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OF THE SCANDINAVIAN SOCIETY FOR PARASITOLOGY

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

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 Níðhöggr 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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EDITORIAL WELCOME

The Nordic countries are sparsely populated, and there are not many parasitologists within each field of science in any of them. For 25 years the Scandinavian Society for Parasitology has served as a fruitful meeting place for all of us; without this many of us would have felt a lot more isolated than we do today. It has also been very stimulating that so many from countries outside the Nordic regions have joined the SSP. Because of this we have switched our mutual communications from the Scandinavian languages to the more commonly accepted one, English. And now we are taking another step, by presenting the Bulletin. With this, we hope to increase the flow of information both between ourselves and with the surrounding world.

This is our first step towards a publication. The final shape of it is not yet quite clear, and the result is very much dependent upon how you, members of the SSP and participants at the SSP symposia respond to our invitation to participate in this new venture. We hope that you will eagerly become contributing authors in the future, and we would also welcome criticisms, comments and suggestions, which could improve the Bulletin.

The first issue presents the proceedings from the 15th Symposium of Parasitology in Uppsala, Sweden. From 1992 there will be two issues per year, which constitute one volume per year. The scope of the ordinary issues is stated on the inside of the front cover, but I would like to stress that we want to present articles from all the various fields of parasitology which are of interest to parasitologists in this northern corner of Europe. This broad contact between the many areas of parasitology, as experienced at the SSP symposia, has proved to be very fruitful. The SSP has now also established more formal ties with our neighbours across the Baltic Sea, and a section called Baltic News, edited by Professor Peter Nansen, is one of the results of these new, closer bonds with the Baltic states.

On behalf of members of the Editorial Board and the Scandinavian Society for Parasitology, the Editor-in-Chief welcomes the readers of the Bulletin, and invites you to participate with contributions to upcoming issues.

Jorun Tharaldsen,
Editor-in-Chief

Proceedings

15th SCANDINAVIAN SYMPOSIUM

# of PARASITOLOGY

Ultuna • Uppsala • Sweden
4-5 October 1991

15th Scandinavian Symposium of Parasitology
4 - 5 October 1991

Venue:

Swedish University of Agricultural Sciences
Ultuna
S - 750 07 Uppsala
Sweden

Themes:

Arthropods and arthropod-borne parasites

Diagnostics and treatment of parasitic infections in humans

Toxoplasmosis - diagnosis and prevention

Parasites of pigs

Epidemiology of fish parasites

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Preface

This volume contains abstracts of 140 communications, including five invited plenary lectures, presented at the 15th Scandinavian Sympoium of Parasitology in Uppsala, Sweden, 4 - 5 October 1991. The conference was attended by more than 170 registered participants, the highest number so far in the history of these symposia.

Most of the submitted abstracts have been accepted, some after minor revisions.The members of the scientific/organizing committee have mainly tended to editorial amendments, authors themselves are responsible for the scientific contents of their abstracts.

The economical support provided by our sponsors is gratefully acknowledged. Such support was received by:

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September 1991

Arvid Uggla
Editor

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WELCOME BY THE PRESIDENT OF THE SCANDINAVIAN SOCIETY FOR PARASITOLOGY

Hans-Peter Fagerholm
Åbo Akademi University, Åbo, Finland &
National Veterinary Institute, Helsinki, Finland

Dear friends - on behalf of the Scandinavian Society for Parasitology I wish to welcome you all to this, the 15th, symposium of our Society. This year we celebrate the 25th aniversary and it is thus a special privilege to acknowledge the presence of so many parasitologists here today in Uppsala, not only from our region but also from numerous other countries. A special welcome is directed to the invited guests from the Baltic republics, as well as to our invited speakers and to our honorary members.

It has for many of us become a valuable tradition to participate in the Scandinavian meetings. It appears that this tradition has also been well adopted by our more recent members as the number of participants at our symposia has steadily increased. One reason for the popularity of our symposia is certainly the opportunities they give to meet new colleagues. These are also some of the few occasions where we can learn what is going on outside our own laboratories, not only in Scandinavia, but also in other regions. We wish, as has been the case so far, to widen the numerous aspects of parasitology dealt with at our symposia. It is, for instance, hoped that more results from investigations utilizing methods of molecular biology with relevance eg. to diagnostics, treatment and systematics will be presented.

The proceedings of the meetings, which in recent years have

been printed in *Information of the Institute of Parasitology, Åbo Akademi University*, have represented an important mirror of research interests in parasitology in Scandinavia. This year you will find the abstracts printed in the first issue of our new *Bulletin of the Scandinavian Society for Parasitology.* We hope that this new forum will provide a more efficient spread of information on our activities, not only in Scandinavia, but also further afield. The Bulletin is the outcome of the work of the "publishing committee", skilfully headed by Prof. Peter Nansen, which was elected by the general assembly at the Elsinore symposium in 1989, and of the board of the Society. As the success of the Bulletin will depend on the activities of all of us I am convinced that all are willing to make an effort to ensure the Bulletin will flourish. We wish to thank Dr. Jorun Tharaldsen (Oslo, Norway) who has accepted the editorial responsibility for the Bulletin. Thanks are also due to the local editors, one in each of the five Scandinavian countries (Tor Bakke, Flemming Frandsen, Lars-Åke Nilsson, Sigurdur Richter, Tellervo Valtonen), and the editor of the 'Baltic News'- section (Peter Nansen), for accepting this task and to Prof. Flemming Frandsen for the design of the front page.

Today, parasitology is an active branch of science in many countries. However, in Scandinavia, the number of positions in this field is unfortunately low. There are also marked regional differences. For instance in the veterinary field there are a total of some 20 academic positions in the other Scandinavian countries but as yet not a single one in Finland. It is urgently hoped that appropriate measures will be taken by the relevant authorities to change this situation. In some Central European countries where the political situation has changed in recent years, the position of numerous prominent parasitologists is at present very difficult due to the economical situations. However, it is hoped that these scientists will get possibilities to continue their scientific work. I would also like to reiterate our appreciation that several guests from the Baltic Countries have come here and I want to express our wish to build more

permanent contacts with scientists in these regions.

Highlights of our present symposium will be the adresses of our invited speakers. We are happy that they have accepted to come and allocated the valuable time needed for preparing their presentations, which deal with themes of substantial interest to us. I also wish to thank all who have submitted abstracts.

Uppsala is known for L. or Linn., as the name of the famous scholar is used by taxonomists in the realm of Biology. We consider it an honour to have the opportunity to participate in this symposium arranged in the renowned surroundings where Carl v. Linné pursued his life. We wish to acknowledge the numerous sponsors. Their support is much appreciated. Finally we wish to thank the organizing committee (Arvid Uggla (chairman), Dan Christensson, Thomas Jaenson, Inger Ljungström, Lars-Åke Nilsson and Jan Thulin) for their great effort, which ensures that this symposium will be successful both scientifically and socially, living up to the high standards of excellence for which Uppsala is known far abroad.

TOXOPLASMOSIS: DIAGNOSIS AND PREVENTION

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While toxoplasmosis has been recognised as an important infection of man and animals for many years, it has come into greater prominence in recent times because of the danger it poses to sufferers from AIDS and because of the attention given by the media to congenital infections of children in some countries.

Toxoplasma gondii is a protozoan parasite capable of infecting all warm-blooded animals. For over fifty years it has been known to cause serious congenital disease in children and occasional fatal illness in adults (Frenkel, 1990).

It is also an important cause of abortion in sheep, goats (Buxton, 1991), and pigs (Dubey, 1986) but not cattle, horses or deer. It can also be an important infection of zoo and captive animals and birds (Frenkel, 1980; Dreeson, 1990), particularly marsupials (Canfield, Hartley and Dubey, 1990) and New World monkeys (McKissick, Ratcliffe and Koestner, 1968; Borst and van Knapen, 1984).

In the 'sixties it was discovered that the parasite could undergo a sexual life cycle in the intestinal lining of susceptible cats, resulting in the excretion of vast numbers of oocysts. This breakthrough permitted scientists to explain many epidemiological features of toxoplasmosis.

A successful diagnosis of toxoplasmosis requires the application of specific laboratory tests. However the value of being guided towards the application of the correct tests by astute clinical evaluation of a case cannot be underestimated.

Clinico-pathological findings in man

Primary acquired infection. It has been estimated that worldwide 500 million people are infected with T. gondii but clinical toxoplasmosis in immunocompetent people is much

less common (Dubey and Beattie, 1988). Signs include enlarged lymph nodes, malaise, aching muscles and joints, a headache and sometimes a maculopapular rash. Symptoms may last a week or so and the patient may complain of a general lack of energy for several months (Frenkel, 1990). Diagnosis with such non specific symptoms is difficult, although lymphadenopathy suggests the possibility of toxoplasmosis. Serology would confirm a tentative clinical diagnosis.

Immunocompromised individuals, on the other hand, risk developing serious illness if exposed to T. gondii. People "at risk" include patients receiving chemotherapy for cancer, individuals with certain tumours of the lymphoid system, immunosuppressed recipients of transplants, and victims of AIDS. In these cases severe acute toxoplasmosis develops following a primary infection or as a result of a recrudescence of a subclinical, chronic infection established before immunosuppression. The patient may have a fever and evidence of pneumonitis, hepatitis and acute necrotising encephalitis.

Diagnosis is made difficult as serological tests tend only to demonstrate low Toxoplasma titres which do not rise during the acute phase, IgM antibody is detected rarely and in patients with neurological signs the parasite is difficult to detect in cerebrospinal fluid. Thus diagnosis in immunosuppressed people relies on clinical findings, the demonstration of low Toxoplasma antibody titres which show that an infection exists (Luft and Hafner, 1990), computed tomography (CT) scanning which can reveal the foci of necrosis and oedema in the brain when present and on a good clinical response to Toxoplasma drug therapy (Luft and Hafner, 1990). The detection of Toxoplasma antigen in either cerebrospinal fluid or in the circulation (Frenkel, 1990) may prove to be a useful method in the future as might the application of the polymerase chain reaction (PCR) (see below).

Congenital Infection. Congenital infection may occur when a woman becomes infected for the first time while pregnant. With primary infection of the mother early in pregnancy transmission to the

foetus is less likely than if infection had occurred later in gestation. However the risk of delivering a severely handicapped infant is greater the earlier infection occurs. Thus about 10% of children with congenital toxoplasmosis have severe damage, such as hydrocephalus and intracranial calcification, while 90% may initially appear normal but be infected and go on to develop retinochoroiditis and other less well defined neurological illness during childhood and teenage years (Couvreur and Desmonts, 1983; Couvreur, Desmonts, Tournier and Szusterkac, 1985; Hohlfeld, Daffos, Thulliez, Aufrant, Couvreur, MacAleese, Descombey and Forestier, 1989; Roberts and Frenkel, 1990). Infection of the mother during pregnancy is not readily recognised without specific tests (Frenkel, 1990; Holliman, Barker and Johnson, 1990).

Clinico-pathological findings in animals

Sheep and goats. Primary infection of the dam with T. gondii in early gestation allows the parasite to invade and kill the foetus which is then resorbed so that the mother may be mistakenly thought to be infertile. Infection in mid gestation may be fatal for the foetus but because it is more developed only partial resorption occurs. The mummified foetus that remains is often born alongside a live sibling although the latter may sometimes be weak and may not survive. Infection in late gestation does not normally cause any mortality although the lambs will be infected. Infected ewes do not normally show any other clinical signs, apart from abortion (Buxton, 1991). In cases of abortion characteristic necrotic foci in placental cotyledons are often visible macroscopically as white spots 2 to 3 mm in diameter and these are readily confirmed by microscopy (Beverley, Watson and Payne, 1971). Histological examination of brains of lambs that die at birth will often reveal focal leukomalacia in the cerebrum and foci of gliosis in many areas of the brain (Buxton, Gilmour, Angus, Blewett and Miller, 1981).

Pigs. The incidence of clinical toxoplasmosis in pigs following exposure to infection is variable and its nature difficult to predict (reviewed by

Dubey, 1986). Primary infection during pregnancy may or may not result in congenital infection. When infection of the developing foetus does occur piglets may be stillborn. Liveborn piglets may or may not be infected. If they are they may remain healthy or may die in the first few weeks of life. Prior to death fever, ataxia and diarrhoea may be evident and at necropsy hepatitis, lymphadenitis and pneumonia may be present. Pulmonary oedema, fibrinonecrotic alveolitis, non suppurative encephalitis, granulomatous nephritis and necrotising hepatitis have all been recorded histologically (reviewed by Dubey, 1986).

Other animals. The relative susceptibility of New World monkeys to T. gondii may be manifest as sudden death or acute illness of a few days followed by death. Clinical signs include dyspnoea, neurological symptoms and sometimes diarrhoea, while the consistent pathological change is necrosis, particularly in the liver, spleen and lungs but also in lymph nodes, cardiac muscle and brain (McKissick et al., 1968). Old World monkeys, on the other hand, appear to be relatively resistant to T. gondii. Infection readily occurs but clinical illness is less common (McKissick et al., 1968). Marsupials also show a generally increased susceptibility to the parasite (Canfield et al, 1990), particularly wallabies (Obendorf and Munday, 1983) and kangaroos (Patton, Johnson, Loeffler, Wright and Jensen, 1986; Dubey, Ott-Joslin, Torgerson, Topper and Sundberg, 1988).

Immunological tests

Immunocytochemistry. Immunocytochemistry (Buxton, Miller, Finlayson and Wallace, 1981; Jeffrey, O'Toole, Smith and Bridges, 1988; Uggla, Sjoland and Dubey, 1987) is particularly useful because it highlights the parasite in lesions where it may be very scarce as well as permitting specific differentiation from different organisms of similar morphology.

Serology. Many serological tests have been developed but the dye test (DT), despite requiring a source of live parasite and not readily lending itself to automation, remains

the reference test for man (Sabin and Feldman, 1948). It is also of value for diagnosis in sheep (Waldeland, 1976), goats (Dubey, Desmonts, Antunes and McDonald, 1985) and pigs (Dubey, 1986).

The indirect fluorescent antibody test (IFAT) correlates well with the DT and as both use intact organisms, as does the direct agglutination test, all three detect antibodies directed against surface antigens. Tests using soluble extracts of T. gondii tachyzoites include the latex agglutination test (Tsubota, Hiraoka, Sawada, Watanabe and Ohshima, 1977), the indirect haemagglutination test (Jacobs and Lunde, 1957), as well as the ELISA (Voller, Bidwell, Bartlett, Fleck, Perkins and Oladehin, 1976). The advantages of the latter are that not only does it permit automation but it can also be set up to detect either IgM or IgG.

Cell mediated immunity can be detected in man and animals with the relatively crude delayed type hypersensitivity test (Frenkel, 1948) or by the more up to date lymphocyte transformation assay (Derouin, Rabian-Herzog, Ballet, 1989) but neither appear to be used for routine diagnosis.

Detection of Toxoplasma nucleic acids. The detection of Toxoplasma DNA or RNA by the PCR (Burg, Grover, Pouletty and Boothroyd, 1989; Savva, Morris, Johnson and Holliman, 1990; Weiss, Udem, Salgo, Tanowitz and Wittner, 1991) still needs to be fully evaluated. The specificity and high sensitivity of this technique will make it very valuable, although stringent sampling and laboratory technique are essential to avoid false positive results.

Isolation of Toxoplasma. Toxoplasma-free mice can be inoculated with suspect material and examined for serological evidence of the parasite 4 to 6 weeks later. No further passage is necessary if the mice remain seronegative. Another option is to inoculate tissue cultures. This has the advantage that it avoids the use of animals and is more rapid but whether it is as sensitive is uncertain.

Prevention of human infection

Advice on avoiding infection applies to everyone but especially to those at-risk. As a high proportion of meat-producing animals are exposed to Toxoplasma it is best to assume that they are a source of infection (Sacks, Delgado, Lobel and Parker, 1983). Meat should therefore only be eaten after adequate cooking, for example when red meat has turned brown. Good kitchen hygiene is essential. Irradiation or freezing and thawing of meat either eliminates or greatly reduces the viability of Toxoplasma tissue cysts (Dubey, Brake, Murrell and Fayer, 1986; Dubey, 1988). It has been shown experimentally also that the parasite can be present in unpasteurised goats milk (Dubey, 1980) but it is not transmitted in eggs (Dubey and Beattie, 1988).

Oocysts can be found where cats defaecate, therefore cultivated soil and childrens' sandpits are a potential source of infection. Ingestion of soil or sand directly or indirectly must be avoided. Domestic sandpits should be covered when not in use to prevent cats gaining access to them, and public sandpits should be avoided because they may also harbour other infections such as toxocariasis.

People at-risk should wear rubber gloves while gardening and vegetables should be washed free of soil. Cat litter trays should be cleaned at least once a day, but not by a pregnant or immunosuppressed person, to remove any Toxoplasma oocysts before they sporulate and become infective. Flushing infected faeces down the toilet may lead to large volumes of salt and fresh water becoming contaminated and thus it is probably better to encourage the hygienic disposal of cat litter in domestic refuse bins. Pregnant women should not assist with lambing ewes or the care of newborn lambs, not only because of the risk of toxoplasmosis but also because sheep may also carry other zoonotic infections such as Chlamydia psittaci and Coxiella burnetii. Farrowing sows and kidding goats may also pose a risk.

Prevention of animal infection

Sheep and goats, being herbivores, become infected after ingesting oocysts, whereas pigs can also pick up infection from undercooked swill containing viable tissue cysts. Zoo animals can become infected with either oocysts or tissue cysts depending on their diet, so that great care needs to be taken in selecting, preparing and storing their specialist meals. In this context it should also be remembered that the feeding of raw Toxoplasma infected meat to any members of the cat family can result in the excretion of Toxoplasma oocysts (Jewell, Frenkel, Johnson, Reed and Ruiz, 1972; Miller, Frenkel, Dubey, 1972; Ocholi, Kalejaiye and Okewole, 1989).

Perhaps the most common source of infection for herbivores is contaminated pasture. Fields treated with manure and bedding from farm buildings where cats live can cause infection (Faull, Clarkson and Winter, 1986) and careless storage of farm feeds may also pose a risk (Plant, Richardson and Moyle, 1974). Fifty grams of infected cat faeces may contain as many as 10 million oocysts (Dubey and Frenkel, 1972). Hypothetically if this was dispersed evenly throughout ten tonnes of concentrated animal feed then each kilogram would contain between five and twenty five sheep-infective doses (McColgan, Buxton and Blewett, 1988). The extent of environmental contamination can thus be related to the distribution of cats.

To limit environmental contamination by oocysts the number of cats capable of becoming infected and shedding oocysts should be reduced. Since these tend to be young animals, breeding should be controlled to favour a small healthy population of mature animals. Feed should also be kept covered to prevent its contamination by cat faeces.

No killed Toxoplasma vaccines are currently available for use in animals or man. However prevention of toxoplasmosis in sheep by vaccinating with Toxoplasma tachyzoites of the incomplete S48 strain was pioneered in New Zealand (O'Connell, Wilkins and TePunga, 1988; Wilkins, O'Connell and TePunga, 1988). Recent

research in the UK confirms the efficacy of the S48 strain (Buxton, Thomson, Maley, Wright and Bos, in press).

Whether the use of this vaccine becomes more widespread remains to be seen but in the meantime the ionophore, monensin, which has significant anti-Toxoplasma activity in sheep, may be used to control infection in countries where legislation permits its use (Buxton, Donald and Finlayson, 1987; Buxton, Blewett, Trees, McColgan and Finlayson, 1988).

The long term aim both in human and animal medicine must be to reduce the risk of infection by improved education and to work towards the development of an effective and safe killed vaccine. Alongside this we need to strive for better means of diagnosis, particularly in immunosuppressed patients, and for improved methods of treatment.

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QUANTITATIVE EPIDEMIOLOGY OF PARASITIC INFECTIONS IN FISH

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The statistical properties of prevalence, intensity, abundance and of morphometric variables used in the study of parasite populations of fish are examined in detail. Research articles published in the last five years are reviewed for their use of quantitative methods.
It appears that many publications depict mostly qualitative taxonomic studies or geographical surveys of parasite distribution. Parasite life-cycle and epidemiological studies make use of statistical methods more frequently, while studies of the population dynamics of parasitic infections, in the laboratory or in the field, are often quantitative.
Sampling theory is systematically overlooked in the planning of field surveys and host or parasite population dynamics are rarely discussed beforehand. Surveys are therefore plagued with too few fish, too often, in too many places.
The advantage of exploratory data analysis using graphics, data transformation, hyphothesis testing or simple statistical models is illustrated. The discussion focusses on the importance of sampling design and a dynamic approach in epidemiological studies of both host and parasite populations.

THE ECOLOGY OF LYME BORRELIOSIS

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Abstract: Lyme borreliosis is a tick-transmitted spirochetal infection of immense worldwide significance. Wherever the infection is intensely transmitted, the principal tick vectors all belong to the genus *Ixodes* and most display similar host relationships; adult ticks require a large vertebrate to maintain populations while small rodents serve as principal spirochetal reservoirs. Dynamical analyses of the interrelationships between vector ticks and their hosts and reservoirs can be useful in understanding distributions of ticks, especially those infected ticks which create human risk. Such analyses can also direct development of strategies to predict and suppress human risk.

INTRODUCTION

Lyme borreliosis, or Lyme disease as it is known throughout North America, is a tick-transmitted spirochetal infection that occurs nearly worldwide, principally in north temperate climates. In just the past decade, since discovery of the causative spirochete, *Borrelia burgdorferi*, Lyme borreliosis has become the leading arthropod-borne disease in both North America and Europe. Wherever transmission of the Lyme borreliosis spirochete is found to be endemic, ticks of the genus *Ixodes* are present to serve as vectors. As expected, the risk for human infection is related to man's exposure to infected ticks; this exposure may occur wherever the specific complex of zootic conditions are found which support enzootic transmission of *B. burgdorferi*. Typically, wooded and brush-covered landscapes create "suitable habitat" for this zoonosis. However, in many afflicted regions Lyme borreliosis appears to be emerging as a "back-yard" disease in response to both social and ecologic factors enhancing human contact with infected vectors *(1)*.

The ecologist is charged with unraveling the complex of entomologic and zoologic interrelationships that result in

particular distributions of infected ticks. He must describe what he observes, including vector relationships with both pathogen and host, the reservoir potential of tick hosts, and the influence of both physical and biologic factors on tick distributions. He must then interpret these observations in a dynamical way because each observed factor or attribute may influence the system in such a way as to yield an unexpected result. The ecologist also has an opportunity to identify foci vulnerable to disruption in the natural transmission cycle or even in the cycle of human risk; strategies for suppressing transmission and protecting human and animal health may come from developing a comprehensive understanding of the natural ecology of Lyme borreliosis.

VECTORS

The principal vectors of *B. burgdorferi* have been identified as those anthropophilic ticks belonging to the *Ixodes ricinus*-complex *(2)*. These include *Ixodes dammini*, *I. scapularis* and *I. pacificus* in North America, *I. ricinus* in Europe and *I. persulcatus* in Eurasia *(3)*. These vectors all exhibit a broad host range although in the adult stage all appear to require that a large animal serve as host while the immature stages more frequently encounter and take their blood meal from smaller vertebrates, especially small rodents. Thus, wherever transmission of *B. burgdorferi* is found, a representative "deer" tick exhibiting a similar host feeding behavior also occurs. Moreover, these ticks all possess low tolerance for dessication and thus, will be found in microhabitats of extremely high humidity.

In addition, zoophilic vectors capable of enzootic transmission of *B. burgdorferi* include the North American rabbit tick *I. dentatus (4)*, the European *I. hexagonus*, and possibly the European mouse tick *I. trianguliceps*. These ticks may maintain or even amplify the spirochete in certain zootic hosts. In the case of *I. dentatus*, a parallel but largely non-overlapping transmission cycle is maintained among cottontails (*Sylvilagus floridanus*) *(4)*; *I. dammini* rarely feeds successfully on rabbits while *I. dentatus* rarely attacks man. Nevertheless, the existence

of such zoophilic vectors create the possibility of maintaining high levels of enzootic transmission even in the absence of human infection. Introduction of an anthropophilic tick species into such a zoonotic transmission cycle could serve as a "bridge" creating human risk where none previously existed.

Other species of ticks and other biting arthropods appear either incapable of, or are otherwise unlikely to serve as vectors of *B. burgdorferi*. Experimental evidence shows that other vectors, including *Amblyomma* and *Dermacentor* ticks *(5,6)*, and mosquitoes *(7)* may become infected with spirochetes but these infections are short-lived and usually do not survive to be transmitted.

SPIROCHETE INFECTION OF VECTORS

Spirochetal infection of *Ixodes* will depend upon a vector's host feeding behavior. Because transovarial transmission of *B. burgdorferi* in *Ixodes* rarely occurs *(8)*, larvae hatch from eggs without spirochetes and must acquire an infection during blood feeding. Larvae and nymphs, thus acquiring spirochetes, pass the infection transstadially, where it can be transmitted by bite from either the nymphal or adult stages. Adult ticks, having had two previous opportunities to become infected, are more likely to be infected than are nymphs. However, risk of infection is greatest from exposure to nymphs *(9)*, since their minute size make them difficult to find and remove before transmission may occur.

From a public health perspective, human risk for infection will be related to the abundance of infected ticks. The proportion of infected ticks in a population, or the tick "infection rate", will largely be determined by that proportion of ticks taking their blood meal from infective hosts, called reservoirs. Although any host bitten by an infected tick may become infected, not all hosts are reservoirs and all reservoirs are not equally infective to those ticks feeding on them. Thus, infectivity to feeding ticks, and not merely seroconversion or isolating spirochetes from host animals would be required to prove reservoir competence. Infectivity can only be measured appropriately by tick xenodiagnosis *(10)* conducted on

populations of wild-caught hosts.

Besides its infectivity, for a reservoir to contribute in a significant way to infecting ticks it must also exhibit a high degree of contact with vector ticks (particularly the immature stages) and be abundant in tick-infested habitats. Each of these host attributes (infectivity, vector contact & host abundance) contribute independently to the overall potential for a particular species to serve as reservoir. However, these parameters can also be linked in an analytical form to compare the relative contribution each host makes to infecting the tick population *(11)*. The "reservoir potential" (R) exhibited by a particular species (s) can be expressed as:

$$R_s = \frac{N_s D_s}{\Sigma_s (N_s D_s)}$$

where N_s is the number of infected nymphs produced by species (s), and D_s is the overall density of species (s) at a given location. In the equation's most simplified form, feeding and molting success of ticks from various hosts can be assumed as equal so that the average number of larvae per host species yields the number of resultant nymphs. Moreover, by including specific species' infectivity, the result when expressed as a percentage will be the proportion of infected ticks derived from a single species, or its reservoir potential. In this manner, it is possible to determine the sources of infected ticks and estimate the relative contribution each reservoir makes towards infecting the tick population *(11)*.

Preliminary analyses to determine the relative contribution made by potential reservoirs in coastal New England (USA) confirms that small rodents, and in particular, white-footed mice (*Peromyscus leucopus*) serve to infect most ticks with spirochetes *(11)*. Indeed, wherever *B. burgdorferi* is intensely transmitted, similar rodents including the European *Apodemus spp. (12)* appear to serve as principal reservoirs. Moreover, large ungulates such as deer, while serving as critical hosts for adult stage ticks, appear incompetent as reservoirs as they are incapable of infecting feeding ticks *(13)*. Thus, in such sites the tick "infection rate" may be largely determined

by the relative proportion of rodent- and deer-fed ticks because most ticks probably acquire a bloodmeal from either deer or rodents. Of course, other hosts also produce non-infected ticks just as other reservoirs may produce infected ticks. Where *I. dammini* is the vector, the "host mix" found at any particular site ultimately determines the tick infection rate, while the abundance of critical hosts, such as deer, appear to determine tick abundance. In other locations, where other *Ixodes* serve as vector, temporal patterns in tick feeding behavior may also serve to regulate the level of infection in the tick population *(2)*.

Despite similarities in vector/host associations throughout the range of *Ixodes* vectors, tick infection rates are not uniform. In western and southern USA, and perhaps in some areas of Europe where tick infection rates are typically lower than in the northeastern USA, certain species of lizards compete with mice as significant hosts of immature *Ixodes*. Moreover, some lizards, notably in the genus *Sceloporus* have been found incapable of infecting feeding ticks *(14)*. Diversion of immature tick feeding on non-competent reservoir hosts will provide a "zooprophylaxis" *(15)*, serving to further dilute the incidence of infection in tick populations. The extent of diversionary feeding by immature ticks will determine the degree of zooprophylaxis. Thus, because lizards appear to serve as important hosts of immature ticks in certain areas, tick infection rates reported from areas with an extensive lizard fauna typically are considerably lower than those reported from the northeastern USA *(16)*.

APPLICATIONS OF ECOLOGICAL STUDIES

Numerous descriptive ecologic studies have documented a wide variety of hosts for *Ixodes* ticks as well as listing those animals and ticks infected with *B. burgdorferi* (Ref. 3). Although many mammals and birds are tick-infested and spirochete-infected, a dynamic analysis, such as comparing the reservoir potential of tick hosts *(11)* may identify particular key reservoirs. In addition, spatial analysis may be useful in placing such reservoirs and their associated ticks in specific habitats. In coastal New England (USA), mice (*P. leucopus*) have been found to

contribute more than 90% of all infected *I. dammini (11)*. Moreover, these important reservoirs concentrate along ecotones between woods and brush or open grassland. Included in this preferred ecotonal habitat in more suburban landscapes are shrub and flower gardens, rockwalls bordering or extending from woodlots and woody buffer screens between house lots. Ticks may be generally distributed on such properties but would occur in greater abundance in that habitat described as ecotone or wooded. Presumably human risk for encountering infected ticks would be greatest in such habitat types, and recognizing these higher-risk foci on properties may permit targetted tick-reduction through directed chemical applications or implementation of cultural modifications to the landscape.

Dynamic analyses documenting the importance of white-footed mice (*P. leucopus*) as principal reservoirs contributing most infected ticks has also stimulated the development of host-targetted strategies for reducing populations of infected ticks. The use of permethrin-treated cotton fiber *(17)* (Damminix®) as nesting material for mice usually results in the virtual elimination of tick infestations on treated animals. Moreover, in sites where mice contribute most infected ticks, the abundance of such ticks has been reduced by as much as 97% *(18)*. This strategy works best when the product is applied to all mouse habitat in an area and competing nesting material is absent. Similar analyses, if employed under different enzootic conditions might direct the implementation of other host-targetted strategies, or suggest an integration of interventions to attack ticks produced by key reservoirs.

Developing an ability to predict risk for Lyme borreliosis transmission could have several useful outcomes not the least of which would be in structuring appropriate tick management strategies. Towards this goal, we have recently been evaluating the predictive capability of the reservoir potential model (RP) to generate expected tick infection rates based on prior-year zoologic parameters. It will still be necessary to critically assess this model under a variety of situations, especially to test the potential for geographic variation in critical parameters such as infectivity. However, in preliminary results from studies

at one location, we have successfully correlated tick infection rates with particular zoologic parameters.

As an example of the predictive capacity of the RP model, spirochete infection rates in nymphal *I. dammini* were measured on Naushon Island in Massachusetts (USA) between 1985 and 1989. The annual May-July infection rate was far from constant, ranging from a high of 24.5% in 1986 to a low of 12.5% in 1987. Using simulations of the RP model, we demonstrated that considerable variation (> 40%) in tick infection rates might be expected as a result of fluctuations in the abundance of white-footed mice (*P. leucopus*) since this animal exhibits such a high degree of reservoir potential on Naushon Island. However, even gross fluctuations (> 50% of average values) in the abundance of the next two most important reservoirs (shrews and wrens) resulted in less than a 2% variance in the expected tick infection rate. Thus, annual mouse densities were evaluated to explain the observed variation in Naushon Island tick infection. Mouse density had been determined between 1985 and 1989 using grid trapping and mark-release-recapture methods. Mice were abundant in 1985, were extremely rare in 1986, and increased to an intermediate density in 1987-1989.

