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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.

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

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PARASITES AS BIOLOGICAL INDICATORS[1]

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Abstract

Parasites can be used as indicators of various aspects of the biology of their hosts. This paper deals with two of these aspects: parasites as biological tags in population studies of aquatic organisms, and parasites as indicators of pollution. Three types of population study are considered: stock discrimination, recruitment migrations, and seasonal migrations. A method of using parasite transmission processes to provide early warning of change in environmental conditions and to monitor the biological effects of marine pollution is proposed. Criteria are suggested for the selection of appropriate indicator parasites for both aspects. Examples are given of successful biological tag studies and of specific parasite transmission processes which are likely to be particularly sensitive to environmental change.

Introduction

Parasites can be used as biological "tags", "indicators" or "markers" to provide information on various aspects of the biology of their hosts. This paper deals with two of these aspects: (1) parasites as biological tags in population studies of aquatic organisms, with the emphasis on marine species; and (2) parasites as indicators of environmental change with particular reference to marine pollution. A new approach to the latter aspect is proposed, with the aim of providing an early warning system for marine pollution by suggesting how to identify and monitor the transmission stages in parasite life cycles that are most vulnerable to changes in environmental conditions.

Parasites as tags in host population studies

The basis of using parasites in host population studies is that the geographical range of a host species overlaps the endemic areas of certain of its parasites. If a host population (or individual) sampled from outside the endemic area of one of these parasites is found to be infected with that parasite, the conclusion can be drawn that the infected host population (or individual) had occupied the area of overlap at some time previously. This is biological tagging in its simplest terms; details of its application to different

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types of population study are discussed below.

Biological tagging of aquatic organisms using naturally occurring parasites has certain advantages over "mechanical" tagging using artificial tags. Biological tagging is a natural process which avoids the stress associated with capture and handling with its attendant doubts concerning the possible abnormal behaviour of tagged individuals. It is much cheaper to implement because samples of host specimens can usually be obtained from commercial catches or from routine research vessel cruises without the need to devote expensive sea time specifically to the process of tagging. It is also probably the most appropriate method of tagging for delicate host species, such as small clupeoid fish, for deep-water species which cannot survive the reduction in pressure involved in their capture, and for crustaceans that shed artificial tags when they moult. However, biological and mechanical tagging should not be regarded as alternatives, but rather as complementary methods that should be used together where practical, possibly in combination with other forms of identification such as meristic and biochemical methods.

Selection criteria

Sets of criteria for selecting appropriate tag parasites have been proposed and discussed by Sindermann (1,2), Kabata (3), MacKenzie (4,5), Lester (6) and Moser (7). As we acquire more information about the biology of parasites of aquatic organisms, it is becoming clear that some of the early criteria proposed are unnecessarily restrictive and that we must be more flexible in applying them as general guidelines rather than as strict rules. The most important of these guidelines are as follows:

1. The parasite should have significantly different levels of infection in the target host in different parts of the study area.
2. The parasite should have a long lifespan in the target host. (The minimum acceptable lifespan will depend on the type of population study, as discussed below).
3. The parasite should be easily detected and identified, otherwise time can become a limiting factor.
4. Finding the parasite should involve the minimum of dissection. Site-specific parasites are time-savers.
5. The parasite should have no marked pathological effects which might change the behaviour of the host.

Different types of population study

1. Stock discrimination

This type of study aims to identify distinct intraspecific populations which are distinguished by different patterns of behaviour, usually in relation to spawning and migrations, but sometimes to feeding.

a) Marine fish

A good example of the use of parasites in stock discrimination of marine fish is the work of Kabata (3,8) who used four species of myxosporean protozoans parasitic in the gall bladder as tags for gadoid fish in the Northeast Atlantic Ocean. Infections are long-lived and, because the parasites are so

site-specific, gall bladders could be preserved at sea and returned for later examination in the laboratory. Large numbers of fish could thus be examined in a short period of time. In the North Sea, Kabata was able to distinguish separate northern and southern stocks of whiting, *Merlangius merlangus*, by marked differences in the relative proportions of fish infected with different myxosporean species. Similarly, around the Faroe Islands he was able to distinguish different stocks of haddock, *Melanogrammus aeglefinus*, on the main plateau and its outlying banks. The basic reasoning was that, if these populations of fish mixed indiscriminately, such marked differences in the geographical distributions of the different parasite species would not exist.

b)Marine mammals

The first study to indicate the potential of parasites as tags for marine mammals was published in 1991. Dailey and Vogelbein (9) showed that larval acanthocephalans of the genus *Corynosoma* may be of value in identifying regional Antarctic stocks of sperm whales, *Physeter catodon*. *Corynosoma mirabilis* was found in whales in the Indian Ocean sector from Africa to Australia, *C. bullosum* was found only in whales from the southern Pacific, and *C. singularis* was found only in the southwestern Atlantic.

c) Marine invertebrates

Although little use has yet been made of parasites as tags for marine invertebrates, there is enormous potential. Commercially important molluscs and crustaceans serve as intermediate hosts for a wide range of helminth parasites and as definitive hosts for protozoans and crustaceans. A search of the literature has revealed three biological tag studies on prawns (Mathews et al. (10), Owens (11), Thompson and Margolis (12)), two on lobsters (Brattey and Campbell (13), Uzmann (14)), two on squid (Bower and Margolis (15), Smith et al. (16)), and one on euphausiids (Dolshenkov et al. (17)).

Owens (11) used the bopyrid isopod *Epipenaeon ingens* to distinguish breeding stocks and to follow migrations of banana prawns, *Penaeus merguiensis*, in the Gulf of Carpentaria in northern Australia. Prawns initially acquire infections as juveniles on their estuarine nursery grounds and Owens calculated that the lifespan of the parasite covered the period of growth, offshore emigration and maturation of the prawns. The parasite was only found on prawns from estuaries and offshore grounds in the southern part of the study area, showing their limited migratory range along the coast.

2. Recruitment migrations

These are migrations made by aquatic organisms from nursery areas to adult feeding and spawning grounds. The range of potential tag parasites for investigating these migrations is much narrower than that available for stock discrimination because they are subject to the following additional selection criteria.

i) The parasite must infect the young host on the nursery ground and the host should not be susceptible to further infection as an adult.

ii) The parasite must have a lifespan long enough for it to remain identifiable in the mature adult host.

a) Anadromous fish

The biology of anadromous fish makes them particularly suited to the use of parasites as tags and there have been many successful studies. The literature includes the classical work of Margolis (18), who used two helminth parasites to follow the migrations of sockeye salmon, *Oncorhynchus nerka*, from fresh water nurseries in North America and Asia to adult feeding grounds across the North Pacific. Plerocercoids of the cestode *Triaenophorus crassus* were found only in juvenile salmon in North American rivers, and the nematode *Cucullanus* (=*Dacnitis*) *truttae* was only found in those from Asian rivers. Both parasites were long-lived and survived the transition from fresh water to marine conditions. Margolis (19) later used two myxosporean protozoan parasites to trace sockeye caught in a marine fishery to their lakes of origin within a single river system in British Columbia.

b) Marine fish

Many marine fish, particularly pelagic species, have nursery grounds located considerable distances from the feeding and spawning grounds of the adults. The Atlantic herring, *Clupea harengus*, is such a species. MacKenzie (20) used the metacercariae of two species of renicolid digeneans and a cestode pleroceroid to follow the recruitment migrations of herring from two nursery areas to the main commercial fishing grounds for adult herring in the North Sea and adjacent waters. The digeneans infect herring only in the first summer of life. The cestode can be acquired by adult as well as juvenile herring, but the fishing grounds investigated lay outside its endemic area. Once infected with these parasites as juveniles, herring are tagged for life.

3. Seasonal migrations

Many aquatic species make annual migrations from feeding to spawning areas, and the routes and timing are often characteristic features of different intraspecific stocks. From the biological tagging point of view, the Baltic Sea is particularly interesting because the range of salinity from purely marine in its western part to almost purely fresh water in the east has such an enormous influence on the parasite fauna. Grabda (21) used a marine parasite to separate a stock of herring which migrates to feed in the high salinity waters of the eastern Baltic and North Sea from three others which remain to feed in lower salinity waters within the Baltic. Larvae of the nematode *Anisakis simplex* can only be acquired through feeding in areas of high salinity, so its presence in herring caught in the southern Baltic identified the migratory stock. Later Grabda (22) used the same nematode, together with a cestode plerocercoid, to prove the North Sea origin of a population of garfish, *Belone belone*, in the southern Baltic.

Parasites as indicators of marine pollution: a proposed early warning system

Good biological indicators to monitor the effects of pollutants on marine organisms must be exceptionally sensitive to environmental change so that a significant reduction in their numbers can be used as a warning of deteriorating conditions before the majority

of less sensitive organisms are seriously affected. There are good reasons for focusing on parasites in the search for highly sensitive indicators. In the first place, there are more parasitic than free-living species (23, 24) and parasitic organisms show enormous biological diversity, reflecting adaptations to the parasitic way of life in different types of host and in diverse sites and environments in these hosts. In metazoan parasites with complex life cycles, the different developmental stages have widely differing biological requirements, so that each stage must be assessed separately for sensitivity to environmental change, thereby further widening the choice of potential indicators. The effects of pollution on parasites of aquatic organisms were reviewed recently by Khan and Thulin (25).

Attempts made so far to use parasites as indicators of marine pollution have concentrated on the pathology associated with parasitic infections. McVicar (26) pointed to lack of sensitivity as one of the main limitations of pathology as a monitoring tool. That is, most obvious pathological conditions are the end result of a sequence of events that may have occurred over a prolonged period and therefore record changes in environmental conditions dating back some considerable time. This is particularly true of pathology resulting from infections with metazoan parasites, which have longer generation times than micro-organisms such as viruses, bacteria and protozoans, and consequently take longer to build up their populations to levels likely to cause disease. Some of the free-living transmission stages of these metazoan parasites, particularly the helminths, are delicate short-lived larvae that are highly sensitive to changes in environmental conditions. A more rapid response to environmental change is therefore likely to be observed by monitoring certain aspects of the transmission of metazoan parasites.

Transmission routes and "windows"

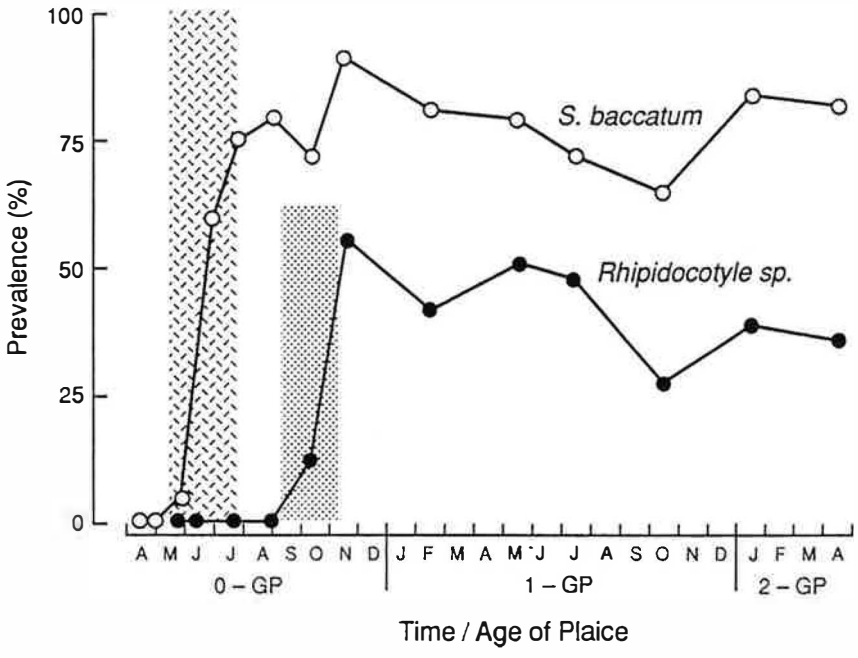
A transmission route is the pathway through which a parasite progresses from one developmental stage to the next and from one host to another. Most parasites have a range of possible host species resulting in a number of possible transmission routes. The number of routes is greatly increased in parasites with complex life cycles involving developmental stages in two or more hosts. Such parasites also show different degrees of host specificity at different stages in their life cycles. For example, a digenetic trematode usually infects only a single species, or a group of closely related species, of primary host (usually molluscan), but may have a wide range of intermediate or final host species. At each stage in the life cycle the range of host species can be arranged along a continuum from those to which the parasite is best adapted and which provide it with the highest probability of successful transmission to the next stage, to "dead-ends" or "ecological sinks" through which no further development is possible (27). Many cestodes show a similar pattern. For example, the trypanorhynch cestode *Grillotia smaris-gora* (= *Grillotia angeli*) infects a number of teleost fish intermediate hosts, including the pelagic species mackerel *Scomber scombrus* and

scad *Trachurus*. However, MacKenzie et al. (28) showed that it is highly unlikely that pre-adult worms in these hosts will ever complete their life cycles because the final host, the monkfish *Squatina squatina*, is a bottom-living ambush predator which is unlikely ever to prey on pelagic fish. Transmission through demersal fish hosts such as the sea-breams (Sparidae) are likely to be much more successful.

