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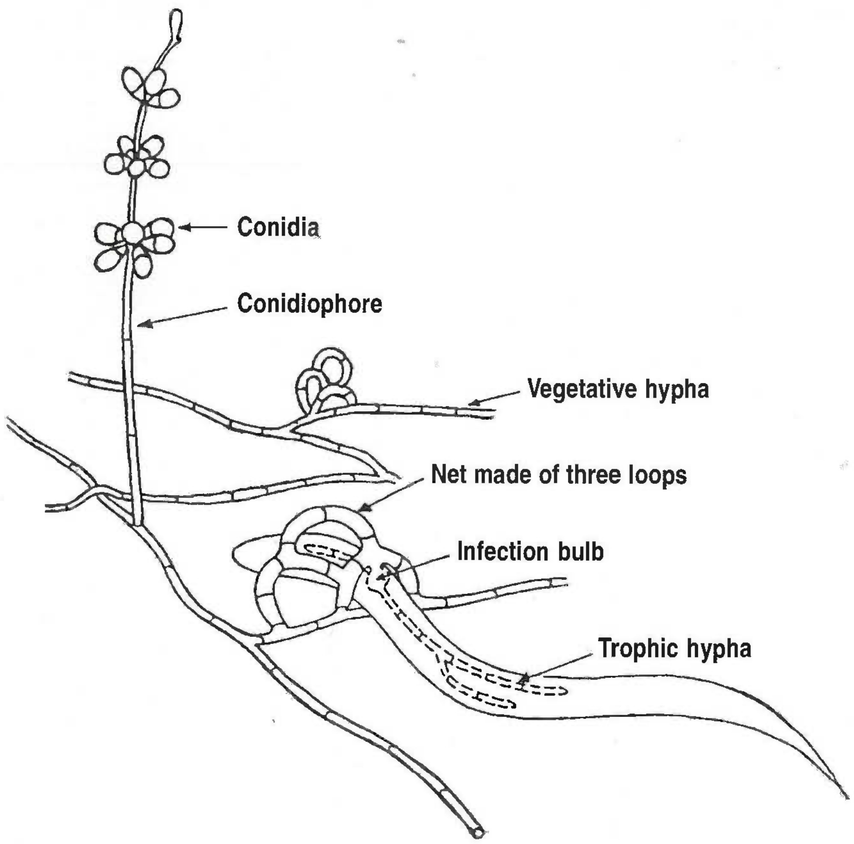
141

Biological control of gastro-intestinal nematodes of ruminants using predacious fungi



Food and Agriculture Organization of the United Nations

Biological control of gastro-intestinal nematodes of ruminants using predacious fungi

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FOREWORD

Gastro-intestinal nematode parasitism is one of the most important disease constraints to small ruminant production in the sub-tropics and tropics, where the environment provide near-perfect conditions for the development and survival of these parasites. Control of the gastro-intestinal nematodes particularly *Haemonchus contortus* and *Trichostrongylus* species is a prerequisite for profitable small ruminant production. Strategies for the control have up till now relied almost entirely on the use of anthelmintics.

The frequent use, often combined with mismanagement of the drugs, have led to wide-spread resistance of the parasites to one or more of the major groups of anthelmintics particularly the benzimidazoles and the levamisole/morantel group. The situation is alarming in many parts of the world, where the exhaustion of almost all chemotherapeutic options for nematode control in sheep and goats is likely to result in serious economic consequences.

There is, therefore, an urgent need for developing alternative sustainable strategies. These include grazing management, breeding for resistance/resilience, better utilization of existing drugs through the understanding of the pharmacokinetics and the use of predacious fungi for biological control of the nematode parasite larvae on pasture.

The predacious activities of a number of fungus species in the soil, compost and manure have been known for more than hundred years, but strangely enough, this knowledge was not utilized in the context of nematode control until much later and initially only in the control of parasitic nematodes of plants. It was not until the beginning of the 1980ies that this principle was systematically investigated and tested, and through the continuous effort of Professor Peter Nansen and Dr. Jorn Gronvold at the Royal Veterinary and Agricultural University, Copenhagen, Denmark the fungi now offers a viable option for biological control of gastro-intestinal nematodes expanding the arsenal of control methods. It should be stressed, that it is unlikely that biological control can completely replace other strategies, and it should be viewed as a component in integrated parasite control.

These Proceedings are a compilation of working papers prepared for the FAO/DCEP workshop on "Biological Control of Gastro-Intestinal Nematodes of Ruminants Using Predacious Fungi" which was held 5 - 12 October 1997 at the Veterinary Research Institute, Ipoh, Malaysia. The main objective of the workshop was, through technology transfer, to increase the capacity of selected scientists and their laboratories to utilize biological control of parasitic nematodes of ruminants in the future. In view of the potential of biological control becoming an important component in integrated parasite control strategies, FAO would like to make the compiled information available to a wider audience.

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PARASITE EPIDEMIOLOGY, RESISTANCE AND THE PROSPECTS FOR IMPLEMENTATION OF ALTERNATIVE CONTROL PROGRAMS

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INTRODUCTION

Livestock are a vitally important agricultural commodity in developing countries of the world. They are reared under a wide variety of production systems ranging from large-scale intensive commercial enterprises to traditional small-holder and village production systems. They can be based on sedentary systems, through an infinite variety of schemes that employ some form of animal movement, to the other extreme of transhumance or nomadic husbandry. Most of the developing countries of the world lie within the limits of the Tropics of Cancer and Capricorn, namely the tropical and subtropical regions of the world. It is here that the problems with helminth parasites are unquestionably much greater than in countries of the more temperate regions. The environment in the tropics/subtropics is favourable more-or-less continuously throughout the year for the free-living stages of parasites to hatch and develop. The limiting environmental variable controlling the development of worm eggs to infective larvae is rainfall, because temperatures are always warm enough to facilitate this process. Consequently there is a direct correlation between the severity of helminth problems and rainfall in those regions where livestock are raised in the developing countries of the world.

PARASITE EPIDEMIOLOGY

Parasitic nematodes of ruminants are among the most common and economically important disease entities of livestock particularly in small ruminants in the developing world. They have simple, direct life-cycles, normally with the following pattern: nematode eggs are passed out to the environment in the faeces of the host. On the pasture the eggs develop through a series of larval stages to reach the 3rd or infective larval stage. These infective larvae migrate onto herbage and are ingested by the grazing animal to commence the parasitic phase of the life cycle. Development to mature, egg-producing adult worms occurs in the gut of the animal. The exposure of livestock to helminth parasites will differ according to the production system, but a common aspect of nearly all husbandry systems practiced in these countries is their dependence on grazing. This of course places the host animal at risk to nematode infection as they are exposed more-or-less continuously to larval pick-up during the wet periods of the year in these regions.

Ecology of the Free-living Stages

In the temperate regions of the world, large number of recommendations to improve efficiency of parasite control in livestock based on combining anthelmintic treatment with some form of grazing management have been developed (Barger, 1996). In comparison, there are relatively few examples of such schemes in the tropics/subtropics, but the potential for grazing management is possibly even greater in these regions. This is due to the fact that the development of the free living stages being generally much faster and more successful than in the temperate regions, the longevity of the larvae is much shorter. Studies in the wet tropical climates show that peak larval concentrations occur on pasture within one week after contamination, but fall to barely detectable levels within 4 - 6 weeks (Banks *et al.*, 1990; Barger *et al.*, 1994; Sani *et al.*, 1995).

Host Specificity

Alternate grazing between sheep and cattle can be a very effective form of parasite control for both livestock species, provided that the grazing alternations are linked with the seasonal reductions in larval availability on pasture. From a practical standpoint, cross-transmission of parasites from one host to the other is of little consequence. In the temperate regions of the world, excellent control of parasites from both species of livestock can occur from very infrequent pasture interchange (Barger and Southcott, 1978) and if the timing is epidemiologically precise, pasture changes need not be accompanied by anthelmintic treatment (Donald *et al.,* 1987). However, care must be exercised in simply transferring such schemes to the tropics and subtropics, without first conducting ecological studies on the free living stages of parasites in these environments. While similar benefits may result from interchange grazing, the grazing intervals will, almost certainly, need to be shorter. Also, control of *H. contortus* may prove difficult. In the more temperate regions this species can cycle in calves but they rapidly acquire natural immunity to become refractory to infection by 12 months of age (Southcott and Barger, 1975). In the tropics this age resistance is slower to, or may never, develop. For example, in Paraguay there was no indication that cattle had acquired significant immunity after two years of grazing (Benitez-Usher *et al.*, 1984).

Mixed grazing of sheep and cattle is a much more commonly practiced procedure. The benefits are mainly seen as being agronomic, with better pasture utilisation and the maintenance of pasture species composition, rather than parasitological. However there have been some reports in the temperate regions (Barger, 1996) and in the tropics (Aumont *et al.*, 1995) that grazing sheep and cattle together can improve performance, particularly of sheep, which was attributed to better parasite control. It may be expected that farmers that practice mixed grazing systems would also tend to use anthelmintics less frequently, however surveys and questionnaire results in Uruguay provide no evidence for this, as levels of anthelmintic resistance on such properties was as great as on sheep enterprises (Nari *et al.*, 1996).

Anthelmintic Resistance in the Tropics/Subtropics

There is no doubt that for the sheep and goat industries at least, anthelmintic resistance is the greatest threat to continuing production throughout the tropics/sub-tropics. The following examples illustrate this point.

- **South America.** Recent surveys in the humid sub-tropical region of South America, which encompasses the northern provinces of Argentina, the southern state of Rio Grande du Sul in Brazil and all of Paraguay and in temperate Uruguay, which collectively account for approximately 50 million head of sheep and goats (Waller et al., 1996), showed that resistance existed to all the commercially available broad spectrum anthelmintic groups. High levels of resistance (exceeding 80% of farms) to both the benzimidazole and the levamisole/morantel groups, and their combination, were recorded in all countries. Although resistance to the macrocyclic lactone group was only at a low level (1.2%) in Uruguay (Nari *et al.*, 1996), it was the only generally efficacious group for most farmers. However, sheep producers in the neighboring countries are in a much more parlous state. Ivermectin resistance was estimated to be present on 6% of farms in Argentina (Eddi *et al.*, 1996), 13% in Brazil (Echevarria *et al.*, 1996) and on approximately 70% of sheep farms in Paraguay (Maciel *et al.*, 1996). In addition, 20% of farms surveyed in Brazil showed resistance in Haemonchus contortus to the salicylanilide anthelmintic, closantel - the highest ever recorded for this drug anywhere in the world.

- **South-East Asia and the Pacific**. The number of small ruminants in South-East Asia and the Pacific nations has increased rapidly in recent years and with this the problems of nematode parasite control. About a decade ago, Banks (1988) warned that anthelmintic resistance was one of the greatest threats to the future of small ruminant production in the South Pacific. This prediction seems to be becoming a reality. Recent surveys showed that combined resistance to the benzimidazoles and levamisole occurred on approximately one third of commercial farms in Fiji and resistance to ivermectin was also emerging (Manueli, 1996; L.F. LeJambre personal communication). Furthermore survey work in Malaysia (Dorny *et al.*, 1994) has shown high levels of resistance, particularly to the benzimidazoles, in parasites of small ruminants and multiple resistance involving all broad spectrum anthelmintic groups, as well as closantel has been recently reported (Sivaraj *et al.*, 1994). Anthelmintic resistance is also increasing on the sub-continent of India (Gill, 1993).

- **Africa**. In the high rainfall regions of Africa, anthelmintic resistance has been detected in virtually every instance when it has been specifically investigated. Unfortunately resources to conduct this work are very limited and largely restricted to Kenya, Tanzania and South Africa. Surveys in these countries show that the situation is grim. In Kenya, resistance is widespread, particularly to the benzimidazoles, and there exists a major problem of poor quality drugs commanding a major share of the market (Wanyangu *et al.*, 1996). Recent surveys in South Africa show that around 90% of sheep farms have parasite strains resistant to compounds from at least one anthelmintic group and approximately 40% of farms now have to confront the problem of multiple anthelmintic resistance (van Wyk *et. al.*, 1997). There are also a number of instances recorded in South Africa where farmers have had to abandon sheep farming because of failure to control worms by chemotherapy (van Wyk, 1990). The situation with regards to anthelmintic resistance, outlined above, makes rather depressing reading. But, it does not overstate the case with regards to this problem in nematode parasites of small ruminants in the tropics/subtropics.

Approaches to Parasite Control – Now and in the Future

Effective helminth control is a major element in ensuring the sustainability of animal production, especially as part of current livestock development plans of increasing the volume of locally produced livestock products being embraced by many countries in the developing world. Herein lies the crux of the problem, namely the sustainability of the helminth control practices themselves upon which overall sustainability of grazing livestock enterprises in developing countries depends.

There are now a number of alternative, non-chemotherapeutic approaches to parasite control which offer benefits, or the promise of great opportunities in the future – not least of which is the focus of this workshop, namely biological control. Parasite control has been almost exclusively focussed on the parasitic phase within the host – particularly in the use of anthelmintics, attempts to develop vaccines and breeding animals with natural resistance/resilience to worms. However in most situations and for most of the time, the greatest proportion of the biomes of the parasite population is not in the host, but on pasture. Research activity should now pay greater attention to control of parasites in the free-living stage, in the pursuit of truly sustainable methods of parasite control.

Effects of Pasture Plants

Direct effects of herbage, or plant extracts, on parasites have been known for a long time and many traditional de-worming preparations currently used by livestock owners in the tropics and subtropics are based on such material (Hammond *et al* 1997). Pasture plants such as legumes, have been reported to have anthelmintic properties, and at certain stages of growth, grasses and forage crops appear to act as vermifuges (for review see Anderson *et al.*, 1987). Recently there has been considerable interest generated following the studies of Niezen and co-workers (1995) which suggests that forages containing condensed tannins provide sheep with the ability to withstand parasite infection. This may be due to direct anthelmintic properties of tannins, or most probably due to the role of tannins in protecting dietary protein from ruminal degradation and thus animals are on a better plane of nutrition. This work has some exciting possibilities for the tropics/ subtropics as the majority of forages high in condensed tannins are found in these regions of the world. These have been generally looked upon with disfavor as excess condensed tannins in the diet of animals have detrimental effects on health and productivity. It may be that there is an optimum level, or type, of tannin in the diet – too little and there is no beneficial effect of ruminal bypass of protein – too much induces anti-metabolic, toxicity problems in animals. This is obviously an area requiring closer investigation.

ATTACKS ON THE FREE-LIVING STAGES

Grazing Management

Pastures provide the link between the free-living and parasitic phases of nematode parasites of grazing livestock. At different stages of growth, pasture species may facilitate, or impede the survival of the free-living populations, the establishment of parasite burdens and lessen or intensify the effects of parasitism in livestock. In theory, competent management of pastures provides the efficient conversion of herbage to animal products and the effective control of nematode parasites. There are a number of good examples of grazing management, which offer efficient and practical adjuncts to parasite control.

Grazing systems have been developed for the Pacific Island countries (Barger *et al.*, 1994) and Malaysia (Sani *et al.*, 1995) to exploit the short survival time of free living larvae. These involved the subdivision of pasture into small plots and allowing sheep or goats to graze for no more than 4 days before movement to a new plot.

Animals were moved around the suite of plots, returning to the original plot after approximately 30 days, thus avoiding auto-infestation whilst efficiently managing pasture productivity. Subdivision fencing was easily and cheaply erected by using solar-powered electrical fencing, or using readily available, cheap fencing materials. The need for anthelmintic treatment is drastically reduced, or can possibly even be eliminated (Barger *et al.*, 1994), which is in stark contrast to the routine of treating sheep and goats every 3-4 weeks with anthelmintic which is commonly practiced in these countries. Unfortunately, these simple and practical grazing management systems tend to be abandoned, not because they ultimately fail, but because livestock owners consider that they require more effort compared with simply suppressively drenching their stock (P.Manueli; P. Chandrawathani pers. comm.). The message of impending total anthelmintic failure and the need to adopt sustainable parasite control practices is not being heeded or reinforced.

Biological Control

By definition, biological control does not assume to be a substitute for chemotherapy where the expectation, if not the reality, is that parasites may be eradicated by the frequent use of drugs with efficiencies approaching 100%. Biological control agents rarely eliminate the target organism, but reduce the numbers to acceptable levels and maintain a balance between the pathogen and the antagonist. In contrast also to chemical control of nematode parasites which is directed entirely at the parasitic stage within the host, biological control will almost certainly be focused on the free-living stages on pasture. Within this environment, the pre-parasitic stages of nematodes are subject to a variety of both abiotic and biotic factors that can profoundly influence their development and survival. The most important abiotic factors are temperature, oxygen and humidity – extremes in these can be lethal on these free-living stages. With regards to biotic factors, there exists a vast assemblage of living organisms that can affect the success of worm eggs developing to infective larvae. From these may emerge candidate(s) for biological control of worm parasites.

Before considering these, it is useful to briefly describe the general concepts of biological control. Essentially, it can be divided into two broad categories.

- *Natural biological control.* Control produced by native (or co-evolved) natural enemies in the normal environment. Although such organisms certainly exist against worm parasites, under most livestock grazing enterprises they are likely to have little impact, otherwise there would not be a problem with worm parasites in the first instance. It has been argued that the major ecological disturbances that followed the intensification of livestock grazing systems, have tipped the balance in favor of parasites by providing an abundance of susceptible hosts and favorable pasture micro-environments for the free-living stages.

- *Applied biological control.* Control produced by human intervention. This is further divided into classical biological control, which is effected by the introduction of exotic natural enemies, or augmentative biological control which is brought about by the enhancement of natural enemies already present in a given environment. Most people associate biological control with the former. Although there have been some examples of classical biological control that have been spectacularly successful, such examples in Australia are the use of the *Cactoblastis* moth to control prickly pear and *Myxomatosis* to control rabbits, there have also been some spectacular disasters. Again Australia can provide an example, with the cane toad introduced to control cane beetles, but it has now spread widely, causing inestimable damage to both beneficial invertebrate and vertebrate fauna alike. As a result, regulatory authorities in many countries insist on thorough environmental assessments to be conducted before they sanction field release of introduced organisms. Control of nematode parasites of livestock is likely to be by the augmentative approach, either by manipulation of the environment or of the existing natural enemies of parasites.

Biological Control of Parasites by Manipulation of the Environment

There are good examples of environmental manipulation, or management, for the biological control of insects. These include changes in land use, habitat provision, reducing natural enemies of beneficial species, and improved pesticide utilisation – particularly more selective use. There is good reason to consider that it is possible to lessen the effects of worm infections in livestock by similar environmental manipulation. In support of this, there is evidence that organic farming practices increase the abundance and variety of dung-dwelling microrganisms, particularly fungi, which may include nematophagous species (Bell, 1983). These findings may partly account for the good levels of parasite control in organically reared lambs in New Zealand (Niezen, *et. al.*, 1996). There is also some evidence that the type of plant species used in pastures can influence the species and type of fungi that colonise the dung of livestock that graze on the pastures (Hay and Niezen 1995).

The practice of "green manuring" of land, by the ploughing-in of various crops, as a replacement for synthetic fertilizers, is now being strongly advocated in Western Europe. This is not only more ecologically responsible, but another "spin-off" benefit is that it encourages the proliferation of earthworms which can have an important influence on the free-living stages of parasites, as described in more detail below.

Biological Control of Parasites by Manipulation of the Organisms

Direct manipulation of natural enemies of parasite larvae consists of mass production and field release of individuals of a given species of organism. There are two types of release, namely inoculative and inundative. Inoculative release refers to the release of relatively small number of individuals where the expectation is that the progeny of these individuals will provide long-term pest suppression. In contrast, inundative release is the release of massive number of individuals with the aim of providing immediate pest suppression. It is in this latter category of augmentative, inundative release that future biological control of worm parasites of livestock will be developed.

Candidates for Biological Control of Worm Parasites

A. Dung removers

- *Dung beetles*. Dung beetles are found throughout the world and these are often capable of rapid and often complete dung removal and thus are indirectly responsible for significant reductions in the number of free-living stages of parasites (Waller and Faedo, 1996). However, such dung dispersal activity is notoriously labile, being dependent on ideal weather conditions, therefore little opportunity exists to exploit these organisms in attempts to achieve cost-effective and reliable biological control of nematode parasites.

- *Earthworms.* These take over the role of dung beetles in the cool, moist regions of the world. In northern Europe for example, earthworms play an important and often dominating role in removal of cattle dung from pastures and can be responsible for significant reduction of infective larvae on pasture (Grønvold, 1987).

B. Parasite antagonists

A number of organisms have been identified that exploit the free-living stages of parasites as a food source. These include microarthropods, protozoa, predacious nematodes, viruses, bacteria and fungi (Waller and Faedo, 1996). Although all are of intrinsic interest, it is from the latter two groups of organisms that breakthroughs in biological control are likely to emerge.

- *Bacteria.* Many species of bacteria are associated with the cuticle, body cavity and gut of nematodes and some of these are pathogenic. *Bacillus penetrans* is a promising candidate for the control of parasitic nematodes of plants. It produces highly resistant spores, which attach to the cuticle and then invade the nematode host. This bacterium is highly host-specific, which is both a good and bad thing. It is good from the standpoint that only the target nematode pest will be affected, but bad insofar as the search for the specific *B.penetrans* pathogen for each of the whole range of nematode pests would be most laborious, expensive and fruitless in many cases. Another factor that is hampering the exploitation of this organism is the difficulty in culturing large quantities of *B.penetrans*, which is an absolute pre-requisite for commercialisation. Many bacteria and closely related organisms, the *Actinomycetes*, produce important secondary metabolites, which include antibiotics, insecticides and anthelmintics. As such they should be regarded as microbial control agents, rather than true biological control agents.