There was a significant correlation between mouse density in year X and the tick infection rate in year X+1 ($p = 0.04$), suggesting a causal relationship between mouse density and subsequent spirochete infection of nymphs. Reservoir potential analysis over the 5 year period were simulated using average parameter values for all hosts with the exception of mouse density; mouse densities actually observed were used for each simulation. Each of the 5 simulations resulted in predicted tick infection rates which were significantly correlated ($p=0.02$) with the observed infection rate for each repective year. Thus, at this site, the RP model ably predicted tick infection rates based soley on mouse density estimates.

CONCLUSIONS

Despite specific differences in transmission cycles of the Lyme borreliosis spirochete worldwide, this infection is

transmitted by *Ixodes* ticks whose populations are maintained by bloodfeeding on large animals but which acquire infection principally from small rodents. Because these ticks exhibit little off-host vagility, their distributions will be closely correlated with the habitat of tick hosts but may be modified further by physical constraints such as moisture. Moreover, the abundance of infected ticks and thus the greatest risk for human infection will likely be found in the habitat frequented by critical rodent reservoirs. Studying the ecology of Lyme borreliosis, especially the potential for certain hosts to serve as reservoirs of infection, may lead to novel interventions, such as directed landscape manipulation, host-targetted acaricides and an ability to predict risk in advance of the transmission season. Without a doubt, strategies to suppress human infection must include a variety of ecologic components.

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HELMINTHS IN PIGS - PRESENT STATUS AND FUTURE TRENDS

by

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The spectrum of species of helminth parasites infecting swine is rather narrow compared with that of other livestock. Considering the situation in Northern Europe only a handful of worm species is of major importance, i.e. Ascaris suum, Oesophagostomum dentatum, Oesophagostomum quadrispinulatum, Strongyloides ransomi and Trichuris suis. This is partly explained by the tradition of indoor rearing of pigs, which precludes development of certain parasites, and makes transmission more or less independent on natural environmental conditions of the various geoclimatic zones. - Much of our recent knowledge on pig parasites in this part of the world stems from studies conducted as part of a joint Scandinavian research project, and the present review is to a large extent based on its results (presented in NKJ-rapport no. 59, Scandinavian Contact Agency for Agricultural Research 1991, - and elsewhere in the present proceedings).

PREVALENCE

Survey studies indicate that the prevalence of helminth parasites, e.g. in Denmark, has decreased considerably since the 1960's along with a strong intensification and modernization of the pig industry. As production units have grown bigger and labour has become more expensive, construction of new buildings and equipments has occurred on a large scale, although some traditional units are still left. Slatted floors have replaced concrete floors with straw bedding, and tethering of sows, early weaning etc. have been established. In a comprehensive joint Scandinavian herd survey (Roepstorff and Nilsson, NKJ-rapport no. 59, see above), prevalence figures of the various helminths were by multi-variable analyses related to management factors, and this indirectly explained the cause of the decreasing prevalence rates over the past 30 years. Among the two prevailing worms, i.e. A.suum and Oesophagostomum spp., the latter is more influenced by housing and management than the former. Evidently, both housing type, early weaning, drug treatment and hygienic measures significantly depress parasite transmission and explain that this formerly very common parasite may be completely absent in some modern herds. In contrast, A.suum is less vulnerable to the

above factors, and this worm still prevails in many modern pig units, yet at lower prevalence rates than in traditional herds. The difference between the two worms in their response to the above extrinsic factors may be explained by differences in the resistance of their external stages, in reproductive potentials and in host regulatory responses. S.ransomi and T.suis both have low and sporadic occurrence in housed pigs. One general and important conclusion that can be drawn from the above studies is that the most efficient control includes combinations of housing and hygiene factors unfavorable to helminth transmission, rather than just an uncritical belief in the virtue of the routine use of anthelmintics alone.

ECONOMICAL LOSSES

Relatively few reliable data are available on economical losses caused by pig helminths. A generally-held perception is that losses are marginal and associated with chronic infections. This may perhaps to some extent be correct seen on the background of the prevaillance of long-standing subclinical parasitism, although examples of acute and marked deleterious effects have been reported from scattered observations. - Economical losses due to worm infections have been estimated in two ways, i.e. either by comparing the performance of naturally infected animals in which the parasite is controlled with that of comparable animals in which no control is attempted, or by comparing the performance of experimentally infected an uninfected animals. The first approach is hampered by the fact that naturally infected pigs often have highly variable and largely unknown burdens of several species of worms present at the same time, and furthermore the drug treated pigs often remain in the contaminated environment and thus get continuously reinfected with unknown numbers of infective larvae. The second approach is weakened by the difficulty in choosing infection doses and regimes reflecting the natural situation in the field. In a recent large-scale experimental study (Eriksen et al., NKJ-rapport no. 59, see above) groups of pigs were trickle-inoculated with A.suum eggs at two different dose levels to simulate long-term exposure over the period from weaning until slaughtering as baconers. Only the low level exposed pigs showed a slight, yet statistically significant, growth depression and increased feed utilization just enough to justify control investment, whereas the high level exposed pigs were statistically unaffected possibly due to higher acquisition of immunity at an earlier stage. One important feature that has arisen from recent experimental studies is the apparent lack of positive correlations between parasite findings at slaughtering of pigs and production losses, e.g. number of which spots in the liver and adult A.suum burdens on the one side, and performance data on

the other. This explains the misleading and conflicting results from a series of production trials made in the past. In the future there is a great need for performing comprehensive and well-planned production trials on the basis of the knowledge recently gained. Thereby herds in particular need for implementation of control can be better defined, and overuse or misuse of antiparasitic drugs avoided. One should also be aware of diseases and losses associated with supervening microbial infections. The experimental provocation of bacterial lung lesions in A.suum infected pig and mice and the associations between this helminth and pleuritis in naturally infected pigs should stimulate further investigations.

HOST RESPONSE

The host response play an important role in regulating the parasite population in terms of preventing establishment of pathogenic worm loads and in terms of lowering contamination of the environment with infective stages. In other words, the host response is an integrated part of the over all epidemiological pattern and is of decisive importance for the success of control attempts. This holds true not least for ascariasis in the pig, which is known to be associated with a certain strong immune response. Recent experimental studies monitoring worm kinetics as influenced by a developing host response have shown that immunity may be more solid and develop earlier that hitherto believed. Even in relatively young pigs may acquisition of immunity lead to killing of larvae already at a prehepatic site. This may explain, why numbers of hepatic white spots often diminish during the course of continuous, chronic exposure, and further, as discussed previously, why white spots and intestinal worm burdens at the time of slaughtering of pigs may be poor indicators of preceding exposure levels. Recent studies have also jointed to the existence of a genetically determined variability in the ability to raise an effective immune response against A.suum, as reflected in a highly aggregated worm distribution with the majority of worms being harboured in a minority of the host population. However, with the subclinical nature of ascariasis it is at present doubtful whether this phenomenon will stimulate genetical studies aiming at selection for resistance.

PREVENTION

The future prevention and control of helminthiasis in the modern pig industry should be based on better insight into the parasitological status of the individual herd. With the close interrelations already established between extrinsic factors and parasite prevalence and intensity it should be possible on the basis of herd records and routine samplings to select the

proper implementation of additional control e.g. in the form of strategical drug treatments. Or one might even reveal herd situations where treatments are not feasible at all. The anthelmintic solution promoted by drug manufacturers or salesmen often appeals to the farmer because it demands little contemplation and labour investment. However, used uncritically there is a danger of overuse, misuse and development of anthelmintic resistance. Furthermore, as the global pig anthelmintic market is relatively small there is limited incentive for the pharmaceutical companies to invest major research resources in this area, compared with the interests in ruminant anthelmintics. Actually, there have been some examples where e.g. dose rates and recommendations for pig anthelmintics have been wrongly stated at marketing. This calls for future closer collaborations between researchers, drug companies and agricultural advisors.

NEW CONCERNS

During the last decade pig production systems have been of growing concern to the producers as well as to non-agricultural sections of the population. In western countries in particular, there is increasing public criticism from consumers and from animal protection societies having growing political influence. For these reasons pig production units designated ecological, "green" or just extensive are growing in numbers. This management may be characterized as a reversion to the traditional farming decades ago with outdoor rearing of animals and poorer hygiene. Inevitably, the impact of parasitism will increase and if drug use at the same time is excommunicated parasitologists will be confronted with interesting difficult but not necessarily insoluble, problems concerning the choice of correct prophylactic strategies.

PARASITE VACCINES FOR HUMAN USE - PRESENT STATE OF THE ART

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Vaccination against viral and bacterial diseases has resulted in several notable contributions to improved human health. But - although we have a handful of vaccines against eukaryote parasites of domestic animals, we have none in general use against the parasites of man.

Several factors are responsible for this unsatisfactory state of affairs:

1) Protozoan and helminth parasites most commonly occur in poor tropical countries which cannot afford the true economic cost of vaccines.

2) Eukaryote parasites are antigenically much more complex than viruses and bacteria. Identifying key immunogenic antigens is a formidable task.

3) Many eukaryote parasites have complex life cycles with more than one host. They are very difficult to grow in vitro.

At least some of these problems are being overcome with the aid of molecular genetics and peptide synthesis. We are approaching a time when relatively large amounts of immunogenic parasite antigens become available - although they will be expensive!

But a major problem remains: eukaryote parasites have evolved a number of devices which enable them to evade or circumvent host immune attack. Examples are the extreme antigenic diversity of African trypanosomes and the molecular mimicry evolved by schistosomes. These mechanisms, which cause immunoconfusion in the host, will be discussed and an attempt made to realistically assess the prospects for effective vaccination of man against eukaryote parasites.

THE EPIDEMIOLOGY OF TRYPANOSOMA THEILERI ON A SWEDISH DAIRY FARM AND EXPERIMENTAL INFECTION OF CALVES

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On a dairy farm, on which a year previously Trypanosoma theileri was recovered both in the cattle and in 2 species of Tabanids, 16 dairy cows, 5 heifers and 18 calves were screened for trypanosome infections. All the cows, 3 heifers and none of the 18 calves were found infected with trypanosomes during the indoor season. Parasitemias were minute. No trypanosomes were found in thin or thick stained smears nor in the buffy coat layer using the microhaematocrit centrifugation technique. Trypanosomes were only recovered from NNN cultures in which 1 ml of blood was inoculated and which was incubated at 27^{o}C for 5-10 days. In mid June 17 of the 18 calves aged 1-3 months were transfered out to pasture for their first time. Three weeks later 6 out of the 17 calves (35.3%) were found infected with trypanosomes. Two weeks later 12 calves (70.6%) and after a further two weeks all the 17 calves (100%) were found infected. No trypanosomes were detected in the blood from the control calf, which had remained indoors. Two of four uninfected calves born to cows, which were shown to be Trypanosome infected, were injected subcutaneously at two weeks of age with 8.000 trypanosomes from a three-day-old culture. The calves were bled every second day for trypanosomes the first 14 days, thereafter once a week during 10 weeks using the above mentioned methods. No trypanosomes were isolated despite stressing the calves for 10 days between the seventh and eight weeks post inoculation with Dexamethasone 5 ml (1 mg ml^{-1}) im per day. When inoculating the same calves 4 weeks later with 50.000 trypanosmes of 5 days NNN-cultures, trypanosomes were isolated from both the inoculated calves 2 weeks later.

THE PROBLEM OF MECHANICALLY TRANSMITTED TRYPANOSOMES

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Trypanosomiasis is one of the major constraints on livestock development in many parts of the world. In Africa, though the possible occurence of mechanical-transmission can not be ruled out, most of the salivarian trypanosomes pathogenic for livestock are transmitted by tsetse flies, in which they undergo cyclical development. But some trypanosomes have adapted to mechanical-transmission by bitting flies other than tsetse. The mechanically-transmitted trypanosomes include T.(T.) evansi, T.(T.) equiperdum and T.(D.) vivax viennei. It is widely believed that these trypanosomes originated from the tsetse transmitted parasites of tropical Africa.

This adoption of mechanical-transmission, has allowed these trypanosomes to gain wide geographical distribution spreading outside the confined area of continental Africa and has increased their range of mammalian hosts. Despite their economic significance, research on these parasites has been relatively neglected. The present paper highlights the economic losses in livestock production in areas endemic for the diseases caused by these trypanosomes. The need to understand the degree of genotypic and particularly antigenic diversity amongst parasites circulating in the endemic areas is stressed. Factors affecting the pathogenicity of the diseases, the use of modern techniques to improve diagnostic and the possible control methods to curb the spread of these diseases are discussed

MALARIA IN GOTHENBURG 1985-90

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Eighty-nine cases of malaria were diagnosed during the period. The annual incidence increased from 8 cases 1985 to 31 cases 1990.. Plasmodium falciparum caused 50 of these. Africa was the predominant source of infection and more than 50% of the patients were either recently arrived refugees or emigrants returning to malarious regions after a long stay in Sweden. The mean-time of exposure was 2 months in Africa compared to 4 months in South East Asia. The patients with falciparum malaria fell ill on average 10.3 days after leaving the malarious region compared to more than 100 days for patients with vivax malaria. Six patients were severely ill (by Plasmodium falciparum) but all survived. Parasite counts for 62 patients varied from 100/microliter to 500.000/microliter with no significant difference between the species. A high parasite count was associated with longer duration of fever and lower platelet counts.Patients presumed to have chloroquine resistant malaria were treated with either quinine (with or without Fansidar) or mefloquine. A comparison in therapeutic response did not show any significant difference between the two treatments concerning time to defervecence or clearing of parasites. Recrudecence occurred in one patients with quinine-treated falciparum malaria and in three patients with Pl. vivax. Malaria drugs for prophylaxis according to Swedish recommendations had b een adequately taken by only 26/81 patients.

FIRST DOCUMENTED CASE OF HUMAN BABESIOSIS IN SWEDEN

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Babesiosis is a tick-borne protozoan disease of several wild and domestic animals. It is manifested by hemolytic anemia and the prescence of the parasites in host erythrocytes. The species Babesia divergens of bovine origin, has occasionally been reported to cause disease in humans, with a severe and often fatal outcome in splenectomized individuals. Several treatment regimens have been tried, but with limited success. Recent reports of a favourable response to blood exchange transfusions and a combination of quinine-clindamycin have been most promising.

Case report. A 34-year old man, who had undergone splenectomy five years earlier due to traumatic injury, presented with a 2-day history of fever 40°C, myalgia, malaise, dysuria and dark urine. On admission the patient was alert. Laboratory findings included Hb 142 g/l, platelets 210 x 10^9/l and creatinine 130 umol/l. The following day the patient rapidly deteriorated with anuria (creatinine 568), hemolytic anemia (Hb 96), trombocytopenia (platelets 73) and massive fibrinolysis and was transferred to the intensive care unit. Peripheral blood smear revealed intraerythrocytic parasites consistent with B. divergens in 40% of the erythrocytes. Diagnosis was confirmed by gerbil inoculation and by significant increase in B. divergens antibodies. The patient underwent exchange transfusions and his parasitemia was reduced to 1%. Immediate iv therapy was instituted with 600 mg quinine and 600 mg clindamycin every 12 hours. After 10 days parasites were no longer seen on smears. The patient received quinine for 11 days and clindamycin for 20 days. On this regimen symptoms slowly improved. Because of renal failure he had to be dialyzed for 4 weeks. At follow-up he was recovered and his blood tests had returned to normal.

Conclusion. Human babesios is a rare disease, but with a potential fatal outcome. B. divergens is widely distributed in cattle in the south of Sweden. Natural transmission occurs through the bite of an infected tick (Ixodes ricinius). This case report illustrates the successful treatment of a severe and life-threatening infection of Babesia in man. Babesiosis should be considered as a diagnostic alternative in splenectomized and other immunocompromised patients with severe infections.

CLINICAL MANIFESTATIONS IN HUMAN LYME BORRELIOSIS

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Lyme borreliosis may affect different organs and the clinical picture and course may be highly variable.

EARLY LYME BORRELIOSIS

Erythema migrans (EM), the cutaneous hallmark, starts at the site of borrelial inoculation. Typically it is a homogeneous or annular erythematous lesion spreading centrifugally.

Borrelial lymphocytoma presents as a bluish-red nodule with a dense polyclonal lymphocytic infiltrate. Predilection sites are the ear lobes and the breast.

Multiple EM-like lesions, appearing as a result of hematogenous spread, are the cutaneous markers of disseminated disease.

Neuroborreliosis may involve both the peripheral and the central nervous system. The triad of a lymphocytic menigitis, cranial neuritis and radiculoneuritis is a typical feature.

Cardiac manifestations such as atrioventricular conduction distrubances and **ocular involvement** may also develop.

Lyme arthritis characteristically tends to affect only one or a few large joints, most commonly the knee.

LATE LYME BORRELIOSIS

Acrodermatitis chronica atrophicans is a chronic skin lesion generally present on the extremities. It starts with an inflammatory phase with bluish-red discoloration which years to decades later may be followed by an atrophic phase.

Neurologic, rheumatic or other organ manifestations may sometimes be chronic and persist or remit for more than one year.

TICKS AND TICK-BORNE INFECTIONS IN NORWAY

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Introduction. This review is a compilation of results published by several authors. The most important works were done on ticks and Babesia on cattle by H. Tambs Lyche, and on ticks, arboviruses and vector borne bacteria by R. Mehl, T. Traavik and coworkers at the Nationalt Institute of Public Health, the University of Tromsø and the Norwegian Defence Microbiological Laboratory.

Methods. Host relations and geografical distributions of ticks were investigated by collection of host animals and by flagging. Isolations of viruses and bacteria was attempted from ticks and host organs. Serum from birds, wild and domestic animals, and man were examined for antibodies against these microorganisms.

Results. Eleven species of ticks are recorded in Norway: On birds: *Ixodes arboricola*, *Ixodes caledonicus*, *Ixodes frontalis*, *Ixodes lividus*, *Ixodes uriae* and *Hyalomma marginatum*, on mammals: *Ixodes hexagonus*, *Ixodes trianguliceps*, *Rhipicephalus sanguineus*, *Argas vespertilionis*, on birds, mammals and reptiles: *Ixodes ricinus*. Microorganisms transmited by *Ixodes ricinus* in Norway: Tick-borne encephalitis virus (TBE) (louping ill virus ?), Kemerovo-group virus and Uukuniemi group virus, *Erlichia phagocytophila*, *Borrelia burgdorferi*, *Francisella tularensis*, *Babesia divergens*, *Babesia microti* and *Trypanosoma*. Microorganisms transmitted by the seabird tick *Ixodes uriae*: Uukuniemi and Kemerovo group viruses, Tyuleniy virus of B group, orbivirus-like virus and Runde-virus (coronavirus like).

Conclusion. *Erlichia* and *Babesia* cause serious diseases in sheep and cattle in the range of *I.ricinus*. *Borrelia* causes a numbre of cases of disease in man each year. Antibodies against arboviruses are widespred in man and animals, but it appears that the TBE virus seldom causes clinical manifestations in man and cattle. Tyuleniy virus can produce pathogenic canditions in seabirds. Ticks have very little importance as vectors for tularemia in Norway.

THE EPIDEMIOLOGY OF LYME BORRELIOSIS IN DENMARK

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Objective. To determine 1)the prevalence of Borrelia burgdorferi in Ixodes ricinus and other species of Ixodes, 2)the prevalence of B. burgdorferi in wild mammals (rodents, fox, deers), 3)the population dynamic of the rodent reservoir and the ticks and 4)the seasonal variation in prevalence of Lyme Borreliosis in the mammalian reservoir.

Methods. I. ricinus were collected by blanket dragging in one locality in Northern Zeeland, dissected and examined for spirochetes. The mammalian hosts were examined for Lyme Borreliosis by immunofluorescence. The rodents - Apodemus flavicollis and Clethrionomys glareolus - were caught with Uggla-traps arranged in quadrant measuring 10x10 meters and inspected in the early morning. All mice were examined for ticks and blood samples were collected. Ticks as well as blood samples have also been collected from Dama dama and Capreolus capreolus in the hunting season autumn and also May-June for the bucks only.

Results. The project is running from 1990-1992 and the preliminary results indicate a spirochete infection rate in adult ticks up to 25 % in the autumn, and a lesser rate in nymphs. The serodiagnosis for A. flavicollis (n=36) and C. glareolus (n=113) demonstrated in autumn average percentages on 30.6 and 10.6, respectively. Since the population of the small rodents were dominated by the new generation the figures are minimum. The infection rates calulated on adults and young ones were 16.2 and 7.9, respectively for C. glareolus. A serodiagnoses carried out on blood samples from 24 D. dama and 16 C. capreolus demonstrated prevalence about 50.

Conclusion. Small rodents and dears appear to be important hosts for B. burgdorferi with I. ricinus as the vector. The transmission seasons seem to be in April-June and September-October.

THE DANGER AREAS OF TICK-BORNE ENCEPHALITIS IN ESTONIA

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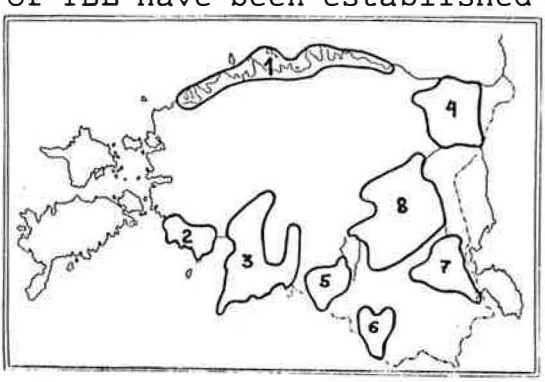
Objective. Determination of danger areas of tick-borne encephalitis (TBE) in Estonia, their epidemiological characterisation.
Methods. The circumstances of the infection of 755 cases of TBE occurred in Estonia in 1950-1990 have been studied.
Results. Eight danger areas have been revealed which account for about 20% of the territory of the Estonian mainland and almost 93% of the registered cases of TBE. The danger areas differ on the season of tick attacks, the age and sex of the patients, the reasons of visiting the danger area.

The danger area of TBE	% of cases of TBE	Dominating tick species	The season of tick attacks, maximum
1.Northern coast	9,3	I.ricinus	1st ten-days period (tdp) Aug
2.Varbla	2,9	I.ricinus	1st tdp Aug
3.Pärnu	32,5	Both equally	2nd tdp May and 3d tdp Aug
4.Kurtna	7,1	I.persulcatus	1st and 2nd tdp June
5.Tarvastu	3,6	mostly	3d tdp May
6.Kaagjärve	3,2	" - "	3d tdp May
7.Järvselja	13,5	" - "	2nd tdp May
8.Tartu	27,9	" - "	2nd tdp May

Conclusion. Eight epidemiologically different danger areas of TBE have been established in Estonia.



SPECIES IDENTITY AND MORPHOLOGY OF BOVINE DEMODECTIC MITES IN FINLAND

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Species of the genus Demodex are common parasites of the skin of many animals, including man. Their extremely small size and marked morphological similarity makes species identification difficult. Although some reports have been presented on the occurence of bovine demodectic mites in Finland, further studies were needed. The slaughter houses and tanneries have estimated their losses to be as high as 30 million FMK:s per year(1988), due to the damaged hides. An investigation started in 1989 with the long-term objective to reduce this problem. The immediate objectives were to identify the species' of demodectic mites found in cattle in Finland and to define the morphological features of the mites, using both scanning electron microscopy (SEM) and light microscopy (LM) in combination with image analyzing equipment.

Samples (n=503) were obtained from different parts of the country during one year. Most of the samples were stored in formalin, but some fresh material was also included. Mites were stored in 70% alcohol until prepared for SEM or LM and image analysis. Morphologically identifiable demodectic mites were found in 69 out of 503 cysts sampled. One female from each cyst and the 8 males that were found, were identified. In addition two cysts were selected, one from the northern and one from the southern part of the country. 30 females from both of these cysts were measured and analyzed.

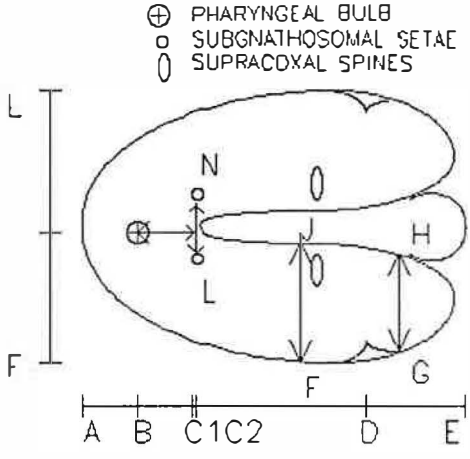
All the mites conform to Demodex bovis, which thus was the only parasite species encountered in the material. They were morphologically compared with Demodex canis.

Because of their potential taxonomic significance, a detailed morphometric analysis was made of the head structures (gnathosoma) of D. bovis (see Fig. 1). It is suggested that this information can be used in further studies in differentiating species' of the genus.

Fig. 1. D. bovis. Morphological features of the head. Measurements, in μm, based on LM drawings (average and SD). (Total length of females: 203-271 μm.)

	N-Finland n=30	S-Finland n=30
A-E	33,6 (2,1)	34,0 (3,9)
A-D	25,2 (1,5)	24,8 (3,1)
A-C2	9,1 (1,5)	12,3 (2,5)
B-C2	5,3 (2,0)	5,3 (0,6)
A-B	3,6 (0,1)	6,6 (1,2)
F-L	23,0 (3,4)	24,8 (2,1)
F-J	9,6 (0,9)	8,5 (0,8)
G-H	6,4 (0,8)	7,1 (0,5)
B-C1	4,8 (0,2)	4,1 (0,4)
L-N	7,2 (1,6)	6,3 (3,1)

C1:crossing point of line from B to line LN. C2:caudal point of bay like structure in the middle of head.



CONSEQUENCES OF ARTHROPOD PARASITES FOR SEXUAL SELECTION IN BIRDS

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Objective. The hypothesis by Hamilton and Zuk (1982) that elaborate secondary sexual characters may have evolved in vertebrates as a signal of health and thus resistance to parasites, has led to extensive research efforts and debate in evolutionary ecology. In this discussion the term 'parasites' has often been used indiscriminately, disregarding differences in biology of each taxa and consequences of these for the host. The objective of this investigation is to point out that species diversity and natural history should be of great concern in parasitological surveys.

Methods. Nests of starlings, *Sturnus vulgaris,* from South Sweden were put in separate small plastic bags a few days after the nestlings had left the nestbox. Each little bag, still open, was put in a large one, which was closed by tying a perspex tube sealed by a finemeshed net to the opening. The bags were left at room temperature for a week, and the outer bags were inspected for flies, fleas, and mites daily. The nests were then put in Berlese funnels, in order to extract remaining arthropods.

Results. Three parasitic species of three different orders were found in the nests, i e Diptera: *Carnus hemapterus*, Siphonaptera: *Ceratophyllus gallinae*, and Mesostigmata: *Dermanyssus gallinae*. The only species among the mesostigmatic mites who was demonstrated to be a predator was *Macrocheles penicilliger*. Two other mesostigmatic mite species, probably scavenger feeders, were *Hypoaspis* sp. and *Proctolaelaps pygmaeus*.

EFFECTS OF AFRICAN DUNG BEETLES ON SPLASH DISPERSAL OF INFECTIVE LARVAE OF *Cooperia* sp.

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Objective. To evaluate the effect of dung-burying beetles on dispersal of infective larvae of the cattle parasite *Cooperia* sp. from cow pats to the surroundings.
Methods. The experiment was performed in Zimbabwe in Africa. From a carefully mixed lot of cattle faeces containing *Cooperia* sp. eggs, 1-kg portions of faeces were placed in 6 small buckets (upper diam: 23 cm; lower diam: 19 cm; height: 25 cm) containing 6 kg of soil each. The faecal portions were shaped as cow pats (diam: 20 cm; height at centre 3 cm), which covered the entire soil surface.Ten dung beetles, *Diastellopalpus quinquedens*, were added to each of three buckets. The other three buckets were controls without beetles. The buckets were kept in the laboratory at approximately 24°C, and the cow pats were watered artificially with between 2o and 80 ml at daily intervals to keep optimal conditions for larval development. When it was raining the cow pats, still in the small buckets, were placed in the centre of wider and higher big buckets (diam: 32 cm; height: 38 cm) and placed outdoors. Infective parasite larvae spread with splash droplets during the rain, were collected in the big buckets and counted.
Results. During the experimental period of 33 days, more than half of the faeces disappeared in the buckets with beetles compared with the controls without beetles. The dung-burying activity of the beetles did not result in an increased number of infective larvae in the soil under the cow pats, which indicate that many of the parasites in buried faeces were destroyed. Moreover, the activity of dung beetles in cow pats resulted in a 7o% to 90% reduction in spash dispersal of infective larvae of *Cooperia* sp.
Conclusion. It must be expected, that dung beetles have a significant reducing influence on the survival and transmission of infective *Cooperia* sp. larvae and related parasites in Africa.

DEGRADATION OF DUNG AFTER TREATMENT OF CATTLE WITH IVOMEC

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Objective: To clarify whether ivermectin residues in the faeces of cattle treated with IVOMEC® have an impact on the native fauna in the dungpat and/or the rate of faecal degradation under typical central European conditions.

Methods: Four trials were conducted in north Germany by the Veterinary College of Hannover and in south Germany by MSD, Rosenheim. Degradation of dung pats from untreated cattle (Hannover) or from cattle treated with levamisole (Rosenheim) was compared with degradation of pats produced by cattle after injection with ivermectin subcutaneously at 0.2 mg/kg bodyweight. The rate of degradation was calculated by measuring the size of pats and by analyzing the content of organic substances at regular intervals. The activity of dung fauna was evaluated in a second series of dung pats, deposited at the same time. At intervals up to 63 days, one eighth of each of these dung pats was taken and the arthropods and nematodes present were recovered, identified and counted.
Results: The rate of dung degradation was similar for pats from ivermectin treated and control animals at both sites. The variety of beetle species recovered was similar for both locations and consisted of 66 species in Hannover and 68 species in Rosenheim. Treatment with **IVOMEC® (ivermectin)** had no effect on adult or immature beetle populations nor on earthworms, although the development of some diptera and of dung nematodes was influenced in pats up to approximately 3 weeks after treatment.

THE IMPACT ON DUNG FAUNA OF FECAL IVERMECTIN RESIDUES EXCRETED AFTER SUBCUTANEOUS INJECTION OR POUR-ON TREATMENT OF HEIFERS.

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Objective. To estimate toxic effects of fecal ivermectin residues on cow dung fauna i.e. Aphodius dung beetles and Diptera Nematocera and Cyclorrapha.
Methods. Heifers were given 0.5 mg kg^{-1} bw by pour-on, 0.2 mg kg^{-1} bw by subcutaneous injection or remained untreated, and dung was collected from the animals at various days after treatment. Experimental pats made of the dung were exposed up to 45 days in an ungrazed field plot. Insects from the pats and underlying turfs were counted, and a general linear model was used for separate comparison of insect numbers from pour-on or s.c.inject. pats with insect numbers from control pats for each "day after treatment". The qualitative effect of treatment or no treatment and the first and second order quantitative effects of days of field exposure were included in the model. The experiment was performed in late summer.
Results. The ivermectin excreted in dung voided up to 13-14 days after pour-on treatment and up to 28-29 days after s.c. inject. treatment significantly reduced the number of dung pat inhabiting Cyclorrhapha. Development of Aphodius spp. larvae was inhibited in pats made of dung collected 1-2 days after both treatments, but no effect was seen in pats made of dung collected at later dates after treatment. Nematocera larvae were unaffected.
Conclusion. Treatment by the 2.5 times higher pour-on dose did not result in excretion of toxic dung for a prolonged period compared to treatment by s.c. injection. This agrees with measurements of lower ivermectin concentrations in pour-on dung than in s.c. inject. dung, when both dung types were voided one week or later after treatments.

