral *T. gondii* infection were identified (8.0%). Six of these 14 patients had this complication as their first AIDS defining event. The mean T4 value at diagnosis was 0.08 x 10^9 cells/l (median 0.02, range 0.00-0.28). All but one patient had T4 levels of less than 0.2 x 10^9 cells/l.

Conclusion

In a Norwegian AIDS population we found that 8.0% of the patients had developed cerebral *T. gondii* infection. This complication usually occurred at low T4 values (mean: 0.08 x 10^9 cells/l), and it may be the first AIDS defining event (in 6 out of 14 patients).

Prophylaxis should be considered in HIV positive patients with T4 levels of less than 0.2 x 10^9 cells/l.

LOCALIZATION OF *TOXOPLASMA GONDII*-BOUND COMPLEMENT C3 DURING HOST CELL INVASION

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Objective

The purpose was to localize complement C3 bound to *T. gondii* tachyzoites after contact with normal human serum by immunocytological staining.

Methods

The RH strain of *T. gondii* was used to infect mammalian cells in tissue culture. Cells were fixed in acetone or in paraformaldehyde. Normal human serum was added to fixed parasite cultures and bound C3 visualized using fluorescent anti-C3 antibodies. *T. gondii* tachyzoites were visualized using monoclonal antibodies against *T. gondii* P30 and P32 antigens. Polyclonal rabbit anti-*T. gondii* antibody was used as a reference.

Results

Staining of extracellular parasites was seen with anti-C3 antibody conjugate after serum incubation. No staining of intracellular parasites was seen. In addition, granular extracellular material binding C3 was seen. P30 and P32 were present in both intra- and extracellular parasites, but did not react with the extracellular, granular, C3-binding structures. However, the latter reacted with the rabbit anti-*T. gondii* antibodies. Failure of intracellular parasites to bind serum C3 was not due to lack of accessibility, as antibody-mediated complement binding was readily visualized. Also isolated parasites proved to be either complement-binding or not.

Conclusion

The results suggest that extracellular *T. gondii* tachyzoites bind C3, and that the bound C3 is released together with components of the tachyzoite surface upon entry of the organisms into host cells. The C3 bound to the surface of tachyzoites may contribute to their attachment to host cells.

SEROSURVEY FOR ANTIBODIES AGAINST BORRELIA BURGDORFERI IN DEER IN DENMARK

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Objective

The objective was to estimate the prevalence of antibodies against *Borrelia burgdorferi*, the Lyme Disease spirochaete, in deer in Denmark.

Materials and methods

A total of 145 blood samples were collected from male and female roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*) and red deer (*Cervus elaphus*). Sampling took place in connection with game shootings from November 1990 to December 1991. The presence of deer IgG-antibodies to a *Borrelia burgdorferi* strain (DK ECM 1) was examined by an indirect immunofluoroscense assay (IFA) at serumtiter 1:64.

Results and conclusion

Antibodies to *Borrelia burgdorferi* were found in 51% of the roe deer, 38% of the fallow deer and 27% of the red deer. The absolute numbers of seropositive animals are shown in Table 1. Differences between the 3 species were significant. There were no significant differences in seroprevalences between males and females.

The relatively high antibody prevalence indicates that deer are exposed to the Lyme Disease spirochaete in Denmark.

Table 1

Prevalence of antibodies against *Borrelia burgdorferi* in three species of deer in Denmark (number of seropositive animals/number of deer examined).

Species	Males	Females	Total
Roe deer	20/51	20/28	40/79
Fallow deer	5/15	6/14	11/29
Red deer	5/16	5/21	10/37
All species	30/82	31/63	61/145

MICROTIDAE AS A RESERVOIR FOR *BORRELIA BURGDORFERI* IN DENMARK

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Introduction

Studies in the USA on the reservoir of Lyme borreliosis clearly indicate that the most important animal reservoir in that country are rodents belonging to the family Muridae, and particularly to the genus *Peromyscus*.

Objective

The objective of this study was to identify the animal reservoir of *B. burgdorferi* in Denmark.

Methods

696 Microtidae and 360 Muridae were caught in Ugglan traps in 1990-1992 at various biotopes. The IFA technique was employed to demonstrate seropositive mice.

Results

The prevalence of antibodies against *B. burgdorferi* in the examined Microtidae and Muridae was 23% and 33%, respectively. The actual number of infected Microtidae and Muridae was 160 and 119, respectively.

Conclusion

The higher prevalence of *B. burgdorferi* in the Muridae as compared to the Microtidae, indicates that the first mentioned rodents are the most important mammalian reservoir of *B. burgdorferi* in Denmark. The prevalence in the Microtidae was, however, at a remarkably high level in comparison with the results of similar studies of others. But since the actual catch of Microtidae was 1.9 times higher than the catch of Muridae, from an epidemiological point of view, *Microtus agrestis* and *Clethrionomys glareolus* (belonging to the Microtidae) must be just as important as, or even more important than Muridae (in Denmark represented by *Apodemus sylvaticus* and *A. flavicollis)* as a reservoir for *Borrelia burgdorferi* in Denmark.

CRYPTOSPORIDIA IN RODENTS IN DENMARK

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Objective

Earlier investigations of cryptosporidiosis in Denmark have focused on the effects on humans and livestock (e.g. Holten Andersen *et al.*, J Infection 1984; 9:277-82; and Henriksen and Krogh, Nord Vet-Med 1985; 37:34-41). This study is the first report on the prevalence of cryptosporidiosis in rodents, and therefore the first indication of a possible reservoir group in Denmark.

Methods

727 rodents (453 Microtidae and 274 Muridae) were caught live in Ugglan traps from April 1991 to September 1992. Faecal smears were taken and fixed in methanol/1% HCl and thereafter stained by a modified Ziehl-Neelsen technique (Henriksen and Polenz). Specimens were considered positive with *Cryptosporidium* sp. when typically coloured oocysts were found. This gives a minimum estimate of the actual prevalence. Positive control slides were kindly provided by the State Veterinary Serum Laboratory.

Results

Rodent examined	Uninfected	Infected	Prevalence
Microtidae	416	37	8.2 %
Muridae	259	15	5.5 %
Both families	675	52	7.2 %

The overall prevalence of *Cryptosporidium* sp. was 7.2%. There was no statistically significant difference in prevalence between the families Microtidae and Muridae ($0.10<p<0.95$).

Conclusion

This study indicates that rodents might serve as a reservoir group for cryptosporidia. There are some problems with the exact identification of the *Cryptosporidium* species. The method used in this study did not stain a specific species. More investigations are needed before we can say whether the species found in rodents can infect humans and livestock or not.

EXPERIMENTAL *EIMERIA ALABAMENSIS* COCCIDIOSIS IN CALVES: ATTEMPTED CONTROL WITH TOLTRAZURIL

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Objective

Overwintered oocysts of *Eimeria alabamensis* have been identified as a cause of diarrhoea in calves in Sweden during their first two weeks on pasture. This study was designed to test the efficacy of toltrazuril in the prevention of *E. alabamensis* coccidiosis in experimentally infected calves.

Methods

Fifteen calves, aged three to six months, were divided into three groups (A, B, C) of five. Each calf was inoculated orally with 150 million sporulated oocysts of *E. alabamensis* per day for three consecutive days (on days 0, 1 and 2). Calves in groups B and C received a single oral dose of toltrazuril at 20 mg/kg on days ÷1 and 2, respectively. Group A calves served as untreated controls. The oocyst output, growth rates and clinical signs of the three groups of calves were compared during a 16-day-period.

Results

All fifteen calves developed clinical diarrhoea starting on day 3 (groups A and C) or day 4 (group B). The calves showed depression, poor appetite and weight loss. The oocyst output of group C calves (treatment on day 2) was, however, significantly lower than that of the untreated calves (group A).

Conclusion

In calves dosed with large numbers of *E. alabamensis* oocysts daily on three consecutive days, the administration of a single oral dose of toltrazuril (20 mg/kg) one day before or two days after the first inoculation did not prevent development of coccidiosis.

PRESENT DAY STATUS OF CHICKEN COCCIDIOSIS IN SWEDEN

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Introduction

Seven species of *Eimeria* may occur in the intestinal mucosa of domestic fowl, *Gallus gallus*. Four of these species, *E. brunetti*, *E. maxima*, *E. necatrix*, and *E. tenella*, are highly pathogenic, while the remaining species may cause subclinical disease with some economical losses. Coccidiosis is one of the most serious diseases in intensive chicken rearing, i.e. under crowded conditions. To prevent this disease, management systems have been introduced where the layers are raised in cages, while replacement and broiler chickens are given coccidiostats in their feed. However, the occurrence of coccidiostat-resistant strains of *Eimeria* is increasing, and in Sweden a new law may be implemented which may forbid the raising of layer birds in cages. Therefore, the adoption of new countermeasures against coccidiosis seem to be required in the future. To define these countermeasures, a good knowledge of the epidemiology of chicken coccidiosis in Sweden is necessary.

