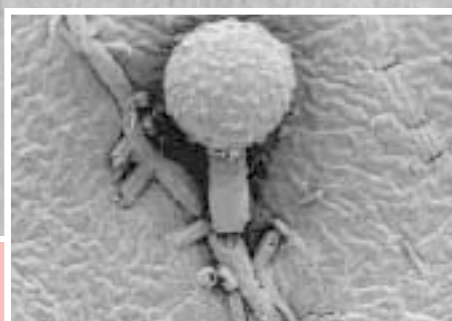


Biological control of nematode parasites of small ruminants in Asia



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FOREWORD

As incomes rise and population grows, people in the developing world are diversifying their diets to include a variety of meats, eggs and dairy products. In developing countries the demand for meat has grown rapidly over the past 20 years at a rate of 5.6 percent a year. The trend towards the consumption of livestock products is likely to continue and currently it is estimated that some 360 million cattle, 560 million sheep and goats and 190 million more pigs will be necessary to satisfy the nutritional world demand. This growing demand for livestock products offers an opportunity to the 675 million rural poor who depend on livestock to improve their livelihoods. It is likely that the greatest beneficiaries will be the small and medium scale market oriented farmers, but only if a suitable environment is created, including access to credit, development of infrastructures including animal production and health services. Farmers need access to information on disease control and livestock management to help decide where to invest their resources in order to increase production.

Globally, parasitic and other endemic diseases continue to be a major constraint on profitable livestock production. They are rarely associated with high mortality and easily identifiable clinical signs, usually characterised by lower outputs of animal products, by-products, manure and traction, which all contribute to production and productivity losses. Ready access to anthelmintics and the ease by which they could be applied, combined with improved diagnostic tests and the immense progress made in the knowledge of the epidemiology of parasites of ruminants led to a period of relative success in the control of gastrointestinal nematodes, particularly in more commercial livestock production systems. However, the false assumption that parasite control is easily accomplished by the use of chemical means alone have led to the development of anthelmintic resistance which complicates the efficiency of current and future parasite control programmes.

In view of the importance of effective helminth control in ruminant production systems throughout the world, FAO promoted partnerships with research institutions that systematically investigated and tested other alternatives of control. Now it is clear that **Biological Control**, as a component of Integrated Parasite Control, will help to move away from reliance on anthelmintic treatment to more sustainable forms of gastrointestinal nematode control.

These proceedings are a compilation of working papers edited by Dr Peter Waller, Swedish Agricultural University, Sweden and Dr Michael Larsen, Royal Veterinary and Agricultural University, Denmark for the FAO Regional Workshop on "Biological Control of Nematodes Parasites of Small Ruminants in Asia" which was held 16-20 September 2002 at the Veterinary Research Institute, Ipoh, Malaysia. The document also includes a summary of the results of the FAO-TCP Project 0065(T) carried out by the Veterinary Research Institute at Ipoh, Malaysia.

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FAO PERSPECTIVES AND GOALS

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INTRODUCTION

Globally parasitic diseases continue to be a major constraint for poor developing countries. They are rarely associated with high mortality and their effects are usually characterised by lower outputs of animal products, by-products, manure and traction all contributing to assure food security. The development of increasingly effective drugs capable of controlling a large number of parasite species has, apparently, been one of the most impressive technological achievements of the twentieth century. The systematic use of relatively inexpensive parasiticide agents that are effective and easy to apply has made it possible to control pests which affect wide range of production systems. The continuous development of compounds by the pharmaceutical industry has been as exciting as it has been disturbing. Stimulating, because of the whole range of possibilities for preventive and/or curative application to control economically important pests, but at the same time worrying, because of the possibilities of developing parasite resistance, creating ecological imbalances and leaving residues in meat, and milk (food safety). Indeed, the development of resistance is intricately linked to increased treatment frequency. This enhances the possibility of residues, which could turn into a non-tariff barrier to trade between countries. Another situation preventing trade, both within and between countries, is the possibility of introducing resistant parasites through the transfer or import of live animals. This is a widely recognised fact in the case of arthropods and is becoming more frequent in helminths.

The projections of the International Food Policy Research Institute (IFPRI) using the IMPACT (International Model for Policy Analysis of Agricultural Consumption) are that the consumption of meat and milk in developing countries will grow by about 3% per year between now and 2020 (Delgado et al. 1999). It is likely that this will improve the livelihood of small and medium scale market oriented farmers but only if an enabling environment is created including access to credit, development of infrastructure and animal production and health services. Farmers need access to information regarding disease control and livestock management supporting their ability to decide where to invest their resources to increase production and productivity. In this decade, almost one century after the first reports of arthropod resistance to pesticides for agricultural use, we are seeing an almost exponential increase in new cases of resistance

in the parasite species affecting agriculture and public health in various geo-climatic areas of the world (OIE/FAO, 1998). This transformation in the genetics of parasite populations has developed within a world context of far-reaching political, social and economic changes, which must be taken into consideration when attempting to implement sustainable systems of livestock production (Nari and Hansen 1994). The scenario for the twenty-first century will be characterised by meat, wool and milk markets that are ever more globalised, competitive and demanding, especially with regard to residues and environmental contamination. Governments and industry will not have the same operational freedom as in the past and it is unlikely that there will even exist a drug to which parasites cannot develop resistance. In 2001 the global market for Animal Health products was of 11,050 million dollars. During the same period the demand for parasiticides represented 28.1 percent of the total sales even though it differs from one geo-economic area to the other depending on production systems and the composition of parasite populations. Cattle and sheep with a 33.3 percent of participation still represent an important portion of the market of animal health products (IFAH 2002).

Within this frame of reference, FAO consideration is given to possible approaches to be taken at national, regional and international level in order to control the problem of parasite resistance and prevent it from escalating, as well as to secure the sustainability of animal production systems. The economic importance of the resistance phenomenon is intricately linked with the causal agent's distribution, prevalence, incidence and impact on local production. Unfortunately, much of the available information concerning production and economic losses does not yet cover all parts of poorest countries / systems of the world, and accordingly with the last data available from the last study carried out by OIE/FAO (1998) more than 65 percent of the countries surveyed have not carried out any such studies (Nari and Hansen 1994).

GENERAL STRATEGY

Sustainable disease control will not be possible unless there is sustainable national production systems. It therefore appears imperative to require that developing countries have a multidisciplinary approach to manage the parasite problem within the context of their own productive system. The need to involve all of the parties, Governments, the pharmaceutical industry and private and international organizations - in developing a sustainable and economically viable programme to combat parasitic diseases in general, and resistance in particular, has become ever more crucial. Total dependence on a single method of control has proven to be non-sustainable and non-cost effective in the long term. In terms of control of parasite resistance, Integrated Parasite Control (IPC) effectively combines several means of control as a way to destabilise those parasite populations with the largest proportion of individuals that are genetically resistant to parasiticides. For this reason, IPC is generally associated with a reduction in the frequency of treatments since, in a number of studies, a strong association

has been observed between treatment frequency and the development of resistance. Nevertheless, the reverse is not necessarily true, since, at least for gastrointestinal nematodes, the selection pressure exercised by the treatment will depend on the potential for dilution of the refugia populations (van Wyk 2002). So, when implementing an IPC programme, it is not sufficient merely to use fewer treatments; instead, the treatments must be carried out in accordance with seasons/times/animals, which allow greater dilution of parasite populations combined with other means of control. The implementation of an IPC programme involves important elements that are sometimes difficult to achieve in developing countries. These include the availability of results from applied research; a change of policy to foster the application of methods that are less dependent on parasiticides; and the participation of the producer and his veterinary advisor in training programmes.

CURRENT FAO APPROACH

A working group was established in 1997, called the «Working Group on Parasite Resistance» (WGPR). This is a panel of experts, which advises FAO on strategies for IPC and resistance management. The WGPR gathers, organizes and analyses information on the epidemiology, diagnosis and control of parasites and management of resistance to parasites, assisting FAO in creating a “logistical framework” for the application of the available knowledge and by the preparation of guidelines for its diagnosis and control. The WGPR works in constant collaboration with the pharmaceutical industry by means of a FAO/Industry Contact Group. Industry is represented by the Veterinary Parasite Resistance Group (VPRG). The VPRG is a specialised consultative group whose mandate is to advise industry and non-industrial organizations on the implications and consequences of resistance to parasites, and on monitoring and control strategies. The VPRG currently includes ten of the world’s leading companies, which conduct research and development on parasiticide agents. As a follow-up to the WGRP’s recommendations, FAO has promoted and financed the creation of two Regional Reference Laboratories (RRL) on resistance in ecto- and endoparasites diagnosis and control that will be operational by around end-2002. During the first phase, the RRL located in Mexico and Uruguay have the task of developing the region’s technical capability for diagnosing and monitoring parasite resistance to drugs. The activities of the Centres is disseminated through the corresponding Regional Networks coordinated by INTA (Argentina), CORPOICA (Colombia) and University of Pretoria (South Africa) which will also provide timely information on training courses, seminars and working meetings and the availability of duly harmonised and standardised protocols. In short, the aim of all of these measures is to provide the required conditions and information for the development of sustainable IPC and the appropriate management of resistance to parasites by using the current knowledge and the available data originated by FAO Technical Cooperation Projects in Member Countries. (TCP/ BRA/6713; TCP/ PAR/8821; TCP/ URU/ 8921; TCP/ KEN/ 8822; TCP/SAF/8821,TCP/IRA/8923; TCP/

MAL/0065) and/or Agreements with partner research institutions ((i.e. ILRI, DCEP, CSIRO) and / or experts.

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QUESTION AND ANSWER SESSION

Q: Dr P.K.Sanyal

- i) How much feedback was received from Governments of different countries about their helminth problems?
- ii) Overall, how do you perceive integrated worm control to be viewed from a global viewpoint - now and in the future?

A: Dr A.Nari

A significant number of countries still have no idea that they have problems of anthelmintic resistance. For example, it is usual that countries export sheep with resistant parasites without realizing the consequences. Globalization and transport to more distant markets has introduced greater risk for the spread of anthelmintic resistant parasites. From the many thousand requests for assistance (TCP) that FAO receives each year, not many are related specifically to anthelmintic resistance.

Integrated Parasite Control (IPC) as a concept is applicable globally but it needs to be validated under field conditions at the country /regional level. IPC cannot be extrapolated to areas /regions with different animal husbandry and parasite epidemiology. More regional coordination is needed between international organizations to avoid overlapping activities (not only in research) in countries, at the field level. A more synergistic and complementary effort is needed in priority continents like Africa and Asia.

GLOBAL PERSPECTIVES ON NEMATODE PARASITE CONTROL IN RUMINANT LIVESTOCK: THE NEED TO ADOPT ALTERNATIVES TO CHEMOTHERAPY, WITH EMPHASIS ON BIOLOGICAL CONTROL

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INTRODUCTION

Despite the remarkable achievements in the discovery and development of anthelmintic drugs, with ever - increasing levels of potency and ever - increasing spectra of activity, nematode parasitic disease remains one of the greatest limiting factors to successful, sustainable ruminant livestock production, worldwide (Perry and Randolph, 1999). It is beyond the scope of this short review to give a comprehensive account as to the multiplicity of reasons behind this, but the short answer is that parasites exhibit remarkable biological plasticity – essentially meaning that they can evolve to counter any constraint (chemical or otherwise) that is used against them.

In a balanced ecological system both host and parasite populations are firmly controlled by a complex array of interacting factors. However domestication of livestock has tipped the balance in favour of the parasites. This is because it is almost always associated with:

- Restriction of livestock movement
- Increasing stocking rate
- Increasing the proportion of susceptibles in the herd / flock
- Increasing productivity demands on the animals

Domestication of livestock has been with us since pre-biblical times. However the global threats imposed by nematode parasites on ruminant livestock production never have been so great as at the present point in time.

IMPERATIVES FOR CHANGE IN NEMATODE PARASITE CONTROL

1. Evolution of Anthelmintic Resistance

Since the first reports of resistance to the broad spectrum anthelmintics were made some four decades ago, this phenomenon has changed from being considered merely as a parasitological curiosity to a state of industry crisis in certain livestock sectors. This extreme situation exists with the small ruminant industries of certain countries in the tropical/sub-tropical regions of the world where resistance to the entire broad spectrum anthelmintic arsenal now occurs.

It appears that because of the very high costs and risks associated with taking a new active drug down the development track to marketing, that the pharmaceutical industry has, in general, turned away from this activity. By implication, the international small ruminant industry is too small for these companies to make the necessary investment. This begs the question: What is the fate of the sheep (and goat) industries in those parts of the world where resistance is rampant and immediate ameliorative parasite control options are required?

Resistance in cattle nematode appears to be spreading, but the reports are localised isolations. Most concern surrounds ivermectin resistance that has been reported in *Cooperia* spp. The macrocyclic lactone anthelmintics are so widely used for parasite control in cattle that the treatment failure because of resistance would be serious indeed.

2. Consumer Demands and the Organic Farming Movement

In recent years there has been an increasing demand by consumers that agricultural products should be “clean and green”. Impetus for this has been the contamination of human food products with substances that had tragic adverse embryogenic effects, and the development of super-resistant human microbial pathogens. The threat of adverse effects on the environment on the use of any chemicals in agricultural production has also driven this agenda.

Countries in Europe lead the world in the move towards organically produced livestock products. For example in Sweden, there is an active government policy to foster organic farming and the country is aiming to have 20% of its agricultural commodities organically produced by 2005. Legislation now proclaims that for organic farming, the prophylactic use of drugs (including of course, anthelmintics) is prohibited. As a consequence, new and serious animal welfare issues are starting to emerge throughout Europe, that are caused by distress suffered by animals due to uncontrolled parasite infections.

Therefore these two quite disparate issues, namely anthelmintic resistance and organic farming, have brought the matter of maintaining effective parasite control to the forefront of grazing livestock management enterprises throughout the world.

THE RUMINANT LIVESTOCK INDUSTRIES

To put anthelmintic resistance and organic farming into perspective, one must consider these matters in relation to the need, or the applicability, of adopting alternative nematode parasite control in ruminant livestock, not only for individual countries where ruminant livestock are raised, but also globally. As a very broad generalisation, livestock are maintained under the following categories:

A. EXTENSIVE, NOMADIC LIVESTOCK INDUSTRIES

The vast tracts of marginal agricultural land ranging from rugged mountainous country and deserts to natural grasslands which encompass Asia, the former USSR and the Sahel region of Africa account for more than 700 million small ruminants. Production systems are largely based on traditional transhumance activities and shepherding small flocks, which graze on different pastures with each season. Nematode parasitism is regarded as causing important losses in production in these regions (Anon. 1992), but this is exacerbated by severe and often lengthy seasonal shortages in nutrition. Anthelmintic treatment tends to be *ad hoc* and haphazard, but generally infrequent, so the selection for anthelmintic resistance may be considered to be low. There is likely to be very few instances where any opportunities for alternative parasite practices would be applicable, and even less likelihood of their adoption.

B. BROAD ACRE, PERMANENT GRAZING LIVESTOCK INDUSTRIES

Typical of this form of husbandry are the ruminant livestock industries of Australasia, southern Latin America and Southern Africa. Here livestock are grazed all year round on permanent pasture. Improvements in pasture production by the introduction of superior plant cultivars and the application of fertiliser, led to an expansion, particularly in sheep numbers. This was accompanied by an increasing risk of nematode parasitic disease, but coincided with the revolution in control by the use of chemotherapy. This in turn meant that livestock producers, relied almost exclusively on the use of anthelmintics to control parasites in their flocks. As a result some of the greatest problems of anthelmintic resistance are found in these regions of the world (Waller 1997a; Sangster, 1999). Much work has been done to investigate alternative, non-chemotherapeutic parasite control options (Barger, 1997) including in Australia, the use of *Duddingtonia flagrans*, as a biological control agent against sheep parasites (Faedo et al, 1998; Knox and Faedo, 2001; Waller et al. 2001a; 2001b).