Associated with each transmission route is a transmission "window" - the period during which transmission of the parasite from one host to another can take place. The length of the window depends upon the concurrence of infective stages of the parasite with hosts that are susceptible to infection. In some transmission routes these windows are open virtually throughout the entire life span of the host so that infection can take place at almost any time. In others the windows are very narrow and represent weak links in parasite life cycles at which routes through some host species may be easily disrupted by changes in environmental conditions.

Some marine digenetic trematodes with fish intermediate hosts provide good examples of narrow transmission windows. MacKenzie and Gibson (29) showed that all infection of plaice, *Pleuronectes platessa*, with metacercariae of the digeneans *Stephanostomum baccatum* and *Rhipidocotyle* sp. occurs in the first year of life, with *S. baccatum* only during June and July and with *Rhipidocotyle* sp. only from about the middle of September to the end of October (Fig. 1).Transmission of each of these parasites to plaice is therefore limited to a period of 6-8 weeks in a maximum host lifespan of over 20 years. In a year when emergence or viability of the

Figure 1. Changes with time in the prevalence of two species of digenean metacercariae in juvenile plaice on a nursery ground on the west coast of Scotland. The transmission windows are shaded.



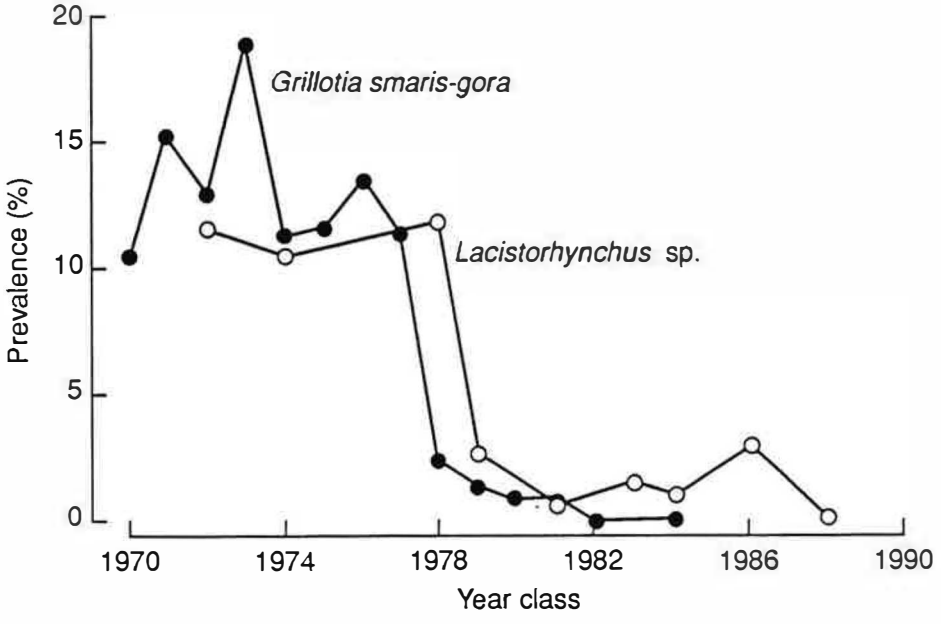
cercariae of these digeneans and/or spawning and growth of plaice are disrupted by a change in environmental conditions, an entire year class of plaice might show a significant reduction in infection. Such a situation was described by Kennedy (30) when he showed how annual variations in the length of the transmission window determined the abundance of metacercariae of a fresh water digenean in different year classes of perch, *Perca fluviatilis*.

Examples of parasite transmission failures have been recorded from the marine environment. MacKenzie (31) described how transmission of the trypanorhynch cestodes *Grillotia smaris-gora* and *Lacistorhynchus* sp. to mackerel and herring respectively in European waters was suddenly greatly reduced in 1979 and has not recovered since (Fig. 2).

Nagasawa et al. (32) described how transmission of the parasitic copepod *Pennella* sp. to saury, *Cololabis saira*, in the northwest Pacific failed in 1985, no infected saury were found in this region in 1986, but the parasite's reappearance in 1990 was reported by Honma and Imai (33). The reasons for these marine transmission failures are unknown, but they are probably related to natural environmental changes. It may be significant that the study areas referred to are close to the limits of the geographical distributions of these parasites. Populations at the fringes of a species' range are likely to be the most vulnerable to even very minor changes in environmental conditions.

Some of the examples given above are known to involve transmission routes through less important host species. Plaice

Figure 2. Long-term changes in the prevalence of Grillotia smaris-gora in mackerel and Lacistorhynchus sp. in herring from European waters.



are less important than other species of flatfish in the life cycle of *S. baccatum*, and mackerel are less important that demersal fish in that of *G. smaris-gora*. Because they tend to be particularly vulnerable to changes in environmental conditions, narrow transmission windows associated with less important hosts in the life cycle of a parasite could provide a highly sensitive early warning system for marine pollution. The non-appearance of a parasite in a particular year class of one of its less important host species does not necessarily mean that the parasite has disappeared from the area. It may still maintain its population by continuing to infect its more important host species. From the point of view of using the parasite as an indicator the important aspect is that a vulnerable link in the transmission process has been broken and a warning bell has sounded.

Selection criteria for host/parasite systems to be monitored

These criteria are intended primarily for application to marine locations, hitherto relatively free from pollution, that are likely to be affected by new industrial or recreational developments. Surveying of the site and the selection of host/parasite systems to be monitored should ideally commence while the site is in a relatively clean state so that monitoring can get underway as the development proceeds.

1. Attention should be focused on sedentary host species, many of which will be invertebrates, and on juvenile fish for which the study area serves as a nursery ground. Most adult fish, particularly pelagic species, are migratory to some extent and therefore should not be considered unless the study area is known to cover their full migratory range.
2. Attention should also be focused on parasite development stages, such as eggs and free-living larvae, which are directly exposed to the external environment. Pollutants tend to accumulate in sediments, so good indicators are likely to be found among parasites which infect benthic or demersal organisms and whose free-living transmission stages are indirect contact with the sea bed.
3. Selected parasites should occur commonly in targeted host species and should be easily identified.
4. Selected host/parasite systems should have narrow transmission windows.
5. Special attention should be paid to parasites living close to the limits of their geographical distributions.

The emphasis in this paper is on marine pollution monitoring, but the principles apply to both the marine and fresh water environments and to detecting natural as well as man-made changes. Minor changes in hydrographic conditions may have major effects on the transmission of some parasites, so it is important that possible effects of natural change are eliminated before pollution is considered. This, however, is a problem that is common to the use of any living organism as an indicator of environmental change.

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SCREENING OF 22 ANTHELMINTICS FOR THEIR EFFICACY AGAINST THE GILL-PARASITIZING MONOGENEANS *PSEUDODACTYLOGYRUS* SPP. FROM THE EUROPEAN EEL (*ANGUILLA ANGUILLA*).

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Abstract

A total of 22 anthelmintics were tested in a standardized laboratory system for their parasiticidal effect on *Pseudodactylogyrus* spp. and the short term adverse effects on the host *Anguilla anguilla.* Drug exposure of infected eels, 24-25 hours at approximately 25°C, showed the benzimidazoles, especially mebendazole and luxabendazole, to posses the highest efficacy. The only high-effect non-benzimidazole eliciting no acute toxic reactions in the host was praziquantel. Many anthelmintics displayed acute toxicity to eels.

Introduction

The gill parasitizing monogeneans *Pseudodactylogyrus anguillae* and *P. bini* from the European eel *(Anguilla anguilla)* have caused considerable morbidity and mortality in European intensive eel farming enterprises based on recirculated heated water (1, 2). These parasites originally described in Japan (3) and in China (4), were also previously reported to cause problems in eel farming (rearing of Japanese or European eel) in the Far East (5, 6, 7, 8, 9). The two congeneric monogenean species were probably imported to Europe by transfer of live eels from the Pacific area (10, 1, 11).

Formaldehyde addition to the fish tank water was formerly the preferred treatment against this parasitosis. However, this method failed to eradicate all the parasites and more efficacious methods were desirable (1). Mebendazole was later found to exhibit excellent anthelmintic activity towards the gill monogeneans (12, 13, 14), and a number of other drugs have been investigated by various authors using different experimental approaches. However, a strict comparison of the results is hampered due to the varying experimental design. It has been shown that temperature has a notable effect on the drug efficacy (15) and other factors might influence such trials. Therefore, in a series of experiments using a standardized water bath method, we have screened a total of 22 anthelmintics for their effect on *Pseudodactylogyrus* spp. and their fish host *Anguilla anguilla.*

Materials and methods

Parasites and eels:

Small pigmented specimens of the European eel (*Anguilla anguilla)* naturally or experi-

mentally (16) infected with anthelmintic naive populations of *Pseudodactylogyrus anguillae* and/or *P. bini* were used. At least 10 eels were used in each batch but only surviving eels were examined for the presence of parasites (Table 1).

Drugs:

A total of 22 different chemicals (Table 1) were investigated for their anthelmintic effect.

Experimental design:

Batches of infected eels were exposed to different concentrations of drugs at temperatures between 24 and 27°C for a period of 24-25 hours. Eels were incubated in 7 l drug solution (aerated) in 19 l aquaria. Such a procedure prevented these small eels from escaping the aquarium. After drug exposure, the eels were transferred to non-medicated water at the same conditions as outlined above and examined for parasites after additional 3-5 days.

Statistical analysis:

The abundance of parasites (17) in each experimental group after treatment was compared to the abundance in the appropriate control group employing the Mann-Whitney procedure at a 5 % significance level.

Results

Drugs with high parasiticidal effect, low acute toxicity.

A number of drugs used in water bath for 24 hours at approximately 25°C had a high parasiticidal effect on *Pseudodactylogyrus* spp. (Table 1). Thus mebendazole (only insignificantly better than luxabendazole), was efficacious in concentrations of 1 mg/l, and albendazole, luxabendazole and praziquantel eradicated all parasites when the drugs were used in a concentration of 10 mg/l. No acute adverse reactions in eels exposed to mebendazole (1 mg/l), albendazole (10 mg/l), luxabendazole (10 mg/l) and praziquantel (10 mg/l) were recorded. However, any long term adverse drug effect on eels would not be recorded in these studies as they were terminated within five days after the 24 hour treatment.

High parasiticidal effect, high toxicity.

Niclosamide (1 mg/l) eradicated all parasites, but was associated with high eel toxicity, and Rafoxanide (1 mg/l) reduced the worm burden notably, but showed a high acute toxicity (Table 1).

Moderate parasiticidal effect, low to moderate toxicity.

A relatively large number of anthelmintics elicited only a moderate reduction of the infection level of *Pseudodactylogyrus* spp. even when they were used in higher concentrations. Thus the benzimidazoles fenbendazole, flubendazole and oxibendazole (10 mg/l), parbendazole (5 mg/l), triclabendazole (10 mg/l), and the tetrahydropyrimidines pyrantel (100 mg/l) and morantel (10 mg/l) did not remove all parasites from the host, although the parasite number was significantly reduced (Table 1). The toxicity of these drugs is as yet undetermined. Some mortality of eels was observed in connection with exposure to these drugs, but a strict association between mortality and drugs or other

factors was not established. Metrifonate (1 mg/l) reduced the infection level significantly, but toxicity (spasms of eels) was recorded during the 24 hours exposure. Higher concentrations (10-50 mg/l) killed all exposed eels. The transfer of small pigmented eels to 3 per cent sodium chloride was shown to reduce the infection level considerably, but this sudden exposure was associated with some host mortality.

