BULLETIN

OF THE SCANDINAVIAN SOCIETY FOR PARASITOLOGY

Vol. 2 No. 1 1992

BULLETIN OF THE SCANDINAVIAN SOCIETY FOR PARASITOLGY

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

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

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

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

BULLETIN OF THE SCANDINAVIAN
SOCIETY FOR PARASITOLOGY

VOL. 2 No. 1 APRIL 1992

The Scandinavian Society for Parasitology is celebrating its 25th anniversary this year, and one of our founding fathers, Professor emeritus Bo-Jungar Wikgren, who is also honorary member, kindly accepted our request to write the history of the society, which is presented here:

Bull Scand Soc Parasitol 1992; 2: 1-8

The Scandinavian Society for Parasitology 1967-1992

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The Beginning

A small, newly founded Institute for Parasitology in Finland hatched the idea to organize a Scandinavian, or more exactly a Nordic collaboration in the field of parasitology. There was not much parasitology in Finland in those days and the institute felt lonely.

The Institute first made a survey of the parasitological research and education in the Scandinavian countries (1). According to this survey, at least 90 persons in 51 institutes were involved in parasitological diagnosis or research. The number of parasitologists and institutes in the Scandianavian countries were: Denmark 9/7, Finland 32/17, Norway 10/8 and Sweden 39/19. However, only two institutes were fully committed to parasitology (The Danish Bilharziosis Laboratory, and the Parasitological Institute of the Finnish Society for Sciences).

Parasitologists from all Scandinavian countries were invited to a meeting, a Symposium in Turku (Åbo), Finland, in December 12-14, 1966. Thirty-four persons from the Scandinavian countries, except Iceland, participated. Nineteen scientific reports were presented (2).

Only nineteen participants stayed until the last day when a debate of the future forms of the Scandinavian collaboration in the field of parasitology was announced. These participants elected a working committee ("arbetskollegium"), headed by professor Bo-Jungar Wikgren, to act in order to obtain permanent funding from the Nordic Council, and to assess the interest in a Scandinavian Society for Parasitology.

I have two memories from this Symposium. One is the joy of meeting and making the

acquaintance of so many enthusiastic Scandinavian parasitologists. The other stems from the closing party: we had Finnish white currant sparkling wine as the welcome drink, with the first dish, with the second dish, and with the dessert. That was odd. But, of course, the alcohol had the same pleasant effect as ever. The Symposium also had a special treat: a postsymposial four-day trip to Leningrad, a city that was in those times visited with awe and curiosity.

The Foundation

During the first Symposium, Docent Holger Madsen, Copenhagen, kindly invited us to a second Symposium in Denmark. This was arranged in December 13-16, 1967, in the small town Hillerød, not far from Copenhagen. The place was some kind of boarding school, very secluded and with short beds. The 70 participants had a very rewarding Symposium, with 45 contributions and a cosy atmosphere. To the closing party, Dr. Greta Hedenström, Gothenburg, made a song, a habit that was to become a tradition for several symposia. I cite the first verse in Swedish:

> Icke blott i varma länder är det gott om vilda djur.
> Ock så här, där vi har vant oss vid en mer steril kultur
> är det gott om parasiter, icke blott i fisk och får.
> Har vi tur så kan vi möta dem varhelst vi går och står
> kan vi skönja deras spår,
> oftare ju mer vi lär oss av att träffas år från år
> i den vinterkalla Nord.
> Vi får kasta överbord
> våra dagliga bekymmer och ta till oss nya ord.

The constituting meeting of the Scandinavian Society for Parasitology was held on the last day of the Symposium, December 16, 1967. The 34 persons attending this meeting decided to found the Society. They elected the first, interim board and a scientific council. The first, interim board consisted of professor Bo-Jungar Wikgren, Åbo, chairman, professor August Brinkmann, Bergen, vice-chairman, Dr Johan Reuter, Åbo, secretary, vet.med.lic. Olle Nilsson, Stockholm, treasurer. The founding meeting further elected members to a scientific council: Professor Bertel von Bonsdorff, Helsingfors, docent Holger Madsen, Copenhagen, professor Otto Ronéus, Stockholm, and professor Rolf Vik, Oslo.

The Early Symposia

The third Symposium was held in Stockholm in December 1968. Its physical environment, the Bergendahl castle, was different from that of the previous Symposia, but the atmosphere was the same; that of a closely knit league of Scandinavian parasitologists.

The Society elected its first regular board. Professor Rolf Vik from Oslo became the new chairman. The meeting also accepted 88 applications for membership. It set forth some intents for further activities. The foremost was to create its own journal. More than twenty years were to elapse before this goal was reached! Another ambition took only two years to fulfil: the affiliation with the European Federation of Parasitology.

The fourth Symposium was arranged at the hotel Voksenåsen, Oslo in December, 1969. The Society accepted 20 new members. It asked the old grand man of Scandinavian parasitology, professor Orvar Nybelin, Gothenburg, to accept the first honorary membership of the Society. The emerging internationalization was reflected in the decision to adopt "Scandinavian Society for Parasitology" as the English translation of "Nordisk förening för parasitologi". Due to doubts expressed by some members, the plan to create a journal was dropped.

The next Symposium, the fifth, was arranged at an educational center in the outskirts of Helsinki in December, 1970. At its previous meeting, the Society had delegated the acceptance of new members to the board. It reported 25 new members, thus raising the total to 126 members. The board was rearranged with Dr. Halvorsen, Oslo, as the new chairman. The meeting also resolved to join the World Federation of Parasitologists. Professor Bertel von Bonsdorff, Helsinki, was asked to accept a honorary membership.

Arranging symposia every year was fast becoming a burden to the few organizers available. Also, some repetitiousness could not be avoided. It was becoming apparent that the Nordic parasitological community could not support symposia every year. It was a pity, since frequent meetings gave a special identity to the scattered parasitologists in the Scandinavian countries, and were a valuable addition to the scanty parasitological education. I am sure that the rapid rise of parasitological research in the Scandinavian countries in the sixties and seventies is partly due to the activities of the Society. Faced with the realities, the 1970 meeting made a significant decision: symposia should hereafter be arranged only every second year. Because the Society could hold its councils only in connection with the symposia, the annual meetings were renamed general assemblies (1972).

Growing Ambitions

The Society had thus established its routines: symposia and general assemblies every other year (with one exception), and some activities in between. Symposia were arranged in Copenhagen 1972, Lindingö, Stockholm

1974, Fantoft, Bergen 1976, Åbo 1979, Køge 1981, Stockholm 1983, Tromsö 1985, Hanaholmen, Helsinki 1987, Helsingør 1989, and Uppsala 1991.

The proceedings from the first two symposia were printed in the series Information from the Parasitological Institute in Åbo (2,3). The proceedings from the symposia in 1968 and 1969 were published in Nytt Magasin for Zoologi (4). The 1972, 1974, and 1976 proceedings were published in this same journal (5,6,7) which had been renamed Norwegian Journal of Zoology. Unfortunately, the proceedings from the 1970 meeting in Helsinki were never printed. From 1979 to 1989 the proceedings were again printed in the Information from the Parasitological Institute in Åbo (8-13). And then, at last, the proceedings of the XV Symposium (1991) were printed in their proper place, the Bulletin of the Society (14).

We may here note a very important change: Beginning in 1987, the proceedings were printed prior to the symposia and could thus be distributed to the participant.

The Society grew slowly but steadily, in 1976 it had 150 members, in 1991 209. About half of the members are zoologists, the other half veterinarians and physicians. Some parasitologists from other countries became members, a census in 1981 showed 144 members from Scandinavian countries and 11 from other countries, chiefly Britain. New honorary members were professor Bo-Jungar Wikgren, Åbo (1976), and Professor Elias Bengtsson, Stockholm ((1983).

The ambition to arrange larger symposia with more guest speakers from abroad grew rapidly. To provide some idea of the development of the symposia, and thus also the activities of the Society, let us compare the second Symposium in Hillerød, Denmark, in 1967, with the tenth Symposium in Køge, Denmark, in 1981:
In Hillerød we had 70 participants, all of them from the Nordic countries. Forty-five research reports were presented. There were no guest lecturers, no parallell sessions, no poster exhibition. Fourteen years later in the Køge Symposium we had 180 participants, a considerable part of them from countries outside Scandinavia. We had 5 guest lecturers: professor Bossche from Belgium, professor Laarman from The Netherlands, professor Ogilvie from England, professor Over from The Netherlands and professor Urquhart from Scotland. Ninety lecturers and reports were presented in 20 separate sessions. In addition, 12 posters were presented by their proud makers.

Contacts with other Parasitological Societies were made: World Federation of Parasitolo-

gists, British Society for Parasitology, European Federation of Parasitologists, Deutsche Gesellschaft für Parasitologie, and the Polish Parasitological Society.

The internationalization raised a debate of the official language of the Society. In the early years all contributions were given in one of the Scandianavian languages. This, of course, contributed to the "Nordic" atmosphere, but also caused some difficulties. Some Finnish members had problems understanding contributions in Swedish or Norwegian, and especially in Danish. Rapidly spoken Danish could impose difficulties also for members with Swedish or Norwegian as their native language. Beginning with the Symposium in Stockholm in 1974, contributions were given also in English. This created some opposition. The council resolved in 1974 to recommend English as the language of the symposia. In 1981 it resolved that the activites of the Society should be communicated in one of the Scandinavian languages, but the secretary should provide a reasonable service in English to members outside of Scandinavia. Yet, the use of English has increased, and the Scandinavian languages are chiefly used in the official papers only.