In broad acre, permanent grazing regions of the world, there are seasonal peaks in the availability of infective nematode larvae on pasture. The bulk of these peaks are invariably derived from relatively short periods of time, rather than from a progressive accumulation, of prior contamination by animals grazing on the pastures. In the regions that receive predominately winter rainfall, the seasonal peak in infective larval availability is generally in spring, derived largely from contamination the previous late summer / autumn. For those regions that receive summer rainfall, the larval peak occurs in late summer / autumn, derived from spring / early summer contamination. For those regions that receive rainfall more-or-less evenly distributed throughout the year, then larval numbers on pasture tend not to oscillate from very high to very low levels, but are of more even amplitude throughout the year, with the magnitude of the overall infectivity determined by the amount of rainfall.

B.1 Opportunities For The Use Of Biological Control (Bc) – Broad Acre Permanent Regions

Because of the numerical and economic importance of the sheep industries in these regions of the world, the following broad recommendations apply to this industry – but are equally applicable to goats run under similar conditions. The obvious time to deploy BC is at the time when contamination produces the seasonal peaks in larval numbers.

B.1.1 Sheep Flocks – Ewes

- late summer /early autumn - in the winter rainfall regions.
- spring / early summer - in the summer rainfall regions.

These times generally coincide with lambing and thus the peri-parturient ewe would invariably be excreting a relatively large number of nematode eggs.

- lambing to weaning - in the uniform rainfall regions.

As the timing this event varies in these regions, it would be a good policy to make this a blanket recommendation.

As the objective in all three instances is to reduce the number of infective larvae on pasture at a time when pasture growth is at its maximum and when weaning generally occurs, it would provide the opportunity to keep the young weaned lambs on the lambing pasture. However, although this advice is theoretically sound, there is justifiable concern that it simply is not good management practice to leave weaners on the lambing pastures. Extensive work in Australia has shown that irrespective of the level of parasite control that is practiced (anthelmintic treatment implied) in lambing flocks under conditions of permanent grazing, the most important strategy

is to move lambs off the pastures that they were raised with their dams at weaning when they are approximately 3 months of age (Waller et al. 1987a; 1987b). Under these circumstances the following strategy of using BC could be implemented:

B.1.2 Sheep Flocks – Weaner Lambs

Young, recently weaned sheep are highly susceptible to parasite infection. The objective is therefore to graze these animals on pastures of low infectivity. Unless weaning is accompanied by treatment with a highly effective anthelmintic and then moved to helminthologically clean pastures, weaner sheep are highly likely to contaminate their pastures. Providing BC for a relatively short period of time (2-3 months) immediately after weaning, will help ensure that pastures will remain lightly infected. Provided that nutrition is not limiting, these animals will grow well and gradually acquire a natural immunity to parasite infection on the same pastures during the following months.

B.1.3 Cattle

As a generalisation, it can be said that very little, if not zero, parasite control is practiced for adult beef cattle in these regions. The only class of animals that receive attention in this respect are weaned animals up to approximately 18 months of age. For these animals, there can be considerable anthelmintic medication given – either in the form of sustained release boluses (blanket anthelmintic cover for up to 4 months), or frequent anthelmintic treatment (usually injections). This is particularly so in the humid regions of southern Latin America and to a lesser extent in Australasia. It is in cattle managed under these latter circumstances, that reports of anthelmintic resistance in cattle nematodes have emerged (Vermunt et al. 1995, Fiel et al. 2002; Echevarria et al. 2002).

For the dairy industry, there still remains a debate as to whether anthelmintic treatment improves productivity in adult cows. However to achieve this implies that the animals need to be maintained essentially worm-free. Irrespective of the whether the productivity benefit is achievable, it is unrealistic and arguably counter-productive in the long-term. Essentially this type of management will prevent the animals mounting and maintaining an immune response to parasites, through the acquisition of modest numbers of infective larvae. BC would not claim to emulate such conditions, so the only justification for using BC in dairy cows would be to render pastures that they graze (and contaminate) of very low (zero) infectivity. However the fact that adult dairy cows are large, and they produce voluminous quantities of faeces with invariably negligible nematode egg counts, would mean that the benefit/costs of using BC in dairy cows is highly questionable. Additionally, it has been shown that pastures previously grazed by adult cows, which had received no previous worm treatment, are of very low infectivity,

thus suitable for grazing young stock (Dimander et al. 2002). The matter of using BC in young dairy cattle, is equally applicable as that described above for beef cattle.

B.2 Possible Means Of Deployment Of Bc – Broad Acre, Permanent Regions

B.2.1 Sheep and Goats

The suggested target groups of animals are lactating ewes and/or weaned lambs. The recommendation is for the deployment of the BC agent for a limited time, with a maximum of 3 months in any circumstance. Also, the suggested times of BC coincide with those of short-term targeted supplementary feeding, which are established management practices in these classes of sheep on many farms found in these regions. The recommendation would be to co-administer the fungal spores of *D. flagrans* with the feed supplement. However, because of the extensive nature of sheep production in these regions, a strict adherence to daily supplementary feeding, is often not possible or practical. It must be recognised that feeding less often than each day, will result in an uneven shedding of fungal spores in the dung of animals, thus allowing the opportunity for some nematode eggs to develop to infective larvae, in a faecal environment with sub-optimal concentrations of *D. flagrans*.

Clearly much greater opportunities for BC would exist in these regions if effective methods of *D. flagrans* depot delivery were available, such as the use of supplementary feed blocks, or controlled release devices. Although work has been conducted for such systems of sheep management in the broad acre permanent grazing, no fungal block (Waller et al, 2001a), or fungal controlled release device (Waller et al 2001b) has been developed which provides the effective parasite control for the minimum required time of at least 2 months.

In accordance with general recommendations for BC, it should be used in conjunction with other forms of parasite control, with the aim to maintain effective parasite control for the foreseeable future. Other such adjuncts that are applicable to sheep production in the broad acre, permanent pasture regions of the world include:

- Alternate grazing strategies with other species, or classes of stock.
- Exploiting aftermath grazing where applicable.
- Use of specific aids, such as the Famacha system (Malan and van Wyk 1992).
- Long-term selection, both within and between breeds, for innate resistance to parasites.

B.2.2 Cattle

Substituting BC for blanket anthelmintic treatment is an obvious possibility although this, to my knowledge, has not been trailed. One obvious proviso is that in situations where managers

have found it necessary to treat intensively with anthelmintics to prevent parasite induced losses, and where anthelmintic resistance has emerged, BC alone will almost certainly not work. Substantial changes in management, including other adjuncts to control parasites, will need to be made.

The use of a fungal controlled release device for use in young cattle is certain to have considerable appeal in cattle raising enterprises in these regions.

C. INTENSIVE LIVESTOCK INDUSTRIES

These relate to essentially two quite distinct regions. Firstly the livestock industries of the humid tropics /sub tropics, which are typified by small-holder farmers. Secondly are the grazing ruminant industries of Western Europe; although certain sectors of the sheep industries in France and the United Kingdom would be more akin to the broad acre production systems outlined above. Both share similar characteristics insofar as there are many fewer animals per owner than in the broad acre permanent grazing regions, much greater contact occurs between the owner and his stock, and housing of stock is a feature.

C.1 Possible Means of Deployment of BC – Intensive Regions

The contrast in intensive livestock management systems, is not between the animal species (sheep and cattle) but between geographic regions, that the possible means of deployment of BC should be examined.

C.1.1 Tropics / SubTropical Regions

From the outset, it is important to recognise that in extreme situations of subsistence farming, which are an unfortunate feature in these regions of the world, technological developments are either unaffordable or inappropriate. For example reputable brand anthelmintics are either too expensive, or locally manufactured products are of such inferior quality that they are not used by the stock-owner. As a consequence, massive mortalities of young stock caused by internal parasites are still, tragically, a commonplace phenomenon, particularly in countries of Africa and Asia. At the other extreme where anthelmintics have been used intensively, high levels of multiple resistance have developed to such an extent that total chemotherapeutic failure to control worm parasites of small ruminants is now a reality (Waller, 1997a). Between these two extremes, farmers face the continual battle with attempts to prevent mortalities, to contain clinical disease and reduce production loss due to worms as much as possible. This almost always requires the frequent use of anthelmintics and, as a consequence, they are also moving inexorably down the path to total anthelmintic failure.

The major environmental variable that controls the severity of nematode parasites in the tropics / sub tropics is rainfall, as it is almost always warm enough to facilitate the rapid development of the free-living stages on pasture. In addition, the most pathogenic nematode parasite of small ruminants, *Haemonchus contortus*, is endemic throughout the whole region. Thus in situations where it is virtually wet the entire year, clinical outbreaks and mortalities due to Haemonchosis in sheep and goats of all ages, can occur at anytime. In areas with distinct rainy (wet) and dry seasons, discontinuities in the larval availability pattern on pasture can occur.

Because of the invariable development of high levels of multiple anthelmintic resistance in those countries where farmers can afford to drench in the tropics / sub-tropics, it is critical to understand that no alternative parasite control method on its own can be an effective, practical alternative – even for the short term. The message that must be heeded by owners of small stock, is that a combination of control methods must be adopted – of which one is the possibility of BC.

The almost universal occurrence of night housing of small ruminants in the tropics / sub-tropics means that the opportunity of using BC, incorporated in a feed supplement, is a practical possibility. Additionally, the incorporation of *D. flagrans* spores into feed blocks used for immediate and rapid consumption is an additional option. Both these methods of deployment have been tested in the control of nematodes of sheep and goats in Malaysia with success (Chandrawathani et al. 2002). But the potential of creating unnecessary problems in making fungal blocks (eg. short “shelf-life” spore viability, palatability), suggests to me that the additional complication of formulating blocks – rather than simply offering the fungal material as a daily feed supplement – is unwarranted. As mentioned above, such deployment of BC must be accompanied by other parasite control measures. By far the most effective, sustainable, consumer and user “friendly”, is short-term rotational grazing. This has been shown to be spectacularly successful in the humid tropics (Barger et al 1994, Sani and Chandrawathani, 1996). The combination of BC + rotational grazing is currently being trailed in Malaysia and results to hand have proved to be very encouraging (Chandrawathani P. pers. comm.). Other strategies that need to be incorporated include:

- Selection (purchase) of breeds with high levels of natural resistance to parasites.
- Improving nutrition eg. adopting the low-cost, farm manufactured urea-molasses technology (Knox and Zahari 1997).
- Utilising medicated urea-molasses blocks on a selective, strategic basis.
- Use of anthelmintics in this way (commonly the benzimidazoles) generally shows that drug efficacy is restored (Knox 1996).
- Use of Famacha (Malan and Van Wyk 1992).

Another possibility of BC which has very limited (but locally very important) applicability is in the control of *Strongyloides papillosus*, a parasite responsible for the “sudden death syndrome” reported in intensively reared young calves on the southern sub-tropical islands of Japan (Taira and Ura 1991). This parasite is ubiquitous in young ruminants, but of particular importance in the tropics / sub-tropics. It is unique in having the ability to reproduce in the free-living environment as well as in the parasitic stage within the host. With the ability of *S. papillosus* to replicate in bedding, infective larval numbers increase exponentially and young animals that are kept in pens can be exposed to massive larval challenge by percutaneous infection within 5-7 days. The outcome of which maybe the “sudden death syndrome”, referred to above. A study in Malaysia (Chandrawathani et al. 1998) showed that the nematophagous fungus *Arthrobotrys oligospora*, was capable of greatly diminishing the numbers of *S. papillosus* in ideal culture conditions. Because the size of pens used in these intensive raising units are small, this suggests that fungal material could simply be applied directly to the bedding. This opens up the possibility of using *A. oligospora*, or any other readily available easily cultivated voracious nematophagous fungus, to control of this important parasite, as the need to feed to animals does not apply.

C.1.2 Temperate Regions

Except for the United Kingdom and France, the small ruminant industries of Europe are numerically insignificant by world standards. However they provide products for the protected local, or niche, markets and consequently the value of individual animals is high. In addition, lucrative premiums for organically produced livestock products are also largely responsible for the surge in popularity of organic farming in Western Europe.

Typically, European ruminant livestock are raised on farms that have a variety of agricultural enterprises. This high degree of diversification potentially allows for efficient worm control practices whereby maximum benefit can be achieved by movement of animals to “low-worm” pastures produced by a variety of means. In addition, animal housing which is seasonal (winter) in Europe, but a daily occurrence in the tropics, allows for great opportunity to manage parasite populations in grazing livestock.

Clearly if animals are genuinely worm free at the time of pasture turn-out in spring (this is still possible to be achieved in Europe with the prior use of macrocyclic lactone anthelmintics) and that pastures are new leys – then there is a genuine “clean animals onto clean pasture” situation. In these circumstances, no other worm control measure is necessary. However in practice, this ideal state of affairs occurs very infrequently. Thus there is a great opportunity for BC for livestock in the intensive livestock industries of the temperate region. The simplest, and easiest to achieve at the present point in time, is daily supplementary feeding. The obvious time to employ this is at pasture turn-out in spring. The assumption being that either the pasture has

some level of infectivity of larvae that were derived from livestock grazing (and thus contamination) the previous year and have survived overwinter, or that the animals have residual infections (predominately arrested populations) that have remained *in situ* during the housing period.

In sheep enterprises in western Europe, lambing most commonly occurs within 1-2 weeks prior to turn-out. Ewes with young lambs at foot are allowed access to high quality pasture, but if they are not treated anthelmintic, or if they graze on pastures used by sheep the following year, then they will become infected with parasites. Inevitably they will excrete high numbers of nematode eggs during the lambing to weaning period (the post-partum rise). It is this contamination that gives rise to the peak of infective larval numbers later in the season. This can be catastrophic to the growth of young lambs if they remain, or are reintroduced, to this pasture after mid-summer. In a farmer trial where BC was applied as a daily fungal supplement for just 6 weeks following turnout, showed superior weight gains, earlier turn-off (marketing) and fewer lambs that had to be carried over the following winter due to parasite-induced loss of productivity, when compared with a flock that was traditionally managed (Waller et al 2002).

Similar studies with young, first-year grazing cattle in northern Europe have also met with success. Feeding of BC as a daily supplement for 2 - 3 months following turnout, showed the this reduced herbage larval counts towards the end of the season, thereby limited the risk of parasitic gastro-enteritis and showed improved weight gain over calves not receiving the fungal supplement (Denmark - Larsen et al. 1995; Nansen et al. 1995; Lithuania - Sarkunas et al. 2000; Sweden - Dimander et al. 2002). The arguments as I see them, on the use of BC in adult cattle have been covered under the section on broad acre, permanent livestock industries. Basically I believe that there would be little merit, economically, or from the parasite control standpoint, of using BC in adult cattle – whether beef or dairy.

CONCLUSION

Results are now steadily accumulating to show parasitological, but more important from the standpoint of farmers, animal performance benefits by the strategic use of BC against nematode parasites of livestock. Work has moved from the concept phase and there are now companies actively involved in this technology. It appears that the hurdle of producing commercial quantities of *D. flagrans* spores has been conquered. It is now critical that for BC product registration and generating farmer interest in using this technology, further practical field trials, which yield positive results, are immediately forthcoming.