Moderate toxicity, no parasiticidal effect.

Exposure of eels to levamisole (10 mg/l) was not associated with any parasite eradication, but adverse reactions in a few eels (balance disturbances) were detected. Likewise, phenolsulfonthalein (10 and 100 mg/l) and toltrazuril (10 and 20 mg/l) elicited toxic reactions in eels and no significant reduction of their parasite burdens (Table 1). The benzimidazole thiabendazole (100 mg/l) (Equizole, commercial preparation with additives), showed no convincing parasiticidal effect but elicited adverse reactions in eels.

High toxicity, no parasiticidal effect.

Ivermectin (Ivomec) even in very low concentrations (0.05-1.0 mg/l) elicited heavy mortality in eels. The LD (50) of ivermectin at 25°C was determined to 0.05 mg/l (24 hours exposure time). This dosage did not cause any reduction in the infection level. Bithionol (1 and 10 mg/l) killed all exposed eels within 24 hours. The slightly toxic concentration (spasms in few eels) of 0.1 mg/l did not cause a significant reduction in the number of parasites. Bunamidine (1 and 10 mg/l) showed high toxicity, and no parasiticidal effect was recorded in the experimental group with survivors (0.1 mg/l).

Discussion

The standardized screening of these 22 anthelmintics revealed that some of the benzimidazoles (especially mebendazole) posses the highest efficacy against *Pseudodactylogyrus* spp. and at the same time they seemed to elicit minimal acute toxic reactions in eels. Mebendazole is now the drug of choice in eel farms based on recycled heated water (13, 14) but also luxabendazole has promising effects. These anthelmintics belong to the benzimidazole group, and the main target of these drugs is the microtubule system (18). Unfortunately, the risk for selection of MBZ resistant strains has been reported to be rather high (15). Due to the common mode of action of benzimidazoles, side resistance of MBZ resistant parasites to other benzimidazoles is likely to occur (19). In such cases luxabendazole would be an unsuitable alternative, whereas drugs with a different mode of action should be chosen. Also a number of low effect benzimidazoles would be unsuitable due to this fact, and because the selection for MBZ-resistant parasite strains would be enhanced by the use of such drugs (15). The only non-benzimidazole showing complete parasite eradication at relatively low concentrations (10 mg/l) is praziquantel. A satisfactory effect of this drug against *Pseudodactylogyrus* spp. in large scale systems has also been demonstrated (20). The exact action mechanism of praziquantel in *Pseudodactylogyrus* spp. is unknown, but the use of this drug against other monogeneans was found to be associa

Table 1. Parasiticidal effects of various anthelmintics on Pseudodactylogyrus spp. Trials were conducted as 24 - 25 hours water bath exposure (24-27°C). Eels were examined 4-5 days post treatment. Only surviving eels were examined. Different control groups were used as indicated.

Drug	Concentration mg/l	Parasite abundance post treatment x (S.D.)	Compared to control group no.	P	Reference
Organophosphates					
1) Metrifonate	0.5	38.8 (27.9)	5	N.S	33
(Neguvon)	1.0	17.1 (8.7)	5	<0.5	33
Benzimidazoles					
2) Albendazole	1.0	28.3 (26.2)	1,2	<0.5	16
(Valbazen)	10.0	0.0 (0.0)	1,2	<0.5	16
	100.0	0.0 (0.0)	1,2	<0.5	16
3) Fenbendazole	1.0	45.6 (43.0)	1,2	<0.5	16
(Panacur)	10.0	6.0 (10.5)	1,2	<0.5	16
4) Flubendazole	1.0	16.7 (8.9)	1,2	<0.5	16
(Flubenol	10.0	10.9 (17.5)	1,2	<0.5	16
5) Luxabendazole	1.0	0.1 (0.4)	1,2	<0.5	16
(pure subst.)	10.0	0.0 (0.0)	1,2	<0.5	16
	100.0	0.0 (0.0)	1,2	<0.5	16
6) Mebendazole (Vermox)	1.0	0.0 (0.0)	1,2	<0.5	16
Mebendazole (pure subs.)	1.0	0.0 (0.0)	3	<0.5	15
	5.0	0.0 (0.0)	3	<0.5	15
7) Oxibendazole	1.0	35.3 (15.7)	1,2	<0.5	16
(Loditac)	10.0	16.6 (10.1)	1,2	<0.5	16
8) Parbendazole	5.0	50.2 (47.8)	1,2	N.S.	16
(Helmatac)	50.0	4.9 (4.7)	1,2	<0.5	16
9) Thiabendazole	10.0	87.0 (28.8)	1,2	N.S.	16
(Equizole)	100.0	60.2 (26.6)	1,2	N.S.	16
10) Triclabendazole	1.0	74.2 (40.9)	1,2	N.S.	16
(Fasinex)	10.0	58.0 (44.8)	1,2	N.S.	16
Tetrahydropyrimidines					
11) Pyrantel citrate	10.0	49.1 (27.7)	4	N.S.	30
(Banminth vet.)	100.0	17.2 (8.4)	4	<0.5	30
12) Morantel tartrate	10.0	38.6 (25.8)	4	N.S.	30
(pure subst.)	100.0	32.5 (37.1)	4	<0.5	30
Avermectins					
13) Ivermectin	0.005	56.0 (64.4)	4	N.S.	30
(Ivomec)	0.05	127.0 (71.1)	4	N.S.	30
Phenols					
14) Bithionol (pure subst.)	0.1	8.3 (9.7)	7	N.S.	33

Table 1, continued

Drug	Concentration mg/l	Parasite abundance post treatment x (S.D.)	Compared to control group no.	P	Reference
Salicylanides					
15) Niclosamide	0.1	13.8 7.9)	8	N.S.	31
	1.0	0.0 (0.0)	8	<0.5	31
16) Rafoxanide	0.1	5.8 (4.0)	8,10	N.S.	31
	1.0	0.5 (0.7)	8	<0.5	31
Naphtamidines					
17) Bunamidine-hydrochloride (Scolaban vet.)	0.1	9.1 (5.5)	9	N.S.	32
Isoquinoline-pyrazines					
18) Praziquantel	1.0	8.8 (11.9)	9	N.S.	32
(pure subst.)	10.0	0.0 (0.0)	9	<0.5	32
Imidazo-thiazoles					
19) Levamisole-hydrochloride (Ripercol vet.)	10.0	10.5 (8.9)	9	N.S.	32
Sulfonphtaleines					
20) Phenolsulfon-phtalein	10.0	12.4 (11.5)	8	N.S.	31
(pure subst.)	100.0	10.2 (5.7)	8	N.S.	31
Triazinones					
21) Toltrazuril	1.0	7.2 (3.6)	8,11	N.S.	31
(pure subst.)	10.0	3.3 (2.6)	8,11	N.S.	31
22) Sodium-chloride	10000.0	39.5 (18.4)	6	N.S.	33
	20000.0	34.0 (25.5)	6	N.S.	33
	30000.0	9.9 (8.9)	6	N.S.	33
Control 1 (no solvent)		86,6 (50,4)			16
Control 2 (no solvent)		77.4 (27.9)			16
Control 3 solvent (formic acid 11 mg/l)		17.1 (25.6)			15
Control 4 (no solvent)		63.8 (27,2)			30
Control 5 (no solvent)		50.3 (27.1)			33
Control 6 (no solvent)		30.0 (17.2)			33
Control 7 solvent (DMS 10 µl/l)		11.6 (7.5)			33
Control 8 (no solvent)		7.9 (5.2)			31
Control 9 (no solvent)		4.7 (3.9)			32
Control 10 solvent (acetone 0.3 ml/l)		8.2 (5.1)			31
Control 11 solvent (triethanol 240 mg/l)		7.7 (4.1)			31

N.S.: $P > 0.05$

ted with tegumental damage of the affected parasites (21).

Niclosamide inhibits the formation of mitochondrial energy (22), and the strong effect of this salicylanilide on *Pseudodactylogyrus* spp. indicates the importance of this energy source for the monogeneans. Impact of niclosamide on other monogeneans was found (21), but the high toxicity of the drug to eels excludes it as a potential anthelmintic in eel farms.

Also the other salicylanilide, rafoxanide, shows a similar mode of action. It exhibits, however, not only a significant effect on the eel monogeneans, but also toxicity towards eels, and thus should be avoided in eel farms.

The tetrahydropyrimidines morantel and pyrantel are known to affect the neuromuscular systems in target organisms (22). The moderate effect of the drugs on the gill parasites indicates the presence of such systems in *Pseudodactylogyrus* spp. However, because high concentrations of morantel and pyrantel (100 mg/l) were not associated with total parasite eradication, these pyrimidines seem less suitable as anthelmintics in eel farms using recycled water.

A significant parasiticidal effect of the cholinesterase inhibiting drug metrifonate (1 mg/l) was demonstrated. This is consistent with the presence of cholinesterases in *Pseudodactylogyrus* spp. as demonstrated in *P. bini* (23) and in *P. anguillae* (24). Metrifonate has been suggested as a control agent against pseudodactylogyrosis (25, 8) and the drug is used in semi-extensive eel farming in Japan. However, due to the slight toxicity of 1 mg/l, which only showed partial effect on the parasite level, of parasitism, the use of this drug in intensive eel farm systems in Europe could prove hazardous.

Transfer of *Pseudodactylogyrus* infected eels to high concentrations of sodium chloride (3 per cent) elicits a significant reduction of the parasite number. Unfortunately, this also resulted in eel mortality. A partial parasiticidal effect of sodium chloride has previously been reported (25, 8).

Levamisole interferes with a neuromuscular mechanism and the fumarate reduction systems in target organisms (22). An effect of this anthelmintic on the tegument of other monogeneans were previously reported (21). However, no reduction of the infection level of *Pseudodactylogyrus* spp. was recorded in our study after 24 hours exposure of infected eels to levamisole (10 mg/l). The host seemed to be affected (balance disturbances), suggesting that levamisole is unsuitable for control of pseudodactylogyrosis.

Cestocidal effects of phenolsulfonphthalein have been reported (26), but we were unable to demonstrate any effect on *Pseudodactylogyrus* spp. with this sulfonphthalein used in concentrations of 10 and 100 mg/l. Although no mortality of eels was recorded, balance disturbances of the hosts were found associated with exposure to phenolsulfonphthalein.

The symmetric triazinone toltrazuril is an anticoccidial drug which on the basis of TEM observations appears effective against monogeneans including *Pseudodactylogyrus bini* (27). However, our screening of the

drug for effect on *Pseudodactylogyrus* spp. revealed no parasiticidal effect. In addition toltrazuril was toxic to small eels when it was used for 24 hours.

Thiabendazole differs from a number of other benzimidazoles with known effect on *Pseudodactylogyrus* spp. by the lack of the methyl carbamate in the 2-position of the molecule (26). This chemical difference could be suggested to account for the apparent lack of thiabendazole activity against these monogeneans.

Ivermectin, a widespread efficacious anthelmintic, primarily acts on the GABA-system in nematodes (28). As this avermectin in a concentration of 0.05 mg/l proved lethal to 50 % of the exposed eels within 24 hours, it is suggested that this host possesses such a transmitter system. The same concentration did not reduce the infection level of *Pseudodactylogyrus* spp., suggesting they lack or have a relatively insensitive GABA-system. In all cases this drug should be avoided in eel farms due to the high toxicity. Similar toxicity of ivermectin to other fish species were previously reported (29).

Also the cestocide bunamidine and the flukicide bithionol were without effect on the eel monogeneans in the tested concentrations. Bithionol has been suggested to act on ATP-synthesis, glycolysis and oxidative metabolism in worms (22), and effects on *Pseudodactylogyrus* spp. cannot be excluded when the drug is used in higher concentrations than 0.1 mg/l. Unfortunately, such higher concentrations are lethal to the host.

Conclusions

Among the 22 tested anthelmintics, mebendazole showed the highest efficacy against the gill parasitizing monogeneans *Pseudodactylogyrus* spp. from the European eel. Likewise, a number of other benzimidazoles demonstrated a high efficacy, but are, due to the common mode of action in this drug group, less suitable alternatives if mebendazole resistance should appear. The only non-benzimidazole showing total eradication of the gill parasites without eliciting acute toxic reactions was praziquantel. In order to delay emergence of benzimidazole resistance, alternation between mebendazole and praziquantel treatments is a theoretical possibility. Water temperature has been shown to influence the drug efficacy notably, which should be taken into account when trials under different conditions are compared.