THE PROGRAM TO ERADICATE THE NEW WORLD SCREWWORM, COCHLIOMYA HOMINIVORAX FROM NORTH AFRICA USING THE STERILE INSECT TECHNIQUE: A STATUS REPORT

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The New World Screwworm, Cochliomyia hominivorax, is the most destructive livestock insect pest in Latin America. The larvae are obligate parasites that causes primary myiasis in preexisting wound on mammals, including humans. Successful eradication of the New World Screwworm (NWS) from the Southern USA and Mexico has been accomplished using the Sterile Insect Technique (SIT). SIT involves releases of large numbers of mass-reared radiation-sterilized flies to compete with the wild flies for mates. Wild females mated with sterile males fail to produce offspring. In 1988 NWS-infested animals were discovered in Libya. In 1990 more than 12000 NWS infested animals were reported. This was the first record of an established NWS population outside the Americas. If this pest spreads throughout Africa it would pose an enormous threat to livestock and wild animals. Responding to this threat, FAO established a program to eradicate the NWS from the 25000 sq km infested zone. Since December 1990 sterile pupae have been shipped by air from the production plant in Mexico to Tripoli. Dispersion over the entire infested area started in early February 1991. This was extended to a 40.000 sq km in May to indlude a protective barrier with releases of 40 million flies per week. Quality tests measuring emergence, longevity, flight ability and other parameters are conducted in Mexico and Libya to detect any

quality change. Additional tests have been developed in Libya to assure that optimum fly quality remains after holding pupae/flies in different temperature regimens which is required to control emergency for the weekly 4 dispersion days. The success of SIT is dependent on quality of the released flies. Thus quality control tests are essential in SIT program. As of 1 August 1991, only 6 cases on NWS myiasis have been detected during 1991. Interpretation of the field data are strong evidence that releases of good quality sterile flies, once again, successfully eradicated C. hominivorax.

ARE HOSTS ADAPTED TO THE LOCAL POPULATION OF PARASITES?

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Objective. As parasites are capable of rapid evolutionary change, they can trace local conditions. As a result, individual species may have very different characteristics in separate localities. To answer the question if parasites from a foreign population have greater negative effect on a host, compared with a locally occuring parasite population, a study was performed outside Uppsala. A nest box breeding population of great tits (*Parus major*) was used together with fleas (*Ceratophyllus gallinae*) collected from Uppsala and from the isle of Gotland, situated about 300 km from Uppsala.
Methods. When the egglaying period of the great tits had started, nest-boxes were randomly divided into three groups: 1. addition of 100 fleas from Uppsala 2. addition of 100 fleas from Gotland 3. spraying with an insecticide to remove all fleas. During the breeding period the length of incubation and of the nestling period were noted. When the nestlings were 13 days old, their weight, tarsus length and wing length were measured. After the nestlings had left the nest, the remaining nest material was collected and the amount of fleas in them was counted.
Results. The foreign fleas affected the nestlings more severely than the local ones in all of the measured parameters. Furthermore, the foreign fleas had survived better or multiplied more quickly than the local fleas.
Conclusion. The foreign fleas can exploit the host better, since the host does not have as good defence against foreign genotypes of parasites as for local genotypes. As the host has an inherited resistance to the local flea population but not to the foreign ones, the nestlings have to develop immunological factors. Investment in an effective immune system is costly and divert resources away from other metabolic demands, which shows up in the smaller size of the nestlings in group 2.

SERODIAGNOSIS OF SARCOPTIC MANGE IN DOGS

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Humoral antibodies directed against Sarcoptes scabiei var vulpis were studied in an experimental infection of dogs. Positive ELISA OD-values were seen 2-3 weeks after infection. Sera from dogs infested with other ectoparasites as Ctenophalides cati and C. canis, Trichodectes canis, Linognatus setosus, Otodectes cynotis and Demodex sp did not show any reaction against the Sarcoptes scabiei antigen.

Serum-samples of 634 dogs, tested at the Dept. of Clinical Chemistry, SLU, Uppsala for various reasons, were tested for antibodies against S. scabiei. These sera served as a representative sample from the swedish dog population. Three percent of these showed positive OD-values.

In a separate study focused on dogs with suspected sarcoptic mange 709 sera were tested. Forty percent of these sera showed positive OD-values.

Sera from dogs all over Sweden sent for routine testing of antibodies to S. scabiei showed positive OD-values in approximately 20 %.

In all 3 groups tested there were no difference regarding age, sex or race. Positive reactions were more frequently found in sera of dogs from Nothern Sweden and the county Småland than from other parts of Sweden.

FUNGAL PATHOGENS OF IXODES RICINUS - THE VECTOR OF LYME BORRELIOSIS

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Objective. Pathogenic fungal species have been found to be important mortality factors of insect populations. The objective in the present study is to evaluate the importance of pathogens on Ixodes ricinus with emphasis on fungi.

Methods. Ticks were collected either from systematic blanket dragging or from small rodents and deers. The ticks were incubated at room temperature for several weeks. Dead ticks covered with fungal mycelium were examined. The fungi were identified and cultivated on artificial media. Further, ticks were subjected to infection from pure cultures of the pathogens grown on agar to prove eventual pathogenicity.

Results. The preliminary results are as follows: proportion of both adult ticks and nymphs were covered by fungi after their death. The following species have so far been identified and isolated on agar: Beauveria bassiana, Verticillium lecanii and Aspergillus spp. B. bassiana and V. lecanii are known as pathogens on both insects and mites. Both species were able to infect ticks in the laboratory.

Conclusion. Ticks are infected by various species of fungi which may be of importance in the regulation of the tick population. The fungi are potential biological control agents of various species of ticks.

MOSQUITOREPELLENTS FROM ACHILLEA MILLEFOLIUM L.

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Objective. Since mosquitoes are known to be vectors of several different diseases it is therefore of intrest to prevent this transfer. One way to do this is the use of repellents. An ethanolic extract of yarrow, Achillea millefolium L. (fam. Asteraceae), was found to have mosquito repelling activity (Thorsell, 1978).
The aim of this study was to identify the mosquito repelling components that obviously occur in the extract.
Methods. The extract was fractionated into basic, neutral and acidic compounds of different hydro-lipophilicity. This was done using liquid-liquid extraction at different pH. The obtained fractions were evaluated for activity in a repellent test using Aedes aegypti L. Further separation and identification was performed using TLC, HPLC and MS. Identified compounds were subjected to further studies to measure their mosquito repellent activity.
Results. It was found that repelling activity occurred in several fractions. One fraction containing carboxylic acids (e.g. caffeic acid, chlorogenic acid, ferulic acid, mandelic acid and salicylic acid), the second one nitrogenous compounds (e.g. betonicine, cholinechloride, homostachydrine, prunasin, stachydrine and trigonelline) and the third phenolic substances (pyrocatechol, quercetin and tannic acid). Most of these have already been isolated from yarrow (Karrer, 1976-1985).
Conclusions. These results indicate that its possible to isolate mosquito repelling compounds from plant material, e.g. Achillea millefolium L. The identified substances were of acidic, nitrilo or quarternary nitrogen character and are thus different from the most abundant repellents that are of amide, ester or alcohol nature.
Most of the identified compounds possessed a contact repelling effect and some of them had an activity of the same magnitude as DEET.

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TWO GROWTH FACTORS IN THE GULL-TAPEWORM

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Characteristic for many adult pseudophyllidean tapeworms is their special mode of growth. During their life span, which for *Diphyllobothrium dendriticum* reared in hamster may dure up to some months, there is a constant production of new tissue in the neck region and a corresponding loss of old tissue in the tail end. Between the producing neck and the dying tail is a gradual maturation of tissue, especially of tissue belonging to the genital organs. In the same individual all stages from the tiniest aggregation of germinative cells forming the first sign of genital anlage in the medullary parenchyma of the neck to the fully developed egg-filled ovaries and oviducts in the tail can be found. Everything happens as on a conveyor belt. When the worm reaches maturity no netto growth takes place any more.

The guiding principles behind this *never ending growth* are still unknown. Mitogenic factors have been searched for. With immunocytochemical technique basic fibroblast growth factor (FGF) and epidermal growth factor (EGF) have been localized in the gull-tapeworm. FGF immunoreactivity occurs solely in the central nervous system. EGF immunoreactive cell bodies were localized in the main nerve cords and furthermore in the mature genital organs. The effect of FGF was studied by cultivating plerocercoids *in vitro* in media containing anti-FGF and protamine (a blocker of the bFGF receptor) and by counting the number of cells in mitosis.

The rapid and constant growth of tapeworms makes them interesting model organisms in the studies of cell multiplication and cell differentiation (Gustafsson 1990).

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SEASONALITY IN PREVALENCE AND INTENSITY OF *Gyrodactylus salaris* MALMBERG, 1957 (MONOGENEA; GYRODACTYLIDAE) ON PARR OF ATLANTIC SALMON *Salmo salar* L. IN RIVER BATNFJORDSELVA, NORWAY

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Abstract

In Norway, the viviparous monogenean *Gyrodactylus salaris* Malmberg, 1957, is highly pathogen to parr of Atlantic salmon *Salmo salar* L. The parasite was probably introduced to Norway with infested smolts in the early 1970s, and has mainly been spread with infested salmon parr stocked in the rivers. So far, the parasite has been found in 35 rivers, and in all, except one, the salmon parr population has been reduced to a minimum within 3-5 years after the parasite was introduced to the river. In River Batnfjordselva, located at the north-western coast of Norway, the percentage of survivors is much higher. Consequently this river was chosen to study the seasonality in prevalence and intensity of *G. salaris* on Atlantic salmon parr. The fish were collected at roughly monthly intervals, from june 1985 to july 1987 by means of electrofishing. 422 parr were collected; the number in each sample varied from 5 to 43. In total, approximately 203,500 *G. salaris* were found on 403 infested parr (mean 505); the number of parasite specimens per fish varied from 1 to about 12,500. The prevalence was 100% in most samples, except at the lowest water temperatures (0.0-4.0°C) when the prevalence could decrease to 60%. The mean intensity at each sample varied greatly. From October 1986 to February 1987 the mean number of *G. salaris* dropped from 2.030 to 34. At the same time the variance-to-mean ratio dropped from 4,251 to 68. The rapid reduction of parasite intensity in the late fall and early winter months can probably not be explained by parasite population dynamics or host induced parasite mortality, but mainly because of a parasite induced host mortality.

RESISTANCE TO GYRODACTYLUS SALARIS MALMBERG, 1957 (MONOGENEA) IN SALMO SALAR: A GENETIC COMPONENT

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Objective. Differences in host susceptibility and resistance to Gyrodactylus salaris between Salmo salar from the Baltic sea and Norwegian watercourses have recently been reported (Bakke et al. 1990). The aim of the present study is to examine the genetics of the susceptibility/-resistance traits in F_1 crosses between Baltic and Norwegian salmon.

Methods. The Gyrodactylus salaris strain used originated from the river, Lierelva. The Baltic S. salar originated from the river, Neva (USSR), the Norwegian S. salar from the river Imsa. F_1 crosses were made between: (1) Neva male x Imsa female and (2) Imsa male x the same Imsa female. The progeny (0^+, 5.3-7.9cm) was exposed to infected salmon for 2 days (isolated fish) and 6 days (grouped fish) at 12^oC. Experiments: (1) Two replicate groups of 10 Imsa x Imsa and two replicate groups of 10 Neva x Imsa were individually isolated in boxes (11x17x5 cm water level); (2) Two groups, 50 Imsa x Imsa and 50 Neva x Imsa, were kept in grey plastic tanks (100x100x20 cm water level). The number of G. salaris was counted on anaesthetized (2 min, 0.04% Chlorbutanol) fish.

Results. In all the laboratory epizootics, G. salaris infrapopulation growth was highest in the Imsa x Imsa F_1 progeny. Mean intensity on isolated (1) and grouped (2) fish: (1) Imsa x Imsa: 26/101/315/493 (day 0/16/30/36), Neva x Imsa: 20/65/152/156 (day 0/16/30/36); (2) Imsa x Imsa: 57/220/420 (day 0/14/28), Neva x Imsa: 43/168/236 (day 0/14/28). The relative daily population growth on pooled individually isolated parr was significantly higher on Imsa x Imsa progeny between days 30-36 ($p<0.05$).

Conclusion. The growth of Gyrodactylus infra-populations was continuously slower on Neva x Imsa F_1 progeny. This indicates an inheritable component in G. salaris resistance in male Neva salmon.

HUMORAL IMMUNE RESPONSE OF THE EUROPEAN EEL (ANGUILLA ANGUILLA) AGAINST MAJOR ANTIGENS IN ANGUILLICOLA CRASSUS (NEMATODA).

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Objective. To isolate and partially characterize major antigens in the swimbladder nematode Anguillicola crassus parasitizing the European eel (Anguilla anguilla).
Methods. Adult female specimens of A. crassus were removed from the swimbladder of naturally infected eels and sonicated. SDS-PAGE (10 % polyacrylamide) was performed under reducing conditions of the parasite sonicate. The separated proteins were electroblotted onto 12 nitrocellulose membrane strips, which were incubated separately in serum (diluted 1:50) from 6 infected and 5 non-infected eels or blocking buffer alone (control). To detect immuno-reactive bands the strips were incubated in a rabbit anti eel Ig serum (diluted 1:1000) followed by an incubation with a peroxidase conjugated swine anti rabbit Ig (diluted 1:2000). To visualize immunoreactive protein bands the strips were reacted in dioctyl-sulfo-succinate, tetramethyl benzidine, citrate phosphate buffer and hydrogen peroxide.
Results. More than 12 distinct protein bands with MW ranging from app. 14 kDa to more than 94 kDa could be distinguished in the Coomassie blue stained SDS-PAGE slab with the parasite sonicate run under reducing conditions. The sera from infected eels reacted with at least two of these proteins with MW of app. 25 kDa and 40 kDa. Sera from non-infected eels did not show reaction to any of these proteins. Both infected and some non-infected eels reacted to a 93 kDa protein.
Conclusion. The European eel is capable of mounting a humoral immune response to distinct antigens from Anguillicola crassus, and our immunoblot method provides a serodiagnostic tool for detection of Anguillicola infection in eel.

ADVANCED TECHNIQUES IN THE TAXONOMY OF SPECIES OF *GYRODACTYLUS* (MONOGENEA) PARASITISING BRITISH FRESHWATER SALMONIDAE

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Several species of the ectoparasitic monogenean genus *Gyrodactylus* infect populations of wild and farmed salmonids in northern Europe, where in some cases they are thought to be responsible for high mortalities in juvenile fish. Species determination based on subtle differences in hook morphology has posed a taxonomic problem when carried out at the level of the light microsope.

Geographically distributed populations of *Gyrodactylus* parasitising four hosts of the family Salmonidae were collected and their sclerites compared using scanning electron microscopy following enzymatic digestion. This revealed the amount of morphological variation that exists within a population sample and between species from other biogeographical locations outside the UK.

Marginal hooklets and hamuli collected from enzymatic digestion, viewed under the scanning electron microscope, were measured using the classical feature of hook shape. These were then compared to both novel parameters measured using a digital image analysis system and to measurements taken with the light microscope. The image analysis system enables the measurement of new parameters of hook shape and the removal of some of the unreliable taxonomic criteria used previously. Collected data were then subjected to a computer analysis of variance package to isolate and confirm different species of *Gyrodactylus*.

In addition, sputtercoated sclerotized portions of the haptoral attachment apparatus, extracted and purified by sonication and serial centrifugation, were examined by scanning electron microscopy. By this technique the precise morphology of the accessory bars and the posterior membrane of the ventral bar can be described: these are lost using present enzymatic digestion techniques.

THE CESTODE EUBOTHRIUM SP. IN FARMED MARINE SALMON - INFECTION PATTERN AND TREATMENT.

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Objective. To study in marine farmed salmon, Salmo salar L. 1) the infection pattern over time with the cestode Eubothrium sp. and 2) the results of treatment with anthelminthics.
Material and methods. Salmon smolts produced at MOWI's hatchery at Varaldsøy, Hardanger, transferred to MOWI's marine aquaculture unit on the outer coast at the end of May 1990, were sampled at intervals during summer-fall 1990 to follow the build-up of the infection with this cestode. In cooperation with BP Nutrition Aquaculture, at the end of October 1990 the fish in 6 pens were treated with anthelminthics: 5 were given praziquantel regimens, 1 fenbendazol, plus one control group. By glass-plate compress we searched for, collected and weighed the worms.
Results. Fresh-water smolt were not infected; after about 4 weeks in the sea a single fish, in a batch of 29, harboured 10 tiny specimens. During the summer the infection increased gradually, by the middle of August prevalence reached about 80 %, abundance 14, maximum number in a single fish 164. By the middle of October, prior to treatment, the over-all infection in 281 fish was: prevalence 85 %, abundance 6.67. Early in the summer the specimens were very small, hardly more than scoleces, but gradually the specimens grew large and became sexually mature with eggs.
The anthelmintic treatment gave as best result a 85 % reduction in prevalence, obtained with 5 mg praziquantel/kg fish given on each of two consecutive days. Scoleces were found in sample of voided worms. The infection in 40 fish from the control group and 40 from the "best result"-group, sampled on 13. March 1991, showed a prevalence of 82.5% and 4.5 % respectively, abundance being 6.1 and 0.45, respectively.
Conclusion. Salmon becomes infected with a marine Eubothrium species in sea water in the summer and autumn, but little or no infection seems to occur during the winter.

DISTRIBUTION OF X-CELL DISEASE IN THE COMMON DAB (*Limanda limanda* L.) IN THE MORAY FIRTH

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Objectives. X-cell disease in common dab is characterised by the development of pale, swollen gill filaments. Histological examination of the lesions show X-cells to be present in the area between secondary gill lamellae. X-cells are of an unknown type, however, most recent studies suggest they are amoeboid like unicellular parasites (Diamant and McVicar, 1989). There is some evidence to suggest that the distribution of X-cell disease is highly patchy (Diamant and McVicar, 1989).
This paper attempts to further describe and interpret the distribution of X-cell disease.

Methods. An intensive survey of the prevalence of X-cell disease is being carried out in the Moray Firth on the east coast of Scotland. Prevalences have been determined from hauls off the southern shore of the Moray Firth and from a series of hauls forming a grid covering an area of *c.* 3 nm^2 situated within Burghead Bay (south coast, Moray Firth).
Experimental investigations into the aetiology and dynamics of X-cell disease are also being carried out.

Results. The results of the field survey show X-cell disease to be restricted to a narrow band of inshore water (*c.* 3 nm wide) with further extensive variation of prevalence within this patch.

Conclusions. That a potentially parasitological condition shows such marked spatial variation over a limited area clearly has implications for the design and interpretation of future surveys for this and other parasites.

References. Diamant, A. and McVicar, A.H. (1989). Distribution of X-cell disease in common dab, *Limanda limanda* L., in the North Sea, and ultrastructural observations of previously undescribed developmental stages. *J. Fish Dis.*, **12**:25-37.

COCCIDIANS OF FOUR MARINE FISH SPECIES FROM SCOTTISH WATERS

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Objectives. To determine whether *Goussia clupearum* only or other species infect the livers of herring (*Clupea harengus*), mackerel (*Scomber scombrus*), Norway pout (*Trisopterus esmarkii*) and poorcod (*Trisopterus minutus*).

Methods. Squash preparations of fresh or frozen livers were examined with Normanskii Interference Contrast. Some liver samples were processed for light and electron microscopy using standard methods.

Results. Each sporulated oocyst contains four sporocysts and has a thin rounded wall. There is no polar granule or oocyst residuum. The sporocysts are oval, with two banana shaped sporozoites divided by a suture, and each with a round refractile granule. The sporocyst residuum is fine-grained. Between herring and mackerel on the one hand and Norway pout and poorcod on the other there was a significant difference in sporocyst size. The ultrastructure of both developing and mature macrogamonts is similar. These stages are limited by a single unit membrane and lie inside a single or double walled parasitophorous vacuole, depending on stage of development. Mature sporocysts from herring, mackerel and Norway pout had a three-layered membrane.

Conclusions. Based on the morphology of both oocysts and sporocysts and on the ultrastructure of some of the developmental stages, it is concluded that the four fish species are infected by the single species *G. clupearum*.

INFESTATION BY APIOSOMA AND ICHTHYOBODO IN PIKE-PERCH FRY REARED IN TWO DIFFERENT TYPES OF NATURAL-FOOD PONDS

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Objective. The aim of the present study was to examine whether parasites have an effect on the production of natural-food ponds stocked with pike-perch (Stizostedion lucioperca) fry.
Methods. Pike-perch fry were studied for parasites twice a month from June to mid-August in two different types of natural-food ponds in southern Finland. The fry were not fed or treated during the summer.
Results. In the pond at Lohja, Ichthyobodo necator occurred on the skin and gills of the fry from June to mid-July, infestation being worst at the beginning of July. At Rauma, slight infestation by I. necator occurred only in the middle of August. The fry from the pond at Lohja were very strongly infested by Apiosoma sp. from July onwards. E.g. the fins of the fry were filled with these protozoans. At Rauma Apiosoma sp. appeared in the middle of July, with the peak at the end of the month, but the level of infestation was clearly lower than at Lohja.
Conclusions. The massive infestation by Apiosoma sp., together with the occurrence of I. necator, was evidently a consequence of the poor nutritional state of the fry in the pond at Lohja. Both the growth rate of the fry and the production of the pond were clearly poorer at Lohja than at Rauma.

HENNEGUYA SP. (MYXOSPOREA) ON THE GILLS OF PERCH (PERCA FLUVIATILIS) IN CENTRAL FINLAND, SEASONALITY AND PATHOGENICITY

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The occurrence and developmental stages of Henneguya sp. were studied from the gills of perch (Perca fluviatilis) between February 1986 and November 1987 from four lakes of differing in water quality and pollution level in Central Finland. The prevalence of Henneguya cysts was highest (40 %) in the oligotrophic and lowest in the two eutrophic lakes (26.5 and 33.5 %), while in the polluted lake it was 36 %. The most distinctive change between the two years was found in the prevalence of infection in the polluted lake, where infection was 57 % in 1987 and 18 % in 1986.

Distinct seasonal variation was found in all lakes; the highest prevalences of Henneguya cysts were found in February-May in the oligotrophic lake and mainly in February-April in other lakes and the lowest occurred during the summer.

The developmental stages of Henneguya sp. in cysts on the gills ranged from very early stages to fully-developed spores. Early developmental stages were encountered throughout the year, but fully mature spores and developing spores occurred mainly in spring. All of the developmental stages were present and often concurrently in the same fish in spring.

No host reaction, apart from cyst formation was found against Henneguya.

PARASITES AS INDICATORS OF SEWAGE SLUDGE DISPERSAL IN THE FIRTH OF CLYDE

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Objective. To explore the relationship between levels of pollution and the prevalence of the digenean *Zoogonoides viviparus* (Trematoda: Zoogonidae) in the prosobranch mollusc *Buccinum undatum* on an sewage sludge dump site in the Firth of Clyde.

Methods. *Buccinum* were sampled monthly at sites along a pollution gradient over an 18 month period. Data were collected on parasite prevalence and seasonal variability, host condition, growth and infection with age. Laboratory studies have examined the effects of sewage sludge on the parasitic and free-living transmission stages in the life-cycle of *Zoogonoides*.

Results. There is a positive correlation between the prevalence of *Z. viviparus* in *B. undatum* and distance from the centre of the sewage sludge dump site. Parasite prevalence decreases significantly along a gradient of increasing sewage pollution. The observed correlation is not due to other environmental or host-related factors. Sewage sludge was found experimentally to affect the survival of *Zoogonoides* miracidia and to significantly increase the mortality rate of *Buccinum*. Mortalities were significantly higher in infected than uninfected whelks, leading to a reduction in parasite prevalence among the surviving individuals.

Conclusions. The *Buccinum*-parasite system appears to provide a good bioindicator for monitoring sewage sludge dispersal in the Firth of Clyde, and this model offers support for the use of parasites as biological indicators in routine monitoring studies.

PARASITES AS BIOLOGICAL TAGS FOR COD, *Gadus morhua* L., IN NORTHERN NORWAY

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Objective. To investigate the feasibility of using parasites to separate stocks of cod in northern Norway.

Methods. Samples of cod, totalling 103 fish, were taken from three locations in the southern Barents Sea and the fjords Balsfjord and Ullsfjord. Samples were preserved by deep-freezing. Each cod was later examined for protozoan and metazoan parasites and sample prevalences and abundances were compared for statistical significance.

Results. Sixteen species of parasite were found, seven of which showed statistically significant differences in prevalence between locations. The greatest differences were between the Balfjord sample and the other two. Potentially useful biological tags include the protozoan *Myxidium* sp. (from the gall bladder), the larval nematodes *Pseudoterranova decipiens* and *Phocascaris* sp., the digenean *Hemiurus levinseni* and the acanthocephalan *Echinorhynchus gadi*.

Conclusion. Cod in Balsfjord may comprise a largely self-contained population. An extension of this study is likely to contribute significantly to our knowledge of the stock structure of cod in northern Norway.

SUSCEPTIBILITY OF ARCTIC CHAR (SALVELINUS ALPINUS) TO GYRODACTYLUS SALARIS MALMBERG (MONOGENEA)

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Objective. Gyrodactylys salaris is suggested imported to Norway on salmon from the Baltic area (Malmberg 1988). Knowledge of the host range of the parasite, especially the eastern immigrants, is in this respect important. Previous laboratory tests (Tanum 1983) and field records (Mo 1988) indicate a potential for S. alpinus to be host for G. salaris. Our aim was to study the susceptibility and resistance of S. alpinus to G. salaris.
Methods. The G. salaris strain used originated from the river, Lierelva. 150 S. alpinus (anadromous Hammerfest stock, fulsib, 0^+, unfed, mean length 6.9cm(5.9-7.6); mean weight 2.6g(1.7-3.5)), previously unexposed to G. salaris, were exposed for 11 days to 26 infected salmon at 12°C. Experiments: (1a) Two replica with 50 grouped fishes in grey plastic tanks (100x100x20cm water level), (1b) one group reduced to 22 after 96 days of infection; (2a) 21 individually isolated fishes in floating boxes (11x17x5 cm water level); (2b) 19 isolated fishes after 96 days of grouped infection. The number of G. salaris was counted on anaesthetized (2 min, 0.04% Chlorbutanol) fish.
Results. Mean intensity (range) after one week of exposure (n=21): 55.8(10-151). (1a) Prevalence >80%, mean intensity fluctuating during 281 days of observation, peak infection individual fish, ca. 1500 (after 255 days); (1b) Decline and parasite elimination by day 60; (2a) Increasing intensity up to day 22, decline and parasite elimination by day 159; (2b) Decline but no parasite elimination after 150 days. S. alpinus were individually variable: (1) Innately resistant; (2) Initially susceptible, aquired respons, infection eliminated; (3) Initially susceptible, aquired respons, infection not eliminated.
Conclusion. Prolonged reproduction of G. salaris on anadromous S. alpinus is demonstrated under laboratory conditions. S. alpinus may accordingly physiologically function as a host for G. salaris in the field.

SUSCEPTIBILITY OF GRAYLING (THYMALLUS THYMALLUS) TO GYRODACTYLUS SALARIS MALMBERG (MONOGENEA)

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Objective. The epidemics of G. salaris on Salmo salar in Norwegian watercourses is explained as a consequence of a recent anthropogenic introduction of the parasite. This depends on host specificity and the species taxonomy. The riverine Thymallus thymallus is known to have crossed the present-day east-west watershed in Scandinavia and colonized for example rivers in northern Norway. Gyrodactylus thymalli Zitnan, 1960 described from T. thymallus in Czechoslovakia, is close to G. salaris (Zitnan 1960) and the two are difficult to distinguish morphologically (Malmberg 1987). The aim of this study is to examine the potential of T. thymalli as host for G. salaris.

Methods. The Gyrodactylus salaris strain used originated from the river, Lierelva. Thymallus thymallus (0^+, mean weight 2.5g, range 1.4 - 4.4g; mean length 7.2cm, range 6.2 - 8.5cm) previously unexposed to Gyrodactylus, originated from the lake, Femunden. 150 fish were exposed for 1 week to 20 infected salmon at 12°C. Experiments: (1) Two replica with 50 grouped fishes in grey plastic tanks (100x100x20cm); (2) Nine individually isolated fishes in floating boxes (11x17x5cm). Parasite number was counted on anaesthetized (2min, 0.04% Chlor-butanol) fish.

Results. Mean intensity (range) after one week of exposure (n=44): 12.7(3-25). Pooled results: (1) Increasing intensity up to day 22, then a decline; (2): Increasing intensity up to day 7, then a decline. Maximum observed duration of infection 50d (+7d exposure). The susceptibility of individual fish was variable: (1) Innately resistant; (2) Initially susceptible, aquired response after one week; (3) Initially susceptible, no response.

Conclusion. G. salaris transfer to and reproduction on T. thymallus occurs under laboratory conditions. T. thymallus may accordingly physiologically function as a host for G. salaris in the field. The results support the possibility of conspecificity between G. salaris and G. thymalli.

PATTERNS IN OCCURRENCE AND DISTRIBUTION OF FRESHWATER FISH PARASITES

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Current literature maintains that the distribution of freshwater fish parasite species among lakes is the result of chance or stockastic processes which makes it impossible to predict the probable occurrence of any particular species of parasite in any particular lake. This problem is related to the commonness and rareness of species and the delimination of communities where comparisons must be made within defined scales. We study this by comparing the parasite component communities of roach (Rutilus rutilus) and bream (Abramis bramae) in a number of lakes within an area of 100 km x 100 km. We assume that degree of similarity among freshwater fish parasite component communities is a function of degree of discontinuity and distance among (assumed) communities. To describe the within and among lake variation in sample composition, samples have been drawn from one large lake and two sets of interconnected, smaller lakes where the larger lake and the two sets are not connected with eachother. This is intended to give an estimate of how different are "different" and how similar are "similar" freshwater fish parasite component communities on this scale.

THE USE OF LOGIT MODELS IN ANALYSING INFECTIONS OF ERGASILID COPEPODS IN FISH FROM CENTRAL FINLAND

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Logit models provide a useful statistical method for analysing multivariate frequency data, in which variation of a dichotomous variable (e.g. infection) is analyzed using categorized variables.

Rutilus rutilus (n=1255) and Perca fluviatilis (n=866) from four neighbouring lakes in Central Finland of different trophic status and pollution level were studied for parasitic ergasilid copepods between 1986 and 1988. Four ergasilid species were found: Ergasilus briani (total prevalence 16.9%), Neoergasilus japonicus (15.6%) and Paraergasilus longitigidus (2.1%) in roach and Ergasilus sieboldi (9.9%) and P. longitigidus (4.9%) in perch.

The effects of the water quality of the lakes, season and year on the prevalence of E. briani, E. sieboldi and N. japonicus infections were studied by constructing logit models. Generally, the prevalence of E. briani on roach depended on the season only, with more frequent occurrence in summer. The prevalence of E. sieboldi in perch was mainly dependent on the study lake, occurring most frequently in the oligotrophic lake. All three parameters affected the prevalence of N. japonicus in roach. This more complicated system is explained by the long duration of its free living stages, its loose attachement and different location on the host, being found on the fins, as opposed to the gills.

INFECTION PATTERN OF ANISAKIS SIMPLEX IN SAITE (POLLACHIUS VIRENS), COD (GADUS MORHUA) AND RED FISH (SEBASTES MARINUS).

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From the Vega area, on the west coast of Norway, about twenty specimens of each of the three fish species were sampled monthly and examined for A. simplex larvae over a period of one year. The location in the fish and the size of the larvae was noted. The infection rate with A. simplex, although different in the three fish species, was found to be more or less constant throughout the year without distinct seasonal fluctuations neither in prevalence nor in intensity. The location of the larvae within the fish also turned out to be somewhat different in the three different fish species while the length of the larvae tended to vary with fish size and time of the year.

INTERACTIONS BETWEEN COMMON SEALS AND LOCAL FISH POPULATIONS IN THE HVALER AREA, OSLOFJORD.

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The population dynamics of fish is described from historical data and bi-annual trawl catches. Individual 'sealworm' burdens (*Pseudoterranova decipiens*) are determined for demersal and benthic fish species caught in trawls or traps. The diet of the local common seal (*Phoca vitulina*) colony is established by a detailed study of faeces samples.

A model is proposed, to explain how the population dynamics of both hosts and parasite have been affected by the epizootic which killed most of the seals colony in Hvaler in 1988.