The scientific council of the Society was planned to strenghten the scientific reputation of the Society, and to make suggestions for new activities. In addition to the original members, Professor August Brinkmann Jr., Bergen, Professor Gunnel Huldt, Stockholm, Dr. Wolmar Nyberg, Helsinki, Professor Otto Ronéus, Stockholm, Professor J.C Siim, Copenhagen, and Professor Bo-Jungar Wikgren, Åbo, were members of the council. The council did, however, never function properly, chiefly due to lack of funds to arrange meetings. It was discontinued in 1982.

The Planning of a Nordic Collegium for Parasitology

In the sixties there was a total of about one hundred persons in Scandinavia with some involvement in parasites. Of these only a handful were dedicated and truly knowledgeable parasitologists. Most of the others, however, longed to learn more about parasites.

The chief task of the working committee elected in 1966 was to establish a Scandinavian organisation with permanent funding to promote parasitological research and education. The model was the Nordic Collegium for Marine Biology, founded a few years earlier. These collegia are permanent organisations, funded by the Nordic Council. As an even more ambitious alternative, the foundation of a Nordic Institute for Parasitology was suggested.

The Finnish Nordic Council delegation made

a proposition to found a Nordic Collegium for Parasitology. A recommendation of this proposition was given to the Nordic governments during the general meeting of the Nordic Council in Oslo in 1968. It was then remitted to the different authorities in the Nordic countries. Forty-two out of 47 institutions reacted positively. The rejecting verdicts of the Swedish Medical Research Council and the Norwegian Scientific Research Council (Norges almenvitenskaplige forskningsråd) were, however, enough to upset the initiative. The working committee was terminated during the 1972 meeting.

The issue of a Nordic Collegium attracted renewed interest in 1974. Then a new Nordic Secreteriat in Arctic Medicine (Oulu, Finland) had recently been founded. The board of the Society discussed the matter, and concluded that the time was not ripe for any new action by the Society.

Other Activities

The first educational activity arranged by the Society was "Nordic Course in General Parasitology". It was held at the Zoological Laboratory of the University of Copenhagen in August 11-21, 1970. Its organizers and chief lecturers were Dr. Holger Madsen and Mag. Scient. Jørn Andreassen.

In 1975 the Society together with the Nordic Collegium for Marine Biology arranged a 10-day course in Marine Parasitology in Bergen. Professor August Brinkmann Jr. and University Lecturer Björn Berland were organizers. In 1979 Dr. Erling Bindseil arranged a course in Veterinary Parasitology in Copenhagen. The course: "Use of Labeled Antibodies in Parasitological Research" was held in Stockholm, in August 30 to September 5, 1982. The credit goes to Professor Gunnel Huldt and Dr Inger Ljungström. Further the Society arranged a Nordic postgraduate course in Bergen, June 2-10, 1986 on the theme "Aquaculture and Parasitic Diseases". Organizers were Dr. Emmy Egidius, Dr. Jan Thulin and University Lecturer Björn Berland. A course in Parasitic Zoonoses is planned for 1992.

Funds from the Nordic Culture Foundation made it possible to arrange a Special Symposium on the theme "The Dissemination of Parasites". It took place in connection with the VI Symposium of the Society (Copenhagen, 1972). In 1987 the Society arranged a seminar on the theme "Vectors and Vector Bound Diseases" in Copenhagen.

In 1976 a "Nordic research project on gastrointestinal nematodes in cattle" was started with participants from all the 5 Nordic countries, headed by Dr. Peter Nansen.

The Finnish physician Dr Liisa Jokipii re-

ported in 1983 the creation of a Nordic circle in Clinical Parasitology. In 1986 Dr. Peter Nansen established a Nordic research project on the parasites of pigs.

It may be true that there is little reason to establish new scientific journals. Nevertheless, a Society of some standing is lacking something essential without its own publication. At the XIV Symposium in 1989, the general assembly constituted a group for the planning of a Bulletin for the Society. The group consisted of Dr. Peter Nansen, Dr. Hans-Peter Fagerholm, and Dr. Arne Skorping. The first issue of the Bulletin of the Scandinavian Society for Parasitology contained the proceedings of the XV Symposium in Uppsala in 1991. Two issues per year are planned. The editor-in-chief is Dr. Jorun Tharaldsen. In addition, there are national editors in each country.

Overview

Shared history and common cultural traditions make the Scandinavian countries a very special region in Europe. It is obvious that this also should have its own Society for Parasitology. This was founded in 1967. It was an immediate success. The positive development of Parasitology in the Scandinavian countries in the last two decades is much due to the activities of the Society. The Society has an decisive role also in making the Scandinavian Parasitology known abroad.

The drive of the enthusiastic and unpaid members of the board is the life force of the Society. The lack of regular funding is its Achilles' heel. The Society has evolved dramatically during its quarter of century of existence. It definitely fills a niche in the Scandinavian scientific system.

Chairmen

Professor Bo-Jungar Wikgren, Åbo (1967-68), Professor Rolf Vik, Oslo (1968-70), Dr. Odd Halvorsen, Oslo (1970-72), Professor Gunnel Huldt (1972-76), Dr. Tor Pettersson, Helsingfors (1976-81), Dr. Jørn Andreassen, Copenhagen (1981-85), Dr. Peter Nansen (1985-89), and Dr. Hans-Peter Fagerholm (1989-).

Honorary members

Professor Orvar Nybelin, Gothenburg (1969), Professor Bertel von Bonsdorff, Helsingfors (1970), Professor Bo-Jungar Wikgren, Åbo (1976), and Professor Elias Bengtsson, Stockholm (1983).

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Bull Scand Soc Parasitol 1992; 2: 9-29

Anthelmintic resistance in parasitic nematodes of domestic animals. A review with reference to the situation in the Nordic Countries 1992

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Summary

This contribution outlines the problem of anthelmintic resistance in parasitic nematodes of domestic animals. As an introduction, the available anthelmintics for animals are summarised, including the essential pharmacology and mode of action and resistance mechanisms. The drug classes and the parasite species affected are mentioned. The geographical distribution of anthelmintic resistance in the world, Europe and the Nordic Countries is reviewed. Factors of importance to the development of anthelmintic resistance are treated in detail. Recommendations for the prevention of the development of AR are outlined, and methods to detect anthelmintic resistance are described.

Introduction

The history of modern anthelmintics, recently reviewed by Bjørn (1), dates back to the 1920's. Early reports indicate that sodium arsenite was in common use against nematode parasites in sheep. Later, cupper sulphate was claimed to have some effect wich could be enhanced with sodium arsenite, mustard or nicotine (2). These compounds were claimed to be effective against stomach worms, but as means of control, these chemicals were insufficient and often toxic to the animals. In 1938 American researchers discovered anthelmintic properties of phenothiazine. This was the first anthelmintic with a relatively low toxicity to sheep and cattle. In the late 1950's the organophosphorus compounds appeared. Many of them had been developed as insecticides. They proved to be highly efficacious against most helminth parasites. In this decade, the effect of piperazine was discovered. This drug showed a wide margin of safety.

The modern broadspectrum anthelmintics

The anthelmintics available today are characterized by a wide safety margin and a broad spectrum of activity against helminth species. On the basis of the mode of action, these drugs can be subdivided into 5 classes of anthelmintics. They are listed in Table 1.

Table 1. Classes of broadspectrum anthelmintics

Class I Benzimidazoles and pro-benzimidazoles

Class II Neuromuscular acting compounds

Class III GABA acting compounds

Class IV Salisylanilids and substituted nitrophenols

Class V Acetylcholine esterase inhibitors

In the following, only the essential characteristics of the 5 drug classes are summarized. More extensive reveiws of the of the anthelmintics in different animal species are given in the reference list(,3 4).

Table 2. Class I anthelmintics, benzimidazoles and pro-benzimidazoles

Benzimidazoles	***Pro-benzimidazoles***
thiabendazole fenbendazole oxfendazole albendazole parbendazole mebendazole flubendazole oxibendazole cyclobendazole luxabendazole	*febantel thiophanate netobimin*

In table 2 anthelmintics belonging to class I are listed. The development of thiabendazole launched a new area in the history of anthelmintics. This drug had a very broad spectrum of activity and an extraordinary wide safety margin. It was found to be highly efficacious against most gastrointestinal nematodes in many animal species and man. This drug formed the basis for the development of a number of related drugs, also known as benzimidazoles (BZ). The parent compounds of the pro-benzimidazoles do not have anthelmintic properties. However, after being administered to the host, these drugs are metabolized in the liver to anthelmintically active BZ (4). The metabolites are BZ themselves, and effective against a broad spectrum of helminths.

Mode of action: The benzimidazoles exert their effect on the intracellular polymerization of the tubulin molecules to microtubules (5). These intracellular protein elements play a very important role as a cytoskeleton and as intracellular channels of

transport. The cellular functions are disrupted, and the worm dies. The best effect of BZ is achieved by high concentrations over extended periods of time (6).

Resistance mechanisms: BZ adheres to certain binding sites at the ß-tubulin molecules (5). Recent research has established that the binding sites at tubulin from benzimidazole resistant nematodes are changed in a way so that the drug cannot combine with the site of action, i.e. the benzimidazoles will not induce the above mentioned effects. As all benzimidazoles share the same mode of action, it implies that once resistance has developed against one of the BZ, resistance at some degree will also be present against the rest of the benzimidazoles. This phenomenon is also known as *side resistance*.