- *Fungi.* Fungi that exhibit anti-nematode properties have been known for a long time. They consist of a great variety of species which include nematode-trapping (predacious) fungi, endoparasitic fungi, fungi that invade nematode eggs and fungi that produce metabolites that are toxic to nematodes (Barron, 1977). The most important groups of nematophagous fungi are the first two, namely:

  - *Nematode- trapping fungi.* These fungi produce specialised hyphal trapping devices, such as adhesive networks, knobs, and constricting or non-constricting rings. Fungi in this class may also produce nematode chemoattractant and/or chemotoxic substances (Waller and Faedo, 1993). Within a short period of time following capture of the nematode, the fungus penetrates the worm and destroys it.

  - *Endoparasitic fungi.* These fungi invade the nematode from adhesive spores that stick on the cuticle, from spores that are ingested by the nematode, or from motile spores in water.

Fungi from these two classes are found in all environments throughout the world, but are particularly abundant in rich agricultural soils. Under laboratory conditions, where fungi are grown as a monoculture on standardised, generally nutrient-poor media and are provided with a nematode prey that cannot escape, results can be spectacularly successful. Total capture and destruction of nematodes can occur within a matter of hours. However this type of work provides little relevant information as to how these fungi would perform as practical biological control agents against animal parasitic nematodes. Testing needs to be done to determine the limitations and opportunities for parasite control associated with the livestock production systems being considered.

CONCLUSION

Modern control of nematode parasites needs to move away from reliance on anthelmintic treatment to a more integrated form of pest management. This will have as essential components various non-chemotherapeutic methods of control. To achieve this requires technology transfer and education programs to be implemented and financially supported. The task will not be easy and there is no common strategy that can be recommended because of the inherent diversity in the political, economic, educational and animal management structures in countries of the developing world. However, there are now clear signs that policy and decision makers in these countries are becoming increasingly more sensitized to the importance of helminth parasites as major causes of productivity loss in grazing animals as well as to the significance of problem of anthelmintic resistance. It is hoped that this workshop on the biological control of helminth parasites in livestock in the tropics and subtropics, will be the catalyst to a comprehensive evaluation of the potential of this alternative technology in this region of the world.

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POSSIBLE MEANS OF USING NEMATOPHAGOUS FUNGI TO CONTROL NEMATODE PARASITES OF LIVESTOCK

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INTRODUCTION

Although direct application of fungal spores to animal bedding has the potential to control important parasites, such as Strongyloides papillosus (Chandrawathani et.al., in press), it seems that for most circumstances in which nematode-destroying fungi can be used in the prophylactic control of nematode parasites of livestock, is to incorporate fungal material into the diet of animals. For this purpose, the nematophagous fungus, Duddingtonia flagrans is an ideal choice. This is because of the superior ability of this fungus to survive passage through the gastrointestinal tract of livestock, as the resting, chlamydospore stage. This stage can survive the harsh environmental conditions of gut passage (anaerobic, enzymatic and thermal) in livestock (Larsen et. al., 1992; Grønvold et. al.,1993; Waller et. al., 1997), to which fungi that produce thin-walled conidia, succumb (Faedo et. al., 1997).

However, before fungal material can be used in this manner, local isolations of fungi need to be made, then cultured in sufficient quantities to enable testing to be conducted firstly in the laboratory and then ultimately under field conditions.

Methods for Selecting Fungi as Biological Control Agents

The most important principle for selecting candidate fungi as possible biological control agents is to obtain isolates from the field in the region, or country, where this work is to be performed. This is important for several reasons. Firstly, it has been observed that laboratory stocks of fungal isolates lose various attributes, which may include nematode-destroying capacity, following repeated passage. Secondly, most countries have stringent requirements regarding the importation and field release of exotic living organisms. These two drawbacks would apply if strains of fungi with known nematophagous activity were obtained from the major fungal collections or repositories in Europe or North America. Fungal species that have evolved to survive under local environmental conditions would be much better strains to work with than those derived from centralised fungal collections.

The most relevant sites for sampling would be the environments where the fungi are expected to exert their effects, notably fresh faecal deposits, but in intensive animal production systems, animal bedding may also be appropriate. The reason for restricting the sampling to these sources is simply to save unnecessary labour at a later stage, because in almost all circumstances, future fungal deployment will be in ways which require it to survive passage through the gastro-intestinal tract of animals and then to trap nematodes in freshly deposited faeces. Almost certainly, a large number of nematophagous fungi would be isolated from other sources such as soil, pasture etc. However almost all would fail the most important test of gut survival and thus their isolation (and any other testing) would be wasted effort.

The use of animals as a stringent screening procedure means that the number of occasions on which isolations can be expected is very few. Therefore, if a serious attempt is to be made, a large number of small samples should be collected per rectum, from livestock found on a comprehensive range of farms in a region. Suitable procedures have been described (Larsen et. al., 1994). Following isolation by these means, pen trials should be carried out to confirm the gut survival and nematophagous capabilities of the fungal strains

POSSIBLE FUNGAL PRODUCTS

Direct Application

This could only be considered in the most intensive forms of animal production where animals are closely confined, and of course, where internal parasitism is a problem. Such an example would be the intensive calf-rearing units in the southern islands of Japan where Strongyloides papillosus can cause sudden death in massively infected animals in the hot summer months (Taira and Ura, 1991). A practical solution to this problem may be the direct application of fungal elements to the bedding (Chandrawathani et. al., 1997). Therefore, the requirement for fungi to survive gut passage is not relevant in this circumstance. All that would be required is for the fungi rapidly to colonise the bedding and to reduce the overwhelming number of S.papillosus larvae responsible for the sudden death syndrome, but to allow sufficient numbers to survive to provoke the normal, rapid acquisition of immunity which characteristically occurs against this parasite.

However, apart from similar forms of highly intensive livestock production, it is beyond the bounds of reality to conceive of a practical means of applying fungal material, especially to the grazing environment, to produce reliable and substantial reduction in the free-living stages of parasites.

Supplementary Feeding

Danish workers have demonstrated that a daily supplement of barley grains supporting the growth of D.flagrans will reduce parasitism and increase productivity in grazing cattle, pigs, horses and sheep (Larsen et. al., 1996). These results are especially exciting as they demonstrate that the principle of biological control of nematode parasites using nematophagous fungi is very robust, being applicable across the whole spectrum of grazing livestock species. Clearly then, the transfer of this technology to those production systems where long-term daily supplementary feeding is a common management procedure, would be relatively straightforward. The major impediment would be the need to scale-up production to satisfy the commercial requirements for the fungal grain supplementary feed option for biological control of nematode parasites.

Feed Blocks

Block administration, developed mainly for mineral supplementation and to a lesser extent for anthelmintic medication, is now undergoing a resurgence of interest as a means of low-cost nutrient supplementation of livestock. These blocks can be manufactured using simple, low-cost technology and generally incorporate surplus plant by-products as the nutrient source. These by-products may well prove to be suitable growing substrates for locally isolated strains of nematophagous fungi. A range of block formulations containing D. flagrans chlamydospores have been tested and the results are very encouraging (P.J. Waller & M.R. Knox, unpublished data). These blocks have also been shown to have a shelf life of at least 6 months. Fungal blocks could prove to be a particularly important control option in the humid tropics and sub-tropics where tethered husbandry and night housing with stall feeding are common animal management practices and where anthelmintic resistance is a serious problem.

Controlled release devices

Intra-ruminal sustained or controlled release devices are a modern advance in anthelmintic medication. Although the unit costs of these devices is high, they allow great flexibility in animal management insofar as they provide protection against parasite infection for an extended period of time. Rather than using anthelmintic compounds, devices containing fungal spores could provide this extended prophylactic effect. The objective would be to develop a device which would release sufficient spores for an extended period (60 days or more) to result in a substantial reduction in the number of infective larvae which succeed in migrating to pasture over the same time period. These devices could be administered at epidemiologically critical times to reduce seasonal peaks in larval numbers but would allow sufficient larvae to escape and thus provoke the development of naturally acquired immunity in grazing livestock.

Investigations have shown that chlamydospores of D. flagrans can withstand tabletting pressures required for manufacture of these devices. The devices have a good shelf life and can release optimum concentrations of spores for effective parasite control in vivo (P.J. Waller & K. Ellis, unpublished data). Further work is required to test the time/release profiles of fungal chlamydospores in these prototype devices and to verify the long-term in situ viability of spores in devices administered to livestock. Although it is premature to speculate as to whether commercially attractive, fungal controlled release devices will be developed, they have an enormous potential market as a non-chemotherapeutic, environmentally benign form of parasite control to all the grazing livestock industries throughout the world.

CONCLUSION

Modern control methods of worm parasites of livestock need to shift away from the reliance on anthelmintics to a more integrated approach to pest management. Biological control is a major component of integrated pest management (IPM) of insect pests and there are grounds for optimism that this will also apply to animal nematode infections in the near future. With the current move towards "sustainable" agriculture, biological control can be expected to play an even more substantial role in IPM of worm parasites. However, this view must be tempered with the inescapable fact that the commercial, financial and animal management dependence of anthelmintics is too great to allow for any rapid change. But the goal will be the spectre of widespread, high level anthelmintic resistance.

In comparison with other non-chemotherapeutic approaches to parasite control in livestock, progress in biocontrol using nematophagous fungi in recent years has been remarkable. Although commercial interest is high, there has been a general reluctance by companies involved in the anthelmintic business to invest in this research. Part of this is due to the fact that as distinct from the anthelmintic discovery and development, where new drugs can be tightly protected by patents, this is not the case for naturally occurring organisms. However, there is an ever increasing interest worldwide, by potential users of biological control products which are in tune with the move towards the sustainable, ecologically and environmentally acceptable systems of livestock management and disease control. Therefore there is a clear market for biological control products against worm parasites of livestock.

Biological control has many obvious attractions and advantages over other non-chemotherapeutic means of worm control. For example, it will be applicable to the range of worm parasites not only within, but also between, species of livestock, which is one of the major shortcomings of the worm vaccine approach. It will provide the opportunity for livestock producers to capitalise on the increasing demands of consumers for chemical-free livestock products. Finally, it is also difficult to envisage the development of resistance mechanisms which casts a dark shadow over the future of anthelmintics.

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NEMATOPHAGOUS FUNGI, NEW AGENTS FOR BIOLOGICAL CONTROL OF NEMATODE PARASITES OF LIVESTOCK - ECOLOGY, IDENTIFICATION AND CULTIVATION

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INTRODUCTION

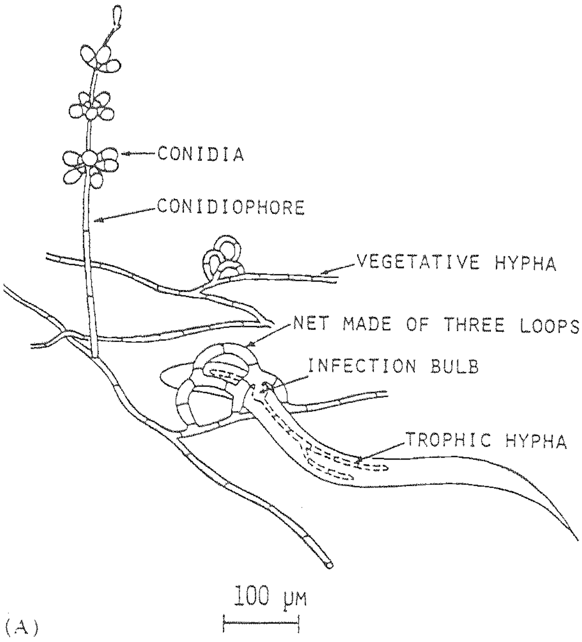
Parasitism with gastrointestinal nematodes in grazing livestock is a major problem facing farmers globally. Control is currently achieved by the use of anthelmintics which include a number of broad spectrum and narrow spectrum compounds. An indiscriminate use of anthelmintics has led to development of resistance particularly in gastro-intestinal nematodes of sheep. Consequently, research is focused on developing alternative interventions and strategies to drenching, among which are biological control with nematophagous fungi.

ECOLOGY OF NEMATOPHAGOUS FUNGI

Nematophagous fungi are a taxonomically diverse group utilising nematodes as a food source. They are commonly grouped as egg parasitic, endoparasitic or nematode-trapping (see Figure 1 A-C).

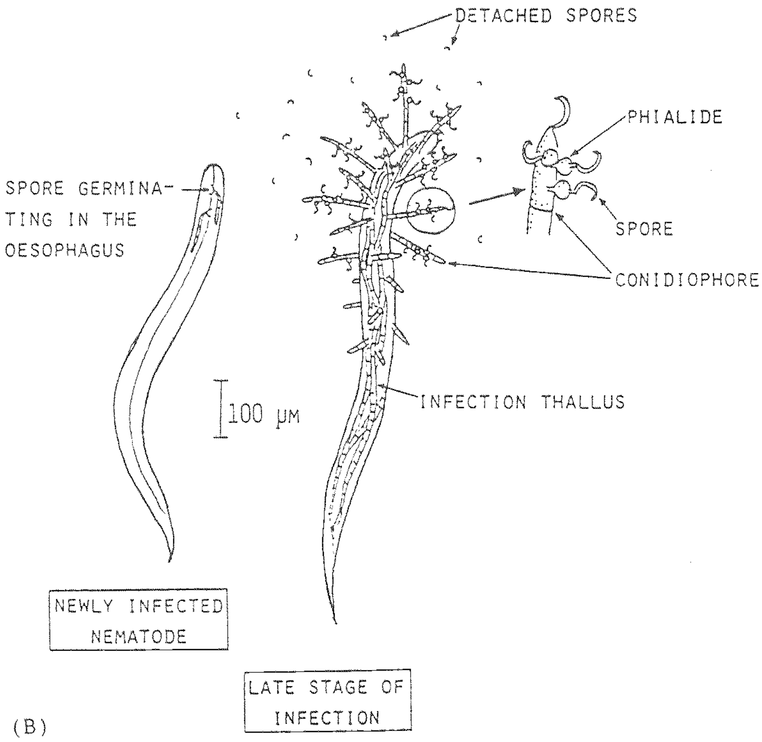
Nematophagous fungi are ubiquitous fungi and are commonly found in decaying plant material, dung and soil (Duddington, 1951; Barron 1981; Peloille 1981; Gray 1983; Nordbring-Hertz 1988). The optimum temperature for growth of most nematophagous fungi has been found to be between approximately 20E and 25EC (Feder, 1962; Cooke, 1963; Olthof & Estey, 1965; Pandey, 1973; Peloille, 1979). Predacious nematophagous fungi normally forms traps on the growing hyphae, but when exposed to various stimuli, such as dead or living nematodes, soil, compost, or dung-extract, some of these fungi are also able to produce traps directly from conidia (Mankau 1962; Cooke, 1964; Dowe, 1987; Dackman & Nordbring-Hertz, 1992). Although nematodes are utilized as a food source, there does not appear to be a simple equilibrium between the number of nematodes present and nematophagous activity of the fungi. Cooke (1962a & 1962b) found that in response to changes in the organic environment, nematode numbers in soil rose to quite high numbers but independent of that nematophagous fungal activity rose and fell.

The distribution of the fungi within the soil varies according to their trapping mechanism and is to some extent correlated to the type of soil. Gray (1985) found that predatory nematophagous fungi that produce ring trapping devices, were associated with high moisture, high organic matter content soils. These soils would most likely contain high nematode numbers. In contrast, fungi producing adhesive networks were associated with soils containing low moisture and low organic matter. With respect to the fungi producing constricting rings, Cooke (1963) found that they had slower growth rates compared to fungi producing adhesive networks. In a recent study by Persmark et al (1996) there appeared to be seasonal fluctuations in both nematode numbers and the



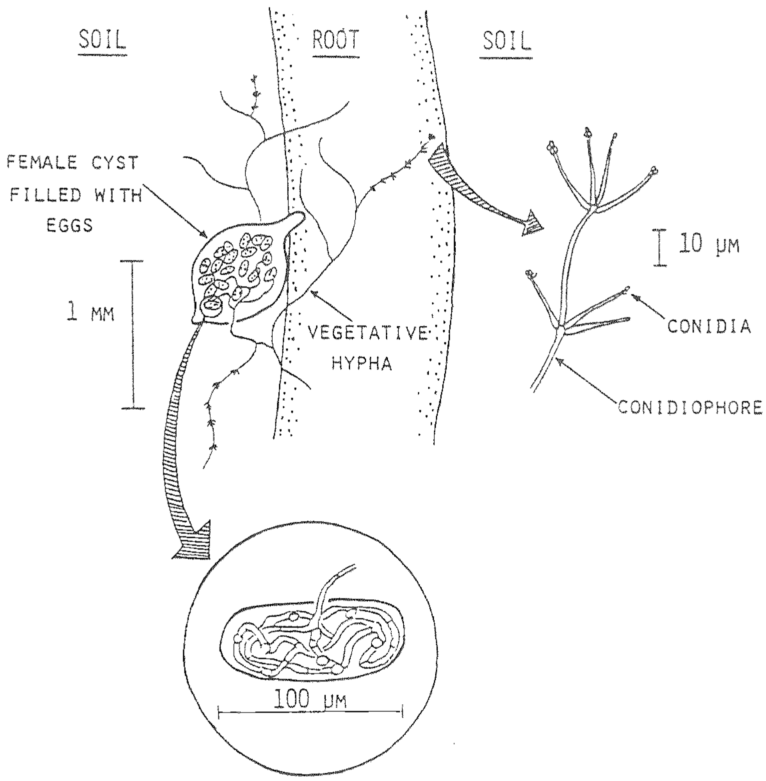
CONIDIA
CONIDIOPHORE
VEGETATIVE HYPHA
NET MADE OF THREE LOOPS
INFECTION BULB
TROPHIC HYPHA
100 μM

(A)



DETACHED SPORES
PHIALIDE
SPORE GERMINATING IN THE OESOPHAGUS
SPORE
CONIDIOPHORE
100 μM
INFECTION THALLUS
NEWLY INFECTED NEMATODE
LATE STAGE OF INFECTION

(B)



(C)

Fig.1. **(A)** *Arthrobotrys oligospora*: a representative drawing of the nematode-trapping type. A nematode has been caught in a net, made of three loops. Inside the nematode, the fungus has formed an infection bulb from which trophic hyphae grow out, and fill the body of the nematode. Uniseptate conidia, borne in clusters at intervals along the length of an erect conidiophore, is also shown. (Drawing by J. Grønvold.) **(B)***Harposporium anguillulae*: a representative drawing of the endoparasitic type. Nematodes are infected by consuming crescent-shaped spores when they move into areas infected with spores. The spores lodge in the muscle tissue of the oesophagus, if nematodes try to swallow them. A germ tube penetrates the oesophageal muscle and enters the body cavity. Inside the victim, the fungus develops an infection thallus. In the late stage of infection, short unbranched conidiophores break out through the host cuticle at numerous points. On the conidiophores, philides produce crescent-shaped spores successively. After Barron (1977). **(C)** *Verticillium chlamydosporium*: a representative drawing of fungal parasites of cyst and root-knot nematodes. The dead female, i.e. the cysst, still attaching to the root, is filled with eggs. Some of the eggs have been infected by vegetative hyphae, penetrating the cyst wall and the egg shells. A conidiophore with conidia is also shown. After Dackman (1988).

presence of nematophagous fungi, in particular endoparasitic fungi. However, no such correlation could be demonstrated between the saprophytic nematode-trapping fungi and nematode numbers, despite their parallel decrease in numbers in winter and increase in Autumn. The author concluded that although the saprophytic nematode-trapping fungi were able to utilize the soil nematodes, they were not a regulating factor of these nematodes, but both fungi and nematodes were dependent on the same regulating environmental factors.

Mahoney & Strongman (1994) and Hay et al. (1997) recently demonstrated that all types of nematophagous fungi (endoparasitic and predacious) quickly invade dung when deposited on the ground. Potentially all types of fungi present on pasture could be picked up and passed in the faeces of the grazing animals. This is apparently not the case. In the two published surveys regarding isolation of nematophagous fungi from fresh dung of sheep, cattle and horses (Larsen et al. 1991, Larsen et al. 1994) the fungi isolated appeared to be not only almost exclusively predacious fungi, but also of the net-trapping kind only, producing three dimensional sticky nets. These findings appears to be contradictory to what is often suggested regarding potential candidates for biological control. Based upon the degree of parasitic behaviour (obligate to saprophytic survival), it has been suggested that the endoparasitic fungi would constitute much better candidates for biological control compared with fungi that are able to survive and grow as saprophytes, in particular the net-trapping predacious fungi. The discrepancy could possibly be due to the fact that the net-trapping fungal species often produce a lot of conidia and/or resting spores (chlamydospores). The probability for spores of these fungi to be picked up in sufficient numbers by grazing livestock and subsequently survive the passage through the gastrointestinal (GI) tract of these animals appears to be much higher than for fungi producing very few spores or fungi, that are spread only in close association with infected nematodes. Maybe more important, the production of large numbers of resistant chlamydospores by some of the fungi might be the key factor determining their success of survival through the GI-tract of the grazing animals.

Isolation and identification of fungi

A number of different techniques have been described for the isolation of endoparasitic and predacious fungi, respectively (Gray 1984; Bailey & Gray 1989; Dackman et al. 1987; Persmark et al., 1992). For the isolation of nematode-trapping fungi, the soil sprinkling technique has been found to be most efficient and widely used. Larsen et al (1991) used a modified sprinkling technique to isolate nematophagous fungi from faecal and soil samples. The sample was crushed and 1-3 g spread onto 2% agar plates in a cross pattern. Antibiotics can be added to the media to suppress bacterial growth. The plates are then baited with a large inoculum of nematodes, either a pure culture of a soil nematodes (i.e. *Panagrellus redivivus*) or infective, third stage larvae of a parasitic nematode. The soil nematode, in addition to stimulating activity of present nematode-trapping fungi, will also stimulate the activity of the endoparasitic fungi. If infective third stage larvae are used as bait, only the nematode-trapping fungi will almost exclusively be stimulated almost exclusively. It is very important that there are living, moving nematodes present on the plate during the entire observation period (normally 3 to 4 weeks for isolation of fast growing fungi). Frequent monitoring of larval numbers and re-baiting is necessary to ensure stimulation of nematophagous fungi. When a positive observation of a nematode-destroying fungus has been made it is important to follow the development of conidiophores and subsequently pick freshly produced conidia for pure cultures and identification. Endoparasitic fungi often do not produce conidiophore and conidia above the surface of the agar and thus it is not possible to isolate the fungus for development of pure cultures.