All trials so far conducted in widely dispersed localities aimed at assessing the possible adverse impact (basically on beneficial soil nematodes) of using BC, show that this technology is

environmentally benign. Further work has shown that dose rates of fungal spores can be substantially reduced from those selected in the initial trials, in both sheep and cattle. This is a very important economic issue, because it will reduce the cost of production, which will ultimately be passed on to the consumer (livestock owners who use the technology). This review has focussed on ruminant livestock, but there is ample evidence to show that the same BC technology has a place in grazing pig production, and most particularly for the control of nematode parasites of horses.

It is my view that is in Western Europe, where the greatest initial opportunities for a commercial BC product will occur. This is because of the high value of individual animals, the impetus towards organic farming, the low number of animals / operator, and the relatively easy management of ruminants in this region. However the threats of unmanageable anthelmintic resistance provides a goad towards seeking viable alternatives to parasite control of small ruminants in the intensive livestock systems of the tropics and sub/tropics. Likely consumer resistance to paying for this new technology may be overcome, as there are examples (eg Malaysia) of government financially underwriting the costs establishing and supporting local livestock industries. The threats posed by the problem of anthelmintic resistance of course apply to the sheep industries in the broad acre, permanent grazing regions. Therefore maybe it is the sheer weight of numbers (sheep) in this region that will drive the commercial agenda as to where the first “glittering prize” of a marketable BC product will occur.

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QUESTION AND ANSWER SESSION

Q: Dr Chandrasekaran

- i) Do you foresee biological control as being able to control helminths on its own? Is there a possibility of developing resistance due to prolonged use of MUMB?

Q: Dr Sanyal

- i) Apart from fungus, are there any other organisms that can affect the infective stages of worms in the dung pat?

A: Dr P. Waller

Biological control needs to be integrated with other methods of worm control to maintain its long-term effectiveness. Reliance on one specific control option alone imposes selection pressure in favor of individual parasites that can evade the controlling agent – whether chemical, or non-chemical. In relation to the specific question of MUMB, it has been shown

that incorporation of an anthelmintic into a feed block (commonly a benzimidazole) generally restores drug effectiveness, even when resistance to single-dose oral formulations of the same anthelmintic group occurs. However with time, the parasites will develop resistance to the drug and thus prolonged MUMB use alone will become ineffective.

The dung pat is the haven for a huge number of organisms and therefore a complex dynamic situation exists between them all. Nematode-destroying fungi are just one group of organisms that can prey on nematodes, but it is important to remember that the predator for one organism can be prey for another. This certainly applies to the nematode-destroying fungi. There are other organisms that use these microfungi as a food source. This explains why nematode-trapping fungi do not assume dominance in the dung/pasture mat microenvironment, when they are used as a biological control agent of nematode parasites of livestock.

BIOLOGICAL CONTROL IN A GLOBAL PERSPECTIVE – A REVIEW WITH EMPHASIS ON *DUDDINGTONIA FLAGRANS*

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INTRODUCTION

Parasitic nematodes cause serious infections in small ruminants and, as one of the greatest causes for loss of productivity plus compromised welfare in grazing ruminants throughout the world, constitute a serious problem for small ruminant livestock producers (Perry & Randolph 1999). Beside the direct losses due to drop in production and deaths of animals, most of the economic losses are due to sub-clinical effects and although not immediately noticed by the owner, these can be substantial. Lanusse and Prichard (1993) estimated that worldwide 1,7 billion US\$ is spent annually to combat helminth parasites in cattle. Although the amount spent on small ruminants is much less it is still very substantial. In Australia the estimated costs to control worms in sheep is between 220 mill. (McLeod 1995) to 500 mill. US\$ (Emery & Wagland 1991). And when we consider the reported large problems in the sheep industry in South & Central America plus South Africa due to anthelmintic resistance, the costs of treatment in these countries will also be major.

The anthelmintic resistant gastro-intestinal (GI) nematode populations constitute a major problem especially in small ruminants in the subtropics and tropics, but are also a serious threat to livestock in the rest of the world (Conder & Campbell, 1995; Waller, 1997; Sangster, 1999). Work to overcome these problems has been going on with increased intensity for more than a decade. The reason for this interest is multi-faceted but primarily driven by the serious development of anthelmintic resistance (AR) in parasite populations. Other reasons are e.g. restrictions by rules to control parasite problems in organic livestock production, regulation of conventional drug use by legislation, plus a strong and increasing demand by politicians and consumer alike to reduce or completely avoid chemical residues in agricultural products. Among the various new approaches are:

- **Grazing strategies**, primarily used as a tool to secure availability of grass (Thamsborg, Roepstorff & Larsen 1999), but implementation of specific grazing strategies resulted in alleviation of the impact of gastrointestinal nematodes in livestock (Stromberg & Aeverbeck 1999, Barger 1999).
- **Smart use of anthelmintics**. This strategy has been proposed to preserve available, still efficacious drugs, and at the same time postpone the onset of resistance (Hennessy 1997 a + b).
- **Diet**. Besides work showing the importance of nutrition and ability to cope with parasites (Coop & Kyriazakis 1999), various tanniferous plants have been investigated for potential effect against either incoming parasite larvae and/or already established worms (Niezen et al. 1995, 1996, 1998; Waghorn et al 1995). Last, in monogastrics (pigs) it has been demonstrated how a diet containing easily degradable fibres compared to one containing indigestible fibres can affect the establishment of nodular worms (*Oesophagostomum* spp) in the intestine (Petkevicius et al., 1999).
- **Breeding** for animals with a higher resistance to parasitic GI nematodes has reached some level of success, but is far from widely implemented since often such improvement has some trade off with respect to productivity (Kloosterman, Parmentier & Ploeger 1992; Woolaston & Baker 1996; Gray 1997).
- Researchers in South Africa (Van Wyk, Malan & Bath 1997) have developed a very practical and promising technology, **FAMACHA**. This is a colour score chart, which shows the eye

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BIOLOGICAL CONTROL (BC)

1. Principles of BC

The philosophy behind BC is to utilize one of the natural enemies of the nematodes, making it possible to reduce the infection level on pasture to a level where grazing animals avoid both clinical and sub-clinical effects of the parasitic nematodes. It is important to emphasise that BC performed by a nematode-destroying fungus is a preventive measure with absolute no effect on the adult stages inside the host and its primary aim at reducing pasture larval infectivity, and subsequently reduce the overall parasitism of grazing livestock. But since no biological control agent will/should eliminate the number of infective stages to zero, the grazing livestock will constantly receive a small amount of parasitic larvae and thereby be able to develop a natural immune response.

2. BC trials – other fungi

Of the potential BC agents (natural enemies of nematodes such as mites, collembolans, predacious nematodes, tardigrads, amoebae, bacteria, virus, and fungi) almost exclusively the group of nematode-destroying micro fungi has been investigated, and in particular one, namely *Duddingtonia flagrans*, have shown real promise when tested in animals. The nematode-destroying fungi are using nematodes, and in the case of animal parasitic GI nematodes the free-living larval stages on pasture, as a food source. The fungi are naturally occurring in rich organic soil, decaying plant material or dung, and are ubiquitous. Recent studies have shown that deposited fresh faeces are quickly colonised by various species of these fungi (Hay *et al.* 1997 a+b; Bird *et al.* 1998). It has also been found that the fungi are picked up by grazing livestock (cattle, sheep and horses) and subsequently excreted in the voided faeces (Larsen, Faedo & Waller, 1994; Manuelli *et al.* 1999; Saumell *et al.* 1999). These fungi belong to a taxonomically diverse group, with both egg parasitic, endoparasitic and predacious nematode-trapping fungi. Fungi belonging to the first two groups have not been studied to a great extent due to the apparent lack of natural ability of the spores to survive in sufficient numbers through the GI tract of e.g. ruminants. This low degree of survival subsequently impairs their ability to infect and kill a sufficiently high number of developing parasite larvae in the dung environment. Within the group of nematode-trapping predacious fungi several species have been tested against the free-living infective stages of various ruminant parasitic nematodes (see Table 1).

3. BC trials – *Duddingtonia flagrans*

Among the predacious fungi the species *Duddingtonia flagrans* have been displaying superior abilities with respect to GI tract survival as well as subsequent destruction of developing parasite larvae in dung (Larsen *et al.* 1991, 1992; Faedo, Larsen & Waller, 1997; Grønvold *et al.* 1993;

Waller *et al.* 1994). In comparison with many other species of predacious fungi, *D. flagrans* is a relatively slow growing fungus (Grønvold *et al.* 1996), and as with other predacious fungi, growth is strongly influenced by temperature (Larsen 1991; Fernandez *et al.* 1999d). Laboratory, plot and field trials with spores of *D. flagrans* fed to the animals have shown a good reduction of free living larval stages of the parasitic nematodes *Cooperia oncophora*, *Ostertagia ostertagi* and *Dictyocaulus viviparus* in cattle (Larsen *et al.* 1992, 1995; Grønvold *et al.* 1993a; Wolstrup *et al.* 1994; Nansen *et al.* 1995; Fernandez *et al.* 1998, 1999b+c; Faedo *et al.* 2002b), *Haemonchus contortus*, *Trichostrongylus colubriformis*, *Teledorsagia circumcincta*, *Nematodirus* spp. in sheep (Peloille, 1991; Larsen *et al.* 1994, 1998; Faedo *et al.* 1997, 1998, 2000; Githigia *et al.* 1997; Llerandi-Juarez & Mendoza de Gives 1998; Mendoza de Gives *et al.* 1998; Knox & Faedo 2001; Fontenot *et al.* 2002; Chandrawathani *et al.* 2002; Waller, Knox and Faedo 2001; Waller, Faedo and Ellis 2001), *Oesophagostomum dentatum* and *Hyostrogylus rubidus* in pigs (Nansen *et al.* 1996; Petkevicius *et al.* 1998), plus cyathostomes, *Strongylus vulgaris* and *S. edentatus* in horses (Larsen *et al.* 1995a, 1996; Fernandez *et al.* 1997, 1999a; Baudena *et al.* 2000).

Cattle

Titration in calves have shown that at the level of 1 million spores per kg liveweight there is more than 85% reduction in number of larvae in faecal cultures. Besides the effect of different spore doses, there are some indications that the trapping efficacy might be influenced by the initial number of eggs (and hence number of larvae) in the dung. This has been found in two in-vitro dose titration studies (Furmonavicious 1998; Waruiru 1998) where an increasing trapping activity of *D. flagrans* was observed with increasing number of eggs per gram of faeces (epg). With approximately 1000 *D. flagrans* spores /g faeces the trapping activity increased with increasing epg within the range of approx. 50 to 1600 epg for all of the 4 parasites tested. The results also indicated that for *Oesophagostomum radiatum*, the stimulus of the larvae on trapping activity by the fungus was markedly lower than for the remaining species tested. This result is in line with what has been found earlier for a related fungus *A. oligospora*, against *Oesophagostomum* species of pigs (Nansen *et al.* 1988), the larvae of those also being very sluggish, slow moving and subsequently, not stimulating trapping activity of the fungus to the same degree as very active infective larvae of other nematode species. Full scale field studies using doses of 1 million spores per kg live weight have shown not only prevention of clinical outbreak, but also suppression of sub-clinical effects with significant production gain (live weight gain). Although potential for control has been demonstrated in laboratory and plot trials (Henriksen *et al.* 1997; Fernandez *et al.* 1999c) more work seems to be needed when it come to attempts to control lungworm (*Dictyocaulus viviparus*) in cattle.

TABLE 1: Predacious fungi,(excluding *Duddingtonia flagrans*), and animal parasitic nematodes tested 1985 - 2002. NB! The publications mentioned are not listed to match the fungus-parasite combination listed.

FUNGAL SPECIES	PARASITE	PUBLICATIONS	
Endoparasitic fungi:			
<i>Drechmeria coniospora</i>	<i>Ostertagia ostertagi</i>	Jansson et al. 1985	
	<i>O. circumcincta</i>	Santos & Charles 1995	
<i>Harposporium anguillulae</i>	<i>Haemonchus contortus</i>		
	<i>Trichostrongylus colubriformis</i>		
	<i>H. contortus</i>	Charles et al. 1996 Roque et al. 1998	
Predacious fungi:			
<i>Arthrobotrys oligospora</i>	<i>Cooperia punctata</i>	Gomes et al. 2001	
	<i>C. oncophora</i>	Grønvold 1989a	
	<i>C. curticei</i>	Grønvold et al. 1985, 1987, 1988, 1989	
	<i>O. ostertagi</i>	Chandrawathani et al. 1998	
	<i>Dictyocaulus viviparus</i>	Nansen et al. 1986, 1988	
	<i>Strongyloides papillosus</i>	Hashmi & Connan 1989	
	<i>H. contortus</i>	Murray & Wharton 1990	
	<i>O. circumcincta</i>	Wharton & Murray 1990	
	<i>T. colubriformis</i>	Dackman & Nordbring-Hertz 1992	
	<i>Oesophagostomum dentatum</i>	Waller & Faedo 1993	
	<i>O. quadrispinulatum</i>	Mendoza de Gives et al. 1994	
	Cyathostomes	Mendoza de Gives & Vasquez Prats 1994 Charles et al. 1995	
	<i>A. superba</i>	<i>H. placei</i>	Araújo et al. 1999
		<i>C. punctata</i>	Gomes et al. 2000, 2001
	<i>A. oviformis</i>	<i>H. placei</i>	Gomes et al. 2000, 2001
	<i>C. punctata</i>		
<i>A. conoides</i>	<i>H. placei</i>	Araújo et al. 1993, 1999	
	<i>C. punctata</i>	Gomes et al. 2000, 2001	
<i>A. robusta</i>	<i>H. placei</i>	Araújo et al. 1993, 1999	
	<i>H. contortus</i>	Gomes et al. 2000	
	<i>C. punctata</i>	Mendoza de Gives et al. 1992	
	<i>S. papillosus</i>	Mendoza de Gives & Vasquez Prats 1994 Gonzales Cruz et al. 1998	
<i>A. tortor</i>	<i>H. contortus</i>	Grüner et al. 1985	
<i>A. musiformis</i>	<i>O. circumcincta</i>	Grüner et al. 1985	
	<i>H. placei</i>	Gomes et al. 2000, 2001	
	<i>C. punctata</i>		
<i>Dactylaria candida</i>	<i>O. circumcincta</i>	Grüner et al. 1985	
<i>Monacrosporium eudermatum</i>	<i>H. contortus</i>	Mendoza de Gives & Vasquez Prats 1994	
<i>M. ellypsosporum</i>	<i>H. placei</i>	Araújo et al. 1992, 1999	
<i>M. thaumasium</i>	<i>H. placei</i>	Araújo et al. 1999	
	<i>O. ostertagi</i>	Hindhede & Jensen 2002	
	<i>C. oncophora</i>		
<i>M. sinense</i>	<i>H. placei</i>	Araújo et al. 1999	
<i>M. gephyropagum</i>	<i>S. papillosus</i>	Gonzales Cruz et al. 1998	
<i>Pleurotus pulmonarius</i>	<i>O. ostertagi</i>	Larsen & Nansen	
	<i>C. oncophora</i>	1990, 1991	
	<i>O. quadrispinulatum</i>		
	Cyathostomes		

Horses

In horse trials it has been found that in a titration study a dose at 1 million spores per kg live weight resulted in more than 85% reduction in number of developing cyathostome larvae in faecal cultures (Larsen et al 1995a). Plot trials have also demonstrated significant reduction of number of infective larvae in herbage surrounding faeces from animals fed fungal spores (Fernandez et al 1997; Baudena et al 2000). Only one field study has been published, and here feeding fungus to infected yearlings provided a pasture, which in turn was significantly reduced with respect to number of infective cyathostome and *Strongylus* spp. larvae on herbage. This was also reflected in the worm burdens of grazing tracer foals. The numbers of both large and small strongyles were significantly reduced in the tracers that had been grazing the pasture previously grazed by fungus treated yearling (Larsen et al 1996).