Table 3. Class II anthelmintics. Neuromuscular acting compounds

Imidazothiazoles	***Tetrahydropyrimidines***
tetramisole levamisole butamisole	*pyrantel morantel oxantel*

Both types of drugs are formulated as powders, and levamisole is also found as an injectable. Some formulations may have a bitter taste. After administration these anthelmintics are readily absorbed to the blood circulation, with a peak concentration after approximately 30 minutes (7). The major part of the dose is eliminated within 24 hours.

Mode of action: These anthelmintics induce their effect via the acetylcholine receptor in the nervous system of the worm. The drug combines with the α-unit of this receptor, causing persistent depolarization of muscle cells (8). This leaves the worm in a spastic paralysis, and the worm will be removed by gut motility. The side effects are attributable to a similar effect on the host's nervous system.

Resistance mechanism: The mechanism of resistance against the neuromuscular acting compounds has not been solved completely, but recent investigations indicate a loss of receptor binding sites or a change in a specific binding site (9). In this class of anthelmintics, primary levamisole resistance generally also confers resistance to pyrantel/morantel. However, in two instances it has been found that pyrantel resistance in *Oesophagostomum dentatum* of pigs and *Trichostrongylus colubriformis* of sheep (11) displayed full susceptibility to levamisole. These observations may indicate that the receptor bind-

ing site is slightly different, hence selection for pyrantel resistance does not automatically select for levamisole resistance.

Table 4. Class III GABA-acting anthelmintics

Avermectins	***Piperazines***
ivermectin	*piperazin salts*
moxydectin	
doramectin	
milbymycin	

Pharmacological properties: This class consists of two distinct types of drugs. The piperazines are old, wellknown compounds with less broadspectrum activity. These drugs are easily dissolved in water, but only slightly soluble in organic solvents. They are formulated as powders, and have a slightly acidic taste. After administration they are readily absorbed, metabolized and eliminated by the host. Piperazines have a wide safety margin (3).

The avermectins represent the newest type of anthelmintics. They are produced by the fungus *Streptomyces avermitilis,* a fungus isolated from Japanese soil samples in the late 1970's (2). The avermectins are macrocyclic lactones insoluble in water, but soluble in some organic solvents and dimethyl sulphoxide. They are stable under normal storage conditions. The ivermectins are marketed as an injectable formulation, as a mixture for pour-on on the back and as a paste. After subcutaneous injection, the drug is slowly absorbed from the injection site to the bloodstream. Maximum concentration is achieved after 48-72 hours. Elimination is slow and takes place over several weeks. Ninety eight percent is excreted almost non-metabolized in the faeces. If ivermectin is administered orally, elimination is terminated within few days (13). In addition, it will be degraded in the rumen of cattle, sheep and goats, hence a substantially reduced effect will be observed (14). Dermal application results in a similar pharmacokinetic profile as after injection. The ivermectins have a very wide safety margin. However, young calves and some breeds of dogs have become paralysed after injection. Avermectins have an extraordinarily broad spectrum of activity at low concentrations, including effect on common ectoparasites. There is no effect against liverflukes or tapeworms. Due to the long persistence in body compartments, a treated host can be regarded as protected against reinfection with nematode parasites for 2-3

weeks.

Mode of action: Avermectins act on receptors in the nervous system of the worm. Presynaptic neurones are stimulated to release τ-aminobutyric acid (GABA), a neurotransmitter that causes stabilization of the post synaptic nerve. In addition, avermectin opens the chloride channels wich allows chloride-ion influx. The GABA and the Cl-influx effect brings the worm into a flaccid paralysis, it looses its ability to localize at the predilection site, and is removed by gut motility (12).

Mechanism of resistance: There are only few reports on resistance against ivermectin. The mechanism of resistance is under study and needs still to be clarified. A loss of a receptor binding site has been proposed.

Class IV Salisylanilids and substituted nitrophenols

bromsalan	*disophenol*
c l o s a n t e l	*nitroxynil*
n i c l o s a m i d	*brotianid*
oxyclosamid	
rafoxan	

Class V Acetylcholine esterase antagonist

dichlorvos	*coumaphos*
trichlorophon	*haloxon*
naphthalophos	

The anthelmintics in class IV and V are only used to some extent, probably because of their narrower spectrum of activity. Class IV consists of salisylanilids and substituted nitrophenols. These drugs are typically used against bloodsucking parasites, because the compounds bind tightly to plasma proteins after absorption. This feature is also responsible for a long time of action, because the elimination of these drugs is very slow.

Some of these anthelmintics are now finding more extended use in areas where multiresistant *H. contortus is* present. Organophosphorus compounds are only used to a limited extent and will not be discussed.

Anthelmintic resistance

Definition of anthelmintic resistance:

Australian research workers have formulated the following definition of anthelmintic resistance:

Anthelmintic resistance is defined as a significant increase in the ability of individuals within a strain to tolerate doses of a compound which would prove lethal to the majority of individuals in a normal population of the same species.

Anthelmintic resistance (AR) has been found against drugs in all five anthelmintic classes. Table 5 summarizes the magnitude of the problem within each class.

Table 5. Anthelmintic classes affected anthelmintic resistance

Class I, BZ and pro-BZ	+++++
Class II, NM compounds	++++
Class III, GABA-acting drugs	++
Class IV, Salisylanilids	+
Class Y, Ach-ase antagonists	+

As seen in Table 4, BZ anthelmintics are the most frequently affected drugs. One could speculate that helminths more easily may develop resistance against this class than any other class of anthelmintics. However, laboratory studies do not support such a hypothesis. The explanation for the high prevalence of AR in this drug class is mainly that BZ, since the marketing of thiabendazole have been the most widely used anthelmintics (4). Further, all BZ share the same mode of action, which means that once resistance has developed against one BZ, some degree of resistance against the rest of the BZ will be present. In some countries BZ-R became a real problem in the beginning of the 1980's. Then farmers shifted to the class II anthelmintics, but in the late 1980's the same farmers faced severe problems with resistance against this drug class as well. In some countries levamisole and morantel resistance is just as widespread as BZ-R (4). In 1988 AR was found against ivermectin in *H. contortus* of sheep in South Africa (16) and reports from this country now state that resistance against this drug is widespread (17, 18). AR in class IV is now spreading (18), but in class V, AR is patchy and not very common (18).

Helminth species affected by anthelmintic resistance

AR is primarily confined to trichostrongyle nematodes of grazing ruminants, but the problem has also been detected in horses and pigs. Table 6 summarizes the species in which AR has been found.

Table 6. Helminth species affected by anthelmintic resistance

Sheep and goats	***Cattle***
Haemonchus contortus	*Ostertagia ostertagi*
Trichostrongylus colubriformis	*Cooperia onchophora*
Ostertagia circumcincta	
Nematodirus battus	
Cooperia curticei	
Fasciola hepatica	
Pigs	***Horses***
Oesophagostomum dentatum	*Cyathostominae*
Oesophagostomum quadrispinulatum	

The largest number of resistant nematode species have been found in sheep and goats. This reflect the circumstance that these animal species in many parts of the world are the most important source of protein. To ensure a high level of productivity, stock is subjected to frequent anthelmintic mass treatments. As an exception, AR was recently AR was detected in *Fasciola hepatica* against rafoxanide and closantel, a class IV anthelmintic (19). In cattle resistance has been detected in the important abomasal nematode *Ostertagia ostertagi* against morantel (20). However, resistance first appeared after 4 generations of selection in the laboratory. Resistance against *Cooperia oncophora* has also been described. In Denmark AR was found in the nodular worms of pigs, *Oesophagostomum dentatum* and *Oesophagostomum quadrispinulatum (21)*. The largest and probably the widest spread problem of AR is found in the small strongyles *(Cyathostominae)* of horses (22). In countries where horses are kept for sport and leisure, owners are very anxious to keep the horses clean of worms, which is done by means of frequent anthelmintic treatments. The problem is highly prevalent all over the world, but luckily confined to the less pathogenic small strongyles.

Geographical distribution

The first report on anthelmintic resistance (AR) stems back from 1957 when resistance against phenothiazine in *Haemonchus contortus* of sheep was diagnosed in Kentucky, USA (23). In the same area, resistance against thiabendazole was found in the same nematode in 1964, 4 years after this new anthelmintic was marketed. During the next decade a number of field reports on benzimidazole resistance (BZ-R) in sheep nematodes, like *Ostertagia circumcincta, Trichostrongylus colubriformis,* appeared in various parts of the world. AR is now found on all con

tinents (4). The highest prevalence of AR is found in the Australian sheep industry. Other countries, like New Zealand, South Africa, Brazil, and Argentina, are severely affected by AR (4). On the northern hemisphere reports on AR have emerged mainly during the 1980's. AR has been documented in Great Britain, Germany, Belgium, The Netherlands, France, Denmark,Sweden, Norway, Switzerland and Austria (24). In most countries AR has been diagnosed after specific investigations, rather than after experiencing brake down of control. This is probably attributable to the subclinical course of most helminth infections in ruminants in the temperate areas of the northern hemisphere.

In Scandinavia only few cases of AR have been detected. In horses AR is evidenced in Norway (25) and Sweden (26). In Denmark a survey established benzimidazole resistance in 13 of 16 studs studied (27). This study also established that horses on average were treated 7.1 times/year. A study on the occurrence of AR in sheep nematodes has been performed in Denmark (28). Twentytwo farms were studied, and AR was detected on 7 farms. Levamisole and benzimidazole resistance was found on 3 and 4 farms respectively. The first instance of Ar in pigs wasdetected in pigs in 1987 (21). An isolate of nodular worms consisting of *Oesophagostomum quadrispinulatum* and *Oesophagostomum dentatum* was found to be highly resistant to pyrantel. This was confirmed later by in vivo studies (29). Levamisole resistance and cross resistance to pyrantel was found in the same species in another isolate (10). The prevalence of AR in pig nematodes in Denmark is not known. However, a 3-year epidemiological survey initiated in 1992 will establish the distribution and the significance of AR in Danish swineherds.

