Bulletin

of the

SCANDINAVIAN SOCIETY FOR PARASITOLOGY

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BULLETIN OF THE SCANDINAVIAN SOCIETY FOR PARASITOLGY

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

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

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PROCEEDINGS

of the

20th SYMPOSIUM OF THE SCANDINAVIAN

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Stockholm-Sweden

4-7 October, 2001

Editor: Jorun Tharaldsen and Johan Lindh

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Local organizing committee: Johan Lindh, Staffan Svärd, Jens Mattsson and Inger Ljungstrom

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WELCOME BY THE PRESIDENT OF THE SCANDINAVIAN SOCIETY FOR SOCIETY.

By TELLERVO VALTONEN

Department of biological and environmental science, University of Jyväskylä, Finland

Dear friends, on the behalf of the Scandinavian Society for Parasitology, I wish to welcome you all to this meeting. This is a Jubilee meeting; being the 20th symposium of the society, which was founded nearly 34 years ago in Denmark . We will celebrate this now in Stockholm, among other things by nominating several honour members, the enthusiasm and voluntary work of whom have kept the society going all these years.

Parasites are ubiquitous and common, they are thought to be important in maintaining sexual reproduction, regulating the dynamics of populations of free-living organisms, and known to cause severe losses in agriculture, aquaculture, as well as immeasurable human suffering, among others. Changes in any level of an ecosystem may cause unexpected disturbances in the prevailing host-parasite relationships. This may lead to problems and cause severe losses and harm in host populations. Our aim is to understand and to learn to predict what happens and in this way to be able to minimize the harm caused by parasites.

The aim of the SSP meetings is to bring together specialists; biologists, microbiologists, veterinarians and medics who share common interests in studying parasites and problems caused by them. Our invited, highly qualified guest lecturers enrich the discussions. Indeed, SSP meetings are known as being in the front line of new and modern ideas in parasitology. An essential feature of the SSP is also that it is small enough to let young scientists and PhD students discuss with experienced scientists . Such discussions have often lead to collaboration between different disciplines and countries.

A special welcome is directed to the invited speakers. During this Symposium we will listen to aspects on antigenic variation, cell biology, diagnostics of parasites, parasites in aquaculture, development of vaccines and drugs and zoonotic parasites etc. A special session on nematodes is also included.

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

Finally, I will thank the local organizing committee, headed by professor Mats Wahlgren and Dr. Johan Lindh, they have performed a formidable task by arranging this symposium in Stockholm. It is my honour and privilege to thank you on behalf of all of us.

INVITED PAPERS

NEMATODE PARASITE CONTROL OF LIVESTOCK IN THE TROPICS/SUB TROPICS: COPING WITH CHEMOTHERAPEUTIC FAILURE

By PETER J WALLER

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Livestock raised in the humid tropics are exposed to a wide range of helminth parasites, which are responsible for enormous losses in productivity, and often high levels of mortality, particularly in young animals. Control methods rely almost exclusively on the use of anthelmintic drugs. Frequency of use depends on the farmers desire to treat his animals and/or his ability to pay for the drugs. However there is an increasing tendency for farmers to resort to treatment with a greater understanding of the losses caused by parasites and particularly due to the fact that cheap, generic drugs are becoming more common. As a result of this, anthelmintic resistance in helminth parasites has developed, but its importance varies between parasite taxa, between parasites of different host species and between different regions/countries in the tropics. By far the greatest problem with anthelmintic resistance is associated with the nematode parasites of small ruminants, particularly with *Haemonchus contortus*, a major pathogen of sheep and goats throughout the humid tropical/sub-tropical regions of the world. However the level of anthelmintic resistance in nematode parasites of sheep and goats varies within this region. The worst problem is generally associated with the large-scale sheep farming countries within these regions (continents of Africa, South America, and Australia). Here, high levels of resistance to a wide range of both broad and narrow spectrum anthelmintics are commonplace. Anthelmintic resistance has also frequently been reported in other important parasites of sheep and goats, notably *Trichostrongylus* spp. and *Teladorsagia (Ostertagia)* spp. Generally speaking, resistance in the Asian countries is not as rampant, although individual countries have very serious problems (Malaysia, Fiji). Anthelmintic resistance in nematode parasites of cattle and horses has also been reported, but generally these are isolated case reports, or associated with parasite species of minor importance. Apart from nematode parasites, anthelmintic resistance has only been reported in the trematode parasite of ruminants, *Fasciola hepatica.*

Evidence is clear-cut that anthelmintic resistance is increasing - in prevalence, magnitude and spectrum. Unfortunately there is a tendency to seek a solution when it is too late, when total anthelmintic failure has occurred. Then there are no immediate alternatives to keep the livestock operation in business. However, alternatives are available, but none in isolation will solve the problem. The way to sustainable nematode parasite control in the future is to incorporate a range of control options into an Integrated Pest Management (IPM) package for nematode parasites of livestock.

The following speakers are invited, but there were no available abstracts at the time of printing:

COMPARING NEMATODE GENOMES
By Mark Blaxter

NEMATODE MOLECULAR SYSTEMATICS:
APPLICATIONS, PITFALLS, AND FUTURE DIRECTIONS
By Steven Nadler

MALE MORPHO-GENETICS: SORDID TALES OF ASSORTED TAILS
By David Fitch

COMPUTER DATABASE PROGRAMS -
TAXONOMY OF PLANT PARASITIC NEMATODES
By Alexander Ryss

CELL BIOLOGY-*GIARDIA*
By Heidi Elmendorf

IMMUNOLOGY-*GIARDIA*
By Steve Singer

TRYPANOSOMA, ANTIGENIC VARIATION
By David Barry

PNEUMOCYSTIS
By Jim Stringer

RICKETTSIA, ANAPLASMA - GENOMICS
By Guy Palmer

PLASMODIUM - ANTIGENIC VARIATION
By Kirk Deitsch

SUBMITTED PAPERS - ORAL PRESENTATIONS

A PROTEASE INHIBITOR ASSOCIATED WITH THE SURFACE OF *TOXOPLASMA GONDII*

By JOHAN G. LINDH, SILVIA BOTERO-KLEIVEN, JUAN-IGNACIO ARBOLEDA & MATS WAHLGREN

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Toxoplasma gondii has a broad host-range including man and a variety of warm-blooded animals. The ability to infect and survive in this wide spectrum of hosts suggests highly evolved mechanisms to handle the harsh environments encountered. Here we show that extracellular tachyzoites are resistant to milligram levels of trypsin and describe the presence of an inhibitor of trypsin associated with the surface of *T. gondii,* TgTI. TgTI has an estimated molecular mass of 37 000 dalton and is encoded by the TgTI-gene which is found at low abundance as an expressed sequence tag (EST) in both the bradyzoite and tachyzoite stages. The inhibitory binding region was found to be in the N-terminus of TgTI where aminoacid-alignment to earlier described protease inhibitors demonstrates 75% similarity. In functional analysis, recombinant TgTI-protein inhibits the activity of trypsin approximately 10 times more efficiently than an inhibitor isolated from soybean. In contrast to other known trypsin inhibitors, TgTI also possesses a predicted membrane-binding region. Polyclonal antibodies raised against recombinant TgTI bind to the surface of the tachyzoite stage as seen both by immunofluorescence and immunoprecipitation of surface labelled parasite proteins. The high survival rate of the parasite in the upper gastrointestinal tract may be enhanced by the presence of the TgTI-molecule.

PLASMODIUM FALCIPARUM; A CORRELATION BETWEEN PARASITE BINDING TO MULTIPLE RECEPTORS AND SEVERITY OF *P.FALCIPARUM* MALARIA

By F. PETTERSSON[1], A. HEDDINI[1], O. KAI[2], J. SHAFI[2], J. OBIERO[2], Q. CHEN[1], A. BARRAGAN[1], M. WAHLGREN[1] & K. MARSH[2,3]

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[3] Nuffield Dep. of Clin. Medicine, University of Oxford, Oxford, UK

Binding of *P.falciparum* infected erythrocytes (pRBC) to endothelial cells in the blood vessels, and to infected/uninfected erythrocytes, are known properties of *P.* -

falciparum malaria. It has been suggested that parasites of a specific adhesive phenotype may bind in particular organs causing severe disease. Here we have examined the pRBC of 111 fresh clinical isolates of children with malaria for a number of adhesive features in order to study their possible coexpression and association with severity of disease. The ability to bind to multiple receptors as well as the ability to form rosettes and giant rosettes was found to be more frequent among isolates from children with severe compared to mild malaria ($p=0.0015$). Whereas previous thinking has tended to emphasize the role of a single, specific adhesive, phenotype, these results support the idea that several receptor-ligand interactions work synergistically in bringing about severe disease.

Acknowledgments

We thank all the staff at KEMRI, Kilifi, Kenya, for critical input and an excellent collaboration. This work was supported by grants from the Swedish International Development Authority SIDA-SAREC, the Karolinska Institute, the Swedish Medical Research Council, KEMRI and the Wellcome Trust.

IDENTIFICATION OF IMMUNODOMINANT PROTEINS IN *GIARDIA LAMBLIA.*

By MALIN WEILAND[1], J.E. DANIEL PALM[1] , WILLIAM J. GRIFFITHS[2] & STAFFAN G. SVÄRD[1]

[1]Microbiology and Tumor Biology Center
[2]Department of Medical Biochemistry and Biophysics, Karolinska Institutet

Colonization of the small intestine by the parasite *Giardia lamblia* is the major cause of parasitic induced diarrhea in humans. Around 250 million people all over the world are currently infected. Humoral immunity has been shown to be important for clearing the infection, but little is known about the immunoreactive antigens. The variable surface antigen (VSP) coat has drawn a lot of attention but the diagnostic and immunological value of these proteins has been questioned. In order to identify novel proteins and new vaccine targets we have studied the immunoreactivity of sera from Swedish patients with diagnosed acute giardiasis. Sera from 90 patients were first screened on one-dimensional western blots to determine the weight distribution and reactivity pattern. A few sera reacting to different molecular weights were further analyzed on 2D Western blots of total trophozoite extracts. From 2D gels the corresponding immunoreactive proteins were cut out and digested with trypsin before they were analyzed on a QTOF tandem mass spectrometer. Generated peptide masses and peptide sequences were compared to data from the ongoing *Giardia lamblia* genome project. Totally twelve immunoreactive proteins have so far been identified.