Identification must be performed directly on specimens taken from the original plate. Important features for fungal identification are the trapping mechanism used, as well as the morphology of the conidiophore and conidia. The presence/absence of chlamydospores has also been used as a secondary character in the identification of these fungi. However there is a lot of variation in the appearance of chlamydospores mostly due to environmental changes such as, type of media, and temperature. Thus, this feature can not be considered of key importance. Furthermore, it should be noted that the length of conidiophores can vary, depending on type of media for cultivation. Measurements of conidiophore length should be made either on material from the original plates or from a pure culture of the fungus on a defined media, shortly after isolation. For the identification of the fungi, the key by Cooke & Godfrey (1964) is still very useful, in addition to the keys by van Oorschot (1985) on *Arthrobotrys* and allied genera plus by Rubner (1996) on *Dactylella-Monacrosporium* species. Each identification should be confirmed by checking the original description in the literature. In case of doubtful identification, one of the major culture collections (i.e. American Type Culture Collection or Centraalbureau voor Schimmelcultures) could be consulted for confirmation of the identification. Prices for identification are variable, often ranging from approx. US$50, but those interested should directly contact such fungal repositories mentioned above, for the current price of this service.

Molecular techniques

Incubation of fungi for identification using traditional methods may require up to 10 days. During culturing, rapidly growing microorganisms obscure the slow growing nematophagous fungi. As outlined above, nematophagous fungi are subject to mycostasis. That is, samples containing microorganisms present in high concentrations will inhibit the growth of the less abundant nematophagous fungi. It is essential that a technique be developed to overcome these difficulties and allow researchers to accurately monitor nematophagous fungi in environmental samples.

Molecular techniques present several advantages over traditional identification using morphological characteristics. Nucleic acid sequences unique to particular organisms can be found which do not depend on gene expression. Molecular techniques do not require culturing of samples and isolation of fungal species. These techniques are more frequently used to detect and characterise fungi in natural and man-made environments (MacNeil et al, 1995; Muyzer and Ramsing, 1995; Persson et al, 1996). A simple method for extracting DNA from mycellia grown on petri dish cultures is outlined by Lecellier and Silar (1994). For environmental samples, such as soil or faeces a more rigorous extraction technique is necessary to ensure complete removal of any possible inhibitors. This is outlined in Porteous et al (1997).

Molecular techniques for the identification of fungi often rely on the 18S gene rRNA or DNA. The DNA sequence that separate the genes coding for each individual rRNA molecule, called intergenic transcribed spacer sequences (ITS), are highly variable (White et al, 1990). The ITS region often varies between species, variety and even isolates. Surrounding the ITS is the well conserved regions of the 18S, 28S and 5.8S genes. The polymerase chain reaction (PCR) can be used to amplify the ITS region using primers designed from the conserved regions of DNA. There are often hundreds, even thousands of copies of the rDNA in eukaryotes making it an ideal region for designing probes. However, probes for fungi are not limited to this region. Research is required in designing probes for key nematophagous fungi before this technology can be applied.

Cultivation of fungi

As mentioned previously, cultivation of endoparasitic fungi in pure culture can be very difficult, but descriptions for mass production of spores of specific species of these fungi has been published (Lohmann & Sikora, 1989). For small experiments in the laboratory with a limited number of faecal cultures the production of necessary amount of fungal material could be performed on agar media. When dealing with larger trials such as with housed animals, plot or field trials, it is necessary to mass culture the fungal spores. Production of fungal material on a semi solid media has been described by Larsen (1991). A modified version was described by Gronvold et al. (1993). In principle, cultivation is performed on various types of media such as barley, wheat, corn or whole rice. It is important to establish the right balance between the selected media, water content and cultivation temperature. For experiments involving a limited number of grazing animals, cultivation of fungal material on a semi-solid media in Erlenmeyer flasks (or similar types of container that can be autoclaved) is simple and not too laborious. Cultivation of *Duddingtonia flagrans,* the most promising fungus for biological control, has been performed on barley (20° g + 20° ml water in 1 l Erlenmeyer flasks) for 2-4 weeks at 201 - 301C. When the culture is older than 3-4 weeks a high number of resistant resting spores, chlamydospores, are produced ready for use in different tests, such as *in-vivo* passage trials on housed animals or in field trials. The cultivated material can either be used fresh or dried.

Preservation of cultures (short/long term)

Since different species/genera of nematode-destroying fungi might respond differently to various storage techniques, it is very important to store replicates of the isolates and to use more than one technique. For details on how to best preserve living fungal material, see Smith & Onions (1994). For a limited collection of fungi, used in small laboratory experiments or similar, routine sub-culture on an agar medium is possible and practical. Cultures can also be kept in tubes on agar slants, with or without added sterile oil. Another simple technique is storage of a small block of culture on nutrient medium agar in sterile water. Although there is no guarantee for success one could try also to store replicates of the agar cultures in the freezer.

For long term storage and preservation one of the best and most affordable methods is storage of fungal material on silica gel. It is a simple technique, preserving most fungi for many years and keeping the material genetically stable.

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UREA-MOLASSES BLOCKS FOR PARASITE CONTROL

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ABSTRACT

Throughout the developing world the productivity of ruminant livestock is severely constrained by inadequate nutrition and gastrointestinal parasitism which interact to restrict growth and reproduction rates and contribute to high mortality in some flocks and herds. Where nutritional deficiencies are likely to exacerbate the detrimental effects of parasitic infection, the use of low cost supplements such as urea-molasses blocks (UMB) can enhance the animal's ability to utilize the available diet and assist the animal to withstand infection. Such supplements should therefore be considered an integral part of husbandry practice in these areas, in order to reduce the debilitating effects of parasitism and minimise the requirement for alternative means of control. When parasite challenge is high or during periods of low host immunocompetence caused by immaturity or physiological stresses such as reproduction, it is likely that alternative means of control will be necessary. During these times UMB can be employed to deliver chemotherapeutic or biological parasite control agents. Future parasite control will depend on the successful integration of all available means of control into strategic programs for each production system being targeted and UMB will have an important role to play in many of those systems.

INTRODUCTION

Throughout the developing world the demand for ruminant livestock products is increasing but limited land resources limit substantial expansion of the ruminant livestock industries unless previously underutilised feed resources are exploited. The productivity of ruminants in this region is severely constrained by inadequate nutrition and gastrointestinal parasitism which interact to restrict growth and reproduction rates and contribute to high mortality in some flocks and herds. By reducing these constraints, considerable productivity gains can be made without increasing overall flock and herd sizes.

The available feed resources in these areas are often low in quality, fibrous and deficient in components which promote efficient rumen function. Supplementation, particularly with high quality protein, is often necessary to maintain adequate productivity, but high cost limits widespread adoption of this approach. Nematode parasites can cause high mortality in ruminant livestock and losses from clinical and subclinical infections probably equal the value of present output in some areas. Up to the present time, control of nematode parasites has either relied on frequent anthelmintic treatment or has not been undertaken due to the high cost or unavailability of anthelmintics. Where anthelmintics have been frequently used, anthelmintic resistance is emerging as a major problem (Sivaraj *et al*, 1994; Waller *et al*, 1996) and ruminant industries must seek alternative control methods.

This paper firstly describes low cost nutritional approaches which can be used to manipulate rumen function and help optimise efficiency of microbial growth in the rumen from available feed resources. A summary of the pathophysiological effects of nematode parasites with regard to the protein nutrition of host sheep then precedes a brief review of evidence supporting the beneficial effects of nutritional supplementation of infected hosts. The role of strategic nutritional supplementation in future parasite control programs is then discussed in relation to the use of low cost supplements as delivery vehicles for nutritional and other components which have direct nematocidal activity.

MANIPULATION OF RUMEN FUNCTION WITH NON-PROTEIN NITROGEN

In livestock rearing enterprises which are reliant on low quality roughage for the provision of digestible carbohydrate, the most critical nutritional deficiency is nitrogen. In situations where other dietary components are not limiting, efficient rumen function is dependent on having sufficient degradable nitrogen in the diet to provide adequate ruminal ammonia for microbial growth. In situations where deficiency occurs, provision of additional nitrogen can have a dramatic impact on digestibility and productivity from low quality diets. Protein sources can provide additional nitrogen through rumen fermentation but unfortunately in most developing countries, supplies are inadequate and costly. Non-protein nitrogen supplements are less expensive, readily available and can assist the animal to meet its nutritional requirements from low quality roughages.

Non-Protein Nitrogen Supplements

Non-protein nitrogen (NPN) has long been identified as being able to provide the nitrogen requirements for microbial growth in ruminants. A broad range of compounds can provide NPN (Doyle, 1987) but since the 1920's most research has concentrated on the use of urea as a NPN source since it is relatively inexpensive and readily available in most agricultural situations. In animals offered low quality diets, supplementation with urea increases microbial decomposition of feed carbohydrates, which results in increased feed intake and increased protein availability for intestinal absorption (Preston & Leng, 1987). Delivery of urea supplements to grazing animals has been successfully attempted by many means including in drinking water (Stephenson *et al.*, 1981), in molasses mixtures (Nolan *et al.*, 1975; Langlands & Bowles, 1976; Mulholland & Coombe, 1979; Coombe & Mulholland 1983) and in feed supplement blocks (Leng *et al.*, 1991; Taiwo *et al.*, 1992). The latter means of delivery appears most practical for widespread use and will be discussed further below.

Urea Supplementation and Efficiency of Microbial Growth

When urea is fed to the ruminant animal it is rapidly metabolised to ammonia in the ruminal fluid. Numerous studies have attempted to determine the optimal concentration of ammonia for microbial synthesis in the rumen. Satter & Slyter (1974) suggested that between 50 and 80 mgN/l was optimal for microbial synthesis in the rumen while several other authors have suggested concentrations of 150-200 mgN/l to be more appropriate in animals fed low quality fibrous diets (Leng & Nolan, 1984; Boniface *et al.*, 1986; Perdok & Leng, 1989). Recent studies by Balcells *et al.* (1993) established that by increasing the amounts of urea (3, 6, 9 & 12 g/day; giving rumen ammonia levels of 50-110mgN/l) infused into the rumen of sheep, increased concentrations of allantoin were excreted in the urine. Prior studies had established that allantoin excretion could be used to determine the response of microbial yield to changes in rumen-degradable N supply (Chen *et al.*, 1990; Balcells *et al.* 1991). Therefore, with increased urea supplementation more microbial protein became available to the sheep and it was suggested that most of this increase was due to enhanced feed intake stimulated by an improved rate of rumen fermentation (Balcells *et al.*, 1993).

Further studies conducted with similar methodology but restricting feed intake to 800g/day showed that the greater the rumen ammonia concentration, the greater the influence on microbial protein production (Kanjanapruthipong, 1995). With increased rates of urea infusion digestibility increased, protozoa numbers increased and then declined, fungal sporangia numbers increased then gradually declined while urinary purine production increased as rumen ammonia levels increased (see Figure 1). It was suggested that, at the higher concentrations of ammonia, increased microbial protein availability resulted from decreased turnover of microbial cells within the rumen due to lower numbers of protozoa (Kanjanapruthipong, 1995). From this study it can be concluded that for situations where low quality forages are to be utilised, supplementation with NPN to give a ruminal fluid ammonia concentration of at least 200mgN/l would be appropriate.

UREA-MOLASSES BLOCKS (UMB)

Composition of UMB

One means of delivering NPN supplements in areas where low quality feeds are the primary feed resource is through feed supplement blocks. These blocks have varied compositions (Kunju, 1986; Sansoucy, 1986; Leng, 1986; Leng *et al.*, 1991; Wan Zahari, 1990; Taiwo *et al.*, 1992; Hadjipanyiotou *et al.*, 1993; Sansoucy, 1995) but the primary ingredients are urea to provide NPN and molasses to provide energy and attract the animal to eat the supplement. Molasses is also a good source of various macro and micro-minerals and can be further fortified with minerals appropriate to the particular production system during the preparation procedure (Leng, 1986; Kunju, 1986; Preston & Leng, 1987). A generalised formulation for the preparation of UMB is presented in Table 1. Since quality and price ingredients can vary considerably between localities it is recommended that various formulations be tested prior to embarking on larger scale production. Studies of UMB formulation conducted in Malaysia have concentrated on the utilisation of local agricultural byproducts with considerable success (Wan Zahari, 1990) and this approach should be followed where possible.

Application of UMB

Urea-molasses blocks have been shown to increase feed intake and improve the digestibility of roughage-based diets (Krebs & Leng, 1984; Kunju, 1986; Sudana & Leng, 1986; Soetanto, 1986; Soetanto *et al.*, 1987). Field trials have established that access to UMB results in productivity increases in small and large ruminant livestock in the developing nations of Asia and the Pacific Islands (Kunju, 1986; Hendratno *et al.*, 1991; Leng *et al.*, 1991; Manueli *et al.*, 1995; Salman, 1996; Wan Zahari *et al.*, 1996a,b) and in Australia (Butler *et al.*, 1994). It is expected that further use of UMB will occur throughout the developing nations as the human population expands and demand increases for ruminant products from the limited feed resources available, since UMB enables rumen function and ruminant productivity to be optimised cost effectively

(Leng *et al.*, 1991).

Consumption of UMB

Variability of consumption of block supplements is commonly observed when individual animals within a flock or herd are compared (Lobato & Pearce, 1980; Lobato *et al.*, 1980; Kendall *et al.*, 1983) and this can cause some concern for livestock producers who desire uniform productivity from all animals. Factors such as block constituents, particularly nitrogen content, and hardness can affect rate of consumption. Intakes of UMB by Malaysian sheep varied considerably when urea and salt concentrations were varied (M. Wan Zahari, unpublished). Of equal importance to block characteristics are animal-related factors such as exposure time, previous experience and social behaviour and factors relating to pasture quality and availability (see review by Bowman & Sowell, 1997). It is desirable for the majority of animals to consume sufficient block supplement to compensate for deficiencies in the available diet and therefore blocks should be tailor made to the local environment and animals conditioned to consume them. Inadequate block intake does not appear to be as great a problem in developing nations livestock production systems where supplements are usually readily consumed. Over consumption should also be avoided to reduce the risk of urea toxicity (Preston & Leng, 1987) and to maintain supplementation cost at an economic level.

UMB as a delivery vehicle

In situations where UMB are regularly used to deliver deficient nutritional components they can also be employed to simultaneously deliver chemotherapeutic agents. Continuous low dose anthelmintic medication has been successfully delivered this way resulting in efficient control of nematode parasites (Knox, 1995; Sani *et al.*, 1995; Chandrawathani *et al.*, 1996; Tan *et al.*, 1996; Wan Zahari *et al.*, 1996a,b; Yahya *et al.*, 1997). Similarly UMB have been used to deliver ionophores to increase feed efficiency in grazing cattle (Bransby *et al.*, 1992). Recent work to investigate the use of UMB for the delivery of chemicals to suppress rumen protozoa populations has been inconclusive and investigations are continuing (S.H. Bird, personal communication). Of greater relevance to the primary theme of this workshop is the potential for the delivery of biological control agents by UMB particularly in situations where grazing animals are confined for at least part of the day since initial investigation of this possibility has been very encouraging (P.J. Waller and M.R. Knox, unpublished results). A more speculative possibility is the future use of UMB to deliver protective vaccine antigens against helminth and other disease causing organisms.

PARASITE EFFECTS ON PROTEIN NUTRITION OF HOST SHEEP

Nematode parasite infections can cause anorexia, maldigestion and malabsorption of feed, gastrointestinal loss of endogenous protein and initiation of immune responses in sheep. These factors will be considered independently with regard to their effects on protein nutrition in infected sheep but it is important to remember that these factors are often interdependent when considering their influence on host protein nutrition.

Anorexia

Voluntary reduction in feed intake is commonly observed during infection with parasitic nematodes and can severely influence the protein economy of the host by significantly reducing the amount available for anabolic processes (see review by Symons, 1985). The degree of anorexia may be affected by the species of parasite and its site of infection and by the breed, age and resistance status of the host sheep. More importantly, level of infection also affects anorexia as clearly demonstrated by Steel *et al.* (1980) where infection levels below 3000 infective *Trichostrongylus cloubriformis* larvae per week failed to reduce feed consumption but increasingly higher infection levels produced greater reductions in feed intake. Increased availability of protein in the diet can reduce the degree of anorexia in infected sheep as demonstrated in pen experiments with *Haemonchus contortus* (Abbott *et al.*,1986, 1988; Datta *et al.*, 1996) and *T. colubriformis* (van Houtert *et al*, 1995).

It has been suggested that anorexia may result from pain and discomfort associated with infection or be the result of hormonal feed back mechanisms from disrupted gastrointestinal function (Symons, 1985). More recently it has been recognised that increased cytokine activity associated with inflammatory responses to infection may be a major causitive factor of anorexia (Grimble, 1989).

Protein digestion and absorption

Results of studies attempting to determine the effects of nematode infection on the protein nitrogen digestibility of feed resources are somewhat equivocal with some studies demonstrating a reduction (Owen, 1973; Barger, 1973; Dargie, 1980; Steel *et al.*, 1980) but other studies showing no effect (Roseby, 1973; Sykes & Coop, 1976; Poppi *et al.*, 1981). Methods used in estimating the apparent N digestibility of feed rely on measurements of all dietary matter entering and leaving the sheep under observation. While dietary matter entering the system is easily recorded, faecal matter leaving the system comprises not only the residues of digestive processes but also remnants of endogenous protein loss due to infection. Such measurements may therefore overestimate the N digestion of infected animals. Digestive absorption can be reduced at the site of infection due to tissue damage or increased local inflammation but this is compensated for by increased absorption at sites distal to the affected area (Bown *et al.*, 1991) so overall effects on absorption are negligible.

Gastrointestinal losses of endogenous protein

Infection with *H. contortus* and *T. colubriformis* can result in substantial losses of endogenous protein in the form of whole blood, plasma, sloughed epithelial cells and mucous. A considerable proportion of these proteins require redigestion before being absorbed at sites distal to infection. Unresorbed residues will either be excreted with the faeces or be further digested in the large intestine, absorbed as ammonia and excreted as urea in the urine and therefore represent a major drain to the overall nitrogen economy of infected sheep (Rowe *et al.*, 1988; Poppi *et al.*, 1986; Kimambo *et al.*,1988). Synthesis of specific proteins for repair, replacement and reaction to damage of the gut wall, to mucous production and to plasma or whole blood loss can however, impose a significant drain on resources which would otherwise contribute to the synthesis of muscle, bone and wool (MacRae, 1993).

Nutritional cost of immune function

Inflammation and other immune responses occur locally and systemically as a result of the presence of, and damage caused by, gastrointestinal parasites. These responses can represent a significant drain on the nutritional resources available to the host and redirection of protein away from normal body processes may result. Mucus contains high concentrations of threonine, serine and proline (Neutra & Forstner, 1987) and increased mucus production may result in deficiencies of these amino acids for other processes. Mucus also is reported to be resistant to digestion and resorption from the small intestine (Lindsay *et al.*, 1980) so once formed its component amino acids are effectively unavailable for synthesis of other protein. It has also been shown in laboratory studies that production of the immunological mediators leukotrienes (MacRae, 1993) and cytokines (Grimble, 1990) require specific amino-acids, particularly sulphur-amino acids, which may reduce their availability for other processes. Wool production in particular is highly dependent on the availability of sulphur-amino acids and parasitic infection can cause significant reduction in the amount and quality of wool produced (Barger, 1982).

THE BENEFITS OF ENHANCED NUTRITION IN PARASITISED RUMINANTS

Several reviews have concluded that sheep offered a high plane of nutrition are better able to withstand the detrimental effects of nematode parasite infection than those less adequately nourished (Parkins and Holmes, 1989; Coop & Holmes, 1996; van Houtert & Sykes, 1996). It has been shown that an adequate supply of dietary protein enables infected sheep to withstand the pathophysiological consequences of infection through compensating for parasite-induced protein deficiency resulting from increased endogenous protein loss from the gastrointestinal tract. Improved dietary protein supply improves the capacity of infected sheep to mount an effective immunological response to infection and enhances the onset of parasite rejection (Steel *et al*, 1982; Abbott *et al*, 1988; Roberts and Adams, 1990). The following recent studies further demonstrate the importance of dietary protein supply.

Post-ruminal infusion of protein

The studies of Bown *et al* (1991) clearly confirmed the importance of additional protein through post-ruminal infusion of sodium caseinate which markedly reduced the debilitating effects of *T. colubriformis* infection whereas isocaloric amounts of glucose had no effect. Availability of protein aided parasite rejection as evidenced by lower faecal egg counts and lower total parasite counts at slaughter in those lambs receiving the infusion. Similar results were achieved by Coop *et al.* (1995) with infused lambs infected with *Ostertagia circumcincta* having lower egg counts and worm burdens. Worm size was also reduced and mucosal mast cell numbers and protease levels increased indicating a higher degree of immunity in infused lambs.

Bypass protein supplements

A similar nutritional result to post-ruminal infusion can be achieved by feeding a protein source which escapes or "bypasses" rumen fermentation but is available for digestion in the intestines. A proportion of most feed protein is bypass protein and chemical and heating processes involved in modern vegetable oil extraction and feed manufacture increases this proportion dramatically. These include feed resources like soya bean, coconut, cotton seed and fish meals and oil palm kernel cake while some leguminous plants with high tannin levels also are a good source of bypass protein. Bypass protein feeds are now commonly available and have been utilised in recent studies on parasite-nutrition interactions.