Small ruminants

It is in particular within the area of small ruminants (sheep and goats) that the interest for BC and *D. flagrans* has picked up over the last approximately 5 years. Research has been or is on-going in many countries in Europe, USA, Central and South America, South East Asia, Southern Africa, and Australasia. In many of those countries anthelmintic resistance is a major constraint to the production, and new tools for the future integrated control of the parasites are in great demand.

Plot trials involving faecal material from sheep fed spores of *D. flagrans* have shown a good reduction of the free living larval stages of various parasitic nematodes (Peloille, 1991; Faedo *et al* 1998; Knox & Faedo 2001) and the BC potential has also been clearly demonstrated in field trials (Githigia *et al.* 1997; Knox & Faedo 2001; Fontenot *et al* 2002). The initial field tests with calves mentioned earlier showed that daily feeding of fungal spores (chlamydo spores) for 2 to 3 months to the grazing animals prevented build-up of dangerous levels of infective larvae on pasture, when the calves were given a daily dose of approx. 1 million chlamydo spores per kg live weight or higher. But recent dose-titration tests show that this dose level can be lowered for the small ruminants (Pena *et al*, 2002; Waller, Knox & Faedo, 2001; Terrill *et al*, unpublished observations). Using between 5×10^4 and 10^5 spores/kg body weight more than 90% reduction was found in the faecal cultures used for these tests with housed animals. With respect to these figures for the efficacy of the fungus in faecal cultures, which is most often used as a quantitative as well as qualitative measure for the trapping capacity and vitality of the spores that survived passage through the animal GI tract, it is important that somehow in the future there will be a harmonisation of the techniques used by the different investigators so that results obtained can be compared on an even level. At present many, very different types of faecal culture techniques are employed around the world. Some of issues that need to be

addressed are the sample size incubated, moisture of culture, length of incubation, temperature, and use of climatic chamber or similar standardised incubation set-up.

The dose-titration trials mentioned have all been based upon a daily dose of spores, and a trial by Husted (2000) showed that it is important that the selected dose is provided daily. In a in-house feeding trial a group of goats were given a dose of 1 million chlamydospores in the feed supplement either daily, every second or third day and the number of larvae developing in faecal cultures were compared to the number in similar cultures from animals infected with parasites, but not fed any *D. flagrans* spores. Daily feeding provided a good, high level of reduction after some initial fluctuation due to the switch of diet in the animals (goats taken from pasture just before housing and fed different diet), while feeding every day resulted in oscillations with a dip in percentage reduction every second day reaching approximately the level of reduction seen with daily feeding. Feeding every third day was clearly the least effective way of treatment, leaving two days with very low to no reduction in larval numbers. In conclusion daily feeding is necessary, especially where and when dealing with a parasite such as *H. contortus*, with its very high egg producing potential.

The expectation of the effect of the fungus also in field trials has been fired by the calculations made earlier by Barnes, Dobson & Barger (1995). With their mathematical model they predicted the level of control when using biological control in their model set-up. The predicted activity of the nematode-destroying fungus was calculated based on a set standard management norm of 30 dead lambs out of 2000 under normal grazing conditions in New South Wales, Australia, with 3 drug treatments per year and move of lambs to safe pasture at weaning. In the simulation it was predicted that the reduction in lamb deaths would be 73% if the fungus caused 75% reduction in the number of infective larvae on pasture during a 90 days treatment period. If the fungus caused 90% reduction in larvae, the reduction in deaths would be 87% for treatment over 90 days and 43% reduction if the treatment period were only 60 days.

The few field trials performed until this day seem to confirm the good reduction capacity of *D. flagrans* when measured as potential to reduce larvae in faecal cultures of the animals fed fungal spores daily, and also to reduce the number of larvae on herbage, picked up by tracer animals (Githigia et al. 1997, Pena et al 2002). In the most recent trials in Australia (Waller, Knox & Faedo 2001) and Malaysia (Chandrawathani, see page 44) good control has been obtained both by daily feeding chlamydospores in supplement (more than approx. 90% reduction in number of developing larvae in cultures) but also after providing the BC agent in blocks (urea molasses/mineral blocks).

4. Vehicles and principles for deployment of *D. flagrans*

The plot and field trials mentioned above involving *D. flagrans* were all performed with spores given with a daily supplement, but some initial work on feed blocks (Waller, Knox & Faedo 2001; Chandrawathani *et al*/2002) as well as slow release devices (Waller, Faedo & Ellis, 2001) seem to give some strong indications of the potential of employing these technologies for deployment of spores to grazing animals. Although these techniques need to be developed much further the preliminary results give us a clear indication of the potential of using the biological control element in future integrated, sustainable parasite control programs. And with a diverse battery of ways (supplement including also incorporation of spores into feed pellets, block, and slow release device) to deliver spores to the animals, the role of BC in such programs will be significantly strengthened, as well as its adaptability to the very diverse types of management in livestock production systems world-wide.

For detailed discussion of background and possible principles of implementation of BC with *D. flagrans* under a range of management systems for different types of livestock worldwide, see pp. 5 by Dr. Peter Waller. Here, I would just like to mention a few possible basic principles on the potential use of *D. flagrans*. One very important feature is to acquire information on the epidemiology of the parasites in question in the system in question. For BC to work best it is important that the start of treatment coincides with the time(s) of the year just before an expected increase (not the peak of larvae) in herbage larval numbers appears. And that the feeding period is sufficiently long enough to cover most of the anticipated 'average' life expectancy (months) of adult worms of the parasites in question. With the right dose, and timing it should be possible to subsequently significantly reduce the number of infective larval stages on pasture and thereby provide the best environment to protect young stock from heavy infections and disease. When young stock (calves, lamb or kids) are going to graze pasture previously grazed by older, infected animals it is possible to let them start grazing for up to 3 weeks and then clean them out with an effective anthelmintic (based on knowledge of AR status), let them graze for another 2 weeks and then start fungus treatment. This should guarantee that when larvae start to develop in excreted faeces from these individuals, the fungus is present and will trap and kill them to keep pasture infectivity minimal. If young animals graze together with older, infected stock – such as ewes or does with lambs or kids at foot – then it would be wise to start the fungus treatment 2-3 weeks before time of birth, due to the peri-parturient egg rise seen in the small ruminants. Feeding should possibly continue until weaning or at least 3-4 months. The latter is still an open question, due partly also to the fact that the price of a future commercial fungus product is not known, and a high price could be prohibitive for extended feeding periods. In certain systems it might be necessary to use differential dosing, which means that during the first 1/2 or 1/3 of the anticipated fungus treatment period, a high daily dose is used, and then during the remaining time it is reduced, but overall during the entire fungus feeding period, the animals get an average daily dose known to be very efficacious.

While a regime involving use of anthelmintics can be implemented in most of the production systems worldwide, it is not possible in organic farming systems. Here the producer must try to reduce the pasture level of infective larvae pre introduction of the BC principle. That could be done i.e. by grazing with other types of livestock (co-grazing or rotational grazing between species or age groups) that would remove many of the infective stages. Of course the same principle could be used on conventional farms as well, which would provide them another option for reduced chemical use. Again, if young naïve stock are introduced on an infected pasture they should graze for 2 weeks and then start to receive fungal treatment. With young stock born on pasture or brought out together with their mother, dosing with fungal spores should start immediately.

5. Environmental Impact

Besides being safe for animals and man, it is imperative that new technologies such as biological control using fungal spores need to prove to be of no negative impact to the grazing environment. Although work on this is still on going, some published results seem to point to just that no-effect of the fungus on beneficial soil/pasture organisms. Short time test with earthworms (Grønvold et al. 2000) showed no negative impact, and a study looking at the population of soil nematodes on plots/pastures where fungus treatment was used, did not find any significant negative impact (Yeates, Waller and King 1997). This could probably be explained by recent findings showing that if deposited inside faecal material even at very high concentration, the fungal spores are spreading to the surrounding soil/pasture (Faedo et al 2002a).

CONCLUSIONS

If we try to summarize all the results on BC using *D. flagrans* as the control agent against parasitic nematodes in livestock we have found very good effect observed in faecal cultures of fungus treated animals, there is a significantly reduced transmission of infective larvae to herbage from deposited dung containing fungus, and in cattle, sheep and horses (tracers for the latter two) we have seen subsequent reduced worm burdens of the grazing animals. In the calf trials performed we have seen good production effect, which has also been reported by Waller, Knox and Faedo (2001) in sheep but results of other trials have not been able to show the same positive effect. Whether or not the small ruminant system is inherently so different from say cattle remains to be seen, but with the on-going activity it should be possible to clarify this issue within the next few years.

In the future to cope with the problems caused by the parasitic nematodes in grazing small ruminants in both the organic and conventional production systems, it will necessary to implement integrated, sustainable control strategies. These will have to consist of both existing

non-chemical options (grazing management), as well developed new approaches (biological control, vaccines, FAMACHA, resistant animals, or bioactive forages) in combinations with clever use of the existing drugs. Parasites are here to stay, but with biological control using the nematode-destroying fungus *D. flagrans* as a tool in future integrated control strategies (with or without the element of anthelmintics) it seems possible to safely manage the loss causing parasitic nematodes. And at the same time we will be able to do this with a reduced use of chemicals, preserving these for when really needed, to treat animals that fall sick due to parasites.

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QUESTION AND ANSWER SESSION

Q: Dr Rehana Sani

- i) It may not be practical to put spores in UMB as the moisture in the blocks may stimulate the spores to germinate. Are the block conditions suitable for spore germination?

Q: Dr P.K. Sanyal

- i) *Arthrobotrys* produces a lot conidia but not chlamydospores. Could *Arthrobotrys* and *Duddingtonia* spores be combined to give an additive effect for larval control on pasture? In India, a mixture of *Arthrobotrys* and *Duddingtonia* gives a positive response when used for larval control when sprayed on dung pats.

A: Dr M.Larsen

Trials have shown that the fungal blocks lose their effect if kept too long. This would be especially so under tropical humid conditions. The best effects are obtained within a week of production of UMB containing fungal spores i.e. about 5 days, not more.

The problem of triggering the *Arthrotrysto* to produce spores needs to be overcome. Mixing the 2 species of fungi may be useful only if spores could be produced in large numbers.

MEETING FARMER NEEDS FOR WORM CONTROL - A PARTICIPATORY AND REGIONAL APPROACH

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INTRODUCTION

Livestock are important elements of the pathway out of poverty for millions of the rural poor in Asia and the Pacific. Livestock have a special role to play in the conversion of feed that is unsuitable for humans into food and other useful products. Small livestock, especially goats, sheep, pigs and poultry are especially important for the poorest livestock keepers and for the landless who could start to raise livestock.

Helminth parasites of all livestock are important for two reasons. First, their impact on health reduces the level of output of meat, milk, traction and manure, and reduces their asset value through increased mortality, especially of young stock. Infection with the intestinal helminth *Toxocara vitulorum*, for example, is the largest single cause of mortality of Asian buffalo calves. Secondly, a sustainable approach to the control of gastrointestinal nematodes, which affect all species, has been to integrate many interventions involving feeding, breeding, grazing management and health control. Thus, for example, integrated control of nematode helminths in goats in South East Asia has become a springboard for a wide range of improvements in goat production, driven mainly by a reduction in mortality and better use and management of local resources.

The explanation for this may lie in the biology of some helminths whose life cycles are so closely linked to nutrient cycling in a grazing system. Good management of the nutrient cycle may simply lead to good management of parasites transmitted by parasitic larvae or cysts in the feed supply. Alternatively, it is possible that an integrated and holistic approach to any of the major constraints facing livestock production, for example, quality and quantity of feed, endemic and epidemic diseases, inadequate access to markets, may lead to overall improvements in productivity and security. Success may be as closely linked to the approach to the problem as it is to effectiveness of the technical components in the solution. In the context of this meeting on biological control it is encouraging to see that the focus is not only on assessing the technical effectiveness of fungal control but also on the myriad of environmental, economic and social factors that will influence its successful adoption.

AN INTEGRATED APPROACH TO WORM CONTROL IN GOATS IN SOUTH EAST ASIA

The starting point for this program was to develop breeds of goats and sheep in South East Asia that had genetic resistance to nematode infections, with a focus on the Philippines. This built on successful research in a number of tropical countries where breed differences had been demonstrated in sheep and, to a lesser extent, in goats. The concept was to develop resistant genotypes and then integrate this genetic component into a comprehensive worm control program. However, as ideas were discussed among the research group over many months, two major changes to the concept emerged.

We agreed that there had been sufficient research on a number of options for worm control in sheep and goats for a group of 'experts' to design effective control programs without waiting for new genetic information. These 'best-bet' options (including smarter drenching, rotational grazing and use of medicated feed blocks) would be validated with farmers as needed and, as new data became available and new options developed, these would be added to the mix of control measures. This formed the basis of a successful project funded by Australian Centre for International Agricultural Research in the Philippines and Indonesia.

The second conceptual change was based on the simple observation that over many years there have been many technologies developed for worm control in large and small ruminants but that the uptake of these technologies was very low. A classic example is the drenching of buffalo calves at 2 weeks of age to completely break the life cycle of *T. vitulorum*. It then became a clear objective of the group to understand why there was so little adoption and that farmers had to be intimately involved in the research and development. We concluded that no technologies could be effective without an understanding of the real needs of small ruminant farmers. This led to a second regional project funded by International Fund for Agricultural Development with major components in Cambodia, the Lao PDR, Indonesia, Philippines and Vietnam.

Thus the thrust of these two projects, and a regional objective of International Livestock Research Institute, became research on appropriate technologies and to do the research with the full range of partners. These partners are the farmers, extensionists, biological, social and economic scientists, government officials and investors, whose support and commitment are needed to develop an effective program that removes internal parasites as a constraint on production. The end-product may not be a control program in the conventional sense but a set of changes that minimizes the impact of worms on mortality, income and the livelihood of farmers.

IS BIOLOGICAL CONTROL AN OPTION FOR WORM CONTROL IN SOUTH EAST ASIA?

The technical options included in our current program in the Philippines include:

- *Use of effective chemicals*, incorporating knowledge on anthelmintic resistance and use of alternative delivery methods such as feed blocks. Quarantine drenching is an appropriate strategy for institutions and large commercial suppliers of stock.
- *Improved nutrition* including the use of tree and shrub leaves to reduce intake for ground-based and contaminated feeds; plants with possible direct or indirect anthelmintic effect and cut-and-carry methods especially during times of heavy rain or heavy pasture contamination.
- *Grazing Management*. improved housing to reduced stress through better ventilation, shelter, manure and feed management, rotational grazing and management of contaminated areas around housing.
- *Controlled Breeding*. includes timing of breeding to have young susceptible kids and lambs when worms can be best managed; considering possible increase or decrease in genetic resistance when deciding to use new genetics, especially 'upgraded' or 'improved' bucks and rams.

All of these options are based on experimentation that has been conducted with farmers, extensionists and local scientists. Vaccines, selective breeding and other 'upstream' methodologies are not discussed at the farm level, and neither, so far, has biological control. If biological control using fungi can meet the needs of small farmers who have restricted access to technologies, limited cash for inputs and a wide range on uses for their goats and sheep then it would an interesting addition to the 'basket of technology options' available for the control of nematodes.