GENOME PLOIDY AND DISC STRUCTURE IN DIFFERENT STAGES OF THE *GIARDIA LAMBLIA* LIFE CYCLE

By DANIEL PALM[1], ROLF BERNANDER[2] & STAFFAN SVÄRD[1]

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[2]Department of Cell and Molecular Biology, Biomedical Center, Uppsala University, Uppsala, Sweden

The early diverging eukaryotic parasite *Giardia lamblia* is unusual in that it contains two apparently identical nuclei in the vegetative trophozoite stage. We have recently determined the nuclear and cellular genome ploidy of *G. lamblia* cells during all stages of the life cycle. During vegetative growth the nuclei cycle between a diploid (2N) and tetraploid (4N) genome content and the cell, consequently, cycles between 4N and 8N. Fully differentiated cysts contain four nuclei, each with a ploidy of 4N, resulting in a cyst ploidy of 16N. The newly excysted cell, for which we suggest the term "excyzoite", contains 4 nuclei (cellular ploidy 16N). In a reversal of the events occurring during encystation, the excyzoite divides twice to form four trophozoites containing two diploid nuclei each. To extend these studies we decided to study the regulation of the adhesive disc structure during *Giardia* differentiation. A monoclonal antibody reacting with the disc was raised. Characterisation showed that the monoclonal antibody reacted specifically with beta-giardin. Immunoflourecence analysis of cells in all stages of the life cycle showed that the disc is put down into fragments late in encystation, at the time when the nuclei are divided. The mature cyst contains four tightly packed fragments of beta-giardin. Early in excystation, the cell has no functional disc but the disc is quickly unpacked and reconstructed at the exyzoite surface. The disc is completely assembled when the excyzoite has divided into two trophozoites

A PLEA FOR OLD SCANDINAVIAN ZOOLOGICAL LITERATURE ON THE WEB OR CDS

By B. BERLAND

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In biology it is essential to identify organisms correctly. For any modern work the old systematic/taxonomic literature is still very important, but only in or near the "old" universities, with good libraries, are the old classical works available, and are for all practical purposes not available to workers "in the bush". In fish parasitology, Scandinavian authors such as P. C. Abildgaard, O. Fabricius, O. F. Müller, H. Krøyer, C. Linné, T. Odhner, P. J. Olsson and G. O. Sars wrote classical works. Key European

works by Rudolphi, Dujardin and van Beneden are referred to, but seen by few. The veterinarians and wild-life parasitologist also have their classical favourites. Electronic publishing is now a reality; entire encyclopediae and modern textbooks are now available on paper and on CDs. In principle the old classical works can be converted to electronic format, and be made available either on the web or on CDs. I ask the SSP board to take the initiative to approach the Nordic research councils or "Nordisk Råd" to take steps to make the pre-1900 Scandinavian treasures, which are not covered by copyright, available in electronic form, either on the web, or on CDs, or both. If a success, other European countries may join in making their classical biological treasures available in electronic format, either on a national basis or in a common pool.

CHARACTERIZATION OF A POLYPYRIMIDINE-BINDING PROTEIN IN *TRYPANOSOMA CRUZI*

By MAJA NYSTRÖM, ANH-NHI TRAN & LENA ÅSLUND

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In trypanosomes, gene expression is mainly regulated post-transcriptionally through RNA processing mechanisms such as trans-splicing and mRNA stability. Both these mechanisms have been shown either to require or be affected by a polypyrimidine tract located upstream of the 3\' splice site directing the trans-splicing (Huang & Van der Ploeg, 1991), and in the case of mRNA stability, located in the 3\'-untranslated region of the mRNA (Hotz *et al.*, 1997).

We have, from our EST project, identified a gene in *Trypanosoma cruzi* with homologies to a polypyrimide-binding protein (PTB), present in other eukaryotic cells and involved in cis-splicing and mRNA stability. The gene for *T. cruzi* PTB (TcPTB) encodes a protein of 490 amino acids corresponding to a size of approximately 45 kDa, slightly smaller than the PTB in other organisms. The protein resembles PTBs in other organisms harboring several RNA recognition motifs (RRMs). The TcPTB has a short C-terminal extension of 29 amino acids, which might be involved in interactions with other factors. A second gene, putatively encoding a shorter variant of PTB has been found located upstream of TcPTB. The *T. cruzi* polypyrimidine-binding protein has been expressed as recombinant protein and its binding properties and functional role in the RNA processing machineries of the parasite are being investigated.

References

Huang J, Van der Ploeg LH. EMBO J 1991; 10: 3877

Hotz H-R, Hartmann C, Houber K, Hug M, Clayton C. Nucl Acid Res 1997; 25: 3017

INTERRELATIONSHIPS OF CRABS, LEECHES, FISH AND TRYPANOSOMES IN COASTAL WATERS OF NORTH NORWAY. PART 2

By K. MACKENZIE[1], W. HEMMINGSEN[2] & P.A. JANSEN[3]

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The red king crab, *Paralithodes camstchaticus*, was introduced to the Barents Sea from its native North Pacific by Russian scientists in the 1960s and 1970s. A thriving population now established in the Barents Sea is steadily spreading westwards along the coast of Finnmark. The leech *Johanssonia arctica* lays its eggs on the carapace of the crabs and is known to be a vector for a fish trypanosome.

In October 1999 and 2000 we investigated the occurrence oftrypanosomes in cod, *Gadus morhua*, haddock, *Melanogrammus aeglefinus*, and long rough dab, *Hippoglossoides platessoides,* in blood samples taken at a series of stations along the coast of Finnmark. In both years we found two foci of infection in cod: one in Varangerfjord in eastern Finnmark, where there has long been a dense population of red king crabs, and another in western Finnmark where there are no king crabs. Sample prevalences varied from 0 to 96%. Haddock were infected at about the same level as cod, but no infections were found in long rough dabs. Prevalences and mean abundances of infection in the same areas did not differ significantly between the two successive years samples, with one exception - Tanafjord, which showed a significant increase in trypanosome infection of cod from 1999 to 2000, and where the population of red king crabs has increased sharply in recent years.We discuss the interrelationships of crabs, leeches, fish and trypanosomes in the study area, including the possibility that we may be dealing with two species of trypanosomes with different leech vectors.

REGIONAL DIFFERENCES IN PARASITE ABUNDANCES OF WILLOW GROUSE, *LAGOPUS LAGOPUS* L. USEFUL AS BIOLOGICAL TAGS?

By PER R. HOLMSTAD & ARNE SKORPING

D*epartment of Zoology, University of Bergen, Norway*

In 1992 a total of 151 willow ptarmigan (*Lagopus lagopus*) and 119 ptarmigan (*Lagopus mutus*) were collected from six different areas in Troms county, Northern Norway, comprising a gradient from coast, via fjords to inland localities. In addition, 185 hosts from one of the coastal localities has been collected and examined for parasites between 1992-1996. Nine species of parasites were found. Microparasites and cestodes did not show any notable difference in abundance between localities, but significant, regional differences in abundances were found for the nematodes *Ascaris*

compar and *Trichostrongylus tenuis*. Their potential use as biological tags is discussed.

IS THERE A TRADE-OFF BETWEEN THE IMMUNE-RESPONSE AND GROWTH IN YOUNG ATLANTIC SALMON?

By O. MOBERG, A. NYLUND & A. SKORPING

University of Bergen

The establishment and maintenance of an immune response is likely to be costly in terms of metabolic resources. This might present us with a trade-off situation where immuno-competence is given a higher priority, at the cost of growth.

This hypothesis was tested using young salmon (*Salmo salar* L.). To challenge the immune-system, two groups of fish were vaccinated, using six-factor vaccines, while untreated groups from the same family-groups were kept as controls. The fish were given reduced food-rations from the time of vaccination to avoid any over-compensation in food-intake, which could mask differences in growth. Individual changes in weight and length, as well as various immune-parameters, were measured every month

Preliminary analyses of the results indicate that the untreated groups had significantly higher growth-rates than the vaccinated groups, suggesting that growth-rates in salmon are reduced as a consequence of the stimulation of the immune-system. The implications of this result to general fish parasitology will be discussed.

CAN PARASITE-INDUCED WINTER-MORTALITY IN GROUSE (*LAGOPUS LAGOPUS* L.) BE DETECTED BY A DECREASE IN AGGREGATION PATTERNS?

By ESPEN SCHEI, PER HOLMSTAD AND ARNE SKORPING

Department of Zoology, University of Bergen, Norway

Several studies have shown that a significant fraction of the grouse present within an area in autumn will die during the winter. In our study area there tend to be negative relationships between parasite intensities and grouse body condition, suggesting that the most infected birds will have a nutritional disadvantage at the start of the winter season. If parasites affect winter mortality, we would expect mortality to be correllated with parasite intenstities, which should cause a decrease in parasite overdispersion.

This study consists of 3 different samples collected during the winter 99/00, one in September/October, one in November/December and one in February/March,

within an island location close to Tromsø, Norway. The number of birds examined were 85, 30 and 60, respectively. All birds were aged, sexed and searched for parasites within the digestive tract and in the connective tissue around the crop/oesophagus.

So far the most abundant parasite is the cestode *Hymenolepis microps*, which shows a decreasing degree of aggregation during the winter period. We are currently examining the colic caecae where we expect to find the nematode *Trichostrongylus tenuis* and the coccidian *Eimeria* sp. The results for all parasites will be presented and discussed.

VARIATION IN SUSCEPTIBILITY AND BODY GROWTH BETWEEN HOSTS IDENTIFIED BY THEIR INFECTION HISTORY

By D.A. LYSNE[1] & A. SKORPING[2]

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[2] *Department of Zoology, University of Bergen, Norway*

Six hundred Atlantic cod (*Gadus morhua* L.) were individually marked and caged for 19 months. During this period each cod was inspected for *Lernaeocera branchialis* (L.) at several intervals. During the caging 79 % of the cod remained uninfected, 8.5 % were infected, but lost the parasite, 8 % were infected with one parasite, and 4.5 % were infected with more than one parasite. One group of hosts developed significantly higher infection rates than the others. These were fish which had been infected previously, but had lost their infection.

Furthermore, growth rates in the four groups of cod, identified by their infective history, were compared. The highest rate of increase, both in body mass and body length, was recorded in the group of male fish infected with one parasite throughout the experimental period. Conversely, the males which were free from infection, showed significantly lower growth rates. The observed differences in growth rates could not be explained by changes in parameters related to life history strategies. The alternative explanation to these results is that resistance to *L. branchialis* was associated with costs in terms of reduced growth rates of body mass and length.

GENETIC AND FUNCTIONAL CHARACTERISATION OF THE TRYPANOTHIONE SYNTHETASE IN *TRYPANOSOMA CRUZI*

By ANH-NHI TRAN, BJÖRN ANDERSSON, ULF PETTERSSON & LENA ÅSLUND

Dept. of Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Sweden

Within the *Trypanosoma cruzi* genome project, we have previously generated Expressed Sequence Tags (ESTs) corresponding to about 1/4 of all genes present in the parasite. Among these ESTs, we have identified several interesting genes involved in unique metabolic pathways in trypanosomes. One of these encodes the trypanothione synthetase (TRS), which together with other enzymes involved in the trypanothione metabolism have been suggested to be important drug targets. We have cloned the trypanothione synthetase gene which encoded a protein of 647 amino acids. The deduced sequence revealed an overall sequence identity of ~60% to the orthologous enzyme identified in the apathogenic parasite *Crithidia fasciculata*. The TRS gene is present as single copy in *T. cruzi*. However, a high sequence polymorphism of 1/38 bp within the coding region was found between allelic copies of the gene. The gene was found to be located on a chromosome pair with an extremely large size differerence of about 2 Mbps.

To study the function and importance of the trypanothione synthetase in *T. cruzi*, we are creating in vivo knockouts and/or parasite mutants over-expressing the enzyme. Further studies to investigate whether the highly sequence polymorphism has any functional consequence, are also in progress.