Studies by van Houtert *et al.* (1995) used three levels of fish meal supplementation (0, 50 and 100g/day) to demonstrate the benefits of bypass protein supplements in young Merino lambs offered a nutritionally balanced ration based on oaten chaff and essential minerals while infected with 3000 *T. colubriformis* L_3/week. Establishment rates of worms were not affected by the supplement, but rejection of established worms occurred earlier in those fed the higher level of fishmeal, as indicated by reduced faecal egg counts and worm numbers at slaughter. The rejection of infection by those on the higher level of supplementation coincided with increased eosinophil levels and increased mucosal mast cell protease levels. Liveweight gain was also influenced by level of fishmeal supplementation with those receiving no supplement showing a 43% lower rate of gain than uninfected controls whereas the 50 and 100g fishmeal/day groups lost 18% and 11%, respectively.

To compare the effects of protein supplements of different rumen degradability on parasitism in young merino sheep, Smith *et al.* (1996) supplemented the basal diet of oaten chaff with either lupins (35% protection) or canola meal pellets (57% protection) while being infected with 5000 *T. colubriformis* and 3000 *O. circumcincta* per week. After the 12 week experiment the canola group had lower faecal egg counts and were consuming more food than those offered the lupin supplement. The authors suggested this was probably due to a more rapid and effective immune reaction by those sheep offered the less rumen-degradable protein supplement.

An experiment to determine the level of bypass protein supplementation required to elicit improved production and immunity in young crossbred sheep infected with *H. contortus* has been undertaken by Datta *et al*. (1996). Using different levels of a high bypass protein supplement (cotton seed meal) these authors were able to describe a significant relationship between level of supplementation and faecal egg output with those receiving the higher levels of supplement having the lowest faecal egg output. Feed intakes and weight gains were maintained on higher levels of supplementation but substantial reductions occurred at lower levels.

Supplementation with urea

Unfortunately the provision of high quality protein is usually not an economic proposition for small ruminant producers due to high cost and is therefore not often practised. Recent research has indicated that provision of low cost supplements enhances the ability of infected hosts to overcome the detrimental effects of nematode parasitism. In controlled pen studies with young Merino sheep, Knox *et al*. (1994) showed supplementation of a low quality roughage diet of oaten chaff and essential minerals with urea reduced the effects of parasitic infection by reducing faecal egg output and parasite burden and increasing weight gain and wool production (see Table 2). Further studies (M.R. Knox and J.W. Steel, unpublished) using a UMB supplement with a similar basal diet showed similar production responses to supplementation in parasitised young Merino sheep but not when feed intake was restricted to that of the unsupplemented group (see Table 3).

In both these studies supplemented animals with mixed species infections of nematode parasites showed similar or greater weight gains and wool production than their respective unsupplemented uninfected control groups. Such supplements can, therefore, assist sheep to overcome the detrimental effects of nematode parasite infection. This response is attributed to a greater intake of the basal diet, presumably due to increased digestibility arising from enhanced rumen NH_3-N levels and their effect on microbial fermentation, which results in increased post-ruminal microbial protein availability .

Manueli et al (1995) investigated the impact of UMB supplementation in young Fiji sheep and found that access to UMB almost doubled the numbers of lambs born and the weight of lambs reared to weaning. The requirement for salvage treatment due to high (>4000 epg) faecal egg count was also halved in those sheep with access to UMB compared to those with no supplement. Later experimentation has confirmed that the greater lamb growth and survival was due to increased milk production in the ewes receiving the UMB supplement (P.R. Manueli, M.R. Knox and F. Mohammed, unpublished).

UMB AND STRATEGIC PARASITE CONTROL

Clinical and sub-clinical nematode parasitism cause substantial production loss in ruminant livestock production systems in terms of reduced growth of meat and fibre, reduced reproductive performance and increased mortality (Barger, 1982). Attempts to control this problem have relied heavily on the use of anthelmintic chemicals and has resulted in the widespread emergence of strains of nematodes resistant to these chemicals (Rolfe, 1997; Waller, 1997). In some sheep production systems anthelmintic resistance has developed rapidly to all classes of chemicals (Sivaraj *et al*, 1994; Waller *et al*, 1996) and since no new anthelmintics are likely to be available in the near future, continued production is under severe threat. Alternative means of control are now being sought which will reduce the reliance on chemical intervention and maximise the host's natural ability to resist infection.

Host nutrition is one factor which has not been fully investigated with regard to its interaction with nematode parasitism but which may play an important role in future integrated control strategies. Substantial evidence exists that supplementation of young sheep enhances their ability to overcome the detrimental pathophysiological effects of infection with nematode parasites. This response is partly due to direct compensation for parasite-induced protein deficiency which results from increased endogenous protein loss and also due to an increased capacity to mount an effective immunological response to infection. In situations where persistent subclinical infection occurs, strategic nutritional supplementation of young animals, particularly in the period immediately post-weaning, may reduce the requirement for alternative methods of control. Recent evidence also suggests that the benefits derived from tactical use of supplements persist long after the period of supplementation ceases and would have a major influence on the lifetime performance of the animal.

Rumen function can be dramatically changed by optimising the rumen environment with mineral and/or NPN supplements (Leng, 1991) and UMB are a practical and popular means of NPN delivery. Recent research has shown the beneficial effects of urea supplementation on the ability of sheep fed a low quality roughage diet to resist the effects of gastrointestinal nematode parasites (Knox *et al.*, 1994). Such supplements should therefore be considered an integral part of husbandry practice in these areas, in order to reduce the debilitating effects of parasitism and minimise the requirement for alternative means of control (Knox & Steel, 1996). When parasite challenge is high or during periods of low host immunocompetence caused by immaturity or physiological stresses such as reproduction, it is likely alternative means of control will be necessary. During these times UMB can be employed to deliver chemotherapeutic or biological parasite control agents. Future parasite control will depend on the successful integration of all available means of control into strategic programs for each production system being targeted and UMB will have an important role to play in many of those systems.

Figure 1 Effects of levels of ruminal fluid ammonia obtained through urea supplementation on ruminal fluid protozoa numbers, fungal sporangia, *in sacco* digestibility and urinary excretion of purine derivatives in sheep fed a low protein roughage-based diet (adapted from Kanajanapruthipong, 1995).

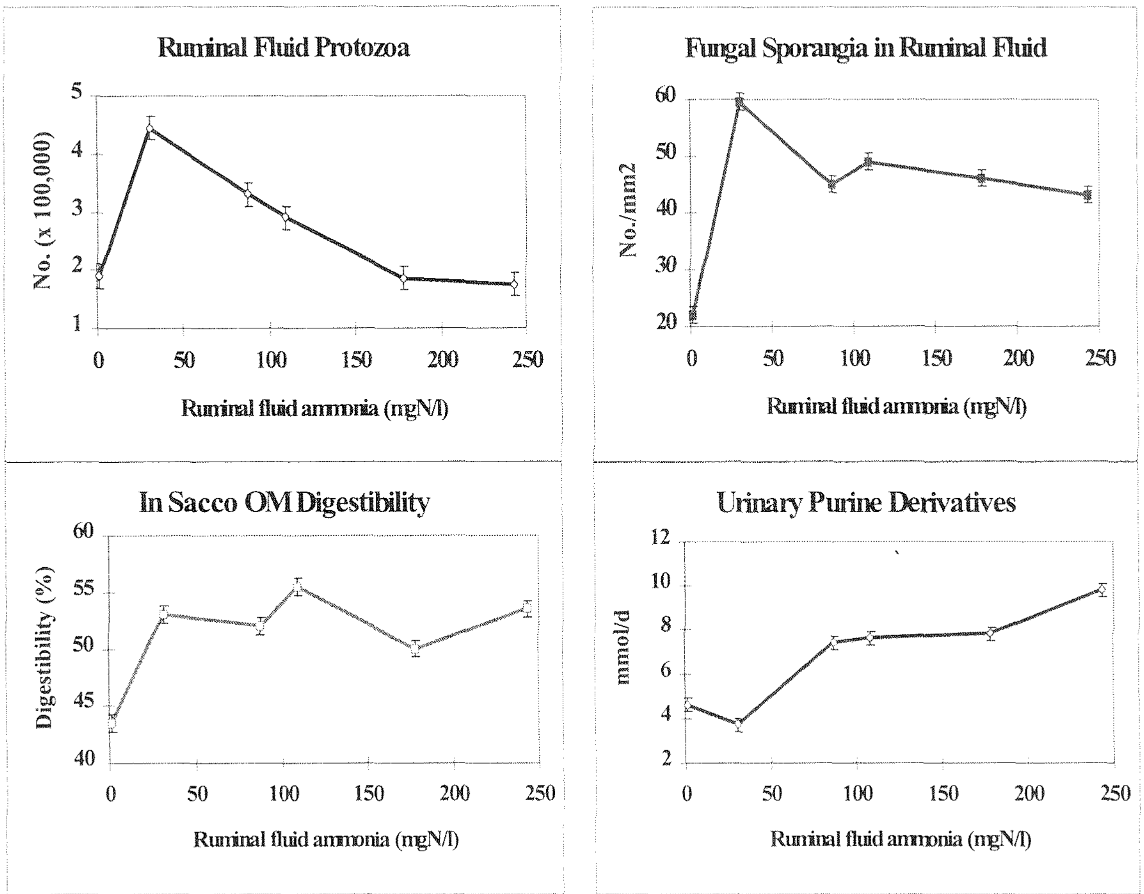

Table 1 - Generalised formulation for the preparation of urea-molasses blocks.

Component	Common Ingredients	Quantity (%)	Example (%)
Non-protein nitrogen	Urea, poultry manure	5-10	10 (Urea)
Molasses		20-40	25
Salt	NaCl	1-10	10
Binder [1]	CaO, $Ca(OH)_2$, MgO, cement, gypsum, dolomite, Na bentonite	5-15	10 (CaO)
Water [2]		0-30	15
Mineral Premix	Commercial products or tailor made to meet local requirements	1-2	1
Protein meal [3]	Fish meal, coconut meal, cotton seed meal, palm kernel cake, leucaena leaf meal	5-25	10
Filler	Wheat bran, rice bran, rice husks, chopped oil palm fronds	5-25	19

[1] Local cost and availability will determine most appropriate binder or combination of binders.
[2] FAO formulations recommend the use of water (see Hadjipanyiotou *et al.*, 1993).
[3] Locally available agricultural byproducts (prefer those high in Phosphorus).

Table 2 - Mean liveweight and wool production over 18 weeks of merino weaner sheep on a basal oaten chaff diet or supplemented with 3% urea and infected with nematode parasites.

Dietary Treatment	Parasite Treatment	Liveweight gain (g/day)	Wool production (g/day)
No urea	Nil	30	4.2
No urea	*H. contortus* [1]	29	4.2
No urea	*T. colubriformis* [2]	32	4.4
No urea	Mixed [3]	22	3.8
3% urea	Nil	50	5.3
3% urea	*H. contortus*	40	5.7
3% urea	*T. colubriformis*	37	5.2
3% urea	Mixed	39	5.3

[1] 200 L_3 thrice weekly.

[2] 1000 L_3 thrice weekly.

[3] 200 *H. contortus* L_3 and 1000 *T. colubriformis* L_3 thrice weekly.

Table 3 - Mean liveweight and wool production over 20 weeks of merino weaner sheep on a basal oaten chaff diet or supplemented with a urea-molasses block and infected with nematode parasites.

Group	Chaff	Block	Parasite	Liveweight gain (g/day)	Wool production (g/day)
1	*ad libitum*	No	Nil	69	6.4
2	pair fed to 1	Yes	Nil	69	6.4
3	*ad libitum*	Yes	Nil	90	8.6
4	*ad libitum*	No	Mixed[1]	41	6.2
5	pair fed to 4	Yes	Mixed	39	6.0
6	*ad libitum*	Yes	Mixed	59	7.5

[1] 200 *H. contortus* L_3 and 1000 *T. colubriformis* L_3 thrice weekly.

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THE ECONOMIC IMPORTANCE OF GASTRO-INTESTINAL NEMATODES TO THE LIVESTOCK INDUSTRY IN ARGENTINA

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INTRODUCTION

Argentina's natural environment provides an excellent setting for extensive livestock farming, where animals graze on pastures all year around. The animal stock distribution in Argentina up to 1991 was as follows: Cattle, 48 million head; sheep, 24 million head; swine: 4 million head; and horses, 2.8 million head. These figures may have changed since the 1991 census. The Humid Pampa, the region formed by the provinces of Buenos Aires, Córdoba, Santa Fe, and east side of La Pampa, covers an area of 500.000 km^2 (about 20% of the country) where 65% of the total livestock is produced, i.e. 31 millions head of cattle. (Doyle, C. 1992)

Gastrointestinal helminthosis is a major problem in both cattle and sheep but studies on the economic effects on grazing livestock has largely involved the beef cattle industry. It was established that this is the foremost problem in postweaning and fattening cattle system, second in importance in dairy production systems, and ranked as the sixth most important problem in breeding systems. (Entrocasso, C.M., 1987)

Therefore, it is now well known that the gastro-intestinal helminthiosis is one of the most important diseases with a high economic impact on beef production systems, and it has been established that parasite control is the variable which has the highest correlation with increasing production. (Fiel, C.A., Steffan, P.E., 1994)

Losses in Argentina are estimated at US$22 million/year due to mortality, and US$170 (89%) million/year due to subclinical losses, of which US$92 (48%) correspond to losses in the Buenos Aires province. (Entrocasso, C.M., 1988) To this should be added the cost of anthelmintics.

ECONOMIC IMPACT STUDIES: EFFECTS OF HELMINTHOSIS ON CATTLE PRODUCTION

Liveweight gains

Gastrointestinal parasitism causes significantly reduced weight gain and occasionally bodyweight losses in both dairy (Fernández, A.S., et.al, 1992; Fernández, A.S., et.al., 1994) and beef calves and yearlings, mainly in the form of subclinical infections. In beef cattle, these reductions commonly amount to 20% (20-40 kg) of the bodyweight gain in a short period of time (30 - 60 days) and losses may rise up to 30-40% (30-60 kg) if symptoms become clinically apparent. (Entrocasso, C.M., 1988). The reduction in growth rate is neither recovered after treatment or following provision of better nutrition or the combination both (Stefan, P.E.; Fiel, C.A., 1986). The price can be reduced to nearly 20% per kilogram of live weight in groups of animals after suffering parasitic infections (Entrocasso, C.M., 1988)

Autumn and winter are crucial seasons due to the age of the animals, the lack of nutrients and the high availability of infective larvae on pasture ingested by calves consequently leading to the high adult worm burdens. Those, in turn, produce decreased weight gains / losses of weight as indicated above. Therefore, GI nematodes produce devastating effects on young animals from weaning (March) until 18-20 months of age.

Heifer Reproduction

Another important effect of the GI nematodes is on reproduction in heifers. It has been clearly demonstrated that nematode infections can significantly delay the sexual maturity of 15-16 month old heifers. Significant differences in both weight and length of the uterus and the number of *corpus lutea* in the ovaries between treated and non-treated animals heifers were recorded (Stefan, P.E., et.al., 1990). It was also demonstrated that the pelvic areas of 24 months-old heifers exposed to GI nematodes are smaller than those of heifers without parasitic infection (Stefan, P.E., 1991).

Milk Production

Two trials have been conducted on milking cows in the east-central area (Cuenca Mar y Sierras) of Buenos Aires province to investigate the effects of the GI helminthosis. The first trial has shown that anthelmintic-treated cows produced 200 kg more milk than non-treated animals after 305 days of lactation ($P<0.05$) (Biondani, C.A.; Steffan, P.E., 1988). The second study also showed average differences (57 kg milk) at day 120 of lactation between treated and non-treated groups of cows, although no significant differences ($P>0.05$) were found (Fiel, C.A., et.al., 1991).

Carcase composition

It has been demonstrated that nematode infections affect tissue quality. In yearlings suffering from GI nematode infection, the muscles with high or moderate high growth patterns responsible for good carcase conformation, in parasited stock were most affected. Fat deposition was also adversely affected (Garriz, C.A., et.al., 1987).

Current parasite control practices and problems encountered in their implementation

Control programmes are widely used in beef production systems with treatments carried out at weaning time and at the end of winter. The scale method is often adopted in order to decide whether or not to apply anthelmintic treatment.

In many farms it has been possible to achieve good nematode control by integrating the use of safe pasture e.g. new pasture, and frequent anthelmintic treatment during autumn-winter (Entrocasso, C.M., 1988; Stefan, P.E., et.al., 1993). It is not unusual with intensive 4-6 weekly treatment of young cattle in the humid Pampa. Implementation of good control practices are hampered by the lack of extension service - at least in some areas - leading to farmers treating animals only when clinical signs are evident. Important economic losses are therefore inevitable. The lack of epidemiological knowledge by practitioners to recommend adequate methodologies of control is also a serious limitation.

Anthelmintic resistance is not known to occur in cattle parasites but it has been shown to occur in sheep in the northern provinces in Argentina (Eddi, et.al.) Extensive studies are necessary in order to get a clear picture about this topic in Argentina.

OPPORTUNITIES FOR BIOLOGICAL CONTROL

Only one report of isolation of nematophagous fungi in Argentina has been made so far. At the present time all ongoing work about the subject is carried out abroad, where Argentinian researchers are working in order to achieve specialisation in the subject. At this stage it is obvious that the implementation of biological control in large areas with livestock grazing all-year around needs to be extensively studied in Argentina. The fact that animals are exposed to parasitic infective larvae continuosly is a major challenge regarding the method and the timing for the administration of the fungal material. This requires epidemiological knowledge of the areas where biological control will be implemented, in order to achieve successful results. The obvious target area will be the Humid Pampa region. Studies on Biological control could be directed in two ways: natural or artificial. In the first case, epidemiological studies are needed to know the natural behaviour of fungi and its nematophagous characteristics regarding environmental conditions and animal species. In artificial biological control, native or introduced species could be used. In either case, characterization of the species as well as environmental impact need to be evaluated.

Much work has to be done before biological control could be a reality in Argentina, with a large range of activities contemplating not only research but extension and training. First of all, the notion of biological control as an alternative parasite control method needs to be widely accepted at several levels, i.e. university, farmers, practitioners, polititians. Secondly, an extensive evaluation of why biological control is needed, i.e. anthelminthic resistance, residues in food, ecotoxic effects of anthelmintics, development of new drugs- and its understanding will lead to elaborate research projects in order to achieve the necessary results for a future successfully use of nematophagous fungi as biological control method in this country.

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STUDIES ON NEMATOPHAGOUS FUNGI TO REDUCE PASTURE INFECTIVITY WITH FREE LIVING STAGES OF TRICHOSTRONGYLID NEMATODES

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INTRODUCTION

In Brazil, ruminants are raised mainly on permanent pastures in Brazil. Gastrointestinal nematodes are an important limiting factor for livestock production of these animals due to ideal conditions for parasite development and transmission which occurs almost all year round. This is particularly the case for small ruminants, which are highly susceptible to the main parasite present in the Brazilian flocks, namely *Haemonchus contortus*. (Honer and Bressan, 1992; Bianchin, 1996; Echevarria, 1996; Padilha, 1996).

To avoid losses associated with parasitic gastroenteritis, farmers generally apply several dewormings per year. In a survey conducted in Rio Grande do Sul, in which 31 sheep farmers were interviewed, the number of anthelmintic applications varied from 6 to 12 per year. (Echevarria and Pinheiro, 1989). Interviewed farmers in Ceara State reported the use of 1-2 dewormings a year in goats (Cavalcante, 1995). In dairy cattle, 3-4 dewormings are generally used in the Southeast Brazil. Most farmers reported that they did not use the recommended strategic control programmes developed during the last 15-20 years in Brazil but used anthelmintics empirically or diagnostically (Charles and Furlong, 1996).

Anthelmintic Resistance

The frequent use of anthelmintics often at insufficient dosages to prevent losses due to trichostrongylid infection in ruminants have resulted in the development of anthelmintic resistance in the worm population, specially in the State of Rio Grande do Sul, where most of the sheep are raised. A recent survey in the region showed severe problems with anthelmintic resistance: 90%, 84%, 72%, 13% and 20% of the examined farms were resistant to albendazol, levamisole, a mixture of levamisole and albendazole, ivermectin and closantel, respectively. (Echevarria, et. al., 1995). In the goat population, mainly raised in the Northeast region, anthelmintic resistance is suspected to be present. It is important to note that although goats have a different metabolism of anthelmintics and require a different dosage, they are dewormed at the recommended dosage for sheep. Continuous underdosing could be the reason for the report of reduced efficacy or could be the reason for anthelmintic resistance. (*Charles et al.*, 1989; *Charles and Medeiros*, 1993; Santos et al., 1993). In cattle, there has been no surveys to clarify the picture of anthelmintic resistance. However, there are some reports of reduced efficacy. (Pinheiro and Echevarria, 1990).

New Approaches to Parasite Control

Since the middle of the 70s, EMBRAPA and some universities in Brazil have implemented studies to develop parasitic control programs for the most important production areas of the country. These programs were developed, and tested both on experimental farms, in computerized farm models and in private farms and shown to be efficient in reducing losses. (Charles, 1992; Furlong, et. al., 1993; Bianchin, 1996; Echevarria, 1996; Padilha, 1996). Surveys of parasite control management conducted at cattle farms in Brazil revealed that farmers are aware of the importance of worm control. However, few farmers use anthelmintics according to the recommended control procedures (Charles and Furlong, 1996). A study conducted among extension veterinarian, which deal with dairy cattle, for example, revealed that few of them are aware of the information developed in the last few years regarding worm control. (Charles, 1995). Neither farmers nor extension veterinarians are aware that resistance can develop and become a great problem. The result of both surveys indicate a need for continuing education courses for extension veterinarians on worm control emphasizing management of resistant nematodes and ways of preventing its occurrence.

Continuing educational courses for extension veterinarians and the correct use of anthelmintics by farmers will improve worm control and retard the development of resistance in the areas in which it has not yet occurred. However, in some areas such as the State of Rio Grande do Sul, where the worm population have developed multiple resistance (Echevarria, et. al. 1996), control alternatives should be developed.