OCCURRENCE IN SOUTH-BALTIC FISH OF ANISAKIDAE LARVAE DANGEROUS TO MAN

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Objective. Long-term investigations were carried out to describe the occurrence of Anisakidae larvae in 4 most frequently caught fish species.
Methods. The investigations covered fish caught in all Polish economic exclusive zone fishing grounds. A total of 17 545 herrings, 1857 cods, 5660 sprats and 649 flounders were examined. Routine ichthyological / body length, weight, sex, gonad condition, age, spawning group of herrings/ and parasitological / number and place in host of larvae, their taxonomy / investigations were performed.
Results. Herrings were found to be infected with Anisakis simplex larvae. The parasite were most frequently found in fish / 86 % in assortment "D" / from western fishing gro - unds / No. 107 - 105 /. In other fishing grounds the rate of infestation of fish did not exceed 10%, except Gulf of Gdańsk fish where 30% were recorded. A clear seasonality of herring infection could be seen. Larvae were mostly detec - ted in spring and sporadically in summer. The herrings in - fected were members of the coastal spring spawning group . Larvae were mainly found on the mesentery and on pyloric processes. In or upon herring gonads and muscles, the main sources of infection to man, larvae were detected in her - rings infected in 15 % and 2,4 %, respectively.
In cods mainly in the liver, Anisakis, Contracaecum and Hysterothylacium larvae were found, with 3 % parasitic prevalence.
The intensity and prevalence of the infestation by parasi - tes were found to increase with body length and age of her - rings and cods.
No Anisakidae larvae were found in sprats and only 0,9 % in flouders.
Conclusion. In comparison with data from the 1960s to 1970s a higher infestation rate of herrings by A. simplex larvae was recorded. The larvae were present even in Vis - tula Coastal Lagoone fish. A decrease of parasitic infes - tation was noted in cods in which larvae representing 3 Anisakidae genera were detected.

INFECTION BY *CRYPTOCOTYLE* SPP. ON ATLANTIC COD *(GADUS MORHUA)* IN FISH FARMS.

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Marine fish living close to the coast or in the fjords often suffer severe attacks from cercariae of the digenean *Cryptocotyle* spp. emerging from periwinkles. Cysts containing metacercariae are seen as black spots in the skin due to host response. This project is designed to clarify if the caging of cod increases infection due to their continual exposure to cercariae.

Atlantic cod, about 40 cm. in length, were caged about 150 meters from shore at a research station in North Norway. Fifty fish were sampled randomly every third month for two years, and compared to wild cod in a nearby fjord. The infection was quantified by means of a skin digestion technique.

Infection prevalence rose to 100% during the first year. The intensity increased quickly at first, but thereafter decreased. In wild cod infection remained unchanged. The degree of overdispersion increased rapidly as intensity rose the first year, but then started to decrease.

It is concluded that the caging of cod leads to an increase in infection level of *Cryptocotyle* spp. and that the infection occurs nonrandomly.

DACTYLOGYRUS-COMMUNITIES ON THE GILLS OF ROACH: MICROHABITAT DISTRIBUTION AND INTERSPECIFIC RELATIONSHIPS

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Dactylogyrus-communities on the gills of 293 roach were studied in three interconnected lakes in Central Finland between February 1988 and April 1989. The water quality of the lakes differs, but the differences between the Dactylogyrus component communities are small. Nine Dactylogyrus species were found: D. crucifer, D. nanus, D. suecicus, D. micracanthus, D. similis, D. caballeroi, D. fallax, D. sphyrna, and D. vistulae. D. crucifer and D. nanus were considered as core species. D. sphyrna and D. vistulae were rare satellite species which were not used for further studies, and the remaining five were secondary species. All seven Dactylogyrys species studied showed significant species specific preferences for certain gill-arches. Due to the pronounced seasonality of these species the months of May and June with the highest species diversity and abundance, were used for analysing interspecific relationships. It could be seen that, although changing abundances had some effect on niche breadth, the occurrence of other species was also significant, indicating interaction between some species. The limiting effect of some species, most probably D. similis, on D. micracanthus was most obvious. It was also evident that the overlap between some species was lower when species abundances were higher, indicating concurrence during peak abundances.

ON THE SPREAD OF *GYRODACTYLUS SALARIS* MALMBERG, 1957 AND *G. DERJAVINI* MIKAILOV, 1975 (MONOGENEA) IN SWEDISH SALMON RIVERS

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Since 1988, Baltic and Atlantic strains of salmon and brown trout in Swedish rivers have been investigated for species of *Gyrodactylus*. Until May 1991, *G. salaris* Malmberg, 1957 was found on salmon parrs in 2 out of 8 rivers in the Baltic region and in 2 out 5 rivers in the Atlantic region. Corresponding figures for *G. derjavini* Mikailov, 1975: on brown trout parrs were 3 out of 7 and 3 out of 4 rivers respectively. A more northerly distribution area limit for *G. salaris* than for *G. derjavini* is assumed. Different areas of distribution and different macroenvironments may explain the absence of *Gyrodactylus* species in certain rivers. However, an unevan distribution of the two species in Sweden may have arisen from an earlier spread to new areas by stocking rivers, by fish transports and by infected water from fish farms with salmonids, including the rainbow trout. Two salmon smolts from one river (Atlantic region) had approximatly 100 and 600 *G. salaris* specimens respectively, while other smolt specimens were uninfected or contained one or two, up to 60 worms. An increased infestation intensity during springtime in older parr specimens, especiallally smolts is assumed. Migrating smolts and "precocious males" are assumed to be responsible for the effective spread of *G. salaris* throughout a river. In two rivers in the Baltic region and one river in the Atlantic region *G. derjavini* was present on salmon parrs. Possibly this "wrong" host" has been temporarily utilized as a transport host for further spreading among brown trout parr specimens. Similarly, the rainbow trout, but not the brown trout, can be utilized for the spread of *G. salaris* among salmon parr specimens.

PARASITE INFECTIONS OF THREE-SPINED STICKLEBACK (*G. aculeatus*) IN A SUBARCTIC LAKE

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Lake Takvannet in Troms, Northern Norway, is an oligotrophic, dimictic lake with a dense population of arctic char (*S. alpinus*).
The lake is also inhabited by three-spined stickleback (*G.aculeatus*) and a small population of trout (*S. trutta*).

Before 1984, the char population was dense, stunted and heavy parasitized. Since 1984 an intensive fishing programme has been carried out.
Expected effects (among others) were
1) reduced competition for food and consequently better growth
2) a dietary shift based on less copepodes and more cladocerans/benthic prey.

This dietary shift was expected to reduce some of the parasite infections, since copepodes are intermediate hosts to several of the parasites occuring in the lake. *Diphyllobothrium* are one of the dominating genera. However, this reduction has been difficult to detect, and it is hypothesized that three-spined stickleback may maintain the transmission-rates of allogenic char parasites like *Diphyllobothrium* spp.

To study the importance of stickleback in parasite transmission, samples of *G. aculeatus* were collected in plexiglass traps once a month during the ice-free season. In the lab, length, weight, sex and macroparasites were registered.

The results show that the stickleback in L. Takvannet harbours most of the parasite species also found in the char. *Diphyllobothrium* spp., especially *D. ditremum*, is dominating the parasite fauna together with *Schistocephalus solidus*, and it is suggested that the combination of these two parasite species may cause some of the heavy infections of *Diphyllobothrium* seen in the biggest char. The results also indicate that the stickleback in L. Takvannet may function as a "reservoir" host for parasite species common to both stickleback and char.

ON SOME PARASITES OF SALMONIDS FROM A TARN IN WESTERN NORWAY

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Introduction. Parasites of wild salmonids in Norway are poorly understood both from an epidemiologic and population perspective. As part of recent research we have examined a small mountain lake, Vengsvatn in Fusa, Hordaland, Norway with respect to both ecto- and endoparasites.

Material and Methods. 49 trout (Salmo trutta) and 30 charr (Salvelinus alpinus) were taken by gill net. Blood samples were taken from those fish still living. The fish were weighed, sexed, individually bagged and taken on ice to the laboratory for examination.

Results and Discussion. The following parasites were found in their respective hosts.

Trout: Eubothrium crassum (65%); Diphyllobothrium dendriticum (49%); Crepidostomum sp. (35%); Eustrongyloides sp. (06%); Haemohormidium sp. (43%).

Charr: Eubothtrium salvelini (03%); Diphyllobothrium dendriticum plerocercoids (40%); Proteocephalus sp. (97%); Crepidostomum sp. (10%); Salminocola edwardsii (53%).

Of the five helminths, single copepod and single piroplasmid species found here, only two, the cestode D. dendriticum and the trematode Crepidostomum sp., were shared by both the trout and charr. The piroplasmid, Haemohormidium sp. is a first record in salmonids and is probably a new species.

STUDIES ON THE POSSIBLE MIGRATION OF ANISAKIS SIMPLEX LARVAE FROM THE VISCERA INTO THE FLESH OF THE HERRING, CLUPEA HARENGUS, AFTER CAPTURE.

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Objective. Earlier examinations (Smith, J.W. & R. Wootten, 1975. Int. J. Parasit., 5, 133-136; Smith, J.W., 1984. Int. J. Parasit., 14, 491-495) have shown that a significant part of Anisakis larvae after capture of the hosts migrate from the body cavity into the flesh of fatty species, including herring, while this migration do not occur in lean species. As preliminary Danish examinations have failed to show such migration in the herring, the present experiment was carried out to reevaluate the phenomenon.

Methods. In 1990 two identical trials were carried out on spring (2.5% fat) and summer (14.6% fat) herrings from the North Sea. On a purse seine vessel the herrings were immediately placed in either refrigerated sea water/chilled sea water (-1.0 - 0°C), on ice (0°C), in 10°C sea water or in 10°C sea water for 12 hr, whereafter they were cooled on ice (0°C). Immediately after capture and after 1½ and 5½ days of storage, the herrings were divided into 3 fractions: the fillets, the belly flaps (the surroundings of the body cavity, representing what is being discarded commercially), and the viscera. These fractions were seperately digested in pepsin-HCl.

Results. The overall prevalence of Anisakis-larvae was 90% and 92% in the winter and summer trial, respectively. The abundance was 8.5 and 6.6 larvae per herring, in the two trials respectively, and the individual worm burdens were strongly overdispersed (range 0-75).

The abundance of larvae in the fillets was 0.06 and 0.09 larvae per herring immediately after capture, in the two trials respectively, while the corresponding values of the belly flaps were 0.19 and 0.24 larvae per herring. Irrespective of season, storage conditions and duration of storage, there was no significant increase (and not even a tendency for any increase) in the number of larvae in the fillets or in the belly flaps.

Conclusion. The present study shows that Anisakis-larvae are very common in the North Sea herrings, that few of the larvae are present in the fillets already at capture, and that no significant migration of larvae from the viscera into the fillets take place after capture, irrespective of storage conditions of the herrings and fat content of the flesh.

A PRELIMINARY STUDY: INFECTION OF *PSEUDOTERRANOVA DECIPIENS* IN DIFFERENT FISH SPECIES.

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A total of twenty marine fish species were examined for 'sealworm' *Pseudoterranova decipiens*. The fish were collected in the Oslofjord in 1985/86 and in 1990/91, and form Vega (northern Norway) in 1990/91. In the Oslofjord only common seals (*Phoca vitulina*) are present, at Vega both common seal and grey seal (*Halichoerus grypus*) are present.

In the Oslofjord sculpin (*Myoxocephalus scorpius*) and Atlantic cod (*Gadus morhua*) are the most heavily infected fish species. Nearly 95 % of the sculpins were infected, some specimens had up to 300 *P. decipiens* in the flesh. The abundance of *P. decipiens* in cod was much more variable than in sculpins.

In Vega area, tusk (*Brosme brosme*), sculpin and cod had the highest worm burdens. Tusk was haviest infected species.

The data is analysed to link infection levels in fish to the local abundance of seals and the distance between fish samples and seal haul out sites.

Macroparasites of cod, *Gadus morhua*: the effect of caging on infection rates of parasites transmitted by food and by freeliving transmission stages.

In cod fed artifically, food-transmitted, heteroxenous parasites would be expected to decline. Parasites transmitted by free-living transmission stages, however, should increase since caged fish are less mobile and in denser populations. This hypothesis was tested by a longitudinal epidemiological study comparing infection levels of macroparasites in caged cod and in "natural" wild cod.

A total number of 1000 cod were caught in Altafjord, North Norway and transferred to a cage (85 m^3) at Finnmark Research Center. During a period of two years, every third month 40 fish were sampled at random from the cage and examined for macro-parasites. For one year parallel samples were taken from the wild fish-stock within the same area.

The experiment is still running, and here we report preliminary results for ascaroid larval nematodes (food-transmitted) and *Cryptocotyle lingua* (transmitted by free-living stages). In natural cod populations the intensities of ascaroid nematodes are reported to increase with fish size. No such increase was observed in caged fish. It is suggested that this may be explained by very low mortality rates of these nematodes and a decline in the transmission of infective stages due to artifically feeding. *Cryptocotyle lingua* showed a significant increase in mean intensity among the caged fish. So far, the hypothesis of the effect of caging on transmission rates is therefore confirmed.

Raphidascaris acus larvae in roach (Rutilus rutilus) from Central Finland: occurrence, seasonality and histopathology

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The prevalence of Raphidascaris acus larvae in the inner organs of roach varied between 19 and 63 % in four lakes studied between September 1985 and August 1986 in Central Finland. The highest infections were found in the polluted and the two eutrophic lakes (63, 42 and 38 %, respectively), while in the oligotrophic lake the prevalence was 19 %.

Distinct seasonal variation was found in three of the lakes, the highest values being found in late autumn and early spring, and the lowest in April-June. The seasonal variation may be explained by the increased response of fish against these nematode larvae in late spring and early summer. This is indicated by the histopathological changes; infiltration of different white blood cells and fibrocytes and forming of typical parasitic granuloma around many dying and dead R. acus larvae especially in the liver and the pancreas of roach. This in turn suggests that the roach is not the most favoured second intermediate host of R. acus.

R. acus larvae were also found in the intestine of roach throughout the year in all of the lakes (the prevalence varied between 23 and 42 %). No clear seasonal variation was found, indicating a continuous recruitment of worms.

A SIMPLE AND RAPID STAINING TECHNIQUE FOR SOME COMMON FISH PARASITES.

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Introduction. When clearing and/or mounting very small and delicate nematodes it is common to stain them lightly - cotton blue being recommended in the literature - in order to see them. One of us (BB) has for years used a textile dye (Dylon "Mexican Red" cold water dye, DYLON international, London, GB) for this purpose to stain nematodes and crustaceans; a few grains are added to the fixative or clearing medium. One of us (AL) has successfully tried this method, slightly modified, on monogeneans.

Procedure. Freshly collected gyrodactylids are placed for a period of 15-30 min. in 3o % ethanol to which a few grains of the Dylon stain are added. They are then cleared and studied in lactophenol, and may be mounted in glycerol jelly.

Results. In Gyrodactylus spp. specimens, from both fresh-water and marine fishes, some taxonomically important structures in the opisthaptor - ventral bar, ventral bar processes, proximal folds on anchor roots and ventral bar membrane - stain metachromatically in different shades of red. The anchor shafts, anchor points and the marginal hooks do apparently not absorb the dye. However, the latter structures too are clearly seen by ordinary light microscopy in specimens mounted temporarily in lactophenol.

The same stain technique has also been sucessfully employed for other monogeneans, such as Entobdella hippoglossi and Kuhnia scombri, and various parasitic copepods.

Conclusion. Textile dyes may be used for staining parasitic helminths and crustaceans.

PEPTIDERGIC INNERVATION OF SENSORY STRUCTURES IN NEMATODES

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The presence of neuropeptides in nerves of the sensory structures in two species of parasitic nematodes, Ascaris suum (order Ascaridida, family Ascarididae) and Cystidicola farionis (Order Spirurida, family Cystidicolidae), was studied. Immunocytochemical methods were used for localization of FMRF-amide-like neuropeptides in the nervous system. In this preliminary study, immunoreactivity to FMRF-amide, RF-amide, and the related neuropeptide SALMF-amide was detected in the central nervous system of the species studied, and also in the cephalic papillary nerves, in axons of the amphids, and in nerves innervating caudal papillae. Because of the systematic and taxonomic importance recently attributed to caudal structures in male worms of the superfamily Ascaridoidea, it is of interest to define the morphological and functional aspects of the innervation of the caudal sensory structures, and to study possible differences between various nematode groups.

HAEMATOLOGICAL RESPONSES OF THE EUROPEAN EEL (*ANGUILLA ANGUILLA*) PARASITIZED BY THE SWIMBLADDER NEMATODE *ANGUILLICOLA CRASSA* IN A THERMAL EFFLUENT.

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Haematological responses in the European eel, *Anguilla anguilla*, infected with the blood feeding nematode *Anguillicola crassa* have been studied in an area of the Baltic receiving thermal discharges from a nuclear power station. The following haematological parameters: erytrocyte count (B-EPK), haematocrit (B-EVF), leucocrit (B-LVF), haemoglobin concentration (Hb), mean corpuscular volyme (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and differential counts of blood smears, were studied both in relation to intensity of infection (number of parasites per host) and parasitization index (weight of parasites per host in relation to the weigth of the host). The haematological parameters of the infected fish indicate anaemic changes characterised by a reduction haemoglobin content, total erytrocyte count, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration. An increase in leucocrit was also noted in infected fish. No consistent effects were observed in haematocrit values and mean corpuscular haemoglobin. It is concluded that measurement of the haemoblobin concentration is a rapid and sensitive test in order to monitor anemic responses in the European eel infected by *Anguillicola crassa* in the wild.

EXPERIMENTAL STUDIES ON THE SALINITY TOLERANCE OF GYRODACTYLUS SALARIS MALMBERG, 1957 (MONOGENEA)

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Objective. A possible route for Gyrodactylus salaris dispersal is via migration of infected fish through brackish water. Some preliminary results from a study on the salinity tolerance of G. salaris infecting Salmo salar parr are presented.

Methods. The G. salaris strain used originated from the river, Lierelva. The hatchery reared S. salar (1+, unfed, dim illumination, mean weight 3.0g (0.6-6.3); mean length 6.6cm (4.4-8.6)) originated from Lierelva. Filtered seawater was diluted with dechlorinated laboratory water, and measured by an YSI 33 salinometer. Twelve laboratory infected S. salar were individually isolated in aerated grey plastic boxes (9x12x12 cm water level) at different salinities (0.0, 5.0, 7.5, 10.0°/oo) and temperatures (6°C, 12°C). G. salaris counts were made on anaesthetized (2 min in 0.04% Chlorbutanol) fish.

Results. The mean extinction time (50%) of the G. salaris infrapopulations in 10°/oo was ca. 29h at 12°C, and ca. 55h at 6°C (direct transfer from freshwater). The max. survival time of infrapopulations at 6°C was twice as long as at 12°C (ca. 120/60h). The mean extinction time in 7.5°/oo was ca. 41h at 12°C, and ca. 95h at 6°C. The max. survival time at 12°C was 54 days (6°C is still under study). The infrapopulational growth rate in 5°/oo equalled freshwater at both 6 and 12°C. The survival time of G. salaris populations was inversly proportional to temperature.

Conclusion. Gyrodactylus salaris infrapopulations can survive for days in 10°/oo and for months in 7.5°/oo. Although there is decreased viability of G. salaris in saline water, a potential for dispersal through brackish water is present, especially at low temperatures.

AN OUTBREAK OF TRICHINOSIS IN LEBANON

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In 1982 in south Lebanon, an outbreak of trichinosis occurred in an area consisting of four villages, in all including 6440 persons. In 252 families, involving 2318 persons, typical clinical signs of *Trichinella spiralis* infection could be recognized among family members. Twenty-one of these families, including 194 persons, were randomly choosen for further studies during the outbreak. Nearly half of the family members (46%) had clinical symptoms consistent with acute trichinosis.
12/16 tested persons had high anti-*Trichinella* antibody titres (IFA positive in 12/16 and ELISA in 11/16). Class-specific antibodies, IFA-IgG, IgM and IgA, were detected in most tested cases. Of the remaining 4 seronegative cases 3 had a duration of clinical symptoms $\leq$ 12 days. High peripheral eosinophilic counts (> 20%) were noted in 8/16 cases. In total 15/16 cases were positive in any of these tests. Control serum samples from other Lebaneses and from United Nations staff were antibody positive in 6/130 (4.6%), of these 5/67 (7.5%) were from Lebanese persons.
The total number of infected persons could be calculated to be around 1000 persons. The cause of this outbreak could be attributed to consumption of raw pork meat, which is an ingredient of "Kebbeh Nayyeh" - a favourite Lebanese dish.

FUCHSIN POSITIVE *CRYPTOSPORIDIUM PARVUM* OOCYSTS ARE FREE OF INTACT SPOROZOITES.

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Objective. To stain infectious sporozoites within oocysts. *Cryptosporidium parvum* is a protozoan responsible of chronic disease in congenital or acquired immunodeficient patients. Acid fast staining with carbolfuchsin is the most usual method to reveal the parasite; however immunofluorescence staining gives better results. As regard to the number of oocysts, infectious inoculum has been reported to vary from 10^4 to 2 x 10^7.

Methods. Specimen of faeces were collected from 3 infected patients (AIDS patients) and one experimentally infected lamb (Dr M.Naciri INRA Tours France). Faeces were preserved in 2.5 % potassium dichromate and stored at 4° C. for 0.5 to 3 months. Ether extracted faeces were concentrated by saccharose gradient. A smear of washed supernatant was air dried on a slide, then fixed in methanol. Fuchsin staining was applied for 30 min then 5% sulphuric acid for 20 sec. After washing with water, specific fluorescein conjugated monoclonal antibodies (FITC-mAbs) (Diagnostic Pasteur France) were incubated for 30 min. After washing, 10 µl of propidium iodide (PI) (100µg/ml) was added before mounting slides in glycerine. Slides were analysed by optical and fluorescence microscopy:

Results. Only 5% of dichromate preserved oocysts were fuchsin stained while all the oocysts were stained with FITC-mAbs. Treatment with 10% formalin or 0.5% sodium hypochlorite incubation increased the proportion of fuchsin positive oocysts up to 90% with sodium hypochlorite for 2 hours. Sporozoites could be stained with PI after excystation. Ethanol fixed oocysts stained with PI showed four spots within the oocysts. By combination of the 3 staining procedures, we showed that fuchsin positive oocysts are free of intact sporozoites with a pale homogenous PI staining. Negative fuchsin oocysts are either empty of sporozoits or full of 4 intact sporozoites.

Conclusion. A simple method to visualize intact sporozoits within *Cryptosporidium parvum* oocysts has been developed. This method will be useful to appreciate infectiousness of oocysts before culture or animal model infestation. We therefore assumed that sodium hypochlorite increased non infectious oocysts. On the other hand, for parasitological diagnosis, faeces will have to be formalin preserved to increased positive detection with acid fast staining.

TRYPANOSOME kDNA-BINDING PROTEINS AS POSSIBLE TARGETS FOR DRUGS (SSP XV)

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Objective: To identify proteins associated with the single mitochondrion (kinetoplast) of trypanosomatids with a view to inactivating them with drugs.

Introduction: The unique single mitochondrion (kinetoplast) of trypanosomes contains some 30% of the cell's DNA in a network of thousands of catenated rings. The network is highly structured, its division is controlled stringently, and kinetoplast DNA (kDNA) transcripts are precisely and extensively edited to restore proper translational reading frame. It is accepted that all these functions require proteins, but reports of such proteins are very few.

Results: We reported a class of proteins, from the trypanosomatid parasite *Crithidia fasciculata* , which aggregated exogenous kDNA preferentially in an *in vitro* assay (Tittawella, I. 1990. FEBS Lett. 260, 57-61). We recently found that these proteins copurify with an endogenous kDNA component and that they protect parts of the copurifying kDNA from DNase attack. We have defined a 25 kD protein responsible for this aggregation/protection of kDNA. We propose that the protein may be involved in maintaining the vital architecture of the kDNA network and/or in network replication. The protein's ability to preferentially retard kDNA in agarose gel electrophoresis offers a convenient assay for screening for drugs that would prevent aggregation.

INDUCTION OF ANTI-SECRETORY FACTOR IN MICE BY A NON-INTESTINAL PARASITIC INFECTION- SCHISTOSOMIASIS MANSONI.

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Objective. A group of proteins, known as antisecretory factors (ASF), have recently been described and characterized in humans, pigs and rats. These proteins, probably being synthesized in and released from the central nervous system, counteract the intestinal hypersecretion induced by e.g. cholera toxin. It has also been shown that intestinal challenge with e.g. cholera toxin induces the production of ASF in the central nervous system, resulting in elevated levels of ASF in the pituitary gland and in the blood. Since induction of ASF is not confined to intestinal bacterial infections but has been shown to occur during infection with the intestinal parasite *Entamoeba histolytica* it was considered to be of interest to study the possible induction of ASF also during other parasitic infections. As a model system we chose mice experimentally infected with the blood helminth *S. mansoni.*

Method. Swiss mice were infected percutaneously with 150 cercariae of *S. mansoni.* Groups of three mice were killed at 6h, 1, 7, 14, 21, 28, 35, 42, 49 and 56 days after infection. Blood and pituitary glands were collected and ASF was prepared from these materials using affinity chromatography on Sepharose 6B followed by elution with 1 M methyl-alfa-D-glucoside. The antisecretory activity was tested in rats by iv injection of the isolated test material 30 seconds before challenge with 2 μg cholera toxin into a ligated intestinal loop. After 5 h the accumulated fluid secretion was measured and antisecretory activity was expressed as per cent inhibition of the secretion induced by cholera toxin.

Results. Mice infected with *S. mansoni* had increased ASF levels in both blood and pituitary gland in contrast to uninfected control mice which did not display any significant antisecretory activity. The pituitary gland, but not the blood ASF level, was increased already 6 h after infection. Both levels increased successively during day one and day three and then declined to low levels after one week. Two peaks were then noted during the period between four and eight weeks after infection when the experiment was terminated.

Conclusion. The results show that ASF is induced in mice after infection with *S. mansoni.* The blood ASF levels noted during certain phases of the infection were probably high enough to be protective against enterotoxin induced diarrhoea. Peak levels of ASF were noted during the skin phase and when the parasites were established in the portal system, entering the phase of oviposition. The biological significance of the present findings are still unclear but it may be speculated that a possible lower mortality due to lower sensitivity of the host to enterotoxic diarrhoea should also favor the survival of the parasite and therefor constitute one of the factors in the complex host-parasite relationship. Another possibility is that the ASF response is part of an acute or chronic inflammatory reaction as suggested by the coincidence in time between ASF peaks on the one hand and the migration of schistosomulae in the skin and excretion of parasite eggs into the intestinal lumen respectively, on the other.

PROTOZOA AS POTENTIAL RESERVOIRS AND VECTORS OF MAMMALIAN MYCOPLASMAS AND VIRUSES

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Objectives. The fact that many species of protozoa, including parasites, can have many different kinds of endo- and ectobionts, has already been known for a long time but up to now organisms living on and in protozoa have mostly attracted the attention of biologists. However, during the recent years in addition to protozoologists-biologists also medical doctors and veterinarians have shown greater interest in this problem because some well-known mammalian pathogens have been found to act as endobionts of protozoa which via hosts may be transmitted to animal organisms or change its host's biological properties, including pathogenicity.
Methods and Results. In favour of both variants testify the results obtained in our department. By electron microscopy we have repeatedly detected that mammalian mycoplasmas may penetrate into the cell of Trichomonas vaginalis where they can persist over a long period of time. According to literature (Pindak, F.F. et al. 1989. Genitourin. Med., 65, 366 - 371) the same species of protozoa may also acquire genital herpes simplex virus which is detectable in the cytoplasm of trichomonads for at least six days after being "fed" on human rhabdomyosarcoma cells (RD) infected with these viruses. Convincing data have also been gathered in support of the hypothesis about the role of free living protozoa as reservoirs and vectors of mammalian viruses. Using axenic cultures of Tetrahymena pyriformis as a model in the investigation of protozoon-virus relationships we could establish by virological methods that depending on the type of virus this species of free living ciliates can either inactivate viruses, be their hosts or remain indifferent to them. As to the viruses studied by us, T.pyriformis could inactivate besides human-type influenza virus also the swine-, seal- and bird-types and remained indifferent to ECHO-11 whereas Coxsackie B-5 virus could penetrate into the cell of T.pyriformis and persist there.
Conclusion. Many years of investigation in this department have convinced us that protozoa may be the vectors of many mammalian viruses, among others also the virus of hepatitis and HIV. We have good reason to suppose that viruses which have penetrated into protozoa may considerably change the biological properties of its host, including pathogenicity (resp. virulence).

DIAGNOSIS AND FREQUENCY OF TRICHOMONIASIS OF THE RESPIRATORY TRACT OF MAN

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Objectives. As more and more often one may come across data on findings of trichomonads in cases of various pulmonary pathologies of man which even end lethally, we decided to find out the frequency of those protozoa in cases of inflammations of the respiratory tract.

Methods. The research material was taken directly from the bronchi with the help of a bronchoscope applying the methods for determining the microflora of bronchi. For detecting trichomonads we always used bephase egg serum medium LSV-2 (Hallmann, L. 1953. Stuttgart. Georg Thieme Verlag) without the addition of antibiotics whereas the intensity of trichomonads' reproduction was observed at 37° during 10 days.

Results. The material from the bronchi of 504 patients was examined. Trichomonads were detected in 53, i.e. 10.5 per cent of the investigated patients but the isolation of these protozoa did not depend on their occurrence in the oral cavity, neither were they always simultaneously found in the sputum. In all the 53 cases trichomonads were detected by the culture method. The trichomoniasis of the respiratory tract cannot be diagnosed by serological methods because specific antibodies can also be found in the blood sera of those patients who have trichomonads in the oral cavity only Most frequently trichomonads were detected in patients with chronic pneumonia, chronic bronchitis and tuberculosis of the lungs (17, 18 and 10 cases respectively).

Conclusion. Although the mere detection of trichomonads in the bronchi does not allow us to draw far-reaching conclusions about the etiological role of trichomonads in pulmonary pathologies of man, we can still suppose that these protozoa may play an important part in the pathogenesis of inflammations of the respiratory tract because treatment with metronidazole leads to full or partial recovery of patients.

INVOLVEMENT OF MHC PRODUCTS IN THE RESISTANCE AGAINST EXPERIMENTAL CHAGAS DISEASE.

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The aim of the present study is to analyze the relevance of H-2 molecules in the control of parasite load during Trypanosoma cruzi infection. We measured the effect of biweekly ip administration of 1mg anti IA^d monoclonal antibodies (mo- ab)(HB3) on the resistance of BALB/c mice against the infection with 200 bloodstream forms of T. cruzi, Tulahuen strain. Anti- class II treatment dramatically increased the susceptibility of mice to the parasite, as measured by parasitemia and mortality, when compared to mice treated with an irrelevant mo-ab (F9-53) or non treated controls. This treatment inhibited the "infection driven" induction of class II and Ig molecules in spleen and lymph node cells from mice at 13 dpi, but it did not alter the constitutive expression of these surface molecules in lymphoid cells from non infected mice. Furthermore, anti-IA administration resulted in lower levels of anti-T. cruzi IgM and IgG specific antibodies. Hosts chronically infected with T. cruzi develop specific immune responses that confer resistance to new acute infections and contribute to reduce the number of circulating parasites. The presence of MHC-restricted and unrestricted mechanisms operating during the chronic phase of T. cruzi murine infection was studied in an adoptive transfer model. 2.5×10^7 T- cell enriched nylon wool non adherent populations from spleens of BALB/c ($H\text{-}2^d$) mice chronically infected with T. cruzi (immune T-cells) were iv transferred to syngeneic or to congenic BALB/B ($H\text{-}2^b$) naive recipients, which were challenged with 200 bloodstream trypomastigotes 24h later. Transfer of immune T-cells conferred protection to the MHC incompatible congenic strain: A significant delay in mortality and lower parasitemias than non transferred or control counterparts (non transferred or transferred with allogeneic non-immune T cells) was observed. However, later during the infection, the congenic recipients displayed higher susceptibility to the parasite as measured by mortality and parasitemia when compared to the syngeneic recipients of immune T cells. BALB/c recipients displayed higher titer of anti-T. cruzi IgG, than the non-transferred counterparts, whereas protected BALB/B allogeneic recipients did not show an enhancement on antibody titer in comparison to their non transferred controls.

Our data indicate that the enhanced expression of Ia molecules is relevant in the resistance of mice against T. cruzi, and involved in the generation of an "early" specific antibody response. In a later phase of infection, we show that both MHC restricted as well as unrestricted mechanisms are involved in the T-cell mediated transfer of protection against Trypanosoma cruzi.

SOLUBLE IL-2 RECEPTOR IN LEISHMANIASIS.