SCHISTOSOMA JAPONICUM: HISTOPATHOLOGY OF INFLAMMATORY LESIONS IN THE LIVER OF CONGENITALLY INFECTED PIGLETS

By T. IBURG[1], M.V. JOHANSEN[2], P.S. LEIFSSON[1] & R. LINDBERG[3]

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[2]*Danish Bilharziasis Laboratory, Denmark*
[3]*Department of Pathology, Faculty of Veterinary Medicine, SLU, Sweden*

The inflammatory response in liver tissue from piglets with a congenital *Schistosoma japonicum* infection was examined at two times post infection. The piglets, which were offspring of 3 sows infected intramuscularly with 9000 S. japonicum cercariae in 10th week of pregnancy, were allocated in to 2 groups (n = 9 and 17), killed and perfused at 5 and 11 weeks, respectively, after birth. All piglets excreted eggs in low amounts and had low liver tissue egg counts with no significant difference between the 2 groups. Inflammatory lesions in the liver consisted mainly of granulomas in portal areas, often obliterating the portal venules, and often with central eggs or egg remnants. The granulomatous reaction consisted of epitheloid cells and occasional

giant cells with layers of lymphocytes, eosinophils and various amounts of collagen and fibroblasts. These findings show that the piglets are able to mount an inflammatory response to an intrauterine infection. The volume of circumoval granulomas with single, mature, intact eggs decreased ($p = 0,12$) as the infection progressed, and the number of eosinophils in granulomas with eggs or egg remnants decreased slighty as well, suggesting a tendency toward modulation over time of the inflammatory response to a single exposure in utero.

THE *TRYPANOSOMA CRUZI* GENOME PROJECT

By ESTEBAN BONTEMPI, CAROLE BRANCHE, KIMBERLY EDWARDS, HAMID DARBAN, DELAL ÖNCÜ, SINDY KLUGE, LENA ÅSLUND, ULF PETTERSSON & BJÖRN ANDERSSON

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Trypanosoma cruzi causes Chaga's disease, which affects 16-18 million people in South- and Central America. Large-scale mapping and sequencing of the *T. cruzi* genome is carried out in our laboratory in collaboration with TIGR and the SBRI using a clone by clone approach. We have as a pilot project sequenced the third smallest chromosome of the CL Brener strain of *T. cruzi*, and parts of its longer homolog. The sequence difference between the two homologous chromosomes has been found to be more than 5%, and to consist of single base changes and small insertions and deletions, The 400 kb size difference appears to be due to a chromosome rearrangement resulting in a large insertion in the longer chromosome. Most genes on chromosome 3 are single-copy, and organized in long stretches where all are transcribed on the same strand, but several repeated gene regions have been found. The repeats identified include *T. cruzi* repeat elements and five tandemly repeated gene loci. The latter include two loci of house-keeping genes that are present in more than 20 copies in tandem, a repeated pseudogene locus and repeated surface antigen family members. The surface antigen genes, which are thought to be involved in the evasion of the immune system, are particularly interesting, since their untranslated regions are more conserved than the coding regions. Thus, the *T. cruzi* genome has several unique features that will present difficult challenges for the characterization of the genome.

ON SPECIFIC AND NON-SPECIFIC ECTOPARASITES OF HUMANS IN ICELAND

By K. SKIRNISSON

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Totally, 20 invertebrates have been reported to cause skin irritation among humans in Iceland. Four species are host-specific; the sarcoptic mange *Sarcoptes scabiei*, the pubic louse *Phthirus pubis*, the head louse, *Pediculus humanus capitis* and the closely related body louse, *P. h. humanus*. The last mentioned species has already been eradicated but the other species are still endemic, but rare. Further two species are non-specific; the human flea, *Pulex irritans* and the bed bug, *Cimex lectularius*. The human flea was eradicated by the middle of the 20th century but the bed bugs in the seventies. All these ectoparasites are regarded to have been endemic in Iceland since the country was settled more that 1100 years ago, except the bed bug, which was introduced by the end of the 19th century. Non-specific ectoparasites, able to cause skin irritations among humans, include three species of freshwater leeches (*Theromyzon* spp.), which normally parasitize on waterfowl, a previously undescribed cercaria of an avian blood fluke (Schistosomatidae), which in recent years has caused repeated outbreaks of cercarial dermatitis among children playing in a certain wading pond in Reykjavík, the bird ticks Ixodes uriea and I. ricinus, the rat tick *Ornithonyssus bacoti*, the cat and the dog mites *Cheyletiella parasitovorax* and *C. yasguri*, respectively, two bird fleas (*Ceratophyllus gallinae* and *C. garei*), two rodent fleas (*Nosopsyllus fasciatus* and *Ctenopthalmus agyrtes*) and finally the black fly *Simulium vittatum*.

PREVALENCE AND PATHOGENICITY OF THE GILL ARTERY WORM *PHILOMETRA OBTURANS* (NEMATODA, DRACUNCULOIDEA) OF PIKE (*ESOX LUCIUS* L.) IN A LAKE IN SW FINLAND

By SARA KÅLL[1], JOUKO SARVALA[2] & HANS-PETER FAGERHOLM[1]

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Philometra obturans (Prenant 1886) has been reported from the northern pike (*Esox lucius*) in Europe maily. The adult female, attaining a maximum length of even 21 cm, occupies the large arteries of the gills and may reach backwards even to the heart. Its pathogenicity has not been studied previously. Fifty-nine pikes (mainly 2-4 years of age) were sampled by fish lure in the Littois lake (1.5 km2) in SW Finland. Preliminary results (samples taken in late spring) show the prevalence of the parasite in the pike to be as high as 33.9%. The mean intensity of the infection was 2.0 worms. When worms were present, as a rule, one or both of the two outermost left and right

gill arches were infected. Blood values, blood cell counts and histopahtology of the gill arches are to be investigated. Differentiated fish behaviour during fish storage in a water tank suggests that the parasite is a serious threat to the pike, and that higher infection loads may be fatal for the pike. This is in line with previous observations (unpubl.) made on pike in the lake studied. While 90% (n = 11) of large (29-40 cm) perch studied had larvae (mean intensity 5.8) identified as P. obturans moving actively in the retina of the eyes, no adults or larvae were present in the eye or serosa of the pike. The results give additional support to previous observations suggesting that several spirurid species of the family Philometridea are highly pathogenetic to their fish hosts.

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EXPERIMENTAL INOCULATION OF 13 DOGS WITH THE CANINE NASAL MITE, *PNEUMONYSSOIDES CANINUM*

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Introduction: The canine nasal mite, *Pneumonyssoides caninum*, is a parasite of the nasal cavity and sinuses of dogs. Findings of *P. caninum* have been reported from throughout the world. Clinical signs associated with *P. caninum* infection are mainly unspecific signs of the upper respiratory tract, such as sneezing, reverse sneezing, facial pruritus and, also, impaired scenting ability.

A recent investigation in Sweden showed that 111 (21%) of 534 dogs examined in connection with necropsy were infected with *P. caninum.* Although its life-cycle is unknown, it is assumed that the mite transmits through direct contact betweeen dogs. However, an indirect mode of transmission cannot be excluded.

In this paper we describe experimental inoculation of 13 dogs with *P. caninum.*

Materials and methods: Thirteen 6-month to 2-year old purpose-bred Beagles were sedated and inoculated with 20 to 41 P. caninum mites each. The mites had been collected from naturally infected dogs at necropsy. Inoculation was done 1 to 2 days after the donor dogs had been euthanasied. In one case, however, inoculation did not take place until 3 days after the death of the donor dog.

Results: At necropsy, performed 6 to 42 weeks after inoculation, between 2 and 23 *P. caninum* mites were found in 12 of the dogs. The dog in which no nasal mites were found, had been inoculated 3 days after the death of the donor dog.

SEROEPIDEMIOLOGICAL SURVEY ON *DICTYOCAULUS VIVIPARUS* INFECTION IN CATTLE IN SWEDEN

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The seroprevalence of *Dictyocaulus viviparus* was established in Swedish cattle, with emphasis on the role of adult animals as silent carriers. A survey was performed in 10 herds between 1999 and 2000. All had a history of dictyocaulosis in 1998 and accordingly three farms were treated with ivermectin, febantel or morantel. All cattle on these farms that had been out on pasture during 1998 and 1999 were sampled at the beginning and the end of the grazing period 1999 and 2000, respectively. From each farm, blood samples were collected from 23 to 118 animals, serum was obtained and antibodies were measured with an ELISA. The overall seroprevalence was 8.9% (216/2437). In 1999, elevated levels of antibodies were identified in 4.8% (28/587) and 12.2% (77/630) of the sera at turnout and housing, respectively. The corresponding figures in 2000 was 2.8% (16/565) and 14.5% (95/657). The seroprevalence varied between 0% to 15.1% at turnout 0% to 29.9% at housing. The seroprevalence in calves <1 year and in older cattle >2 years was 14.9% and 7.0%, respectively. Thus, despite that there was a significantly greater proportion of calves than older cattle infected, seropositive animals were found in all groups. These results suggest that arrested larvae in older cattle play an important role in the over-wintering survival for lungworm infection in Sweden.

A COMPARISON OF THE rDNA INTERNAL TRANSCRIBED SPACERS OF *HAEMONCHUS* IN SMALL RUMINANTS

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The genus *Haemonchus* consists of blood-sucking parasitic nematodes in the abomasum of small ruminants. Worms of this genus are responsible for extensive losses, particularly in tropical and subtropical regions but are found in temperate regions as well. In Sweden *Haemonchus contortus* has been found close to the Artic circle. Accordingly, it has been speculated as to whether this is the same species as the one found in the tropics. In this study we examined the genetic differences in the internal transcribed spacers (ITS-1 and ITS-2) of ribosomal DNA (rDNA) in *Haemonchus* sp. The rDNA region spanning the ITS-1, ITS-2 and the 5.8S rDNA gene was amplified by PCR from each of four worms from Swedish sheep, Swedish goat

and Kenyan sheep. The fragments were sequenced and examined with restriction enzyme analysis. The intraspecific variation in the ITS-1 of the isolates varied between 0.8% and 1.8%. In the ITS-2 the variation within each isolate ranged from 0.9% to 1.8%. All studied worms were identical in the 5.8S rDNA gene. Modeling of the ITS-2 secondary structure also identified a new putative long-range interaction. As the variation within each isolate was greater than the variations between them, this indicates that the *Haemonchus* analyzed in this study all belong to the same species. However, one fixed difference was observed between the Swedish and the Kenyan isolate in position 164 of the ITS-1.

NON-CHEMICAL APPROACHES TO NEMATODE PARASITE CONTROL OF CATTLE IN SWEDEN

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Background: Control of nematode parasitic infections in young cattle is becoming an increasingly important issue in Sweden. Factors tof impact on this are economic, consumer concerns, government policy and maintenance of open landscape.

Method: A grazing trial was carried out between 1998 and 2000. Each year, first-year grazing cattle were used on improved pasture with the following 5 treatment comparisons: 1) rotational grazing using pasture grazed by older cattle the previous year as spring pasture and a mid-summer move to aftermath; 2) biological control using the nematophagous fungus *Duddingtonia flagrans*; 3) ultra-low dose copper; 4) untreated; and 5) Ivermectin bolus treated animals. Apart from the rotational strategy, all treatments were set stocked. Treatments 4 and 5 represented negative and maximum parasite control respectively.

Results: Rotational grazing proved to be as effective as the bolus treatment. The biological control resulted in a weight gain advantage of 40 kg to the untreated set stocked animals. Copper treatment had very limited benefit.

Conclusion: These results clearly illustrate the importance of subclinical nematode parasitism in Sweden. In this trial the untreated, set-stocked animals showed no overt signs of parasitism. However, substantial benefits (40 - 60 kg weight gain) can result from effective parasite control. Importantly, this has been shown to be achievable and sustainable without resorting to the use of chemical drugs.