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ACQUISITION OF INHIBITED EARLY FOURTH STAGE *OSTERTAGIA OSTERTAGI* LARVAE IN TRACER CALVES GRAZED IN LATE SUMMER AND EARLY AUTUMN

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Inhibited development of the fourth stage larvae of *Ostertagia ostertagi* undoubtedly plays an important role in the epidemiology of *Ostertagia ostertagi*. This phenomenon may perhaps help the parasite to avoid both immune effects of the host and unfavourable seasonal conditions for the free-living stages (1). Occurrence of inhibited larval development has been reported in many countries in Northern Europe, e.g. Scotland (2), Denmark (3), Sweden (4), and Norway (5). These reports indicate that a high proportion of *O. ostertagi* larvae ingested in late autumn and early winter become inhibited at the early fourth stage (EL_4). Thus, relatively higher numbers of inhibited EL_4 are recovered at necropsy of calves grazed in October/November than in calves grazed in the preceding summer months.

In an experiment conducted in the 1991 grazing season, we investigated the size and composition of *O. ostertagi* worm burdens in tracer calves grazed in early autumn. The primary purpose of the experiment (to be published elsewhere) was originally to study the effect of early season treatments with an anthelmintic on set-stocked calves naturally exposed to trichostrongyles. This experiment was conducted in Vemmetofte situated in South-East Zealand, Denmark. It involved two groups of first season cross breed Limousine X Red Danish heifer calves (groups T_1 and T_2). The two groups were turned out on May 6 and grazed separately on two plots until they were housed on October 8. Animals of group T_2 were treated with an anthelmintic at turn out and 10 weeks later, while the other group (T_1) served as an untreated control group. On August 13 and again on September 24 groups of each three parasite-free 'tracer' calves were turned out and allowed to graze together with each experimental group of animals in order to monitor pasture infectivity. All together twelve tracer calves were examined during this experiment. Following two weeks grazing, the tracers were housed and subsequently slaughtered 2 weeks later to determine worm burdens.

The majority of worms recovered at necropsy of the tracer calves belonged to *Ostertagia ostertagi*, while *Cooperia oncophora*, *Nematodirus helvetianus* and *Capillaria spp.* made minor contributions. The total number of *O. ostertagi* and the percentage of inhibited *O. ostertagi* larvae (EL_4) found in the tracer calves are shown in Table 1. With the exception of one calf grazing plot T_2, the results clearly showed that calves grazed September/October had significantly

higher percentages of inhibited larvae than calves grazed only 6 weeks earlier (August). No previous studies have been made in Denmark on the expected gradual increase in numbers of inhibition prone *O. ostertagi* larvae in the abomasal mucosa, and it is unknown, so far, whether the dramatic shift in proportions of EL_4 within a relatively short late summer period (6 weeks) may be a general feature or an occasional finding.

In studies by Armour et al. (6) in Scotland and Borgsteede (7) in the Netherlands, the rise in number the of EL_4 during the late season grazing was less marked and took place over a longer period of time. As inhibition may be induced particularly at low temperatures (8,9), the observed difference between e.g. our results and those of Armour et al. (6) and Borgsteede (7) may be explained by a more rapid onset of low temperatures in the Danish autumn. But genetic differences between strains of *O. ostertagi* in their capability to enter inhibition may also exist. Thus, differences on the capacity of *O. ostertagi* strains to enter inhibition were demonstrated between a laboratory-maintained and a field strain of *O. ostertagi* (10), and between different field strains (11,12). Furthermore, the beef cattle management system, which in fact was practiced in the farm where our experiment was performed, may play an additional role in selection for inhibition prone *O. ostertagi*. Smeal and Donald (13) demonstrated much lower proportions of inhibited larvae in tracer calves grazed on pasture contaminated with *O. ostertagi* of dairy cattle origin than on pas-

Table 1. Ostertagia ostertagi burdens and percentage of early fourth stage larvae (EL_4) of tracer calves grazed in late summer and early autumn

Date grazed	Plot T_1			Plot T_2		
	Calf no.	Total number of *O. ostertagi*	EL4 stage as % of total number	Calf no.	Total number of *O. ostertagi*	EL4 stage as % of total number
Aug. 13-Aug. 27	114	480	11.46	143	1755	25.64
	115	5030	4.57	144	1865	1.88
	116	7610	5.91	145	635	0.79
	Mean	**4373**	**7.31**	**Mean**	**1418**	**9.44**
	SEM	**(2084.3)**	**(2.11)**	**SEM**	**(392.95)**	**(8.11)**
Sept. 24-Oct. 8	116	6910	97.54	146	190	73.68
	117	12,600	89.21	147	580	86.21
	118	8790	88.74	148	610	0
	Mean	**9433**	**91.83**	**Mean**	**460**	**53.30**
	SEM	**(1673.8)**	**(2.86)**	**SEM**	**(135.28)**	**(26.89)**

tures contaminated with the parasite of beef cattle origin. Dairy calves usually spend their first grazing season together, segregated from their dams. This system ensures availability of highly susceptible animals on pasture, and pasture contamination may be rather high throughout the late part of the season, thereby providing sufficient numbers of larvae for cycling of the parasite in the following year. In this situation, inhibition may be of relatively less importance for the survival of the parasite, in contrast to the situation with mixed grazing of pasture born beef calves and their more or less parasite immune dams (13).

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LOCALIZATION OF ACID PHOSPHATASE IN THE RHOPTRIES OF *SARCOCYSTIS CRUZI*

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Invasion of coccidian protozoans into host-cells plays a central role in the life cycles of these intracellular parasites guaranteeing their further development. It has been shown that three types of organelles of the apical complex (rhoptries, dense granules and micronemes) participate in the host-parasite interaction during the host-cell invasion (1,2). Rhoptries appear to act as extrusomes (3) excreting their content during the host-cell invasion (4). Little is known about the rhoptry proteins of *Sarcocystis*. However, the presence of different proteins has been demonstrated in the rhoptries of the related sporozoan *Toxoplasma gondii* (5) and in haemosporidians of the *Plasmodium* genus (6). Bernard and Schrevel (7) have proposed that rhoptries of *Plasmodium berghei* contain a neutral endopeptidase. The presence of acid phosphatase has been demonstrated in the rhoptries of *T. gondii* (8). No cytochemical characterization of *Sarcocystis* rhoptries has been carried out.

The ultrastructural localization of acid phosphatase (3.1.3.2., AcPase) was examined in the cyst merozoites of *Sarcocystis cruzi*. The characterization of the material used has been described previously (9). Pieces of bovine myocardium containing tissue cysts were fixed in 3% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH-7.3) immediately after the cattle had been slaughtered. The material was taken in parallel from three different animals. Three to five cysts were prepared from each animal. The cytochemical reaction was carried out according to Mehlhorn et al. (10). The specimens were postfixed in 1% OsO_4, embedded in Epon 812 or Epon-Araldit. Serial sections were stained with uranylacetate and examined on a Jem-100 C-X electron microscope within two weeks after embedding.

A strong reaction to AcPase was observed in the rhoptry ductules (Fig. 1 and 2). Inside the rhoptry lumen a very intensive reaction was detected in the upper part of the organelle adjacent to the ductule orifice. The reaction was comparatively weak inside the main body of the rhoptry. A significant amount of reaction product was localized on the membranes surrounding the organelle. The localization of AcPase in the rhoptries of *S. cruzi* resembles that of a 225 kD protein (11) and the AMA-I antigen (6) in the rhoptries of *Plasmodium falciparum*. The protein with AcPase activity in *Sarcocystis* might be associated with the rhoptry ductules and rhoptry membranes having some role in promoting the excretion of the rhoptry contents, or this protein might have a function

Fig. 1. Reaction to acid phosphatase (arrows) in the rhoptry ductules (D) of Sarcocystis cruzi. x 920.

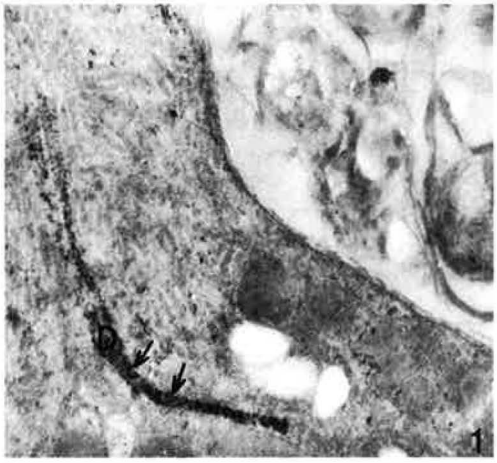
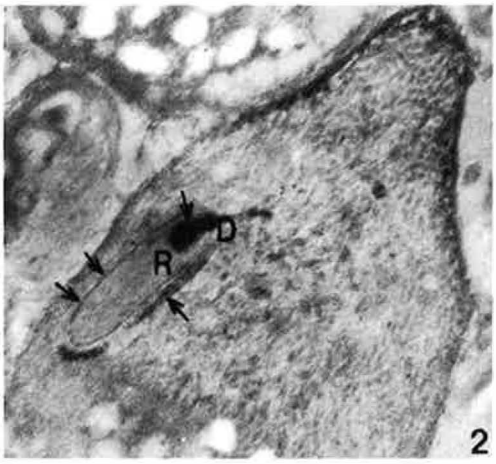


Fig. 2. Reaction to acid phosphatase (arrows) in the rhoptry ductules (D), in the upper part of the rhoptry (R) adjacent to the orifice of the ductule and on the membrane surrounding the rhoptry. x 920.



in the host-parasite interaction during the early stages of host-cell invasion. The first hypothesis may be suggested by the fact that AcPase was detected on the membrane surrounding the organelle. To test the second hypothesis, a detailed study of the rhoptry extrusion during the host-cell invasion must be carried out.

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Announcement

SECOND NORDIC TRAINING COURSE IN PARASITIC ZOONOSES

Epidemiology of zoonotic diseases

The objective of the course is to give an introduction to the epidemiology of parasites shared by animals and man, with emphasis on the interdisciplinary aspects of these infections. The course will include infections common in the Nordic countries as well as in the tropics. The course will give an introduction into transmission, epidemiology and control of these infections in both animals and man.

The lectures will include basic concepts in the epidemiology, transmission biology, from both the veterinary and medical point of view. The infections covered will include toxoplasmosis, cryptosporidiosis, trichinellosis, leishmaniosis and schistosomosis. The course will among others include workshops on planning and design of epidemiological studies on parasitic zoonoses including intervention studies. Legislative public health measures and their effect on the control of parasitic zoonoses will be discussed.

The course is open to veterinarians, biologists and medical doctors. The course is supported by NORFA, Nordic Forskerutdanningsakademin under the Nordic Council. The course is free, and travel and lodging expenses will be reimbursed.

Place	National Veterinary Laboratory, Copenhagen, Denmark
Time	May 24 to May 28, 1993
Organizers	Danish Centre for Parasitic Zoonoses
Application	Before April 15

Information and Application:

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IN VITRO EFFECTS OF IMMUNE MOUSE SERUM ON THREE SPECIES OF *HYMENOLEPIS* TAPEWORMS.

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Abstract

Precipitates occurred *in vitro* on newly excysted parasites in the presence of serum collected 2 weeks p.i. with 10 *Hymenolepis microstoma*, but did not develop on all 4-day-old worms until exposed to serum collected 4 or more weeks p.i. and on all 35-day-old worms until exposed to serum collected 6 or more weeks p.i. Precipitation titres on 4-day-old worms reached 1:512. Immune sera producing precipitates reduced growth of worms *in vitro*. IgG and IgM, but not IgA were found in the precipitates. IgE or complement does not seem to be involved in this formation. Precipitates developed also on worms of *H. diminuta* and *H. nana*.

Introduction

It has been shown that a primary infection of the three cestodes *Hymenolepis diminuta*, *H. microstoma* and *H. nana* in mice confers resistance, although to different degrees, to a challenge infection (1-3), and that one species stimulates cross-resistance to another species, e.g. *H. microstoma* to *H. diminuta* (4) and *H. diminuta* and *H. microstoma* to *H. nana* (5,6).