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Leishmaniasis are diseases caused by protozoa of the genera Leishmania. In the visceral leishmaniasis (VL), antigen-specific and non-specific immunosuppressions are found. The underlying mechanism is not well known. In the cutaneous (CL) and mucocutaneous leishmaniasis (MCL) the immunological alterations are relatively mild. We searched for soluble IL-2 receptor (sIL-2R) in sera from leishmaniasis patients using ELISA test and we studied the relationship between sIL-2R levels and CMI response in order to examine the possible role for sIL-2R in immunopathology of leishmaniasis.

The levels of sIL-2R (U/ml) were: a) 4107 ± 5040 (mean ± SD) in 22 VL; b) 1382 ± 618 in 10 MCL; c) 1192 ± 555 in 15 CL and d) 439 ± 110 in 5 normal controls. Duging evolution of the disease in 11 VL we found: 3511 ± 3346 before beginning of treatment, 3023 ± 1706 in the 2nd. week, 1391 ± 447 in the 3rd. week of treatment and 804 ± 333 one month after treatment. In 10 MCL we had 1382 ± 618 before treatment and 1134 ± 271 soon after treatment.

In CL we observed a correlation between sIL-2R level and time of evolution of the disease which was not seen either in VL or MCL. Higher levels of sIL-2R were observed in patients with recurrent forms of CL. In MCL there were no correlation between number of lesions, extension of destruction of mucosa and sIL-2R level. There were no evident immunosuppression in CL but we found a correlation between higher sIL-2R level and lower L.(V) panamensis antigen-induced lymphoproliferation index. In VL (11 patients studied during evolution), we saw that the PHA-induced lymphocyte proliferation were depressed in most of them independent of sIL-2R level before treatment, but in the 2nd. and 3rd. week of treatment we observed lower response when sIL-2R level were above 2000 U/ml.

In a binding assay designed to evaluate IL-2R expression using CTLL cells and biotinylated IL-2, we observed increase of IL-2 binding when VL sera with high sIL-2R level were added.

In different forms of leishmaniasis we observed high level of sIL-2R in sera that correlates to chronicity and systemic involvement. Further studies are necessary to understand the implications of high level of sIL-2R to the immunological alterations in the leishmaniasis and the significance of this increased binding of IL-2 in the presence of high sIL-2R level.

IMMUNOLOGICAL CROSS-REACTIVITY BETWEEN A RECOMBINANT ANTIGEN OF *ONCHOCERCA VOLVULUS* AND THE RETINAL PIGMENT EPITHELIUM

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The aetiology of posterior segment eye disease associated with onchocerciasis (causative agent *Onchocerca volvulus*) patients is unclear. One hypothesis suggests that autoimmune responses resulting from cross-reactivity between *Onchocerca volvulus* and eye tissue may be a contributary factor.

Using a pool of human infection sera to screen a cDNA library constructed in lambda gt11 using adult *O. volvulus* mRNA, a recombinant antigen, designated OV39, has been identified. Antiserum raised against the recombinant antigen immunoprecipitates a 22,000 Mr antigen from an homogenate of adult parasites and, also recognises a 44,000 Mr component of bovine retinal pigment epithelium (RPE) in Western blotting experiments. A 44,000 Mr peptide is also precipitated from a triton X-100 extract of cultured human RPE metabolically radiolabelled with S^{35}-methionine. A rabbit antiserum raised against whole *O. volvulus* extract also precipitated a 44,000 Mr human RPE antigen.

The antigen recognised by the anti-recombinant serum was localised within the RPE by light and electron microscope immuno- histochemistry. Similar experiments performed on *Onchocerca* nodules localised the corresponding parasite antigen in the wall of the uterus. Uterine microfilariae did not express this antigen but new-born microfilariae did bind the anti-recombinant serum.

Identification of these cross-reacting antigens and the production of the reagents described should enable us to test the hypothesis that autoimmunity contributes towards the pathogenicity of eye disease seen in some onchocerciasis patients.

CELLULAR RESPONSES OF DBA/2 MICE TO PRIMARY, SUPERIMPOSED AND SECONDARY INFECTIONS OF THE BILE DUCT TAPEWORM, HYMENOLEPIS MICROSTOMA.

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Objectives. The purpose of this study was to elucidate the development of a primary, a superimposed and a secondary infection of 10 H. microstoma and the effects on number of peritoneal exudate cells (PEC's), bile duct diameter and weight of the mesenterial and portal lymph nodes (MLN & PLN) and the spleen in inbred C5-deficient DBA/2 mice.
Methods. Thirteen week old male and female DBA/2 mice were each infected with 10 cysticercoids of H. microstoma and autopsied at weekly intervals up to 13 weeks post infection (p.i.). Furthermore, one group of mice were treated with an anthelmintic 4 weeks after a primary infection and either followed for 4 weeks or reinfected up to 8 weeks after treatment. Another group of mice were given a superimposed infection 1, 2 or 4 weeks after a primary infection. At autopsy, the number and weight of worms per mouse, the bileduct diameter, the dry weights of the MLN, PLN and spleen and the number of PEC's were measured.
Results. The primary infection of 10 H. microstoma was established and persisted for 13 weeks with a mean of 7.2 ± 2.2 worms. The mean worm weight/mouse increased to about 11 mg dry weight after 3 weeks. The mean diameter of the bile duct increased 10-13 times after 3 to 5 weeks to about 5 mm. The dry weight of the MLN increased in the 2nd-4th week p.i. to nearly 300% of the size in uninfected mice and the PLN in the 2nd week to 4-500%. The spleen increased about 100% during the 2nd week p.i.. An anthelmintic treatment with Praziquantel given 4 weeks after a primary infection resulted in a dramatic decrease in bile duct diameter and weights of the MLN and the PLN, but no decrease in the number of PEC's one week after treatment was noted. However, 2 weeks after treatment all parameters measured were nearly back to the level of uninfected mice.
When a secondary infection was given 1, 4 or 8 weeks after Praziquantel treatment, a high degree of resistance was achieved 1 week after treatment, but resistance had disappeared 8 weeks after treatment.
Mice receiving a concomitant (superimposed) infection 2 or 4 weeks after a primary infection and autopsied 1 week later showed a complete resistance against the superimposed infections, while primary worms were still present.
Conclusions. It was shown that DBA/2 mice exert concomitant immunity with complete resistance against a superimposed infection 2 weeks after an infection with 10 H. microstoma, while the primary infection was not affected. A removal of the primary infection by Praziquantel resulted in a return to normal level of parameters investigated within 2 - 4 weeks and in an increase in establishment of a secondary infection. Further studies are needed to find out which cells are involved in the short-lived resistance and why long-lived resistance is not stimulated.

A PRELIMINARY REPORT ON CHARACTERIZATION OF *THEILERIA HIRCI*-INFECTED OVINE MONONUCLEAR CELL LINES

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Objective: The tick-borne protozoan parasite *Theileria hirci* infects sheep and causes an acute disease with high morbidity and mortality. Ovine mononuclear cells infected and transformed by this parasite have been cultivated *in vitro* (Hooshmand-Rad, P. & Hawa, N.J. Trop. Anim. Hlth. Prod. 1975, 7, 121-122), however, it is not known which group(s) of cells it prefers. Here we report the results of work to phenotypically characterize three established ovine mononuclear cell lines infected by *T. hirci.*

Methods: Surface antigens on cells from three cell lines harbouring, respectively, schizonts of three different strains of *T. hirci,* isolated in Iran, were analysed. This was made by indirect immunofluorescence using a panel of murine monoclonal antibodies for detection of lymphocyte antigens (Mackay, C.R. et al. Vet. Immunol. Immunopathol. 1987, 17, 91-102) and rabbit anti-sheep Ig (Dakopatts, Denmark) for detection of surface Ig.

Results: In all three cell lines, more than 95% of the cells expressed histocompatibility class II (MHC II) antigens. None of the cell lines expressed surface Ig or the T cell antigens CD4, CD5 or CD8.

Conclusions: This is the first report on characterization of cells infected by *T. hirci.* It appears that the three cell-lines subjected to investigation neither belonged to B-cell nor to T-cell lineages. Whether these cells originally possessed either B-cell or T-cells surface marker antigens but supressed the expression of them after being infected, or if they entirely belonged to other mononuclear cells not searched for in our work, remains to be elucidated. The high proportion of cells expressing MHC II antigen could be interpreted as that the parasite preferentially infects MHC II expressing cells or that this antigen is expressed after infection. Thus, further studies are needed to clarify if *T. hirci* modifies the antigen expression of mononuclear cells, or if it preferentially infects certain cell type(s). Such knowledge will be crucial in the development of efficient immunization regimes, based on application of subunit vaccines, against *T. hirci.*

TOTAL AND SPECIFIC IgE IN HUMANS WITH DIFFERENT DEGREES OF EXPOSURE TO *SCHISTOSOMA MANSONI*

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Total and specific IgE were determined by ELISA using human sera from a schistosomiasis endemic area in the Sudan. The following groups were investigated : Chronically infected canal cleaners (n=18), newly recruited cleaners (n=16), normally exposed idividuals, mainly farmers (n=64), school-children (n=44), Sudanese negative control (n=44) and an European negative control (n=10). Blood samples were collected at the same time as parasitological examinations were performed.

A sandwich ELISA using monoclonal mouse anti-human IgE for coating and HRP conjugated rabbit anti-human IgE was used for the determination of the total IgE. An indirect ELISA was performed using soluble egg antigen of *Schistosoma mansoni* for coating and HRP conjugated rabbit anti-human IgE for the detection of specific IgE.

Chronically infected canal cleaners had the highest geometric mean (<xg>) of faecal eggs/g (232), followed by the new cleaners (150), children (58) and the normally exposed individuals (9.3).

Chronically infected cleaners had the higest level (<xg>) of total IgE (9750 IU/ml), followed by the new cleaners (3120), normally exposed individuals (1450), children (480), Sudanese controls (180) and European controls (31).

In contrast, the highest mean ELISA absorption for specific IgE was found in the new cleaners (0.90, range 0.078-2.86) followed by children (0.67, range 0.031-2.39), chronically infected cleaners (0.35, range 0.09-2.53), normally exposed individuals (0.17, range 0.038-0.81). The cut-off point was 0.147 (mean+ 2SD for Sudanese controls).

Three months after praziquantel treatment faecal egg counts were reduced (<xg>=3.9 and 7.3 for chronically infected and new cleaners respectively). In spite of this, no drop was observed either in the total or specific IgE of the last two groups.

There were considerable variations of total and specific IgE within the groups. Specific IgE level in chronically infected cleaners may be affected by desensitization; alternatively the determination may be blocked by other immunoglobulin classes.

SPECIFIC IgE and IgG SUBCLASS ANTIBODIES TO Ascaris AND Toxocara IN SERUM FROM EAST ASIAN REFUGEES.

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Blocking antibodies are known to interfere with immunoassays for specific IgE and histamine release assays performed on whole blood. Using sera from East Asian refugees with specific IgE to Ascaris suum and/or Toxocara canis excretory/secretory (ES-) antigen of the second stage larvae, we studied IgG blocking antibody activity and its subclass association by IgG-depletion on Protein A columns and antigen degradation.

Indirect ELISA's for IgE and IgG subclasses were established using coated ES-antigens, diluted serum (1:5 for IgE, 1:1000 for IgG_1 and 1:50 for IgG_2 and IgG_4). Detecting reagents were HRP-conjugated rabbit-anti human IgE, monoclonal anti human IgG_1/anti human IgG_2 followed by HRP-conjugated rabbit-anti mouse Ig and HRP-conjugated monoclonal anti human IgG_4.

IgE responses from 10 patients to Ascaris ranged from 0.06 - 0.76, responses to Toxocara ranged from 0.19 - 2.28. Response from pooled normal serum was 0.04 for both antigens. Passage of serum through Protein A columns before ELISA led to significant increases for most patients to Ascaris (range 6-73%, mean 38%) and Toxocara (range 11-150%, mean 52%). Increases in IgE-response of >25% were associated with high levels of IgG_4 in 3/5 reactions to Ascaris and 5/6 reactions to Toxocara. For one patient Protein A passage resulted in a significant drop for specific as well as total IgE indicating the presence of mixed immune complexes or IgG anti-IgE autoantibodies.

Boiling (30 min.) of antigens before serum-reaction typically led to substantial reductions (50-90%) in the binding of IgE and IgG_4 antibody, whereas binding of IgG_1 and IgG_2 was less affected. Periodate treatment gave very variable reductions in the binding of the investigated isotypes to Ascaris ES, but mainly affected the IgG_1 and IgG_2 response to Toxocara ES.

IgG_4 antibodies exert a major blocking effect on specific IgE in roundworm infections.

THE ANTIBODY RESPONSE TO *DRACUNCULUS MEDINENSIS* (GUINEA WORM) IN HUMANS LIVING IN AN ENDEMIC AREA OF NORTHERN GHANA

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Objective. To elucidate basic aspects of the antibody response of humans to Guinea worm infection, with the long-term objective of developing an immunological technique for diagnosis of early Guinea worm infection.

Methods. Guinea worm material and blood samples were collected in the Northern Region of Ghana. A crude Guinea worm antigen was prepared from adult female worms and sera used were obtained from individuals with an open (patent) infection, from individuals previously but not presently infected and from individuals claiming never to have been infected. In addition, sera from individuals coming from outside areas endemic for Guinea worm, infected with *Onchocerca volvolus*, *Wuchereria bancrofti* and hookworm, and sera from non-endemic Danish controls were also analysed. All sera were tested for antigen specific IgA, IgM, IgG1 and IgG4 antibodies using SDS-PAGE, immunoblotting and ELISA technique.

Results. Generally, techniques measuring IgA, IgM and IgG1 antibodies to Guinea worm antigens lacked specificity and failed to differentiate between past and present infections. Most promising results were obtained with the IgG4 antibody subclass. Cross-reactions were thus only observed with *O. volvolus*, in that sera from individuals infected with the other non-Guinea worm parasites and sera from the Danish controls were almost non-responders. Sera from persons claiming never to have been infected were medium-responders, while persons carrying an open infection and persons previously infected were high responders. Future studies will also include the use of purified antigens and tests for circulating antigens.

SEROLOGY IN CHAGAS' DISEASE

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Serum samples from honduran cardiac patients and from Leishmania braziliensis, L. mexicana, L. donovani and T. rangeli infected individuals, were analysed in four serological tests: Indirect Immunofluorescence Assay (IFA) using T. cruzi amastigote and epimastigotes stages as antigens, ELISA using alkaline extracted epimastigotes as well as the amastigote stage specific glycoprotein (Ssp4) . The IFA using amastigote was shown to be a sensitive assay and no cross reaction was recorded. When epimastigotes were used in the same test , a pronounced cross reaction was observed with visceral Leishmaniasis sera .The ELISA technique using epimastigotes extract showed a cross reaction mainly with Leishmania donovani. Contrary, the SSp4-ELISA had a high specificity for T. cruzi infections and the cutaneous and visceral leishmania patients had no recognition of this glycoprotein. Reactivity was also absent in T .rangeli serum samples. This suggest that Ssp4 is conserved in many strains, and could be a suitable candidate in the search for improvement the serological tests. Taking four positive serological reactions to ascertain a T. cruzi infection, 53% of the 118 cardiac patients showed positive serology. From this we selected 21 serum samples that could be regarded as chronic cardiac Chagasic patients and they were tested in an ELISA technique using synthetic peptides, based on recombinant T. cruzi antigens. 5% of the sera recognised peptide 7 that resemble the recombinant antigen SAPA (Shed-Acute-Phase-Antigen) while 90 % and 62 % recognized peptides 2 and 1, a reactivity that earlier was shown to be present in the chronic phase of the infection. One serum failed to recognised any peptide, while others recognised up to four peptides. None of the sera from individual infected with other protozoa recognized the peptides. In preliminary analyses of Chagas' sera from Uruguay, similar percentages of reactivities in the peptide-ELISA was obtained. Results from studies using the peptide-ELISA to analyze potential cross reactivities with african trypanosomiasis sera is under way.

COMPARISON OF COMMERCIAL ANTI PNEUMOCYSTIS MONOCLONAL ANTIBODIES

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Objective: To compare immunofluorescence (IFL) staining patterns of three commercially available anti Pneumocystis (PC) monoclonal antibodies (moab).
Methods: Indirect immunofluorescence according to the manufacturers descriptions - Northumbria Biologicals Ltd (N) Meridian diagnostics Inc (M) and Dakopatts (D) - were used to stain human PC positive sputum and bronchoalveolar lavage samples as well as rat lung derived PC samples (rat material kindly provided by Olga Guerrero, University of Leon and Anti Sukura, School of Veterinary Medicine, Helsinki). Double staining was performed by using first one moab followed by the corresponding FITC conjugate, thereafter another moab followed by the corresponding TRITC conjugate.
Results: Cysts and trophozoites were detected by both D and M whereas mostly scattered cysts and only smaller groups of cysts were detected by N. When the same speciemens were double stained it was clear that the N reagent failed to stain some of the cysts. All conjugates did also unspecifically stain fungi in certain speciemens.
Rat derived PC in smears was stained properly only by N. The same antibody did however not stain rat PC in paraffin sections .
Conclusion: The N reagent stains uniform PC cysts which makes it easy to interpret. It is also applicable to rat PC. Trophozoites and a some cysts are not stained and N is not applicable to paraffin sections. In contrast the D and the M reagents stain all known stages of the *P. carinii* organism and are applicable to smears as well as paraffin sections.
To avoid confusion with fungi a conjugate control for each speciemen is recommended.

PNEUMOCYSTIS CARINII IN CORTICOSTEROID-TREATED VOLES

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Objective. Pneumocystis carinii (PC) is an opportunistic pathogen which causes clinical disease in immunocompromised hosts. The purpose of this study was threefold: 1) to find the most suitable staining method for PC when studying large population samples of voles, 2) whether wild and laboratory raised Clethrionomys voles harbour PC and 3) whether the immunosuppression protocol with methylprednisolone used in experimental studies on PC in rats, also works for voles.
Methods. Three different staining protocols were employed to detect the organism in lung samples of 53 voles (26 bank voles and 27 grey-sided voles) to find out a suitable method for a large scale screening. The employed procedures were: Grocotts methenamine silver (GMS) stained paraffin sections, toluidine blue O stained impression smears and methenamine silver stained frozen sections. Immunosuppression regimen of 0,4 mg of methylprednisolone injected subcutaneously once a week (DepoMedrol', The Upjohn Co), was used for 0, 1, 3, 8 and 20 weeks periods for groups of five animals in each species.
Results. GMS-stained paraffin sections were relatively easy to interpret and gave more positive samples than the other two methods. It thus seems a satisfactory method for large scale population analyses. PC-organisms were detected from both vole species, but no interspesific differences were seen. An unexpected result was that methylprednisolone treatment of voles did not induce a similarly fatal PC infection as occurs in corticosteroid treated rats. All positive voles had mild infections and no severe or lethal PC-pneumonia (PCP) were found. In the positive samples only a few cysts could be seen on individual microscopic fields. Thus, corticosteroid treatment did not cause fatal or severe PCP in voles. This might be due to species dependent differences in metabolizing methylprednisolone.

OCCURRENCE OF PNEUMOCYSTIS CARINII COMPARED TO PARASITISM BY GASTROINTESTINAL HELMINTHS IN THE SHREWS SOREX ARANEUS AND SOREX CAECUTIENS

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Pneumocystis carinii (PC) is an opportunistic pulmonary pathogen which causes clinical disease in immunocompromised hosts. The prevalences and intensity of PC infection in different functional groups of two species of shrews Sorex araneus (n=63) and S. caecutiens (n=60) were compared using GMS staining for the detection of PC cysts. The possible role of PC as an indicator of immunodeficiency was investigated by comparing the prevalences and intensity of PC infection to the previously studied helminth burden of the same shrews. The shrews were trapped in 1988-1990 at Pallasjärvi, western Finnish Lapland.

The prevalence of PC in S. araneus was 70% and in S. caecutiens 17%. The interspecific differences in prevalence were significant in every functional group except adult males. In S. araneus, the prevalences did not differ significantly between any functional group. In S. caecutiens, the prevalence in females was considerably lower than in males, mature males being more often infected than juvenile ones. The interspecific difference in the prevalence of PC was similar to the difference in the helminth burden of these species.

The relation of the intensity of PC infection to the size of the shrews was significant only in juvenile females. Heavily infected juvenile females were lighter and shorter than less heavily infected ones.

The total number of helminths or the number of helminth species did not correlate with the intensity of PC infection in any functional group. The possible value of PC as an indicator of immunodeficiency remains unclear.

DNA AMPLIFICATION FOR DETECTION OF *PNEUMOCYSTIS CARINII* IN HUMAN RESPIRATORY SAMPLES

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Objective: The currently used method for diagnosing *P. carinii* infection is based on immunocytology which is not suitable for large scale screening. By using the polymerase chain reaction (PCR) we have developed a diagnostic procedure for detection of *P. carinii* DNA in human respiratory tract samples.

Methods: The primers used direct amplification of a 403 bp fragment of the thymidylate synthase gene of *P. carinii.*
The PCR method was tested on 42 clinical sputum and 44 broncho-alveolar lavage (BAL) samples. The latter category included samples from both immunosuppressed and immunocompetent patients.

Results: A single band of the expected size was detected in agarose gel electrophoresis in samples containing *P. carinii.* The result was confirmed by hybridization with a *P. carinii* specific radiolabelled probe. The primer sequences did not detect human DNA or DNA from fungi and bacteria. Fourteen samples were positive using the PCR but negative by the immunocytological method. The PCR missed one positive case.

The result suggest that *in vitro* amplification by PCR of *P. carinii* DNA may be an alternative method for detection of *Pneumocystis* parasites.

Probes complementary to rRNA for identification of *Sarcocystis*

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Introduction

Sarcocystis is a genus of cyst-forming coccidian parasites in the phylum of Apicomplexa. *Sarcocystis* is recognized as a cause of subclinical and clinical diseases in animals and of economic loss in farm animal production. Specific diagnostic methods on species as well as on genus level are needed.

Methods

Cystozoites of *S. cruzi* and *S. tenella* were recovered from heart muscle tissue of naturally infected cattle and sheep, respectively, using pepsin-HCl digestion and purification by gradient centrifugation in Percoll. Whole cysts of *S. gigantea* were isolated from oesophagus of naturally infected sheep. Stable RNA was extracted from the different *Sarcocystis* species. Small ribosomal subunit RNA (srRNA) was partially sequenced using reverse transcriptase and DNA primers complementary to evolutionarily conserved regions of srRNA. The sequences were compared by computer alignment.

Results

Sequence comparisons revealed a high degree of similarity between the three *Sarcocystis* species studied. The highest degree of similarity was obtained for *S. cruzi* and *S. tenella*. However, sequence differences between these two species were found in the V4 region. Probes were constructed for identification of *S. cruzi* and *S. tenella* and they were tested in hybridization experiments.

Conclusions

Ribosomal RNA sequences from different *Sarcocystis* species can be of great taxonomic value and probes complementary to rRNA will most certainly be valuable tools in the specific diagnosis of sarcocystosis.

POLYMERASE CHAIN REACTION (PCR) FOR DETECTION OF *ENTAMOEBA HISTOLYTICA* DNA

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The **objective** was to establish a method for the identification of *E. histolytica*-specific components. Current studies have focused on the detection of amoeba DNA from the *E. histolytica* cyst stage for detection of parasite DNA in stool samples.

Methods: Synthesized complementary DNA primer sequences, designed from the published DNA sequence encoding for the *E. histolytica* actin gene (Huber et al, 1987), were used for detection of DNA from four axenically grown pathogenic *E. histolytica* strains.

Results: The PCR result shows positive reaction with trophozoites from all of four amoeba strains and no reaction with *Giardia lamblia* or human DNA. A number of clinical stool samples containing different protozoa were used as controls for specificity of the primers.
Preliminary results indicate that DNA can be extracted from cysts by freeze-thawing and prolonged incubation with proteinase K and SDS.
The results thus suggest that PCR may detect intestinal protozoa as a supplement to microscopy or as a primary screening method.

GENES ENCODING CYTOLYTIC FACTORS FROM *ENTAMOEBA HISTOLYTICA*

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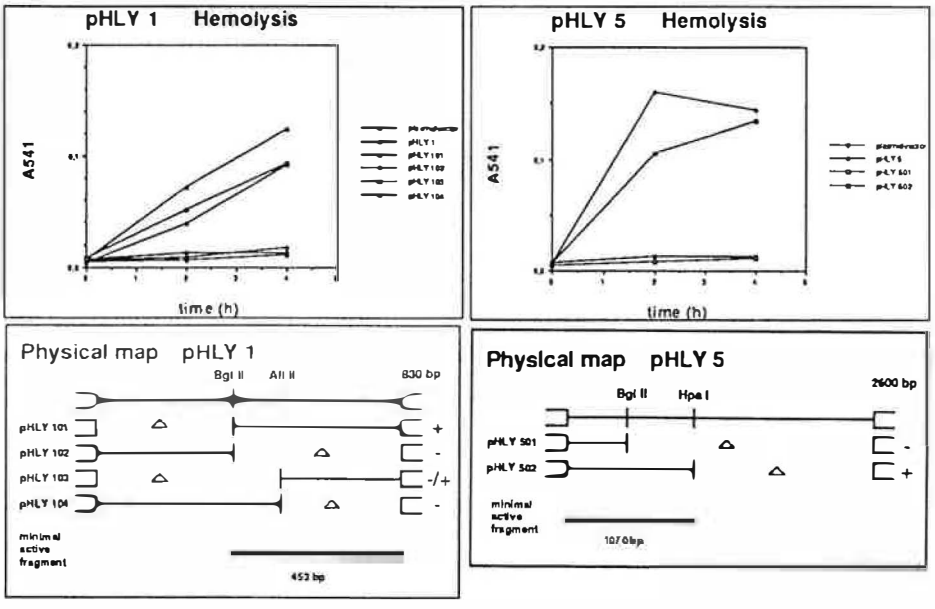
Entamoeba histolytica is the causative agent of amoebic dysentery. This pathogen colonizes the large intestine of humans and afflicts tissue damage to the colonic mucosa. In this process the amoebae lyse the cells of the epithelium. A number of lytic factors have been described in *E. histolytica*. These include proteases, phospolipases and amoebaporin. We have attempted to isolate genes encoding cytolytic activities from *E. histolytica* and further characterize these genes.

To isolate cytolytic genes from *E. histolytica* we screened a genomic library for clones with the ability to disrupt red blood cells (RBC:s). Isolated clones were subjected to restriction enzyme analysis and based on this analysis it was possible to divide the inserts into five unique DNA segments. The clones were expressed in *Escherichia coli* and crude extracts were tested in hemolysis assays. All five clones were positive in this test with crude extracts (se below).

Deletion derivatives of the clones were constructed and their ability to lyse RBC:s were tested. By this analysis it was possible to deduce minimal fragments from which active hemolytic factors could be expressed. In the figure the data from two of the clones, pHLY1 and HLY5, are shown in the the left and right panels, respectively. Also shown are the physical maps of the respective clones and derivatives.

We have isolated several clones from *Entamoeba histolytica* which encode hemolytic functions and, hence, are putative cytolysis genes. We intend to functionally characterize these factors and we hope that this will lead to a better understanding of amoeba pathogenesis.



MONOCLONAL ANTIBODIES AGAINST 67 kDa *ENTAMOEBA HISTOLYTICA* ANTIGEN

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Objective: The objective was to identify *E. histolytica* -specific antigens recognized by sera from patients with invasive amoebiasis. To isolate the antigen(s) and to develop monoclonal antibodies against it.

Methods: A crude homogenate of *E. histolytica* was fractionated using ion exchange chromatography and the generated fractions tested for reactivity with patient sera by ELISA and western blotting. Reactive fraction was used for generation of monoclonal antibodies.

Results: Several antigenic parasite components were seen. However, antibodies against a 67 kDa component were seen only in sera from patients with *E. histolytica* infection.
Two monoclonal antibodies were obtained by immunization with the fraction containing the 67 kDa component. One (3G2) reacted with 67 kDa component and two additional lower molecular weight components in immunoblotting. The antigen moved as a single band in non-reduced gels. The other monoclonal antibody (3E3) failed to react in immunoblotting but both antibodies reacted in dot blotting. The *E. histolytica* target antigens recognized by 3G2 and 3E3 were intracellular based on immunocytochemical staining.

ANTI-TUBULIN REACTIVITY OF ENCYSTING *GIARDIA LAMBLIA*

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The **objective** of this study was to observe the possible changes of tubulin distribution during the encystation process by indirect immunofluorescence using a monoclonal anti-tubulin antibody.
As shown before, (1), the ventral flagellae of *G. lamblia* trophozoites can be selectively stained with the monoclonal antibody originally raised against *Pneumocystis carinii.* (2) .
Metods: *G. lamblia* cysts were induced in axenic culture, in the BIS medium supplemented with porcine bile and lactic acid calcium salt, washed in PBS and distributed on microscope slides. Acetone fixed smears containing cysts taken at different moments of encystation, were incubated with monoclonal antibody and the anti-tubulin reactivity was tested by indirect immunofluorescence.
The **results** show that the strong positive reaction of trophozoites disappears during encystation. Early cysts display only scattered positive dots in the cytoplasm and faint staining of the cyst wall.
These results suggest that expression of the tubulin-specific epitope in the ventral flagallae is stage-specific and associated with motility.

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THE VIABILITY OF *IN VITRO* INDUCED CYSTS OF *GIARDIA LAMBLIA*

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G. lamblia cysts obtained by an *in vitro* encystation procedure represent two morphological types which can be distinguished in the light microscopy. *Type 1*: oval shaped, with well defined walls and cytoplasmic structures, yellow-brown when stained with iodine in direct preparations and *Type 2*: smaller, irregular shaped, with granulated cytoplasm, yellow-blue when stained with iodine, having no visible structures.

Objective: The aim of this study was to find out if there is any correlation between the different morphological appearance of Giardia cysts and their viability.

Methods: Cysts of different human isolates were raised *in vitro* by supplementing the culture medium with porcine bile and lactic acid calcium salt. Samples taken at 24, 48, 72 and 96 hours of encystation, were washed in PBS, water-treated, counted and diluted to obtain the desired concentration. To determine viability, the preparations were counterstained with two fluorogenic dyes: propidium iodide (PI) and fluorescein diacetate (FDA). FDA stains selectively viable cells. Passing through intact cell membranes, it is metabolized by intracellular esterases to produce fluorescein which accumulates inside the cell and exhibits green fluorescence when excited by blue filter. PI, excluded by intact cells, passes through damaged cell membranes and interacts with DNA and RNA in the nuclei of dead cells, forming red stained complexes when blue exciter filter is used.

Results show differences between strains in their capacity to produce cysts, which ranged from 5 to 20%. The viability measured by the positive FDA staining was time dependent. Within the first 24h of encystation the majority of cysts was viable. After subsequent 72h, the number of viable, FDA positive cysts decreased significantly to less then 10%. Interestingly, cysts produced by one of the strains, mainly *Type 2*, appeared to be nonviable; negative by FDA and positive by PI staining. Even after 8h of encystation, we failed to detect FDA positive, viable cysts.

A COMPETITIVE EIA FOR TESTING ANTIBODY AGAINST ENCEPHALITOZOON CUNICULI

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Objective. To develope a simple and sensitive serologic test for detecting antibody against Encephalitozoon cuniculi (Ec) in different animal species and human sera which should relay on the same testing procedure regardless of the species tested.

Methods. A monoclonal antibody against Ec was developed. Mice (BALB/c) were hyperimmunized by intraperitoneal injections of killed Ec spores 3 times with 2 weeks interval. Spleen cells from the mice were fused to sp2/0 myeloma cells according to the method of Köhler and Milstein (1975). After 10 days the supernatants were screened for production of anti-Ec antibody by indirect EIA. Positive clones were subcloned by limiting dilution and grown in F-DMEM (SVA, Uppsala) with 2% Ig-minimized FCS. The antibodies were purified on Protein A Sepharose (Pharmacia, Uppsala). The IgG fraction was conjugated to $NaIO_4$ activated horse radish peroxidase (HRP) (Nakene and Kawoi, 1974) and stored at 4°C with 0.05% NaN_2 as preservative. Microtiter plates were coated with supernatant antigen from mechanically disrupted Ec spores (Waller, 1977).