Material and methods

We have examined faecal samples obtained regularly from farms where the chickens were raised on the floor. The number of oocysts per gram faeces was determined after flotation and counting in McMaster counting chambers. Oocysts were isolated and stored in potassium dichromate, and purified either by single oocyst infection (*E. acervulina* and *E. maxima*) or inoculation of merozoites via the cloaca (*E. tenella*). When suspected outbreaks of coccidiosis occurred on the farms, the entire gastrointestinal tract of the birds were examined for coccidia.

Results

During a period of 12 months, 290 faecal samples were examined, of which 238 were found to contain one or more species of *Eimeria*. During this period, 13 outbreaks of coccidiosis were detected, of which 50% were in chickens 21-24 weeks old. In at least two of the outbreaks, the birds had been given coccidiostats in their feed. The occurrence of *E. necatrix* was suspected, but only *E. acervulina*, *E. maxima*, and *E. tenella* have been isolated and purified.

Conclusion

It is now clear that at least three of the pathogenic species of *Eimeria* occur in Sweden. The occurrence of coccidiostat-resistant strains is now a reality. Other measures than chemoprophylaxis, such as vaccination, must be sought. Although limited in number, our observations indicated that no outbreaks of coccidiosis occurred on farms where other systems than battery-cages were in use.

SEROLOGICAL DIAGNOSIS OF *NEOSPORA CANINUM* INFECTION IN DOGS

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Neospora caninum is a *Toxoplasma*-like cyst-forming coccidian parasite of dogs, cattle and other animals. In dogs the organism is a cause of congenital infection, resulting in central nervous system and muscular disease. Diagnosis is based on clinical signs and is verified by serological assays such as the indirect fluorescent antibody test. The identity of organisms observed at post-mortem investigation is established by immunohistochemical tests or by electron microscopy.

The use of the indirect fluorescent antibody test for establishing the diagnosis of clinical canine neosporosis will be presented, as well as preliminary results from a serological survey on the prevalence of *N. caninum* antibodies in dogs in Sweden.

SEROLOGICAL RESPONSE OF SHEEP EXPERIMENTALLY INFECTED WITH *THEILERIA HIRCI*, AS MEASURED BY THE INDIRECT FLUORESCENT ANTIBODY TEST (IFAT)

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Theileria hirci is a tick-borne protozoan parasite which is highly pathogenic to sheep. The parasite initially infects the lymphocytes, and subsequently the erythrocytes. *T. hirci* is closely related to *T. annulata* and *T. parva*, both of which may cause serious disease in cattle.

Objective and methods

Although the use of the IFAT for the detection of *Theileria* infections has been described, information about the level and duration of the antibody response in *T. hirci* infection is very limited. To study these aspects, six adult sheep were inoculated with an *in vitro* culture suspension of lymphoid cells infected with *T. hirci* schizonts. The same type of cell was used for the preparation of acetone fixed antigen slides. Sera were tested in two-fold dilutions in PBS, starting with 1:10. Negative control sera were obtained from the experimental animals prior to inoculation. The serum from a sheep experimentally infected with *T. hirci* and subsequently reinoculated with autologous infected cells, was used as a positive control serum.

Results

Reciprocal antibody titres at different days after inoculation are shown below:

Sheep	Days post inoculation												
No.	0	11	15	20	27	34	39	47	54	61	67	85	99
40	0	0	40	80	320	640	1280	320	640	640	640	640	320
41	0	0	320	320	640	1280	1280	1280	1280	1280	640	320	320
43	0	0	80	1280	640	1280	2560	1280	640	640	320	320	320
44	0	0	160	640	640	640	640	640	320	160	320	320	160
46	0	0	160	320	640	640	640	640	640	320	320	320	160
47	0	0	40	80	320	320	320	320	320	160	160	160	80

Conclusion

Bright fluorescence specific to the intracellular schizonts could be demonstrated in all experimental animals from day 15 onwards. All animals were still positive at the end of the examination period. No fluorescence was detected with the negative control sera.

CLINICAL PICTURE AND HISTOPATHOLOGICAL LESIONS IN RATS NATURALLY INFECTED WITH *NOTOEDRES MURIS*

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Introduction

Notoedres muris causes ear mange in rats. The skin response to the infection has been described and the life cycle of *N. muris* was first studied in the 1940ies. There are, however, no information on the sequence of events with regard to the clinical and pathological changes occurring throughout the course of the infection in rats.

Methods

Four litters of rats were studied. Three of them were born to dams chronically infected with *N. muris*, and one litter was born to uninfected parents. At three weeks of age the neonates were separated from their parents, and at five weeks of age they were subdivided according to sex. The animals were observed daily and any clinical signs of skin lesions were recorded. Skin biopsies from the pinnae and tails were taken at regular intervals after birth and were fixed in 10% formalin, sectioned and stained with haematoxylin-eosin and toluidine blue.

Results

Pruritus was first noticed when the neonates were 16 days old (dpp). Pinpoint large red spots were seen on the pinnae of some of the rats at 18 dpp. After another two weeks most rats showed red spots on the pinnae and tails. The lesions gradually increased in size. At two months of age some rats showed cauliflower like crusts on their pinnae.

The first microscopical changes were seen at 18 dpp on the pinnae and at 23 dpp on the tails. Slight acanthosis, hyperkeratosis, spongiosis, epidermal hydropic degeneration, crust formation and a mixed inflammatory cellular infiltrate with predominance of neutrophils in the dermis were seen. Mites were present in epidermal pockets in the stratum corneum. After 50 dpp all the lesions, except the acanthosis, became more pronounced. Lymphoid cells and eosinophils became the dominant inflammatory cells. Pustules, exocytosis and slight parakeratosis were seen. No increase in the number of dermal mast cells was found.

Conclusion

Neonatal infections were first seen clinically from 16 dpp and histologically from 18 dpp on the pinnae. Lesions on the tails were found a few days later.

CILIOPHORAN SYMBIONTS OF *GASTEROSTEUS ACULEATUS* L. FROM WESTERN NORWAY: WITH COMMENTS ON THE OBSERVED UPTAKE OF HOST CELLS BY TRICHODINIDS

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In a previous study of the parasites of three-spined sticklebacks from western Norway, emphasis was placed on trichodinid ciliates (1). Other ciliates recorded in that investigation were not identified to species level. Peritrich ciliates are usually regarded as ectocommensals, which may become harmful when the host resistance is reduced by other factors. Lom (2) demonstrated the mechanism by which trichodinids mechanically damage the host epithelium. However, to date there is no evidence that these ciliates are capable of taking in host cells or tissue fragments.

Objectives

The primary aim of this investigation was to identify the ciliophoran symbionts of three-spined sticklebacks from western Norway. Secondly, we wished to obtain further information concerning the ability of peritrich ciliates to act as parasites.

Methods

Numerous *G. aculeatus* were taken by clear plastic traps from 9 freshwater and 2 brackish water localities in western Norway. After capture, the fish were transported to the laboratory and kept alive in aerated plastic tanks before examination. In order to identify the symbionts, observations, measurements and photographic work were made on: 1) living specimens, 2) silver impregnated material, and 3) Diff-Quik stained gill smears.

Results

A total of 5 ciliophoran species belonging to 3 different subclasses have been identified so far: *Chilodonella* sp., *Capriniana piscium*, *Apiosoma amoebae* var. *cryptomicronucleata*, *Trichodina domerguei* subsp. *domerguei* and *T. tenuidens*. The finding of *Chilodonella* sp. on *G. aculeatus* seems to represent a new host record for this parasite. The uptake of host blood cells was observed in the gill trichodinid *T. tenuidens*.

Conclusion

Five ciliophoran species have been identified from three-spined sticklebacks from western Norway. A *Chilodonella* species was apparently recorded for the first time on *G. aculeatus*. *Trichodina tenuidens* was shown to be capable of taking in host blood cells.

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PARASITES AS BIOLOGICAL TAGS FOR COD, *GADUS MORHUA* L., IN NORTHERN NORWAY

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Objectives

Our objectives were to examine whether parasites could be used as biological tags (1) when studying the migration of cod between the Barents Sea and the fjords of northern Norway; and (2) when studying if a silled fjord may contain a stock of cod separate from the stock in adjacent areas.

Methods

A total number of 517 cods were taken in spring and autumn samplings from three different localities in northern Norway: the Barents Sea, one silled fjord (Balsfjord), and one open fjord (Malangen). The cods were examined for seven parasite species: the protozoans *Myxidium bergense*, *M. oviforme* and *Zschokkella hildae*, the acanthocephalan *Echinorhynchus gadi*, the digenean *Hemiurus levinseni*, the cestode *Abothrium gadi*, and the copepod *Lernaeocera branchialis*.

Results

There were statistically significant differences in the prevalence of most of the parasites between the Barents Sea and both fjords, while there was little difference in the prevalence between the two fjords. The results are interpreted and discussed in terms of stock discrimination and migration of cod in the study area.