Immunological factors appear to be involved in this resistance, although the exact contributions of humoral and cellular immunity in these host-parasite relationships have not yet been clarified. Murray, Foster & Passmore (2) demonstrated antibodies in both serum and washings from the intestinal lumen of mice infected with *H. microstoma*, but no direct effects of antibodies on the parasite were noted. However, antibodies produced in rabbits against *H. nana* or from mice infected with *H. nana* have been shown to produce precipitates on the tegument of adult *H. nana in vitro*, respectively (7,8). Similar precipitates also occur on protoscolices and adults of *Echinococcus granulosus* (9), on the metacestodes of *Spirometra mansonoides* (10) and *Mesocestoides corti* (11), and on excysted metacercariae of the trematode *Fasciola hepatica* (12,13) when these parasites are exposed to immune serum *in vitro*.

When 4-day-old worms of *H. nana* and *H. microstoma* were cultured *in vitro* in 50% serum from mice infected with 1, 2 or 4 cysticercoids of *H. microstoma* on day 0, challenged with 30 *H. nana* cysticercoids on day 39 and killed on day 49, precipitates developed on both species of worms (6).

The purpose of this investigation was to elucidate the dynamics of precipitate formation on hymenolepids *in vitro* and to determine the effect of serum dilution and para-

site species and age on the response. In addition, the rôle of antibody and/or complement on the formation of the precipitate and subsequent effect on parasite growth was determined.

Materials and methods

Parasites and incubations:

Hymenolepis diminuta and *H. microstoma* originating from the Wellcome Laboratories for Experimental Parasitology, University of Glasgow, have been maintained in our laboratory since 1970 and 1978, respectively. Eggs of *H. nana* were kindly supplied by Dr. A. Ito (The Medical School, University of Gifu, Japan) in 1986. Parasites were maintained by passages through the flour-beetle, *Tribolium confusum*, and rats, mice and nude mice, respectively.

Excystation of cysticercoids obtained from infected beetles and incubation procedures for newly excysted worms were as described by Bøgh, Christensen & Andreassen, (14), except that the incubation medium used was Medium 199. Four- and 35-day-old worms were recovered from NMRI nu/nu mice and +/nu mice respectively, infected orally with 25 or 50 cysticercoids. At autopsy, the small intestine was opened and the worms transferred to Petri dishes and washed two times in modified Hanks BSS (15) and once in Medium 199 without L-glutamine containing the antibiotics, benzylpenicillinsodium (2000 units/ml) and streptomycin sulphate (2.0 mg/ml). Addition of fungicides was not necessary. The 35-day-old worms were cut approximately to the length of the 4-day-old worms (5-10 mm). The newly excysted worms and the 4- and 35-day-old worms were incubated separately, one per well, in flatbottomed microtiter plates (Nunc). However, in the experiment to determine the cross-reactivity between sera and the three species of *Hymenolepis*, three 4-day-old worms (one of each species) were used per well. In all experiments control worms were incubated in medium alone, fresh normal mouse serum or heat-inactivated (56°C/30 min) normal rat serum (hiNRS). In none of these controls were precipitates found on the surface of the parasites after 72 h incubation.

Serum:

Blood was obtained from uninfected Kyoto rats or NMRI mice either uninfected or infected with 10 *Hymenolepis sp.*, and serum separated, as described by Bøgh *et al.* (14). Serum was stored at - 18°C until required.

In all experiments parasites were exposed to a total of 100 µl solution consisting of 50 µl test serum and 50 µl Medium 199 with antibiotics. In the dilution experiments the test serum was further diluted with hiNRS so that in all cases the final medium contained a total of 50% serum and 50% Medium 199.

Observations and measurements:

Observations were carried out using an inverted Zeiss microscope placed in an observation chamber at 37°C and the experiments finished after 72 h. The length of the worms was measured in the microscope by using a calibrated object micrometer one hour after the worms had been placed at 4°C. To determine the effect of dilution of the serum, the precipitate titer is expressed as the reciprocal of the highest serum dilution giving a precipitate.

Immunofluorescence test:

To visualise any antibodies present on the surface of the parasites, an indirect immunofluorescence test was carried out according to the technique used by Simonsen and Andersen (16). Worms were incubated for 30 min at 37°C in 6 µl fresh immune mouse serum (fIMS) and 94 µl Medium 199 with antibiotics, washed in PBS and observations made on an Olympus CH microscope with an Olympus LH50A with a halogen lamp acting as a light source.

RESULTS

Serum precipitation titers on 4-day-old H. microstoma

Neither precipitates nor lysis of the tegument were found associated with 4-day-old worms after 72 hours incubation *in vitro* with serum from uninfected mice and mice infected for 7 days with 10 *H. microstoma.* However, serum from three of five mice infected with *H. microstoma* two weeks previously formed precipitates on the surface of the parasite. Serum from mice infected for 3-12 weeks all produced precipitates on the surface of the worm and an increasing amount of precipitates occurred at the bottom of the microtiter well after 24h in culture. The precipitation titre increased with increasing time post-infection and from week 5 to 12 all serum titers were between 128 and 512 (Fig. 1).

Precipitation of serum on different ages of H. microstoma

Serum from mice infected with 10 *H. microstoma* for one week did not produce precipitates on any of the three ages of worms. However, on parasites exposed to serum from mice infected for two weeks, precipitates occurred on all the newly excysted worms, about half of the 4-day-old cestodes, but on none of the 35-day-old parasites. Serum collected three weeks after infection also produced precipitates on 25% of the 35-day-old worms. Precipitates were not present on all 35-day-old worms unless the serum was collected from mice that had been infected for six weeks (Table 1).

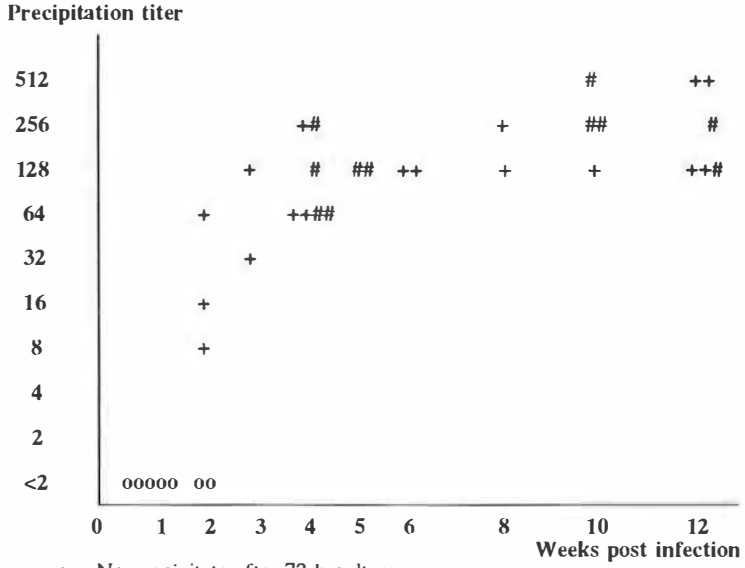
Effect of serum and precipitates on the growth of newly excysted and 4-day-old H. microstoma

Newly excysted worms (experiment a) and four-day-old worms (experiments b1 and b2 with worms of different length) incubated 72h *in vitro* with serum from mice infected with 10 *H. microstoma* for 2 weeks to 3 months all developed precipitates and grew less (6-25% increase in length) than control worms kept in fresh serum from uninfected mice (fNMS) or from mice infected for only one week (FIMS 1 week), where no precipitates developed (36-71% increase in length). The greatest growth occurred in other control worms incubated with heat-inactivated normal rat serum (hiNRS) (79-97% increase in length) (Table 2).

Presence of specific immunoglobulin in the precipitates

All worms kept in sera from mice infected with 10 *H. microstoma* from 2 - 24 weeks developed precipitates and fluorescence with anti-mouse immunoglobulin (Ig), IgG and IgM. In contrast, fluorescence was not detected with anti-IgA (12 sera tested). Immunofluorescence was also found on worms incubated in hiIMS. However, fluor

Figure 1. Serum precipitation titers on 4-day-old Hymenolepis microstoma



o = No precipitate after 72 h culture
+ = Highest serum titer causing precipitate
# = Precipitate at this titer, but serum not titrated further

Table 1. Percentage of worms of three different ages of Hymenolepis microstoma with precipitate after 72 h incubation in serum from mice infected with 10 H. microstoma for up to 12 weeks.

Week number after infection	0-day-old worms (newly excysted)	4-day-old worms	35-day-old worms
1	0 %	0 %	0 %
2	100 %	55 %	0 %
3	100 %	83 %	25 %
4	100 %	100 %	79 %
5	100 %	100 %	75 %
6	-	100 %	100 %
8	-	100 %	100 %
10	-	100 %	100 %
12	-	100 %	100 %

- = not tested
Test made with serum diluted 1:2, 1:4, 1:8 & 1:16.
Positive sera always gave precipitate up to titer 16.

Table 2. Increase in length of a) newly excysted worms (1 experiment) and b) 4-day-old worms (2 experiments) of Hymenolepis microstoma after 72 h in 50% serum cultures.

a)

Type of serum	Number of worms	Mean length at 0 h	Average increase in %	+ or - precipitate
hinrs	10	0.175 mm	89 %	-
FNMS	10	0.164 mm	56 %	-
FIMS (3 months)	10	0.165 mm	19 %	+

b-1)

Type of serum	Number of worms	Mean length at 0 h	Average increase in %	+ or - precipitate
hinrs	5	7.49 mm	79 %	-
FNMS + FIMS (1 week)	10	7.99 mm	36 %	-
FIMS (2-12 weeks)	15	9.03 mm	6 %	+

b-2)

hinrs	6	0.88 mm	97 %	-
FNMS	6	0.88 mm	71 %	-
FIMS (12 weeks)	6	0.92 mm	25 %	+

hinrs = heat inactivated Normal Rat Serum
FNMS = fresh Normal Mouse Serum
FIMS = fresh Immune Mouse Serum
(weeks or months) = Time after infections of mice with 10 *H. microstoma*

Table 3. Precipitates of immune sera on 4-day-old worms of Hymenolepis microstoma, H. diminuta and H. nana.

		Serum from mice infected 3 to 12 weeks with the worms, i.e. with antibodies against:		
		H. microstoma	*H. diminuta*	*H. nana*
4-day-old worms of:	*H. microstoma*	+	-	-
	H. diminuta	+	+	-
	H. nana	+	+	+

+ = presence of precipitates after 72 h in culture
- = no precipitate found after 72 h in culture

escence and precipitate formation was not detected on parasites kept in Medium 199 alone, in fresh normal mouse serum (FNMS), 1 week FIMS, fresh normal rat serum (fNRS) or hinrs (12 sera tested). Since worms incubated in hiims (56°C, 30 min) stimulated the development of precipitates, it can be concluded that IgE and complement, which are sensitive to inactivation at this temperature, are not involved in the precipitates.

Cross-precipitates between H. microstoma, H. diminuta and H. nana

Four-day-old worms of the three *Hymenolepis* species were incubated with 50% serum from mice infected with 10 worms of one of the three species 3 to 12 weeks previously. Although precipitates occurred on all three species of worms 72 h after incubation in serum from mice infected with *H. microstoma*, they were not observed on *H. microstoma* when exposed to serum from mice infected with *H. diminuta* or *H. nana*, and anti-*H. nana* serum only produced precipitates on *H. nana* (Table 3).

On *H. microstoma* the precipitate was first visible on the suckers but proceeded to the rest of the scolex, the neck region and the strobila. On *H. diminuta* the precipitate started on the strobila and then later occurred on the scolex. On *H. nana* the precipitate started in the neck region and then became visible up to the scolex and further down the strobila.

Discussion

The relationship between time of infection of NMRI mice infected with ten *H. microstoma* and the serum precipitation titer (Fig. 1) was very similar to that observed by Murray *et al.* (2), for worm-specific serum and intestinal antibody titers measured by ELISA in AKR and C3H mice also infected with ten *H. microstoma*. In the latter study, however, the serum titers were higher than observed in our observations.