Factors of importance in the development of AR

The development of AR may at first sight seem to be a matter of the less susceptible worms surviving treatment and hereafter passing eggs which ultimately will give progeny to more resistant worms. It is probably true that this is the concept of selection for AR, which is depicted in Figure 1. However, many constraints are facing the parasite during its lifecycle and these factors will also influence the success of resistant individuals reaching the reproductive state. In Table 7 the most important factors in the development of AR are summarized, and their significance will be discussed in the following.

Frequency of anthelmintic treatments:

A number of surveys on AR univocally conclude that the more frequently parasitized animals are treated with anthelmintics, the higher the risk for development of AR. If the distance between treatments approaches the pre-patent period, development of resistance may be very fast as only resistant individuals surviving consecutive treatments will mate.

Table 7. Factors of importance in the development of AR

Non-parasitic factors

Frequency of anthelmintic treatment
Time of treatment
Use of the same class of drugs for several years
Size of dosage used
Administration and pharmacokinetic behavior
Management of livestock

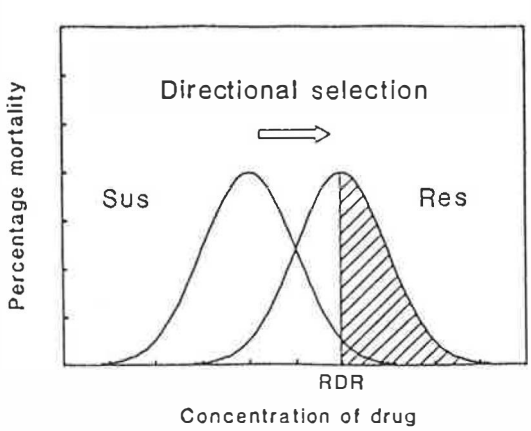
Parasitic factors

Biological factors
Size of refugia
Fecundity of helminth
Generation interval
Regulation and turn over of host population

Genetic factors

Initial number of resistant alleles in population
Mode of inheritance
Sex dependent susceptibility to anthelmintics
Interaction between resistant alleles and parasite genome

Figure 1. Concept of anthelmintic resistance selection



The progeny of such matings will probably have a higher resistance status compared to matings between susceptible and resistant individuals. In the racing horse enterprises it is not unusual that all horses are treated every 4-6 weeks. This is probably the main reason why the problem of AR is so intense in this animal species. In some intensive sheep productions the frequency of treatments may approach the situation in horses, but in general, lambs in most countries will receive 2-5 treatments per year. In intensive goat farming animals are often treated with 4 weeks intervals. This combined with the generally high susceptibility to helminth infections and the lower efficiency of anthel-

mintics in goats has caused AR to develop very quickly and to serious levels in this animal species. With respect to cattle, the generally lower frequency of anthelmintic treatments may be the explanation of the few instances of AR in helminths of this species.

Time of treatment: On permanent pastures without relocation of stock, anthelmintic treatments traditionally have been induced when clinical symptoms appeared. Such treatment would have been instituted when the pasture level of infection was high, leading to disease inducing populations of worms. Wiewed with population biological eyes, the total worm population at such times will be at its highest in numbers, with the greatest proportion of them at pasture. Resistant worms surviving treatments will produce resistant progeny, but due to the high number in the total population, the resistant fraction will be "diluted", i.e. the resistant fraction will be very small compared to the susceptible population. In the northern hemisphere this situation will be present late in the grazing season, typically from August and onwards. Treatments at the beginning of the grazing period, the socalled *strategic treatments,* have been advocated by parasitologists in order to reduce pasture contamination later in the grazing period. Such treatments may theoretically be dangerous with respect to the development of AR. At this time the entire parasite population is at its lowest level in numbers. The greatest proportion will be overwintering larvae on pasture. They will form the first adult population subjected to anthelmintic treatment. Survivors of such a treatment will be the sole source of re-contamination, and this "resistant" fraction will constitute the largest proportion compared to the susceptible fraction. These larvae may become adults and subject to a new treatment, and a new strong selection will take place and result in more resistant offspring which will form the overwintering population. The adoption of such a worm control program may result in rapid development of resistance.

Use of the same class of drugs: From the introduction on anthelmintics, it follows that anthelmintics belonging to the same class share the same mode of action and also resistance mechanism. This means that if drugs from the same class are used over successive years, their common resistance mechanism will be selected. Surveys on anthelmintic usage and anthelmintic resistance clearly substantiate that usage of anthelmintics from the same class is a risk factor in the development of resistance(30). To minimize AR development, anthelmintics from different classes should be used on a rotational basis. This will be discussed later under prevention of development of AR.

Size of anthelmintic dosage: Viewing back on Figure 1 it is evident that the concentration of anthelmintic to which the parasite is exposed is of ultimate importance for the effect on the host confined population. Unexposed helminth populations show a tolerance distribution against anthelmintics, i.e. a varying degree of susceptibility to the drug. This is also recognized by drug companies when the recommended dose for effective treatment is established. Until recent times an anthelmintic dose had to be able to eliminate 95% of the host population of worms to be recognized as an efficient anthelmintic. Now there is a general agreement that all worms should be eliminated by treatment. This sharpening of the criteria for an efficient anthelmintic has the following background: In the early days when thiabendazole was marketed, it was found to be highly efficacious, and parasitologists and farmers' advisers recommended to halve the dose rate in order to save the farmer for costs when purchasing anthelmintics. However, this advice may in fact have contributed to the high frequency of AR in the class I anthelmintics. Underdosing is a risk factor. This is supported by studies on anthelmintic usage at Australian sheep farms. They revealed that farmers seriously underestimated the bodyweight of the sheep (30). As anthelmintics must be administered according to the bodyweight, a number of farmers gave too low doses of anthelmintic to their sheep. Detailed epidemiological analyses have established that farmers who did underestimate the bodyweight of his sheep to the highest degree, also had the most severe problems with AR. This is in fine consonance with the theory of selection of anthelmintic resistance as outlined in Figure 1.

Pharmacokinetic behavior and mode of action: After administration, anthelmintics show varying pharmacokinetic behavior, i.e. levamisole reaches a peak concentration in 30 minutes, BZ after 36 hours, ivermectin after 48-72 hours. Based on the mode of action, it is important to obtain specific profiles in order to get the optimal anthelmintic effect. For class I anthelmintics it is crucial to get a high level of concentration at the infection site for an extended period of time in order to allow the interference of BZ with the microtubuli synthesis. When the full effect is obtained, it is important that the drug is eliminated quickly. For Class II anthelmintics it is important to get a high peak concentration, instantly follow by a rapid elimination. Such a profile will secure the paralyzing "knock down" effect on the worms. Even after prolonged high concentrations worms can regain motility and migrate back to the predilection site. A number of factors can influence the pharmacokinetic profile. It

has been shown that if the socalled oesophageal groove reflex of ruminants is acting at drenching, a substantial part of the dose will pass the forestomachs straight into the abomasum (31). This will reduce the effect of BZ, because the residence time of the drug at the parasite location will be reduced. Studies have shown that administration of BZ directly into the rumen enhances the effect on the parasites. Recent studies in Australia underline that the content of dietary fibers influences the concentration of BZ's at the infection site. High contents of fibers bind BZ particles and thereby reduce the effect on the parasite.

Formulation of anthelmintic: In recent years various anthelmintics have found new formulations in the form of boluses, either as sustained release or pulse release devices. It is important that these formulations give rise to the above mentioned profiles. One of the first boluses released on the market was the morantel sustained release bolus. This bolus gave rise to high peak concentration shortly after administration, but the concentration slowly declined during the rest of the active period of the bolus. Such a profile may in theory be a strong selector for resistance. Once the concentration reaches the discriminating level, the less susceptible worms survive and reproduce, and resistance may develop. The same concern applies to ivermectin after subcutaneous injection. Some BZ have been formulated into pulse release boluses, releasing a full dose of BZ with 3-4 weeks intervals. If the released dose does not give rise to an optimal effect and worms survive, devices with the release intervals mentioned may induce a strong selection pressure, as only surviving resistant worms will reproduce. However, so far there are no confirmed reports on AR developed after usage of bolus formulations.

Management practices: A number of grazing systems have been developed for sheep and cattle in order to improve pasture utilization and production. In some of them control with anthelmintics against parasites have been incorporated. Such systems are termed "integrated control". An example of such a control scheme is the "dose and move system", where grazing animals are moved to a fresh pasture with low infection after treatment. This has proved to be very efficient with respect to production gain, but it has also been shown that this control method can select for AR, especially if the method is used in a pasture rotation scheme. The explanation is simple. If worms survive treatment, they will produce resistant progeny which will be the sole source of infective larvae on the new pasture after move. If the same procedure is carried out successively with rotation between 3 or 4 paddocks, lar-

vae on all paddocks will be the survivors of resistant worms. Examples from England (33) and Denmark have shown that AR may develop very quickly in such integrated control schemes.