Test performance. 1) 50 ul diluted serum to be tested and 50 ul HRP-conjugated monoclonal antibodies in optimal dilution were added to the antigen coated wells and left for 1 hour at 37°C. **2)** Washing 3 times with PBS-Tween. **3)** TMB substrate was added to each well and left for 10 min at room temp. **4)** The result was read by a photospectrometer at 450 nm.

Results. The results were compared with results from testing the same sera in indirect EIA and Carbon Immunoassay. There was no significant difference regarding positive and negative sera between the 3 tests.

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BLUE-GREEN ALGAE; A NEW AGENT CAUSING DIARRHOEA ?

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During recent years a novel microorganism has been reported in patients with the AIDS syndrome and travellers with diarrhoea. The organism resembles an unsporulated coccidian oocyst or "a large *Cryptosporidium*". In light microscopy the organism is seen as a sphere of about 8-9 μm in diameter with refractile inclusions. It does not take up iodine stain but is stained with modified acid-fast staining (Ziehl Neelsen). It has been preliminarily classified as a blue-green algae (cyanobacterium).

We have found the organism in five stool samples from patients, four of whom had diarrhoea but none of them had signs of immunodeficiency. They have been travelling in various parts of the world (Vietnam, Eastern Europe, Turkey, Colombia and Lebanon). The organisms were slightly larger than *Cryptosporidium* (4-6 μm) and showed varying staining intensity with Ziehl Neelsen. For definite differentiation of the organism from *Cryptosporidium* we showed absence of reactivity by immunofluorescence with anti-*Cryptosporidium* monoclonal antibody. In follow up stool examinations 2 weeks after the initial sampling two out of four were still positive. These five cases are to our knowledge the first report of this organism diagnosed in Europe.

VISCERAL LEISHMANIASIS IN SOMALIA

Clinical and immunological findings in hospitalized patients and in villagers in an endemic area.

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Visceral Leishmaniasis (VL) is a systemic intracellular infection with high mortality if left untreated. Main clinical symptoms are wasting, diarrhoea, anemia and splenomegaly. The disease is transmitted by sandflies. Little is known about its prevalence and distribution in Somalia, a poor country with limited medical resources.

Objective. To find methods suitable for clinical diagnosis as well as for epidemiological population studies in Somalia.

Materials. Patients from Mogadishu hospitals treated for leishmaniasis (n=26) or other diseases (n=63). Inhabitants of a village in an endemic area (n=306). Healthy residents of Mogadishu (n=162).

Methods. Questionnaires containing personal and medical data for hospital patients and villagers. The same groups were subjected to physical examination including body weight and spleen size.
Serology: direct agglutination test (DAT) and immunofluorescence (IF) for serum antibodies. For delayed type hypersensitivity leishmanin skin test.

Results. Among the 26 hospital patients treated for VL the male /female ratio was 2.7 and 85% were below 16 years. The diagnosis was confirmed parasitologically in 13 cases. All patients had symptoms of wasting and all had splenomegaly. We found a good correlation between DAT and IF results. and both methods showed high sensitivity and specifity.The antibody titres recorded in VL patients were significantly higher than in other hospital patients and in healthy Mogadishu residents. All VL patients had DAT titers ≥800 and IF titers ≥100 in IgG and IgM. Such titers were not found in any of the two control materials.
Of the 254 villagers tested for antibodies 20 had DAT titres of the same level as the VL patients. The male female ratio in this group was 2.3 and 60% were below the age of 16 Two subjects had very low body weight and seven had splenomegaly. Leishmanin skin test was performed in 246 persons, 63 (26%) of whom were positive. Only 5 (8%) of the latter had significant antibody titres indicating that the majority of the skin test positives were infected in the past.

Conclusions. Together with clinical symptomatology the two serologic tests employed seem to be useful in clinical diagnosis of VL. The DAT test is easy to perform and does not require any sophisticated equipment and reagents. Its therefore preferable in Somalia. The leishmanin skin test is not suitable in clinical diagnosis but may be useful in epidemiological population studies.

TREATMENT OF PLASMODIUM FALCIPARUM MALARIA WITH MEFLOQUINE

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Objective. At the department of Communicable and Tropical Diseases, Rigshospitalet, Denmark, mefloquine has been used since 1982 to treat patients with verified or suspected cloroquine and sulfadoxine- pyrimethamine resistant P. falciparum malaria. 81 patients treated with mefloquine are reviewed with respect to efficacy of treatment and side effects.

Methods. All patients with P. falciparum malaria treated with mefloquine during the period 1982-1988 were reviewed.

Results. 40 patients had complicated malaria. 18 patients were initially treated with i.v. quinine. Mefloquine dose for adults was 1500 mg in one dose or divided in two with six hours' interval. Gastrointestinal side effects were common, but in only 10 cases the dose had to be repeated because of vomiting. No neurological side effects were recorded in relation to treatment or during the follow-up period (30 days). Temperature subsided with a mean of 2.7 days after initiation of treatment. Trophozoites disappeared after a mean of 3.6 days. Resistance to mefloquine was not observed except in one case from Tanzania. Mefloquine is recommended for the treatment of worldwide acquired P. falciparum malaria, although patients should be monitored closely to disclose resistance and possible long-term neuropsychiatric side effects.

SCANNING ELECTRON MICROSCOPIC OBSERVATION TO SEE THE EFFECT OF CDRI COMPOUND (CODE 80/53) FOR ITS GAMETOCYTOCIDAL ACTION ON MALALRIA PARASITE

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Objective. To standardize CDRI compound (Code 80/53) as a safe gametocytocidal agent for malaria parasite.
Methods. The CDRI compound (code 80/53), synthesised as a safe gametocytocidal agent to substitute the primaquine, was tested for its gametocytocidal action. Different doses viz. 1.25 mg base/kg, 2.50 mg base/kg and 3.75 mg base/kg of water soluble CDRI compound (80/53) was administered orally to the rhesus monkeys (Macaca mulatta). Different batches of Anopheles stephansi mosquitoes were fed on these monkeys, 0 hr, 5 hr and 24 hr after drug administration. The oocysts on midgut of mosquitoes were observed under Philips 515 Scanning Electron Microscope.
Results. The control batch of 1.25 mg base/kg drug showed very high infectivity while the batch after 5 hr of treatment of the same drug showed considerable reduction in oocyst number and the oocyst growth was found completely stopped after 24 hr of drug treatment. There was observed very sharp decline in oocyst count at 5 hr after 2.50 mg base/kg drug treatment but no oocyst could be observed in the mosquitoes of 24 hr post treatment batch. In case of 3.75 mg base/kg drug, the oocysts were not developed at all in both 5 hr and 24 hr post treatment batches of mosquitoes.
Conclusion. The CDRI compound (code 80/53) was found to have good gametocytocidal action.

SPECIFIC IgG_4 ANTIBODIES IN DIAGNOSIS AND FOLLOW-UP STUDIES OF TREATED HYDATID DISEASE CASES.

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The performance of an ELISA for E. granulosus specific IgG_4 antibodies in hydatid disease was critically evaluated, using serial serum samples from 9 patients with infection imported from the Mediterranian area. Eight cases were surgically verified, one was a relapse, occuring 20 years after cyst operation and responding to chemotherapy. Control sera were from Danish normal persons (n=10), from East Asian refugees (n=14) and from chronically S. mansoni infected canal workers from Sudan (n=13).

Indirect ELISA's were established, using crude sheep hydatid cyst fluid (SHCF) for coating and horseradish peroxidase (HRP-) conjugated, monoclonal mouse anti-human IgG_4 and anti-human IgG_1. An ELISA for SHCF-specific IgE employed HRP-conjugated rabbit anti-human IgE. Optimal serum dilutions were 1:200 for IgG_4 and 1:1000 for IgG_1. IgE was tested at serum dilution 1:20.

The mean IgG_4-reaction from 27 individuals with other helminth infections was 0.022 ± 0.025, giving a cut-off value for seropositivity (mean + 2x s.dev) of 0.072, corresponding to 4.5x the reaction from a pooled serum (NSP) from 10 normal Danes.

For 8 patients with a cyst location to the liver, samples taken at or around ($\pm$ 3 mo.) surgery/start of chemotherapy gave SHCF-specific IgG_4-responses ranging from 6.5x to 136x that of NSP. The corresponding figures for IgG_1 were 0.7x - 4.4x. One patient with cysts in the femoral muscle was negative for IgG_4 at the time of sampling (5 mo. post surgery). Two patients had significant specific IgE compared to controls. Their IgE response was up to 5x increased after depletion of IgG with Protein A Agarose.

After operation, specific IgG_4 levels gave steeper drops than specific IgG_1 during 6 mo.- 2 years follow-up periods.

SHCF-specific IgG_4 has an interesting potential as a diagnostic parameter and as an indicator of succesfull elimination of hydatid liver cysts.

COMPARATIVE ANTIGEN ANALYSIS OF DIFFERENT LIFE STAGES OF *SCHISTOSOMA MANSONI*.

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Objective. Parasitic trematodes present a complex antigen mosaic and a large number of individual antigens have been demonstrated by various techniques. It has been reported that some of the schistosome antigens are of allergenic nature, some are binding schistosomicidal drugs, some display lectin reactivity and some have enzyme activity. In this study, we characterized and compared, by means of crossed immunoelectrophoresis (crossed IE), the antigen mosaics of adult worm, egg and cercaria of *Schistosoma mansoni* and their possible serological relationships.

Methods. A modification of the crossed IE technique was employed using a 1 % agarose gel and Tris-barbital buffer (pH 8.6, ionic strength 0.02). The first-dimension electrophoresis was performed at 5 V/cm for 80 minutes. The second-dimension electrophoresis was run at 2 V/cm for about 20 hours through a gel contaning 5 % antiserum. An intermediate gel, eventually supplemented with 5 % antiserum or 0.3-1.0 mg/ml lectin, was interposed between the first dimension and the second dimension gels for characterization purposes. In order to detect enzyme activity of schistosome antigens, crossed IE plates were incubated with various enzyme staining solutions for at least 2 hours at 37°C. A tandem variant of the crossed IE was used for comparison of antigens.

Results. Seven antigen components were identified as shared between all three life stages of *S. mansoni*. Furthermore, one antigen was common to adult worm and snail, and one other antigen was shared between cercaria and snail. Characterization of enzyme activities revealed three individual precipitating antigens in adult worm of *S. mansoni* possessing esterase, leucyl-glycyl-glycine peptidase and phenylalanyl-leucine peptidase activities respectively. One further precipitinogen with malate dehydrogenase activity was identified for all three life stages. Two individual precipitinogens in adult worm, one in egg and one in cercaria possessed lectin binding reactivity.

Conclusion. The present study shows that variations of the crossed IE technique combined with analysis for enzyme activity and lectin binding might be advantageous for differentiation and characterization of antigens in crude extracts of the various life stages of *S. mansoni*. Such techniques should for instance be useful at purification procedures aiming at identification of potentially protective antigens.

IDENTIFICATION OF A NON-ACTIN TARGET AUTOANTIGEN IN *SCHISTOSOMA MANSONI* MUSCULATURE

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The **objective** was to identify autoantibodies capable of reacting with schistosomes.

Autoantibodies against actin occur in various inflammatory conditions, high titres being present in chronic active hepatitis. Such antibodies react with schistosome musculature and surface spines. The spines consist of actin in a crystalline form.

Methods: By indirect immunofluorescence and Western blotting, we have studied the reactivity of sera from individuals suffering from schistosomiasis and individuals without evidence for schistosomiasis with *S. mansoni* adult worms.

Results: We have identified antibodies which react strongly with both the the musculature and the spines and antibodies which react with musculature, but not with spines. In immunoblotting the target antigen of the latter antibodies was identified as a 100 kDa component, clearly distinct from the 43 kDa actin band and from myosin heavy and light chains.

Discussion: Several muscle proteins (e.g. α -actinin, villin) are of similar size as the autoantibody target antigen. They will be considered in our further attempts to identify the target antigen.

NEW METHOD FOR ELECTROPHORETIC CHARACTERIZATION OF SCHISTOSOME INTERMEDIATE HOST SNAILS.

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A new method for characterizing schistosome intermediate host snails by electrophoresis has been tried at the Danish Bilharziasis Laboratory. The method utilizes the SDS-page technique.

Objectives: Previously starch gel electrophoresis (SGE) has been utilized for characterization schistosome intermediate host snail species and for differentiating different species with good results. SDS-page has been utilized for isolating proteins in antigen studies at the Laboratory for some time, and this elegant techniques was tempting to utilize for studies for schistosome intermediate host snails like Bulinus and Biomphalaria, wherefore such experiments were initiated.

Methods: Selected species, Bulinus globosus, B. abyssinicus, B. tropicus, B. truncatus, B. pfeifferi, with well expressed bands in starch gel electrophoresis as sodium Dodecyl Sulfate polyacrylamid gel electrophoresis, SDS page. The starch gel electrophoresis method utilized has previously been described from the Laboratory (Jelnes, 1980), the SDS-Page method was the one described by Laemmli (1970).

Result: The results of both methods were good. Clear bands were demonstrated. SDS seems to have more sharp bands. In few locations SDS showed to have more bands than SGE.

An advantage using SDS could be that it is faster to use and the results are better reproduced by photographs. However, still experiments are needed to evaluate the possibilities for using this technique in systematics of snails.

TOTAL AND SPECIFIC SERUM IgE IN RATS INFECTED WITH MONILIFORMIS MONILIFORMIS

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Objective:
The aim was to study anti ovalbumin IgE, anti worm IgE and total IgE in serum of old and young Hooded Lister rats given different doses of the acanthocephalan Moniliformis moniliformis (M.m.) and to compare these infections to an Nippostrongylus brasiliensis (N.b.) infection.

Methods:
Ovalbumin, 0.1 mg plus adjuvant, was injected intraperitoneally in Hooded Lister rats on day -24.
On day 0 the rats were given doses of 10 M.m., 50 M.m. or 1000 N.b. infections.
Anthelminthic treatment of the M.m. infection, Oxyclozanid 170 mg/kg rat, was given day 63 and 64.
Challenge N.b. infection was given day 78.
Anti worm IgE and anti ovalbumin IgE titers were measured in Wistar rats by PCA (Passive Cutaneous Anaphylaxis) test using two fold serial dilutions of sera.
Total IgE was measured by PRIST (Paper Radio Immuno Sorbent Test) using a rabbit anti rat IgE prepared in our laboratory.

Results:
IgE potentiation (rise in heterologous IgE) was detected in total IgE as well as anti ovalbumin IgE during the N.b. infection, but not during the M.m. infections.
Host age and M.m. dose influenced the time of detectable anti worm IgE occuring day 10 - 24 p.i. whereas the rate of increase and the maximum level (1024 - 4096) of the antibody titer was dose and host age independent. The anti N.b. IgE titer became positive day 21 p.i. within the time range observed for the M.m. infections and increased with the same rate as in the M.m. infections to a similar high titre level.
In the M.m. infections, the timing and increase of total IgE reflected the anti worm specific IgE.
Anti M.m. IgE was potentiated by an N.b. infection, and anti M.m. IgE quickly decreased after anthelminthic removal of M.m.

Conclusion:
M.m. and N.b. infections induced the same increase rate and a comparable maximum level of worm specific IgE in rats. In contrast to N.b., a M.m. infection did not induce potentiation of (heterologous) IgE antibodies.

COMPARISON OF IgG ANTIBODIES AGAINST *TOXOPLASMA GONDII* IN MATERNAL BLOD, CORD BLOOD, AND ON BLOOD COLLECTED ON FILTERPAPER.

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OBJECTIVE

In a screeningprogram for toxoplasmosis in pregnancy one of the tests has to be carried out after the delivery. Neonatal screening for Phenylketonuria, and hypothyroidism is compulsory in Denmark and is caried out on blodspots on filterpaper. As the analysis is centralised additional test could easily be performed at low cost. We wanted to investigate if the PKU-filterpaper could be used for the detection of IgG-antibodies against *Toxoplasma gondii*.

METHODS

221 coherent samples of maternal serum, cord-serum, and 3-10 mm discs from filterpaper, were examined with an ELISA for anti-Toxoplasma IgG.

RESULTS

(32%) of the samples was found to be IgG positive. Results that compares the antibody response in the three types of samples are presented.

CONCLUSIONS

It is concluded that the filterpaper presently used for the PKU and hypothyroidism screening in Denmark can be used in a screening for anti-toxoplasma antibodies.

SEROLOGICAL DIAGNOSIS OF OCULAR TOXOPLASMOSIS IN CHILDHOOD

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Ocular toxoplasmosis is the most common infectious cause of chorioretinal inflammation in nonimmunocompromised patients. The aquired or congenital toxoplasmosis is one of the most important reason for visual impairment in childhood. The infection can have disastrous effects, causing severe damages in the central nervous system: microchephalus, hydrocephalus, mental retardation, epilepsy.

Currently, the diagnosis of ocular toxoplasmosis is based on the observation of a necrotizing lesion in the fundus. Definitive diagnosis is usually obtained by serological evidence.

We have examined 35 children (age between 2 months and 14 years) divided into four clinical groups according to Jerome J. Kazdan.

Groups were as follows:

1/ active ocular and systemic form (10 children)
2/ recurrent ocular form (6 children)
3/ inactive ocular form with/without systemic signs (7 children)
4/ active ocular form (12 children)

Serological tests we used: copmlement fixation (CF), indirect hemagglutination (IHA), enzyme immunoassay (ELISA) and indirect fluorescent antibody test (IFA).

Our results will be detailed on the poster according to the clinical picture and therapy (Clindamycin, Tindurin, Sulfonamid).

Conclusion: Any titer of antibody is significant because in recurrent ocular toxoplasmosis there isn't strong correlation between the level of *Toxoplasma gondii* titer and activity of the ocular disease. The early diagnosis and treatment of the active form is essential to prevent the more serious ocular and neurological symptoms.

TOXOPLASMOSIS IN BROWN HARE (Lepus europaeus)

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Objective. Toxoplasmosis is a common post-mortem diagnosis in hares in Sweden. The aim of the present investigation was to study, by means of immunodiagnostic methods, the incidence of acute toxoplasmosis as well as the prevalence of latent *Toxoplasma gondii* infection in brown hares (*Lepus europaeus* P.) in Sweden.

Methods. The peroxidase anti-peroxidase (PAP) staining technique, employing mouse anti-Toxoplasma serum, was used as a complement to routine histopathology to detect *T.gondii* infection in liver tissue sections from 388 brown hares subjected to autopsy.

The prevalence of antibodies to *T. gondii* in serum samples from 176 seemingly healthy brown hares was studied, using direct agglutination test (DAT) and enzyme-linked immunoassay (ELISA). Selected sera were also subjected to immunofluorescent antibody test (IFAT) and Sabin Feldman dye test (DT).

Results. Morphological changes in the liver due to acute toxoplasmosis consisted of multiple, irregularly shaped necrotic areas, well defined from the surrounding normal tissue. *T. gondii* organisms were detected on basis of positive PAP staining in 39 (10,1%) out of the 388 cases.

Antibodies to *T. gondii* were not detected in any of the 176 brown hares included in the serological study.

Conclusions. The high incidence of acute toxoplasmosis in combination with a low prevalence of latent infection suggests a high susceptibility for *T. gondii* infection in the brown hare in Sweden.

IN VITRO CULTURE OF *TOXOPLASMA GONDII*

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Objective: To evaluate *in vitro* cultured *T. gondii* trophozoites as a source of antigen for diagnostic use in toxoplasmosis.

Methods: The *in vitro* culture of *T. gondii*, RH strain, has been established using glioma cell line MG 251 as host cells. The yield of parasites was calculated. The *in vitro* growth characteristics of trophozoites serially cultured for about 3 months and trophozoites obtained "fresh" from peritoneally infected mice was compared. The number of free trophozoites in the culture medium was determined at 24 h intervals. NP 40-soluble extracts of *in vitro* cultured and *in vivo* cultured *T. gondii* was run on 10,6% SDS-PAGE, transferred to a nitrocellulose membrane and used for western blotting with rabbit-anti-*T. gondii* sera, sera from patients obtained after infection and the monoclonal antibody 6E2.

Results: The yield of organisms from *in vitro* cultured trophozoites is approximately 200-fold. The comparison between *in vitro* and *in vivo* cultivated trophozoites showed no differences in multiplication rate. In the western blotting some small differences was seen.

Conclusion: Most antigens recognized by our anti-*T. gondii* sera were present in parasites obtained by the two culture methods. The *in vitro* cultured parasites have several advantages over the *in vivo* cultured trophozoites: - on ethical grounds; - it becomes cheaper and the procedure involves no risk of mouse contamination. However the differences in immunoblotting pattern using patient sera may be of importance and will be furthered investigated.

TRIAL OF A LIVE TOXOPLASMA (S48) VACCINE IN SHEEP

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Objective. To test the efficacy of the live incomplete S48 strain of Toxoplasma gondii in protecting pregnant sheep against challenge with a complete (M3) isolate of T. gondii.
Methods. Sixty four pregnant ewes, vaccinated 77 days before mating with live S48 Toxoplasma tachyzoites, were challenged orally with M3 oocysts at 90 days gestation, as were 30 unvaccinated ewes. These animals, along with a further group of 20 unchallenged control ewes, were monitored until after lambing.
Results. Vaccinated ewes were febrile for a few days after vaccination. Antibody to the parasite then rose to high values before declining gradually over the ensuing weeks. After challenge with oocysts vaccinated ewes developed an ealier, lesser and shorter febrile response than unvaccinated animals. Both groups developed high titres of antibody. Only 18% of lambs from unvaccinated ewes survived compared with 76% from the vaccinated animals. The latter also produced heavier lambs although two thirds of them were born infected. All lambs from the unchallenged control group were born live and uninfected.
Conclusions. Live S48 Toxoplasma tachyzoites can induce a short lived infection in sheep which leaves them uninfected but substantially immune to challenge.

IMMUNIZATION OF MICE WITH *TOXOPLASMA GONDII* ISCOMS FOLLOWED BY ORAL CHALLENGE WITH CYSTS AND OOCYSTS

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Objective: The iscom is a highly immunogenic formulation of antigens incorporated into a matrix consisting of the adjuvant Quil A and cholesterol. Immunization with Toxoplasma iscoms has been shown to result in protection against a lethal infection of *T gondii* tachyzoites inoculated intraperitoneally (ip). The aim of this study was to investigate the immunity against a natural mode of infection, namely oral infection with cysts and oocysts.
Methods: Toxoplasma iscoms were prepared from strain RH *T gondii* tachyzoites. The major antigens in the iscoms as seen in immunoblots were the membrane antigens P22 and P30 (identified by monoclonal antibodies), a group of antigens with an approximate molecular weight of 40000-60000 and one antigen of approximately 6000.
25 mice were injected subcutanously three times with 6 weeks intervals with iscoms (5 µg protein/mouse), and 25 control mice were injected with matrix, (10 µg Quil A/mouse). One week after the second immunization three mice from each group were bled and sera were analysed for antibodies to *T gondii* by ELISA. In addition 3 mice were tested for delayed type hypersensitivity (DTH) by a foot pad assay.
17 days after the third immunization, 8 mice from each group were inoculated orally with 10 strain C56 cysts, 9 with 250 strain Me 49 oocysts and 8 were injected ip with 2.5 x 10(4) strain C56 tachyzoites. Mortality was recorded for 30 days and brains of survivors were examined for presence of Toxoplasma cysts.
Results: High titers of antibodies to *T gondii* and a DTH reaction were detected in immunized mice but not in control mice.
Challenge with cysts resulted in death of 6 immunized mice and all control mice. In the group challenged with oocysts only one immunized mouse but all 8 controls died. Of the mice inoculated with tachyzoites all except one immunized mouse died. The conditional probability of survival was significantly higher for the immunized mice challenged with oocysts and tachyzoites, than for the controls. In the group inoculated with cysts there was no statistically significant difference between immunized and control mice.
Toxoplasma cysts were found in brains of all surviving mice.
Conclusion: Immunization of mice with iscoms containing a restricted number of antigens of *T gondii* resulted in protective immunity against lethal oral challenge with oocysts, partial protection against ip challenge with tachyzoites, but no measurable protection against oral challenge with cysts.
The outcome of the tachyzoite challenge is in accordance with previous studies. The different result with oocysts and cysts might be due to differences in virulence between the strains and stages used, or to difference in challenge dose, compared to minimal lethal dose.

A NON-CYSTFORMING STRAIN OF TOXOPLASMA CAN BE SAFELY USED FOR VACCINATION AGAINST OVINE ABORTION

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Infection with Toxoplasma gondii is widely recognized as a major cause of abortion and barrenness in sheep, mostly in maiden ewes which have not yet been in contact with the parasite. As soon as ewes have ingested oocysts produced by cats - the most likely source of infection - they become seropositive and are protected against abortion. This sustained immunity is believed to be caused by the presence of tissue cysts, and will be boosted by later infections.

Strain S48 was isolated in New Zealand from fetal cotyledons of an aborted lamb many years ago and has been passaged through mice ever since in the tachyzoite stage. During those passages it has lost the capacity to form tissue cysts. It also appeared to have lost the capacity to form oocysts in cats. The strain is lethal to the mouse: even a single tachyzoite will kill the mouse.

For safety and ethical reasons one cannot use the mouse as a production animal.

Therefore, we adapted the strain to continuous in vitro cultivation. Masterseed has been frozen down, stored in liquid nitrogen, and tested for absence of a large variety of pathogens.

The safety of this live vaccine has been demonstrated by infecting SPF lambs with a high dose. The vaccine causes a temporary febrile response, but no clinical symptoms. Using the mouse as an indicator no parasites (bradyzoites, tissue cysts) could be detected in the muscles or brain tissue of the lambs. When cats were fed these tissues, they did not produce oocysts.

When ewes are vaccinated 4-6 weeks before mating they develop antibody titres which gradually drop to low levels. A strong and persistent protective immunity against abortion, however, is induced (see paper by D. Buxton et al).

THE INCIDENCE OF TOXOPLASMA ANTIBODIES AMONG 5,531 PREGNANT WOMEN IN DENMARK. A PROSPECTIVE STUDY.

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OBJECTIVE

Infection with Toxoplasma gondii in the pregnancy will in about 50% of the cases be transmitted to the fetus, and can result in fetal death or the birth of a severely handicapped child. Knowledge of the prevalence of antibodies in fertile women will make it possible to estimate the extent of congenital toxoplasmosis. The purpose of this study was to describe the prevalence, incidence and geografical distribution of toxoplasmosis in Denmark.

METHODS

Consecutive sera from 5,531 pregnant women, taken at the first post-conceptional contact was studied. Sera was tested for specific IgG antibodies by enzyme-linked immunosorbent assay (ELISA), and for IgM antibodies by capture-ELISA. The result was related to the womans age, and place of living (area of country and size of town).

RESULTS

27% of the woman were IgG seropositive. A yearly rate of seroconversion at p=o,o116 (SE=0,0016) was estimated, and could be concordant with a constant rate of seroconversion from the age of 0 year and onwards. 32 woman had a positive IgM result, indicating "recent" infection, consistent with the estimated rate of seroconversion. No geografic differences were observed.

CONCLUSIONS

The results indicate that 0,65% of pregnant danish women annually will be infected with toxoplasmosis, and are at the risk of getting a child with a congenital infection.

SEROPREVALENCE OF *Toxoplasma gondii* IN FATTENING PIGS IN FINLAND

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Objective. The presence of antibodies to *Toxoplasma gondii* in pigs is an indicator of infection in the tissues of the animals tested. Since the prevalence of *T.gondii* in pigs in Finland had not previously been reported, a survey was conducted to study to what extent fattening pigs in this country may harbour antibodies to *T.gondii*, indicating that they may serve as a source of human infection.

Methods. Blood samples were collected randomly during spring 1988 from 307 fattening pigs at four abattoirs located in different parts of Finland. The blood was taken from the stick wound after stunning the animals. Following preparation, the sera were kept at -20°C for serological analysis. The samples were initially screened in duplicate by enzyme linked immunosorbent assay (ELISA) at a dilution of 1 : 100 (Uggla, A. & Nilsson, L.-Å.: Develop Biol Stand 1985, 62, 37-42) and samples showing positive absorption values were finally titrated by an indirect fluorescent antibody technique (IFAT) to their endpoint titres (Uggla, A. & Iljort, M.: Acta Vet Scand 1984, 25, 567-576). IFAT titres equal to or exceeding 1 : 20 were regarded as positive.

Results. Only one out of the 307 pigs tested was found to have antibodies to *T.gondii*. The prevalences of seropositive pigs (IFAT ≥ 1 : 20) at the four abattoirs, from south to north, were thus 0 %, 0 %, 1.5 % and 0 %, respectively, giving an overall prevalence of 0.3 %.

Conclusions. The seroprevalence found here was remarkably low as compared to results from other parts of Europe. It should be kept in mind, however, that only a small number of pigs were tested and that only young animals were included, whereas the rate of *T.gondii* infection in domestic animals generally increases with age. Nevertheless, the present results indicate that Finnish pigs are not important as a source of human toxoplasmosis.

A COMPARISON OF SOME COMMERCIAL TEST-KITS FOR DETECTION OF SERUM-ANTIBODIES TO TOXOPLASMA GONDII IN ANIMALS.

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Different diagnostic tests for detection of serum antibodies to *Toxoplaxma gondii* were compared with respect to sensitivity, specificity and rapidity. Fiftyeight ovine, sixty porcine and sixty feline sera were respectively tested by: Indirect fluoresceine antibody test (IFAT, bioMerieux Toxospot), direct agglutination test (DA, bioMerieux Toxoscreen), latex agglutination test (LA ANI Biotech OY Biocard Toxo Ab) and enzyme-linked immunosorbent assay (ELISA), using *Toxoplasma* antigen produced by National Bacteriological Laboratory (SBL), Stockholm.

In general, there was a good agreement among IFAT, DA and LA tests, which all showed expected results. However, a few samples of each animal species showing low titres in the three mentioned tests, were found negative in the ELISA.

The quickest test to perform was LA which took 30 - 50 min for a single sample. Ten such tests took about 1- 1 1/2hs. It took about 2 1/2hs to test 10 samples by IFAT. To run DA and ELISA took between 5 and 6 hs, each for 30 samples.

SELECTIVE BINDING OF *TOXOPLASMA GONDII* ANTIGENS TO HUMAN CELLS *IN VITRO*

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Objective: Components of *T. gondii* with selective binding capacity to the plasma membrane of host cells may be involved in attachment, penetration and protection of the parasite. The present study was preformed to identify such parasite components.

Methods: Homogenate of trophozoites of RH strain of *T. gondii* was incubated with glioma cell line MG 251 grown on slides and the bound antigens were detected by indirect immunofluorescence (IF) and western blottings using monoclonal anti-*T. gondii* antibody 6E2, rabbit antisera obtained by infection with toxo strain 626/62 and sera from patients obtained after toxoplasma infection. As conjugates the appropriate FITC-anti-immunoglobulin was used.
For the western blotting glioma cells were incubated with the parasite preparation, washed, run on a 10,5% SDS-PAGE and transferred to a nitrocellulose membrane. Bound *T. gondii* antigens were detected by incubation with the antisera, the appropriate HRP-conjugated anti-immunoglobulin and developed with diaminobenzidine and H_2O_2.

Results: Binding of parasite antigen to the surface of the cells was demonstrated with IF. Western blotting showed selective binding of a number of *T. gondii* components from 27 to 65 kDa to the cells. One was the 30 kDa antigen to which the monoclonal antibody 6E2 is directed .

Conclusion: Several components including the 30 kDa antigen recognized by our monoclonal antibody seems to bind selectively to the plasma membrane of cultured glioma cells. They remain to be furthered characterized.

IDENTIFICATION OF A SECRETED TOXOPLASMA GONDII ANTIGEN ASSOCIATED WITH THE PARASITOPHOROUS VACUOLE OF THE HOST CELL

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Objective: An attempt was made to produce monoclonal antibodies against putative "circulating antigens" of *Toxoplasma gondii*.

Methods: BALB/c mice were infected with *T. gondi* RH strain and bled from the tail vein into heparinized tubes. After centrifugation 1000 g plasma was filtered through 0.45 mm membrane and injected intaperitoneally into BALB/c mice for production monoclonal antibodies. Screening for anti-toxoplasma antibody producing hybridomas was performed by indirect immunofluorescence.