RECENT ADVANCES IN SEALWORM LIFE CYCLE STUDIES

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Objectives

The life cycle of the sealworm, *Pseudoterranova decipiens*, is not completely understood. This study is part of a larger program aimed at determining which macroinvertebrates are the most important intermediate hosts of the sealworm.

Methods

Amphipods (*Amphiporeia virginiana*) were captured by dipnet from beaches on Sable Island, located 290 km east of Halifax, Nova Scotia in 1991 and 1992. 2020 and 2044 amphipods were captured and fixed in 5% glycerol and 70% ethanol in 1991 and 1992, respectively, dissected and examined microscopically. A sample of 5157 amphipods collected in 1992 was placed in a modified Baermann apparatus fitted with a 1 mm sieve, containing a solution of 7 g pepsin, 4 ml concentrated HCl, and 6 gm NaCl in 1000 ml water. The filtrate was examined periodically with a stereomicroscope and nematodes found were fixed in hot 5% glycerol in 70% ethanol.

Results

Five of the 2020 amphipods dissected in 1991 were infected with sealworm. In 1992, 2 of the 2044 amphipods had sealworm infections. Two sealworms, 1 *Paracuaria adunca*, and 1 *Ascarophis* sp. were found from the sample of 5157 amphipods enzymatically digested.

Conclusions

The amphipod *Amphiporeia virginiana* is a new host record for *P. decipiens*, *P. acuaria*, and *Ascarophis* sp. Previous studies suggest that mysids are more important than amphipods as intermediate hosts for sealworm. However, the amphipod *A. virginiana* inhabits subtidal waters along beaches of Sable Island, in close proximity to basking colonies of grey seals, the most important definitive host for sealworm. Crustaceans located close to seal haul-outs may be exposed to very high levels of infective stages of *P. decipiens*.

SIBLING SPECIES OF THE SEALWORM, *PSEUDOTERRANOVA DECIPIENS*, IN THE NW ATLANTIC

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Introduction

The sealworm, *Pseudoterranova decipiens*, has been shown to have three sibling species (1), two of which occur in the NE Atlantic. Investigation of the life cycle of *P. decipiens* of NW Atlantic origin indicated a difference between worms of grey seal (*Halichoerus grypus*) origin and those of harbour or common seal (*Phoca vitulina*) origin (2). Biochemical characterisation of larval worms in fish, using iso-electric focusing, has shown two different types (3). These two types of larva have been present in fish in different geographical locations in proportions corresponding to the type of seal present, suggesting that one type may reach maturity in the grey seal, the other in the harbour seal.

Methods

Adult *P. decipiens* were collected from grey seals and harbour seals. The uteri of gravid females were removed and placed, individually, into vials for later life cycle studies. The body was cut into two, with one half being examined using PAGE, the other using IEF.

Results

Previous life cycle studies were confirmed in that L_2 of harbour seal origin did not infect *Gammarus* spp. directly, whereas some of those of grey seal origin did infect *Gammarus* spp. directly, while others did not. Biochemical characterisation showed two protein profiles for adult female worms in grey seals and only one (similar to one of those in the grey seal) in the harbour seal.

Conclusion

Grey seals can act as the definitive host to two "sibling species" of *P. decipiens*, whereas harbour seals can be host to only one of these.

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LABIAL DENTICULATION IN ASCARIDOID NEMATODE PARASITES

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The superfamily Ascaridoidea [see classifications adopted by Hartwich (1974), Gibson (1983), Sprent (1983), Fagerholm (1991) and Petter (1992)] accommodates some 49 genera of nematode parasites. The systematic position of several of these still remains to be verified. In 28 of the genera, the species have a rim of labial denticles on the inner anterior labial surface. Labial denticulation is generally present in species of the Ascarididae (Angusticaecinae, parasites of reptiles and occasionally amphibians; Ascaridinae, parasites of land mammals; Toxocarinae, parasites of land mammals and birds) and the Anisakidae (mainly parasites of sea mammals and reptiles, but also occasionally in some birds and fishes). Labial denticulation is rare in the Heterocheilidae (mainly parasites of reptiles) and the Raphidascarididae (parasites of fishes mainly).

In order to carry out a study of homologies, or any evident convergence in different groups, certain species were examined with respect to their labial denticulation by scanning electron microscopy. The aim of the study was to provide additional data for a subsequent cladistic analysis of the superfamily. Particular interest was directed to some anisakids, such as the closely related genera *Contracaecum* Railliet & Henry, 1912 and *Phocascaris* Höst, 1932, which have been found to differ with regard to the presence or absence of labial denticulation. The systematic position of *Acanthocheilus* Molin, 1858, which possesses only a few labial denticles, is also discussed.

REGULATION OF IFN-γ, IL-2 AND IL-2R PRODUCTION AND Vß EXPRESSION DURING A MURINE *T. CRUZI* INFECTION

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Infection with *Trypanosoma cruzi*, the causative agent of Chagas disease, alters humoral and cellular immune responses, including the production of different lymphokines. The aim of this study was to examine the mitogen-induced expression of IFN-γ, IL-2 and IL-2 receptor (IL-2R) during the murine infection with *T. cruzi*.

We have previously described the modulation of IFN-γ, IL-2 and IL-2 receptor (IL-2R) at both mRNA and protein level in Con A stimulated spleen (SC) and lymph node cells (LNC) from *T. cruzi*-infected BALB/c mice.

During the acute stage of infection (21 dpi) the SC, in contrast to the LNC, did not show an accumulation of IL-2 and IL-2R mRNA. The proliferative response of the SC to Con A was also reduced. The ability of the SC to produce IL-2 and IL-2R mRNA was restored during the chronic stage of the infection at 60 dpi. SC obtained at 21 dpi expressed IFN-γ mRNA after 2 or 5 hours of Con A stimulation. Interestingly, IFN-γ mRNA was depressed after 16 hours of stimulation with Con A, showing that the kinetics of accumulation was altered. Preliminary data indicate that murine SC at 21 dpi stimulated with αCD3 or PMA+αCD3, have a pattern of mRNA accumulation similar to that observed after Con A stimulation. These mitogens induced IFN-γ, but not IL-2 and IL-2R mRNA production, as analyzed in Northern hybridisation or RNAse protection assays. Unstimulated cells from control or infected mice showed no expression of these transcripts. SC from mice at 21 dpi displayed a lower proliferative response to αCD3.

Taken together our results show that, during the acute phase of the murine *T. cruzi* infection, SC have a severely suppressed capacity to produce IL-2 and IL-2R mRNA, while the IFN-γ gene can be transcribed.

In order to study the expression of variable segments of T cell receptor at mRNA level, we are now examining the TcR Vß repertoire using a semiquantitative PCR methodology with primers and probes that are specific for 21 Vß TcR families.

IMMUNOFLUORESCENT DEMONSTRATION OF *GIARDIA LAMBLIA* CYSTS BY STAGE-SPECIFIC MONOCLONAL ANTIBODY

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A microscopic diagnosis of giardiasis is often difficult, since demonstration of the parasite may be affected by fluctuation in the number of cysts excreted. The use of immunological methods based on parasite- and stage-specific antibodies in the detection procedures, facilitates the demonstration of individual parasites, which are easily missed by conventional light microscopy.

Objective

The purpose of our study was to attempt to improve the sensitivity and specificity of parasite detection in stools by employing stage-specific monoclonal antibodies for the visualisation of *Giardia lamblia* cysts.

Methods

Monoclonal antibodies were produced by immunization with in vitro induced *G. lamblia* cysts. Indirect immunofluorescence (IFL), using undiluted hybridoma-culture supernatant as a source of monoclonal antibodies, and FITC-conjugated anti-mouse immunoglobulin was used for demonstration of parasites. 150 stool samples submitted for microscopic stool examination based on clinical suspicion of giardiasis, and 50 stool samples containing low number of *G. lamblia* cysts were examined.

Results

Hybridoma line 1E10 secreting IgG1 monoclonal antibody reacting with cyst-specific antigens of 19-22 kD present on the cyst wall and inside the encystation-specific vesicles in trophozoites was chosen for stool examination. Thirty five out of 150 stool samples were positive by IFL, whereas 15 were positive by light microscopy. In four out of 50 samples from the positive control group no cysts could be demonstrated by IFL in repeated examinations. The lowest number of *G. lamblia* detectable by IFL was 3-4 cysts per spot, while 30 cysts was the limit by light microscopy.

Conclusions

Monoclonal antibody-based demonstration of *G. lamblia* cysts in stools proved to be more sensitive than ordinary light microscopy. Specific IFL reaction with the cysts wall allowed identification of *G. lamblia* cysts.

A STUDY ON THE TRANSMISSION AND DISSEMINATION OF A *HEXAMITA* SP. FROM FARMED SALMON IN NORTHERN NORWAY

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Introduction

During the last three years a parasitic flagellate belonging to the family Hexamitidae has been found to infect farmed salmon in northern Norway. This protozoan parasite was sampled from the muscles of naturally infected fish in order to study its survival on different growth media and in cell cultures at different temperatures. Furthermore, we have studied the transmission of this *Hexamita* sp. between its hosts and its dissemination within its hosts.