Parasitic factors

Biologicalfactors, size of refugia: Under an anthelmintic treatment regime, the refugia can defined as the part of the total parasite population that escapes the effect of the anthelmintic. Under normal grazing conditions the largest proportion of the total parasite population will be outside the host. This means that only a small fraction of the population, namely the host confined one, will be exposed to the drug and subject to selection. A large refugia will serve as diluent of the resistant surviving progeny. Experimental studies have shown that the size of the refugia has an important bearing on the rate of development of AR (34). Factors that tend to reduce the size of the refugium may increase the risk of development of AR. Such factors could pasture hygiene measures and pasture rotational schemes.

Fecundity of helminth: The reproductive capacity of helminth parasites is variable. For instance, the large roundworm of pigs, *Ascaris suum,* the female produces between 200.000 and 1.000.000 eggs per day. A female of *Ostertagia ostertagi* lay a few hundred eggs per day. The sheep nematode *Haemonchus contortus,* the nematode that most frequently has developed AR, produces between 20.000-100.000 eggs per day. In general, it can be stated that the higher the fecundity of a parasite, the greater are the chances for the parasite to adapt to environmental changes, i.e. to respond to anthelmintic selection pressure.

Generation interval: The shorter the generation interval is for a parasite species, the faster it can respond to selection pressures by genetic shift. Most trichostrongyle nematodes have a generation interval of 6-8 weeks and they may be able to pass 2-4 generations per year, depending on the climate. Only very few helminths with long generation intervals (1 year), like *Fasciola hepatica* and *Nematodirus* spp., have developed AR.

Regulation and turn-over of host population: The host often has certain mechanisms to control the size of the host worm population. In some species the establishment of incoming larvae is reduced, in others some stages of the worm are allowed to rise to a certain level, and for some worms there is a continuous turnover of some or all stages. The effect of turnover, host worm population size and control mechanisms have not been studied in detail in the light of development

of AR, but it is evident that the longevity of adult or larval stages in the host must have an important bearing on the chances of a worm to develop AR. Worms with a short lifespan in the host will have smaller chances to develop resistance compared to those with extended longevity.

Genetic factors, initial number of resistant alleles: AR is a heritable trait. It is generally considered that the genes responsible for resistance before anthelmintics are used excists in very low numbers, and are not of importance in the survival of the parasite, otherwise they would be isolated more frequently in unexposed populations (35). However, some BZ-resistance genes seem to be linked to genes for cold tolerance, at least in H. *contortus* and some fruit fungi. This may serve as an explanation of the fact that BZ-R has developed very fast in some areas.

Mode of inheritance: Only few studies have been performed on the mode of inheritance of AR. The results are somewhat conflicting. In one study is was concluded that BZ-R was regulated by a major single recessive gene (36), and another study concluded that BZ-R was regulated by several genes, i.e. a multigenic trait (37). The same conclusions have been arrived at in studies of levamisole resistance. However, the experimental design and the techniques used in the studies are not the same, and none of the studies employed methods to examine genotypes or phenotypes of single worm specimens. In theory multigenic inheritance would delay development of AR, whereas monogenic resistance may give rise to a fast and sudden presence of AR.

Interaction between resistant alleles and the parasite genome: There are indications that resistant genes may lower the general fitness of the parasite, at least during the selection process, i.e. the fitness of resistant individuals is only superior in the presence of an anthelmintic. However, if the resistant genes reach a high frequency in the population, and if the recombination with the genome occurs, the fitness without presence of an anthelmintic will be high. In such a situation it is unlikely that any reversion against increased susceptibility will take place. In general it has been found that once field isolates have developed AR at such a level that control failure is apparent, the likelihood of reversion to susceptibility is low.

Prevention of development of AR

Anthelmintics have proved to be very useful in the control of parasite infections, and will also in the future play an important role in efficient production of grazing animals. However, the problem of AR has threatened the use of anthelmintics, and international drug companies have stated that new anthel-

mintics with a novel mode of action cannot be expected on the market within the next 2 decades. Hence the source of anthelmintics in the near future is the already existing one. To increase the lifespan of anthelmintics and to reduce the risk of development of AR, several steps can be taken. The most important are mentioned in Table 7.

Table 7. Prevention of development of AR

Use anthelmintics sparingly, examine the need for anthelmintic treatments in the herd
Use high doses of anthelmintics
Use anthelmintic classes in a rotation scheme
Treat new animals effectively before they are introduced to the herd
Include anthelmintic independent methods in control

Use anthelmintics sparingly: Anthelmintics should be used only when necessary. It is very important that advisers issue the right directions for worm control to the farmers. First of all it is very important to examine the parasite problem in the herd. Faecal samples must be taken from at least 10-30 animals from different age categories. Samples should be examined for the presence of helminth eggs and subsequently cultured to yield third stage larvae for species identification. When the parasite problem has been determined, hygiene and management should be evaluated. Based on the collected parasitic data and information about the herd, a control program should be developed. After institution of the control program, its efficiency should be scrutinised by regular examination of faecal samples.

Use high doses of anthelmintics: From the introduction on development of AR it is very clear that suboptimal or subterapeutic doses select for AR. It is therefore important that animals receive the full recommended dose according to the liveweight. If sheep or cattle have to be treated flockwise, the heaviest animal should by weighed. All animals should then be treated with a dose according to the liveweight of this animal.

Use anthelmintic classes in a rotation scheme: If it is found that anthelmintic treatments are necessary in the control of parasites in a herd, anthelmintics from different classes should be used in a rotation scheme on a yearly basis. Such a program could start with the use of an anthelmintic from class I the first year, then a compound from class II the following year, and thereafter a drug from Class 3 the third year. In the 4th year a benzimidazole could then be used again. Both field studies and theoretical computer models have shown that development of resistance can be delayed by such a strategy.

Treat new animals effectlvely before introduction to the herd: The most important way of geographical spread of AR is by transport of hosts harboring resistant worm popula-

tions. If animals, e.g. new breeding stock are to be introduced to the flock, it is wise to treat them with high doses of anthelmintics, maybe a Class I and Class II anthelmintic in combination, both at the recommended dose rate. The treated animals should be kept isolated from the rest of the flock for 3-7 days in order to prevent any dissemination of resistant individuals.

Include anthelmintic independent methods in the control: Effective worm control can be achieved without use of anthelmintics. As an example, grazing of safe pastures can be mentioned , i.e. pastures with no or negligible numbers of infective larvae. Such pastures can be provided after taking a crop of grass for hay or silage. Mixed or alternate grazing between different host species such as sheep and cattle, since they host different helminth species. Remember, do never graze sheep and goats together.

Methods to detect AR

AR may be suspected if there are signs of persistent worm infection despite correct treatment. This is obvious, especially for parasites causing clinical disease. However, most helminth infections in livestock have a subclinical course, and the effect of treatment is seldom obvious. It is therefore necessary to use specific methods to detect resistance, and to monitor resistance status in a flock. Table 8 summarizes the most important methods used to investigate AR.

Table 8. Methods used to investigate AR

In vivo methods

Faecal Egg Count Reduction (FECR) Test
Controlled slaughter assay
Critical slaughter assay

In vitro methods

Bioassays

Egg hatch assays
Larval development assays
Larval motility assays

Biochemical assays

Tubulin binding assay
Isoelectric focusing
Molecular genetic techniques

In vivo methods: The Faecal Egg Count Reduction Test is the most important test to be used under field conditions. The test is simple to perform. At least 10 animals should be selected for each anthelmintic to be tested. All animals should have been left untreated for at least 8 weeks. A proper estimate of the liveweight of the animals should be obtained by means of a scale or other recognized safe methods. Animals are treated with the anthelmintic in question, and a faecal sample of at least 15 gram is taken directly from the rectum. Samples are marked with the identification of the animal, e.g. eartag number. Samples are stored at

ambient temperatures (4-8°C) and transported quickly to the laboratory. 10- 14 days later faecal samples are taken from the same animals. At the laboratory, the faecal samples are subjected to McMaster Egg Count determination and larval cultures are set up in order to differentiate between trichostrongyle species. After the data are obtained, the percentage reduction in faecal egg output is calculated for individual animals. The mean percent reduction in egg output for all animals tested is then calculated. This percentage is also termed Faecal Egg Count Reduction (FECR). **An anthelmintic is regarded as efficient if FECR > 95 %. If FECR < 95 %, and if the lower confidence limit of FECR is less than 90 %, AR can be declared** (38). Larval cultures will show which species are present before and after treatment and possibly indicate which species that might be resistant.

The slaughter assay techniques should be mentioned. These techniques are only used for research purposes and when AR has to be substantiated. At least 10 parasite free animals are infected with the suspected resistant isolate, and another 10 animals are infected with a known susceptible isolate. After the artificial infections have become patent, 5 animal in each group are treated with the anthelmintic in question at the recommended dose. Seven days after treatment all animals are slaughtered, the gastrointestinal tract is removed, and appropriate sections of the tract are examined for the presence of worms. The mean worm number in treated and untreated groups is calculated, and the so called Worm Count Reduction (WCR) is calculated. **If WCR < 95 %, AR can be declared.** A variant of this assay is the critical slaughter assay. Here animals serve as their own controls. Animals are artificially infected, and when the infection becomes patent, all animals are treated. The following 7 days all faeces passed by the animals are collected and examined for eliminated worms. Then the animals are slaughtered and the intestines are examined for the presence of surviving worms.