ACKNOWLEDGMENTS

A feature of this work is the large number of people involved and it is the interactions among them that make it a sustainable regional and international effort. Special thanks to Marie Alo her team at PCARRD in the Philippines and the farmers, extension workers and scientists working in Pangasinan and Cebu who continue to adapt the Philippines options.

CONCLUDING REMARKS

The need for a participatory approach to combat poor productivity in developing countries is demonstrated in the Philippines. There is a need also to train technical, extension staff to improve services to farmers.

DUDDINGTONIA FLAGRANS FOR CONTROL OF PARASITES IN FARM ANIMALS: A COMMERCIAL PERSPECTIVE

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The nematode trapping fungus *Duddingtonia flagrans* has been extensively tested in the last 8 years in field trials in Europe and has been shown to give excellent control of nematode worms in calves and increased growth rates with treated calves typically weighing 20-30 kg more than the untreated animals at the end of the season.

Trials in small ruminants have however, given more variable results. The reasons for this are unclear at present, but are probably related to the structure of the faecal material which can dry out before the fungus has killed nematodes. In cases where the fungus has functioned and nematode burdens on pasture have been reduced only rarely have production benefits been obtained.

For the nematode trapping fungus to be commercialised, large quantities of spores must be produced at very low cost and then stabilised so they retain their viability during storage. Very few, if any, fungi are produced as spores on a large scale so it has been necessary to develop technology for spore production.

For *Duddingtonia* to be effective, spores must be consumed daily and this can be a problem where animals are fed spores in a feed supplement. The solution would be sustained release device or bolus that would dissolve slowly in the rumen and release spores over several months. The technical challenges are great however, and spores would have to be packed in a small device and survive the manufacturing process and then remain viable under rumen conditions for long periods. Clearly much work remains to ensure a reliable effect of treating animals with *Duddingtonia*.

Before any product based on *Duddingtonia* can be sold, documentation must be obtained that the fungus will not cause any adverse effects on the animals to be treated, farmers handling the spores or on the environment. Registration requirements vary in the different regions of the world, but within the EU the product is regarded as feed additive while in the US it is viewed as a biological pesticide.

Only if reliable efficacy can be obtained, and the large battery of safety tests concluded satisfactorily will the commercial use of *Duddingtonia* become a reality. The ever increasing incidence of resistance to chemical anthelmintics should however, provide the stimulus to ensure that products based on the fungus do become a reality in the not too distant future.

QUESTION AND ANSWER SESSION

Q: Dr P. Waller

- i) How do you perceive your company dealing with the issues of licensing? Is it possible to subcontract manufacture of commercial quantities of spore material to an industrial partner in an Asian country?
- ii) Is it possible to use the indigenous isolates of the country eg. from Malaysia or India, for mass production at your factory?

Q: Dr P.K.Sanyal

- i) Would it be cheaper to produce fungal spores in Asia than Western Europe? Does the safety tests conducted on the fungus, include tests on the production of chemical and antibiotic compounds?

Q: Dr A.Nari

- i) Brazil seems to have a huge market potential for biological control of nematode parasites of livestock, but handling of material by farmers and environmental moisture effects could be a constraint for the storage of fungal material and the final effectiveness of the product. Could you also elaborate on the cost?

A: Dr A.Gillespie

Licensing could be done in the Asian countries as well as with other European countries following the procedures stipulated. To use each isolate to produce fungal spores for each corresponding country would take too much effort and be exceedingly costly, since data on safety, registration etc would need to be repeated for each isolate. To produce the fungal material cheaply, it is better to use one fungal isolate as this will greatly save on costs.

In the current economic situation, the production of spores in Asian countries would likely to be cheaper. The delivery options for parasite control in livestock could vary from country to country. Optimistically one could perceive in the future a bolus form of delivery for better stability and which would be "farmer friendly". However I need to stress that a lot of time and expense would be needed to develop a bolus delivery system, whereas with the fungal supplement as a feed additive we are close to being "at market". However, there may be a problem with dosages needing to be varied for different livestock species. . Of course to be

a commercial success, the price has to be competitive with current chemical control methods. However there is good evidence that livestock producers will pay for such a biological control product if it delivers positive results.

All microorganisms – particularly the microfungi and actinomycetes - produce chemicals and antibiotics. Although these have been recorded for *Duddingtonia*, no specific tests have been done. However, *Duddingtonia* produces 2 types of antibiotic compounds not related to those used in human, or veterinary medicine.

A tolerance test, whereby 30 times the recommended daily dose is administered for several months is required for any product registration. If any untoward side effects are produced then the trial, or the concept, maybe abandoned.

DEVELOPMENT OF BIOLOGICAL CONTROL AS A COMPONENT OF INTEGRATED CONTROL OF NEMATODE PARASITES OF RUMINANTS

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INTRODUCTION

Helminth parasites and respiratory infections are the main causes of mortality and morbidity in the small ruminants in Malaysia. As such worm control programmes has been heavily dependent on the use of anthelmintics, leading to severe drug resistance against the common strongyles such as *Haemonchus contortus*, *Trichostongylus* sp. and *Oesophagostomum* sp.(Chandrawathani et al. 1999). It is thus very appealing to farmers and consumers alike to resort to non chemical means of worm control and a novel approach such as the use of a nematophagous fungi, *Duddingtonia flagrans*, would be able to suit the purpose. The following study describes work that encompasses a survey and isolation of nematode destroying fungi in Malaysia, (Chandrawathani et al. 2002) as well to assess its use as a biological control agent for nematode parasites of small ruminants under local conditions. Pen studies using *Duddingtonia flagrans* as a feed additive and incorporated into a feed block have also been conducted in sheep and goats (Chandrawathani 1997; Chandrawathani et al. 2002).

MATERIALS AND METHODS

Trial 1:

Feeding trials of fungal material produced in the Veterinary Research Institute using the Malaysian isolate of *Duddingtonia flagrans* was conducted on sheep. Naturally infected sheep with *H.contortus* were individually fed for 5 days with fungal supplement at 3 different spore dose rates (1.25×10^6 , 2.5×10^6 and 6.25×10^6) with 2 animals at each dose rate. Daily fecal egg counts, fecal fungal isolations and larval cultures were conducted during the trial (see Figure 1).

Trial 2:

Feeding trial of fungal material (1×10^6 spores) was conducted on 2 artificially infected sheep in individual pens for 5 days. Two animals were non fungal fed controls. The same laboratory analyses was done (See Figure 2).

Trial 3:

A group of 6 sheep, naturally infected with *H. contortus* were fed with urea molasses blocks containing fungal spores for 7 weeks. The sheep have an intake of about 100-120gms of block material per day, which would deliver about 1×10^6 spores per day to each animal. The blocks were prepared by The Nutrition Unit, MARDI. Laboratory analysis was conducted as above, 3 times a week (see Figure 3).

Trial 4:

The small paddock trial in VRI involves 3 groups of 8 animals kept separately in pens under one roof. All the animals are between 12-18 months of age and of mixed sex weighing about 25 kg. They are all grazed on separate paddocks and housed at night. Two tracers are grazed with each group for 3 weeks and slaughtered for total worm count after 2 weeks. Group 1 was fed fungal blocks at 100-120 g/day/animal, Group 2 fed fungal granules at 500,000 spores/kg/liveweight per day and Group 3 fed plain blocks. The fungal material was obtained from Chr. Hansens. Laboratory analysis of larvae recovery from faeces, faecal egg counts, Famacha score of the animals and the PCV was done (see Fig.4).

Trial 5:

The Infoternak Farm is a small government farm situated close to VRI where two groups of sheep, 6-8 months of age are rotationally grazed on improved pastures at a rate of 3-4 days per paddock. Each group of 20 sheep is grazed on a separate suite of paddocks; Group 1 is fed 500,000 spores/kg/liveweight (fungal granules from Ch. Hansens) and Group 2 is the control. Two tracers are put in each group for 3 weeks and slaughtered after 2 weeks for TWC. The PCV and Famacha is done monthly and the faecal egg counts done weekly. This trial is still in progress.

Trial 6:

The Calok Farm is a large government farm on the East Coast of Peninsular Malaysia where the sheep breed is predominantly Barbados Black Belly and its crosses. The trial has 2 groups of 30 sheep each, Group 1 fed with Chr. Hansens fungal supplement (500,000 spores

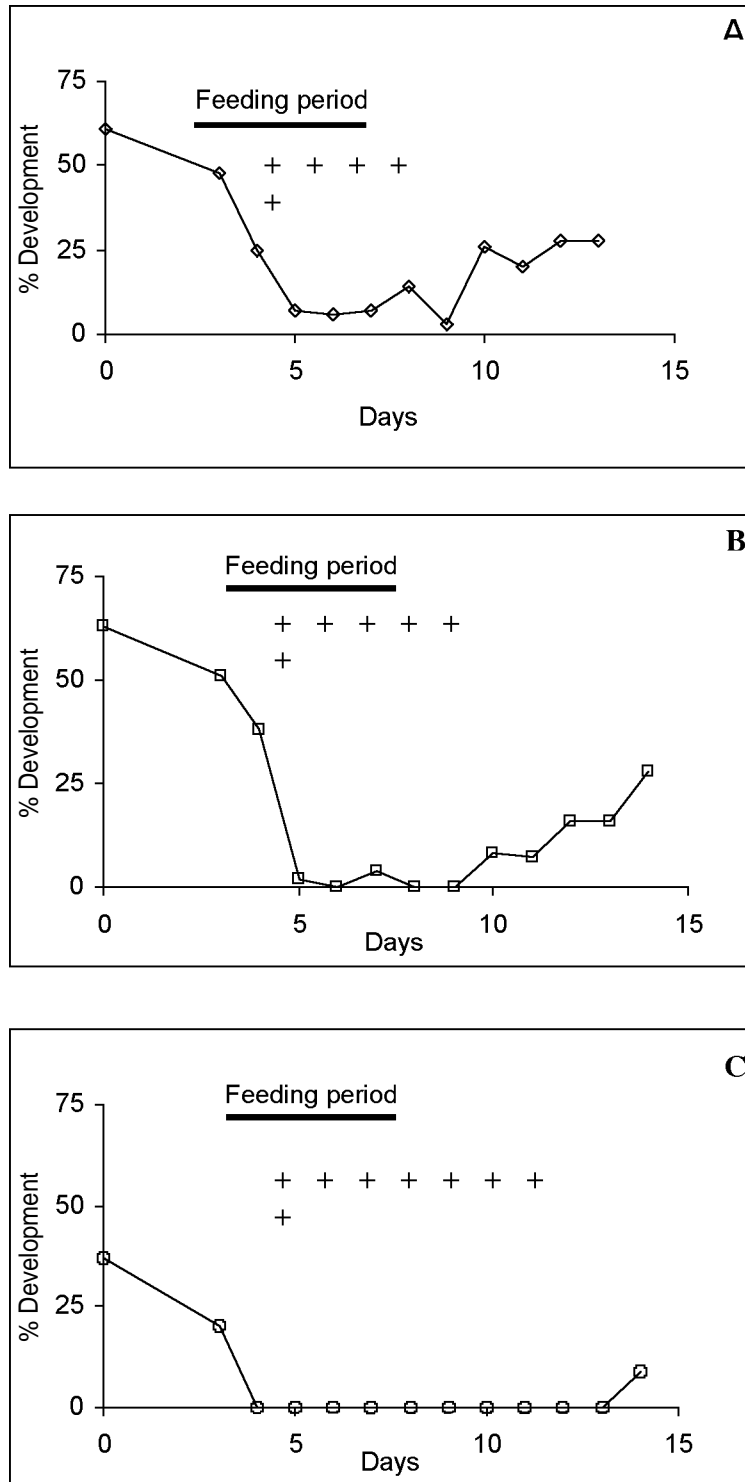
per kg liveweight) and a control group (Group 2). Faecal egg counts, PCV, Famacha score and weight gains are recorded monthly. The 2 groups are rotationally grazed on a separate suite of paddocks. Tracer sheep are put in the groups to assess pasture contamination. This trial is still in progress.

DISCUSSION AND CONCLUSION

The feeding trials with fungal spores (Trial 1 & 2) showed that *D. flagrans* was able to reduce the infective larval stages from nematode eggs in feces. There is evidence of a dose related response in the 3 groups in Trial 1. Trial 2 showed that the fungal treated group has a marked reduction in larval recovery of 97-100%. The fungal block trial (Trial 3) showed that the larval recovery was drastically reduced in weeks 2 through to 4. In week 5-7 there was an increase in larval recovery but this did not reach pre treatment levels. There was a major fluctuation in the egg counts as well as the recovery of fungus from blocks and faeces. This can be attributed to the erratic consumption of blocks throughout the trial. The culture of block material for fungal isolation was also erratic due to the overgrowth of other precocious fungi.

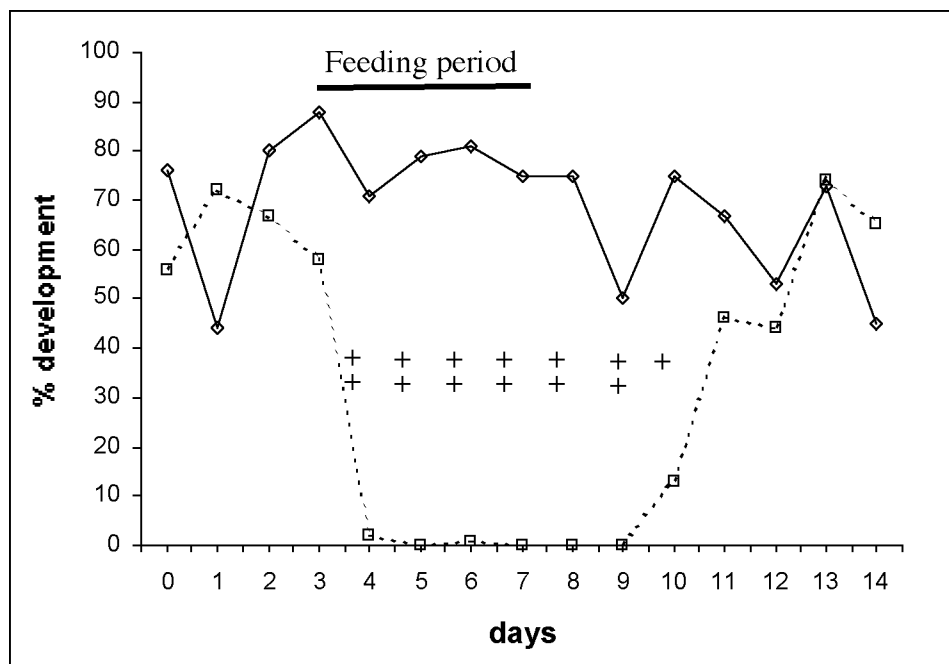
In common with the work in Denmark and Australia, *D. flagrans* was found to be suitable to be used as a fungal supplement. It can also be recommended to be used for urea molasses blocks provided the intake of the block is sufficient for the animals to obtain the required number of spores daily. The incorporation of fungal material in the diet has shown to reduce larval recovery substantially, thereby opening avenues for the control of pasture contamination. With the advent of drug resistance and increased awareness towards a healthy, drug free food product, it is timely to introduce such novel and innovative methods of nematode control among livestock producers to use on their livestock.