It is interesting that the precipitates developed on all newly excysted worms and on some 4-day-old parasites in serum from mice infected for only two weeks with 10 *H. microstoma*, while precipitates against 35-day-old worms did not appear until three weeks post infection (p.i.) and did not occur on all parasites until 6 weeks p.i.. This perhaps indicates the presence of stage-specific antigens and antibodies. Indeed, this is in accordance with the results obtained by Ito, Itoh, Andreassen & Onitake (17), in which BALB/c mice recognized stage-specific antigens of *H. microstoma*.

The reduction of growth of both newly excysted and 4-day-old *H. microstoma* exposed *in vitro* to immune mouse sera which caused precipitates on the worms can be correlated with the *in vivo* observations of Howard (18). In the latter it was found that *H. microstoma* grew more slowly in secondary infections in mice, particularly in the first four days, than in primary infections. Although it would have been wiser if growth of tapeworms *in vitro* was based on an increase in dry weight, protein content and/or DNA content rather than on length of the living worms, the need to obtain measurements before and after cultivation prevented the use of these parameters. Since we have

found a reduction in growth of both newly excysted and 4-day-old worms cultured at the same time and after repeating the experiment with 4-day-old worms, we believe that our results are valid. Although the immune sera, which caused precipitates, also caused stunting in growth of the worms, the parasites were not killed. This is in contrast to the effect of an antibody found in rhesus monkeys hyperimmunized against *Schistosoma mansoni* which was lethal to schistosomula cultivated *in vitro* (19), but it is in accordance with the observation of Herd (9), who found that protoscolices and adult *Echinococcus granulosus* remained healthy and active over a period of 8 days in immune sera in spite of precipitation reactions. Howell, Sandeman & Rajasekariah (12) also found that metacercariae of *Fasciola hepatica* acquired precipitates but survived culturing in immune serum for 36 h, but did not develop when transplanted intraperitoneally to naive rats. Whether newly excysted *H. microstoma* cultured *in vitro* in immune mouse serum, which caused precipitate formation and stunted growth, are also unable to develop after transplantation into the duodenum or bile duct of naive mice needs to be investigated.

Precipitates found *in vitro* have a similar appearance to those described by Howell and Sandeman (13) on the metacercariae of the trematode *F. hepatica.* These workers, who showed that the precipitate, when used to immunize rats against an oral challenge with metacercariae, caused a 50% reduction in worm burden, suggested that the precipitate contained a functional antigen of the parasite. A similar relationship between precipitate formation *in vitro* on *H. microstoma* and impaired viability of newly excysted cysticercoids *in vivo* has yet to be established.

The presence of the mouse immunoglobulins, IgG and IgM in the precipitate on the tegument of *H. microstoma* is similar to what was found in precipitates on metacercariae of *F. hepatica* by Howell and Sandeman (13), who presumed that the immunoglobulins were protective antibodies. It is of interest that we could not find IgA in the precipitates, because Murray *et al.* (2) found that serum IgA levels in the highly resistant AKR mice were higher than in the significantly less resistant C3H strain.

That precipitates are formed in hiIMS is in agreement with the findings on *H. nana* by Nakamura *et al.* (8), and on *F. hepatica* by Howell *et al.* (12), and indicates that neither IgE nor complement is involved in the formation of the precipitate. Further studies on the *in vitro* effects of immune sera deprived of certain immunoglobulin classes and normal sera with e.g. IgG or IgM from immune sera added needs to be carried out.

Although mice infected with *H. microstoma* stimulate production of antibodies, which produce precipitates not only on living worms of *H. microstoma* but also cross-react with *H. diminuta* and *H. nana,* mice infected with *H. diminuta* or *H. nana* did not stimulate antibodies which precipitate on *H. microstoma* (Table 3). These results might indicate differences between antibody production in mice having the chronic infection of *H. microstoma* and mice having the rather shortlived *H. nana* and *H. diminuta* infections.

The precipitates found in the present investigation are also similar to the 'Cercarienhüllen' reaction (CHR) around cercariae of schistosomes after *in vitro* culture in immune serum. Furthermore, it has been shown that the CHR is located in the tegumental glycocalyx of the cercariae (20). Schmidt and Peters (21) have observed a filamentous layer of glycoconjugates at the distal area of the microtriches on *H. microstoma.* We therefore suggest that electronmicroscopical investigations should be carried out in an attempt to elucidate if the precipitates represent immune complexes formed within the microthrix border, and if any damage to the surface of the parasite occurs.

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Announcement

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CELLS AND CYTOKINES IN MALARIA

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Centre for Medical Parasitology at University of Copenhagen held its second annual international mini-symposium on 19 September at the University Hospital (Rigshospitalet) in Copenhagen. The topic of this year's mini-symposium was cell adhesion and cytokines in malaria, and it consisted of invited talks by scientists from Switzerland, England, Sweden and Denmark.

The first, and main, lecture was given by Dr. Georges Grau from University of Geneva, who discussed in detail the pathology of murine cerebral malaria (CM), its causes, and how data derived from mouse studies compares to findings in human CM. Some strains of mice are susceptible to a rapidly lethal syndrome with cerebral pathology following infection with *Plasmodium berghei ANKA*. Murine CM is associated with obstruction of blood circulation in the brain due to massive adhesion of lymphocytes, erythrocytes, and platelets to endothelia in the cerebral microvasculature. The sequestration of all these cell types appears dependent upon adhesion mediated by interaction of LFA-1 with endothelial ICAM-1. In keeping with this, treatment with anti-LFA-1 mAb has a dramatic curative effect in murine CM. The neuropathology in CM is linked to an overproduction of TNF, which is under T-cell control, primarily through production of IFN-γ. The lymphokine production profiles are different in susceptible and resistant mouse strains. T cells from susceptible mice produce mainly IFN-γ, and only small amounts of IL-4, while the reverse pattern was seen in resistant mice. In humans, non-immune individuals produce larger amounts of IFN-γ following malaria-specific stimulation than immune individuals. Dr. Grau suggested that the Th_1-like T-cell profile associated with susceptibility to CM in the mouse may also be associated with CM susceptibility in humans.

In the second talk, I discussed the role of LFA-1 mediated T-cell adhesion in human *P. falciparum* malaria. Peripheral T cells from acutely ill malaria patients are unresponsive to antigenic stimulation, and there is evidence to suggest that this energy is due to a disease-induced redistribution of antigen T cells. T cells characterized by high expression of LFA-1 adhere more effectively to endothelial cells and to purified ICAM-1 than cells with low LFA-1 expression. In acute *P. falciparum* malaria, LFA-1^{hi} T cells are depleted from circulation, and plasma levels of soluble ICAM-1 are increased, suggesting upregulation of endothelial ICAM-1 expression. Taken together, these data suggest that LFA-1/ICAM-1 mediated T-cell adhesion is a feature of acute *P. falciparum* malaria.

The third talk presented by Dr. Mats Wahlgren, Stockholm University, focused on erythrocyte rosetting (i.e. binding of uninfected to infected erythrocytes) and adhesion of erythrocytes to endothelia. Both these phenomena have been shown to be involved in the pathogenesis of *P. falciparum* malaria. All parasite isolates obtained from cerebral malaria patients were rosette-positive, while isolates from uncomplicated malaria cases were either rosette-negative or had significantly lower rosetting rates than parasites from infections with cerebral involvement. Dr. Wahlgren concluded his talk by discussing the prospects of an erythrocyte adhesion-blocking vaccine.

The following three talks dealt with various aspects of malaria-induced TNF production, the parasite antigens involved, and the consequences for the host. Dr. Janice Taverne, University College and Middlesex School of Medicine, London, discussed TNF-inducing antigens of rodent malaria parasites. Such antigens are toxic to mice and induce little immunological memory following exposure (antibodies are predominantly T-cell independent IgM). The TNF-inducing component(s) of such antigens are associated with phospholipids, and are not proteins. The possibility of using detoxified forms of these antigens in vaccination was discussed.

Dr. Dominiq Kwiatkowski, University of Oxford, reviewed the evidence for involvement of TNF in the pathogenesis of cerebral malaria, and viewed investigations of TNF in malaria as an interesting model, as this monokine has beneficial as well as detrimental features, and may thus be well-suited for studies of the immune system's attempts to balance pathological and protective consequences of the response to infection. Dr. Kwiatkowski continued his talk with an intriguing introduction to mathematical models incorporating deterministic chaos as a tool in malaria immunological research.

In his talk, Dr. Palle Høj Jakobsen, Centre for Medical Parasitology, extended Dr. Taverne and colleagues' findings to the human malaria parasite *P. falciparum.* As in rodents, TNF-inducing protein-associated phospholipid antigens capable of inducing host production of TNF have been identified. However, in contrast to findings in murine malaria models, no evidence has been obtained so far indicating a protective role of antibodies against *P. falciparmu* TNF-inducing antigens.

In the final presentation of the symposium, Dr. Marita Troye-Blomberg, Stockholm University, summarized data regarding regulation of B- and T-cell responses to the *P. falciparum* antigen Pf155/RESA, and their role in protection against malaria. She continued to discuss the regulatory role of IL-4, and possible distinct malaria-specific Th_1 and Th_2 subsets akin to those described in various murine and human systems.

The symposium was well-attended, and characterized by informal, lively and rewarding discussions between the speakers and a very attentive and engaged audience. Encouraged by the very positive outcome of this year's symposium, we hope it will be possible to arrange another one next year.

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REPORT FROM A NORDIC SEMINAR: COCCIDIAL INFECTIONS OF RUMINANTS - DIAGNOSIS, EPIDEMIOLOGY AND CONTROL

Abstracts edited by Arvid Uggla

The coccidia are intracellular protozoan parasites causing important diseases in both animals and man. Intestinal coccidia as *Eimeria* and Cryptosporidia proliferate in the epithelium of the gut and may cause severe diarrhoea. Thus, in temperate areas of the world *Eimeria* coccidiosis is regarded as one of the most important causes of diarrhoea in cattle and sheep. Other coccidia, such as *Toxoplasma* and *Sarcocystis*, form cysts in the tissues of their hosts. Some of these infections can be transmitted transplacentally from mother to offspring, resulting in abortion or congenital disease. One well known example is *Toxoplasma*, a cause of abortion in pregnant women and in animals as sheep and goats. Many of the coccidia (e.g. *Toxoplasma* and *Cryptosporidium*) are zoonotic, and an infection can, especially in immunocompromised humans, develop into severe disease.

With the intention to create an internordic network for veterinary parasitologists working in the field of coccidial infections of farm animals, a meeting was arranged in Uppsala, November 16-18, 1992. The meeting opened with a public seminar entitled "Coccidial infections of ruminants - diagnosis, epidemiology and control". Speakers were mainly researchers from the Nordic countries, and a specially invited guest, Dr J.P. Dubey from USDA Livestock and Poultry Sciences Institute, Beltsville, USA. He gave a talk on *Neospora caninum*, a recently described parasite, which has shown to be an important pathogen of cattle in the USA. Abstracts of the presentations given at the seminar are published here.

The seminar was arranged by professor Arvid Uggla, Department of Parasitology, Swedish University of Agricultural Sciences and National Veterinary Institute, Uppsala, and dr Stig M. Thamsborg, Department of Clinical Studies, Royal Veterinary and Agricultural University, Copenhagen, and was financially supported by The Nordic Academy for Advanced Studies (NorFa) and in part by the Swedish Associaton for Veterinary Research.

THE COCCIDIA - CLASSIFICATION AND IDENTIFICATION

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The coccidia are intracellular protozoan parasites with life cycles comprising three phases; asexual multiplication (schizogony or merogony), sexual multiplication (gamogony or gametogony), and asexual reproduction of the zygote (sporogony) leading to formation of infectious sporozoites.

The organisms are grouped in subclass Coccidiasina under class Sporozoasida and phylum Apicomplexa. To this subclass be-

long the order Haemosporidia with important blood parasites of domestic animals and man, such as the malaria parasites (*Plasmodium spp.*) and the piroplasms (*Babesia spp.* and *Theileria spp.*), and the order Eucoccidida with the coccidia proper. The genera of true coccidia of greatest veterinary and medical importance are commonly grouped under suborder Eimeriina in the families Eimeriidae (*Eimeria spp.* and *Isospora spp.*), Sarcocystidae (*Sarcocystis spp.*, *Toxoplasma gondii* and *Neospora caninum*), and Cryptosporidiidae (*Cryptosporidium spp.).*

Eimeria, Isospora and *Cryptosporidium* species are, principally, intestinal parasites of vertebrates with direct life cycles. *Sarcocystis spp., T. gondii* and *N. caninum,* generally referred to as cyst-forming coccidia, have, typically, two-host life cycles involving intermediate hosts harbouring in their tissues a resting stage of the parasite. Most coccidia are strongly host specific, but e.g. *Cryptosporidium spp., T. gondii* and *N. caninum* can be found in different hosts. The life cycle of *N. caninum* is not yet revealed.