In vitro methods, bioassays: A number of laboratory bioassays have been developed to measure anthelmintic sensivity in parasite strains. For testing benzimidazole sensivity, a egg hatch test is available (39). This test is based on the ovicidal effect of benzimidazoles. The test is carried out on isolated nematode eggs and is conveniently performed in flat bottomed micro titre plates. Suspensions of eggs are incubated in serial dilutions of thiabendazole at 27°C for 24-36 hours. The number of first stage larvae and unhatched eggs are counted, and the percentage hatch is calculated for each concentra-

tion. A similar assay is carried out on eggs from a known susceptible strain of the same species. The hatch data are subjected to a probit analysis to establish if there is a significant difference between the lines. This assay is very sensitive to changes in the resistance status and will reflect an early development of anthelmintic resistance. This assay can only measure levels of benzimidazole resistance, however. A modification of the assay, called the egg hatch paralysis assay can measure resistance in Class II drugs (40). A third kind of assay should be mentioned, namely the larval development assay (LDA) (41). In this assay a number of anthelmintics are embedded in agar at serial concentrations. Pure eggs are loaded on top of each agar-anthelmintic-concentration, and the assay is incubated at room temperature for 7 days. During this period eggs may develop into third stage larvae. The number of unhatched eggs, L_1, L_2, and L_3 larvae are counted. The data are analyzed as in the egg hatch assay. This assay offers the advantage of testing all anthelmintics at the same time. It is highly reproducible, and sensitive to changes in anthelmintic sensivity in nematodes.

Biochemical assays: Only few attempts have been made to develop biochemical assays to detect AR. The main reason is probably that some mechanisms of resistance remain unknown. A tubulin binding assay was developed in Australia (41). It measures the amount of radiolabelled mebendazole bound to purified tubulin. The assay has proved to be very sensitive to detecting the BZ resistance status in a number of BZ-R strains. However, the assay is not easy to perform, it requires expensive laboratory equipment and special chemicals. Other researchers have attempted to measure esterase activity by isoelectric focusing, but this assay is not able to differentiate distinctly between susceptible and resistant strains. Currently much work is done to isolate the genes responsible for resistant phenotypes, using molecular techniques (43). Such methods may be useful tools in the near future to identify resistant parasites.

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Bull Scand Soc Parasitol 1992; 2: 30-32

Fibreoptic pharyngoscopy for diagnosing throat bots in the reindeer

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Reindeer throat bots, *Cephenomyia trompe* (Diptera: Oestridae) larvae, are considered parasites of major importance, and, together with those of the warble fly, *Oedemagena tarandi*, main indications for antiparasitic treatment (1). According to Skjenneberg and Slagsvold (2), oedema in the pharynx caused by bots may spread to the meninges and brain and cause nervous symptoms, and mature bots may accidentally enter the trachea and even cause fatal bronchopneumonia. Rehbinder (3) found 1st instar larvae in the affected eyes of about 25% of 90 reindeer suffering from keratitis. While warble fly larvae are easy to detect and the effectiveness of antiparasitic treatment is thus easy to evaluate, throat bots have proved difficult to confirm in live animals, and the assessment of the effect of treatment against them has been based only on autopsies. Thus, throat bots have just been excluded from some evaluations of the effects of antiparasitics on reindeer (4,5), while other authors have performed autopsies (1,6). Basic research into throat bots on reindeer and other cervids has similarly been based on autopsies (7,8,9,10) with no possibilities for monitoring the development of the bots in individual host animals.

In order to detect bots in the nasopharynx of living reindeer, a fibreoptic bronchoscope designed for use with human patients (Olympus BF type B2) was tested on 60 one-year-old reindeer calves and 85 hinds on May 11th and 12th, 1991. The animals were restrained on a bench, with one man holding the head straight. The bronchoscope was slowly inserted into the left nostril medioventrally. In four of the 145 animals, the nostril had to be changed to manoeuvre the bronchoscope into the correct place or to get a better view. Less than ten animals coughed during or immediately after pharyngoscopy. No bleeding was observed in any of the animals. The examinations were performed without prior checking of the animals' antiparasitic treatment earlier in December. All but one of the 49 non-treated animals (98%) were found to harbour throat bots; a mean of 23 individuals (S.D. 11) being counted on 27

calves and 21 (12) on 22 hinds, whereas all the treated animals (given ivermectin as a subcutaneous injection, oral paste or percutaneous pour-on) were found to be free of bots.

Eight bot-positive calves were slaughtered immediately after laryngoscopy, and the larvae were collected from these, counted, weighed and their developmental stage determined according to the criteria given by Bennett and Sabrosky (8).

Endoscopy revealed 20 (S.D. 8) bots i the calves, while they were found upon autopsy to be harbouring 57 (48) bots. The proportion of 2nd instar larvae was 35%, the rest being 3rd instars. Endoscopy showed every animal to be positive, but never revealed more than 30 bots, even though the reindeer harboured up to 136 individuals, 99 in this maximum case being 3rd instars (Table 1). It seems that at this time of the year, when the majority of the bots are 3rd instars, the maximum number detectable is about 20-30, the others being situated deeper in the pouch of the tonsil (Tonsilla pharyngis dorso-medialis) described by Rehbinder and Nordkvist (9).

Acknowledgments

We are indebted to DVMs Henrik Wickström and Kimmo Lampinen for lending us their valuable bronchoscopic equipment, and to the staff of the Kaamanen Reindeer Research Station, especially Martti Tervaniemi, for invaluable help.

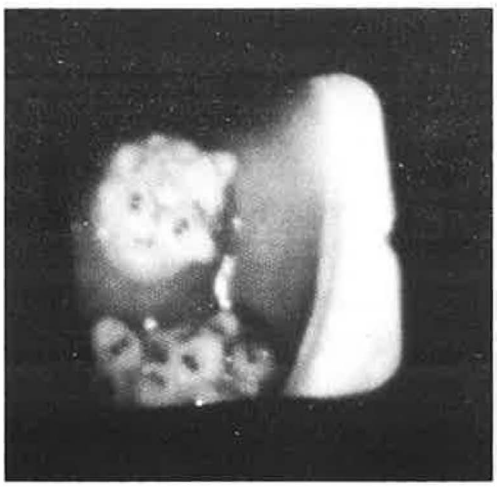

Figure 1. Throat bots in the pharynx of a reindeer observed with fibre-optic pharyngoscopy.

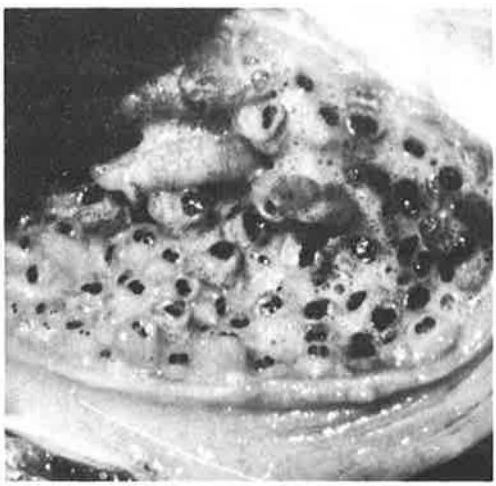

Figure 2. Throat bots in the pharynx of a reindeer calf at autopsy.

*Table 1. Throat bots in reindeer calves examined May 11*th-12th, 1991

Calf number	sex	bots found by endoscopy	bots found at autopsy	bot length min (mm)	bot length max (mm)	2. instars
4383	m	30	55	8	26	11
4388	m	30	136	6	29	37
4389	m	15	18	19	25	0
4396	f	5	7	8	14	7
7974	f	20	20	6,5	27	4
7994	m	20	95	6	30	27
7996	m	20	101	6	24	42
7999	f	20	23	13	23	1
average		**20**	**56,9**	**9,1**	**24,8**	**16,1**

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Bull Scand Soc Parasitol 1992; 2: 33-35

Differentiation between parasitic interstitial hepatitis and mycobacterial lesions in pig livers

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"Milk spots" (interstitial hepatitis) are frequently observed in the livers of slaughtered pigs at meat inspection. *Ascaris suum* larvae are the most common cause of milk spots, though *Toxocara* spp. may also cause similar spots (1). Liver flukes, *Fasciola hepatica* and *Dicrocoelium dendriticum*, and the cestode *Cysticercus tenuicollis* do not seem to be of any importance as a cause of milk spots in pig livers in Scandinavia (1). Due to the failure to demonstrate parasites or parasitic fragments, several workers have doubted that milk spots are in fact of parasitic origin. Sofrenovic (2) observed the presence of small nodules consisting of fibroblasts, histiocytes, and epitheloid and lymphocytic cells. He found no spots with haemorrhages, nor did histological examination in any case reveal parasites. Pallaske (3) suggested in 1931 that liver lesions in swine caused by *Mycobacterium avium* might resemble parasitic interstitial hepatitis. This was confirmed in recent outbreaks of mycobacteriosis in swine caused by *M. avium* (4,5).

Current Norwegian meat inspection regulations for mycobacterial lesions in swine require that carcasses with tuberculous lesions involving organs such as the liver are to be considered as having a generalized infection, and are to be condemned. It is thus very important for the meat inspector to be able to distinguish mycobacterial lesions in pig livers from spots of other origin, especially the "milk spots" caused by ascarid larvae.

Materials and methods

In 1987, approximately 23.000 swine were routinely examined by the local meat inspection authority in Haugesund. All carcasses were examined post mortem according to the current Norwegian meat inspection regulations, including incision of mandibular lymph nodes, and inspection/-palpation/incision of various organs. Samples were taken from livers with suspected tuberculoid lesions when tuberculoid lesions were also found in *lnn. mandibulares* and/or *lnn. jejunales,* and generalized

mycobacteriosis had not been previously diagnosed in the herd, and sent to the National Veterinary Institute, Oslo, for verification. Histological (n=61) and bacteriological (n=12) examination was undertaken according to the methods described by Alfredsen and Saxegaard (6).