Figure 1: Mean % development of nematode eggs to infective larvae in faeces of lambs fed with three spore doses – A: 1.25×10^6 , B: 2.5×10^6 and C: 6.25×10^6 *D. flagrans* clamydospore / animal / day



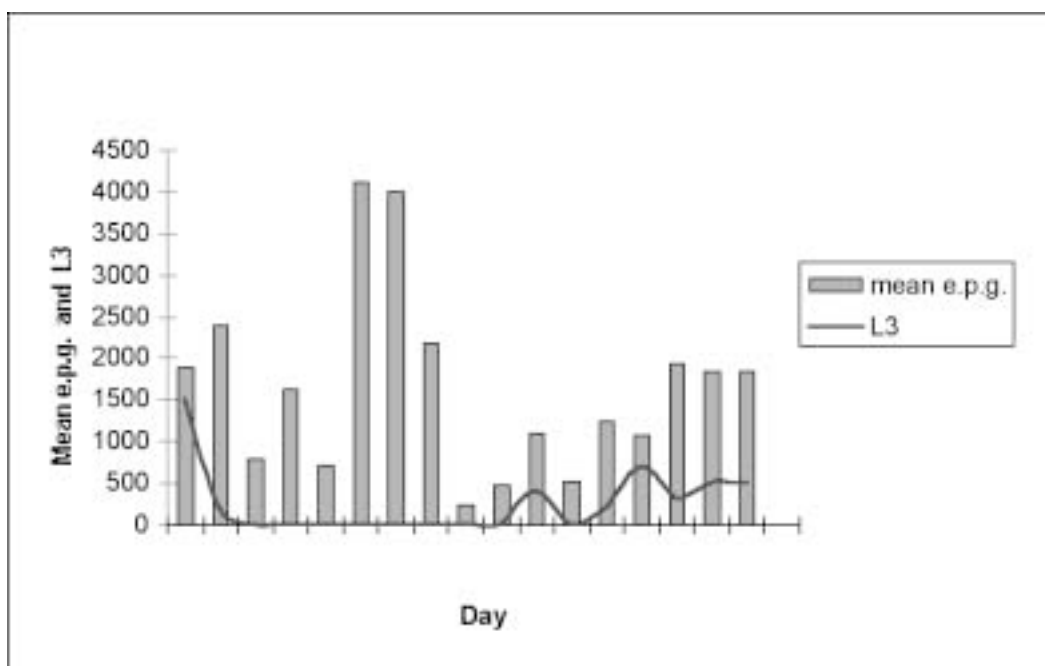
Number of + represent the presence of *D. flagrans* in faeces cultures for each lamb in the fungal treatment group.

Figure 2: Mean % development of *H. contortus* eggs to infective larvae in faeces of lambs, untreated or fed 1×10^6 chlamyospore/animal/day



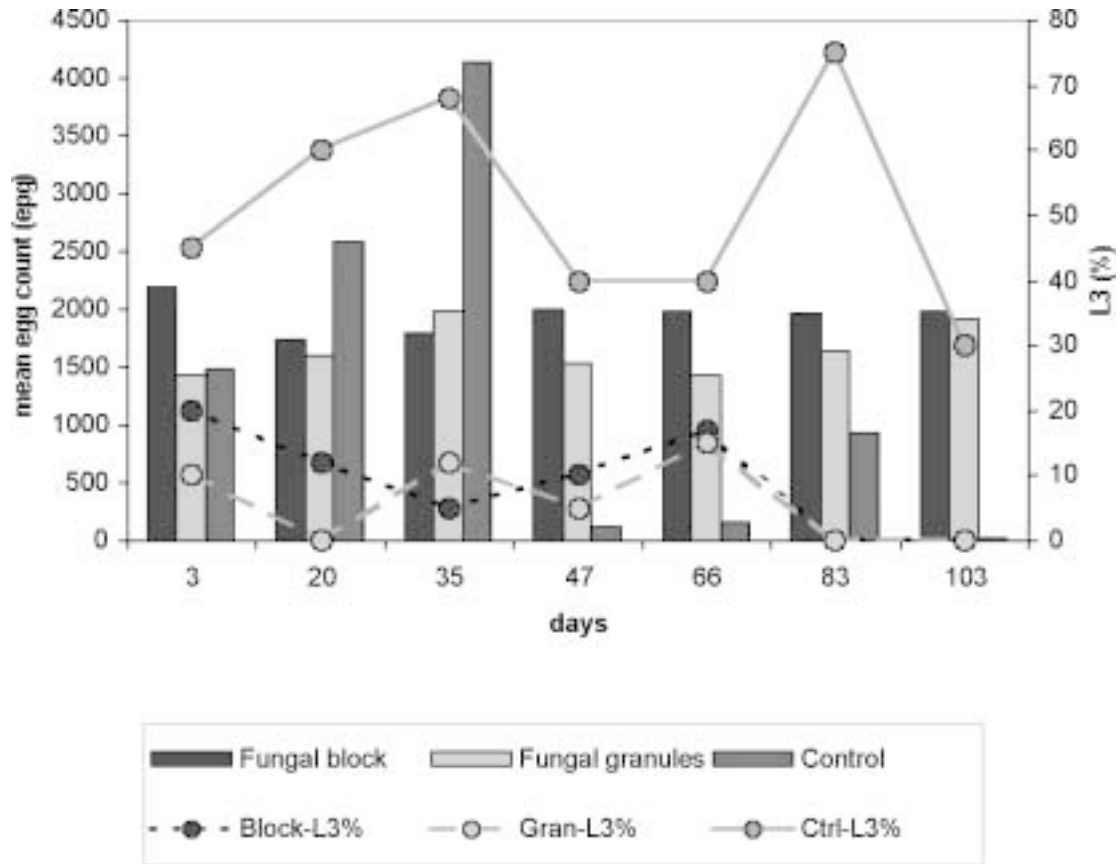
Number of + represent the presence of *D. flagrans* in faeces cultures for each lamb in the fungal treatment group.

Figure 3: Mean faecal egg counts and % larval development in 6 lambs offered supplementary feeding block containing *D. flagrans* chlamyospore for 7 week trial period



Number of + represent the presence of *D. flagrans* in faeces cultures for each lamb in the fungal treatment group.

Figure 3: Small paddock trial in Veterinary Research Institute, Ipoh



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HELMINTHIASIS AND ITS CONTROL IN SMALL RUMINANTS IN SABAH

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INTRODUCTION

Sabah is situated on the northern tip of Borneo Island. It is separated from West Malaysia by the South China Sea and the Philippines by the Sulu Sea. The neighbouring state is Sarawak (also part of Malaysia) and Sabah also shares common borders with Kalimantan (Indonesia), and Brunei. Sabah's population consists of Malay, Chinese, and Indigenous people of Sabah such as: Kadazan, Murut, Bajau, and others. The estimated animal population in Sabah for the year 2000 is: 42,318 head of buffalo, 40,530 head of cattle, 27,839 head of goats, 1,720 head of sheep, and 93,400 pigs. However, the estimated numbers of goats are usually inaccurate. This is because goat products seldom enter the formal marketing system and their contribution to the rural and national economic tends to be grossly underestimated. About 95% of the goat population is reared by small-scale farmers. Most of these farmers are involved with other kinds of economic activity such as government servants, paddy planters, fisherman, and others. Therefore, goat rearing is only considered as a part time activity.

There are two main designs for goat housing: the ground level type, and the stilted type (or raised floor type with a slatted floor). Housing of goats does not take into consideration factors such as separation units for kids, sexes, pregnant does, or health reasons. Goats are usually grazed on the road side, bush land, communal grazing ground, and even in town areas. No special pasture and supplement are given to goats. There is no proper breeding program in the traditional goat rearing. This may lead to indiscriminate mating between bucks and does of the same parents, which may result in poor quality stock. Based on laboratory results, the most common disease in goats are melioidosis followed by pasteurellosis and haemonchosis. Worms are the major problem in goat rearing in Sabah. There is no proper worm control program other than the use of anthelmintics and rotational grazing. The types of anthelmintic used are: valbazen, ivermectin, cydectin, botamak, levitape, flukiver, vermisoole, piperazine, levemisoole, and oxfenthic. Usually the anthelmintic dosage given is based upon the estimated weight. There is a possibility of under dosage or over dosage especially if an automatic drenching apparatus is used. De-worming intervals are usually from one to two months. But, the frequency usually increases during the rainy season to combat worm problems due to wet conditions of

the pasture. De-worming is routinely given and not based on faecal egg counting. Faecal egg counts are usually not carried out as the farms are located in the interior regions far from the diagnostic laboratory.

In mid 2002, a Faecal Egg Count Reduction Test was performed at the Government Sheep Breeding Farm (Ranau). Groups of 10 young sheep were randomly assigned to the following treatment groups: levamisole, oxfendazole, ivermectin and closantel. All anthelmintics were given at the manufacturers recommended dose rate. Ten days later the post-treatment faecal egg counts showed that there was 0%, 0%, 0% and 34% efficacy respectively. This represents total anthelmintic failure. Most seriously is the fact that this farm distributes sheep to small holders throughout Sabah, so inevitably there has been an unwitting distribution of nematodes that are highly resistant to anthelmintics as well. Plans are underway to survey the small holder flocks, as well as the situation regarding goats, in Sabah. In addition strong efforts will be put in place to attempt to rectify the total collapse of worm control on the Ranau farm, by a variety of means.

QUESTION AND ANSWER SESSION

Q: Dr P. Waller

- i) When was the Polled Dorset breed introduced into Sabah and what was the basis of its selection in the importation programme? This breed has no enhanced ability to survive in the tropics, nor is it especially resistant to *Haemonchus contortus*.

A: Dr Norma

This breed was introduced in 1999. However recently it has been proposed that a change should be made to the hair breeds of sheep for importation into Sabah, following the work on Peninsula Malaysia which has shown these to be more tolerant to helminths.

OVERVIEW OF HELMINTH PROBLEMS IN SMALL RUMINANT LIVESTOCK IN SARAWAK

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INTRODUCTION

Goats have been reared in Sarawak since time immemorial and generally as a small scale activity for the livestock owners personal use and as a side income during festivals. Rearing of sheep is a new activity in Sarawak, commencing in the 1960s and increased in the mid 1980s when the Government Livestock Department purchased 202 Poll Dorset sheep from Australia with further purchases of Polled Dorset / Marlin crossbreds from West Malaysia. The breeds of sheep that have been introduced into Sarawak are Malin, Malin Cross, Dorset Horn, Poll Dorset and the Barbados Black Belly. The breeds of goats in Sarawak are Kambing Katjang, Jamnapari, Anglo Nubian, but various crossbreds are common.

Currently, the sheep population is approximately 9,500 head (with 2/3 of these being found on the smallholder farms and 1/3 on government farms). The goat population is approximately 9800, with more than 95% owned by smallholders.

ANIMAL HUSBANDARY SYSTEMS

Free Range Systems

This is a common system practised by the smallholders whereby the sheep and goats are allowed to graze in the morning and are kept at night in simple shelters with little or no fencing.

Semi – Intensive Systems

Under this system, the animals are released during the day and housed at night. Supplementary feeding may be given when necessary. Proper sheds with necessary facilities are provided to accommodate large flock sizes. Fencing is required for open pastures. Shepherds are needed to monitor the flock movement if integrated with crops such as coconuts, oil palm or rubber plantations. This type of animal husbandry practised is generally good as the flock health can be monitored daily in the morning before releasing and in the evening when they return to the housing sheds.

Intensive Systems

In this system, the animals are always kept indoors and stall-fed with concentrate feeds, fodder or other agricultural byproducts. They are provided with good housing facilities and require only minimum land size. However, this system is quite rare and it is not encouraged, but it can be recommended for a fattening project as well as lambs from birth till 3 months old to ensure good health care and better growth rate.

SMALL RUMINANT MANAGEMENT

Management of the Sheep flocks

Sheep flocks can be categorised into several groups for ease of management of the herd. These include newborn lambs, pre-weaning lambs, post – weaning and growing lambs, adult sheep and pregnant ewes. The general management practices that should be adopted in a sheep farm include tail docking, castration, shearing of wool, dehorning , hoof trimming and control of ecto and endo parasites.

Management of Goat flocks

It is recommended that goats on a farm be subdivided into 2 groups for ease of management. The groups include those less than 4 months old and those more than 4 months old.

GENERAL MANAGEMENT PRACTICES

The general management practices for goats are far simpler compared with programs used for sheep. These include releasing the animals to the field and making sure that herd health programs are rigorously followed. Also the physical condition of the animals needs to be monitored, whereby hoof trimming needs to be routinely carried out and dirty skin needs to be cleaned.

FLOCK HEALTH PROGRAMME - SARAWAK

Sheep and Goat Flock Health Programme

Age	Health Programme	Action
1 – 7 days	<p>Treat navel with tincture iodine</p> <p>Obtain enough colostrum for newborn</p> <p>Ensure ewe produces sufficient milk</p> <p>Difficult birth</p> <p>Abortion</p>	<p>Treat everyday until dry.</p> <p>At least for the first 3 days or fostering the lamb to newly lambed ewe or give artificial colostrum if available.</p> <p>Check whether the teats are functional. Calf replacer to be given when necessary.</p> <p>Insert pessaries to the uterus.</p> <p>Collect serum and sent for serology to determine the cause(s) eg. Brucellosis, Leptospirosis etc.</p>
1 – 4 days	<p>Check daily for diarrhoea and other sickness</p> <p>Contagious ecthyma vaccination</p> <p>Multivitamin</p> <p>Check for nasal discharge</p>	<p>Collect faecal sample for parasitology and bacteriology</p> <ul style="list-style-type: none"> • If positive for coccidiosis, treat using sulphamethazine or other sulphur drugs. • If positive for worms, treat using appropriate anthelmintics e.g Synanthic , Systamex etc. • If positive for bacteria, treat with antibiotics e.g Castromycin., Kaopex N, etc. <p>At the age of 1 – 2 weeks.</p> <p>When necessary.</p> <p>Separate them and treat promptly using antibiotics e.g trivettrin, Tylan 50 etc.</p>
2 months	Fecal sample	Check for parasite, deworm flock if worm burden is heavy.
3 months	<p>Weaning</p> <p>Parasite monitoring</p> <p>Castration</p>	<p>To check on parasites, worms, coccidia etc.</p> <p>If necessary.</p>
4 months	Clostridial and pastuerella vaccination	Use (Heptavac) 7 in 1 vaccine clostridial + pasteurella) at 6 months interval or yearly depends on type of vaccines used.
6 months	Check for endoparasites	<p>Deworming interval depends very much on the severity of infestation, normally it is done monthly.</p> <p>For helminths, Ivomectin is effective. As for tapeworm, Mansonil is effective.</p>

Common Diseases of Sheep – Sarawak

Type of Diseases	Description	Symptoms	Prevention	Treatment
Parasitic Gastro-enteritis	Mainly a disease of young sheep and grazing sheep exposed to contaminated grass	Watery diarrhoea Inappetance Rough coat Lambs showed the typical 'tucked – belly' appearance Dehydrated	Shed needs to be clean and dry. For pasture, the most efficient and desirable method is drainage. Drench the herd once for 3 weeks or 2 months.	Systemex, Nilzam, Synantic, Panacur, Ivermectin Injectable
Coccidiosis	Always occurs in lambs. This disease is caused by protozoa.	Watery diarrhea Inappetance Dehydrated Very thin Foul Smell	Give enough space to the animals. Shed needs to be clean and dry.	Sulphur drugs
Bloat	Occur when the animals eat too much young grass and produce gas in the stomach	Difficulty in breathing Extended abdomen		" Bloat remedy"
Mange	Caused by ticks, mites and lice	Dropping of hair Aneamia Typical yellowish crusts on skin Thin and weak	Do dipping according to the programme.	Use Tactic
Foot Rot	Comon occurance during rainy season and in poorly drained farms		Grazing area should be dry.	10% formalin, copper sufphate
Melioidosis	Caused by Pseudomonas pseodomallei bacteria	Fever, Inappetance, Coughing, Limping, Discharge from mouth, Difficulty in breathing	Isolate the sick animals.	No specific treatment
Pasteurellosis	High body temperature Difficult brathing	Coughing, Nasal discharge	Treat the animals.	Antibiotics- Penstrep, Oxytetracyclines
Pneumonia	Occur when the animals had been under the rain for quite some time.	Depression Weak Discharge from the nose Coughing	Treat the animals.	Antibiotics – Ampicillin, Terramycin L.A

Common Diseases of Goats – Sarawak

Type of Diseases	Description	Symptoms	Prevention	Treatment
Contagious Ecthyma	Caused by virus			
Tympany	Stomach became swollen	Always urinate Always restless and making a lot of noise	Made sure the grazing area should contained less than 50% legumes. Give balanced diet, feed should not contain too much nitrogen such as legumes.	“Bloat remedy”
Pneumonia	Could be due to several factors such as bad ventilation, Wetness, cold and crowded conditions	Mucoid discharge from the nose Animal weak and depressed Fast breathing Coughing	Isolate the sick animal. Prevent the animals from getting wet. Made sure the shed is clean, dry and good ventilation.	
Diarrhoea	Caused by several factors such as worms, wrong feed, bacteria etc.	Feces soft and smelly Anal region dirty and wet Depressed Likes to isolate from the herd	Made sure correct feed is given. Practise rotational grazing. Clean the shed to prevent infection.	Give diarrhoea drugs such as koapectin etc. Give athelminthics

QUESTION AND ANSWER SESSION

Q: Dr P.K.Sanyal

- i) Is liver fluke a problem in Sarawak?
- ii) Because wool breeds of sheep, particularly the Merino, are not suitable for the tropics, is there any government programme to change the genotype of breed, for example to the Barbados Black Belly, by importing these stock from Peninsula Malaysia? A concerted effort by parasitologists in Malaysia needs to be made to inform the government and policy makers of the importance of the indigenous breeds of sheep, which are especially adapted to tropical conditions.