Results and Discussion: A monoclonal antibody was obtained which reacts with a 30 kDa cytoplasmic parasite antigen. According to immunofluorescence and ultrastructural immunolabeling studies the antigen is secreted from intracellular dense granules of parasites and incorporated into the membrane of the parasitophorous vacuole of infected host cells. It is absent from the parasite surface membrane. The mechanism for appearence of the 30 kDa antigen in the plasma is not known. The mechanism for appearence of the 30 kDa antigen in the plasma is not known. The immunization procedure makes the 30 kDa antigen a putative circulating toxoplasma antigen.

TOXOPLASMA GONDII: CROSS-REACTING ANTIGENS AND RECEPTORS ASSOCIATED WITH THE RETINAL PIGMENT EPITHELIUM (RPE)

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Objective. To study the mechanisms underlying the tissue damage associated with*T.gondii* invasion of the retina.
Methods. Immunoprecipitaiton of S^{35}-labelled saline extract of human RPE antigens by sera collected from *T.gondii* infected patients with chorioretinitis. Immunoprecipitaiton of I^{125}-labelled saline extract of human RPE antigens by sera collected from *T.gondii* infected and non-infected rabbits. Western blot analysis performed on a saline extract of *T.gondii* tachyzoites using antisera raised against the OV39 recombinant antigen of *O.volvulus* and sera from human toxoplasmosis patients. Analysis of S^{35}-labelled RPE proteins eluted from a *T.gondii*-Sepharose affinity column. Two-dimensional analysis of S^{35}-labelled *T.gondii* protein(s) eluted from a RPE-Sepharose affinity column.
Results. Indication of immunological cross-reaction between *T.gondii* and RPE came from the experiments in which radiolabelled homogenate of RPE was mixed with *T.gondii* positive sera. Four of nine human sera tested precipitated RPE antigens of 17 and 32 kD as did the rabbit antisera, which also indentified a third component of 19 kD. Antisera raised against a 22kD *O.volvulus* recombinant antigen that cross-reacts with a 44kD component of human RPE, recognises two *T.gondii* antigens with m.w. of 33 and 27 kD using Western blotting analysis. Three major components of RPE with molecular weights of 70, 54, and 30 kD were eluted from the *T.gondii*-column. Only the 30kD component was identified after an aliquot of the affinity column matrix was boiled in 1% SDS. The reciprocal experiment revealed the presence of a single protein, m.w. of 30 kD when analysed on single dimension SDS-PAGE. In two dimensional SDS-PAGE/ IEF resolved two components with PI values of 6.5 and 6.8.
Conclusion. Preliminary evidence has been obtained for a specific immunological cross-reactivity between *T.gondii* and human RPE cells. Two RPE antigens with m.w of 17 and 32 kD have been identified. *T.gondii* exhibits a tropism for retinal tissue which suggests existence of specific receptors. The results presented are preliminary and although some of the molecules identified are of similar size to parasite and host proteins already described, we have **no** evidence of homology.

RECENT OUTBREAKS OF BOVINE CYSTICERCOSIS IN NORWAY.

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Cysticercosis is rarely diagnosed in cattle in Norway; in 1989, cysts of *Taenia saginata* were reported in only 0.03 % of all cattle slaughtered. In most cases, they represent stray animals, in which only few cysts are detected, and the cysts are often old and calcified.
In 1990, several cases of cysticercosis were diagnosed, and in many instances, the number of animals infected in each herd was high, and the animals were often heavily infected. Most of these cases were reported from the county Rogaland, in south-western Norway, and several were diagnosed in animals which were permanently stabled, but had been fed fresh grass which had probably been contaminated with untreated sewage. Further details on the possible sources of infection will be dealt with. Our findings indicate that the existing regulations on the use of sewage/sludge are not always enforced. Stricter practices should be applied, and more information given to the farmers on the possible risks of transmitting parasites to their cattle, and eventually to man.

THE EXCRETION OF EIMERIAN OOCYSTS IN CALVES DURING THEIR FIRST WEEKS ON PASTURE

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Objective. Recent clinical experience in Sweden has shown that large amounts of Eimerian oocysts often are excreted in the faeces of calves with diarrhoea during their first weeks on pasture. The aim of this study was to examine the excretion pattern of these oocysts, to identify the species involved, and to get indications of their clinical significance.

Methods. Fecal samples were collected from 79 calves, 4-16 months old, during the last two weeks before and the first three weeks after turning them out on pasture. Ten dairy herds were included. In two of them, outbreaks of clinical diarrhoea in calves during the first weeks of the grazing period, had been reported the preceding years. All pastures had been used for calves the previous grazing season.

Results. In seven of the herds, most of the calves got a transitory diarrhoea after 4-6 days on pasture, which in some of them lead to obvious loss of condition. A significant increase in oocyst output, starting after 6-15 days on pasture and with a peak after 9-15 days, was noticed in eight of the herds. The amount of oocysts had returned to initial levels 15-23 days after turnout. *Eimeria alabamensis* was the quantitatively dominating species in all herds. Oocysts of *E. bovis, E. bukidnonensis, E. cylindrica, E. ellipsoidalis, E. pellita, E. subspherica, E. zuernii, E. auburnensis and/or E. wyomingensis* were found in low numbers. The period from transfer of the animals to pasture until the increase in oocyst excretion corresponds to the prepatent period of *E. alabamensis,* which makes wintering oocysts of this species a conceivable source of infection.

Conclusion. The investigation indicates that *E. alabamensis* is an etiological factor of diarrhoea in first-year-grazing calves in Sweden.

GASTRIN AND GASTRIN-RELATED RESPONSES TO *Ostertagia ostertagi* INFECTION IN THE CALF

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The most important gastro-intestinal parasite of cattle in Northern Europe is the abomasal nematode *Ostertagia ostertagi*. Infection with this parasite has been shown to impair the efficiency of digestion by lowering the production of HCl in the stomach and elevating gastric pH. This increases secretion of the gut peptide gastrin from G-cells in the pyloric antrum which 1) stimulates acid production and hypertrophy of the fundic mucosa; 2) slows down reticulo-rumenal motility and abomasal emptying which leads to a stasis of ingesta and a drop in feed intake. This depression in appetite is, in turn, largely responsible for the poor weight gain shown by parasitised calves. This paper reviews recent progress in elucidating the mechanisms underlying these events.

IN VIVO PASSAGE OF STRESS SELECTED NEMATOPHAGOUS FUNGI IN CALVES.

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Objective. A study was made on the survival and resultant activity of stress selected nematophagous fungi after *in vivo* passage in calves.
Methods. Calves were fed twice daily with fungal material on barley grains. Faeces were collected on the 4th and 5th day after start of the experiment. The capacity of the fungi to reduce the development of parasitic larvae in the collected faeces was tested by mixing the 'fungus' faeces with faeces containing eggs of **Ostertagia ostertagi**. This faecal mixture was used to form 125 g dung pats that were incubated for 3 and 4 weeks. Numbers of infective larvae developed were registered after baerman extraction. Dung pats without fungal material but containing nematode eggs were used as controls. By using the same basic materials conventional vermiculite faecal cultures were set up for the same incubation period and conditions. Recognizable barley grains were washed out of the fresh faeces and incubated on water agar plates.
Results. In dung pats eight out of ten fungal isolates showed a capacity to reduce developing **O. ostertagi** larvae by approximately 85%. In vermiculite cultures the reducing capacity tended to be even higher in that five out of the isolates reduced the numbers of larvae by more than 95%. Comparing the two 'faecal culture techniques' it was evident that the vermiculite culture resulted in more uniform data than the dung pat assay, which showed a higher variability in larval recoveries. Out of ten fungal isolates tested nine could be reisolated from the plates incubated with the barley grains originating from the fresh faeces samples.
Conclusion. The experiment clearly showed a high ability of selected fungi to survive the passage through calves as well as a capacity to subsequently reduce the number of developing parasitic larvae in the faeces.

ESTIMATION OF GRASS INTAKE IN GASTROINTESTINAL NEMATODE INFECTED SHEEP.

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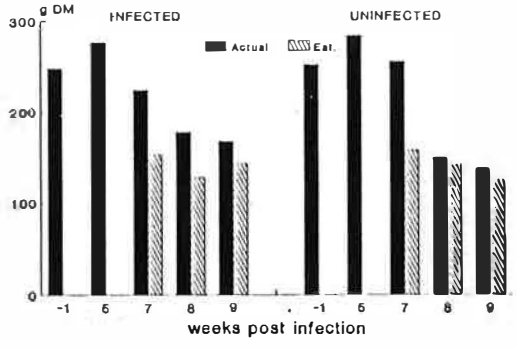
Objectives. Anorexia is an important factor in reducing performance in gastrointestinal nematode infected sheep. Intraruminal boli with constant release of inert chromoxide offers possibilities of estimating faecal output and if the digestibility is known feed intake in grazing animals can be calculated. This method was evaluated in housed lambs in the present study.

Methods. Two groups of each four 5-6 month old lambs from pasture were given anthelmintic and placed in metabolic cages and fed grass stored at -18°C ad lib.. One group was infected with 10.000 L3 3-5x/week (Ostertagia and Trichostrongylus spp.) and the other remained as uninfected controls. Feed intake and amount of faeces were recorded daily, bodyweight and faecal egg counts (EPG) weekly. Faecal dry matter (FDM) and dry matter digestibility (DMD) were calculated week -1, 5, 7, 8 and 9 p.i.. All lambs were dosed with chromoxide boli (CaptecChrome, Nufarm, NZ; release 165 mg Cr_2O_3/day) at start of week 6 and FDM were calculated using the chromoxide content in faeces week 7, 8 and 9. Worm counts were performed at slaugther week 9.

Results. The lambs had moderate EPG's at start of experiment and had thus been previously exposed to infection. This was reflected in a variable susceptibility to the artificial infection. Three of four infected lambs had 2-3000 EPG week 9 while the last lamb had 8280 EPG (the youngest). Total worm counts ranged 45000 to 54000. Mean weight loss of infected and uninfected lambs was 5.3 and 3.6 kg, respectively with large variation within groups. The mean DMD was 68±5 and 68±4 for infected and uninfected lambs, respectively and showed small variation with respect to week. The groups means of actual and estimated FDM were as follows:



Conclusion. In accordance with the literature, the DM digestibility was not found significantly altered in infected sheep as compared to uninfected. The correlation between actual faecal dry matter and an estimated value based on chromoxid content was satisfactory 2-3 weeks after dosing with the boli while the results obtained earlier i.e. 8-12 days after were not. The method offers a reliable potential for estimating feed intake in grazing infected sheep.

HELIGMOSOMUM MIXTUM (NEMATODA) IN THE BANK VOLE *CLETHRIONOMYS GLAREOLUS*: MORTALITY OF THE HOST DEPENDS ON THE NUTRITION

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H. mixtum (Nematoda) is the most common helminth parasite of the bank vole in Finnish Lapland. Based on our long-term monitoring we have earlier concluded that this parasite has a minor impact on the host.
To test this experimentally we live-trapped voles in autumn 1989 and 1990, in the early phase of the annual autumn decline, and kept the voles at diets containing 3% or 6% protein in 1989, and at 3% in 1990. The experiments ran from early October until mid-December. A possibility for the natural transmission cycle in *H. mixtum* was allowed by leaving a part of each cage uncleaned weekly.
Based on our earlier data (10 years, about 1000 voles), the prevalence of lumenal *H. mixtum* in autumn samples of immature voles varies between 12-94% (mean 61%), and the highest intensity from nature is 42 worms in a host. The infection parameters in early October 1989 were high: prevalence was 96% (23 voles) and intensity 7 ± 6 (mean $\pm$ s.d.). Of 19 voles studied, only 5 has 1-2 encysted larvae in the intestinal wall.
Of the 40 voles at low protein diet in 1989, 17 died during the experiment. At higher protein level, 5 of 36 died ($X^2=7.5$, $p<0.01$). The main cause of mortality was the massive infection by *H. mixtum*. The mean number of lumenal worms in dead voles was 472 ± 386 (n=14). The mean for encysted larvae in the intestinal wall was 76 ± 110. The highest numbers were 1581 lumenal worms and 407 encysted larvae in one host. At the end of the experiment, the surviviors had 13 ± 9 lumenal worms (n=38). Only 4 of them had 1-4 encysted larvae.
Macroscopically the small intestines were dilated and the they contained watery ingesta. Parasites and hemorrhages were abundant in the proximal small intestine. In the histological sections hemorrhages, mucosal lesions and larvae could be found. Pathological diagnosis was severe enteritis which had caused most of the deaths. Voles died rapidly, healthy-looking voles could be found dead 12 hrs later. The mortality peaked at 4-6 weeks.
In the 1990 experiment mortality was low. This could be due to the much lower prevalence of *H. mixtum* in the field in 1990 than 1989.
We conclude that the seasonal autumn decline of the bank vole can partly be due to interaction between parasites and impaired nutrition of the host. The autumn prevalence of the parasite seems to be important, too. There were also clear individual differences in the susceptibility between the voles in the same cage. This suggests also for genetic heterogeneity in voles' ability to resist parasites.

PREVALENCE OF *Anoplocephala* IN SOUTHEAST ENGLAND

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The horse tapeworm, *Anoplocephala perfoliata*, is ubiquitous in distribution, yet little is known of its epidemiology or clinical significance. Some equine surgeons believe that a proportion of horse colics, particularly those involving intussusception, are associated with heavy tapeworm infection. This stimulated a survey of the prevalence of this parasite in the area around the RVC equine clinic just north of London. Eighty healthy horses were treated with pyrantel embonate (at 38 mg/kg bodyweight) and expelled tapeworms counted and identified. Infection was found at 60% of surveyed stables and in 31% of grazing horses. There was no obvious relationship between prevalence and sex, age or breed but infection appeared to be absent in permanently stabled animals. Fewer than 3% harboured heavy infection compared with 13% of a series of 130 cases of ileal or caecal obstruction treated at the RVC (Edwards, G.B., 1986, Proc. 16th Congr. Europ. Soc. Vet. Surg. 99-105).

PATHOLOGICAL CHANGES AT THE ILEO-CAECAL JUNCTION OF HORSES ASSOCIATED WITH *Anoplocephala perfoliata* IN AN ABATTOIR IN SOUTHWEST ENGLAND.

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The intestinal tracts from 20 normal horses, killed at a local equine abattoir, were examined for the presence of *A.perfoliata*. The presence or absence of the parasite at the ileo-caecal junction (ICJ) was noted and samples collected in formalin. Tapeworms were not counted in the intestinal contents. In 4 horses no tapeworms were observed; in 9 horses 1-20 tapeworms were seen, and in 7 horses more than 100 tapeworms were found attached to the mucosa. The results were correlated with histopathological changes at the ICJ which included thickening and ulceration of the mucosa and infiltration with eosinophils; the changes being most severe in the intestinal tracts in which the largest number of parasites was found.

EFFECT OF IVERMECTIN TREATMENT ON APPETITE IN REINDEER *(Rangifer tarandus tarandus).*

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Objective: The objective was to test whether a single anthelmintic treatment increased appetite in naturally infected six-month-old reindeer.
Methods: Eighteen six months old reindeer females with naturally-acquired parasitic infections were divided into two equally sized groups, Treatment (T) and Control (C), using the block randomization method. This involved dividing the animals into nine blocks of two, based on a ranking of parasite transmission-propagules and body weight, and then randomly allocating to either T or C. In November, T was injected subcutaneously with one ml. Ivermectin, while C received one ml. isotonic saline solution. The animals were housed in individual enclosures and fed a high protein reindeer diet (RF-80) *ad lib.* Daily feed consumption was measured from three weeks before treatment to eleven weeks after.
Results: There was no significant difference in feed consumption between the two groups at the 95% level (analysis of variance).
Conclusions: Even though this study did not reveal any increase in appetite following treatment, we cannot rule out such an effect under natural conditions. This is due to a higher protein content in the artificial forage which can mask anorexia. Furthermore, anorexia may occur at other times of the year due to time-specific physiological and parasitological conditions. A follow-up study is presently being conducted to test this latter hypothesis.

PANACUR^{R} /AXILUR^{R} AS AN ANTHELMINTIC IN DOGS AND CATS

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Panacur^{R} /Axilur^{R} is frequently used as an anthelmintic in domestic, wild and zoo animals because of its wide spectrum of action, its extremely good tolerability and its versatility of application. In Canidae and Felidae a dose of 50 mg/kg b.w. for 3 days is recommended. Other dosage schemes, including substantially longer regimens, are used, also in zoo animals.

According to our own studies and those of other authors, the recommended dose eliminates nearly 100 % of the immature and adult stages of ascarids, *Ancylostoma*, *Capillaria* and *Trichuris* and to a very large extent also adult tapeworms. With other doses dogs have been cured of infestation with *Filaroides hirthi*, *F. osleri*, *Angiostrongylus vasorum*, and also *Heterobilharzia americana*. The worming of pregnant bitches is of special interest. In trials with 10 pregnant bitches a dose of 25 mg fenbendazole/kg b.w. was administered in medicated feed from the 40th day of gestation until two days postpartum. It was found that the total worm burden of *Toxocara canis* was reduced by 98.4 % in comparison with untreated controls. This dosage completely prevents an *Ancylostoma caninum* infection in puppies.

Fenbendazole's activity in cats is comparable with that in dogs: ascarids, *Ancylostoma*, *Trichuris* and *Taenia* are almost 100 % eliminated by the recommended dose. In other dosages fenbendazole is highly active against *Aelurostrongylus abstrusus* and *Paragonimus kellicoti*.

The initial investigations had already shown that fenbendazole is well tolerated in all animals. In toxicity tests it was not possible to determine the minimum lethal dose because it exceeded the maximum dose that can be administered. In the dog the following oral doses of fenbendazole were well tolerated: > 500 mg/kg b.w. for 1 day, > 125 mg/kg b.w. for 30 days, > 125 mg/kg b.w. for 90 days. In trials with 72 dogs which were treated with fenbendazole in the critical phase of pregnancy there were no side effects, ie neither teratogenicity nor embryotoxicity etc.

Only limited trials were carried out in cats: In groups of 7 cats each the study of clinico-chemical and clinico-haematological parameters over 21 days showed that fenbendazole is well tolerated by cats in the therapeutic dose (50 mg/kg b.w. for 3 days) and three times the therapeutic dose (150 mg/kg b.w. for 3 days). The effect on segmented neutrophils was only transient and these had again returned to the physiological norm 14 days after the first treatment. The serum kinetics of fenbendazole and the metabolites SO-fenbendazole and SO_2-fenbendazole after administration of 1/2 Panacur tablet/cat for 3 days (this is equivalent to 63.0 to 72.8 mg/kg for 3 days; on average 65.8 mg/kg b.w.) were studied. Fenbendazole is only slightly absorbed following oral administration but this is adequate for the treatment of development stages of the parasite in the host to be affected. A limited field trial in which 17 pregnant cats received long-term treatment did not show any abnormalities in the mother animals or their kittens.

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ANTHELMINTIC RESISTANCE IN NEMATODE PARASITES OF SHEEP IN DENMARK

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Objective The purpose of this study was to elucidate the presence of anthelmintic resistance in nematode parasites of sheep in Denmark.

Methods In a field study 22 flocks of sheep were selected for a faecal egg count reduction (FECR) test. The flocks included in this study had used the same anthelmintic or the same class of anthelmintics over the previous five years, 3 times or more per year. At least 15 lambs, untreated with any anthelmintic for the past 6 weeks, had to be available for the FECR test on the farm. Lambs were faecally sampled at the day of treatment, weighed and treated at the recommended dose with the anthelmintic used at the farm during previous year. The same lambs were resampled 10-14 days later. All faecal samples were examined for the concentration of nematode eggs by a modified McMaster technique and a composite faecal sample from before and after treatment from each flock was cultured for the production of third stage larvae for worm species differentiation. From one flock a strain of Ostertagia circumcincta (KYSE) showing a low response to levamisole was isolated and compared to a levamisole susceptible strain of the same species by an in vivo controlled slaughter assay and an in vitro egg hatch paralysis assay.

Results Benzimidazole anthelmintics were tested at 19 farms and levamisole at 3 farms. Benzimidazole resistance (FECR 88-94%) was present at 5 farms and at another 3 farms were found levamisole resistance (FECR 73-94%). Larval cultures showed that resistance was present in Ostertagia spp and Trichostrongylus spp. In the suspected levamisole resistant strain (KYSE) FECR was 44.5% after treatment with levamisole at the recommended dose and the worm burden was reduced by 67.7% in numbers. In the egg hatch paralysis assay the LC_{50} of the KYSE strain was 6.05 ug levamisole/ml and in the susceptible strain it was 0.04 ug levamisole/ml.

Conclusion This study showed that benzimidazole and levamisole resistance were present in the parasitic nematodes Ostertagia spp. and Trichostrongylus spp of sheep in Denmark. Resistance was detected at farms where factors theoretically disposing for the development of anthelmintic resistance were found to be present. The field observations were confirmed in one instance by an in vivo controlled slaughter assay and by an in vitro egg hatch assay.

PROSPECTS FOR BIOLOGICAL CONTROL OF NEMATODE PARASITES OF SHEEP

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Despite recent advances in chemotherapy, nematode parasite infection remains the most important disease problem of sheep throughout the world. Present control, based on anthelmintic treatment, is under threat, not only because of the widespread emergence of resistant strains of parasites, but also because of public concern regarding drug residues in sheep products and in the environment. Although alternative intra-host control strategies, such as vaccines and genetic selection for resistant sheep, are being explored, biological control using fungi has received virtually no attention.

Within the free-living environment on pasture, there is a range of fungi which possess nematophagous properties. Some of these fungi have been comprehensively studied, particularly in Scandinavia, and although they may show good activity *in vitro*, their performance under field conditions has met with limited success. Fungi are also important members of the microbiota within the rumen - some of these have been shown to be closely related to free-living nematode destroying fungi.

Investigations are underway to examine a large range of fungi to determine whether they survive passage through the gastro-intestinal tract of sheep, colonise faeces, capture larvae and/or produce nematoxic substances. Also, it is proposed to carry out manipulation of specific fungi to enhance these abilities and thus provide a basis for biological control against nematode parasites of sheep, either in the free-living or rumen environment.

SERUM BIOCHEMICAL CHANGES DURING EXPERIMENTAL *EIMERIA ALABAMENSIS* COCCIDIOSIS IN CALVES

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Objective: In Sweden *Eimeria alabamensis* is connected with spring diarrhoea in first-year-grazing calves. Since the general opinion elsewhere is that the parasite is of low or moderate pathogenicity, an investigation was designed to study clinical, parasitological and some serum biochemical changes due to *E.alabamensis* infection under experimental conditions.

Methods: Groups consisting of three calves, aged two-three months, were dosed with $10x10^6$, $100x10^6$ and $400x10^6$ sporulated *E.alabamensis* oocysts, respectively, and three calves were kept as controls. The calves were monitored clinically and parasitologically for four weeks. Blood samples were taken before inoculation and twice weekly during the observation period. Serum activity of alkaline phosphatase (AP) and concentrations of bile acids (BA) and total bilirubin were determined.

Results: 3-6 days after dosing the calves developed diarrhoea which lasted for 2-5 days. Excretion of *E.alabamensis* oocysts started 8 days post infection (PI) and reached its highest levels (millions of oocysts per gram faeces) on days 8 and 9 PI. In particular animals receiving high infection doses were dull and showed inappetence during the patent phase of infection. In the infected groups, AP activity decreased between 7 and 17 days PI. The decrease was most pronounced on day 10 PI. Concentration of BA decreased gradually, reaching a minimum on day 7 PI. Total bilirubin concentration reached a peak on day 7 PI. The control animals showed no similar changes in any of the parameters measured.

Conclusions: The induced infection is supposed to be local, but it seems likely that the liver in some respect gets involved. The observed serum biochemical changes were most prominent during the acute phase of the infection. Elevated total bilirubin concentration could be an indication of a disturbed liver function. The decreased serum BA concentration might reflect an impaired absorption of bile acids in the small intestine. The reason for the decrease in AP activity could be either decreased induction of AP synthesis in the liver or, less likely, direct losses of the intestinal isomere of the enzyme from the damaged intestinal mucosa.

ISOLATION AND IDENTIFICATION OF COCCIDIAN PARASITES INFECTING DOGS IN EGYPT

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Stray dogs from different localities in Cairo governorate, Egypt, have been investigated for enteric coccidia. Examination of faecal samples from 110 dogs (1-5 years old) during the period from January to December 1986 revealed that 18.8 % harboured coccidian parasites.

The recorded coccidian parasites were Eimeria canis 1.82 %, Isospora canis 6.36 % Isospora ohioensis 2.72 %, Hammondia heydorni 2.72 % and Sarcocystis species 4.55 %.

E. canis oocysts were ellipsoidal to oval in shape measuring 29 - 36.54 * 17.4-20 µm, with micropyle and without micropyler cap. The oocysts sporulated after 48 hrs in 2.5 % potassium dichromate at 27 °C. I. canis oocysts were ovoid to ellipsoidal, measuring 29 - 34.8 * 34.8 - 40 µm (mean 31.9 * 37.7 µm) and without micropyle. I. ohioensis oocysts were ellipsoidal to ovoid in shape with an average size 19.8 * 23.2 µm. I. canis and I. ohioensis oocysts were sporulated in 2.5 % potassium dichromate at 27 °C after 48 hrs and 36 hrs respectively. H. heydorni oocysts were subspherical in shape had no micropyler cap and measured 10.15 - 11.76 * 11.6 - 13.27 µm. Sporulation time was 3 days at 27 °C. Sarcocystis species sporocysts were 14.5 - 16.9 * 9 - 11.3 µm

Fifty intestinal mucosal smears from 50 stray dogs (1-5 years old) stained with modified Ziehl-Nielsen for Cryptosporidium oocysts recorded negative results.

IMMUNOGENIC CAPACITY OF NATURALLY ACQUIRED HYPOBIOTIC OSTERTAGIA OSTERTAGI INFECTION IN CALVES

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Objective: To investigate the impact of natural hypobiotic Ostertagia ostertagi (O.o.) infection on the course of subsequent summer exposure.

Design and methods. Two comparable groups of parasite-free Jersey bullock calves (nine per group) were grazed for six weeks during October and November 1989 - group A on O.o.-contaminated pasture and group B on non-contaminated pasture. After winter stabling the two groups were grazed together on an O.o.-contaminated paddock from the end of April till mid-October 1990. Parasitological and immunological parameters were monitored at two-week intervals: faecal strongylid egg counts (EPG), body weight (BWT), serum pepsinogen concentration (PSG), and specific serum IgA and IgG_1 (ELISA technique). Finally, individual abomasal worm counts were determined (adult worms from washing abomasal contents and larvae from mucosa digestion), when all the animals were slaughtered mid-October.

Results. Group A acquired a moderate initial infection resulting in some egg excretion declining from around 200 EPG in December to negligible values in April. This limited adult establishment was indicated by PSG values of group A fluctuating at a slightly elevated level, as well as by increases in the IgA and IgG1 responses. During March and April the presence of hypobiotic larvae was revealed by increases in the EPG, PSG, IgA, and IgG_1 responses, possibly indicating reactivation of arrested larvae. BWT was but slightly affected.
During the grazing period 1990 pasture contamination was relatively low due to dry conditions in May and June. In consequence, EPGs of both groups were generally low to moderate (50-100 EPG). However, EPGs of group B were constantly considerably higher than those of group A. PSG values were highest in group B from August onwards, whereas BWT developments of the two groups were comparable. Thus, worm burdens established during summer grazing appeared to be bigger in the previously unexposed group B, an observation which was further supported by the IgA response late in the season. It was eventually confirmed by significantly higher worm counts in group B than in group A.

Conclusion. Autumn exposure of calves to inhibition-prone Ostertagia ostertagi infection induced significant specific immunoglobulin responses and reduced abomasal worm establishment during the following summer grazing.

A THREE STEP IN VITRO SCREENING PROCEDURE FOR SELECTION OF NEMATOPHAGOUS FUNGI FOR BIOCONTROL OF NEMATODES IN RUMINANTS.

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Objective. A study was made to develop a screening technique for the selection of fungal biocontrol agents for studies on the control of parasitic nematodes in ruminants on pasture.
Methods. In step 1 fungal samples were treated with diluted rumen fluid for twentyfour hours. In step 2 the surviving fungi were then tested in synthetic saliva, rumen fluid and in pepsin-hydrochloric acid solution simulating the effects of ruminal and abomasal activity. Finally they were tested in a trypsin solution serving as an example of enzyme activity in the gut. In step 3 fourteen surviving isolates were tested for their capacity to reduce the number of developing larvae of **Ostertagia ostertagi** in 125 g dung pats. Control pats without fungal material were incubated and harvested parallel to the fungal test pats.
Results. Twentyone isolates were obtained after treatment with diluted rumen fluid (step 1). The effect of the rumen fluid alone or a combined rumen fluid and pepsin-hydro-chloride treatment led to a reduction in numbers of surviving fungal isolates (step 2). Six out of originally thirteen isolates of the genus **Arthrobotrys** and seven out of originally eight **Duddingtonia** spp. survived (step 2). The dung pat bioassay (step 3) showed that **Arthrobotrys** spp. fungi reduced the development of **O. ostertagi** larvae by approximately 75% while the **Duddingtonia** spp. isolates were able to reduce the number of larvae by approximately 90% compared to the number of larvae that developed from the fungus-free control pats.
Conclusion. The three-step in vitro screening technique was found to be a feasible method for the selection for fungal biocontrol agents.

HPLC ANALYSIS OF MONOAMINES IN THE CESTODE *DIPHYLLOBOTHRIUM DENDRITICUM*.

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Objective. To investigate the presence of biogenic monoamines in the endoparasite *Diphyllobothrium dendriticum*.
Methods. The investigations were done by HPLC with electrochemical detection. A large amount of monoamines as well as precursors and metabolites of monoamines were included in the standard runs.
Results. DOPA, dopamine and serotonin were detected and preliminary results on some of their metabolites as well as other related substances were obtained.
Conclusion. The finding of these substances in this primitive animal indicates that they appeared early in the evolution of the nervous system.

WORM KINETICS IgE AND EOSINOPHILS IN PRIMARY AND SECONDARY ECHINOSTOMA CAPRONI (GUT-TREMATODE) INFECTED RATS

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Methods:
Groups of 6 adult female Hooded Lister rats were given primary (prim) and secondary (sec) 50 metacercariae (mc) infections (inf) of Echinostoma caproni on day 0.
The secondarily infected rats were previously given 50 mc prim inf on day -117.
Worm eggs were counted in samples of diluted 24h faeces in Sedgewick-Rafter counting chambers.
Serum IgE was measured in Kyoto male rats by PCA (Passive Cutaneous Anaphylaxis) test using two-fold diluted sera.
Eosinophils were suspended in phloxine-propyleneglycol and counted in Bürker-Türk bloodcounting chambers.

Results:
The prim inf reached maximum production of 3688 eggs/rat/24h on day 21 and, thereafter, continuously decreased.
The sec inf established maximum egg production simultaneously with the prim inf but at a lower level (about 1000 eggs/rat/24h). The egg production remained constant until day 70 where both prim and sec inf's showed the same decrease.
One prim and 3 sec rats lost their last worms day 84-87.
The first serum IgE was detected day 7 in sec rats and day 21 in prim rats. Maximum titers of 128 and 256 were measured day 42-56 in prim and sec rats respectively. Relatively high titers (32-64) were still found in both prim and sec rats on day 84.
In both prim and sec rats a continuous rise in concentration of blood eosinophils was seen until peak values on day 42 of 750 and 450 per mikroliter in prim and sec rats respectively. Thereafter, the eosinophil level decreased.
The individual IgE titer was positively correllated to the eosinophil level ($p < 0.05$).

Conclusion:
The present model shows that adult trematodes parasitizing a mammalian intestine induces the type I hypersensitivity reactions characteristic of other helminth host systems.

ECHINOSTOMA CAPRONI IN RATS
WORM CONDITION, DISTRIBUTION AND CLUSTERS IN RELATION TO WORM POPULATION

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Methods:
Adult Hooded Lister rats were given primary infections of 50 or 100 metacercariae (mc) of Echinostoma caproni.
Worm eggs were counted in samples of diluted 24h faeces in Sedgewick-Rafter counting chambers.
Worms were recovered at autopsy day 21.
Worms found outside the range of physical contact (5 mm) were not regarded as participants in a cluster.