Acknowledgement: The Swedish Council for Forestry and Agricultural Research

A SUB-UNIT VACCINE AGAINST *PLASMODIUM FALCIPARUM* MALARIA: STEP BY STEP

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Parasite sequestration and cytoadherence, a key pathogenetic feature of *Plasmodium falciparum* malaria is mediated by *Plasmodium falciparum* erythrocyte membrane protein1 (PfEMP1). The latter is a variant surface antigen implicated in various adhesive interactions and induces variant specific antibodies capable of confering protection from homologous strains. Extracellular portion of PfEMP1 exhibits a semi-conserved head structure comprised of the N-terminal sequence(NTS), the Duffy binding-like(DBL-1a) and the Cys-rich inter-domian region(CIDR1a). Sequence information generated under the *P. falciparum* sequencing project was scrutinized for charting out the PfEMP1 encoding variable(var) genes dispersed over the parasite's 14 chromosomes. All DBl-1 domian encoding sequences were cloned into entry vectors utilising site-specific recombination mechanisms and subsequently sub-cloned into destination vectors for expression in diverse systems. The recombinant proteins will be characterised immunologically and adhesive profiles defined in further detail. In order to anaylse the evolutionary history of the DBl-1 domain, a phylogenetic tree based on sequence divergence was also constructed.

REACTIVITY AGAINST B-CELL EPITOPE OF PARAMYOSIN AND ITS HOMOLOGUE FROM HUMAN A-CARDIAC MYOSIN IN PATIENTS WITH HYDATID DISEASE

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Hydatid disease was diagnosed in patients with multiple (8) or solitary (1) liver, lung, and spleen cysts by imaging technique and ELISA. Patients were operated. Albendazole was administered repeatedly after (4), or prior and after surgery (5). Patient sera were screened for antibodies against aa 457-476 of *E.granulosus* paramyosin (EPM), its homologue, aa 1534-1555 of human a-cardiac myosin known to induce experimental allergic myocarditis (CM), and Limbricoidea paramyosin (LPM). A matching group with allergies served as negative control. All patients with hydatid disease recognized LPM; five prior to albendazole treatment, CM; two with severe multiple damage, also EPM at serum dilutions 100-1600. Reactivity against CM coincided with degree of tissue damage, but was on the average low. That differs from the

data received in patients with acute/subacute Nematoda and Trematoda infections, and correlates to limited damage of the visceral organs. The coincidence of reactivity against EPM and CM in the most severe cases of hydatid disease indicates possible involvement of anti-paramyosin immunity in organic pathology. No anti-CM antibodies were found in patients with multiple cysts after chemotherapy due, possibly, to immunosuppressive action of albendazole (Ozeretskovskaya, 1982; 1997). Further cases of hydatid disease and multilocular echinococcosis should be studied with attention to immunomodulating effect of albendazole.

CONGENITAL SCHISTOSOMOSIS JAPONICA - AN UNKNOWN RISK!

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When illustrating the lifecycle of *S. japonicum,* the percutaneous route of infection is shown as the way to infect the definitive host. However, both per-oral and congenital routes have been demonstrated as important alternatives. Congenital infections have been reported from China and Japan mainly from the beginning of last century but these results seems to have been forgotten. So far, we have positively demonstrated congenital transmission of *S. japonicum* in swine, sheep and rabbits, but only if the mother is infected during the later part of her gestation. In the *S. japonicum*/pig model we have shown that the prepatent period is not prolonged as egg excretion was found 6 weeks after sow exposure. Also, we have shown that a congenital *S. japonicum* infection in piglets affected neither establishment nor fecundity of a postnatal challenge infection given 8 or 12 weeks after birth. In spite of this, the challenge infection gave rise to much less liver pathology than the similarly sized challenge-control infection. Furthermore, we have shown that repeated infections of the sow before pregnancy did not prevent establishment of a congenital infection in the piglets during pregnancy. Piglets born with a congenital infection were still fully susceptible to a challenge infection 12 weeks after birth. Treatment with praziquantel 8 weeks after delivery did not effectively cure the congenitally infected piglets and did neither prevent establishment of a challenge infection given 4 weeks after treatment.

TRICHOBILHARZIA REGENTI; INFECTION OF NONSPECIFIC HOSTS AS A SOURCE OF CNS PATHOLOGIES

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Bird schistosome larval stages are the causative agents of cercarial dermatitis. Contrary to the most species maturing in the viscera of definitive hosts, *Trichobilharzia regenti* develops in the CNS and nasal cavities. In an abnormal host (mammals), the development is incomplete, however, following skin phase the parasites migrate to the host CNS, infection of which leads to certain neuromotor disorders. In order to explain the clinical symptoms in *T. regenti* infections of nonspecific host, the histopathological observation of mouse (*Mus musculus*) skin and CNS during various phases of the parasite development was performed.

Our observation showed the stimulation of immune response in the skin soon after the mouse primoinfection (1,000 cercariae per animal) which was characterised by infiltration of neutrophils (6 hours p.i.) and eosinophils (1 day p.i.). Since day 2 p.i, no schistosomula were detected in the skin, however, most of them were found in the thoracic spinal cord; on day 10 p.i., all parts of the spinal cord and the cerebellum were infected. Parasites were located in menings as well as in the matters and surrounded by cellular infiltration in spongy tissue, perivascular eosinophilic infammation and necrotic neurons. Five and ten times repeated mice infections led to stronger and predominatly eosinophilic inflammation; only in the latter case no parasites were found in the CNS. Presenting results support our view that the histopathological changes arising as a consequence of parasite migration may explain neuromotor symptoms during the first contacts of a nonspecific host with bird chistosome cercariae.

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THE EMERGENCE OF *TAENIA SOLIUM* CYSTICERCOSIS AS A SERIOUS AGRICULTURAL PROBLEM AND PUBLIC HEALTH RISK IN EASTERN & SOUTHERN AFRICA - AN UPDATE

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Objective: Previous reports have indicated that cystercosis caused by the pork tapeworm, Taenia solium, is a serious problem in a rural pig raising area of northern Tanzania. Further surveys in other rural pig raising areas of eastern and southern Africa were conducted to determine whether the problem is a very focal one or more widespread considering that pig keeping and pork consumption are increasing dramatically in the region.

Materials and methods: Surveys were conducted in Kenya, the southern highlands of Tanzania, and Zambia whereby the tongues of pigs were examined for *T. solium* cysticerci. In addition questionnaire surveys were conducted in Tanzania and Zambia to collect information from pig owners concerning factors related to cysticercosis. A retrospective survey of pig slaughterhouse records from western Zimbabwe was also conducted.

Results: Lingual examination of pigs in the areas mentioned revealed the following crude prevalences in the communities surveyed: Kenya (0 - 20%), Tanzania (7.6%), Zambia (5.2 - 20.6%), Zimbabwe (up to 25% of pigs from rural small holders). In addition, questionnaire surveys in Tanzania and Zambia indicated that pig husbandry practices, poor sanitation and ignorance may be the main reason for the situation and also that there are many people in these communities suffering from epileptic seizures of unknown etiology possibly related to neurocysticercosis. Also many farmers informed that they sell their pigs to traders who then transport them to urban centres for slaughter.

Discussion: These studies indicate that *T. solium* is widespread in the eastern and southern Africa region providing evidence that cysticercosis is emerging as an important agricultural problem and public health risk not only in rural areas where pigs are raised under extensive conditions but also in urban centres where pork consumption is increasing in popularity and veterinary public health infrastructure is poor.

Acknowledgement: These studies have been conducted under the auspices of the Livestock Helminths Research Project in Eastern and Southern Africa, funded by DANIDA through its programme for enhancing research capacity in developing countries (ENRECA).

SUBMITTED PAPERS – POSTER PRESENTATIONS

IMMUNIZATION OF RAINBOW TROUT FRY WITH *ICHTHYOPHTHIRIUS MULTIFILIIS* SONICATE: PROTECTION OF HOST AND IMMUNOLOGICAL CHANGES

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Rainbow trout fry (10 weeks post hatch) were immunized with a sonicate of formalin killed trophonts (injected or immersion) of the fish pathogenic ciliate *Ichthyophthirius multifiliis*. Challenge infections 22 days after immunization showed a relative protection. No changes in antibody titers and mucus cell counts were associated with the response. However, a minor change in peripheral blood leukocyte and epidermal cell marker were found.

IMPORTANCE OF THE ALTERNATIVE PATHWAY IN COMPLEMENT MEDIATED KILLING OF *GYRODACTYLUS DERJAVINI*

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Various studies have documented that mucus and sera contain substances highly lethal to gyrodactylids. These substances are proven to be heat labile and it has therefore been suggested that complement play a crucial role in the killing of these monogeneans. In addition, it has been confirmed that complement factor C3 is deposited on certain structures of the parasite during incubation with native sera. Finally, it has been alleged that the alternative complement pathway is responsible for the lethal effect. Using monoclonal antibodies raised against recombinant rainbow trout factor B, we have therefore studied the involvement of this factor central to the alternative pathway.

Living *G. derjavini* were subjected to incubations in water or sera - either native, with EDTA or heat-inactivated. Subsequently, by immunocytochemistry, the possible deposition of factor B was examined with the mAb\'s using a biotin amplification step and a Ni-enhanced DAB reporter system.

Gyrodactylids incubated in native sera died within 20-30 s, whereas parasites subjected to heat-inactivated sera or water all lived at least 4-6 h after exposure. EDTA containing sera had no or little adverse effect. Factor B deposition on various parasite

structures were detected only on *Gyrodactylus* exposed to sera containing functional complement activity.

The findings documents the involvement of rainbow trout factor B in the complement mediated killing of *G. derjavini*.

Acknowledgement: The mAbs were kindly provided by Karsten Skjødt.

INFLUENCE OF CODON USAGE ON THE IMMUNOGENICITY OF A DNA VACCINE AGAINST *TOXOPLASMA GONDII*

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Toxoplasma gondii is the intracellular protozoan parasite responsible for animal and human toxoplasmosis.

In an attempt to develop a DNA vaccine against bradyzoites, the tissue stage of *T. gondii*, a construct encoding the antigen SAG4 was made. SAG4 is an 18 kDa surface antigen expressed only in the bradyzoite stage. The initial experiments with the first SAG4 DNA-vaccine in Balb/c mice showed only a weak response in production of antibodies.

The poor immune response towards the SAG4 vaccine might be due to a low expression of the antigen caused by difference in codon usage between the mammalian host and the protozoan parasite. This has been observed in other species and several studies have showed that optimising codon usage for eukaryotic cells can enhance the expression of antigens and thus the immunogenicity of the vaccine

To explore whether codon bias accounted for the poor immunogenicity of the first SAG4 vaccine construct, a new synthetic SAG4 vaccine is currently being constructed using human codons. Data will be presented on the effect on the humoral immune response of the synthetic SAG4 vaccine in a mouse model.