Classification and identification of coccidia are based on size and morphology of the sporulated oocysts, the life cycle including prepatent periods and the range of hosts, the ultrastructural morphology of tissue stages, antigenic compositions and nucleic acid sequences. Iso-enzyme analysis is an important means in distinguishing similarly sized *Eimeria* oocysts, especially in the chicken.

Sporulated oocysts of coccidia of medical and veterinary importance can contain one (*Cryptosporidium spp.*), two (*Ispospora spp., Sarcocystis spp.* and *T. gondii*) or four (*Eimeria spp.*) sporocysts, each containing four (*Cryptosporidium spp., Isospora spp., Sarcocystis spp.* and *T. gondii*) or two (*Eimeria spp.*) sporozoites. The oocysts are spherical, ovoid or ellipsoidal, ranging in size from 2-5 (*Cryptosporidium spp.*) to about 60 x 80 µm (*E. leuckarti*). The exogenous sporulation time ranges from 0 (*Sarcocystis spp.*) to 3-4 weeks (*Eimeria spp.*).

Tissue cysts of the cyst-forming coccidia can not principally be distinguished from one another by light microscopy although host, localization and the size may give some guidance. Ultrastructural features, especially those of the apical part of the organism, also play a role in the identification of, in particular, intracellular stages. Cyst wall ultrastructure is important in distinguishing the different *Sarcocystis spp.* and other cyst-forming coccidia.

Detection and identification of tissue stages can be done by immunohistochemical techniques including immunoenzyme or immunofluorescent methods employing polyclonal antisera or monoclonal antibodies. However, the specificity of antibodies used must be carefully determined.

Serological analyses for specific circulating antibodies are important in the detection of infections by cyst-forming coccidia and are extensively employed especially in the case of *T. gondii.* The specificity of antigens used is crucial. Crude antigen preparations can supposedly be less specific than preparations based mainly on cell membrane components, although antigenic composition may vary between different stages in the life cycle of the parasites. Detection of circulating antigens and immune complexes can also be attempted in infections by cyst-forming coccidia.

Demonstration of cell-mediated immunity such as delayed-type hypersensitivity reaction (skin test) or lymphocyte transformation can be used as indirect indicators for specific infections. Also, demonstration of specific local immune reactions has been used in coccidial infections.

In the future, nucleic acid probe techniques such as polymerase chain reaction (PCR) and *in situ* hybridization will most certainly become increasingly important for identification of coccidian species and demonstration of coccidial infections.

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SMALL SUBUNIT rRNA AS A TARGET MOLECULE FOR DIAGNOSTIC AND PHYLOGENETIC STUDIES OF CYST-FORMING COCCIDIA

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Sarcocystis is a genus of cystforming coccidian parasites in the phylum of Apicomplexa. *Sarcocystis* species cause subclinical and clinical disease in animals and economic loss in farm animal production (1). In recent years, small subunit ribosomal RNA (ssrRNA) sequences have been used for determination of evolutionary relationships among various cellular life forms, including several protozoan parasites. The ssrRNA is very suitable for this purpose, because it consists of evolutionary highly conserved as well as variable regions, and has a functional constancy in all cells (2). Several rDNA probes based on sequence information from rRNA have been constructed for the identification of microorganisms. For diagnosis of protozoan infections in animals and man there is need for methods which make a specific identification of the causative organism on genus and species levels possible. It is evident that probes based on rRNA sequences could offer unique possibilities for specific identification of infectious protozoa, which has been shown for example in malaria (3).

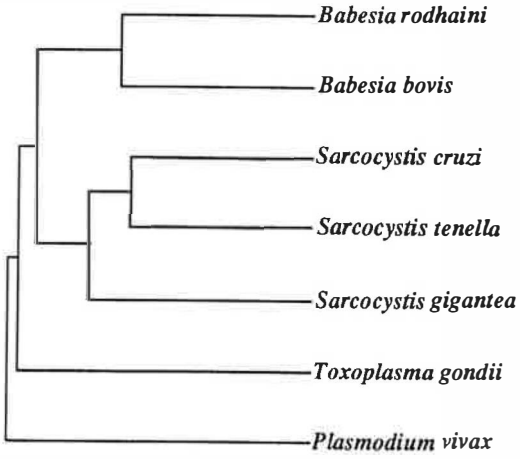
Cystozoites of *S. cruzi* and *S. tenella* were recovered from heart muscle tissue of naturally infected cattle and sheep, respectively, using pepsin-HCl digestion and purification by gradient centrifugation in Percoll™. Whole cysts of *S. gigantea* and *S. fusiformis* were isolated from esophagus from naturally infected sheep and skeletal muscle from water buffalo, respectively. Stable RNA was extracted from the different *Sarcocystis* species as well as from *Toxoplasma gondii*. Small subunit rRNA was partially sequenced from *S. gigantea*, *S. cruzi* and *S. tenella* using reverse transcriptase and DNA primers complementary to evolutionary conserved regions. The sequences were compared by computer alignment. The taxonomical relationships between the species investigated,

and some closely related parasites, were estimated using the computer program PileUp. Three oligonucleotides were designed and used as probes in hybridization experiments.

Approximately 800 bases from ssrRNA of each of *S. gigantea*, *S. cruzi* and *S. tenella* were determined. A high degree of sequence homology was seen in the V5, V6 and the V8 region especially for *S. cruzi* and *S. tenella*. In the V4 region several sequence differences could be found and utilized for construction of an *S. cruzi* specific probe and an *S. tenella* specific probe. The *S. cruzi* probe hybridised exclusively with rRNA from *S. cruzi* whereas the *S. tenella* probe hybridised exclusively with rRNA from *S. tenella*. The data base deposited sequence of ssrRNA for *T. gondii* was computer aligned with the sequences of the *Sarcocystis* species and a *Sarcocystis* genus specific probe targeting the V6 region was made. The probe showed genus specificity compared to *T. gondii* in hybridization experiments. Computer analysis using the program PileUp resulted in a preliminary taxonomical tree based on rRNA sequences (Fig. 1). Results of the analysis suggest a closer relationship between the three species of *Sarcocystis* investigated then any of the *Sarcocystis* species to *T. gondii*. Surprisingly, it also suggests a more close relationship between two species of *Babesia* and the three species of *Sarcocystis* studied than between *T. gondii* and the three species of *Sarcocystis*.

This study demonstrates that probes based on sequences of ssrRNA can be used to identify cystforming coccidia on genus as well as species levels, and that ssrRNA sequences can be used as tools to study taxonomical relationships. Since only part of the sequences have been elucidated, with some sequence ambiguities, additional work is in progress in order to establish the phylogenetic relationships between cystforming coccidia.

Fig 1. A dendrogram showing relationships between some coccidian parasites.



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NEOSPORA CANINUM - AN IMPORTANT PATHOGEN OF RUMINANTS

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Neospora caninum is a recently recognized protozoan parasite of animals. Until 1988, it was misdiagnosed as *Toxoplasma gondii*. The parasite was first recognized in tissues of dogs and thus named *N. caninum*. Subsequently it was found to be infectious to cattle, sheep, dogs, mice and rats. *Neospora caninum* or *Neospora*-like parasites cause paralysis and death in dogs and neonatal mortality and abortion in cattle, sheep, goats and horses. Its life cycle is not known. Tachyzoites and tissue cysts are the only asexual stages known, and the carnivorous definitive host is unknown. Transplacental transmission is the only known natural route of infection. *Neospora caninum* has been transmitted transplacentally in experimentally infected cattle, sheep, dogs, cats and mice.

Neosporosis is emerging as one of the most important causes of abortion in dairy cattle in the United States, particulary in California. In one study, *Neospora* was found in 24.4% of 698 bovine abortions during the 6-year period 1985-1990. Neosporosis can causes *in utero* death, mummification, abortion, stillbirth and birth of weak calves, throughout gestation. The mean age of aborted calves is approximately 5.6 months. There are no specific gross lesions in fetuses or stillborn calves. Microscopically, nonsuppurative encephalomyelitis characterized by necrosis and mononuclear cell infiltrations are the two most important lesions. *Neospora* organisms are few and often difficult to find in H&E-stained sections.

Congenitally infected calves may be born weak, paralyzed, or may become recumbent within a week of birth. One calf was born apparently healthy and developed clinical neosporosis 3 weeks after birth. Cows that have aborted due to neosporosis cycle normally, but repeated congenital infection can occur in the same cow. *Neospora* has occasionally been found associated with neonatal mortality in goats and sheep. There is no antemortem diagnostic test for *N. caninum*-associated abortion in cattle. At present the identification of the parasite in fetal tissues is the only means of diagnosis. The immunohistochemical staining with anti-*N. caninum* serum helps in the identification of the parasite in tissue sections.

CRYPTOSPORIDIOSIS IN RUMINANTS - EPIDEMIOLOGICAL, CLINICAL AND DIAGNOSTIC ASPECTS

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Infections with *Cryptosporidium spp.* are frequently occurring in ruminants, *C. parvum* being the predominant species, but including *C. muris* as well. The host specificity of *Cryptosporidium spp.*, and in particular of *C. parvum*, is low.

Epidemiology: In contrast to *Eimeria spp.* and *Isospora spp.*, autoinfection seems important in the life cycle for *C. parvum*. Therefore, ingestion of oocysts at even low levels may lead to severe infections, as only the development of sufficient immunity will stop the intestinal re-cycling of the parasite

in the infected host. Oocysts of *Cryptosporidium spp.* are fully sporulated when excreted in faeces, and thus ready to initiate infections. The oocysts are highly resistant and may survive for several months under favourable conditions. However, high temperatures (> 65°C), freezing, or to a certain extent desication are detrimental to the oocysts. Transmission of *C. parvum* infections occurs by the faecal-oral route, calf to calf, lamb to lamb, or kid to kid. Subsequently, management systems characterized by low hygiene level and/or crowding of host animals will enhance the risk of clinical cryptosporidiosis. *Cryptosporidium* oocysts may be transmitted from one host species to another, e.g. from infected calves to lambs (*C. parvum*) or from infected mice to cattle (*C. muris*).

Clinical aspects: *C. parvum* is located in the mid and lower part of the small intestine. Damage of the enterocytes and villous atrophy seems to play an important role, when considering the mechanisms by which *C. parvum* produces its clinical effect.

Clinical infections with *C. parvum* in ruminants are strongly related to the age of the hosts, the majority of infections in calves being registrated in the second and the third week of life. In absence of other enteropathogens, cryptosporidiosis in calves is clinically characterized by a transient, mostly mild, diarrhoea. Concurrent infections with rotavirus or other enteropathogens will usually induce more severe clinical symptoms. Jersey and beef breeds may suffer from higher mortality than other breeds when infected with *C. parvum.* Of particular interest are observations which indicate the presence of isolates/strains of *C. parvum* of different virulence to calves.

Clinical signs in lambs and goat kids infected with *C. parvum* are similar to those observed in calves, though usually characterized by a higher mortality rate.

C. muris, frequently observed among cattle of all age-groups in the USA, is located in the abomasum, and the clinical signs will reflect the epithelial damage of this part of the gastro-intestinal tract.

Diagnosis: Several diagnostic techniques are available when searching for oocysts of *Cryptosporidium spp.* in faecal samples, or when doing post mortem examinations. When floated in sucrose solutions, *C. parvum* oocysts will appear in the microscope as 4.5-6.0 µm round bodies (oocysts of *C. muris*: 6.0-8.0 µm), containing 1-4 prominent dark granules. In phase-contrast the oocysts will present themselves highly refractile and easily recognizable.

In smears stained by a modified Ziehl-Neelsen technique, oocysts appear as red-stained (acid-fast) spherical bodies against a green or blue background. Part of the oocysts are usually stained 'erythrocyte-like', while others do not take the dye. Alternative staining techniques include e.g. a modified Köster method, and an auramine/carbolfuchsin method. Negative staining techniques, such as a carbol fuchsin method and a nigrosin method may also be used.