Results and discussion

The gross appearance of both parasitic "milk spots" and tuberculous granulomas showed considerable variation. The most common type of parasitic spots was grey-white and diffuse, and not sharply delimitated. Another type appeared as white to grey, pearl-like nodules with a diameter of 1-2 mm. The tuberculous granulomas were commonly grey-white with a diameter of 1 mm, varying in number from just a few seed-like structures easily overlooked, to extensive and confluent lesions more like the parasitic spots.

Histological examination of tuberculous granulomas showed a non-specific picture with eosinophilic leucocytes and histiocytes. Acid-fast bacteria were observed in only a very few sections. Epitheloid cells and polynuclear giant cells were occasionally seen, though these cell types may also be observed in parasitic lesions (1). However, in contrast to the situation with parasitic spots histological examination of the tuberculous granulomas in no case revealed the presence of haemorrhages. Time-consuming bacteriological examination for mycobacteria was often essential to achieve an exact diagnosis.

The gross appearance and histopathological picture were in accordance with the findings of Roneus (1) and Windsor et al. (4). Based on the histopathologic appearance Roneus (1) divided milk spots into two main morphologically-distinct groups: 1) large and small milk spots of the granulation-tissue type and 2) milk spots of the lymphonodular type. Some of the older, large white spots of the granulation-tissue type showed a tendency to form a lymhocytic nodule, indicating the possibility for the transformation of large white spots of the granulation-tissue type into white spots of the lymphonodular type.

The highest prevalence of livers with verified tuberculous lesions was observed in October and November, when 45% of the cases were found. Milk spot livers are also relatively common at this time of the year, and 28% of the livers were condemned due to parasitic spots during the same period.

Twenty-one of the slaughter pigs from one

of the herds had tuberculous lesions in lymph nodes, liver, and sometimes the lungs (generalized mycobacteriosis). These pigs were older than usual, their mean weight being 83.9 kg, whereas 27 younger pigs from the same herd with lesions confined to the lymph nodes of the head and the mesenterium, had an average weight of 75.4 kg. The development of mycobacterial infection in pigs apparently takes a long time, and tuberculous lesions in the liver are most likely to be detected in the oldest slaughter swine. This is in contrast to the usual situation regarding milk spots. Especially in swine herds with poor cleaning practices, the immunity against *Ascaris* larvae may kill the larvae before they reach the liver, reducing the number of, or completely eliminating, white spots in relatively young pigs (7).

Thorough evaluation of the gross appearance of the liver lesions, sometimes combined with histopathology and bacteriological examination for mycobacteria, together with the detection of other lesions in the carcass, especially in the lymph nodes, resulted in 2651 livers (11%) being condemned because of ascariasis (milk spots), whereas 76 (0,3%) livers (and carcasses) were condemned because of mycobacteriosis.

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FAO Collaborating Centre on Helminthology

Section of Parasitology at The Royal Veterinary and Agricultural University, Copenhagen, Denmark, has been elected as an international collaborating centre for the Food and Agricultural Organization (FAO) of the United Nations, with main emphasis on the epidemiology and control of helminth infections in livestock.

Professor Peter Nansen has been designated head of the centre, which was established on January 1st, 1992.

The functions of the centre include:

Provision of technical advice, expertise and consultants on specific subjects to FAO Headquarters, FAO field projects and FAO Member Countries on request.

Contribution to the development of technical cooperation with and among developing countries by providing them with information services and advice, and by stimulating and supporting research and training.

Assistance in the organization and implementation of some training activities on the subjects through workshops, seminars and individually arranged fellowship training.

DANISH CENTER FOR PARASITIC ZOONOSES

Danish Center for Parasitic Zoonoses was founded on June 13th, 1991, and is an open framework incorporating Danish researchers, working with parasites in both animals and man.

The Danish Center for Parasitic Zoonoses was established with the aim of strengthening the collaboration between different disciplines, and with the aim of providing national and international organizations and foundations with a single institution that represents the combined sum of Danish knowledge on parasitic zoonoses.

Parasitic zoonoses are an economic burden in agriculture and pose a risk of disease in man. Examples of important parasitic zoonoses include: trichinellosis, cysticercosis, hydatidosis, cryptosporidiosis, giardiosis and toxoplasmosis.

Parasitic zoonoses also constitute an important health problem in the third world. The rapid growth of large cities in the developing countries, together with an increase in the number of refugees and water resource development, especially irrigation projects - all influence the dynamics of infections caused by parasitic zoonoses.

In Denmark, the interest in parasitic zoonoses is increasing due to the increased recycling of human and animal waste products in agriculture, and because society demands increasing hygienic quality of agricultural products.

The AIDS epidemic has been followed by a series of infections caused by parasites which were previously regarded as very rare. Several of these infections are zoonotic, i. e. transmitted from animal reservoirs, for instance *Pneumocystis*, *Cryptosporidium* and microsporidia.

These infections pose new problems in diagnostics, treatment, and research in transmission pathways.

Denmark has a long tradition for research of parasitic infections, and has contributed especially to our knowledge of infections such as hydatidosis, cysticercosis, trichinellosis and toxoplasmosis. Denmark is well known for its control of veterinary parasitic diseases through public health legislative measures.

The Danish Center for Parasitic Zoonoses:

- represents the combined knowledge of parasitic zoonoses in Denmark
- provides expertise, advice and consultation

to national and international authorities and commercial companies on diagnostics, treatment, control and epidemiology of parasitic zoonoses
- arranges courses, meetings and seminars on diagnostics, treatment, control and epidemiology of parasitic zoonoses
- encourages and supports interdisciplinary, basic and applied research on veterinary and medical aspects of parasitic zoonoses.

The center has supported a European seminar on congenital toxoplasmosis held in January 1992, and a Nordic seminar on Trichinellosis in Game Animals was organized in February 1992, supported by the Nordic Foundation of Game Research. A Scandinavian course in diagnostic techniques of parasitic zoonoses will be held at the Royal Veterinary and Agricultural University, Copenhagen in May 1992, supported by the Nordic Council of Ministers.

The following organisations participate in the center: Danish Bilharziasis Laboratory; Danish Society for Parasitology; Danish Society for Tropical Medicine; Danish Pest Infestation Laboratory; National Environmental and Food Laboratory; National Veterinary Laboratory; Royal Veterinary and Agricultural University, Copenhagen; State University Hospital (Rigshospitalet), Copenhagen; Statens Seruminstitut, Copenhagen; University of Copenhagen, and University of Aarhus.

The center can be contacted either through one of its member organizations or through the board, which consists of:
Professor Peter Nansen, Chairman, Institute of Veterinary Microbiology, Royal Veterinary and Agricultural University, Phone: +45 35 28 27 80, Fax: + 45 35 28 20 79.
Associate Professor Jørn Andreassen, Vice chairman, Institute of Population Biology, University of Copenhagen, Phone: +45 31 35 41 11, Fax: +45 31 39 54 15.
Dr Eskild Petersen, Secretary, Laboratory of Parasitology, Statens Seruminstitut, Copenhagen, Phone: +45 32 68 36 03, Fax: +45 32 68 32 28.

NEWS Baltic Section

Parasitological Societies in Lithuania

At present, in early 1992, there are two parasitological societies in Lithuania, i.e. Society of Helminthology and Society of Protozoology. Both societies were founded years ago by Institute of Zoology and Parasitology (now Institute of Ecology), under Academy of Sciences, Lithuanian U.S.R. This was in accordance with similar structures in USSR.

Society of Helminthology, chaired by Academician Dr. V. Kontrimavicius, was founded in 1958, and has at present 50 members. Society of Protozoology, chaired by Professor S. Biziulevicius, was founded in 1969, having at present 30 members. Members of both societies represent many scientific disciplines, and comprise university (academy) as well as non-university employees.

The main objectives of the societies are to promote parasitological research and continuing education of scientists, teachers, veterinary and medical practitioners etc. The societies organize 3 - 4 meetings per year, usually with invited speakers, or with round-table discussions on various actual parasitological topics. With intervals of 3 years, the Lithuanian societies also organize parasitological conferences, jointly with the other Baltic countries. The two societies periodically publish 'Acta Parasitologica Lituanica', of which recently the 23rd issue was edited.

At present, it is discussed to fuse both societies into one, namely the Lithuanian Society of Parasitology.

The secretariats of the societies have the following address: *Dr. B. Vosylyte, Institute of Ecology, Academijos 2, Vilnius 2600, Lithuania.*

Professor Vygandas Paulikas

Announcement

DANISH CENTER FOR PARASITIC ZOONOSES

Subject: **Nordic training course in parasitic zoonoses.**
Time: 17-23 May, 1992
Place: Royal Veterinary and Agricultural University, Copenhagen, Denmark.
The course will give introduction to parasites shared by animals and man, with emphasis on the interdisciplinary aspects of these infections. The course will include infections common in Scandinavia as well as in tropical countries, and will give an introduction to transmission, epidemiology, control, diagnosis and treatment of these infections. Approximately half of the time will be practicals in diagnostic procedures (microscopy, antibody assays, DNA (detection etc.). Some of the parasites covered will be: the taeniids, trichinosis, anisakiasis, leishmaniasis, toxoplasmosis, cryptosporidia and other intestinal protozoa and tropical parasitic zoonoses. The lectures and practicals will be given by veterinarians, biologists and doctors. The course is supported by Nordisk Forskerakademi (Nordic Council of Ministers).
Organizers: Danish center for Parasitic Zoonoses.
Professor *Peter Nansen, Henrik Bøg, Bente Ilsøe,* (Royal Veterinary and Agricultural University), *Eskild Petersen* (Statens Seruminstitut).
Deadline for application: 1 April, 1992.
Information: Eskild Petersen, Secretary, Danish Center for Parasitic Zoonoses, Laboratory of Parasitology, Statens Seruminstitut, Artillerivej 5, DK-2300 Copenhagen S, Denmark. Phone +45 32 68 36 03. Fax: +45 32 68 38 68.