DESCRIPTION OF A NEW SPECIES OF *CERVIDELLUS* (NEMATODA: CEPHALOBIDAE) FROM SÉNÉGAL

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The genus *Cervidellus* Thorne, 1937 has become increasingly diverse as new species are described and old species transferred to it. The genus was reviewed by Boström & De Ley (1996), who gave it a provisional and rather broad diagnosis: "Labial probolae bifurcated at one to three levels. Primary cephalic axils with even number of tines, sometimes including two guarding pieces. Tail of both sexes acute." During a survey of plant-parasitic and free-living nematodes in the central regions of Sénégal, soil samples revealed the presence of specimens of a new species of *Cervidellus*. The new species extends the ranges of morphological character states in the genus and is here described from studies by light and scanning electron microscopy. The new species is distinguished from all other species in the genus by the combination of the following characters: prominent longitudinal cuticular ridges (14-18 at midbody), six leaf-like and three-lobed lips, cephalic axils each with two triangular guarding processes, three slender conoid-elongate labial probolae, each with a minute bifurcation apically, cheilostom without sclerotized rhabdia, and vulva in a sunken area surrounded by cuticle ridges.

The authors thank Samba B. Ndiaye for technical assistance during the sampling.

DEPTH PREFERENCE IN MARINE FISH AND INFECTION BY *ANISAKIS SIMPLEX* THIRD-STAGE LARVEA

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The purpose of this study was to get further information on how the preferred depth of fish influenced on the infection of whaleworm *A. simplex* third-stage larvae. The fish used in this study were caught near the island Vestvågøy in northern Norway. Approximately 20 specimens from each of the host species saithe (*Pollachius virens*) living in the upper pelagic waters, cod (*Gadus morhua*) and the deep sea living redfish (*Sebastes marinus*), were sampled on a monthly basis, and the presence of whaleworm L3-larvae investigated. The results showed a stable infection during the year within each of the three fish species, both concerning abundance and prevalence. Saithe had the highest abundance and prevalence, redfish the lowest, and cod taking a medium position between the former two. This results are in accordance with the fact

that the infected cruastacean intermediate hosts mostly seems to occur in the upper levels of the sea, thereby infecting fish living here at a higher rate then fish living at greater depth. This distribution of eggs and hence infected fish hosts, makes biological sense since most whales, the main hosts of the parasite, spend a high proportion of their time in the same few hundred meters of the water column.

PARASITE INDUCED CHANGES IN HOST BEHAVIOUR: STUDIES WITH *DIPLOSTOMUM SPATHACEUM*

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The digenean eye fluke *Diplostomum spathaceum* is a common parasite in many species of fish both in fresh and brackish water. The parasite uses three hosts in completing its life cycle: fish eating birds (definitive host), snails (1st intermediate host) and fish (2nd intermediate host). While in fish, metacercariae of *D. spathaceum* cause a condition known as parasite induced cataract making the lens opaque with metabolic excretions. It is then reasonable to assume that the opacity of the lens also impairs the vision ability of fish, which in turn is likely to make the fish more susceptible to predation. The cataract formation can thus be considered as an evolutionary adaptation by the parasite in order to enhance its transmission. Earlier it has been suggested that impaired vision ability makes the parasitized fish swim closer to the water surface where they are more readily caught by predators. In this study we propose an alternative mechanism, which could increase the predation susceptibility of *D. spathaceum* infected fish. We studied the behaviour of parasitized and control fish in a light-dark gradient and found that there was no difference in swimming depth between groups. However, the reactive ability to artificial predator was significantly reduced with parasitized fish compared to controls. Significance of this phenomenon to actual increase in vulnerability to avian predators is discussed.

AGE DEPENDENT INFECTIVITY AND MORTALITY OF *DIPLOSTOMUM SPATHACEUM* CERCARIAE

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The eye fluke, *Diplostomum spathaceum*, is a common parasite of freshwater fishes. Within the first intermediate host (freshwater snail), the parasite produces free-

living cercariae with finite, non-renewable energy resources to infect the second intermediate host (fish). In this study, we examined what is the mortality rate of cercariae and how the age of cercariae affects their infectivity. We used intensive monitoring of the mortality rate to capture the exact shape of the mortality curve. At the same time, we conducted single fish infections to estimate the decrease in infectivity and to see changes in the shape of parasite distribution in fish. We found out that the amount of active and living cercariae declined with time reaching nearly zero in 36 hours. Infectivity decreased to zero in 27 hours indicating that cercariae lose their infectivity several hours before death. The parasite distribution in fish also shifted towards the normal distribution with the age of cercariae. The results are discussed from the basis of parasite evolution and parasite-host relationships.

EPIDEMIOLOGY OF BOVINE ENDOPARASITES IN THE HIGHVELD AND LOWVELD COMMUNAL FARMING AREAS OF ZIMBABWE

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Between January 1999 and December 2000, 17 500 cattle faecal samples from randomly selected dipping sites in the Lowveld and Highveld communal farming areas were examined for helminths eggs and coccidial oocysts. Strongyle larvae were also identified following the culture of pooled faeces. The monthly presence or absence of metacercariae was noted from grass samples collected around water drinking points and monthly snail densities were also determined. Strongylid nematodes were the most prevalent parasites, followed by paramphistomes species and *Fasciola gigantica* . Infections with other parasites was less prevalent. Calves had highest the prevalence of strongylid nematodes and coccidia while adults had the highest prevalence of paramphistomes species and *F. gigantica.* The prevalence of most common internal parasite infections was higher during the wet season. Data from larval culture indicated that the animals carried mixed parasite burdens. *Cooperia, Haemonchus* and *Trichostrongylus* were the most prevalent genera found. *Haemonchus* was more prevalent in summer, while *Cooperia* and *Trichostrongylus* were present in significant numbers during winter and the hot dry season. Metacercariae were qualitatively found to be present on grass samples collected around watering points from January to August and November to December. Districts with a higher prevalence of *Lymnae natalensis* were noted to have a higher *Fasciola* infection in cattle. With respect to paramphistomes, districts with higher prevalence of either *Bulinus tropicus* or *Bulinus forskalis* or *Biomphlaria* were also noted to have a higher paramphistome infection in cattle. Schistosomosis in cattle was also found to be higher in districts where the prevalence of *Bulinus globosus* was high.

SEROLOGICAL SURVEY OF *TOXOPLASMA GONDII* INFECTIONS IN PIGS SLAUGHTERED IN SWEDEN

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The protozoan parasite *Toxoplasma gondii* is a widespread pathogen infecting both animals and humans. The infection can be acquired either by ingestion of meat containing encysted parasites or oocysts excreted by cats that contaminate the environment. Case-control studies conducted in several European countries have shown that human infection is strongly associated with eating raw or undercooked meat. The aim of the present study was to investigate the prevalence of T. gondii infections in Swedish pigs, so that the risk of ingesting *T. gondii* tissue cysts with raw or inadequately cooked pork could be estimated. During February to May 1999 a total of 807 meat juice samples were collected from 10 different slaughterhouses in Sweden and analysed for antibodies to *T. gondii* by an indirect ELISA (Wingstad *et al,*. 1997). Antibodies to *T. gondii* were detected in 42 (5.2%) of the 807 samples. The seroprevalence in finishing pigs was 3.3%, and 17.3 % in adult swine. These findings agree well with those found in other Nordic countries.

References

Wingstad A, Lind P, Haugegaard J, Henriksen SA, Bille-Hansen V, Sørensen V. Veterinary Parasitology 1997; 72: 129-40

AN IgG AVIDITY ELISA FOR DIAGNOSIS OF BOVINE *NEOSPORA CANINUM* INFECTION

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Neospora caninum is a coccidian parasite which is an important cause of bovine abortion and birth of feeble calves. Demonstration of antibodies to *N. caninum* indicates that an animal is infected with this parasite. However, until recently it was not possible to determine the time of initial infection on the basis of serologic tests. This

situation has changed with our development of an ELISA that measures the avidity (functional affinity) of *N. caninum* specific IgG antibodies (Björkman et al., J. Vet. Diagn. Invest.,1999, 11: 41-44). In this avidity-ELISA low affinity antibodies are eluted by including an incubation with urea after the primary serum incubation. The antibody titres measured with and without urea incubation are then used to calculate an avidity index. When the *N. caninum* IgG avidity ELISA was applied in a herd with abortion outbreaks, we found that cows that aborted had lower avidities, i.e. were more recently infected, than the normally calving cows (McAllister *et al.*, J. Am. Vet. Med. Assoc., 2000; 217: 881-7). Analysis of sequential sera collected in two *N. caninum* infected cattle herds revealed that the herd avidity increased during the years after introduction of infection. The results show that IgG avidity measurements may be a valuable complement to standard IgG assays.

MATERNAL MALARIA: NON-IMMUNE IGG BOUND TO PfEMP1 MEDIATES SEQUESTRATION OF *PLASMODIUM FALCIPARUM* INFECTED ERYTHROCYTES IN THE HUMAN PLACENTA

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Infections with *Plasmodium falciparum* during pregnancy lead to the accumulation of parasitised red blood cells (IE) in the placenta and are an important cause of maternal morbidity and mortality. Certain strains of *P.falciparum* have been found to bind non-immune immunoglobulins onto the surface of the host erythrocyte, which made us investigate their role in sequestration, in particular the possibility that IgG could bridge the IE to Fc-receptors present in the placenta.

We examined the frequency of IgG binding IE obtained from placentas and peripheral blood of six malaria-infected Cameroonese women. The results revealed that IE carrying IgG accumulate in the placenta but are absent from the peripheral circulation of the mothers.

The *P. falciparum* strain TM284S2 cloned for binding to normal non-immune IgG, was found to adhere massively to placental tissue in vitro. The adherence of IE of the parasite clone to cryo-sections of placenta could be drastically and dose-dependently reduced by pre-incubation with immunoglobulin-binding GST-fusionproteins or with protein A of S. aureus, while incubation with glycosaminoglycans (CSA, HA) did not affect the binding.

To map the immunoglobulin binding constructs corresponding to the extracellular domains of the PfEMP1 were expressed as GST-fusionproteins in E.coli. The DBL2b-domain was found to bind non-immune IgG, when tested in ELISA. Several

additional GST-fusionprotein constructs representing fractions of the DBL2b suggested that this domain carries multiple immunoglobulin-binding motifs.

Thus the data indicate that a DBLb is the critical domain of PfEMP1 that binds monomeric non-immune IgG and it presumably acts as a bridge to Fc receptors of the placenta.

DEVELOPMENT OF AN ANTI-SEVERE MALARIA VACCINE

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The possibility of developing a novel malaria vaccine with a virulent factor-*Plamodium falciparum* erythrocyte membrane protein 1 (PfEMP1) was assayed by RNA immunization approach. To maximally mimic PfEMP1 molecule on pRBC surface, mini-var genes were generated by ligation of a transmembrane region (ATS) to DBL1, CIDR and DBL2. The GST sequence was put upstream of the three mini-vars to form GST-DBL1-TM, GST-CIDR-TM and GST-DBL2-TM constructs. The three constructs were cloned in-frame into the SFV4.2 vector. Messenger RNAs encoding the GST-DBL1-TM, GST-CIDR-TM and GST-DBL2-TM were synthesized respectively with in vitro mRNA transcription system. RNA particles were packaged by co-electroporation of BHK cell with each of the mRNAs plus two RNA templates encoding SFV coat and spike proteins. The titre of recombinant virus (with only mini-var RNA genome) was determined by infection of BHK cells followed by IFA.

Four groups of Balb/C mice were injected 106 particles/mouse followed by one or twice boost with 2x 106 particles. One group was boosted with 50μg recombinant protein after two or three times of particle immunization. Sera were collected two weeks after the last boost. The antibody titre of each mouse was determined by IFA. Results suggested that mice immunized with DBL1 construct gave highest surface fluorescence titre. Our immunization approach indicated that PfEMP1 N-terminal domain (DBL1) could be a potential target for further vaccine study.