Methods

Three groups of Atlantic salmon were exposed to a suspension of *Hexamita* sp. by oral injection, anal injection, and infected water exposure, respectively. The dissemination of the parasite within its host was studied by histological examination of the gills, fins, intestine, spleen, heart, kidney, liver and muscles of infected fish. The blood was also examined for the presence of *Hexamita* sp.

Results

The results indicated that the anal injection was the best way of establishing an infection with *Hexamita* sp. for experimental purposes. The parasite appeared to be disseminated in the fish via the blood stream.

FATAL RENAL COCCIDIOSIS IN WILD GOSLINGS - A CASE REPORT

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Renal coccidia are known from various waterfowl. *Eimeria truncata* is recognised as a serious disease agent in domesticated geese, *Anser anser domestica.* It can cause heavy mortality in goslings, particularly in crowded flocks under conditions of poor hygiene.

At the beginning of the waterfowl hunting season in August 1992, a group of hunters visited the small island of Päänpäällinen (surface measure 0.2 ha) located near Oulu, Finland. Päänpäällinen island is known for its nesting greylag geese, *Anser a. anser*. Other suitable nesting and grazing islands are situated at least 10 km from this rather isolated island. On Päänpäällinen island the hunters found 13 dead goslings and sent one of the birds to our laboratory for autopsy. The cause of death was found to be renal coccidiosis.

At a later visit to the island, only one gosling was found, and this bird was brought back to the laboratory for autopsy. In this bird also renal coccidiosis was diagnosed.

In order to recognise which species was responsible for renal coccidiosis, oocysts were put in 2% potassium dichromate solution for sporulation. Only a small portion (1%) of the oocysts was able to sporulate. The morphology of both unsporulated and sporulated oocysts was in agreement with the descriptions of *E. truncata* in the literature.

The greylag goose population density has become high on some small islands in Finland. The nesting season in 1992 started with very warm weather, but after mid-summer the weather turned cool, windy and rainy. Crowding of birds and rainy weather may have led to an accumulation of large numbers of coccidian oocysts from softened droppings, and this, together with inadequate nutrition, may have contributed to fatal renal coccidiosis.

Conclusion: Although no further cases apart from those on Päänpäällinen island have been reported, it is obvious that renal coccidiosis is one of the natural population regulatory methods for geese. Whenever climatic conditions are favourable for this disease, new outbreaks are to be expected in crowded populations.

PARASITES OF FARM MINKS AND FARM FOXES IN ICELAND

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Objective

The objective was to survey the internal parasites of mink pups *(Mustela vison)*, blue fox pups *(Alopex lagopus)* and silver fox pups *(Vulpes vulpes)* on Icelandic fur farms. This is the first organized study of the parasites of farmed minks and foxes in Iceland.

Methods

At the end of June 1991 and in July 1992, fresh droppings from 145 mink pups, 130 blue fox pups and 54 silver fox pups were collected on 19, 15 and 7 fur farms, respectively. The age of the pups varied between 8 and 10 weeks. The faecal samples were examined for the presence of protozoans and helminth eggs using the formalin-ethylacetat concentration method.

Results

The only parasites found were coccidians. There were three species of coccidia in minks and two species in foxes. In the mink pups we found *Isospora laidlawi* (with a prevalence of 19%), *Eimeria mustela* (14 %) and *Eimeria vison* (6%). About one third of the mink pups harboured one, two, or all three of these species.

In the fox pups we found *Isospora canivelocis* and *Isospora vulpina.* The prevalence of *I. canivelocis* was 9% in the blue foxes and 8% in the silver foxes. The prevalence of *I. vulpina* was 19% and 15% in the blue foxes and silver foxes, respectively.

Conclusion

All the coccidians found in this survey, their prevalences varying from 6% to 19%, are well known parasites of mink and foxes in Eurasia and America. However, none of the above mentioned species have been recorded previously in Iceland.

Icelandic mink and fox breeders will be encouraged to pay attention to these new results as some of the coccidian species found in this survey, e.g. *E. vison*, are pathogenic and may cause mortality amongst minks.

LONG-TERM STUDY OF *TOXOPLASMA GONDII* INFECTION IN A SWEDISH SHEEP FLOCK

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Objectives

Toxoplasma gondii is known as an important cause of infectious abortion in sheep. Since only a primary infection during pregnancy will result in foetal death, it is of interest to determine when and where sheep are likely to become infected. The aim of this work was to study the *T. gondii* infection rate over a six year period in a sheep flock kept under traditional Swedish husbandry practices.

Animals and methods

The flock of sheep studied was situated in central Sweden (Uppland) and consisted of 165-235 ewes. The sheep were housed from October to April and lambed in March and April. Individual serum samples were collected twice a year from 1985 until 1990, just before turn-out in spring and after housing in autumn. Sera were analyzed for antibodies to *T. gondii* by ELISA (1).

Results

The seroprevalence and incidence of *T. gondii* infection at each sampling occasion are shown in the table below.

Sampling time*	s-85	a-85	s-86	a-86	s-87	a-87	s-88	a-88	s-89	a-89	s-90	a-90
No. of samples	165	179	172	185	173	188	185	249	202	177	190	235
Prevalence (%)	30	18	18	10	12	16	15	30	31	28	27	45
Incidence (%)	-	6	0	1	0	9	1	22	3	2	0	30

* s=spring; a=autumn

Conclusion

The prevalence of *Toxoplasma* infection varied considerably over the 6 year observation period. The incidence was higher in the summer than in the winter, which indicates that infection with *T. gondii* predominantly was acquired at pasture.

Reference

1. Lundén A, Carlsson U, Näslund K. Toxoplasmosis and border disease in 54 Swedish sheep flocks. Seroprevalence and incidence during one gestation period. Acta Vet Scand 1992; 33: 175-84

TOXOPLASMA GONDII ANTIGENS: P32 IS DISTINCT FROM P30

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Objective

The purpose of the present study was to compare two *T. gondii* target antigens of about 30 kD recognized by monoclonal antibodies using immunoblotting, immunofluorescent staining of various strains, and development of stages. Both antigens have been shown to be constituents of the parasitophorous vacuole membrane (PVM) and to be present in the dense granules of the parasite.

Methods

We have produced monoclonal antibodies against a 32 kD *T. gondii* RH strain antigen by immunization with plasma from infected mice (Linder *et al.* Parasitology Research 1992; 78: 175). Anti *T. gondii* P30 monoclonal antibodies were the same as those described previously (Bonhomme *et al.* Exp Parasitol 1990; 71: 439).

Results

The difference in size between the two antigens was confirmed. P32 is absent from the T626 and M3 *T. gondii* strains. However, both P30 and P32 are present in the S48 vaccine strain. Both antibodies stained the pseudocysts of the RH strain in the livers of tested mice. By immunofluorescence, both target antigens were shown to be located in intracytoplasmic granules, apparently corresponding to the dense granules of the parasite. P30 was seen at the exposed surface of the parasite, but P32 was not. Anti-P30, in contrast to anti-P32, stained all parasite isolates, including the P32-negative T626. P30 also reacted with tachyzoites in infected human samples, which were negative after staining with anti-P32.

Conclusion

The results show that P32 is distinct from the major *T. gondii* surface antigen P30, even though these antigens have a similar size and are both present in the dense granules and PVM.

UNIDENTIFIED PROTOZOAN ORGANISM AS THE CAUSE OF MENINGOENCEPHALITIS IN CAPERCAILLIE (*TETRAO UROGALLUS* L.) IN SWEDEN

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Objective

The objective was to describe meningoencephalitis among capercaillies (*Tetrao urogallus* L.) found dead in Sweden, and make attempts to identify the causative agent.

Materials and methods

Formalin fixed and paraffin embedded tissue samples from 90 capercaillies, autopsied between 1966 and 1985 and diagnosed as probable cases of toxoplasmosis, were sectioned and stained with haematoxylin and eosin. Selected materials were subjected to immunohistochemical and ultrastructural studies.

Results

A group of 53 cases with nonpurulent meningoencephalitis was defined. Pronounced meningitis and encephalitis with perivascular cuffings of mononuclear inflammatory cells and focal gliosis were prominent lesions. Groups of probable protozoan organisms were frequently detected in combination with these changes. The histopathological suspicion of protozoan infection was confirmed by electron microscopy. Organisms in slides processed immunohistochemically showed distinct positive staining reactions when polyclonal rabbit sera against *Sarcocystis cruzi* was used, but no reaction when subjected to *Toxoplasma gondii* antiserum.

Conclusion

Nonpurulent meningoencephalitis is a common finding among wild capercaillies found dead in Sweden. Immunohistochemical and ultrastructural studies indicated that a protozoan parasite related to the genus *Sarcocystis* was the causative organism.