NEWS FROM THE SECRETARY

The SSP-board had a meeting on February 25 (telephone-meeting). The plans for the next symposium, to be held in Oslo during the autumn 1993 were discussed. The meeting will take place during the period 30 Sept. - 2 October, 1993.

The society is also examining the possibility of arranging a workshop in Iceland on marine parasitology. If the practical and financial matters can be arranged, this meeting will take place after the Oslo symposium.

Danish Center for Parasitic Zoonoses will arrange a course in "Parasitic Zoonoses". Time and place: see special advertisement in this issue.

Arne Skorping

ABSTRACTS from the 15th Symposium of the Scandianavian Society for Parasitology in Uppsala, Sweden, 4 - 5 October 1991.

We are here presenting abstract of two communications which were submitted,but not printed with the proceedings in the first issue of the Bull Scand Soc (1991; 1: 1-171).

THE INFLUENCE OF HOST PHYLOGENY AND ECOLOGY ON THE PARASITE FAUNA OF FOUR *PLEURONECTIDAE* FLATFISH SPECIES FROM NORTH NORWAY.

Nora Kh. Lile.
Institute of Biology and Geology, University of Tromsø. N-9000 Tromsø, Norway.

Are parasite component communities in marine fish influenced by host ecology or phylogeny? Four species of flatfish, flounder *Platicthys flesus,* witch flounder, *Glyptocephalus cynoglossus,* American place, *Hippoglossoides platessoides,* and Atlantic halibut, *Hippoglossus hippoglossus,* caught off the North Norwegian coast at 70 degree N, were examined for macroparasites. Although the fish species inhabited the same general areas, they lived under different hydrological and ecological conditions.

The results were analyzed in relation to parasite-host specificity and host ecology (habitat and diet). Most of the parasite species recorded were generalists in choice of host.

The results suggest that the parasite component community of the North Norwegian righteye flounders are influenced mainly by host ecological factors, rather than host phylogenetic relationships.

EXPERIMENTAL CONCURRENT INFECTIONS WITH *OSTERTAGIA OSTERTAGI* AND *COOPERIA ONCOPHORA* IN CALVES

Fadjar Satrija and Peter Nansen
The Royal Veterinary and Agricultural University, Copenhagen, Denmark.

Objectives. An experiment was carried out to study the effect of concurrent infection with *Ostertagia ostertagi* and *Cooperia oncophora* compared with mono-specific infections with either *0. ostertagi* or *C. oncophora.* Also the effects of different infection levels on the course of concurrent infections were studied.
Methods. Six groups of each four three-month old Jersey male calves were formed. Two groups of calves were infected concurrently with 50,000 infective *0. ostertagi* larvae and 50,000 or 200,000 *C. oncophora* larvae, respectively. Three groups received monospecific infections with either 50,000 *0. ostertagi,* or 50,000 *C. oncophora,* or 200,000 *C. oncophora* larvae. One group of calves remained as an uninfected control group. Faecal and blood samples were collected at weekly intervals, and the animals were slaughtered on day 28 after infection.
Results. The faecal trichostrongyle egg counts were significantly higher ($P< 0.05$) in calves receiving concurrent infections than in monospecifically infected calves, but no significant differences were noticed in this respect between the two levels of concurrent infections. Serum pepsinogen levels of the groups receiving *0. ostertagi* increased by week 3 onward, and the levels remained significantly higher ($P< 0.05$ to $P< 0.001$) than in groups with monospecific *C. oncophora* infections or in the uninfected control group. Mean serum pepsinogen levels of the concurrently infected groups were higher than those of the monospecific *0. ostertagi* group, yet, these differences were not significant.
Post mortem *O. ostertagi* counts and establishment rates were largely comparable in the three *0. ostertagi* infected groups. The mean number of *C. oncophora* and the establishment rate was higher in the concurrently infected groups than respective monospecifically infected groups, but the differences were not statistically significant.
Conclusion. The results indicate that concurrent infections may influence establishment of *C. oncophora,* and subsequently enhance pathogenicity.

NORDISK FÖRENING FÖR PARASITOLOGI
SCANDINAVIAN SOCIETY FOR PARASITOLOGY

Protokoll fört vid generalförsamling i Nordisk Förening för Parasitologi fredag den 4 oktober, kl. 17.30 vid Sveriges Lantbruksuniversitet, Ulltuna, Uppsala, Sverige. Ca 35 medlemmar var närvarande.

§ 1

Generalförsamlingen öppnades av ordförande Hans-Peter Fagerholm. Kallelse och dagordning godkändes.

§ 2

Jörn Andreassen valdes till mötesordförande.

§ 3

Till mötessekreterare valdes Inger Ljungström.

§ 4

Till justeringsman valdes Karin Anderssen.

§ 5a

Verksamhetsberättelsen som utsänts till medlemmarna godkändes och lades till handlingarna.
Föreningens ordförande Hans-Peter Fagerholm kommenterade att föreningen etablerat en parasitologisk publikation, Bulletin of the Scandinavian Society for Parasitology och att Jorun Tharaldsen av styrelsen valts till huvudredaktör. Två forskarkurser är under planering, delvis i föreningens regi: "Parasitära Zoonoser", ansvarig Peter Nansen, Danmark i samarbete med Dansk Center for Parasitära Zoonoser och "Monoclonal antibodies in immunolocalization of parasitic antigens", ansvarig Ewert Linder, Sverige. I verksamhetsberättelsen har ordet parasitic i kursens titel tyvärr blivit felskriven.

§ 5b

Föreningens kassör Arvid Uggla föredrog föreningens räkenskaper och påpekade att porto och framförallt adminstrationsavgifter kraftigt höjts under det sista året. Årets överskott uppgick till ca SEK 11 800. Revisionsberättelsen meddelade att räkenskapen efter granskning befunnits i ordning.

§ 5c

Styrelsen beviljades full ansvarsfrihet för den gångna verksamhetsperioden.

§ 6

Arvid Uggla har avsagt sig omval till kassör, men stod till förfogande som styrelsemedlem. Följande förslag till val av styrelse vann understöd och antogs enhälligt av generalförsamlingen.

till ordförande omvaldes	Hans-Peter Fagerholm, Finland.
till vice ordförande omvaldes	Ewert Linder, Sverige.
till sekreterare omvaldes	Arne Skorping, Norge.
till kassör valdes	Birgitte J. Vennervald, Danmark.
till styrelsemedlem valdes	Arvid Uggla, Sverige.

§ 7

Till suppleanter omvaldes Tor Bakke, Norge och Sigurd Richter, Island.

§ 8

Till revisorer omvaldes Göran Bylund, Finland och Sven Nikander, Finland.
Till revisorsuppleant omvaldes Flemming Frandsen, Danmark.

§ 9

Peter Nansen presenterade föreningens nya publikation, "Bulletin of the Scandinavian Society for Parasitology", som skall utkomma 2 gånger per år. Finanseringen skall täckas av medlemsavgifter, annonser och bidrag från Nordisk Ministerråd. Alla medlemmar får publikationen gratis. På förfrågan om möjlighet till prenumeration ex bibliotek och till vilken kostnad beslutades att styrelsen utreder dessa frågor.

§ 10

På styrelsen föreslag höjdes medlemsavgiften till SEK100 (studenter SEK50). Inlösen av checkar från annat land betingar en avgift på SEK 20, varför styrelsen uppmanade medlemmarna att betala för flera år eller direkt till kassören vid exempelvis SSP-symposium. Styrelsen skall utreda huruvida medlemsavgiften i framtiden skall vara i 2-årsperioder. Förslag lägges vid nästa generalförsamling.
Den avgående kassören påpekade att den nya kassören Birgitte Vennervald tillträder omedelbart, varför medlemsavgifter skall betalas till henne.

§ 11

Generalförsamlingen accepterade med tacksamhet en inbjudan, som framfördes av Prof. Odvar Helle, Oslo, att de vid Norges Veterinärskole anordnar nästa symposium i början av augusti 1993.

§ 13

Meddelades att Professor August Brinkman Jr har avlidet.

Stockholm den 22 oktober 1991

Inger Ljungström
mötersekr.

Karin Anderssen
justeringsman

GUIDELINES FOR CONTRIBUTORS

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

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

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

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

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

Books and other monographs:

4. Austin B, Austin DA. Bacterial fish pathogens: disease in farmed and wild fish. Chichester: Ellis Horwood, 1987
5. McFerran JB, McNulty MS, eds. Acute virus infections of poultry: a seminar in the CEC programme, Brussels 1985. Dordrecht: Martinus Nijhoff, 1986. (Current topics in veterinary medicine and animal science 37)
6. Sosialdepartementet. Tsjernobyl-ulykken: Rapport fra Helsedirektoratets rådgivende faggruppe. Oslo: Universitetsforlaget, 1987 (Norges offentlige utredninger NOU 1987: 1)
7. Thornhill JA. Renal endocrinology. In: Drazner FH, ed. Small animal endocrinology. New York: Churchill Livingstone, 1987: 315-39

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BULLETIN OF THE SCANDINAVIAN SOCIETY FOR PARASITOLOGY

VOL. 2 No. 1 APRIL 1992

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