A VAR GENE MICROARRAY

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To elucidate the mechanistic details of the var gene switching, a temptation will be directed towards the study of the whole *Plasmodium falciparum* genome as the sequencing of all the chromosomes is virtually complete. We are planning to design our

custom VAR GENE MICROARRAY and the MALARIA GENOME CHIP (in collaboration with E. Winzeler, GNF, San Diego, USA), together with the generation of phenotypic malaria variants by micromanipulation cloning method, to track the differential gene expression profile under different physiological condition.

OBJECTIVES: (1) To study the differential gene expression profile of clonal parasites at different stages during intra-erythrocytic development: ring-stage parasite (ubiquitous var genes expression) and trophozoite (single and distinct var gene variant) parasite. (2) To correlate var gene switching with different phenotypic variants generated from a clonal parasite population (rosetting parasites and non-rosetting parasites). (3) To characterize var gene expression pattern of different isogenic *P. falciparum* populations with defined adhesive phenotypes to different endothelial receptors. (4) To investigate the expression, regulation and switching of var gene under selective pressure of host's immune system through in vivo study.

TRANSPORT OF RIFINS AND PfEMP1 TO THE SURFACE OF *PLASMODIUM FALCIPARUM* INFECTED ERYTHROCYTES IS VESICLE MEDIATED

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During the asexual stage of malaria infection, the intracellular parasite exports lipids and proteins to the erythrocytic cytosol and plasma membranes. PfEMP1, *Plasmodium falciparum* erythrocyte membrane protein 1, is a protein transported to the host cell surface, which is involved in the pathogenicity mechanisms of the parasite. The protein lacks a signal peptide typical for transported proteins and it is therefor unknown how it gets to the surface. The RIFINS are another group of proteins transported to the erythrocytic surface. These are encoded by the rif genes, repetitive interspersed family, which is the largest gene family of *P. falciparum* and they have a two-exon structure coding for one predicted signal peptide and one ORF of around 1 kb. The signal peptide is directing the RIFINS to the export machinery of the parasite.

We have studied the transport and localisation of PfEMP1 and RIFINS, from the parasitophorous vacuole to the host cytosol and the cell membranes, using immunofluorescence techniques. The proteins are co-localised and the transport mechanism seems to be the same for the two groups of protein. Further using fluorescent lipid tracer shows that the proteins are associated with membranous structures during transport.

Inhibition of parasitic membrane formation also inhibits transportation of the proteins, which indicates that the transportation is vesicle mediated.

THE EFFECT OF REPEATED LEISHMANIN SKIN TESTING ON THE IMMUNE RESPONSES TO *LEISHMANIA* ANTIGEN IN HEALTHY VOLUNTEERS

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Leishmanin skin test (LST) utilizing killed *Leishmania* promastigotes is used in epidemiological studies of leishmaniosis and in evaluating immunogenicity of *Leishmania* vaccines. Since in many instances multiple tests have to be employed, it is important to address their influence on the subsequent immune response. This study aims at evaluating the effect of multiple *Leishmania major* LST on the in vitro and in vivo immune responses in LST non-reactive healthy Sudanese volunteers. Immune responses were measured in individuals at three time points; (a): prior to first LST, (b): 48 hours after first LST and (c) 48 hours after second LST, performed one month after the first LST. None of the volunteers converted to skin test positive during the study period. In vitro immune responses were assessed by measuring levels of IFN-g, IL-4, IL-10 and IL-12 in 72-hour cultures of purified PBMCs or whole blood stimulated with freeze-thawed *Leishmania donovani* antigen. Levels of IFN-g and IL-10 remained unchanged over the three time-points of follow-up. No production of IL-4 and IL-12 was detected in either PBMCs or whole blood. These results show that repetition of LST using *L. major* leishmanin antigen does not modulate the in vivo or in vitro immune responses of healthy Sudanese volunteers to *Leishmania* antigen. The paradox of how heat-killed *L. major* vaccine mixed with BCG enhances the immune response while thimorosal-killed promastigotes does not, is discussed.

PARASITES OF IMPORTED DOGS AND CATS IN ICELAND 1989-2000

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In 1989, import of dogs and cats to Iceland was permitted after decades of restrictions. Altogether 608 dogs and 236 cats from at least 27 and 18 countries, respectively, have been imported during 1989-2000 and subjected to 6-12 weeks of quarantine. The animals are treated against cestodes before arrival and during quarantine against helminths, protozoans and ectoparasites.

Faecal samples, collected at the beginning and at the end of the quarantine period, were examined for the presence of intestinal parasites using the formalin-ethyl acetate sedimentation technique. Ectoparasites detected during isolation were also examined.

The following parasites were found (D=in imported dogs, C=in imported cats, N= already found in native populations): *Giardia* sp. (DCN), *Isospora bahiensis* (D), *I.*

canis (D), *I. felis* (CN), *I. rivolta* (D) *Sarcocystis* sp. (DN), *Opistorchis felineus* (C), *Ancylostoma* sp. (DC), *Capillaria aerophila* (DN), *Strongyloides stercoralis* (D), *Toxascaris leonina* (DCN), *Toxocara canis* (DN), *T. cati* (CN), *Trichuris vulpis* (D), *Uncinaria stenocephala* (DN), *Ctenocephalides felis* (DC), *Ixodes ricinus* (D(N)) and *Ripicephalus sanguineus* (D). Furthermore, *Demodex canis* (D), *Trichodectes canis* (D), *Cheyletiella yasguri* (D) and *C. parasitovorax* (C) were found on animals in connection with this import.

Measures against parasites during quarantine were apparently successful in most cases. However, it is likely that at least three species of ectoparasites (*D. canis*, *C. yasguri*, *C. parasitovorax*) have been transmitted to the native populations.

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THE SUSCEPTIBILITY OF WHITEFISH (*COREGONUS LAVARETUS* L.) TO EXPERIMENTAL INFECTIONS WITH THE MONOGENEAN *GYRODACTYLUS SALARIS* MALMBERG, 1957

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Abstract

The susceptibility of whitefish (*Coregonus lavaretus*) to the monogenean *Gyrodactylus salaris* was experimentally tested in the laboratory after exposure of uninfected yearlings of naive whitefish to the parasite on infected salmon. The results demonstrated that *G. salaris* appeared reluctant to attach to and establish on the whitefish. In addition, only slight parasite reproduction was observed on three (15 %) of the individually isolated fish. The rest of the experimental fish were innate resistant and eliminated the infections with no observable parasite reproduction. Thus, whitefish seem to play no role in the population dynamics of *G. salaris*. However, theoretically whitefish might take part in dissemination of *G. salaris* as transport host.

Key words: Gyrodactylus, host specificity, Monogenea, Platyhelminthes, *Coregonus*

Introduction

Gyrodactylus salaris Malmberg, 1957 is recognised as a serious pathogen in natural Atlantic salmon (*Salmo salar* L.) populations in Norway (Johnsen *et al.*., 1999). Non-salmonid fish species play no apparent role in the population dynamics of *G. salaris* (Bakke & Sharp, 1990; Bakke *et al.*, 1990, 1991a; Soleng & Bakke, 1998), whereas the parasite can reproduce on a number of different salmonid species in addition to Atlantic salmon (Bakke *et al.*., 1991b, 1992a, b, c, 1996, 1999; Jansen & Bakke, 1995). Furthermore, Harris *et al.* (2000) observed that experimentally induced stressors increased the susceptibility of salmonids to *G. salaris*.

Whitefish (*Coregonus lavaretus* L.) can consist of both freshwater stationary and anadromous populations (Jonsson *et al.*, 1988), and is known to occur in lotic habitats as river systems and frequently migrate into brackish water areas in some fjords in Norway (Pethon, 1985). Thus, theoretically whitefish might play a role in dispersal of *G. salaris* both within the freshwater habitats and through estuarine areas (cf. Soleng & Bakke, 1997; Soleng *et al.*,

1998; Soleng, 1999). As the susceptibility and resistance of whitefish to *G. salaris* is unknown, the present laboratory study was conducted to examine the possibility for attachment, establishment and reproduction of *G. salaris* in order to evaluate their potential importance in the parasite population dynamics and dispersal.

Materials and Methods

The *Gyrodactylus salaris* strain used in the experiments originated from wild salmon caught in the River Lierelva (Buskerud County) in Southeast Norway. These infected fish were kept in the laboratory in a fish tank (1 x 1 x 0.3 m water level) at 12 °C for at least four weeks prior to the experiments. The whitefish originated from the River Dokka (Oppland County), and were caught immediately after hatching and transferred to an artificial pond (15 x 4 x 1 m water level) in the Botanical garden at the University of Oslo. The whitefish used in the experiments (age 0+) were raised by natural feeding in this pond through the summer and used in the autumn in the present experiments (mean length: 10.2 cm, range 9.1 - 11.5 cm; mean weight: 7.1 g, range 4.9 - 11.9 g in weight). All fish were acclimated to the laboratory conditions of 11.5 °C (range 11.3 - 11.7 °C) for 7 days prior to the experiments. The fish were not fed during the experiments.

Twenty whitefish were exposed to 18 heavily *G. salaris* infected wild salmon parr for 24 hours in a fish tank. As parasite transmission were restricted after this procedure, the whitefish were subsequently divided into three groups and placed in plastic boxes (0.38 x 0.27 x 0.12 m water level) each containing six heavily infected salmon parr for a further exposure period of 24 hours. Thereafter 20 whitefish were individually isolated in small plastic boxes (0.17 x 0.11 x 0.05 m water level) with mesh bottoms (mesh size 0.5 x 0.5 cm) floating in the fish tanks. The fish tanks received approximately 1 l/min of charcoal filtered dechlorinated Oslo tap water (for chemical composition of water, cf. Soleng *et al.*, 1999).

The course of infection was assessed by counting the parasites on fish anaesthetised in 0.04% chlorobutanol. Details concerning the experimental set-up, anaesthesia, examination of G. salaris on fish, are reported elsewhere (cf. Bakke *et al.*, 1991a; Soleng & Bakke, 1997, 1998). All parasitological terms used follow the definitions given in Margolis *et al.* (1982).