MORPHOLOGY AND OCCURRENCE OF *HEPATOZOON* SP. IN THE LUNGS OF VOLES

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Introduction

Hepatozoon (Apicomplexa: Hemogregarinidae) has sexual stages in blood-sucking arthropods and asexual stages in vertebrates. *Hepatozoon erhardovae* has been reported to parasitize mainly bank voles (*Clethrionomys glareolus*). This study is a part of a research project concerning the role of diseases in population cycles of microtine rodents.

Methods

Specimens of lungs from voles were fixed in 4 % formaldehyde and embedded in paraffin for LM studies, or fixed in 2.5 % glutaraldehyde and embedded in Epon for TEM observations.

Results

Infection with *Hepatozoon* sp. was more prevalent in the lungs of wild rodents collected from Lapland than in rodents from southern Finland. *Hepatozoon* sp. infection was found in 12 % of 66 bank voles and in 0.6% of 158 field voles (*Microtus agrestis*) sampled in southern Finland. In Lapland, 46 % percent of 190 bank voles and 23 % of 30 grey-sided voles (*C. rufocanus*) were positive. Furthermore, 29 % of 35 lemmings (*Lemmus lemmus*) from Norwegian Lapland were found to harbour this parasite. The geographical differences in prevalence might be explained by corresponding differences in definite host densities (arthropods).

Schizonts were found exclusively in lung tissue within parasitophorous vacuoles. The mean size of the parasitophorous vacuoles was 14 x 17 µm (12.7 - 15.3 x 14.9 - 18.9 µm; 95% CI) and of schizonts 9 x 12 µm (8.5 - 10.3 x 10.7 - 13.5 µm; 95% CI). Micronemes, as well as amylopectin granules, were abundant in schizonts. Tubular mitochondria, characteristic of protozoa, were also found in schizonts. Some of the schizonts had one or a few nuclei, while others were filled with nuclear material of developing merozoites. Merozoites and developing gamonts were seen in leucocytes, especially in eosinophilic granulocytes. In thin sections the schizonts had a tendency to be ripped out of the parasitophorous vacuole surrounding them. This might be due to differences in the consistency of the pellicle and the embedding material. Ultrastructurally, the organisms were typical of *Hepatozoon* sp.

IDENTIFICATION OF THE MODE OF TRANSMISSION OF *PNEUMOCYSTIS CARINII*

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Objective

Epidemiological observations and animal studies have suggested that the airborne route is the mode of transmission of *Pneumocystis carinii.* By using the nested variant of the polymerase chain reaction (PCR) for detection of *P. carinii* DNA, we have investigated filters exposed to air collected from rooms in which *P. carinii* infected animals have been housed.

Methods

Two kinds of filter were used, polycarbonate (Millipore) and PTFE (Schleicher and Schüll), both with a pore size of 0.2 μm. After exposure, the filters were washed with physiological NaCl in a Stomacher stirrer and centrifuged at 30.000 g for 20 min. The DNA in 1 ml of the centrifuged material was prepared by conventional phenol extraction and the four PCR primers used for nested amplification were designed from the thymidylate synthetase gene of rat *P. carinii.*

Results

Preliminary results of eluates from both kinds of filter showed, as detected by agarose gel electrophoresis, a single PCR product of 131 bp, which is the expected size of the specific *P. carinii* DNA fragment. The control filters were negative.

Conclusion

It seems possible to investigate the airborne mode of *P. carinii* transmission by using PCR on air filter eluates.

PNEUMOCYSTIS CARINII IN ASYMPTOMATIC HIV POSITIVE PATIENTS

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Introduction

The clinical importance of the presence of *P. carinii* organisms in asymptomatic HIV patients has been questioned (Contini 1992). *P. carinii* was, however, not detected in bronchioalveolar lavages from asymptomatic HIV patients in two small studies (Ognibene 1988, Lundgren 1989).

Objective

Our objective was to determine the presence and importance of *Pneumocystis carinii* in respiratory specimens from asymptomatic HIV infected patients.

Material and methods

The study included 220 induced sputum samples from 50 HIV infected patients with severe immunodeficiency without previous *P. carinii* pneumonia (PCP) and with no prophylactic treatment. The patients were monitored for 18 months (or until they developed PCP, died, or left the study for other reasons), with clinical and laboratory examinations, including sputum induction every second month, or more often if necessary. Microscopical detection of *P. carinii* was done after indirect immunofluorescence (Moab 3F6) and toluidine blue O staining.

Results

P. carinii was found in 15 patients. All 15 patients contracted clinical PCP (from 0 days to 8 months after *P. carinii* was detected).

Conclusion

P. carinii was present in some asymptomatic HIV patients with severe immunodeficiency. The presence of the organism in patients without a history of PCP should be regarded as a pathological condition and prophylaxis or treatment initiated.

GENDER-RELATED BIASES IN DIAGNOSIS AND MORBIDITY ASSESSMENT OF SCHISTOSOMOSIS AND THEIR IMPLICATIONS FOR CONTROL OPERATIONS

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A synoptic inventory developed by us (Feldmeier and Krantz, 1992) was used for systematically reviewing existing data as to how and to what degree gender- and sex-related factors influence the validity of the diagnosis of schistosomosis in women.

Diagnostic sectors comprising survey methodology, parasitological methods, immunodiagnosis, detection of pathology and diagnosis of schistosomosis in the female genital tract have thus been scrutinized. In this way we have identified important gaps in the scientific knowledge of diagnosis of an important parasitic infection. Socio-cultural and gender-related determinants have never been studied systematically, and it is mostly by circumstantial evidence that we can point at biases - sometimes for sex but more often for gender - in much of the published material concerning diagnostic categories suitable for schistosomosis. These errors in diagnostic procedures and the ensuing lack of validity deserve attention from the fields of biomedicine and social science, preferably in a collaborative effort.

MODULATION OF THE IMMUNE RESPONSE IN MURINE SCHISTOSOMOSIS MANSONI BY VACCINATION WITH SCHISTOSOME ANTIGEN IN COMBINATION WITH CHOLERA TOXIN

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Objective

Schistosomosis is a helminth infection which affects many millions of people in the tropics. In the present study on *Schistosoma mansoni* we have investigated the possible adjuvant effect of cholera toxin (CT) in producing protection in mice against a subsequent challenge infection with cercariae.

Methods

Mice were sensitized intradermally or intranasally with adult worm antigen (SWAP) or cercarial antigen (CERC) in combination with CT. For comparison, mice were intradermally sensitized with SWAP in combination with BCG. In all experiments, the worm burden was evaluated 6 weeks after the challenge infection.

Results

Mice vaccinated with SWAP in combination with BCG showed a significant reduction in worm burden (55-73%). Mice vaccinated intradermally with SWAP in combination with CT did not display a significant reduction in worm burden. However, the intradermal vaccination triggered a strong anti-SWAP antibody response and induced a strong DTH response to SWAP.

In order to test a possible adjuvant effect of CT further, mice were sensitized intranasally with SWAP or CERC in combination with CT. Also in these experiments, no significant reduction in worm burden was revealed. Surprisingly, mice given CT alone intranasally revealed a significantly increased worm burden (38-83%). Furthermore, the same group of mice showed a significant peripheral blood eosinophilia at 4 weeks after challenge infection with cercariae. Analysis of the IL-5 response of spleen cells from the same group of mice revealed a higher production of IL-5 at 6 weeks after challenge infection with cercariae as compared with infected control mice not pretreated with CT.

Conclusion

Our findings suggest that cholera toxin may influence the host-parasite relationship in a way which favours parasite survival.

SCHISTOSOMA JAPONICUM INFECTION IN PIGS AFTER SINGLE AND REPEATED EXPOSURE TO LOW DOSES OF CERCARIAE

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Objective

The objective was to assess the parasitological and pathological aspects of porcine *Schistosoma japonicum* infection in order to generate information on the population dynamics of the parasite in a natural host.

Methods

Fifteen young pigs were divided into three groups of five pigs as follows:
Group A pigs served as a control group and were not infected.
Group B pigs were each exposed to 200 cercariae weekly for 8 weeks (at weeks 0-7).
Group C pigs were each exposed to 200 cercariae at week 0 only.

Exposure to cercariae was via the leg immersion technique. Blood and faecal samples were collected every two weeks for haematological examination and faecal egg counts, respectively. All of the pigs were killed and necropsied during week 8. Gross pathological lesions were noted and perfusions performed on the hepatic and mesenteric vessels to determine worm burdens of the liver and intestines, respectively.