A: Dr Nicholas & Dr Chia P.C.

Flukes are present only in cattle. Regarding importation of hair breeds from Peninsula Malaysia, the minister has banned the imports of livestock due to the presence of FMD and Nipah virus. However, Sarawak is planning to import hair breeds from other countries this year in an effort to promote better use of the helminth tolerant breeds.

CONCLUDING REMARKS

In Sarawak, the community-based projects are not successful as there is a tendency for small holders to depend on government subsidies and help at every level. Now there is a concerted effort to encourage the diligent and conscientious farmers who are ready to embark on a commercial enterprise and to be largely independent of government assistance.

ENDOPARASITE IN SMALL RUMINANTS IN THAILAND

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INTRODUCTION

According to the Department of Livestock Development, the population of goats and sheep in Thailand is 125,000 and 75,000, respectively. Approximately 88% of total population of goats is found in southern Thailand, particularly in five provinces close to the Thai-Malaysian border. In contrast to goats, the sheep population scattered over other parts of the country. One of the likely constraints to goat and sheep productivity in Thailand is the effect of internal parasites on mortality and growth of young animals and on the performance of adult animals. The objective of this review is to compile the results of research relevant to control of internal parasites in small ruminants in Thailand. Since, studies on endoparasite in sheep are limited, this review will emphasize endoparasites in goats, particularly, gastrointestinal nematodes.

PREVALENCE OF ENDOPARASITES IN SHEEP

Research studies on prevalence of endoparasites in sheep in Thailand are very few. Only 4 publications have been located (Sukapasana, 1987; Chumnanpood et al., 1988; Sachit et al. 2002a, 2002b). Under grazing conditions on a research station, eggs in feces of lambs of the following endoparasites were found: *Strongyloides papillosus*, *Cooperia*, *Haemonchus*, *Oesophagostomum*, *Trichostrongylus* spp. and *Moniezia benedeni* (Sukapasana, 1987). Oocysts of coccidia were also found. All of these parasite eggs and oocyst of coccidia were found in all sampled sheep except eggs of *S. papillosus* and *M. benedeni* that only 20 and 60% of the sampled sheep, respectively, were found. Moreover, the first eggs/oocyst detected for coccidia, *Strongyloides*, other nematodes and tapeworms in the feces were 19.1, 22, 36.7 and 75.5 day after birth. These results suggest that lambs were infected with parasites and coccidia immediately after lambing.

Chumnanpood et al. (1988) reported a case of paramphistomiasis in sheep from 40 herds in Nakornswan Province, central Thailand, with the morbidity and mortality rate of 50-90%. The

infected animals showed the following clinical signs; submaxillary edema, dullness, inappetance, diarrhoea and deaths after 5-7 days of illness. Sick animals did not respond to broad-spectrum antibiotics and sulfa drugs, or to anthelmintic drugs such as nitroxynil and levamisole. Post-mortem and histological examination were done and large numbers of immature flukes of *Paramphistomum* spp. were found in the upper part of the small intestine. Concentration of serum total protein was low (2.9 mg/dl) that indicated hypoproteinemia.

Recently, Sachit et al (2002a) reported that average egg per gram of faeces (EPG) of gastrointestinal nematodes for Longtail sheep and Barbados-Longtail crossbred sheep raised on a government farm in southern Thailand were 2041 and 1502, respectively.

Sachit et al. (2002b) studied the efficacies of four anthelmintics, namely: albendazole, fenbendazole, ivermectin and levamisole on controlling gastrointestinal parasites in sheep. These anthelmintics had been used on the farm for 5 years. The efficacies, calculated by faecal egg count reduction test using arithmetic means of EPG, for all anthelmintics were low, with the values of 32.47, 47.4, 39.3 and 56.2% for albendazole, fenbendazole, ivermectin and levamisole, respectively. Moreover, 30, 63, 80 and 83% of sheep treated with levamisole, ivermectin, fenbendazole and albendazole had the efficacies less than 95% which indicating anthelmintic resistance (Table1).

Table1: Distribution of sheep according to the efficacies of different anthelmintics.

Anthelmintic	No. of sheep	Drug efficacy			
		<50%	50-90%	90-95%	>95%
Albendazole	30	47(14)	33(10)	3(1)	17(5)
Fenbendazole	30	47(4)	33(10)	0(0)	20(6)
Ivermectin	30	26(8)	30(9)	7(2)	37(11)
Levamisole	30	17(5)	13(4)	0(0)	70(2)

*Numbers of animals for each category are in parenthesis

Source: Sachit et al. (2002b)

PREVALENCE OF ENDOPARASITES IN GOATS

Studies on the prevalence of endoparasites in goats in Thailand reported stomach round worms (*H. contortus* and *Trichostrongylus* spp.), threadworm (*Strongyloides* spp.), whipworm (*Trichuris* spp.), tapeworm (*Moniezia* spp.) and coccidia (*Eimeria* spp.) (Suttiyotin, 1987; Kochapakdee et al, 1991). However, the degree of infestation of these parasites should be compared in terms of percentage of infection and EPG both mean and range. Suttiyotin (1987) found that

the frequency of animals infected with stomach round worms, coccidia, *Strongyloides*, *Trichuris* and *Moniezia* were 91.7, 82.57, 55.1, 11.0 and 0%, respectively. Kochapakdee et al. (1991) also found the infected percentage with coccidia, stomach round worm, *Strongyloides*, *Trichuris* and *Moniezia* were 96, 95, 62, 19 and 4%, respectively.

Faecal egg (or oocyst) counts are simple and basic value to quantify the degree of parasite infection. In a report by Kochapakdee et al. (1991), an average EPG of stomach round worm in village goats, was 1,264, with 33% of sampled animals had EPG greater than 1,000. Also in this report, an average oocyst per gram of faeces (OPG) of coccidia was 2,293 and 58% of the animals had OPG greater than 1,000. *Strongyloides* eggs in the faeces were low (295 eggs/gram) and 88% of the sampled animals contained EPG less than 500. These findings suggest that only stomach round worms, coccidia and *Strongyloides* are commonly found in goats in Thailand. However, based on EPG data, only stomach round worms and coccidia might affect productivity of goats.

Several factors affect prevalence of endoparasites in goats. These include season, type of management, genotype and age of the animals. Suttiyotin (1987) found EPG of gastrointestinal nematodes was higher during the monsoon month (October-December) than in the dry period. However, Kochapakdee et al. (1993a) did not find the differences in EPG of gastrointestinal nematodes for monthly sampling during October-January. The values were greater than 1,600 for all sampling times. This was due to heavy rainfall which continued until January in the year of study.

The type of management affects the prevalence of endoparasites through the microenvironment to which they are exposed. Kochapakdee et al. (1991) found that EPG of stomach round worms was greater for goats raised in fishing villages than goats raised in rice/rubber villages (1,415 vs. 1,149). In fishing villages, goats typically graze together in lowland grazing areas with conditions suitable for parasite infestation. In contrast, the majority of goats in rice and rubber villages were raised by tethering with 4-6 animals per family, so that the spread of parasites was lower. In another study, Kochapakdee et al. (1993b) compared EPG of weaned goats raised in two different university research farms. The results showed that EPG of gastrointestinal nematodes at Klong Hoi Kong farm was 3,665 whereas that for Hat Yai farm was only 117. This large difference was due to the difference in pasture condition between the two sites. At Klong Hoi Kong farm, the pasture was wet, tall and dense, providing conditions suitable for larval survival and transmission. In the contrary, pasture at Hat Yai farm was dry and sparse suggesting that few infective larvae survived.

The effect of age on EPG of gastrointestinal nematodes was also reported. Suttiyotin (1987) found that EPG for preweaning kids in the village was greater than weaned kids (370 vs. 208). Kochapakdee et al. (1991) also found that EPG of gastrointestinal nematodes of young goats

(milk teeth) were 1,523, whereas those for mature animals were 1004. This may suggest that there is some form of immunity to nematodes gained as the animals get older, and are exposed to prolonged infection.

Results of the study of the effect of genotype on EPG varied. Kochapakdee et al. (1995b) did not find any difference in EPG of Thai-native (TN) and Thai-native x Anglo-Nubian (AN) crosses grazing together under village conditions. However, Kochapakdee et al. (1993b) found that EPG of weaned goats raised under research farm conditions were 491, 1,982 and 2,320 for TN and AN, respectively.

Choldumrongkul et al. (1997) found that an average EPG of gastrointestinal nematodes during 6-12 week of age for TN kids was 518 whereas that for AN was 1,689. Pralomkarn et al (1997) also found that EPG (log n+1) for weaned kids experimental infected with *H. contortus* were 1.94, 1.67 and 2.98 for TN, 25% AN and 50% AN, respectively. Based on EPG value, it seems that TN goats were found to be more resistant to gastrointestinal nematodes as their EPG values were low.

EFFECTS ON PRODUCTION

The adverse effects of endoparasites on productivity of animals are manifested in a variety of ways. Changes in body weight are the most common feature of infection. Reductions in liveweight gain vary with the level of infection, the species of parasites, age of the animal, nutritional and immunological status of the host (Anderson, 1982). Gastrointestinal nematodes (particularly *H. contortus*) which inhabits the abomasum, suck blood from the host and results in anaemia. The parasites also damage the abomasal mucosa in the stomach and causes plasma protein leakage. Anaemia and hypoproteinaemia are know to be the feature of *H. contortus* infection in goats (Rahman and Collins, 1990; Rahman and Collins, 1991).

Kochapakdee et al (1993b) reported higher pre-weaning growth rate and weaning weight for kids raised at Hat Yai farm than those raised at Klong Hoi Kong farm. One reason of this difference is the effect of gastrointestinal nematodes since EPG of nematode at Klong Hoi Kong farm were higher than EPG of nematode at Hat Yai farm (3,655 vs. 117).

Kochapakdee et al. (1995b) investigated the effect of gastrointestinal nematodes on growth rate of TN and TN x AN weaned goats in a village environment in southern Thailand. Goats grazed on native pasture without supplementation during 0-9 weeks and supplemented with concentrate (15% CP) 300 g/head/day during 9-18 weeks of experiment. Animals were also divided into 3 groups according to anthelmintic treatment (untreated, 3 weeks or 9 weeks interval treatment). The EPG of goats in the untreated group increased to the level exceeding

1,250 but generally remained at this level throughout the experimental period. Goats treated every 3 weeks had greater growth rate than those in the untreated group or the 9 week-treated group. However, without concentrate supplementation, treated goats gained slightly (1.1 g/kg^{0.5}/d). In contrast, goats grew faster with concentrate supplementation, even untreated goats, gained 5.4 g/kg^{0.75}/d (Table 2). There was no significant difference in growth rate among the genotypes during unsupplemented period. However, when goats were fed a concentrate supplement in the second period, TN goats had significantly lower growth rate than did 75% TN x 25% AN or 50% TN x 50% AN (Table 2).

This finding is in agreement with those of Kochapakdee et al. (1993a) and Pralomkarn et al (1994), who found that 50% TN x 50% AN male weaners grazed on native pasture without concentrate supplementation only maintained their weight. In contrast, they gained weight substantially (63-91 g/d) when supplemented with concentrate at 1.2% of body weight. These findings suggested that 1). without adequate nutrition, crossbred goats did not outperform the native animals, 2). anthelmintic treatment alone would not result in an increase weight gain unless the nutritional status was also improved.

However, under improved management, Pralomkarn et al. (1994), found no significant difference in growth rate of female weaners between treated and control groups. An average EPG was low (<848). Egg per gram in this study were low, probably because goats grazed rotationally every 4 weeks. This suggests that, in addition to nutrition, improved management could be means of controlling parasite infestation.

Table 2: Least-square means for growth rate (g/kg^{0.75}/d) of goats with different anthelmintic treatments and genotypes

	Period of Study ¹		
	0-9 week	9-18 week	0-18 week
Treatments			
Control	0.4 ^{ab}	5.4 ^b	2.9 ^b
3 week interval	1.1 ^a	11.5 ^a	6.3 ^a
9 week interval	-0.9 ^b	7.9 ^b	3.5 ^b
Genotype			
Thai Native (TN)	-0.6	5.6 ^a	2.5 ^a
75% TN x 25% Anglo-Nubian (AN)	1.1	10.2 ^b	5.6 ^b
50% TN x 50% AN	0.2	9.0 ^b	4.6 ^b

¹0-9 week: without concentrate supplementation; 9-18 week: concentrate supplementation

^{ab}Means in the same column for each factor with different superscripts differ significantly (P<.05)

Source: Kochapakdee et al. (1995b)

PARASITE INFESTATION AND BLOOD CONSTITUENTS

Blood constituents, particularly packed cell volume (PCV), haemoglobin and total serum protein are the major indicators of gastrointestinal nematodes infestation especially *H. contortus* (Rahman and Collins, 1990; Rahman and Collins, 1991). Change of these values from the normal values mainly depends on the severity of infestation. Four studies had been conducted to investigate the association between parasite infestation and blood constituents in goats in Thailand, one being under village management conditions (Kochapakdee et al., 1995b) and three under improved management (Pralomkarn et al., 1994; Pralomkarn et al., 1997; Choldumrongkul et al., 1997).