Results

All the whitefish became infected (mean intensity 9.1, range 1 - 24) after the second experimental infection procedure with exposure to heavily infected salmon parr. On 17 of the whitefish (85 %) there were no increase in the size of the infrapopulations after isolation of the fish which indicate that no parasite reproduction occurred, and all the infections were eliminated between day 7 and 14 (Fig. 1A). However, one fish was still infected (with only one parasite) on day 21 (the host died before the next examination). On the rest of the hosts (n=3, 15 %) a slight increase in infection intensity indicate the occurrence of parasite reproduction during the first week (Fig. 1B). On one of these hosts the infection was eliminated after 14 days, while the

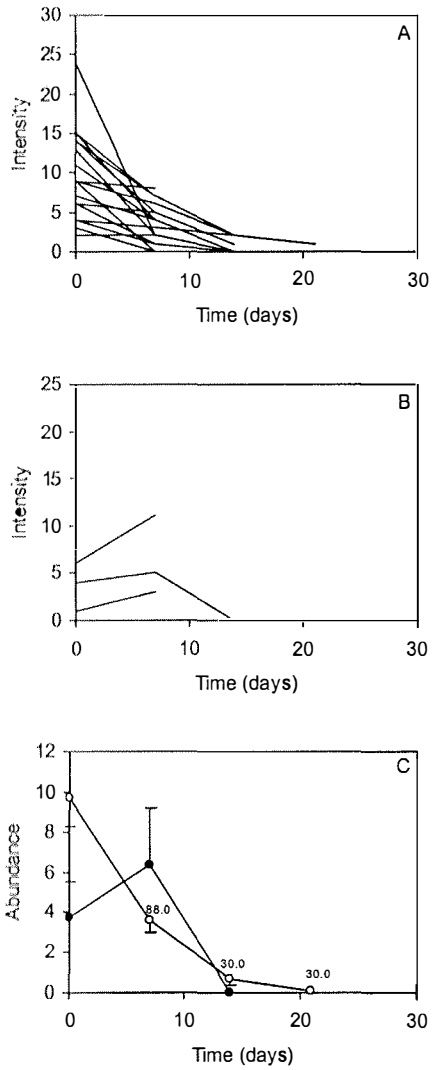

Fig. 1. Course of infection of *Gyrodactylus salaris* on 20 experimentally infected individually isolated whitefish (*Coregonus lavaretus*). (A) Innate resistant fish (n = 17) allowing no parasite reproduction. (B) Slightly susceptible fish (n = 3) allowing some parasite reproduction. (C) Abundance of 3 slightly susceptible fish (●) and 17 innate resistant fish (○). One line represents one fish; termination of the lines indicates host death (in A and B). Bars indicate Standard Error of mean. Prevalence in (C) is 100 % if not indicated on the figure by numbers close to data points.

other two hosts died before the last examination on day 14. Figure 1C presents the average infection of these two groups based on the individual trajectories observed.

Discussion

Gyrodactylus salaris seemed reluctant to attach to whitefish. Most whitefish appeared innately resistant to *G. salaris* with no parasite reproduction occurring. Only three of twenty fish allowed some minor parasite reproduction to occur. The longest duration of a *G. salaris* infection observed on whitefish was 21 days.

These results are consistent with other experiments dealing with experimentally *G. salaris* infected salmonids like Lake trout (*Salvelinus namaycush*) and brown trout (*Salmo trutta*) which appeared unable to support *G. salaris* populations (Bakke *et al.*, 1992c; Jansen & Bakke, 1995). However, there appear to be differences in susceptibility and resistance to *G. salaris* between host stocks of the same salmonid species as found in Arctic char (*Salvelinus alpinus*) (Bakke *et al.*, 1996) and brown trout (Jansen & Bakke, 1995; Bakke *et al.*, 1999). Coregonines display a phenotypic plasticity that far exceeds what is usual in the animal kingdom. The taxonomic recognition among Eurasian populations of *Coregonus* is complicated due to persistent reproductive isolation among morphologically distinct ecotypes, besides numerous cases of polyphyletic and paraphyletic groups with distinct morphotypes genetically more closely related to each other than to identical ones from other regions (see Barnatchez & Dodson, 1994).

Thus, it is difficult to extrapolate from our results on one stock of whitefish to the whitefish "species" as such.

It is recently reported that stressed and immunosuppressed salmonids can sustain a prolonged *G. salaris* infection allowing parasite reproduction to occur (cf. Harris *et al.*, 2000). Theoretically, immunosuppressed whitefish, whatever reason, might therefore play a more significant role in dispersal of this salmon parasite (cf. Soleng *et al.*, 1998; Soleng, 1999). However, based on the present results it can be concluded that whitefish seems to play no significant role in the population dynamics of *G. salaris*.

Acknowledgement

We wish to thank Åge Brabrand for collecting and cultivating the whitefish for the present study.

References

Bakke TA, Sharp LA. Susceptibility and resistance of minnows, *Phoxinus phoxinus* (L.) to *Gyrodactylus salaris* Malmberg, 1957 (Monogenea) under laboratory conditions. Fauna Norv Ser A 1990; 11: 51-5

Bakke TA, Jansen PA, Brabrand Å. Susceptibility and resistance of brook lamprey, *Lampetra planeri* (Bloch), roach, *Rutilus rutilus* (L.) and perch, *Perca fluviatilis* L. to *Gyrodactylus salaris* Malmberg (Monogenea). Fauna Norv Ser A 1990; 11: 23-6

Bakke TA, Jansen PA, Hansen LP. Experimental transmission of *Gyrodactylus salaris* Malmberg, 1957 (Platyhelminthes, Monogenea) from the Atlantic salmon (*Salmo salar*) to the European eel (*Anguilla anguilla*). Can J Zool 1991a; 69: 733-7

Bakke TA, Jansen PA, Kennedy CR. The host specificity of *Gyrodactylus salaris* Malmberg (Platyhelminthes, Monogenea): susceptibility of *Oncorhynchus mykiss* (Walbaum) under experimental conditions. J Fish Biol 1991b; 39: 45-57

Bakke TA, Harris PD, Hansen LP, Jansen PA. Host specificity and dispersal strategy in gyrodactylid monogeneans with particular reference to *Gyrodactylus salaris* (Platyhelminthes, Monogenea). Dis Aquat Org 1992a; 13: 45-57

Bakke TA, Harris PD, Jansen PA. The susceptibility of *Salvelinus fontinalis* (Mitchill) to *Gyrodactylus salaris* Malmberg (Platyhelminthes; Monogenea) under experimental conditions. J Fish Biol 1992b; 41: 499-507

Bakke TA, Jansen PA, Grande M. The susceptibility of *Salvelinus namaycush* (Walbaum) to *Gyrodactylus salaris* Malmberg (Platyhelminthes; Monogenea) under experimental conditions. Fauna Norv Ser A 1992c; 13: 1-7

Bakke TA, Jansen PA, Harris PD. Differences in susceptibility of anadromous and resident stocks of Arctic charr to infections of *Gyrodactylus salaris* under experimental conditions. J Fish Biol 1996; 49: 341-51

Bakke TA, Soleng A, Harris PD. The susceptibility of Atlantic salmon (*Salmo salar*) X brown trout (*Salmo trutta*) hybrids to *Gyrodactylus salaris* Malmberg and Gyrodactylus derjavini Mikailov. Parasitology 1999; 119: 467-81

Bernatchez L, Dodson JJ. Phylogenetic relationships among Palearctic and Nearctic whitefish (*Coregonus* sp.) populations as revealed by mitochondrial DNA variation. Can J Fish Aquat Sci 1994; 51 (Suppl. 1): 240-51

Harris PD, Soleng A, Bakke TA. Increased susceptibility of salmonids to the monogenean *Gyrodactylus salaris* following administration of hydrocortisone acetate. Parasitology 2000; 120: 57-64

Jansen PA, Bakke TA. Susceptibility of brown trout to *Gyrodactylus salaris* (Monogenea) under experimental conditions. J Fish Biol 1995; 46: 415-422

Johnsen BO, Møkkelgjerd PI, Jensen AJ. Parasitten *Gyrodactylus salaris* på laks i norske vassdrag, statusrapport ved inngangen til år 2000. NINA Oppdragsmelding 1999; 617: 1-129 (In Norwegian, English summary)

Jonsson N, Jonsson B, Ruud-Hansen J. Downstream displacement and lifehistory traits of whitefish, *Coregonus lavaretus*, in a Norwegian river. Env Biol Fish 1988; 23: 197-204

Margolis L, Esch GW, Holmes JC, Kuris AM, Schad GA. The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). J Parasitol 1982; 68: 131-3

Pethon P. Aschehougs store fiskebok. H. Aschehoug, Co. A/S, Oslo, 1985 (In Norwegian)

Soleng A. *Gyrodactylus salaris* Malmberg, 1957 (Platyhelminthes, Monogenea): Dispersal potential and environmental stress. Dr. scient.-thesis, Zoological Museum, University of Oslo, 1999

Soleng A, Bakke TA. Salinity tolerance of *Gyrodactylus salaris* (Platyhelminthes, Monogenea): laboratory studies. Can J Fish Aquat Sci 1997; 54: 1837-45

Soleng A, Bakke TA. The susceptibility of three-spined stickleback (*Gasterosteus aculeatus*), nine-spined stickleback (*Pungitius pungitius*) and flounder (*Platichthys flesus*) to experimental infections with the monogenean *Gyrodactylus salaris*. Fol Parasitol 1998; 45: 270-4

Soleng A, Bakke TA, Hansen LP. Potential for dispersal of *Gyrodactylus salaris* (Platyhelminthes, Monogenea) by sea-running stages of the Atlantic salmon (*Salmo salar*): field and laboratory studies. Can J Fish Aquat Sci 1998; 55: 507-14

Soleng A, Poléo ABS, Alstad NEW, Bakke TA. Aqueous aluminium eliminates *Gyrodactylus salaris* (Platyhelminthes, Monogenea) infections in Atlantic salmon. Parasitology 1999; 119: 19

ERRATUM

There is an error in the article "What determines the longevity of mammalian nematodes" by A. Skorping and A.F. Read, Bull SSP 2000 vol 10 no 2. This error is the complete responsibility of the editor, and not an attemp at improving the authors' manuscript.

The whole last paragraph on page 59: "Parasite communities from single host individuals have one undoubted feature: the overwhelming majority of parasite infrapopulations cannot maintain their numbers by self-reproduction. These communities are rigorously defined spatially. The existence of an infracommunity is restricted to its individual host's lifetime. However, at this level parasites only interact or do not interact, compete or do not compete with each other, and either form or do not form guilds. As a result of these interactions isolationist or interactive communities are formed." has been added unintentionally.

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THE PARASITE FAUNA OF AN ISOLATED POPULATION OF GRAYLING, *THYMALLUS THYMALLUS* (L.) IN THE RIVER VEFSNA, NORTHERN NORWAY

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Introduction

The grayling (*Thymallus thymallus* (L.)), a river fish which often spawns in still waters, occur over most of Europe from UK in the west to the Urals in the east (Berg 1932). In Norway, the grayling has spread from Lake Ancylus after last Ice age, via natural watercourses once connected to the Lake: Trysil watercourse, Glomma, Alta and Tana rivers. The River Vefsna is the only watercourse in Nordland county supporting a grayling population.

Based on a literature survey, Mitenev & Shulman (1984) have divided the species of grayling parasites into systematic groups and present their range of distribution. The present paper presents the results of a faunistic eco-parasitological study of the grayling inhabiting the River Vefsna. The composition of the parasite fauna is discussed on the background of the special history of the grayling in the river.

Materials and Methods

The River Vefsna is situated in Nordland county, northern Norway (66°N, 13°E). Average daily flow is 137 m^3/s, and the average spring peak flow is about 550 m^3/s. The total catchment area is 4220 km^2.

The Atlantic salmon (*Salmo salar* L.) ascend through a number of waterfalls and minor obstacles up to 330 m above sea level. Before the outbreaks of *G. salaris*, (cfr. Johnsen & Jensen 1988) the River Vefsna was one of the most important salmon rivers in Norway with a total yearly angling catch of salmon and sea trout (*Salmo trutta* L.) of approximately 15 tons.

In the upper reaches of the Vefsna watercourse, tributaries are redirected so branches originally running eastward into Lake Ancylus (9000 - 7500 years BP), now run into the Atlantic. The connection between the River Ångermannelv in Sweden and the River Vefsna through the flat valley of Harvass-

dalen, permitted the spreading of grayling throughout the main watercourse (Øksendal 1992).