Results

Patency was noted at week 6 post initial exposure, and all exposed pigs had patent infections at the time of perfusion (week 8). Three of the infected pigs had soft or fluid faeces with blood during the last two weeks of the study. Eosinophil counts for the Group B pigs tended to be higher than for the other groups at week 8, yet not at a significant level due to large individual variations. Multiple, disseminated white lesions were noted on the surface of the livers of infected pigs, with 4 of the 5 Group B pigs showing more severe gross lesions than those in Group C. Worm burdens for Group C ranged from 3.5% to 8.0% of exposure dose (200) while the range for Group B pigs was 6.8%-15.3% of total exposure dose (1600), even though schistosomula from the later infections did not have sufficient time to reach their sites of maturation. A significantly higher number of adults from the intestines as well as juveniles from both the liver and intestines were perfused from Group B pigs. There was no significant difference in the number of adults perfused from the livers.

Discussion

Although the established worm burdens were low, some pigs exhibited clinical signs associated with *S. japonicum* infection (i.e. diarrhoea and hematochezia) and all infected pigs displayed gross hepatic lesions commonly seen with the disease. A comparison of the worm burdens of Groups B and C suggests that the early infections of the repeatedly exposed pigs (Group B) did not prevent further infection, but the relatively higher establishment of worms in this group may also relate to differing levels of cercarial infectivity.

COMPARISON OF PRAZIQUANTEL (DRONCIT®) AND EPSIPRANTEL (CESTEX®): EFFICACY TRIALS AGAINST CESTODES IN THE RODENT MODEL AND SERUM KINETICS IN BEAGLE DOGS

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The results of various trials on the efficacy of Praziquantel (Droncit®, Bayer AG, Germany) and Epsiprantel (Cestex®, Beecham, USA) against cestode infestations *in vivo* and *in vitro* are summarized. Furthermore, we have included data on the serum kinetics of both compounds after oral application in beagle dogs.

To compare the efficacies of the potent cestocidal drug Praziquantel and the new compound Epsiprantel against cestodes, both drugs were administered to mice infested with *Hymenolepis nana*. The compounds were tested at dosages of 25, 10, 5 and 1 mg/kg body weight. For each dose and drug we treated two groups of five mice each; one group received a single oral, the other a single subcutaneous application. The same treatment regime was applied to rats infested with *H. diminuta*, using dosages of 5.0, 2.5, 1.0 and 0.5 mg/kg body weight. All animals were sacrified seven days after treatment, and the presence of surviving parasites was determined.

Against *H. nana*, Praziquantel was fully effective at an oral dosage of 25 mg/kg and at a subcutaneous dosage of 5 mg/kg, whereas the animals treated with Epsiprantel at 25 mg/kg body weight still carried intact scolices at day 7 post treatment. Praziquantel eliminated *H. diminuta* at a dosage of 5 mg/kg, either given subcutaneously or orally, while all animals of the Epsiprantel groups remained infested.

To evaluate the serum levels of both compounds, beagle dogs were treated once orally at the recommended dosage of 5.0 mg/kg body weight of Praziquantel or 5.6 mg/kg body weight of Epsiprantel. Blood samples were taken at 30 min, 60 min, 90 min, 2 hrs, 4 hrs, and 8 hrs after treatment. The residues of Praziquantel and Epsiprantel in the respective serum samples were analysed by HPLC and UV-detection.

The oral application of Praziquantel as well as Epsiprantel gave significant serum levels between 1 and 4 hrs after dosing. The highest serum concentrations for both compounds were measured 1 to 2 hrs after application. The serum levels dropped drastically thereafter.

CROWDING OF ADULT *HYMENOLEPIS MICROSTOMA* AND GROSS PATHOLOGICAL CHANGES IN INFECTED MICE

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Objective

One objective with our courses in immunoparasitology during 1991-93 has been to study aspects of the host-parasite interactions in experimental *Hymenolepis microstoma* infection of mice. Particular attention has been drawn to the extraordinary enlargement of the bile duct in which the cestode scolex is anchored. Here we present results combined from these three courses.

Methods

Groups of four NMRI mice were infected with 5 or 50 *H. microstoma* and autopsied on day 21 or 35 p.i. together with uninfected, sex and age matched controls. Bile duct diameter was measured and wet weight of mesenterial lymph nodes and spleen was recorded. Adult worms were dissected free in saline and the total worm wet weight measured.

Results

A clear negative correlation between wet weight per worm and number of worms recovered was seen (figure 1). As a consequence of this, a maximum total worm biomass was evident. The infection induced an increase of the bile duct diameter from the normal about 0.2 mm to a maximum of about 10 mm. This increase seemed to be better correlated to the number of worms than to the total biomass of worms recovered in each mouse. The worms induced an enlargement of the mesenterial lymph nodes and spleen, indicating a sensitization of the immune system of the mouse.

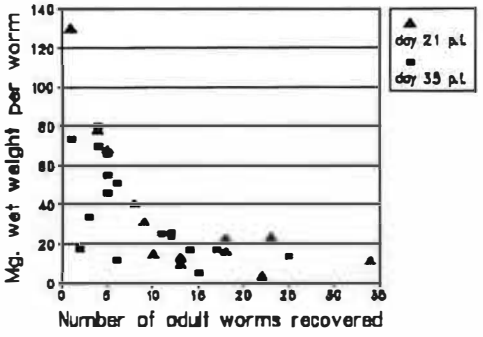

Figure 1: Weight per worm of *H. microstoma* in individual mice in relation to worm number recovered days 21 and 35 p.i.

Conclusions

The adult *H. microstoma* show intraspecific crowding in agreement with studies on other cestode species. The correlation between worm number and bile duct enlargement indicates that the pathological changes primarily are induced locally by the cestode scolices. Central lymphoid organs are enlarged, which is in agreement with other measurements of activity in the host immune system, e.g. circulating IgE antibody.

STRESS EFFECT OF BLOOD SAMPLING ON *HYMENOLEPIS MICROSTOMA*-INDUCED EOSINOPHILIA IN MICE

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Objective: Eosinopenia is a known possible effect of stress (Spry CJF. Eosinophils 1988; Oxford Univ Press). To what extent could this fact influence the eosinophilia induced by a helminth infection?

Methods: Female +/nu NMRI mice were infected with 5 or 50 cysticercoids of *Hymenolepis microstoma* and examined for peripheral eosinophilia on day 26 and 33 post infection.

The mice were immobilized in a cylinder and, without anaesthesia, the tip of the tail was removed by a quick cut with a pair of scissors. Blood samples were taken after about 15 seconds and again after 1, 2, 4, 6, 9, 12, and 15 minutes. Between each of these samples the blood flow was controlled by gentle pressure with the hand. The wound was wiped clean before each sample was collected. The mouse was returned to its housing cage for 15 minutes. The cutting procedure was then repeated and a single blood sample was taken.

The blood samples were prepared and stained for eosinophils by mixing each of them with 45 µl of phloxine propylene glycol solution (Pilot ML. Amer J Clin Path 1950; 20: 870-71). From each sample a 0.8 µl subsample was counted in a Bürker-Türk blood-counting chamber.

Results: In 11 of 12 trials on 8 mice the eosinophil count dropped to a minimum during the first 1 to 6 minutes of blood sampling, but after 9 to 30 minutes, a maximum level higher than the initial value was obtained. A typical result is shown in figure 1.

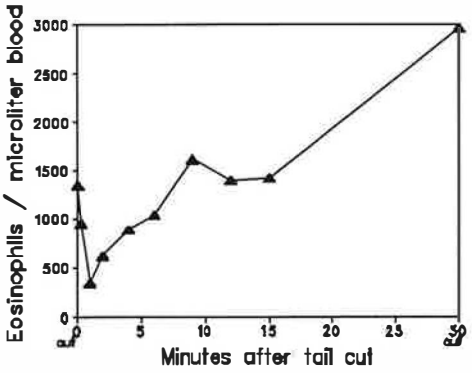

Figure 1: Eosinophilia in a mouse 26 days p.i. with 50 *H. microstoma*.

Conclusion: The *Hymenolepis microstoma*-induced eosinophilia in mice was highly sensitive to the sampling procedure. Within a few seconds of the beginning of blood sampling, a stress-induced eosinopenia occurs and therefore only the very first blood sample is representative of the peripheral eosinophil count. The temporary eosinopenia is followed by a recruitment of more eosinophils than initially observed, and this fact rises the question of which count expresses the best correlation with the helminth induced eosinophilia.

ANALYSIS OF CONSERVED GENES (cDNA) IN THE CESTODE *DIPHYLLOBOTHRIUM DENDRITICUM*, WITH SPECIAL REFERENCE TO ACTIN AND COLLAGEN

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Objective

The objective was to study the structural proteins actin and collagen in a flatworm. These proteins are highly conserved through evolution and have been found in all eukaryotic cells examined so far. It is of particular interest to analyze evolutionarily preserved genes in flatworms, because ancestral flatworms are regarded as the ancestors of all metazoans.

Methods

In order to clone and characterize genes belonging to the actin and collagen multigene families we have screened a *D. dendriticum* cDNA library (Uni-Zap XR, Stratagene). As probes we have used a human γ-actin cDNA and a murine pro-α1 type II collagen cDNA.