The Potential for Biological Control

The use of microorganisms to reduce pasture infectivity as a possibility to control resistant nematodes is being investigated at EMBRAPA-Dairy Cattle Research Center. The first studies with Brazilian isolates were aimed at the identification of nematophagous fungi, which colonize cowpats and sheep faeces, and the speed by which colonization occurs. For this purpose, the faeces of these animals were spread on *Brachiaria decumbens* pastures in the Mata Region of Minas Gerais State.

Cowpats were deposited monthly from April 1995 to April 1996, while sheep feces were spread in the months of July and October 1995 and January and April 1996. Samples of the manure were collected 3, 7 and 14 days after fecal deposition for culture on water-agar containing free-living nematodes as baits. Inoculated plates were examined for three weeks. Isolated fungi were further cultured on corn-meal agar plates for additional studies, classification and characterization.

The colonization of cattle and sheep faeces by nematophagous fungi in these studies was fast and diversified. From the 390 cowpats deposited during the whole period, 293 fungi were isolated. A total of 123 fungi were isolated from the 120 sheep fecal samples deposited on pastures and most fungi were recovered on day 3 and 14 after deposition. Twenty-eight species of nematode-destroying fungi were identified from the isolates: 22 predators and six endoparasitic. Among the predators were a predominance of those fungi, which produce adhesive nets. These were able to colonize faeces in both the dry and wet seasons. *Arthrobotrys oligospora* predominated in the bovine feces while *A. oligospora* and *Monacrosporium eudermatum* predominated amongst the sheep isolates. *Myzocytium* sp. and *Harposporium anguillulae* were the species that predominated among the endoparasitic fungi.

A survey of nematophagous fungi, which pass naturally through the gastro-intestinal tract and pass out with fresh faeces, was conducted from April, 1995 to April 1996. Ten samples from cow and ten samples of heifers were collected directly from the rectum. From the cows, a pool was made of the feces and from which 10 samples of 2 gm each were placed in the center of a water agar Petri dish in which free-living nematodes were added as bait. With the faecal samples from heifers were cultivated for each individual animal. Ten isolates were found in the study: four isolates of the genus *Arthrobotrys*, two of *Monacrosporium*, two of *Harposporium*, one of *Dactylaria* and one unidentified isolate (unpublished results).

In vitro bioassays to check its activity on free-living stages of trichostrongylid species were conducted with some of the fungi species, in which nematophagous activity on free-living nematodes are intense. An isolate of *H. anguillulae, a* very common colonizer of cattle manure and sheep faeces, was tested using a bioassay. The results showed that the addition of 300,000 conidia per gram of feces caused a reduction of 99.5% of the trichostrongle larvae compared to the control group. (Charles et.al., 1996).

The species, which naturally passes through the gastrointestinal tract, have been exposed to further studies such as *in vivo* bioassays to check persistence of nematophagous activity after the feeding of fungi material produced in water agar or chopped corn to calves. Two species, *Arthrobotrys musiformis* and *Arthrobotrys cladodes*, which were found in fresh faeces of cattle and sheep, respectively, were studied. A. *musiformis* were recovered on the third and fourth day after feeding fungi material (culture in chopped corn) to calves and it reduced the number of infective larvae in fecal cultures 72 hours after feeding by 99%. A. cladodes, fed as conidia suspension (23 million and 150 million), were present in faeces 20 hours after feeding but were less successful in reducing the number of larvae. (unpublished results)

Ongoing work is focusing on the search for fungi present in decaying ruminant and equine feces originating from several regions in Brazil, testing the techniques of mass producing fungi in cereal and including feeding of fungi into the integrated strategic parasitic control program for sheep in South Brazil and cattle in Southeast Brazil.

Integration of biological control into parasite control programmes to reduce pasture infectivity in Brazil, where most ruminants are raised on permanent pasture under extensive conditions, should be applied in the seasons of the year where transmission is greatest. Formulations, which could deliver standard doses to animals everyday, if needed, should be developed.

The use of fungi or any other biological agents, which act on the free-living stages of trichostrongylid species on pastures, will change the concept of worm control. Today farmers apply anthelmintic to reduce the worm population in the gut and as a consequence decrease the number of eggs passed in faeces, resulting in a reduction in the number of infective larvae in the herbage. In the future, farmers will use a formulation to be fed to animals, which will act directly on the development of free living stage in the faeces. Extension officers and farmers will need to be made aware of this new concept which will require continuing education courses and worm control campaigns.

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LIVESTOCK PRODUCTION, EFFECTS OF HELMINTH PARASITES AND PROSPECTS FOR THEIR BIOLOGICAL CONTROL IN FIJI

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INTRODUCTION

Gastro-intestinal parasites are the major cause of losses in the Fijian small ruminant industry. They are the largest single cause of deaths on small ruminant farms, and also cause substantial production losses in the case of chronic infections. Currently, parasite control on small ruminant farms in Fiji relies almost exclusively on the regular use of anthelmintics. Given the possible problems that could be caused by the development of widespread anthelmintic resistance there is a need to develop alternative and sustainable methods of parasite control.

Research carried out in the past in Fiji in conjunction with ACIAR has targeted rotational grazing, selection of resistant hosts, nutritional manipulation and the use of medicated feed supplements. However, each of these approaches has been faced with difficulties. In an attempt to widen the range of sustainable parasite control methods the MAFF and ACIAR has begun investigations into the use of a nematophagous fungi in Fiji.

Fiji: Location Geography and Climate

The Fiji archipelago consists of more than 300 islands scattered over 230,000 km^2 of ocean, lying between latitudes 15 and 22 degrees South, and longitudes 174 degrees East and 177 degrees West. Of the total land area of 18,378 km^2 the two main islands of Viti Levu (57%) and Vanua Levu (30%) covers 87%.

The climate is tropical maritime, with three climatic zones: a wet zone on the windward sides of the main islands, a dry zone on the rainshadow sides and an intermediate zone in the transition area between the wet and dry zones. Mean annual rainfall ranges from 1800 mm to 3200 mm (Twyford and Wright, 1965).

The Importance of Small Ruminants

Small ruminants (sheep and goats) play an important role in Fijian agricultural systems. In 1991 there were 24,027 goat farmers holding 187,235 goats (mean flock size of 7.79 head) (Otanez, Narayan and Tubuna, 1992). Since 1991, 88 new sheep farmers have been established holding some 2,842 sheep with a mean flock size of 32.3 head.

Whilst very few sheep and goats enter the formal (abattoir/butcher shop) system of marketing, there is a large demand for live sheep and goats with live male animals fetching premium prices of $3.00 - $3.50 per kg liveweight. The high demand for small ruminants is largely due to the fact that they are the only commodities not subject to religious taboos (unlike pork and beef), a factor of major importance in Fiji's multi cultural society.

Table 1. Goat and sheep meat imports and estimated local production

Year	Goats Local Prod (t)	Goats Imports (t)	Goats Value of prod ($000)	Sheep Local Prod (t)	Sheep Imports (t)	Sheep Value of prod ($000)	Total Value of Local Prod ($000)
1990	624	84.8	3 744	7.8	8 373	46.8	3 790.8
1991	640	114.9	2 840	10.7	9 807	64.2	3 904.2
1992	651	55.1	3 906	16.5	11 129	99	4 005.0
1993	621	137.2	3 726	16	10 378	96	3 822.0
1994	745	129.2	4 470	20	9 533	120	4 590.0
1995	790	101	4 740	24	1 231	144	4 884.0

Note: The value of production is based on a value of $6/kg carcass weight.
Source: Manueli, 1996a.

The Importance of Gastro-Intestinal Parasites in Small Ruminants in Fiji

Studies in sheep carried out in Fiji have indicated that parasites may be responsible for 30 percent of all stock deaths in privately owned small holder flocks and up to 50 percent of all mortalities in intensively managed government flocks (Manueli, 1996b). The most important parasites of small ruminants in Fiji are:

Table 2. Gastro-Intestinal Parasites of Small Ruminants in Fiji.

Species	Site	Frequency of Observation[A]
Haemonchus contortus	Abomasum	+ + +
Trichostrongylus axei	Abomasum	+ + +
Trichostrongylus colubriformis	Small Intestine	+ + +
Strongyloides papillosus	Small Intestine	+ + +
Moniezia expansa	Small Intestine	+ + +
Oesophagostomum columbianum	Large Intestine	+ +

[A] +, Occasional; + +, Common; + + +, Very Common;
Source: Walkden-Brown and Banks (1986)

Investigations into the parasite nutrition interactions in Fiji sheep have clearly indicated that the parasite problem is exacerbated by sub-optimal nutrition. Improvements in nutrition have shown to result in a reduction in the need for anthelmintic treatments in supplemented animals, with an improvement in productivity of 66 – 85% over unsupplemented controls. The effect of parasite control in addition to nutritional supplementation has resulted in a further 30 – 62% increase in productivity measured as total weight of lambs weaned (Manueli, 1996b).

Current Parasite Control

Current control methods range from monthly drenching on large intensive farms (and government farms) to sporadic drenching (2-3 times per year) in small holder and extensively managed flocks. Anthelmintics commonly used are levamisole, albendazole and fenbendazole with Ivermectin usage being restricted to government stations. All anthelmintics are imported into Fiji at considerable cost with supply being subject to the vagaries of shipping services. The high level of anthelmintic usage on the larger farms has resulted in anthelmintic resistance on a number of goat and sheep farms. Limited surveys (Banks, et.al., 1987) have shown the level of resistance to be 44%, 38% and 17% for benzimidazole, levamisole and the combination of both drugs. More than half (54%) of farms have resistance to either benzimidazole or levamisole drugs. At this stage, no ivermectin resistance has been reported. This is of special concerns as the large flocks are the major source of improved breeding animals for the nations small ruminant farmers, thus resistant worms are being distributed along with improved breeding stock.

The problem of the development of anthelmintic resistance is further exacerbated by the lack of knowledge on the part of both farmers and extension officers of correct anthelmintic usage. The tendency of farmers to regularly under-dose their animals due to either a desire to save money, or a genuine unawareness of the importance of dosing according to liveweights.

Non-chemotherapeutic Alternatives to Parasite Control

Alternatives to chemotherapy that have been the subject of research in Fiji include: the development of a rotational grazing system, the selection of resistant hosts, the use of medicated feed blocks and nutritional manipulation. More recently research has begun into the use of the nematophagous fungus *D.flagrans* to control gastro-intestinal parasites of sheep.

Rotational grazing

As a result of research from ACIAR project 8418 it was found that due to rapid hatching of larvae on pasture, infection with infective larvae occurred all year round in both the wet and dry zones. However, larval survival was relatively short with the majority of larvae dying within 10 weeks of hatching (Banks, et.al., 1990). This resulted in the development of an 8 paddock, 28 day rotational grazing programme (Lawrence 1992); however, uptake by farmers of the rotational grazing systems has been slow. This has been due largely to the high fencing costs associated with the establishment of the rotational grazing system and the small land areas and flock sizes of most small holder farmers.

Genetic resistance

The enhancement of host resistance through the selection of resistant hosts is currently being pursued. An estimate of the heritability of faecal egg counts of 0.27 ±

0.03 has been calculated for Fiji sheep (Woolaston, et.al. 1995). However, the breeding of resistant sheep is a long-term programme and the benefits will not become apparent for some years.

Improved nutrition

Nutritional supplementation has some promise as a large number of small holder farmers regularly provide some sort of feed supplement to their livestock each evening. However, high costs of supplements and poor distribution systems for feeds have resulted in low level of utilization of supplementary feeding.

The introduction of Urea Molasses Blocks (UMBs) and Medicated Urea Molasses Blocks (MUMBS) has proved popular with many farmers; however, farmers have been facing problems with obtaining ingredients for small scale on farm manufacture of the blocks. The use of UMBs and MUMBs is an area that has been the subject of extensive research during ACIAR/CSIRO Project 9132 (Manueli 1996b). The use of MUMBs in conjunction with UMBs (1 wk MUMB, 4 wks UMB) has proven to be effective both on research stations and on private farms. However, the acceptance level of farmers has been limited due to the problems mentioned above sourcing ingredients for on farm manufacture of blocks.

The problem of the supply of feed ingredients is likely to prevent the widespread uptake of the UMB, MUMB technology in Fiji as long as individual farmers are required to mix their own feed blocks. In an attempt to overcome this problem the Division of Animal Health and Production will, over the next 18 months, establish six small scale, Urea Molasses Block manufacturing plants at various locations around the country. The plants, which will be based on cement mixer technology, will be used for the manufacture of UMBs and MUMBs for sale to farmers.

Research Into Nematophagous Fungi In Fiji

Research into the use of nematophagous fungi in Fiji began in July of 1996 in conjuntion with the CSIRO under the aegis of ACIAR. This involved the collection of some 2,459 faecal samples from sheep and goats from a 26 farms in Fiji. The samples were then cultured in an attempt to detect the presence of nematophagous fungi. A number of fungi were detected and identified. Twelve separate isolations of Astrobotys/Geniculifera were made, but unfortunately no local isolate of *D.flagrans* was obtained. The lack of success with isolating *D.flagrans* was thought to have been due to a number of factors. These included: low numbers of larvae developing in the samples due to low faecal egg counts in the animals sampled; faeces were not cultured on agar which may have inhibited larval movement on the petri dishes and thus reduced the chances of observing trapping; the large numbers of other saprophytic fungi that grew in the cultures may have prevented the development of *D.flagrans* in the cultures. As a result another attempt to isolate nematophagous fungus was carried out in 1997.

The second attempt involved the collection of a further 253 samples from sites that had produced trapping fungi in the past. However, this attempt was also unsuccessful despite the fact that the samples this time were cultured on agar (2% agar, 0.02% Tetracycline) and with additional larvae supplied.

Subsequently a Queensland strain of *D flagrans* was imported and quantities of fungal material have been grown on sterile, moist cultures of rice for experimental evaluation of this fungus in controlling parasite infections of small ruminants under local pen and subsequently, field trials. Preliminary results of a pen trial being carried out in Fiji indicate that *D.flagrans* is reducing larval recovery rates in ewes being fed *D.flagrans* chlamydospores.

POSSIBLE WAYS OF USING NEMATOPHAGOUS FUNGI IN FIJI

At present the best possible mechanisms for the use of *D.flagrans* in Fiji is through its inclusion in Urea Molasses Blocks and feeding of rice with fungal cultures as a feed supplement.

a) Feeding in Urea Molasses Blocks

The establishment of the Urea Molasses Block plant for the commercial manufacture of UMBs represents an opportunity for the incorporation of *D.flagrans* in feed blocks for distribution to farmers. As previously mentioned, it is planned to establish six commercial urea molasses block plant in Fiji in late 1997 and early 1998. It is possible that rice grains, on which *D.flagrans* has been grown, could be incorporated into the urea molasses Blocks and fed as a feed supplement to livestock at night in night sheds. However, there will be a need to investigate the stability of the chlamydospores in the blocks. This will require that research be carried out into the development of appropriate block formulations.

b) Feeding of Fungus on Rice

Since a culture of supplementary feeding of livestock currently exists in Fiji, there is a possibility that the fungus could be fed directly on rice grains as a feed supplement. However, the success of this approach will be subject to the development of a satisfactory distribution system and the acceptance of rice as a supplement by the sheep. This approach is the one being used in pen trials that are currently being carried out in Fiji. Unfortunately there are no results available at this time.

c) The Incorporation of fungi into pelleted rations

The possibility of incorporating fungus into compounded feeds represents a viable option as the largest local feed miller has expanded feed production to include the production of ruminant diets.

d) Incorporation into a Reduced Rotational Grazing Programme

Studies to be carried out in Fiji will investigate the use of the fungus in a reduced rotational grazing programme. The current programme requires a minimum of 8 paddocks and a 28-day rotation. The use of the fungus to reduce larval survival and availability on pastures may allow the use of an abbreviated rotational grazing programme. However, this approach will require that epidemiological studies be carried out to investigate the trapping ability and survival of the fungus on pastures.

CONCLUSIONS

Small ruminants will continue to play an important role in the Fijian farming system because of the high demand for live animals for home consumption. The problem of gastro-intestinal parasites however remains the major problem facing small ruminant farmers causing mortality and reduced productivity that is often exacerbated by sub-optimal nutrition.

The problem of widespread anthelmintic resistance in Fiji is the major threat facing the small ruminant industries in Fiji. This threat coupled with the relatively high cost of imported anthelmintics, has made it necessary to explore all possible methods of parasite control. Previous research has highlighted rotational grazing, nutritional supplementation together with the feeding of medicated feed supplements and the selection of resistant hosts as possible alternative methods of control. However, due to the small size of both herds and holdings in Fiji, the long time scale required for the development of resistant hosts, and problems faced by farmers in obtaining materials for the manufacture of UMBs and MUMBs the adoption of these control methods has been limited.

The use of nematophagous fungi for parasite control in Fiji represents an attractive method of parasite control with potential to be incorporated into the local farming system. However, this will require that difficulties faced in growing *D.flagrans* in large quantities be overcome. If the fungi can be produced locally using locally available materials and is shown to work well under Fiji conditions this may result in a reduction in the imports of expensive anthelmintics. The fungi also represent a possible way of modifying the rotational grazing programme allowing for a reduced rotation and thus making it more acceptable to local farmers. However, any decision to release the fungus for widespread use by private farmers will require that a thorough environmental impact study be carried out.

At the conclusion of the current experimental programme in Fiji we will have generated knowledge that will allow us to determine the optimal method of use of the nematophagous fungus in Fiji. However, the fungus remains only one of a number of alternatives to chemotherapy available to us in Fiji. Any future sustainable parasite control in Fiji will have to rely upon a number of different control measures.

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INTEGRATED PARASITE MANAGEMENT IN RUMINANTS IN INDIA: A CONCEPT NOTE

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ABSTRACT

Parasitic gastroenteritis, dominated by haemonchosis, is one of the major constraints to profitable ruminant production in India. The hot and humid tropical climate is very favourable for the development and survival of pre-parasitic stages of nematodes and infective larvae are available on the pasture at all times of the year in most parts of the country. Owing to favourable climatic conditions for development and survival of preparasitic stages and in absence of conventional or alternate control strategies, control of ovine, caprine, bovine and bubaline parasitic gastroenteritis is primarily attempted by the frequent use of anthelmintics at short intervals, particularly in intensive and semi-intensive management systems, which has been shown to result in the emergence of anthelmintic resistant parasite strains. Efforts are now being made to develop and use environmental friendly alternatives to chemotherapy, viz., grazing management, host resistance to parasites etc. There is also a growing awareness about the importance of parasitism and need for integrated management for sustainable parasite control.

Although in India biological control using mycological agents is restricted to the plants, there is need for research on nematophagous fungi mediated parasite control as an alternative to chemotherapy. Although anthelmintic resistance is not as rampant as reported in other developing tropical countries, particularly in the southern hemisphere, there is need to have other alternative means to combat this menace, which will not only reduce the use of chemicals but also the cost of animal production as a whole. Further, investigation to alternates to chemical control should be continued against which parasites may not develop defenses as the kinds of changes the parasites will have to make to avoid the newer methods of control are inherently more difficult and hence slower than the task of developing resistance to anthelmintics.

INTRODUCTION

According to FAO (1994), there are 44.8 million sheep and 118.3 million goats in India with a large genetic diversity as reflected by 40 species of sheep and 20 of goats which account for 0.5 to 5 per cent of the value of total output of the livestock sector (Singh, 1995). Indian goats have adapted to the variety of climatic and agroeconomic situations prevalent in different parts of the country and cost little to maintain as they feed on harvested or fallow fields, canal banks or overgrazed commons. Sheep in India are kept by people who have traditionally taken to sheep farming and are reared in the migratory system, which is dependent on season and availability of pasture to graze. The sheep breeding regions of India are classified as (1) The northern plains, (2) The semi-arid western region, (3) The southern humid region and (4) The temperate and subtemperate mountains. Both sedentary and migratory system of sheep production are common in India. Flocks of Central Institutes and

Government sheep breeding farms engaged in research on sheep production are primarily sedentary flocks. Integrated sheep development programmes are feasible for the sedentary flocks which are located in areas with sufficient grazing/forest land.

There are 193.0 million cattle and 78.8 million buffaloes in India (FAO, 1994). Though India is poised to become the World's number one milk producing country, the per capita productivity of the dairy stock in the country is very low and far below the world averages. The need to optimise productivity in dairy animals in Indian context is further aggravated by liberalised economic policies favouring a market driven economy confronting the Indian dairy farmers with global competition on a scale hitherto not known to them.

PARASITIC GASTROENTERITIS (PGE)

Animal diseases are conservatively estimated to reduce production of animals in India by 30-40 percent. Indeed, much of the national animal diseases control efforts during last three decades have been devoted almost exclusively against bacterial and viral diseases, which are responsible for major epizootics. Parasitic gastroenteritis is now regarded as one of the major causes of low productivity in small and large ruminants. Further, parasitic infection is believed to be the reason for poor immunological response to the vaccines used to control major bacterial and viral epizootics. Efforts for effective control of parasitic gastroenteritis (PGE) in domestic ruminants in India have not been made due to paucity of information on the epizootology of the disease, its variability depending on the agroclimatic conditions, shortage of trained personnel and traditional practice of keeping and management of animals.

Small Ruminants

Haemonchus contortus is considered to be the most pathogenic nematode parasite of sheep and goats (Sood, 1981) followed by *Trichostrongylus colubriformis* (Sanyal, 1988a). However, *Bunostomum*, *Oesophagostomum*, *Cooperia*, and *Strongyloides* spp. are also common, particularly during rainy and post-rainy months.

Large Ruminants

Haemonchus spp. is the predominant nematode recorded in buffalo while, *Haemonchus* and *Mecistocirrus* spp. head the list in cattle (Sanyal and Singh, 1995a). However, *Cooperia*, *Oesophagostomum* and *Trichostrongylus* spp. are present in low numbers particularly during monsoon and post-monsoon months.