So far, immunological techniques, e.g. IFAT, have been introduced only in a few laboratories. Immunological techniques will undoubtedly gain more attention in the years to come, due to their generally higher specificity.

The severity of clinical signs in ruminants and the level of oocyst shedding are usually correlated. Thus OPG of 10^6 or higher are often found in clinical infections. When doing herd diagnosis it seems relevant to examine faecal samples from calves suffering from diarrhoe as well as from healthy calves due to the potential occurrence of low-virulent strains/isolates of *C. parvum.*

Reference list will be submitted by request to the author.

EIMERIA COCCIDIOSIS IN SHEEP IN NORWAY: A HISTORICAL REVIEW

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In most parts of Norway the sheep are housed at least for some period in the winter, and the lambs are born when the ewes are indoors. It is common to keep the sheep on slatted floor, where the lambs will have limited contact with the dung of the mothers. The lambs are in most cases turned out at 2 - 4 weeks of age.

For many years problems with scour in lambs on spring pastures have occurred in many parts of Norway. Gastrointestinal helminths were first suspected to be important in this connection. As the diarrhoea in lambs occured as early as 2-3 weeks after turnout, it was suggested that parasite eggs or larvae which had survived the winter in the pasture, to a large degree were responsible for this condition.

From 1959 to 1963 some investigations on the winter survival of free-living stages of parasites in the pasture were carried out, using wormfree lambs which were kept for two weeks in paddocks where sheep had grazed the previous year. It was then revealed that some helminth species could survive the Norwegian winter in the pasture, and also that they could be the cause of a softening of the faeces of the lambs. It was further demonstrated that lambs were heavily infected with coocidia in these paddocks. One of the experimental lambs died of coccidiosis after it had been housed for one week after a two-weeks' stay in the paddock. These observations initiated several investigations on the importance of overwintered *Eimeria* oocysts in the pasture as a source of diarrhoea in lambs on spring pastures.

It was demonstrated that lambs grazing pastures on which sheep had been kept the previous years, developed severe coccidiosis during the third week after turnout. It was also demonstrated that lambs that grazed such a pasture for two days only, and then were housed again, or transferred to a clean pasture, developed symptoms of coccidiosis similar to those of the lambs that continued to graze the contaminated pasture. Lambs kept on pastures where no sheep had grazed the preceeding years, showed no symptoms of coccidiosis and excreted low numbers of oocysts in the faeces.

It was observed that lambs were eating soil during their first days on pasture. It was then suspected that this habit could explain how the lambs could become so rapidly infected with coccidia on pasture.

The role of contaminated soil as a source of coccidia for the lambs were further examined by giving each of 7 lambs a water suspension of 50 g. of soil from an area on a permanent pasture where the sheep used to rest.

Another group of 7 lambs was tube-fed with a similar suspension of soil from a neighbouring area were no sheep had grazed earlier. A third group of 7 untreated lambs was kept as controls. The lambs were kept indoors on slatted floor. All the 7 lambs given soil from the contaminated pasture developed severe diarrhoea, with large numbers of oocysts in the faeces. Two of these lambes died of coccidiosis. The lambs given soil from the uncontaminated area and the control lambs had low numbers of oocyst in the faeces, and no clinical symptoms of coccidiosis was observed.

The proposal that overwintered oocysts were the main source of coccidiosis in lambs was in contrast to the general view that coocidiosis was due to a mother to offspring transmission. It was, however, demonstrated that the ewes could be the source of coccidia infection in lambs indoors on slatted floor, but this was of minor importance compared to the infection from contaminated spring pastures.

During a series of trials it was observed that coccidiosis in lambs on spring pastures showed a very regular pattern under Norwegian conditions, and that the disease mainly developed during the third week on pasture. In some lambs the symptoms appeared as early as 12 to 14 days after turnout.

On the basis of these observations one tried to prevent the outbreak of the disease by treatment with sulphadimidine, a drug which had been widely used as an anticoccidial drug. Treatment at different times in relation to turnout was tried, and the administration of sulphadimidine at a dose of 200 mg/kg body weight at 12, 14 and 16 days after turnout was generally found to prevent the outbreak of coccidiosis, and significantly lower numbers of oocysts occurred in the faeces of treated lambs. This treatment was widely practiced in commercial farms for several years. It was later advised to give the treatment on three consecutive days beginning on day 12 after turnout.

The treatment with sulphadimidine could, however, not totally prevent diarrhoea in all lambs, and even deaths from coccidosis could occur.

Treatment with other sulfonamides, and treatment with amprolium compounds on the same days as practiced with sulphadimidine were also investigated, but these drugs proved to be less effective than sulphadimidine.

From 1984 onwards several experiments with treatment of lambs on spring pastures with the new anticoccidial toltrazuril were carried out. This drug is effective against all stages of *Eimeria*, and seems to be very effective in the control of coccidiosis in lambs. It was shown that a single treatment with toltrazuril at a dose of 20 mg/kg on day 7 after turnout could prevent the outbreak of coccidiosis, and the excretion of oocyst in treated lambs remained at a low level for several weeks. The lambs were kept on the contaminated pasture for 6 weeks after turnout, and nearly no symptoms of coccidiosis occurred in the lambs during this period. Thus the lambs apparently had acquired a sufficient level of immunity against coccidia to withstand the natural reinfection in spite of the treatment. Treatment on day 10 after turnout did not prevent a softening of the

faeces in the same manner as treatment on day 7. In recent years treatment of lambs with toltrazuril has been widely used with great success in commercial farms in Norway.

The investigtions also showed that the nematode *Nematodirus battus* in some years might cause outbreaks of diarrhoea in lambs at about the same time as the coccidia, i.e. about 2-3 weeks after turnout. Such concurrent infections with *N. battus* might obscure the benefical effect of preventive treatment with troltrazuril against coccidiosis.

EIMERIA ALABAMENSIS COCCIDIOSIS IN CATTLE IN SWEDEN

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Diarrhoea in calves shortly after they are first turned out to pasture is a common clinical problem in Sweden and has traditionally been attributed to change of diet. However, it has recently been discovered that some of these early appearing pasture diarrhoeas are actually due to *Eimeria* coccidiosis.

The species involved is *Eimeria alabamensis*, an intranuclear coccidium which develops in the villar epithelial cells of the lower part of the small intenstine. It was first described in 1941 by Christensen in Auburn, Alabama, USA and is now considered to be widely distributed. The oocysts measure 13-25 x 11-17 µm and are usually ovoid with uncoloured or slightly yellowish wall. The prepatent period is six to eight days.

Calves have been seen to excrete small numbers of oocysts of *Eimeria alabamensis* from two to three weeks of age and reinfections occur commonly. *E. alabamensis* coccidiosis on pasture has been observed in calves which have excreted small numbers of oocysts of this species during the preceeding stable period.

Overwintered oocysts ingested during the first days on pasture have been shown to be the source of infection. Infected calves usually develop watery diarrhoea within four to six days after they are turned out, and large numbers of oocysts (800 000 to several million per gram faeces) are observed in their faeces three to four days later. Affected calves often show a few days of inappetence and are often reluctant to rise. *Eimeria alabamensis* coccidiosis in Sweden is associated with the use of permanent pastures to calves aged 6 to 16 months.

STUDIES ON AN EXPERIMENTAL *TOXOPLASMA* ISCOM VACCINE

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The protozoan parasite *Toxoplasma gondii* is capabable of infecting a wide range of animals, including man, and although the infection in most cases is subclinical, it can, when contracted in utero or by immunocompromised individuals, cause serious disease. In veterinary medicine it is recognized as a cause of abortion in sheep, goats and pigs. Moreover, latent infection in food-producing animals is an important source for human infection (1).

The immunostimulating complex (iscom) is a highly immunogenic formulation of amphi-

patic antigens incorporated into a matrix consisting of the adjuvant Quil A, cholesterol and phospholipids (2). Iscoms prepared from detergent extracted antigens of *T. gondii* tachyzoites contain a number of antigens. Two of the major antigens have been identified by monoclonal antibodies as the membrane antigens P30 and P22 (3). Immunization of mice with *Toxoplasma* iscoms resulted in both humoral and cell mediated immune responses, the latter detected as delayed type hypersensitivity measured by a footpad assay. Challenge experiments have revealed protective immunity, expressed as reduction in mortality or longer survival after a lethal infection with *T. gondii*. Such results have been obtained after intraperitoneal injection of tachyzoites as well as with oocysts given per os. However, since cysts were found in the brains of surviving mice, immunization did not prevent dissemination of the parasite throughout the body (3, 4).

In sheep vaccination with *Toxoplasma* iscoms resulted in a substantial antibody response. After challenge an apparantly reduced lamb mortality and longer gestation period was recorded in immunized ewes as compared to controls, although the differences were not statistically significant. Lambs born alive had precolostral antibodies to *T. gondii*, which indicated they were infected *in utero* (5).

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VACCINES AGAINST AVIAN COCCIDIOSIS AND OVINE TOXOPLASMOSIS

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Coccidiosis in broilers causes considerable losses in the poultry industry. Despite the use of anticoccidial drugs subclinical coccidiosis is caused by drug tolerant strains of *Eimeria acervulina* and *E. maxima*. In the Netherlands the prevalence of these species in broiler houses varies between 50 and 100% (M. Vertommen, pers. communication), leading to average costs of 10 cts = 0.3 SEK per bird (increased feed conversion rate and reduced weight gain). Moreover, the consumer favours the concept of the 'organic' chicken, that has not been fed any antibiotic or anticoccidial drug. Live, oral vaccines are currently on the market, but are not yet widely used, certainly not in broilers: Coccivac and Immucox use low doses of vi-

rulent oocysts, Paracox uses attenuated (precocious) strains.

Experiments aiming at the development of subunit (recombinant DNA) and vector vaccines started about 10 years ago, when Dr. Danforth (USDA, Beltsville) developed a series of monoclonal antibodies against various parasite antigens and organelles. A number of pharmaceutical and biotechnological companies took up the challenge and started screening genomic cDNA libraries and characterizing and isolating protective antigens or epitopes, mainly of the immunogenic sporozoite and merozoite stages. Also work on gametocyte antigens was taken up. Now, ten years later, a range of antigens, mainly from *E. tenella* and some from other species, have been characterized and their genes cloned, sequenced and, partly, tested as fusion proteins through viral or bacterial vector (e.g. Fowl pox virus or *Salmonella sp.*) In most cases protection (i.e. reduction of oocysts or lesion score) is only marginal, but occassionally significant.

A great deal of improvement can be expected from using the right adjuvant and/or obtaining good expression by the vector *in situ.* Problems may occur when the genetic background of the chicken breed is involved in the induction or effectiveness of immunity. Therefore, more studies are required to reveal the immune effector mechanisms underlying protection. Since new drugs are not being developed, one has to deal carefully with the existing ones or use live vaccines.

Toxoplasmosis is recognized as an important cause of abortion and perinatal lamb losses in some parts of the world. Moreover, infection early in pregnancy often leads to barrenness. Cats contaminate cereals, hay and bedding by excreting oocysts in huge numbers, although this happens only during a very short period in their lifetime. Prophylactic treatment of ewes during pregnancy with monensin is effective but expensive. Controlled infection before mating includes a serious risk for public health.

In 1983 a field trial was conducted in New Zealand by Wilkins and O'Connell (Wallaceville, Anim. Res. Inst.) whereby ewes were vaccinated with tachyzoites of an 'incomplete' strain S48. This strain does not produce oocysts in cats, or tissue cysts in intermediate hosts. It was continuously passaged in mice. At Intervet S48 has been adapted to continuous *in vitro* cultivation, and batches of vaccine are produced from frozen master seed material.

It has been demonstrated that the S48 tachyzoites survive (and multiply?) in vaccinated sheep for about one week, causing a febrile response, and then disappear without forming tissue cysts. The vaccine does not prevent the formation of tissue cysts after challenge with a field strain, but protects very well against abortion for at least two mating seasons. Its performance in the field was tested in the UK during the season 1990/91, under supervision of the Moredun Research Institute. The trial showed that the vaccine is indeed effective and safe.

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.

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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

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