In the period 1995 – 1997, 55 grayling were caught between the two waterfalls Laksfors and Fellingfors, partly by net fishing and partly by sport fishery. In 1998, 37 grayling were caught by sport fishery on a 1 km long river stretch just downstream the waterfall Laksforsen.

In 1995, 14 fish were preserved in alcohol and brought to the laboratory for further examination for *Gyrodactylus*. Fresh matrial from 1996, 1997 and 1998, of all fish were examined with a binocular for occurrence of *Gyrodactylus* on the fins. In addition, parasitological studies according to Dogiel's method (Bykhovskaya-Pavlovskaya 1985), including studies of all organs, were conducted on 15 specimens in 1996, 9 specimens in 1997 and 15 specimens in 1998.

The material which were age-determined consisted of 57 fish which were 4 years old or younger and 26 fish of age 6 to 8 years. No 5 year old fish was caught (Table 1).

Table 1. Age and size of the grayling (*Thymallus thymallus* L.) caught in the River Vefsna in 1995 - 1998. (n: number of fish, L: length in mm (range).)

Year	n	L
1995	14*	110 - 234
1996	21	192 – 472
1997	11	255 - 470
1998	37	129 - 327
Sum	83	110 - 472

* in addition 9 grayling older than 3+ (0.4 – 0.7 kg) were caught.

The terms prevalence, intensity and mean intensity are used as recommended by Margolis et al. (1982). The length of the fish was measured as natural tip length (Ricker 1979), and the age of the fish was determined by reading of scales.

Results

No *Gyrodactylus* specimens were found on 55 graylings caught upstream the waterfall Laksforsen. However, seven other different parasite species were found in 24 grayling (from 1996-1997) which were examined according to Dogiel's method (Table 2).

Among the parasitic protozoans, *Hexamita truttae* occurred most frequently. The highest infestation values among helminths were recorded for *Crepidostomum farionis* and *Diplostomum volvens* (Table 2), where especially the previous species dominated.

Gyrodactylus specimens were, however, found on each of 3 grayling (in the mouth, on the pectoral and anal fin) of a total of 37 specimens ranging in age from 1 to 4 years collected in the river downstream the waterfall Laksforsen in 1998. This occurrence of *Gyrodactylus* on grayling is of special interest since it has been shown in laboratory experiments that grayling can be a host for *G. salaris* for some period (Soleng & Bakke 2001). However, unfortunately the specimens were not possible to identify due to their condition after preservation.

Of the seven parasite species found on the grayling from the river upstream the waterfall Laksforsen, the species *D. sagittata* and *P. ovatus* were not recorded below the waterfall (Table 3). But in addition to the protozoan found upstream the waterfall Laksforsen, also

Table 2. Occurrence of parasites on 24 specimens of grayling caught upstream the waterfall Laksforsen in the River Vefsna in the periods 10. – 15. September 1996 and 19. – 22. September 1997

Parasite species	Infected organ(s)	No. of fish infected	Prevalence (%)	Mean intensity (range)
Hexamita truttae	gall bladder	14	58.3	+
Myxobolus neurobius	brain	1	4.2	+
Apiosoma sp.	mouth, skin	1	4.2	+
Discocotyle sagittata	gills	1	4.2	1
Crepidostomum farionis	intestine, gall bladder	24	100.0	25.3 (1–239)
Diplostomum volvens	vitreous body (eye)	14	58.3	3.5 (1–9)
Paracoenogonimus ovatus	heart	1	4.2	1

one unidentified *Myxobolus* sp. was recorded (in the urinary bladder) downstream. The most common helminths recorded in both river regions were *C. farionis* and *D. volvens*, but, however, most frequently observed upstream of the waterfall Laksforsen (Table 3).

Discussion

Both the fish sampling activities and the skin diving observations indicate that the population of grayling in River Vefsna between the waterfalls Laksfors and Fellingfors, is not numerous. Our observations and informations from local fishermen indicate that the region just below the waterfall Laksforsen is the relatively most populated area of grayling in the River.

In general the grayling parasite fauna is usually rich of species, but varies greatly from one water body to another. It is observed to be most diverse in Lake Onega with 32 species (Rumyantsev et al. 1984), and in the River Ponoi, Kola Peninsula, with 33 species (Mitenev & Shulman 1984). The most impoverished parasite fauna is observed in the upper reaches of the rivers that flow into the Caspian Sea, in western Baltic Sea basin rivers and in English water bodies. For example, nine species have been found in the River Uzyan (Zakhvatkin 1946) and the same number in the River Oder as well as in Polish water bodies (Dyk & Lucky 1957, Grabda 1971, and others cited in Mitenev & Shulman (1984) and 14 species in English lakes and rivers (Chubb 1970).

Table 3. Occurrence of parasites on 15 specimens (37 specimens were investigated for occurrence of *Gyrodactylus* sp.) of grayling caught downstream the waterfall Laksforsen in the River Vefna in the period 19. – 24. August 1998

Parasite species	Infected organ(s)	No. of fish infected	Prevalence (%)	Mean intensity (range)
Hexamita truttae	gall bladder	5	33.3	+
Myxobolus neurobius	brain	5	33.3	+
Myxobolus sp.	urinary bladder	1	6.6	-
Apiosoma sp.	mouth, skin	4	26.6	+
Gyrodactylus sp.	mouth, anal fin, pectoral fin	3	8.1	1
Crepidostomum farionis	intestine, gall bladder	12	80.0	2.4
Diplostomum volvens	vitreous body (eye)	5	33.3	1.8

Species that have an indirect life cycle are predominant. They account for about 75% of the entire fauna. Direct life cycle species are far less abundant. However, they are generally slightly more numerous in lakes than in rivers (Mitenev& Shulman 1984, Rumyantsev at al., 1999).

Mitenev & Schulman (1984) analyses of the parasite fauna of the grayling indicated 5 species to be typically found in this host species: *Chloromyxum thymalli*, *Tetraonchus borealis*, *Gyrodactylus thymalli*, *Proteocephalus thymalli* and *Salmincola thymalli*. Eight species: *Myxosoma cerebralis*, *Myxobolus neurobius*, *Discocotyle sagittata*, *Phyllodistomum umblae*, *Crepidostomum farionis*, *Crepidostomum metoecus*, *Cystidicola farionis and Argulus coregoni* parasitize a wide range of salmonid species, and close similarity in the parasite fauna of grayling and other salmonid species has been shown for many zoogeographical zones (Mitenev & Schulman 1984). Infestation with *C. farionis* occurs as grayling feeds on insect larvae, which are the intermediate host of the parasite. *D. volvens* infects the fish as cercaria actively penetrate through the body covers. None of the five parasite species considered most typical for grayling, were recorded in the fish from the River Vefsna. Instead the diversity of the parasite fauna consisted of species wide-spread in other fish species.

The paucity of the parasite fauna of the grayling from the River Vefsna in relation to localities in Russia, may indicate both the relatively restricted age of the river ecosystem and low density of grayling, and the limited fish diversity in the river. For example plerocercoids of *Triaenophorus crassus* and larvae of *Raphidascaris acus* were not found, due to the absence of pike. The paucity of plankton as well as presumably the absence of a diversity of small (relict) crustaceans, may be another reason for the low species diversity of parasites and e.g. the absence of some specific parasite species widely spread in Fennoscandia (*Cyathocephalus truncatus, Cystidicola farionis, Metechinorhynchus salmonis* etc.).

Our studies failed to reveal infestation with *Gyrodactylus* in grayling caught upstream the waterfall Laksforsen. The salmon ladder in the waterfall was closed in 1995 to prevent salmon from spawning upstream Laksforsen. The number of salmon parr and *G. salaris* has been strongly reduced in this part of the river. In the river downstream the waterfall Laksforsen we found accidental occurrence of *Gyrodactylus* only on three specimens of grayling though the parasite is quite widespread on salmon in this region. By electrofishing one location in the area (1998) where the grayling were caught, we found a density of 33 0+ salmon/100 m² and 18 1+ salmon/100 m². The mean intensity of *G. salaris* on the 0+ and 1+ salmon was 21 and 1325 respectively. If the *Gyrodactylus* sp. found on grayling is as expected, *G. salaris*, the low prevalence and intensity observed is a strong indication that grayling is not an important host for *G. salaris* in the River Vefsna. Experimental infections with *G. salaris* on grayling indicate significant biological differences between the function of Atlantic salmon and grayling as host for *G. salaris* (Soleng & Bakke 2001). Both innate resistant and susceptible grayling were found. In susceptible indivi-

dually isolated fish, parasite reproduction lasted for more than 35 days. Parasite reproduction also occurred among grouped grayling as judged from the duration of infection of more than 50 days. However, the level of grayling susceptibility as judged from the *G. salaris* reproduction, was very limited (Soleng & Bakke 2001).

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References

Bykhovskaya-Pavlovskaya IE. Parasity ryb. Rukovodstvo po izucheniju (A manual for parasitological investigation of fish). Nauka. L. 1985 (In Russian)

Chubb JC. The parasite fauna of British freshwater fish. Symp. Brit. Soc. Parasitol 1970; 8: 119-44

Johnsen BO & Jensen AJ. Infestations of Atlantic salmon (*Salmo salar*) by *Gyrodactylus salaris*, in Norwegian rivers. J. Fish Biol. 1986; 29: 233 - 41

Johnsen BO & Jensen AJ. Introduction and establishment of *Gyrodactylus salaris* on Atlantic salmon, *Salmo salar* L., fry and parr in the River Vefsna, northern Norway. J. Fish Diseases 1988; 11: 35 - 45

Margolis L, Esch GW, Holmes JC, Kurtis AM, Schad GA. The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). Journal of Parasitology 1982; 68: 131 – 3

Mitenev VK, Shulman BS. Ekologo-geograficheskiy analiz parazitofauny evropeiskogo khariusa *Thymallus thymallus* (L.) (Thymallidae) v raznykh chastyakh yego areala. (Ecological and geographical analysis of the European grayling *Thymallus thymallus* (L.) parasites fauna in the area). Voprosy. ikhtiologii, T. 24, vyp. 1984; 5: 843-54 (In Russian)

Ricker WE. Growth rates and models. In: Hoar WS, Randall DJ, Brett JR, eds. Fish Physiology. Vol. 8. Bioenergetics and growth. Academic Press, New York, 1979: 677 - 743

Rumyantsev EA, Permyakov EV, Alexeyeva EL. Parasitofauna ryb Onezhskogo ozera i yego mnogoletniye izmeneniya.(Long-term changes in the fish parasite fauna in the Onega lake). Sb. nauchn. tr. GosNIORH, vyp. 1984; 216: 117-33 (In Russian)

Rumyantsev EA, Ieshko EP, Shulman BS 1999. Formation of the European grayling *Thymallus thymallus* parasite fauna . J. Parasitologia. V 33, 3. P. 136-143 (in Russian)

Soleng A, Bakke TA. The susceptibility of grayling (*Thymallus thymallus*) to experimental infections with the monogenean *Gyrodactylus salaris*. International Journal for Parasitology 2001; 31: 793 - 7

Zakhvatkin VA. Parazity ryb gornoi reki Uzyan (pritok Beloi) (The fish parasites mounting river Uzyan (tributary river Beloi). Uchen. zap. Permsk. univer. 1946; t. 4, vyp. 2: 71-7 (In Russian)

Øksendal KM. The Grayling population in the River Vefsna. Årbok for Norsk Skogbruksmuseum 1992; 13: 130 – 42 (in Norwegian)

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