PERCEIVED EFFECT OF PARASITES ON PRODUCTION

Small Ruminants

Naturally acquired mixed trichostrongyle infection is believed to adversely affect the live weight gains and fibre production in small ruminants. In nomadic migratory flocks nematode parasitism is regarded to be causing major losses of production which is often exacerbated by severe and seasonal shortage of nutrition. Suppressive anthelmintic drenchings result in marked increase in live weight gain and clean fleece yield (Sanyal *et al*, 1986; Maru *et al.*, 1986). In intensive farming system morbidity and mortality rates could be reduced to a great extent by the use of strategic dosing coupled field results in depressed nutrient intake, impaired absorption and utilization. Nutritional supplementation, particularly the use of bypass protein, has been shown to limit parasite induced losses. Anthelmintic-unresponsive diarrhoea and anthelmintic-unresponsive growth retardation as reported by Suttle (1994) has also been observed in flocks maintained in institutional farms.

Large Ruminants

Apart from calves, parasitic gastroenteritis in cattle and buffaloes is present in subclinical form (Sanyal *et al.*, 1992a), but the extent to which this reduces animal productivity was not known before 1992. Peri-parturient anthelmintic treatment of crossbred dairy cattle with conventional anthelmintic formulation resulted in 11.26 per cent increase in milk yield with a gain of 1.42 litres of milk per cow per day. It increased the lactation length by 27 days and time to first oestrus was reduced by 8.2 days (Sanyal *et al.*, 1992b). Similarly, an overall 13.2 per cent gain in milk yield in dairy buffaloes with an increase of 0.96 litre/head/day was observed following periparturient anthelmintic treatment (Sanyal *et al.*, 1993). Strategic long-term low-level administration of fenbendazole in urea molasses block has been reported to increase milk production by 1.2 litres per day in dairy buffaloes (Sanyal and Singh, 1995b), 0.57 litre per day in crossbred cattle (Sanyal *et al.*, 1995) and an additional increase of 60 gm in body weight per day in treated heifers (Sanyal and Singh, 1995c).

EPIDEMIOLOGY OF PARASITIC GASTROENTERITIS

Small Ruminants

Most of the available information on parasite prevalence comes from flocks maintained under intensive management system. Very limited information is available from the migratory flocks. However, it is considered that seasonality of infection in these flocks is likely to be the same as in intensively managed flocks, because the prevalence of parasitic gastroenteritis in tropical areas is largely determined by rainfall and production systems practiced. The epidemiological picture of parasitic gastroenteritis in the country can be divided into 4 major agroclimatic zones of sheep breeding in India:

Northern plains

Limited surveys indicated higher prevalence rate during monsoon and post-monsoon months (June to October) in the states of Bihar (Bali, 1973), Orissa (Mishra *et al.*, 1974), Punjab (Chabra *et al.*, 1976) and Haryana (Bali and Singh, 1977). While studying epidemiologic pattern of parasite prevalence, Gupta *et al.* (1987) reported *H. contortus* and *Trichostrongylus* spp. to be responsible for ovine and caprine parasitic gastroenteritis. The adult parasites persisted throughout the year and there was no indication of hypobiosis. The agroclimatic conditions of the zone revealed that, in general, favourable weather conditions for the development and survival of free living stages of the parasites existed all the year round. However, July to October were the months of maximum parasitism.

Semi-arid western region

Rathore *et al* (1955), Dubey *et al.* (1983) and Sanyal (1989a) indicated June to November were the months of high risk. A retrospective study on the prevalence of ovine parasitic gastroenteritis in the flocks of Central Sheep and Wool Research Institute (CSWRI), Avikanagar, Rajasthan during 1988-1993, indicated equivalent average parasitism rate of 1.96 per cent in adults followed by 1.10 per cent in hoggets and 0.17 per cent in weaners. Peak parasitism was observed during July to September (Swarnkar *et al.*, 1996). The worm egg counts in adult sheep rose to a peak in August while in young sheep, peaks were observed in September. Pasture larval burden was more during monsoon and July to October seemed to be more vulnerable months (Swarnkar *et al.*, 1997).

Southern humid region

Gupta and Mathur (1969) observed that November to January are the months of peak parasitism in Madras, Tamil Nadu which receives North-East monsoon during October-November.

Temperate / Subtemperate region

In the temperate Himalayan region of Kashmir predominant parasites were *Trichostrongylus* and *Strongyloides* spp. An increasing trend of infection from spring (March - May), summer (June - September) to Autumn (October - November) was observed with ambient temperature ranging from 7.4 - 29.5°C (Dhar *et al.*, 1982; Krishna *et al.*, 1989, Makhdoomi *et al.*, 1995).

Results of a study undertaken during 1986 to 1989 on the bionomics of pre-parasitic stages, seasonal availability of infective stages on pasture, per-parturient rise and hypobiosis of parasites in the host in subtemperate Tamil Nadu indicated that the period of June to November was best suited for rapid hatching of eggs followed by moulting (Sanyal, 1989b). Seasonal availability of ovine strongyle larvae on pasture indicated *H. contortus* being active during June to November and rare during December to May. Whereas, *Trichostrongylus colubriformis* was found on pasture throughout the year (Sanyal, 1989c). Peri-parturient rise with resultant higher worm burden in lambs was also recorded (Sanyal, 1988b). Hypobiosis of *H. contortus* during September to February was very common, coinciding with higher worm burden in the hosts during March onward (Sanyal, 1988c; Sanyal, 1989d).

Large Ruminants

Pal and Balakrishnan (1987) recorded bimodal distribution of gastrointestinal parasites in cattle in the Andaman and Nicobar Islands with maximum incidence occurring during March and December. Jagannath *et al.* (1988, 1989) reported higher infection rate in buffalo than cattle in Karnataka with higher incidence during south-west monsoon.

Quantitative epidemiology of bovine PGE organized by the National Dairy Development Board in eight different agroclimatic zones of India during 1990-1994 indicated typical seasonal rise in worm burdens in host and on pasture following monsoon rains (Sanyal *et al.*, 1992a, Sanyal and Singh, 1995a, Mathur *et al.*, 1996).

CONTROL MEASURES IN PRACTICE

Chemotherapy with or without grazing management

In majority of farmer's flocks no anthelmintic treatment is given unless clinical cases of PGE appear. In some flocks tactical anthelmintic treatment is practiced during the months of March-April, June-July, September-December. Based on the observations on parasite epidemiology in sedentary flocks of CSWRI in the subtemperate Tamil Nadu, a control strategy was formulated with the combination of rotational and clean grazing with anthelmintic dosing at strategic points (Sanyal, 1991). It was observed that clean pasture provided after winter drenching in January can be grazed for more than 3 months, whereas clean pasture provided after rainy season drenching in June showed high pasture contamination in the second month of grazing. During most seasons, except winter, monthly rotation of pasture was practiced as pasture larval burden reached above critical level within a month of grazing (Sanyal, 1991). Based on the epidemiologic pattern of parasite prevalence, a control strategy was formulated for sheep flocks of CSWRI stationed in semi-arid climate of Avikanagar involving two strategic dosings during March-April and June-July and one tactical drenching during October-November. This strategy implemented since 1985 onward resulted in reduction in equivalent average morbidity per 1000 animal days at risk from 0.12 to 0 (Maru *et al.*, 1993).

PROBLEMS ENCOUNTERED IN CONTROL OF PGE

Anthelmintic resistance

In migratory farmer's sheep flocks anthelmintic treatment against PGE tends to be ad hoc and haphazard, so the selection pressure for anthelmintic resistance is considered to be low. However, distribution of farm-bred flocks to the farmers under various extension programmes have resulted in the spread of resistant strains generated on the farm to the farmer's flocks. Emphasis on anthelmintic treatment has resulted in use of drugs by the farmers, who not only compromise with dose but also with anthelmintic efficacy. Therefore, the risk of selecting for resistance has increased significantly.

In India Varshney and Singh (1976) first reported resistance to phenothiazine and thiabendazole in *H. contortus* of sheep. Later on, during 1990s many reports of anthelmintic resistance appeared like fenbendazole resistance in ovine *H. contortus* (Yadav, 1990), levamisole resistance in caprine *H. contortus* (Yadav and Uppal, 1993), fenbendazole and tetramisole resistance in ovine *H. contortus* in sheep breeding farm, Rishikesh, Uttar Pradesh (Srivastava *et al.*, 1995), multiple benzimidazole resistance in *H. contortus* in sheep (Yadav *et al.*, 1995) and goats (Uppal *et al.*, 1992), emerging resistance to rafoxanide in *H. contortus* in sheep (Singh *et al.*, 1996), albendazole resistance *H. contortus* strains in CSWRI flocks (Singh *et al.*, 1995) and benzimidazole resistance in sheep and goat in Uttar Pradesh (Yadav *et al.*, 1996a). An outbreak of haemonchosis in sheep associated with fenbendazole and morantel resistance was reported for the first time by Yadav *et al.*, 1993a. The first genuine survey for anthelmintic resistance was conducted by Kumar and Yadav (1994). They surveyed 32 traditionally managed and 22 intensively managed flocks in 3 farms for FBZ resistance. They observed no resistance in traditionally managed rural flocks while, 15 of 22 intensively managed flocks had slight resistance (between 60-88 per cent reduction of egg counts) and 4 had severe resistance (less that 60 per cent reduction). Resistance to albendazole and levamisole were reported in five sheep farms located in different agroclimatic regions in India (Gill, 1993, 1996).

Till date there is only one report of fenbendazole resistance in gastrointestinal parasites of cattle (Yadav *et al.*, 1996b).

SUSTAINABLE PARASITE CONTROL (SPC)

The demand for milk, meat and fibre in India is growing not only because of increasing human population but also due to rise of per capita income. Ruminants play a crucial role in the conversion of low quality plant material and crop residues to high quality human food and in returning valuable plant nutrients to the soil. As sustainable development is a compromise between reducing environmental degradation and positive economic growth, sustainable parasite control should aim towards less intensive, lower input, less risk of parasite induced losses with greater opportunities for integration of chemotherapy, grazing management, biological control, mathematical models and decision support systems. In most of the cases resistance seems clearly to be associated with heavy reliance on chemicals, applied frequently and sometimes haphazardly. Drug resistance increases costs of treatment, reduces the efficiency of production, depletes the stock of effective control tools and increases the risk of environmental contamination, as frequency of use and dose increases with declining effectiveness of anthelmintics. As resistance to newer anthelmintics is emerging, there is a need in India for sustainable integrated parasite management which is multidisciplinary in nature and is under resourced because of its long-term nature. Government must acknowledge the crucial role of research and development and the need for widespread dissemination of its results in the quest for sustainable development.

Use of Anthelmintics with Grazing Management

Natural grazing management is an age old practice of farmers wherein the flocks migrate from one pasture to another depending on availability. In sedentary flocks grazing management are being practiced since late 1980s, with reduction in the frequency of anthelmintic interventions. People have started fine tuning the strategy because of the fact that selection pressure on worms to develop anthelmintic resistance is not simply proportional to frequency of treatment, but depends on how anthelmintics are used in relation to grazing management.

Anthelmintic Delivery Devices

One of the recently developed weapon for efficient integrated parasite management programme is self medicating anthelmintic delivery devices. These consist of adding a targeted dose of anthelmintics in a suitable and palatable vehicle which is consumed by the animal at its own will. Through these delivery devices the cost of labour and spillage of drug can be reduced considerably.

At the Biotechnology Laboratory, NDDB, Anand, under the NDDB-ACIAR collaborative research projects (PN 8523 and 9132) two self medicating devices against parasitic gastroenteritis are currently being evaluated. These are:

Medicated urea molasses block licks

This technology is based on the principle that efficacy and spectrum of activity of benzimidazole anthelmintics are increased following sustained low dose administration. Medicated Urea Molasses Block (MUMB) developed by the NDDB, Anand, contains adequate fenbendazole anthelmintic so as to deliver a minimum daily dose of 0.5 mg/kg body weight when licked by the animals on daily basis. Laboratory and field trials have indicated that these are highly effective for treatment and control of gastrointestinal round worms and during improve animal productivity (Sanyal, 1995). This delivery device can be used as a proactive mean to control parasites by their strategic application based on parasite epidemiology.

Dewormer concentrate feed pellets

Medicated feed pellets were developed based on the observation that divided dose schedule of benzimidazole anthelmintics is more efficacious than the single dose. It contains fenbendazole or albendazole for large and small ruminants, respectively and is offered to the animals for two consecutive days. This delivery device is primarily meant for curative treatment of animals (Sanyal, 1997).

Host genetic resistance

Emergence of resistance in gastrointestinal nematodes to broad spectrum anthelmintics and excessive cost of developing new anthelmintics will warrant development of alternative methods of control. Breeding of stocks genetically resistant to helminth parasites may provide a solution, but no such efforts have been made in India. Very few studies have been conducted on the breed susceptibility to nematode parasitism. Though Yadav *et al.* (1997) reported no significant differences in parasitological, pathophysiological and immunological parameters in native Nali and Merino crosses experimentally infected with *H. Contortus*, it was observed in the flocks of CSWRI, that the native Coimbatore breed excreted fewer worm eggs in faeces and had lower morbidity and mortality rates compared to purebred Merino, Rambouillet and their crosses (Sanyal, 1988a). The first specific study in this direction was conducted by Yadav *et al.* (1993b). Following experimental infection with *H. contortus* they observed significantly less changes in haematological values, body weight gains, faecal egg counts, adult worm counts, abomasal pH and pathophysiological changes in the abomasum with increased eosinophilia in lambs of native Munjal breed (Nali x Lohi) compared to crossbred Hisardale (Nali x Corriedale crosses) lambs. An effort is now being made to study Garole breed of sheep which are naturally resistant to various diseases including parasitic gastroenteritis as a joint research programme involving ACIAR, Goverment of India and NGOs.

BIOLOGICAL CONTROL (BC) AND ITS INTEGRATION IN SPC

As reported earlier, parasitic infection of ruminants in India is governed primarily by rainfall and temperature which favour development and survival of preparasitic stages. Therefore, the prevalence of parasitism is seasonal. Farmers tend to recognise the seasonality of the problem and accordingly treat their animals with anthelmintics. In many instances lengthy periods of high rainfall require regular and frequent treatment. The picture is more severe in sedentary flocks where animals are grazed on permanent pastures throughout the year. It is largely been regarded that parasite control can not be achieved by a single method. The available classical and alternate technologies should properly be integrated similar to those practiced in the integrated pest control in agriculture. Biological control seems to be one such additional alternate control strategy. There are promising reports regarding the potential use of biological control of nematode parasites of ruminants using nematophagous fungi in cattle, horse, pig and sheep from Denmark and Australia.

Though in India biological control of nematodes by mycological means is restricted to the field of agricultural research on the use of nematophagous fungi for animal parasite control is being proposed. Though anthelmintic resistance is not as rampant as in other developing tropical countries particularly in the Southern Hemisphere, it is felt that India should develop alternative methods, so as to reduce the use of chemicals. It is also felt that the kinds of changes the parasites will have to make to avoid the newer methods of control are inherently more difficult and hence slower than the task of developing resistance to anthelmintics.

PREREQUISITES TO THE ADOPTION OF BC

Prerequisites to the adoption of biological control using nematophagous fungi in India can be divided into two basic heads:

Research

1. Isolation of local strains of nematophagous fungi which can survive ruminant gut passage.
2. Development of a device for delivery of fungus to the host.
3. Strategic application of alternative control methods and monitoring worm burden in laboratory and field trials simulating standard husbandry practices.
4. Impact of biological control on user and environment.
5. Impact of nematophagous fungi on beneficial saprophytic nematodes useful in nutrient recycling.

Development

These are essential steps for biological control on commercial perspectives.

1. Development of cheap method to scale up fungal production.
2. Development of formulations in which nematophagous fungi can survive storage.

CONCLUSION

Parasitic gastroenteritis is regarded as a major impediment to profitable ruminant production in India. The haematophagous abomasal parasite *Haemonchus contortus* is the dominant species in both small and large ruminants. Monsoon governs the severity of infection which is more in sedentary flocks of sheep compared to migratory flocks. Frequent anthelmintic interventions have resulted in the emergence of anthelmintic resistance particularly in sedentary sheep flocks.

There is increasing awareness that in future the parasite control programme should reduce the reliance on chemical anthelmintics. It is believed that parasitic control needs an integrated approach involving the use of all available means. Compared to other non-chemotherapeutic approaches to parasite control in ruminant livestock, use of nematophagous fungi has shown promising results in Denmark and Australia. Research and development work on biological control of parasites is desirable so as to develop sustainable parasite control strategies.

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THE EPIDEMIOLOGY AND CONTROL OF GASTROINTESTINAL NEMATODES OF RUMINANTS IN INDONESIA, WITH SPECIAL REFERENCE OF SMALL RUMINANTS IN WEST JAVA

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INTRODUCTION

Ruminant livestock are the second most important source of animal protein in Indonesia, following poultry. Data on livestock population available from the Directorate General of Livestock Services shows that the population of ruminants in Indonesia is approximately 6.5 million and 11.9 million for sheep and goats, respectively. The large ruminant population consists of approximately 11.3 million cattle and 3.1 million buffaloes (Direktorat Jenderal Peternakan, 1995). Small ruminants are important animals for small holder farmers in rural areas of Indonesia. Despite the fact that animals are traditionally raised by farmers as a sideline to the main agricultural activities, they play a significant role in supporting farmer's income. They contribute from 14 to 25% of the total annual income of smallholders (Knipscheer *et al.*, 1983). Large ruminants are raised under more intensive management systems, particularly in the eastern part of Indonesia.

MANAGEMENT OF LIVESTOCK

Most sheep and goats are kept in pens with slatted floors, usually around one metre above-ground. Only a few animals are housed on the ground in some areas. There are three management feeding systems adopted by farmers, depending on the location. In high altitude areas, most animals are housed and feed is brought to them and consequently they are infected with very low level of gastrointestinal parasites (Beriajaya, 1986a). In the coastal areas, most animals graze during the day and generally they are infected with high numbers of parasites (Beriajaya, 1996b). In the medium altitude, animals graze for only a few hours each day, being housed for most of the day. These management systems greatly modify the transmission of parasite infection. Generally the more time spent grazing the higher the level of parasitism (Beriajaya and Stevenson, 1985).

Faeces which accumulates under the pens during 1-3 months, is used as fertilizer for crops and vegetables. Because penned animals have very limited opportunity to naturally contaminate pasture by grazing and that there is only little use of dung to fertilize pastures, the transmission potential of parasites is considerably restricted in this system of management.

THE ECONOMIC IMPACT OF NEMATODE PARASITISM IN LIVESTOCK

Gastrointestinal nematode infection is one of the most common problems faced by sheep farmers in Indonesia (Ronohardjo and Wilson, 1986). A wide spectrum of gastrointestinal nematodes of sheep has been found in this country, including *Haemonchus contortus, Trichostrongylus* spp., *Cooperia* spp., *Bunostomum* spp. and *Oesophagostomum* spp. (Kusumamihardja, 1988). Observations in the field showed the prevalence of the infection ranged between 45 to 89% with *H.contortus* and *Trichostrongylus* spp. as the most predominant species in West Java (Sutama and Beriajaya, 1991: Carmichel *et al.*, 1993; Ridwan *et al.*, 1996).

These parasites are the cause of considerable disease problems and therefore are responsible for major economic losses and inefficiency in production. From a slaughter house survey, He *et al.*(1990) found that the prevalence of gastrointestinal helminth infections in sheep including nematodes is approximately 80%. Furthermore, on the basis of carcase weight differences between infected and non-infected animals, this study estimated an annual loss of 3.2 – 4.4 million kg. of meat (US$4.8 – 6.6 million) due to parasite infections. Another study estimated that US$7.04 million was lost annually due to haemonchosis alone (Ronohardjo *et al.*, 1985). This estimate has ranked haemonchosis as the sixth most economically important livestock disease in Indonesia (Table 1).

Fewer studies have been done on the gastrointestinal nematodes of large ruminants. The importance of these parasites almost certainly exacerbates the effects of liver fluke (*Fasciola gigantica*) which has long been considered the most important helminth parasite of large ruminants (Rohardjo *et al.*, 1985). It has been estimated that nematode infections caused 14% reduction in carcase weight of Ongole cattle (He *et al.*, 1989). Together with other gastro intestinal helminth infection, it caused an annual loss of 19 – 39 million kg of meat (US$38 – 79 million).

Toxocara vitulorum is also one of the most prevalent gastrointestinal nematodes parasitising cattle and buffalo calves at an early stage of their life. Results of our recent study have shown that the prevalence of infection in calves aged below 2 months of age, ranged between 34.5 – 52.8% in some districts of West Java and South Celebes (Satrija *et al.*, 1996). A higher infection rate (51.1 – 61.4%) was found in buffalo calves in West Java (Satrija *et al.*, 1995)

Table 1. The estimated annual loss caused by most important livestock diseases in Indonesia (Ronohardjo et. al., 1985)

N°	Disease	Estimated annual loss (Million US$)
1.	New Castle Disease	40.32
2.	Fasciolosis	32.00
3.	Trypanosomosis	22.40
4.	Foot and Mouth Disease	14.72
5.	Haemorrhagic Septicaemia	8.64
6.	Haemonchosis	7.04
7.	Ascariosis	6.72
8.	Brucellosis	3.52
9.	Sephanofilariosis	3.52
10.	Anthrax	3.20

Control of Gastrointestinal Nematodes

Effective control of parasitic gastroenteritis in ruminants requires accurate understanding of the epidemiological pattern of infection. Ridwan *et al.*(1996) conducted an epidemiological study of nematode infection of sheep in four geographical areas of West Java. These areas have different climatic regions, based on annual rainfall and the length of the rainy and dry season (Oldeman, 1975). Results of this investigation confirmed that the pattern of monthly nematode egg counts over a period of one year was the same in all location. The mean nematode egg counts decreased after the end of the dry season and increased about 3 – 4 months after the beginning of the rainy season. This study also revealed that the intensity and epidemiological pattern confirmed that of a previous study which demonstrated the infective larval contamination level of pasture in the humid area of Bogor/West Java was higher during the wet season than during the dry season. This also reflected the cumulative parasite burden at the end of the seasons (Kusumamihardja, 1988).