Results

Actin: We have isolated several cDNA clones, representing at least three different actin genes. One of the cDNA inserts, *Didact1*, consists of 1392 bp. The deduced amino acid sequence is 376 amino acids long. It is a typical invertebrate actin resembling more the cytoplasmic than the muscular isoforms of vertebrate actins.

Collagen: Northern blot hybridization with the murine type II collagen probe revealed a signal for a 1.5 kb mRNA, as well as for a 4.5 kb mRNA. The cDNA corresponding to the 4.5 kb transcript has been partially sequenced, the deduced amino acid sequence resembling vertebrate fibrillar type I collagen.

THE ROLE OF CALANOID COPEPODS IN THE LIFE-CYCLES OF *ANISAKIS SIMPLEX, CONTRACAECUM OSCULATUM* AND *HYSTEROTHYLACIUM ADUNCUM* (NEMATODA, ASCARIDOIDEA)

Marianne Køie
Marine Biological Laboratory, Helsingør, Denmark

Objective

The objective was to examine the role of calanoid copepods in the life-cycles of some ascaridoid nematodes.

Methods

Ensheathed larvae from hatched eggs of *Anisakis simplex*, *Contracaecum osculatum* and *Hysterothylacium aduncum*, and eggs containing fully developed larvae of *H. aduncum* were exposed to laboratory-reared *Acartia tonsa.*

Results

The copepods became infected with *A. simplex* and *C. osculatum* by ingesting the ensheathed larvae, whereas they became infected with *H. aduncum* by ingesting the eggs. The larvae of all three species exsheathed in the alimentary tract and entered the copepod haemocoel.

The larvae of *A. simplex* and *C. osculatum* showed limited growth and survived for only a few days in the copepod haemocoel. Since euphausiaceans feed on calanoid copepods, these are believed to act as transport hosts for *A. simplex*. Larvae of *C. osculatum* are directly infective to small specimens of fish (stickleback, O-group eelpout), but are apparently under natural conditions transferred via copepod hosts.

The third-stage larvae of *H. aduncum* grew and remained in the copepod haemocoel, which became completely filled by the parasite. Small larvae (less than 2 weeks old) (15°C) of *H. aduncum* do not survive when ingested by fishes, larger larvae (about 3 weeks old) penetrate the fish intestine and remain as third-stage larvae in the fish intermediate host, whereas larger larvae (more than 4 weeks old) moult into fourth-stage larvae and remain in the intestine of the fish definitive host.

Conclusion

The results indicate that calanoid copepods are important transfer hosts for *A. simplex*, *C. osculatum* and *H. aduncum.*

PARAFILARIA BOVICOLA IN CATTLE IN SWEDEN: A COMPARISON OF SEROLOGICAL SURVEYS IN 1982 AND 1992

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Objective

Bovine parafilariosis was first recognised in Sweden in 1978. The nematode *Parafilaria bovicola* was spread throughout Sweden by trade and its vector *Musca autumnalis*. Serum samples collected in 1982 and 1992 were examined for antibodies to *P. bovicola* to determine if any change in the prevalence of the parasite had occurred.

Materials and methods

The country was divided into seven geographical regions: 1) South Sweden, 2) the isle of Gothland, 3) South-east Sweden, 4) South-west Sweden, 5) Middle-east Sweden, 6) Middle-west Sweden and 7) North Sweden (see map below). A similar proportion of the total cattle population in each region was sampled.

In 1982, serum samples were collected from 1354 head of cattle, more than 18 months old, at slaughter houses in regions 1, 3, 6 and 7. No samples were collected from regions 2, 4 and 5, because the parasite was absent from these areas at that time. In 1992, 1481 samples were collected from all seven regions of Sweden from cattle aged two years or more. Not more than two samples were from cattle on the same farm.

Serum samples were examined with a competitive ELISA using a crude "whole worm" antigen and a monoclonal antibody against a protein of the adult worm.

Results

The seroprevalences in different regions are shown in Table 1. In 1982, 22.2% of the serum samples were considered positive for *Parafilaria bovicola*, whereas in 1992 only 2.1% of the samples were positive. In 1982, positive samples were obtained from all four regions examined. In region 3 (South-east Sweden), 39.7% of the samples were positive. In 1992, no positive samples were found in the northern region, whereas 15.5% and 5.7% of the serum samples from regions 2 and 3, respectively, were positive.

Table 1: Seroprevalence (%) of *Parafilaria bovicola* in cattle in different regions (R) of Sweden (see text and map on the left) in 1982 and 1992 (ND=not done).

Year	R-1	R-2	R-3	R-4	R-5	R-6	R-7	Total
1982	14.9	ND	39.7	ND	ND	16.4	10.2	22.2
1992	0.7	15.5	5.7	1.9	5.4	2.3	0.0	2.1

Conclusion

Although somewhat different procedures were used in the investigations in 1982 and 1992, one may draw the conclusion that the overall seroprevalence of *P. bovicola* in Sweden has decreased markedly since 1982, but that the parasite has spread to new areas of the country.

PURIFICATION OF *ANGUILLICOLA CRASSUS* ANTIGEN FOR DETECTION OF SPECIFIC ANTIBODIES IN SERUM FROM THE EUROPEAN EEL *(ANGUILLA ANGUILLA)* BY ELISA

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[2]Department of Immunology, BMC, Uppsala University, Uppsala, Sweden

Objective

The dracunculoid nematode *Anguillicola crassus* has recently been introduced to Europe from eastern Asia. In Europe, the parasite has an indirect life cycle, including an intermediate copepod host, a paratenic fish host, and the European eel, *Anguilla anguilla*, as definitive host. In the eel, juvenile parasites inhabit the swim bladder wall, whereas adults occur in the swim bladder lumen where they feed on host blood. Previous studies indicate that *A. crassus* elicits an immune response in the eel. In this study, the humoral immune response of the eel to *A. crassus* has been investigated using an indirect enzyme-linked immunosorbent assay (ELISA).

Methods

In the assay, adult whole-worm extract was used as antigen and affinity purified polyclonal rabbit anti-eel Ig antibodies coupled to alkaline phosphatase were used as conjugate. The specific antibody levels were measured in pooled sera from: 1) uninfected eels; 2) naturally infected eels; and 3) uninfected eels immunized with adult whole-worm extract.

Results

Using this assay, high background levels were noted. This was probably due to the presence of eel immunoglobulin from the gut of the parasite. Consequently, only small differences in reaction were noted between the different sera. However, by purification of the antigen, including affinity chromatography, gel filtration, and iso-electric focusing techniques, the sensitivity of the assay was increased and it was possible to detect differences in antibody levels between the different sera.

Conclusion

Purification of the whole-worm extract is necessary in order to increase the specificity of the present ELISA system for quantification of specific antibodies to the parasitic nematode *A. crassus* in eel sera.

HELMINTH INFECTIONS IN A FLOCK OF ICELANDIC HORSES AND THE RECOVERY OF AN UNIDENTIFIED THIRD STAGE STRONGYLE LARVA

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Objective

To determine the presence and nature of helminth infections in a flock of horses of different ages by faecal examination.

Material and methods

Faecal samples were collected on a single occasion per rectum from 57 horses (5 mo to 20 yrs old) in October 1991. The animals belonged to a flock of untamed horses grazing permanently on drained fen pastures. These horses are only treated once with anthelmintics when they are one year old. Faecal egg counts and third stage larval (L3) cultures for differentiation of strongyle nematodes were performed.

Results

Strongyle EPG (eggs per g faeces) increased significantly with age (Regression analysis, R=0.352, p=0.0073). Mean strongyle egg counts ranged from 120 EPG in foals to 615 EPG in horses older than 6 yrs. *Parascaris equorum* eggs were found in 90% of foals (mean EPG=285) and in one 3 yr old horse. *Oxyuris equi* eggs were found in several foals and *Anoplocephala perfoliata* cestode eggs in two horses.

The following L3 strongyle species/genera were found: *Cyathostomum, sensu lato* in all horses, *Gyalocephalus capitatus* in 21%, *Poteriostomum* spp. in 53%, *Oesophagodontus robustus* in 20%, *Triodontophorus* spp. in 32%, *Strongylus edentatus* in 46%, *S. equinus* in 12% and *S. vulgaris* in 16% of the horses. The strongyle L3 diversity was greatest in 2-3 yr old horses.

In addition, a strongyle L3 having 24 clearly defined intestinal cells, and which has not been described in L3 identification keys, was recovered from a 3 yr old horse.

Of other nematodes, L3 of *Trichostrongylus axei* were recovered from 9% of the samples, and L3 of *Strongyloides westeri* from one foal.

Cyathostomum, sensu lato accounted for 95% of the total number of L3 recovered, while other species/genera each accounted for < 1.8% of the L3.

Conclusions

Strongyle EPG counts increased with the age of the horses. The eggs found in young and adult horses were almost exclusively strongyle eggs, but foals also harboured other nematode species. A third stage strongyle larva that has apparently not been described previously was found in one horse.