Results:
On day 21 recovery of 50 and 100 mc infections from the same batch did not differ significantly. Recoveries recorded from 50 mc infections of different mc batches varied from 30%-60% but the coefficients of variations were similar (32%-46%).
Individual rat mean worm wet weight and mean worm 24h egg production showed a significant negative corellation to the total number of worms recovered on day 21 ($p < 0.05$).
The day 21 worms tended to be more anteriorly distributed in the gut of rats with high worm burdens (mean position in the small intestine being 70% and 54% in rats harbouring 1-10 and 41-50 worms, respectively).
E. caproni were found aggregated in separate clusters in which distances between worms were less than 1 mm.
The number of clusters (maximum 5) in the individual rat seemed independent of the worm population size while the number of worms in the clusters increased with the total number of worms present in the rat.

Conclusion:
Although influenced by crowding in the worm populations studied, E. caproni showed no upper limit of worm number aggregated in a cluster.

INTESTINAL PARASITES OF HORSES IN ICELAND

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Objective. The studies reported here represent the first attempts to provide comprehensive information on the occurrence, prevalence and abundance of parasites in the gastrointestinal tract of Icelandic horses and on the seasonal reproductive activity of the helminth parasites and the effect of anthelmintic treatment on faecal egg output.
Methods. A post mortem examination was performed on ten young foals and eight adult horses. Faecal samples were taken weekly from ten adult horses over a period of two years. Helminth eggs were counted and third stage larvae were cultured and identified. Other faecal eaminations have been performed in the same manner on different age groups over shorter periods of time. Faecal samples of forty foals were examined specially for the presence of *Cryptosporidium* oocysts.
Main results. The following thirty-two parasitic species have been found in Icelandic horses:
Protozoa: *Cryptosporidium* sp. and *Eimeria leuckarti*.
Cestoda: *Anoplocephala perfoliata*.
Nematoda: Strongylid nematodes; *Cyathostomum catinatum*, *C. labratum*, *C. pateratum*, *Cylicocyclus insigne*, *C. leptostomus*, *C. nassatus*, *C. ultrajectinus*, *Cylicodontophorus bicoronatus*, *C. euproctus*, *C. mettami*, *Cylicostephanus calicatus*, *C. goldi*, *C. longibursatus*, *C. minutus*, *Gyalocephalus capitatus*, *Poteriostomum* sp., *Craterostomum acuticaudatum*, *Oesophagodontus robustus*, *Strongylus edentatus*, *S. equinus*, *S. vulgaris*, *Triodontophorus brevicauda*, *T. serratus* and *T. tenuicollis*.
Other nematodes; *Trichostrongylus axei*, *Parascaris equorum*, *Oxyuris equi*, *Probstmayria vivipara* and *Strongyloides westeri*.
Strongylid nematodes were found in horses of all age. Worm burdens of 50,000-100,000 were common in adult horses but young foals were less heavily infected. *P. equorum*, *O. equi* and *S. westeri*, as well as the protozoans, were primarily seen in foals. The strongylid egg number in faeces of adult horses was lowest in midwinter, followed by a rise in spring and a marked peak in summer in the grazing season. The egg output declined rapidly in autumn. When horses on permanent pastures were given anthelmintic treatment in the spring, no eggs were found in the faeces for five weeks, but then eggs reappeared and soon reached the same level as prior to treatment.
Conclusion. Icelandic horses carry most of the common intestinal parasites found in horses in other countries.

RESISTANCE TO BENZIMIDAZOLE ANTHELMINTICS IN SMALL STRONGYLES OF HORSES IN DENMARK

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Objective The purpose of this study was to establish whether anthelmintic resistance was present in nematode parasites of horses in Denmark.

Methods Sixteen horse farms were selected for a faecal egg count reduction (FECR) test. The criteria for a stud to be included in the study was that the usage of anthelmintics had been recorded for at least the previous two years and a minimum of 10 horses were available for an anthelmintic efficiency test. Two sets of faecal samples were collected per rectum, the first set at the day of treatment and the second set from the same horses 7-10 days later. The following anthelmintics were used at the recommended dose rates: febantel, fenbendazole, mebendazole and pyrantel pamoate. All faecal samples were examined by a modified McMaster technique and a larval culture for the determination of the composition of small (Cyathostominae) and large (Strongylidae) strongyles. In connection with the first visit a questionnaire on anthelmintic usage, frequency of treatments and farm management was filled out.

Results Sixteen farms were subjected to the FECR test and subsequent larval culture. Benzimidazole anthelmintics were used at 15 of 16 farms. On 13 of the 15 farms FECR met the criteria of anthelmintic resistance being present in a flock. At these farms FECR values were ranging from 80% to minus 101.3%. At the remaining 3 farms FECR were higher than 95%. Larval cultures revealed that resistance was confined to the group of small strongyles (Cyathostominae). Results of the questionnaire study showed that horses in this study on average were treated 7.1 times/year. Horse owners did change between preparations of drugs, but almost only within the same class of anthelmintics. Horses in the above studs grazed the same paddock every year and the stocking rate was estimated to be 2.4 horses/ha.

Conclusion Our investigations have revealed resistance to benzimidazoles in small strongyles of horses in Denmark and our observations indicate that the problem may be widespread in our country. Frequent drenching, use of the same class of anthelmintics over years and high stocking rates may have contributed to emergence of this problem. Strategies to avoid development of anthelmintic resistance will be discussed.

PREVALENCE OF BENZIMIDAZOLE-RESISTANCE IN EQUINE CYATHOSTOME POPULATIONS IN SOUTHEAST ENGLAND

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In a structured survey of the occurrence of benzimidazole(BZD)-resistant cythostome populations in horses in the United Kingdom, twenty-seven stables in southeast England were selected according to strict criteria, but with minimum bias, to provide matched groups of 96 horses. One group was treated with fenbendazole (FBZ); one with pyrantel embonate (PYR); while the third group was an untreated control. Treated animals received at least 7.5 mg/kg FBZ or 19 mg/kg PYR. Overall efficacy values, as judged by the faecal egg-count reduction test (FECR), were 56.3 and 95.8 percent for the BZD and non-BZD anthelmintics, respectively. FECR values greater than 90 percent were recorded for PYR at 23 of 27 sites, the corresponding figure for FBZ was three of 27. Cyathostomes accounted for more than 90 percent of larvae cultured from faeces before and after treatment.

Conclusion: BZD-resistance is very widespread in southeast England in the class of stable investigated (ie those using BZD anthelmintics regularly with more than nine horses at grass).

SURVIVAL STRATEGIES OF ELAPHOSTRONGYLUS RANGIFERI IN THE INTERMEDIATE HOST

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Why should an arctic species like *E. rangiferi* have a higher developmental threshold (about 10 °C) than other related species living in temperate regions?
We think this is related to differential survival rates of larval stages. Infected snails tend to develop a strong cellular response towards the parasite. This response is apparently more intense against the second larval stage, where most of the growth takes place. In an arctic climate it is important to the parasite to avoid being trapped at this stage during long periods of time where no development is permitted. This could be ensured by having a high developmental threshold.
To test this hypothesis, an experiment is set up where a number of snails of the species *Arianta arbustorum* is infected, and then randomly divided into three groups known to contain first, second and third stage larvae respectively, and incubated at +2°C.
Every six weeks during the winter subsamples of the snails are being investigated for infection, and death-rates of the different larval stages of the parasite is calculated.
The results of the experiment will be presented in the paper.

PARASITES OF DOMESTIC PIGS AND WILD BOARS IN ESTONIA

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Parasites of domestic pigs have been the subject of study for a number of Estonian researchers (M. Sikkut, J. Kaarde, V. Ridala, O. Plaan, J. Parre, A. Kaarma, E. Peebsen, I. Miller and others). The parasitofauna of the wild boar has been studied by T. Järvis.

The following protozoan parasites have been found in domestic pigs: Trichomonas sp., Eimeria debliecki, E. polita, E. scabra, Isospora sp., Toxoplasma gondii, Sarcocystis miescheriana, Cryptosporidium parvum, Balantidium coli. The fauna of the Protozoa in domestic pigs needs further research.

Domestic pigs in Estonia have been found to host the following helminths: Fasciola hepatica, Cysticercus cellulosae, Cysticercus tenuicollis, Echinococcus granulosus larvae, Ascaris suum, Strongyloides ransomi, Oesophagostomum dentatum, Metastrongylus elongatus, M. pudendotectus, Physocephalus sexalatus, Trichocephalus suis and Macracanthorhynchus hirudinaceus. Hyostrongylus rubidus should also be considered as a possible helminth in domestic pigs in Estonia. The most common permanent arthropod parasites in pigs are: Sarcoptes suis, Demodex suis and Haematopinus suis. The parasitoses frequent on Estonian pig farms are eimeriosis, balantidiosis, ascaridosis, oesophagostomosis, strongyloidosis, trichocephalosis, sarcoptosis and haematopinosis. No pig farm in Estonia can be considered free of parasitoses. In most cases it is a simultaneous mixed invasion by several kinds of parasites. Protozoan parasitoses (eimeriosis, cryptosporidiosis, balantidiosis) are common mostly in piglets, with fully-grown pigs appearing as parasite carriers and sources of contamination. Protozoan parasitoses are treated by medication with specific preparations. As helminthoses are mostly parasitoses of the digestive tract, they are diagnosed by coprological examination. Boars are coprologically examined twice a year, carrying sows two weeks before farrowing, piglets after weaning and before fattening. According to the results of the coprological examination dehelmintization is carried out. Effective imported dehelmintizing preparations are little used as they are not available in the present economic situation in Estonia.

Ivomec injected in a two-week interval has proved effective in sarcoptosis control on Estonian pig-breeding farms. Organic phosphorus compounds, chlorinated car-

bohydrates and other preparations are used to control arthropod parasites.

There are thought to be about 1400 wild boars in the Estonian forests. According to the data gathered by T. Järvis seven kinds of helminths have been found in wild boars. The figures in brackets represent the percentage of parasite infested animals; the smallest and the largest numbers of parasites found in one animal. Metastrongylus pudendotectus (93 %; 5-74), M. elongatus (93 %; 2-85), M. salmi (37 %; 2-12), Physocephalus sexalatus (17 %; 1-12), Ascaris suum (33 %; 1-3), Trichocephalus suis (67 %; 2-22), Oesophagostomum dentatum (10 %; 2-5).

The prevalent parasitosis in wild boars is metastrongylosis. Only mixed invasion simultaneously by 2-7 kinds of helminths was found in all boars examined. About 1-2 % of the wild boars in Estonia have Trichinella invasion. Cases of people infected by eating contaminated meat have been recorded. There have been no domestic pig trichinellosis cases in Estonia. Of parasitic insects Estonian wild boars have been found to host Haematopinus apri. To dehelmintize wild boars anthelminthics are added to their supporting winter feed.

PIG PARASITOSES IN LITHUANIA

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Objective. To investigate the occurrence of pig parasitoses in small private farms and large collective farms.
Methods. During 1985-1988 in different districts of Lithuania, we examined by flotation methods 1865 pigs from 14 collective farms (1, - 3,000 pigs) and 1075 pigs from 125 small private farms (2-20 pigs).
Results. According to the results from our research, 93.4% of the pigs from collective farms, and 79.9% of the pigs from private farms, were infected with Oesophagostomum dentatum, Ascaris suum, Trichocephalus suis, Strongyloides ransomi helminths as mono- or mixed infections.
67.6% pigs were infected with the protozoa Balantidium suis and Coccidia spp.
According to data from our research, antiparasitic drugs are used 20 times more frequently in pigs of collective farms than in pigs of small private farms.
Conclusion. In order to prevent high parasitism and to obtain cleaner products, pigs should preferably be raised in small private farms.

COPROLOGICAL SURVEILLANCE AS AN ALTERNATIVE TO ROUTINE ANTHELMINTIC TREATMENT IN SOW HERDS.

Allan Roepstorff, Peter Nansen and Lis Eriksen
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Objective. The prevalences of helminths in Danish swine have decreased gradually during the last decades, especially in large herds with intensive management and modern housing systems. Therefore, the objective of the present study was to evaluate the need for routine anthelmintic treatment in large sow herds.

Methods. In 1987 all anthelmintic treatments were discontinued in 25 large sow herds with low helminth prevalences. During the following 3 years, faecal samples from 25-30 kg pigs, large fatteners (appr. 90 kg) and sows were examined routinely (spring and autumn) for helminth eggs.

Results. During the study, Ascaris suum was found in 21 herds. Marked increases in prevalence rates of A.suum (to 30-50%) did sooner or later occur among sows in 12 herds. In nine of these herds the breeding stock was then treated with anthelmintics (only when the prevalence rate was high), and the prevalence rates then returned to low levels. Irrespectively how infected the sows were, young pigs were never found infected with A.suum, and the fatteners in general had very low infection levels with no significant increases during the study.

Oesophagostomum spp. were found in 3 herds: In two herds at a constant very low level, while in the third herd a marked increase was observed at the end of the study, certainly because of close contact to an infected herd. Oesophagostomum spp. were never recorded from pigs or fatteners.

Conclusions. The results indicate that transmission of helminths in some large sow herds in Denmark is neglegible, possibly due to environmental and managemental factors. Therefore, in these herds coprological surveillance, on the basis of which eventual treatment may be suggested, may be a realistic alternative to routine prophylactic treatment without monitoring parasites. When routine anthelmintic treatment has to be recommended, a significant part of the large herds only need treatment of the breeding stock (e.g. twice per year) as no transmission of helminths seem to take place to the offspring. The traditional anthelmintic strategy with treatment of sows before farrowing and treatment of young pigs at weaning only seem to be necessary in herds, where the piglets become infected from their mothers (i.e. in the majority of small traditionally managed herds).

AN IN VIVO METHOD TO SEPARATE HYOSTRONGYLUS RUBIDUS FROM OESOPHAGOSTOMUM SPP. OF PIGS INTO A PURE STRAIN

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Objective. Preliminary studies showed that fenbendazole, at a dose rate of 0.35 mg/kg, caused a complete reduction of Oesophagostomum spp. in pigs. The purpose of this study was to examine an in vivo method to separate Hyostrongylus rubidus from Oesophagostomum spp. in pigs.

Methods. Eight SPF helminth naive pigs were inoculated via stomach tube with a mixture of third stage larvae of H. rubidus and Oesophagostomum spp. The helminth status of the pigs were monitored by regular examination faecal samples for the presence of nematode egg and subsequently after faecal larval culture for the determination of species composition. Twenty seven days post inoculation (p.i.) pigs, on the basis of EPG, were allocated into 2 comparable groups of which one group was treated with fenbendazole 0.35 mg/kg (recommended dose 5 mg/kg), and the other group left untreated. Five days after treatment all pigs were slaughtered and the gastro-intestinal tract was removed for determination of worm numbers and species.

Results. Five days after treatment the mean post-treatment faecal egg output in the treated group was reduced by 79.6%. Larval cultures showed that pre-treatment samples were composed of 18.1% H. rubidus and 81.9% Oesophagostomum L_3 larvae, and post-treatment samples showed 99.2% and 0.8% L_3 larvae of the two species, respectively. Worm counts revealed a mean number of 681 and 821 of H. rubidus in treated and untreated groups, respectively. With regard to Oesophagostomum spp. no worms of this species were recovered from the large intestine of treated pigs whereas untreated pigs harboured a mean number of 660 Oesophagostomum spp..

Conclusion. The results show that it is possible to separate H. rubidus from Oesophagostomum spp. in vivo by treating pigs infected with mixed populations of the two species. The use of this technique would also allow establishment of pure strains of H. rubidus from pigs infected with H. rubidus, Ascaris suum, Oesophagostomum spp. and Trichuris suum. This is valuable for research purposes when the isolation of pure strains of H. rubidus is essential. Currently we are exploring three other techniques for separating either H. rubidus or Oesophagostomum spp. of pigs into pure strains.

INTERACTION BETWEEN ASCARIS SUUM AND PASTEURELLA MULTOCIDA IN THE LUNGS OF MICE

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Objective. The present study describes the influence of lung migration of Ascaris suum on the pathogenicity of an aerosol exposure of mice to Pasteurella multocida.

Methods. Four groups each of 15 Balb/c female mice were inoculated orally with 3800 infective A. suum eggs per mouse. Eight days later 2 groups were aerosol exposed to a 7 ml broth culture containing $2x10^8$ bacteria of either toxigenic or non-toxigenic P. multocida, respectively; the mice were killed 4 days later. To evaluate lung lesions caused by A. suum alone, 2 groups were killed on p.i. day 8 and day 12, respectively. Lung lesions caused by P. multocida alone were evaluated on day 4 following aerosol exposure. Groups of 15 non exposed mice were killed as uninoculated controls on the same days as the principals. At post mortem recordings of pathological lung lesions, lung larval recoveries and bacteriological examinations of lungs, livers and spleens were made.

Results. The group of mice which were aerosol exposed to non-toxigenic P. multocida around the time when Ascaris larvae migrated in the lungs showed severe disease and all died from septicemia. The bacterlae could be reisolated from all mice. In the group of mice only exposed to the bacterial aerosol 7 died and P. multocida could be reisolated. Seven of the Ascaris inoculated mice concurrently aerosol exposed to toxigenic P. multocida, died from septicemia, whereas the aerosol exposure to toxigenic P. multocida alone did not cause disease in the mice.

Following Ascaris inoculation alone, small hemorrhages were found in the lungs of all mice on day 8, and on day 12 there were small consolidated areas in the lungs but no bacteria could be isolated. No lesions were found in the uninoculated mice.

Conclusion. The present study clearly demonstrated, that during the period when A. suum larvae migrate in the lungs, mice are more susceptible to pneumonia and septicemia caused by aerosol exposure to P. multocida, of both toxigenic and non-toxigenic strains

Aerogen exposure to respiratory pathogens is common in the modern pig industry and possibly Ascaris larvae in the lung may enhance susceptibility to other pathogens. The results of the present investigation in mice should encourage to controlled experiments in pigs on the possible interaction between migrating Ascaris larvae and airborne infections.

ASCARIS SUUM KINETICS IN TRICKLE-INFECTED PIGS

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Objective. An experimental study on pigs was made to simulate natural, long-term exposure to Ascaris suum during the fattening period. Thus, parasite kinetics were followed in pigs receiving A. suum eggs as repeated 'trickle' infections at two dose levels from 25 kg body weight until slaughtering as baconers.
Procedures and results. In pigs inoculated with 500 eggs twice weekly there was an initial marked rise in numbers of hepatic 'white spots', but already from around week 6 after start of the inoculations and until week 16, when the last pigs were slaughtered, numbers of spots diminished drastically. In pigs, receiving only 25 eggs twice weekly, a low and moderately fluctuating number of spots was seen throughout the experiment. Larvae recoverable from livers and lungs were mainly observed in the beginning of the experiment, in particular in the pigs receiving many eggs. Thereafter larval recoveries became extremely low and sporadic, irrespective of dose level. Before patency, immature worms were found in moderate numbers, which were positively correlated with dose levels, but at the time when adult worms started to appear, immatures were practically no longer present. Adult worm numbers were apparently independent of dose level. Totally only 10 out of 40 baconers harboured adults, and 4 out of these 10 pigs harboured 87 per cent of the total worm population. Uninfected pigs kept in groups together with infected ones, were found to excrete low numbers of eggs due to coprophagia. Worm numbers recorded in the pigs were in line with the overdispersed, negative binomial worm distribution, encountered in other helminth infections.
Conclusion. The results show that acquired dose-dependent host responses play an important role in regulating the worm population along the migratory route of the parasite and that final establishment of adults is dose-independent and highly variable.

LOCAL AND SYSTEMIC IMMUNE RESPONSE TO ASCARIS SUUM IN PIGS

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Objective. The present study was designed to compare the local and systemic immune response of pigs to oral infection with either Ascaris suum third stage larvae or infective eggs.

Methods. Three groups each of 7 castrated parasite free pigs being an age of 12 weeks were used. The group receiving third larval stage (L3) was infected with 4 weekly oral doses of each 50 A. suum larvae, harvested from the lungs of a pig inoculated with infective eggs 7 days previously. Larval migration through liver and lungs therefore did not occur in this group. The group receiving infective eggs was infected with 4 weekly oral doses of each 200 eggs. Two weeks after the last infection 2 pigs from each of the L3 and the egg groups were killed and autopsied together with 2 uninfected controls. Thereafter the remaining pigs were challenged orally with 50 infective A. suum eggs per kg bodyweight.

On post challenge day 7 all pigs were killed and white spots in the liver and larvae in the lung were counted.

Specific antibodies were measured in bronchoalveolar lavage, intestinal lavage, bile and serum by an ELISA method using isotype specific peroxidase labeled antibodies to identify IgA and IgG antibodies against A. suum adult perienteritic fluid and L2/L3 larval excretory products.

Results. Compared to the control group, the egg infected group following challenge showed signs of resistance as evidenced by reduced lung larval counts, but such a resistance was less marked in the L3 infected group. Upon challenge markedly increased liver reaction was demonstrated in the L3 infected pigs compared to both controls and egg infected pigs.

Non migrating third stage larvae and eggs given orally to pigs induced an IgA antibody response locally in the lungs. Furthermore challenge following oral infection of the same pigs caused an IgA antibody response in bile of the egg group and in the small intestine of both immunized groups.

Conclusions. The present experiment demonstrated a local IgA immune response both following egg infection where larvae migrated parenterally as part of a normal life cycle and following an infection with L3 that were transferred from lungs of a donor pig to the small intestine, where they remained locally.

Acquired resistance reducing lung larval numbers was demonstrated in both egg and L3 infected pigs.

CHARACTERIZATION OF A MONOCLONAL ANTIBODY PRODUCED AGAINST THE SECOND LARVAL STAGE OF *Ascaris suum*.

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Excretory and secretory(ES) antigens of the second larval stage of *Ascaris suum* are considered prime target molecules for the aquired immune responce in pigs.

In order to characterize one or more of these antigens, murine monoclonal antibodies(Mab) were produced by infecting BALB/c-mice orally with *A. suum* eggs. B-cells from mesentheric lymph node and spleen were fused with X63-Ag8,653 mouse myeloma cells, in the presence of polyethylene glycol. The hybridomas were grown, selected, and subcloned according to described procedures. Culture supernatants were screened by indirect ELISA with ES from *in vitro* cultured 2. and 3. larval stages, and using HRP rabbit-anti-mouse(RâM) Ig as conjugate. Twelve hybridomas gave positive reactions, seven originating from lymph node B-cells and five from spleen B-cells.

When tested for isotype, 4 hybridomas reacted with HRP RâM IgM, 2 with HRP RâM IgG, 2 with HRP RâM IgE, and 2 with HRP RâM IgA. Two hybridomas did not respond to any of the isotype specific conjugates.

One hybridoma, designated 45D, was chosen for further analysis. It was a "spleen hybridoma" producing IgG. It was concentrated on a protein A agarose column, and eluated at pH 5.0, corresponding to mouse IgG_1. Using immuno-precipitation of biotinylated antigen followed by SDS-PAGE and HRP-avidin immunoblotting, the 45D had specificity for three bands in the range of 45 to 60 kilo dalton (KD). After treating the antigen with periodate, binding of 45D was abolished, suggesting that this Mab is binding to a carbohydrate epitope. The Mab reacted with ES from other stages of *A. suum* and even to ES from *Toxocara canis*. But there was no reaction with *Oesophagostomum spp.* crude antigen. Further investigations will clarify whether 45D can be used as an agent in serodiagnosis of ascarid nematodes.

ANTIBODIES DETECTED BY ELISA, THE CLINICAL COURSE AND SYMPTOMS IN SPONTANEOUS SARCOPTES SCABIEI INFECTED NEONATAL PIGS

Set Bornstein and Göran Zakrisson
The National Veterinary Institute, Uppsala, Sweden

The clinical course, symptoms and humoral antibody response are described of eleven piglets born to a chronically Sarcoptes scabiei var suis (Gerlach. 1857) infected sow. Pruritus started on the fourth day after parturition. Within two weeks acute generalized sarcoptic common mange was evident on all piglets. The first signs of encrustations or hyperkeratotic skin lesions were seen three weeks after parturition (wpp). The acute common mange had disappeared at 9 wpp after which chronic mange with hyperkeratotic lesions was found only on the distal parts of the legs, particularly on the hindlegs and on the luminal surface of the pinnae. Pruritus was intense between 1st wpp and 7th wpp and most intense between the 2nd wpp and 4th wpp. Rubbing index was calculated.

Humoral antibodies of the piglets were detected from 6 hours after parturition (hpp). An ELISA with extract of S. scabiei var vulpis as antigen was used. Within 48 hpp maximum mean OD-value was found, 2.0 and at 1 wpp the OD-value had fallen to 1.5. At 3 wpp the OD-value had levelled out to 0.5 and these same values were found until the end of the experimental period at 13 wpp. Five piglets born to a SPF sow were used as controls. These piglets were free of any skin disease. The mean OD-value of the controls throughout the experiment were 0.05.

EVALUATION OF AN ELISA AND A HISTAMINE RELEASE TEST SYSTEM FOR THE DETECTION OF PIGS NATURALLY INFECTED WITH ASCARIS SUUM

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Ascaris suum in pigs has a worldwide distribution and remains prevalent in pigs in Denmark in spite of the availability of effective anthelmintics. This host-parasite system constitutes an important immunological model for other host-parasite systems of major importance, although the parasite seems of only limited economic importance to the Danish pig industry.

In the present study, an indirect ELISA test and a histamine release test system were evaluated for the detection of pigs naturally infected with A.suum. In a recently developed histamine release assay, histamine is selectively bound to glass microfibres and is subsequently detected fluorometrically following coupling to o-phthaldialdehyde. Two antigens were used in both tests: Adult body fluid (ABF) and $L_{2/3}$ E/S antigens, obtained by in vitro cultivation of hatched infective larvae. Furthermore, differential leukocyte counts were performed together with determinations of the number of worms in the small intestine, eggs in faeces and liver milk spots.

Sensitivity and specificity determinations revealed that the ELISA test was more powerful than the histamine release test using either of the two antigens. A total of 150 pigs were tested. Thirty eight of these pigs had 3 or more liver milk spots and 37 of these pigs could be detected in the ELISA test using $L_{2/3}$ as antigen (97%). The same test gave a specificity of 89 % and this combination gave the highest sensitivity-specificity index in the study. On the basis of the obtained material, it was also possible to estimate the probabilities of the presence of liver milk spots in pigs having a specific ELISA value. This could not be done using the results from the histamine release test.

It was concluded that the ELISA test is superior to the histamine release test when used as a diagnostic for A.suum infection. However, the histamine release test system may be valuable in the study of allergic reactions in pigs.

ELISA AND HISTAMINE RELEASE AS DIAGNOSTIC TESTS FOR *Trichinella spiralis* INFECTION IN PIGS.

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The sensitivity of a Histamine Release (HR) technique, based on selective absorption of histamine to glass fibres during the incubation of cells with antigen, was compared with the sensitivity of ELISA for specific antibody.

HR was carried out on 18 pigs, 6 infected with 200 *T. spiralis* larvae, 6 infected with 5000 *T. spiralis* larvae and 6 non-infected (control group). The results obtained by HR during a 7-week infection were compared with those of ELISA. Three antigens were employed: A crude muscle larva extract, an excretory/secretory (ES) antigen and a purified 45 kD antigen.

The earliest measurable reaction in HR performed on whole blood was found on day 19 p.i., whereas the earliest ELISA seroconversions took place at day 15 p.i. with crude and ES antigens. There was considerable variation between pigs regarding which test was the most sensitive for early detection of infection.

At post mortem examination on day 40 p.i. of selected muscle groups all inoculated pigs were found positive with mean recoveries of 7.9 and 225 larvae/gram of tissue in the low and high dose group, respectively. At this time, all animals of the high dose group and 5/6 animals of the low dose group were antibody positive in ELISA. HR performed on whole blood was positive in 4/6 pigs of the high dose group and 1/6 pigs of the low dose group.

Washing of the blood cells prior to antigen provocation led to a markedly improved sensitivity of HR, all animals of the high dose and 3/6 animals of the low dose group being positive by day 40 p.i. The time course in development of ELISA titres and HR reactivity indicated that this effect is due to removal of blocking antibodies.

OCCURRENCE OF PORCINE HELMINTHS AND COCCIDIA IN THE NORDIC COUNTRIES IN RELATION TO HERD FACTORS.

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**Swedish University of Agricultural Sciences, Uppsala, Sweden

Objective. The present prevalence study was carried out in all Nordic countries in order to obtain comparable surveys, and by joint efforts, a large amount of data, suitable for multivariate epidemiological analyses, were accumulated.
Methods. In 1986-88 faecal samples and information on management, housing system, anthelmintic treatment etc. were collected in Denmark (DK), Finland (SF), Iceland (IS), Norway (N) and Sweden (S). From 594 swine herds a mean of 29 individual faecal samples per herd were processed for copro-parasitological analyses.
Results. The most frequently occurring parasite was Ascaris suum, with highest prevalences recorded in large fatteners (DK: 25%, SF:5%, IS:10%, N:23%, S:32%). Oesophagostomum spp. were most frequently found in adult swine (e.g. dry sows: DK:27%, SF: 8%, IS:0%, N:6%, S:23%). Trichuris suis was found at very low prevalences (0-5%), and Strongyloides ransomi was even more rare and sporadic (prevalences: <1% except for IS:5-15%). With respect to coccidia, Isospora suis occurred almost exclusively in piglets (DK:21%, SF:4%, IS:24%, N:<1%, S:22%), while Eimeria spp. showed the highest prevalence in adult swine, especially boars (DK:9%, SF:2%, IS:16%, N:0%, S:8%). Multivariate analyses showed that the prevalences of all parasites were correlated with 'country'. The prevalence of A.suum was very poorly correlated with herd factors, including housing system, hygiene and anthelmintic routine, while marked correlations were found with respect to Oesophagostomum spp. (most prevalent in traditionally managed herds with poor hygiene and no anthelmintic routine). Also, the prevalence of I.suis in piglets was significantly correlated with several housing and hygienic factors, but interestingly the highest prevalences were seen in large herds, pens with slatted floors and in disinfected pens. Finally, the prevalence of Eimeria spp. was significantly higher in herds with outdoor runs.
Conclusion. In general, the highest parasite prevalences were found in Denmark and Sweden, and the lowest in Norway and particularly in Finland. The multivariate analyses did not reveal any single major factor, determining the size of the parasite infection levels. More likely, the occurrence of parasites depends on a combination of factors, varying from species to species.

THE EFFICACY OF IVERMECTIN IN-FEED AGAINST ENDO- AND ECTOPARASITES OF PIGS

Barth,D.[*], R.Alva-Valdes[+], A.F.Batty[**], A.G.Foster[+], E.M.Heinze-Mutz[*], J.E.Holste[+] and M.A.Sierra[++]
MSD Research Laboratories, Germany[*], United States[+], United Kingdom[**], Spain[++]

Objective: To evaluate the efficacy of an in-feed formulation of ivermectin against endo- and ectoparasites in growing pigs.

Methods: A 0.6% w/w ivermectin premix was mixed in complete pig feed at rates which provided ivermectin at a number of dose levels. Efficacy of the medicated feed, fed for 7 consecutive days, was evaluated against immature and adult endoparasites in 20 controlled trials involving 258 animals while efficacy against ectoparasites was tested in 13 trials including 142 pigs.

Results: The results demonstrate that ivermectin administered in feed at a rate of 2 ppm (providing ivermectin at a dose of approximately 100 mcg/kg bodyweight per day) for 7 consecutive days was highly effective against *Oesophagostomum* spp., *Ascaris suum, Hyostrongylus rubidus, Stephanurus dentatus, Ascarops strongyline* and *Metastrongylus* spp. and against the ectoparasites *Sarcoptes suis* and *Haematopinus suis.*

AUTHOR INDEX

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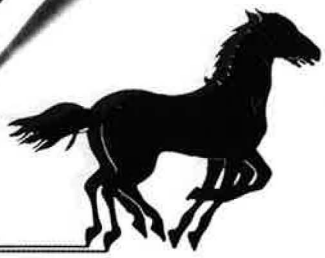

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