The combination of grazing management with anthelmintic treatment remains the best choice for nematode parasite control in ruminants (Fabiyi, 1986). Rotational grazing systems are now well established as one of the most effective measure to control parasites. A small ruminant parasite control project in North Sumatra included a field trial to control haemonchosis using a one-week grazing and a six-week resting system (Ginting *et al.,* 1996). Results of this trial suggest that performance of animals grazed in this system was superior than those grazed in 6 or 12 week rotational cycles. Unfortunately this system is less practicable in traditional farms where unsupervised grazing by several flocks occurs along road sides or verges of paddy fields. In this case, the key to implement the control program is to educate farmers of the benefits of rotation in which stock graze for only a few days on a given area which is then spelled for several weeks (Carmichel, 1993).

Results of parasite control studies elsewhere, have demonstrated that anthelmintic treatment at the time when larval population in pasture is low, may prevent the accumulation of parasites in animals and improve the subsequent performance of grazing animals (Satrija *et al.,* 1996a). An almost continuously high larval availability pattern throughout the year, as shown on previous epidemiological studies, required frequent anthelmintic treatments in an attempt to achieve an effective control. Thus, anthelmintic treatments given to lambs at 4 – 8 week intervals may reduce the level of parasitism and subsequently improve the performance of treated animals (Beriajaya and Stevenson, 1985: Beriajaya, 1986: He *et al.,* 1990).

Except in dairy and feedlot farms, anthelmintic treatment with commercially available drugs is rarely given by farmers to their livestock. The price of modern synthetic anthelmintics is generally too expensive for the small holders, they are not always available, or in small enough packaging at the local markets. These are the main obstacles for implementation of parasite control programs at the village level (He, 1991). Therefore the risk of the development of anthelmintic resistance in Indonesia is considered to be low (Dorny *et al.,* 1995).

Breed Resistance

Breed resistance in sheep against gastrointestinal nematodes has been studied in North Sumatra, from 1990 to 1995. Gatenby *et al.*(1991) and Carmichael *et al*

(1992) reported that some ewes in observed flocks maintained low faecal egg counts. Romjali (1995) studied experimental infections with *H. contortus* in four genotypes of rams aged between 18 and 24 months. The results showed that there was no significant differences in faecal egg counts among the breed types, but there were high variations among individuals within breeds. Another study conducted in North Sumatra with weaned lambs indicated that the Sumatra Thin Tail genotype had lower faecal egg counts than crosses with St. Croix or Barbados Blackbelly (Batubara *et al.,* 1995). The study concluded that the indigenous genotype of sheep may have some degree of resistance against gastrointestinal nematode parasites.

Medicinal Plants

Farmers in Indonesia use a wide range of traditional remedies to treat animal diseases, rather than commercial veterinary drugs (Wahyuni *et al.,* 1992). Ethnobotanical surveys have listed some 25 species of medicinal plants used by farmers in Java to treat gastrointestinal nematode parasites (Sangat-Roemantyo and Riswan, 1991; Murdiati, 1991; Gultom *et al.,* 1991; Ma'sum, 1991). However, the specific anthelmintic efficacy of a number of these plant and herbal preparations has not been experimentally documented. For example, extensive studies have been done to investigate materials from papaya trees for anthelmintic properties. A high efficacy of papaya latex was demonstrated against *Ascaridia galli* in chickens (Mursof and He, 1991) and against *Ascaris suum* in pigs (Satrija *et al.,* 1994). A high efficacy of papaya latex against *Heligosomoides polygyrus* has also been demonstrated in mice (Satrija *et al.,* 1995). When latex was orally administered to sheep experimentally infected with *H.contortus*, it significantly reduced the egg counts of treated animals (Beriajaya and Murdiati, 1977). The results of further investigation will provide the basis for the use of these traditional medicinal plants in the control of gastrointestinal nematode infections in ruminants. The availability of effective and cheap traditional medicines may encourage farmers to increase the animal production in rural areas which will increase the farmer's income and food security.

Biological Control

Work on nematophagous fungi in Indonesia, was initiated with attempts to control some of the important nematode parasites of plants. Several species have been studied, including *Arthrobotrys, Dactylaria, Catenaria* and *Dactylella* spp., and *Arthrobotrys* spp has been used in attempts to control plant parasitic nematodes (Manohara *et al*., 1996). There has also been some studies on the effects of nematode-destroying fungi on animal parasitic nematodes. Two species, *Arthrobotrys* spp and *Verticillium* spp. were isolated near the research institute laboratory at Bogor, but unfortunately the species was not maintained for continuing studies. Presently, work is continuing to find local isolates of nematophagous fungi. Six genera, namely, *Arthrobotrys, Cladosporium, Fusarium, Gliocladium, Paecilomyces, Trichoderma* and *Cephalosporium* spp were found in sheep grazed pastures. These species will be purified and mass cultured for both *in vitro* and *in vivo* studies. *In vivo* studies will be tried in sheep to establish the capacity of these fungi to survive passage through the gastrointestinal tract of ruminants and then to reduce the number of infective parasite larvae in faeces.

It is still too early to determine whether nematode-destroying fungi can be used to control nematode parasites of livestock in Indonesia. Local isolates of fungi are possibly the best candidate, rather than exotic imports, because they have adapted to local environmental conditions and are thus likely to be able to exert there nematophagous effects more effectively. Field studies will determine the efficacy and applicability of these organisms and the best means of delivery. The methods by which this form of control need to be determined. Firstly, the method should be studied in a few smallholder farms, possibly using a feed block supplement. If it works in these systems, then work should continue with large scale farmers.

Conclusion

Ruminants are important livestock kept by farmers in Indonesia. Parasite infections are categorised as major economic diseases of these livestock. Control programs at present are solely based on anthelmintics, which have the disadvantage that resistance is emerging as a significant problem. Nematode-destroying fungi offer an alternative, biological approach to control. However, before they can be practically applied, they need to undergo considerable experimental evaluation under laboratory and finally farming conditions in Indonesia.

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IMPORTANCE OF GASTROINTESTINAL HELMINTHS TO THE LIVESTOCK INDUSTRY IN KENYA

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INTRODUCTION

Gastrointestinal helminths cause considerable losses to the livestock industry in Kenya. These losses are related to clinical parasitic gastroenteritis leading to mortalities especially in lambs, kids, calves and poorly managed and malnourished adults. Greater losses occur due to sub clinical parasitic gastroenteritis leading to chronic production losses. These losses have been reported in all the agroecological zones of Kenya. Although most losses occur in the high rainfall areas, losses have also been reported in the dry and semi arid areas. These production losses are manifested as reduced weight gains, lower milk and wool production and in meat and organ condemnations.

The most common and economically important nematode *Haemonchus contortus* is causing high mortalities in sheep and goats in Kenya (Njanja, 1985; Maingi, 1991; Mwamachi *et.al.*, 1993; Baker *et. al.*, 1993). In the coastal area, the Diani Estate farm lost 81 out of 355 lambs due to helminthosis within a period of 6 weeks after weaning (ILCA, 1993). Helminthosis caused 50.6% of all the deaths in this flock within one year. In the semi arid area of Marsabit, a survey of gastrointestinal helminths of cattle under nomadic management showed that helminthosis was a major constraint to livestock production in this area. *Haemonchus spp* were the most important parasites (Omara-Opyene, 1985). Ulvund *et.al.*, (1984) found that ewes and lambs grazing the cool highland pastures were heavily infected with gastrointestinal helminths and had to be treated every 3 - 4 weeks through out the year. The animals lost considerable weight and *H. contortus* was the main helminth present.

Waruiru *et.al.*, (1993) reported fatal parasitic gastroenteritis in nutritionally stresssed weaner heifers turned out on infected pastures in a medium potential area of Central Kenya. *H. placei* was the main helminth identified.

In a study conducted among small scale farmers in a semi arid area, helminthosis was ranked as the most important disease of livestock and the main cause of mortality in kids (Githigia *et. al.*; 1995 (a)). Current research on goats in the large and small scale farms in a similar area has shown that treatment against gastrointestinal helminths leads to improved weight gains, kid survival, mothering ability and possibly reduces the kidding interval. In the drier areas calves treated against *Toxocara (Neoascaris) vitulorum* were reported by the pastoralists, to be able to survive through the dry season, compared to the untreated calves (initial Kanyari - 1997 Personal communication)

Work by Maingi (1996) in a sheep rearing district in central Kenya has shown that gastrointestinal helminths are a major constraint to the sheep industry. Mortalities due to fasciolosis may reach up to 55% in some farms in the district (Maingi and Mathenge, 1995).

ECONOMIC COST OF HELMINTH INFECTIONS

In a survey covering 5 slaughter houses around Nairobi over a period of 6 weeks, helminths parasites were the major causes of whole carcass and organ condemnations. 11.8% of all cattle slaughtered and 46% of all sheep and goats slaughtered had their organs condemned due to helminthosis (Githigia *et. al.*; 1995 (b)). Although the major documented causes of economic losses in Kenya are due to hydatidosis, fasciolosis and cysticercosis (Cheruyoit,1983), pimply gut (Oesophagostomosis) also causes losses as they render intestines useless for suture and sausage making and cause condemnations of large and small intestines (Githigia *et. al.*; 1995 (b)). In monetary terms, Preston and Allonby (1979) estimated that *H. contortus* caused an annual loss of 26 million US dollars to the agricultural sector. This figure is very conservative and may have doubled during the years.

In the slaughter house survey, about 1200 US dollars were lost within a period of 6 weeks in only 5 slaughter houses. Considering the other slaughter houses and the period the loss could be substantial.

In addition the cost of importing anthelmintics, the primary method for the control of gastrointestinal nematodes places additional stress to the already strained economy of Kenya.

Current Methods of Gastrointestinal Helminth Control in Kenya

These mainly rely on anthelmintics. Anthelmintics are used on a considerable scale in the more important livestock producing areas like dairy farming which is heavily dependent on use of these products (Kinoti, *et. al.*, 1994). Outside these areas they are utilised in intensive livestock operations such as government farms and large commercial enterprises. Use of anthelmintics in small scale and nomadic production systems is limited and is based on rare haphazard anthelmintic treatments (Mbaria, *et. al.*; 1995).

Treatments are usually carried out when the animals show clear signs of helminthosis. The treatment regimes vary, and seem to depend on the regions where research has been undertaken. In the wet highlands treatment is recommended every 3 - 4 weeks through out the year in sheep (Ulvund *et. al.*; 1984). At Diani in the coastal region near Mombasa, treatment is done at weaning. Lambs and kids are monitored for up to 1 year of age. A monitor group is regularly sampled and the whole flock is treated when the monitor animals have a mean faecal egg count (EPG) of between 1500 - 2000 (ILCA, 1992).

Current research findings show that there is a benefit of treating just before and again just after rain for both the short and long rains, reduced pasture infectivity and worm burdens.

Benzimidazole group are the main anthelmintics used. Ivermectins are also in use (Kinoti, *et.al.*; 1994) but their use is restricted due to the cost. Closantel and moxidectin are currently being used in research evaluation prior to registration.

The main problems with anthelmintics is that they are used in a rare haphazard way with no account of epidemiological principles, thus effects are inconsistent (

Mbaria, *et. al.*, 1995).

Anthelmintic Resistance

Benzimidazole resistance has been reported in nematodes of sheep and goats in Kenya (Baker, et.al., 1993; Njanja, *et. al.*, 1987; Maingi, 1991; Maingi, *et. al.*, 1993; Waruiru, *et. al.*, 1991; Mwamachi, *et. al.*, 1993). Levamisole resistance has been reported in sheep and goat nematodes (Maingi, 1991; Waruiru *et. al.*, 1991; Mwamachi *et. al.*, 1993; 1995). The main species of worm involved has been *Haemonchus*. This could be due to its high pathogenicity which makes any control failure more noticeable than in cases with other worm genera.

Resistance is more common among goats than sheep flocks (Wanyangu, *et. al.,* 1994). This could be attributed to the differences in their physiology and drug metabolisms where goats metabolise drugs faster. Little information is available on the dosage rates for goats and they are given the dosage for sheep. This usually does not work as reported by Mwamachi, *et. al.*, (1995).

Another problem is the unavailability and cost of the drugs which preclude many farmers from using them. Also the improper use of the drugs at farm level which is linked to educational levels, community infrastructure and an inefficient veterinary extension service, is another important problem.

Most anthelmintics are sold over the counter with dosage labels in languages that the farmers do not comprehend. The major drug companies also compete for a share in the existing anthelmintic market with focus on sale volumes.

Alternative approaches to Parasite Control

Another control method used in a few areas is grazing management. Pasture management is unknown to most farmers (Mbaria, *et. al.*, 1995). In some areas, animals especially goats are taken out to graze after the dew has evaporated. The purpose of this is that farmers believe that this reduces larval in take however, this has not been verified in experimental evaluation. Other farmers graze the more resistant adults ahead of the less resistant young and lactating animals, in the belief that this improves parasite control.

Host genetic resistance is also a potential gastrointestinal parasite control method. Studies show that the Red Maasai breed of sheep shed fewer faecal eggs under natural and experimental conditions. There is evidence that this breed of sheep is relatively resistant to infection with *H. contortus* (Preston and Allonby, 1978, 1979; Bain, *et. al.* 1993; Baker, *et. al*, 1993). Baker, *et. al.*, (1993) reported higher lamb mortalities from weaning to 1 year among Dorper compared to the Red Maasai sheep breed in a study located near the coast. They suggested that the lower mortalities in the Red Maasai were due to nematode parasite resistance. Several research projects are continuing on the resistance of this breed and the Small East African goat breed to *Haemonchus contortus* at K.A.R.I. , ILRI and the University of Nairobi.

Herbal medicines and plants are also used especially among the pastoralists. They claim that certain plants eliminate tapeworms (their only evidence of helminthosis) but this claim is yet to be verified and also the efficacy of such plants is yet to be determined.

THE FUNGUS WORKS

Possibilities of Integration of Biological Control into Sustainable Control Programmes in Kenya

Following studies in Denmark, which showed that the administration of D. flagrans, by way of a feed supplement to young sheep, significantly reduced infective larval numbers on pasture (Githigia, et.al.,1997), trial work on biological control is planned for Kenya.

It is proposed to integrate biological control with the strategic anthelmintic control programmes. One proposal would be to use continuous fungal administration during periods of heavy pasture infectivity i.e. during the heavy long rains combined with few, tactical anthelmintic treatments. Fungal dosing for selected periods during dry season and the short rains season, also needs to be evaluated.

The objective is to reduce pasture infectivity and total worm burdens and effectively reduce pasture contamination. This in turn will lead to reduced anthelmintic usage and increase the dosing intervals thus saving money and leading to improved livestock production. Hopefully this may lead to a situation where the livestock industry does not depend entirely on anthelmintics and eases the problem of anthelmintic resistance.

Attempts will be made to incorporate fungal material into feed similar to the use of coccidiostats in poultry feeds, into feed blocks or possibly into slow release capsules.

Activities Before Biological Control Concept can be Utilised in Kenya

1. Isolation and characterisation of the nematode destroying fungi from Kenyan soils and dung pats in order to obtain a suitable candidate for biological control. Crop scientists in Kenya have already done some work on plant nematode destroying fungi.
2. Training and development of the necessary manpower to isolate process and handle the fungi.
3. Provision of the equipment and cultivation facilities for the production of sufficient quantities of fungal material for field testing.

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CONTROL OF GASTROINTESTINAL HELMINTHS IN SMALL RUMINANTS THE MALAYSIAN PERSPECTIVE

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INTRODUCTION

Malaysia has a small ruminant population of 227,800 sheep and 258,220 goats. The Katjang breed of goats is the most common followed by imported breeds such as Jamnapari, Anglo Nubian, Alpine, Saanen, Toggenberg, Australian Feral and the Boer breed. The sheep breeds available here are the local Malin, imported Dorset Horn, Wiltshire, Suffolk, Romney, Commercial Merino/Border Leicester crosses as well as the hair breeds such as the Barbados Black Belly and Santa Ines.

The small ruminant industry in Malaysia is mainly in the hands of small holder farmers with individual flock sizes of between 5-50 heads. The animals are housed in raised floor pens at night and allowed to graze on whatever pasture is available during the day. Usually, 6-8 hours grazing is allowed where the sheep and goats would feed on indigenous grasses under tree crops such as rubber, or oil palm, as well as on roadsides. With the high local demand for mutton and goats milk, the price of these commodities have increased thereby encouraging large scale small ruminant operations being integrated with plantation crops. In oil palm or rubber plantations, the natural 'pasture' of *Asystacia* sp. which is a nutritious indigenous grass would make commercial small ruminant farming viable.

However, the productivity of small ruminants is limited by several factors, the most important being diseases. The major disease problems encountered are pneumonic pasteurellosis, endoparsitism, mange, contagious ecthyma, melioidosis and caseous lymphadenitis. In sheep, blue tongue and pink eye have also been reported.

Importance of gastrointestinal nematodes to the small ruminant industry

Over 90% of the endoparasitism cases in small ruminants are due to *Haemonchus contortus* which is a blood sucker found in the abomasum of small ruminants Sani RA & Rajamanickam C. (1990). causing anemia and hypoproteinaemia. Other common nematodes are *Trichostrongylus* and *Oesophagostomum* spp. which damage the intestinal epithelium thus causing protein leakage into the gut. Hence, parasitic gastroenteritis produces a negative nitrogen balance in the animal which is related to innappetance. The post mortem findings in severe cases of parasitic gastroenteritis includes an emaciated carcass, pale muscles and viscera, watery blood, oedema in the lungs, cavities and subcutaneous tissue particularly the neck region and generalised serous atrophy (Sani, et.al.,1986).

Control options for helminthiasis in small ruminants

Chemotherapy has been the most common method of controlling helminthiasis in small ruminants in Malaysia for the past two decades. Benzimidazoles, levamisole, closantel and ivermectin have been in use routinely in smallholder systems as well as in commercial farms often with 6-12 treatments given per year (Chandrawathani P, et.al. 1994). This has led to anthelmintic resistance in many farms nationwide according to the survey carried out by VRI and UPM (Dorny, P. et al., 1994). It was found that 34% of the goat farms showed benzimidazole resistance in *H.contortus* while 2 out of 10 farms were found to have levamisole resistant *H. contortus*. Closantel, being successfully used in the 'wormkill' programme in Australia has been intensively used by smallholders. Studies show that closantel (Chandrawathani, P. et.al. 1994) and ivermectin (Chandrawathani, P. and Waller, P.J. 1997) have an efficacy of 100% in sheep. The faecal egg count reduction test is being conducted routinely on all institutional farms, where drug resistance is most rampant, and at the same time alternative methods for helminth control are being seriously considered.

Parasite Epidemiology and Grazing Management Strategies

As Malaysia experiences a hot, wet tropical climate all the year, the development and survival of the infective larvae on pasture has been determined to facilitate the development of sustainable control programmes. The ACIAR collaborative project PN9132 on the epidemiology of trichostrongyles in small ruminants under rubber and oil palms has revealed valuable epidemiological information. Emergence of infective larvae (L3) on pasture occurs within 4-5 days. These larvae can survive up to 4-5 weeks under our tropical conditions after which only 20% of the larvae would still be surviving (3). This finding has created the possibility of practising pasture management, or rotational grazing, to control helminth infections in grazing animals by minimising the numbers of infective larvae on pasture. With rotational grazing, the need for anthelmintic therapy is drastically reduced (up to 80%) thus overcoming the problem of drug resistance as well as reducing drenching costs. At present rotational grazing is practised in large institutional farms with a large grazing area which can be subdivided and grazed for 3-4 day leaving each paddock spelled for at least 30 days. However, the limitation at this method is that smallholders are not able to practise the system due to limited grazing areas or high cost of fencing.

Epidemiological data also shows that helminth infections in sheep are most evident in the weaners and periparturient ewes (Sam-Mohan, A. 1996) where highest faecal egg counts were observed. This would include animals below the age of 8 months, after which time the animals show some degree of immunity to infections. A marked periparturient rise in egg shedding was observed in ewes 6 weeks before and after parturition. These observations are useful in designing control strategies especially treatment regimes for small ruminants. Thus, it is recommended that sheep under 8 months of age and ewes about to lamb, are dewormed although rotational grazing or other sustainable methods of worm control should be practised where possible to reduce pasture contamination.

Nutritional Supplementation

With the current concerns about indiscriminate drug use, drug resistance and increasingly high cost of newer and more effective drugs, the introduction of the medicated urea molasses block (MUMB) is a welcome reprieve. These are locally made nutritional blocks which has 0.05% fenbendazole incorporated in it. Trials have shown that sheep consuming at least 60-100 grams of MUMB per day are able to maintain a low faecal egg count and at the same time show improved live weight gains

(Chandrawathani, P. et.al. 1997). With this method, labour and drench costs have been reduced as the blocks are placed in the pens where sheep or goats have access to it at night when they are housed. At the same time, the nutritive value of the blocks gives added immunity against concurrent diseases. This method is effective provided existing worms in the animals are removed by drenching prior to feeding the blocks as the amount of anthelmintic being ingested is very low and would not remove existing worm burdens being effective in controlling the incoming larval stage only. The MUMB technology is relatively new and efforts are under way to produce large quantities of cheap blocks so as to making them available to smallholders and commercial farmers alike.

Studies on Biological Control

As the MUMB technology is in place, it would be only right that the next step in sustainable worm control strategies be directed towards the biological control of nematodes by nematophagous fungi. A survey of this fungi from faecal and soil samples over the past 2 years in Malaysia have shown the presence of Arthrobotrys sp. to be present. About 2000 spores of Arthrobotrys sp was able to reduce the Strongyloides larvae from calf faeces by 90% (Chandrawathani, et.al, 1997). This encouraging information has further prompted us to find ways and means to incorporate fungal spores into the existing urea molasses blocks as a way of delivering it to sheep or other ruminants.

In conclusion, in the Malaysian context, the small ruminant industry is stable at the moment and the productivity would be markedly improved if helminthosis could be more effectively controlled. The various control programmes such as strategic drenching, rotational grazing and the use of MUMB are all being practised at different levels depending on the situation and type of farm.