PATTERNS IN THE OCCURRENCE AND DISTRIBUTION OF FRESHWATER FISH PARASITES: COMMON AND RARE SPECIES AND THE RESCUE-EFFECT HYPOTHESIS

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In general ecology it is well-established that there are common and rare species, and that there is a positive relationship between abundance and distribution. It has been discussed and concluded that this is not a sampling-artifact. Several theories have been put forward to explain the positive relationship between abundance and distribution. One such theory is the rescue-effect hypothesis which states that a species' probability of local extinction is balanced by its abundance on the patch and its regional distribution.

We have sampled roach (*Rutilus rutilus*) and bream (*Abramis bramae*) in several localities in south-east Norway. The localities represent different levels of continuity and discontinuity covering several scales. The results of the parasitological investigation will be presented to explore the relationship between common and rare parasite species in relation to metapopulation theory and the gradient of continuous and discontinuous localities on different scales.

THE SINUS-WORM (*LINGUATULA ARCTICA*): A FREAK OF NATURE

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Objective

The parasites of the group Pentastomida are common in the respiratory tract of tropical reptiles. In the Arctic, a ruminant, the reindeer, is parasitised by the pentastomid *Linguatula arctica*. This parasite was called the sinus-worm by Norwegian scientists because the adults live in the palatine sinus of the host (1). The taxonomic position of this odd organism is obscure. Morphologically it is considered to be related to segmented worms (Annelida) and joint-footed animals (Arthropoda). The aim of this study was to elucidate the systematic position of *Linguatula arctica* by studying its ultrastructure.

Material and methods

Sinus-worms were collected in the winter during the slaughter period at Savukoski in the eastern part of the Finnish Lapland. The "worms" were fixed in a mixture of formaldehyde (10%) and paraformaldehyde (2%) and processed routinely for scanning electron microscopy. External and internal surfaces were studied by a Jeol JMS 820.

Results

The transparent, pale yellow, flattened body covered by cuticula, consisted of annulae, or "segments", which were partly scaly on the ventral side. At every annulus there was a lateral sense organ and in the cuticula many small strainer-like holes probably representing openings of the osmoregulatory system. The anterior end had a buccal cavity lined with papillae and two pairs of spiny prominent hooks. In front of the buccal cavity sense receptors (sensilla) were located. The intestine was a straight tube from mouth to cloaca. The uterus in the adult female was a very long, winding tube, ending in the gonopore. The ovary was like a cluster of grapes, and the paired seminal receptacles were packed with long slender spermatozoa. The opening of the male genital organs was in the anterior end on the ventral side. Neither circulatory nor respiratory organs were seen.

Conclusion

The sinus-worm resembles annelids in the morphology of the coelom and by being segmented. However, the ventral nerve cord is typical of annelids and arthropods. Common features with the arthropods are the cuticula, which contains chitin, and the muscles, all of which are striated, and the structure of the gonads. When adding to these morphological findings the crustacean-like embryonal development, it seems justified to consider the sinus-worm as an extremely modified parasitic crustacean.

References

1. Haugerud RE, Nilssen AC. Reinens bihulemark. Ottar 1986; 161: 22-29

THE CUTICULAR MORPHOLOGY OF THE MOUTHPARTS OF *IXODES RICINUS*

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Introduction

In spite of the abundant literature related to the mouthparts of ticks in general, and especially of *Ixodes*, some misunderstandings about the morphology and function of these structures, still exist. We have therefore examined the mouthparts of *Ixodes ricinus* by different methods to reveal some of their secrets.

Methods

Females of *I. ricinus* were collected in the field, and after decapitation, the mouthparts were divided into three lots. One lot was fixed in 2.5% glutaraldehyde in 0.2 M sodium cacodylate, and embedded in Epon to make serial sections, and one lot was prepared for scanning electron microscopy. The mouthparts in the last lot were immersed in lactic acid, kept for one day at 60 °C, and carefully dissected to isolate the hypostome, palps and chelicera, or cut in tangential or cross sections with surgical razor blades at several levels. The sections were then observed and drawn with a drawing tube.

Results

The inner and outer article of the distal part of the chelicerae (cheliceral digits) were found not to be independent of each other, as hitherto supposed. The internal article was provided on the tip with a small pore, to which a narrow channel was connected, with the main chelicera channel open. A complicated valve, not described before, was present between the pharynx and the opening of the salivary duct into the salivarium proper. This valve occludes the salivary duct when the blood is pumping to the stomach, and it may allow for separation of fluids. As known before, the chelicera are covered by a double sheath, but inside this sheath they can protrude, retract or remain in a fixed position aided by a hitherto unknown attachment structure. Some new details of the hypostome are also described.

Conclusion

This study revealed new anatomical details of the mouthparts of *I. ricinus*, as for example a unique type of attachment structure of the chelicera, and the morphology of a valve system in the salivarium. The function of the mouthparts seems to be very complicated.

Erratum

Vol. 3 No. 1

Please observe that the correct top text of page 1, 11, 21, 23, 27, and 35 should be *Bull Scand Soc Parasitol 1993; 3:* (+ page number).

NEWS

Preliminary announcement

A symposium on **Parasites of biological and economic significance in the aquatic environment (SSP PARAQUA-94)** will be held at Westmann Isles, Iceland, 2-6 July 1994.

The meeting is arranged by the Scandinavian Society for Parasitology (SSP) with financial support from the Nordic Academy for Advanced Studies (NorFA), and will include guest lectures by invited speakers on Cestoda, Monogenea, Nematoda (main theme), Trematoda and population dynamics, and there will be free papers (15 min.) and discussions.

The purpose of the symposium is to review parasitic work of the last few decades and analyse future trends in some groups of biological and economic importance. Invited reviews, and abstracts or (optionally) restricted length papers will be published in the Bulletin of the SSP as defined in the final announcement.

Further information on the symposium (including progress of plans regarding workshop on specific topic) will be announced in early 1994.

Scientists who are interested in attending the symposium and who wish to obtain further information, are asked to fill in the form on the following page and send it to the local organising committee.

The participation of a few young scientists from each Nordic country will be sponsored by NorFA. Freely formulated applications including publication list and stated intention to submit a paper should be sent before the end of January, 1994 to Hans Peter Fagerholm, Institute of Parasitology, BioCity, SF-20520 Åbo, Finland.

Send to: Head of local organising committee of SSP PARAQUA-94, Erlingur Hauksson, Icelandic Fisheries Laboratories, P.O. Box 1405, Skúlgatan 4, 121 Reykjavik, Iceland (preferably before December 15, 1993)

Parasites of biological and economic significance in the aquatic environment (SSP PARAQUA-94 symposium) Westmann Isles, Iceland (2-6 July, 1994) sponsored by NorFA.

I am interested in attending the symposium and wish to receive the second announcement when issued.

Name .....
Organization .....
Address .....
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I intend to submit a paper with the (planned) title (or topic):
.....
.....
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GUIDELINES FOR CONTRIBUTORS

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

Short articles/communications should have a maximum length of 2 printed pages, including tables, figures, and references, and may contain a maximum of 2000 words if there are no figures or tables. The first page should show the title of the article, and the name(s) of the author(s). The authors' addresses should be given, and the complete correspondence address with telephone and telefax number (if available). The text should follow, without subheadings, but a short summary, maximum 100 words, may be included.

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

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

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

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

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

References shold be numbered consecutively in the order in which they are first mentioned in the text by arabic numerals within parenthesis marks.

The reference list should follow the style set forth in *Uniform Requirements to Manuscripts Submitted to Biomedical Journals,* Br Med J 1988; 296: 401-05. References to journals should contain names and initials of the authors, article title, the abbreviated name of the journal, year of publication, volume, and first and last page numbers of

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

Examples of correct forms of references are given below:

Standard journal article:

1. 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
2. Horsberg TE, Berge GN, Høy T et al. Diklorvos som avlusningsmiddel for fisk: klinisk utprøving og toksisitetstesting. Nor Vet Tidsskr 1987; 99: 611-15
3. Anonymous. Some facts on small animal practice. Vet Rec 1987; 120: 73

Books and other monographs:

4. Austin B, Austin DA. Bacterial fish pathogens: disease in farmed and wild fish. Chichester: Ellis Horwood, 1987
5. 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)
6. Sosialdepartementet. Tsjernobyl-ulykken: Rapport fra Helsedirektoratets rådgivende faggruppe. Oslo: Universitetsforlaget, 1987 (Norges offentlige utredninger NOU 1987: 1)
7. Thornhill JA. Renal endocrinology. In: Drazner FH, ed. Small animal endocrinology. New York: Churchill Livingstone, 1987: 315-39

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REPRINTS WILL NOT BE AVAILABLE.

In the interest of speed, no proofs will be sent to authors. It is therefore of vital importance that the manuscripts are carefully checked before submission.

BULLETIN OF THE SCANDINAVIAN
SOCIETY FOR PARASITOLOGY

VOL. 3 No. 2 CONTENTS OCTOBER 1993