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PERSPECTIVES IN THE USE OF NEMATOPHAGOUS FUNGI IN THE CONTROL OF GASTRO-INTESTINAL NEMATODES IN THE LIVESTOCK INDUSTRY IN MEXICO

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INTRODUCTION

Mexico possesses an important potential capability in livestock production, due to its extensive grazing areas with suitable characteristics to promote this activity. However, a number of factors limit livestock production in this country. These include, the high costs of supplementary food, the lack of proper management systems, unsuitable grazing regimes, the lack of extension service support and many disease conditions. Parasitic diseases are a very important constraint on the livestock industry in Mexico (Quiroz, 1984; Calderon et.al. 1987). The most economic important parasitic diseases in ruminants have been classified and these include, ticks, blood protozoa, fasciolosis and gastro-intestinal nematodes (P.E.F.G., 1990). Gastroenteritis caused by nematodes in ruminants is considered as a very important problem in animal production systems in Mexico. A large number of species of gastro-intestinal parasites have been identified in cattle and sheep in Mexico. Of which *Haemonchus contortus, Mecistocirrus digitatus, Trichostrongylus sp., Cooperia sp., Ostertagia spp., Oesophagostomum spp., are the most important* (Mejía-García And Orozco de Gortari, 1979; Campos et.al 1990; Hernandez et.al., 1992; Domínguez, et.al. 1993; Sanchez and Quiroz-Romero, 1993; Arevalo et.al 1995. Anaemia decreased weight gain, decreased milk and wool production and susceptibility to other diseases are some of the consequences attributed to these parasites (Quiroz, 1984; Gibbs, 1992; Hayat et.al 1996).

ECONOMIC IMPORTANCE OF GASTRO-INTESTINAL NEMATODES OF LIVESTOCK

There is no information available on the economic impact of the gastro-intestinal nematodes in the livestock industry in Mexico.

However, production costs are affected not only by the reduced weight gain and the subsequent reduced economic return of animal production, but also by the relatively high cost of treatment for parasites.

Current Control

In Mexico, the most common method of control of gastro-intestinal nematodes in cattle and sheep is by anthelmintic treatment (Campos, 1991; Campos et al 1992). This method helps to reduce the parasite burden of animals, however the these products is a considered expensive by many resource limited farmers. The cost of purchasing anthelmintics for livestock in Mexico in 1995 and 1996, has been estimated to US$56 million Mexican pesos (Fragoso, H., 1997 -unpublished-), approximately US$7 million. The environmental impact (Wratten and Forbes, 1996) and the imminent presence of anthelmintic resistance are other disadvantages of their use (Campos et al, 1992). Factors such as the lack of information on epidemiological aspects of gastro-intestinal parasitic nematodosis and the lack of suitable programmes of control of these diseases, especially designed for specific production systems are other problems which may also lead to inappropriate control of parasitic infections.

Studies on biological control of nematode parasites in Mexico

In the National Institute for Research in Veterinary Parasitology, (CENID-Parasitologia Veterinaria), Mexico, a research project to explore the potential use of nematophagous fungi in the control of ruminant parasitic nematodes, has been ongoing since 1989. At the beginning of this project, some in vitro assays were performed and a very high activity of nematophagous fungi was shown against different ruminant parasitic nematodes (Mendoza, 1991; Lozano, 1991; Cruz, 1992; Mendoza et al. 1992; Mendoza et.al, 1994a,b). At the same time, attempts to pass conidia of nematophagous fungi conidia through the gastro-intestinal tract of sheep and recovering them from faecal material, were unsuccessful (Mendoza, 1990). Protection of conidia using different enteric coats against the conditions in the out was considered. Different isolates were screened to test their ability to survive through the gastro-intestinal tract of the animals, and some isolates of Duddingtonia flagrans and Arthrobotrys species from sheep and calf faeces were obtained, some of which displayed a high resistance and remained alive after passing through the digestive tract of animals (Llerandi-Juarez and Mendoza, 1997). In a further experiment a group of sheep infected with Hamonchus contortus was treated orally with an aqueous suspension of conidia which significantly reduced the percentage of larvae in faeces compared with a control group without fungi (Mendoza et.al. 1996).

Evaluation of the use of different mixtures of nematophagous fungi with high activity against nematodes and also different fungal biopreparations to be administered in animals as a method of control of ruminant parasitic nematodes are currently underway.

Experiments which include the evaluation of the production of extracelullar enzymes by nematophagous fungi under different conditions and the influence of nematode cuticle on the nematode-predatory behaviors are also being undertaken.

Although, there are just a few records of biological control of these parasites using nematophagous fungi in Mexico, results are highly promising and in future this method of control could potentially be an excellent alternative to reduce the use of chemicals for the control of ruminant parasitic nematodes.

In further trials, an evaluation of different strategic measures using the nematophagous fungi under field conditions will be undertaken in the most important areas of livestock production in Mexico.

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GASTROINTESTINAL NEMATODES OF RUMINANTS IN PARAGUAY AND THE POSSIBILITIES OF USING PREDACIOUS FUNGI

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INTRODUCTION

Paraguay is a landlocked country located in the centre of South America, covering an area of 406,752 Km^2 and extending between 54° 19' and 62° west, and 19° and 27° 30' south. It is bordered by Brazil, Bolivia and Argentina. The country is divided into two main regions by the Paraguay river, namely the Occidental and Oriental regions.

The western part of the country (Occidental region) accounts for 247,000 km^2 and comprises the Chaco plain which extends to Bolivia and Argentina and in total constitutes one of the largest ecological zones in Latin America covering 1,125,000 km^2 in total. The eastern part (Oriental region) constitutes 39 percent of the total landmass of Paraguay and consists of plains and gentle hills. The highest lands are in the east but even there the hills rarely exceed 500 m in altitude and the highest point in the country is only 850 m. above sea level.

The climate is subtropical with summer rains and dry winters. The average temperature range from 26° C to 37°C during the summer months between October and March. In winter, i.e. from May to July, they range from 16°C to 25°C. Extremes of heat and cold are greatest in the Chaco.

Rainfall is highest in Eastern Paraguay where annual precipitation varies around 1700 mm in upper Paraná to 1200 mm on the lowland of the Paraguay river. Although the heaviest rainfall tend to occur in the summer there is fairly even distribution throughout the year. Seasonal rainfall variation is greater in the Chaco where most of the annual precipitation of 400 to 800 mm falls between October and April and the seasonal effects are greater. Higher rates of evaporation and impermeability of alluvial soils in the Chaco result in periodic droughts, occasional floods and shorter vegetation growing periods than in the east.

LIVESTOCK AND PARASITE PROBLEMS

The sheep population of Paraguay is by South American standards small. The total population of approximately 370,000 is concentrated in the Oriental Region (70 per cent) where the bulk of the 9.0 million cattle in Paraguay are also found. Typically, sheep are maintained in small flocks, rarely more than 200 animals, but often on large farms which carry substantial numbers of cattle. Sheep flocks are commonly confined to small areas where they are maintained at very high stocking rates. Some sheep-cattle mixed grazing occurs in certain districts. British breeds such as Hampshire, Corriedale and Romney Marsh are preferred. Essentially then, the sheep industry is based on European sheep breeds, not renowned for high levels of innate resistance to parasites, in regions of high temperature and at high stocking rates. Many specifically designated sheep pastures are regularly inundated following heavy rains or flooding that favour the build - up of heavy worm infection.

Massive helminth problems are endemic with all important species well represented. The major pathogen is *H. contortus*, but *Trichostrongylus colubriformis* and *Cooperia spp*. in particular, is also a very serious problem.

Parasite Control and Anthelmintic Resistance

Establishment of adequate parasite control programmes have been impeded by a number of factors including insufficient knowledge of the epidemiology of the parasites, the poor to moderate efficacy of the available antihelmintics and the reluctance of both veterinarians and farmers to deviate from the convenience of husbandry-based parasite control programmes. These programmes relied heavily on treating sheep when they were available, or treating only those animals that exhibited clinical signs of parasitism , in many instances on a monthly bases or even every three weeks. (40% of the farms studied).

Attempts to minimize the reliance on anthelmintics for control of parasites have concentrated on developing control programmes for the more susceptible age groups of sheep. Farmers, however, have still been reluctant to adopt the concept of prevention. (i.e. treating animals before they developed clinical parasitism.

Studies carried out from March/July 1994 to determine the prevalence of anthelmintic resistance in the Region with the technical and financial support of FAO. showed that the level of resistance exceeded 50% to all anthelmintics independent at formulations. Resistance to the benzimidazole group was 73%, levamisole group 68%, ivermectin injectable 47%, and oral 73%. There was clear evidence that the three main parasites of the genera *Haemonchus contortus, Ostertagia* and *Trichostrongylus* had a high level of resistance to all three broad spectrum anthelmintic groups.

Clearly the level of anthelmintic resistance in nematode parasites of sheep in Paraguay has reached an alarming level. Farmers and the government are aware of the actual situation which was found in the sheep industry and quickly extending to the goats population. They are eager to find methods to alleviate the problem considering that the sheep/goat production will be impossible in the near future if these circumstances continue. Taking into consideration that there is a governmental program of diversification emphasizing the small ruminant production in an attempt to alleviate the social hardship of the small farmers, it is important that remedial action is taken immediately.

Future Developments

There is an urgent need for the development of other non chemical methods of parasite control. However, in order to undertake any action, it is imperative to get the willingness and cooperation of the Governmental Institutions (Extension Services of the Ministry of Agriculture and Livestock and the different Veterinary Schools) and farmers associations. This is essential in order to implement any activities to promote any control method proposed.

It is well known that biological control will be one of the best chances for controlling gastrointestinal parasites in small ruminants due to the ecological advantages of its application.

In order to implement it, it will be necessary to train the laboratory and field staff in the development of this new methodology.

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CONTROL OF GASTRO-INTESTINAL NEMATODES IN THE FARMING SYSTEMS OF URUGUAY

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INTRODUCTION

Uruguay is situated between the 30° and 35° southern latitudes and 50 and 60 western longitudes, with maritime influence of the River Plate to the south and the Atlantic Ocean to the east. It has a temperate climate with an average rainfall ranging between 1000-1400 mm equally distributed throughout the year.

The prevailing animal production system is based on mixed cattle and sheep grazing natural pastures. The country has the largest sheep stock of Latin America (20 million heads) and is the second tops wool (washed wool) producing country of the world.

Epidemiological studies of nematode parasites of sheep, have shown that the most common species of gastro-intestinal nematodes are represented, including *Haemonchus contortus*, which is the most important parasite except in winter when *Trichostrongylus colubriformis* and to a lesser extend *T.axei* and *Ostertagia circumcincta* populations increase and take on importance.(Nari and Cardozo,1987).

ECONOMIC IMPACT

Recent studies have shown that the potential losses caused by these nematodes in one year old sheep kept in a "non parasite control" scenario amounted to 50% mortality, 25% reduced live body weight gain (LBW) and a reduction of greasy fleece weight (GFW) of 29%. It was also demonstrated that the animals in poor body condition, as a result of the reduced weight gain, did not have full compensatory growth at any time during their production lives. The most affected wool components were the staple length ($p<0.01$) and diameter ($p< 0.05$). No significant influence on the strength of the wool was detected. (Castells et al,1993; Castells and Nari, 1996).

These studies have clearly demonstrated to the farmer's associations the impact of helminth infections and in general there is a high awareness of the problem. The Uruguayan Wool Secretariat (UWS) has estimated the annual production losses caused by parasites in the national sheep flock to be in the order of 18.335.000 kg of greasy wool, which represents a potential annual loss of about US$41,800,000 (SUL, 1996).

ANTHELMINTIC RESISTANCE

The extensive use of anthelmintics for the control of gastro intestinal nematodes of sheep during the last 15-20 years have resulted in widespread anthelmintic resistance. In order to obtain an estimate of the prevalence of anthelmintic resistance throughout all the sheep raising regions of the country, a survey was conducted in 1995 on 252 randomly selected farms. The study only involved farms with more than 600 sheep, which represents 80% of the total sheep population. Three anthelmintic groups were evaluated, namely benzimidazoles, levamisole and avermectins. The results showed that 92.5% farms had some degree of anthelmintic resistance although stock owners with resistance problems often ignore the situation.

Resistance to benzimidazoles was recorded in 86.0 % of the sheep flocks tested, 71% had resistance to levamisole and 1.2 % to avermectins. More than 80 % of surveyed farms had *Trichostrongylus* populations resistant to both benzimidazoles and levamisole. For *Haemonchus,* resistance was confirmed for all three groups of wide spectrum anthelmintics although the resistance to ivermectin is still limited.

A major cause of anthelmintic resistance is the frequent use of treatment and this study showed that almost 50% of the sheep farmers treat their lambs more than 7 times per year with the majority of drenches given during the post-weaning period. However, frequent treatments are not only restricted to the weaned lambs as more than 40 % of farmers drench their ewes a similar number of times. (Nari et al, 1996).

Thus the majority of Uruguayan farms have gastrointestinal nematode populations with anthelmintic resistance and it is, therefore, a realistic end-point prediction, that alternative helminth control will become a major element in ensuring the sustainability of sheep production enterprises in the future.(Nari and Salles,1997).

Diagnosis

The principle of sustainability of nematode control is therefore related to preservation of anthelmintic efficacy through integrated pest management with far less but strategically timed, use of anthelmintics in combination with grazing management and other control strategies.

Considering that only 7.5 % of the tested farms do not have anthelmintic resistance, the starting-point has to be the determination of the problem on a "one farm one problem" basis by using already developed standardized methods for the diagnosis of resistance.

After the diagnosis is accomplished scientists and/or extension service personnel should advise live stock owners according to the epidemiological situation and the practical possibilities of control in each specific farm.

Control Strategies

Promoting and implementing new control strategies are often met with skepticism and the acceptance of perceived complex and "impractical" measures is generally slow However a range of alternative control strategies have been developed based on epidemiological data, more appropriate use of anthelmintics and grazing management.

a) Using the existing anthelmintic groups

Considering the findings of the diagnostic survey these are some of the options available with regard to continuing use of existing groups of anthelmintics

- The annual rotation of wide- and medium spectrum anthelmintic groups. On farms where two or more of the main groups are still working, drugs from each group should be rotated on an annual basis.

- Re-introduction of anthelmintics which were 'forgotten' when new broad spectrum anthelmintics were marketed. After more than 25 years, naphthalophos, was reintroduced in Uruguay at the dose rate of 50 mg/kg which increase its activity against *T. colubriformis*. It is especially recommended on farms where only one wide spectrum anthelmintic group is still effective.(Lorenzelli et al,1996).

- The use of combinations of wide spectrum benzimidazole + levamisole groups. One third of sheep flocks still have nematode populations where the combination usually achieves more than 95.0 % of efficacy. (Nari, et al 1996).

- The use of specific drugs such as the narrow spectrum closantel, for the exclusive control of *H. Contortus* and the other blood sucking parasites.

- Expand the knowledge base of the pharmacokinetics of the anthelmintics. For example, the effectiveness of oral formulations of benzimidazole, and the macrocyclic lactones markedly improves in sheep if they are removed from pasture 12-24 hours before treatment. This of course is only of limited value if resistance is very strong (E. Lorenzelli, 1995).

b) Grazing Management

Grazing management is currently a neglected component of the parasite control strategies in Uruguay. However recent work has demonstrated great potential for parasite control through managing pasture resources either in isolation, or together with reduced use of anthelmintics.

The growth and production potential of the native pastures in Uruguay varies considerably according to the season. During winter, the quality (protein + energy) and quantity of pasture are very low and farms are often overstocked as this usually coincides with the lambing season. In contrast, there is an almost uncontrolled pasture growth during late spring and summer that sometimes precludes the normal grazing pattern of sheep.

The following are the recommendations given to farmers for coping with these extreme differences in pasture production:

- The use of mixed grazing of cattle and sheep during the highest pasture productive periods of the year (namely late spring-summer and early autumn).

- A sheep-rotation grazing scheme for the rest of the year. This programme is based on the demonstrated benefits of resting pastures which resulted in a dramatic reduction of the parasite burden when 90 days elapsed before the lambs returned to the same pasture.(D. Castells and A. Nari, unpub. data,1997).

- The creation of safe pastures for weaner lambs by using alternate grazing between more than two year old cattle and the weaned lambs. The potential of cattle cycling *H. contortus*, was tested by artificially challenging a group of adult cattle with a field strain of sheep *H. Contortus*. No differences between the control and artificially infected groups of cattle could be detected.(A. Nari, 1987). In this grazing system it is not advisable to keep the lambs longer than 2.5-3 months on the same pastures because of their selective grazing (A. Nari and Cardozo,1987).

- When flock rotation in winter is not possible, due to extensive condition of farming and lack of fencing, the use of alternate grazing between cattle and pregnant ewes is another valid strategy. Excellent parasitological results were obtained in ewes after 2.5 and 4.5 months of alternate grazing. (Castells and Nari, 1996).

INTEGRATED CONTROL

A specific modified "Wormkill" Programme, based on the epidemiological data base and the integration of strategic anthelmintic treatments and grazing management has been recently tested on 11.000 sheep of all ages over a period of 26 months. The programme was developed on the basis of a previous diagnosis of anthelmintic resistance in each farm, the knowledge of the local epidemiology, low frequency of drug administration, slow rotation of wide to medium spectrum groups of anthelmintic still being effective combined with closantel and punctual grazing management measures (safe pastures for lambs). It was demonstrated that it is possible to overcome the parasitism of *H.contortus* and *T.colubriformis* and other less important species of nematodes in all age categories of sheep. The results were especially outstanding on one of the farms which previously had a record of using 10 drenches per year, in addition to frequent clinical parasitism being observed. (A. Nari et al 1997).

FUTURE TECHNOLOGY TRANSFER

These sustainable integrated parasite control programmes based on local knowledge developed for Uruguayan conditions of farming should be strongly promoted within a realistic frame. This would secure the necessary time for applied research for the development of more sophisticated non-chemical technology such as genetic resistance, biological control and vaccines.

a) Genetic resistance

Exploiting genetic resistance to diseases in general and to nematodes in particular should without doubt be one of the targets for Uruguayan research institutions. It appears that there is a potential for utilizing both between breed and within breed differences in resistance/susceptibility to nematode parasites. Encouraging results have been achieved in this respect by the UWS and different farmer's associations (R.Cardellino, pers. com.,1997).

b) Biological control

Fungi with a predacious activity against nematodes have shown promising results. It has been demonstrated under experimental conditions that a daily supplement of barley grains supporting the growth of the fungi can lower the larval contamination of pastures and in turn considerably reduce parasitism and increase productivity in grazing calves and sheep. In the context of improved nutrition of sheep, blocks for nutritional and mineral supplements, and the inclusion of the fungi have been tested and could prove to be an important control option in certain management systems. These formulations should be tested to determine if they have a real impact under the extensive sheep farming conditions of Uruguay.

CONCLUSIONS AND RECOMMENDATIONS

The sheep industry is one of the most important sources of income for the Uruguayan economy. The entire sheep population of the country is at present experiencing direct or indirect losses due to gastrointestinal nematodes. Any attemps to increase wool and meat production must be supported through sustainable helminth control programs.

Since a limited survey was carried out in 1992 in one of the more problematic areas of the country which recorded 25% and 60% of levamisole and benzimidazole resistance the problem has rapidly increased up to figures of 71% and 86% respectively.(A. Nari et al 1992; A. Nari et al 1996).

Within the framework of the new epidemiological situation, it will be necessary to remember:

- A sustainable helminth control of sheep livestock will focus on a "one farm-one problem approach" by using a standardized methods for the diagnosis of resistance. To achieve this goal, it is necessary to have a "critical mass" of well trained field veterinarians capable, not only of doing the diagnosis, but also to competently managing a range of technical, integrated control options available in the country. This programme of prevention and control should be promoted not only by the veterinary practitioner but also by other farmers, animal scientists and extension personnel. Courses of continuous education for professionals should be made available.

- Technology transfer for a sustainable helminth control to farmers should be formulated within two well defined levels. One, directed toward key farmers associations by promoting their interest in developing jointly applied research in different production areas of the country. A good example of this philosophy was the joint pilot trial developed for the application of a modified "Wormkill" programme in the northern region of Uruguay. A second but interacting level, should be the massive transfer to farmers of the already available knowledge and technology. The extension personnel of UWS should promote schemes of integrated control by using local information which do not solely rely on use of anthelmintics.

- Research institutions should support applied reset without loosing the link with farmer associations but doing and oriented basic research if it is of importance.

- Due to the fact that the problem of anthelmintic resistance has been exacerbated by the common use of generic products and drugs of dubious quality the official authority (DILAVE "Miguel C. Rubino") should have the proper financial support for a more efficient quality control and monitoring of commercial products.

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Gastro-intestinal nematode parasitism is one of the most important disease constraints to small ruminant production in the tropics and subtropics. Control of gastro-intestinal nematodes, particularly *Haemonchus contortus* and *Trichostrongylus* species, is a prerequisite for profitable small ruminant production. Strategies for control have until now relied almost entirely on the use of anthelmintics. The frequent use of the drugs, often mismanaged, has led to widespread resistance of the parasites to one or more of the major groups of anthelmintics. There is therefore an urgent need to develop alternative, sustainable strategies. Such strategies include grazing management, breeding for resistance or resilience, better utilization of existing drugs through the understanding of pharmacokinetics and the use of predacious fungi for biological control of the nematode parasite larvae on pasture. To further knowledge on the last of these approaches, FAO and the Danish Centre for Experimental Parasitology organized the Workshop on Biological Control of Gastro-intestinal Nematodes of Ruminants Using Predacious Fungi, held from 5 to 12 October 1997 at the Veterinary Research Institute in Ipoh, Malaysia. Fourteen participants from ten countries received theoretical and practical training in the isolation, identification and cultivation of predacious fungi which they will be able to use for biological control of parasitic nematodes of ruminants.

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