Under natural infection in the village environment where goats were continuously grazed in one paddock, Kochapakdee et al. (1995b) found that after 9 weeks of grazing, the EPG of untreated goats was 2,289 whereas EPG of goats treated with anthelmintics every 3 weeks was 46. Consequently, PCV and haemoglobin concentration in untreated goats were lower than those of goats in the treated group. These findings were different from that in goats rotationally grazed on the university farm (Pralomkarn et al., 1994) in which untreated goats had similar blood constituents to those in the treated group. The differences between these two studies are probably due to the severity of infestation. The EPG corresponded to blood constituents in the study by Kochapakdee et al. (1995b), whereas the study of Pralomkarn et al. (1994) the egg counts were approximately 20% of these estimates. Under natural infections on the university farm, Choldumrongkul et al. (1997) did not find the difference in blood constituents between TN and TN x AN kids at 12 week of age. Nevertheless, the EPG between genotypes of kids in this study were different (1,167 and 3,054 for TN and TN x AN kids, respectively).

Pralomkarn et al. (1997) found a decrease in the concentration of PCV, haemoglobin, total protein and albumin in weaner kids artificially infected with 6,750 larvae isolated from sheep, compared with non-infected goats. This occurred in between week 4 and 9 of infection, which is related to the maturity of worms following the infestation. Moreover, weight gain of kids in this study were 36-64% lower than those of control kids.

THE EFFICACY AND RESISTANCE TO ANTHELMINTICS

Anthelmintics are used extensively in Thailand to control gastrointestinal nematodes in goats, especially on research/institutional farms. Three studies (Kochapakdee et al., 1993a; Choldumrongkul et al., 1994; Kochapakdee et al., 1995a) had been conducted to investigate the efficacy of anthelmintics. The first study was conducted under village conditions, using 24 weaner bucks (TN x AN cross-bred) to compare the efficacies of three anthelmintics:

fenbendazole, albendazole and oxfendazole. The second study was conducted in the university farm, using 24 female weaners (TN x AN crossbred) and the efficacies of fenbendazole, albendazole and ivermectin were compared. In the third experiment, 84 goats raised on a university farm were treated with albendazole, fenbendazole, levamisole or ivermectin. In all studies, faecal samples were collected from individual animal on day 0 and again on day 14 (study 2 and 3) or day 28 (study 1) post-treatment. The efficacies of anthelmintics were calculated by faecal egg count reduction test using arithmetic means of EPG. The results of these studies were presented in Table 2. Lower efficacies of anthelmintics in study 1 may be due to the time of faecal sampling instead of the efficacies of the drugs themselves. There was no previous use of any anthelmintics in the village, therefore resistance to anthelmintics should not occur. Faecal samples in this study were collected on day 28 post-treatment. Re-infestation of parasites may have occurred and resulted in high EPG on day 28. In study 2, all anthelmintics were effective in reducing egg counts. However, in study 3, only ivermectin was highly effective whereas the nematodes showed resistance to albendazole and fenbendazole and resistance to levamisole was also suspected.

Table 3: Efficacies of anthelmintics used in goats expressed as fecal egg count percentage

Anthelmintic	Study 1^a	Study 2^b	Study 3^c
Albendazole	58.2	88.0	24.8
Fenbendazole	54.7	-	25.1
Ivermectin	-	93	98.9
Levamisole	-	93	94.1
Oxfendazole	63.4	-	-

Sources:^aKochapakdee et al. (1993a); ^bCholdumrongkul et al. (1994); ^cKochapakdee et al. (1995b)

GENETIC RESISTANCE

There is evidence of genetic variation in resistance to helminths between and within breeds (Gray et al., 1995). Therefore, breeding of animals resistant to internal parasites is an alternative method, complementary to other methods of control. The effect of trickle infection with sheep strain of *H. contortus* in TN% x 25% AN and 50% TN X 50% AN was evaluated (Pralomkarn et al., 1997). The results suggested that TN goats were more resistant to *H. contortus* for parasitological and blood parameters as they had lower EPG, lower worm counts and lower reduction in blood constituents (PCV, haemoglobin, total protein and albumin) compared with the AN crossbred. This may be due to the evolution of Thai native goats in an environment where *H. contortus* is an important parasite. In this study, a large variation among goats within

and between genotypes in parasitological variables was observed. During the course of infection, Thai native goats exhibited less change in blood parameters than their crosses with Anglo-Nubian.

PROPOSED OPTIONS FOR THE CONTROL OF ENDOPARASITES IN GOATS IN THAILAND

Based on the experience in Thailand, we propose that stall feeding, stall feeding and tethering combinations, proper housing, shrub/tree leaves supplementation, by-products supplementation and controlled breeding are the prospect options for the control of the parasites. However, the best choice for the individual farmer depends upon his or her objective of raising goats, available resources or how much resources they are prepared to invest. The following represents the rationale for various proposed methods:

1. *Stall feeding*: This option is already adopted among farmers who raise goats for sale either as live animals for meat or milk. These farmers already know the benefits of this option and willing to invest resources in their goat raising enterprises.
2. *Stall feeding and tethering*: Tethering only could not supply all nutrients animal required even under frequent transferring or adequate feed resources. Therefore, goats should be provided with extra feed, mineral or supplementation during the night in form of stall feeding.
3. *Proper housing*: such as elevated or slatted floor is commonly used and the farmers understand the benefit of this system. Height of roof and wall should also prevent goats from being exposed to drafts, strong wind and rain. Steps should not be too steep and slippery. Feed and water troughs should be protected from goats entering and soiling the feed in the troughs. Some farmers light fires under the house to keep the house warm. Smoke from the fire can also repel insects and other external parasites. Fire also keeps the ground and faeces dry. Supplementation with mineral blocks is advocated as they are cheap, easily available and contain minerals required by animals.
4. *Shrub and tree leaves*: such as Leucaena (*Leucaena leucocephala*) jackfruit (*Artocarpus heterophyllus* Lamk) and *Streblus asper* have been used as a fodder for goats. Many farmers believe that goats that eat grass plus tree leave growth better and are healthier than those that eat grass alone. They believe that goats obtain extra nutrients and medical compounds from tree leaves, However, fast growing trees like Gliricidia (*Gliricidia sepium*), Sesbania (*Sesbania sesban*: *S. grandiflora*) can also be used as a buffer for Leucaena in areas which are not suitable to grow Leucaena. If this option is to be

adopted, it requires adequate number of trees to meet the amount of feed needed by the animals. Trees also need regular lopping and fertilizer application to obtain maximum production.

5. *By-product supplementation*: In southern Thailand, where oil palm are one of the major industries, by-products from the industry especially palm kernel cake (PKC) or palm Kernel meal (PKM) are good source of energy and protein. They can be used as a supplement.
6. *Controlled breeding*: The objectives of controlled breeding are 1). To prevent in-breeding 2). To prevent mating when breeding animals are not ready, 3). to mate the best males and females and 4). To plan on lambing or kidding at times suited to the farmers. To be successful in this option, it requires good record keeping and proper method to keep males and females apart.
7. *Medicated urea mineral molasses blocks*: are not been used in Thailand. However, urea mineral molasses block has already been using in large ruminants. Therefore, it can be easily introduced to small ruminants.
8. *Rotational grazing*: is one of the best options. It not only minimises parasitic infection, but also enhances pasture utilization. However, this option requires a large area and/or more fencing. It seems not to be adopted by small holder farmers.

- One study suggests that Thai native goats are more resistant to gastrointestinal parasite than Anglo-Nubian crosses.
- Based on the experience in Thailand, we propose eight options for the control of endoparasites in goats in Thailand. These are stall feeding, stall feeding and tethering combinations, proper housing, shrub / tree leaves supplementation, by – products supplementation, controlled breeding, medicated urea mineral molasses blocks and rotational grazing.

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QUESTION AND ANSWER SESSION

Q: Dr P. Waller

- i) Studies in Thailand in 1993 showed resistance to the benzimidazole anthelmintics but not to levamisole or ivermectin. Would this be the case now?

- ii) Is anthelmintic resistance being exported from the government breeding farms to the villages with disbursement of animals by policy promoted by the government?. Do institutional farms provide a source of animals to the farmers?
- iii) Is liverfluke a problem in Thailand.

Q: Dr P.K. Sanyal

- i) A FECRT should be sufficient to establish the state of drug resistance in your country.

A: Dr Surasak

The status of anthelmintic resistance is under study in Thailand. Farmers receive animals from institutional farms and thus drug resistance is exported to villages. Liverfluke is not a problem.

WORM CONTROL STATUS IN SMALL RUMINANTS IN THE PHILIPPINES

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ABSTRACT

This paper presents the impact of diseases associated with gastrointestinal helminths in goats. Production losses due to mortalities, susceptibility to diseases, and poor growth are ascribed to parasitism.

The most essential part of worm control programs focuses on the use of chemical anthelmintics. A widespread and growing problem on the development of anthelmintic resistance has been reported. Other management programs are currently being tested experimentally and on-farm to break the life cycle of the parasites and preclude further development of resistance. Studies on the utilization of herbal plants with anthelmintic value proved to be promising, but still require further isolation and testing of the claimed active component.

INTRODUCTION

The small ruminant industry in the Philippines is dominated by the smallholder sector, comprising more than 99.0% (PCARRD, 2001) of goat raisers. Realizing the benefits that can be catered to the countryside by raising goats, the government has made efforts to improve the industry (Dar and Faylon, 1996). Several research investigations for improvement of goat production have been conducted, and the local government units have also been mobilized to prioritize goats as a commodity for expansion.

This paper aims to provide information on the worm diseases that affect goats and their control measures in the Philippines. The ongoing and past research studies related to reports on anthelmintic resistance, and technology options for worm control. The prospect of employing biological method of worm control is also given due consideration.

WORM DISEASES AND THEIR CONTROL

Seasonal prevalence of nematode parasites has been observed, being highest during the rainy months, but infection persists throughout the year (Barcelo and Camalig, 1997; Ancheta *et. al.*, 1998). Mortalities especially in pre-weaning age reaches its peak during the wet season when the health of the animals is compromised by few feed resources available and extensive pasture contamination with infective larvae.

The most important parasites that affect goats are *Haemonchus* sp., *Fasciola* sp., and *Trichostrongylus* sp. Mixed infections are not uncommon. Pneumonia is always associated with *Haemonchus* sp. infections, which in many cases results in death of young animals. In high-risk waterlogged areas, mortalities may reach up to 80%, especially in poorly managed farms. Substantial production losses are also attributed to chronic infections.

Worm control on small ruminant farms relies almost exclusively on the use of chemical anthelmintics. In a recent survey conducted in six of the major small ruminant producing regions in the country, it was disclosed that albendazole has been recommended for use 3—4 times a year for 8 years by over 90% of veterinarians who deal with small ruminants (Gray, Yee, and Ancheta, 1999). Establishment of adequate control programs has been impeded by insufficient knowledge of farmers on the biology of the parasites and their reluctance to adopt new technologies.

ANTHELMINTIC RESISTANCE

Resistance of nematodes of sheep to benzimidazoles was confirmed in Mindanao (Van Aken *et.al.*, 1994) after albendazole was shown to be very poorly effective on an institutional farm. In goats, Ancheta (2001) revealed that 37% of the 167 samples from all over the country have albendazole efficacy rates of >80% while Venturina (2000) reported 53.3% of farms in a province in Luzon to have an efficacy of >95% with a range of 7-100%.

ALTERNATIVE METHODS OF WORM CONTROL

With the looming crisis in regard to anthelmintic resistance, there is an urgent need to develop alternative methods of worm control. It has been realized that there is no single effective method of reducing worm infections. Knowledge of the ecology of parasites will guide the farmer in the integrated and sustainable approach to worm control.

Strategic deworming: Should chemical anthelmintics be used, important considerations has to be made as to what type, how often and how long should the same chemicals be used. Strategic deworming is recommended such that the timing of giving drench is not only 'when needed', but at defined periods of the year when the pathogenic levels of infection is anticipated.

Grazing management: Rapid rotational grazing has been proven to be an effective method to reduce worm load and improve the nutritional status of the animal (Cruz, 2001) but the merits are compromised by the investment required for implementation. Nevertheless, the principle behind the practice is applied by rotational tethering.

Pure confinement: Provision of good housing is advocated as a primary basic requirement in goat raising. Aside from reducing the access of animals to the infective larval stages of worms on pasture, they are protected from inclement weather. Farmers provide cut-and carry grasses to their animals during the rainy season, and feed them in stalls. However, they allow the animals to graze freely for the rest of the year, and house the animals only by night.

Improved nutrition: It must be emphasized that susceptibility of animals to the effects of parasitism depends largely on their immune status. A well-nourished animal has a stronger immune system. One way of improving the health of goats aside from ensuring a continuous supply of good forage, is the incorporation of feed concentrates and mineral blocks. A dewormer (fenbendazole) has been incorporated in the urea-molasses-mineral-block (UMMB) to prevent reinfection after a prior deworming dose to kill off existing adult populations. This is done at peak months of heavy parasite infections to reduce worm load while improving the nutrition of the animals. The economic feasibility of adopting this technology is being assessed.

Use of herbal plants: Several plants have been tested for their efficacy as anthelmintics for goats (Mateo, 1996). *Leucaena leucocephala* (seeds), *Moringa oleifera* (leaves), and garlic (bulb) are few of the plants that show potential as an effective anthelmintic. Most studies conducted on this aspect are preliminary screening only and would require further tests and assays.

Neem (*Azadirachta indica*) leaf has been demonstrated to reduce the growth of infective larvae *in vitro* (Venturina and Dator, 2002), is currently being tested for its efficacy to kill or inhibit development of infective larvae on experimental plots.

Nematophagous fungi: While no previous investigations on this field appear to have been done in the Philippines, it has been observed that some larval cultures infected with unidentified fungi that only few or no larvae are harvested after a seven-day culture period. An investigation may confirm this observation by isolation and identification of the fungi that affects the larvae in routine fecal cultures in the laboratory.

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QUESTION AND ANSWER SESSION

Q: Dr P.K.Sanyal

- i) Are herbal dewormers popular in the Philippines?

Q: Dr P.Waller

- i) There are interesting differences in the way Neem is used in Malaysia and Philippines.

Q: Dr M.Larsen

- i) Are there any negative effects of the Neem concoction on plants (alcohol and water).

Q: Dr Chandrawathani

i) Did you use leaves only for the *Morinda* spp. ?

A: Dr V.Venturina

There is a lot of interest in traditional herbal remedies for livestock in the Philippines. Neem extract in the trials was used on pasture plots as a spray. Research is ongoing using experimental pastures. Morinda leaves are used as an anthelmintic as well as for the treatment of anemia.

A: Dr P. Waller

Worldwide there is a lot interest in herbal dewormers for livestock. These preparations remain an important part of the ancient, traditional, medicinal pharmacopoeia (especially in China and India). Currently there is a lot of research activity going on in many countries, particularly in the tropics. Therefore we can expect some exciting discoveries and developments in the future with regards to herbal de-worming remedies.

WORM CONTROL IN RUMINANTS IN INDIA: PROSPECTS OF BIOLOGICAL CONTROL FOR INTEGRATED NEMATODE PARASITE MANAGEMENT

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PRESENT STATUS

Parasitic gastroenteritis in ruminants in India is governed primarily by rainfall and temperature which favour development and survival of the preparasitic stages. Therefore, the prevalence of parasitism is seasonal (Sanyal, 1996). Veterinarians and animal health workers tend to recognise the seasonality of the problem and accordingly treat the animals with anthelmintics. In many instances, lengthy periods of high rainfall require regular and frequent treatment. Use of anthelmintics over the years has resulted in the emergence of anthelmintic resistance, more commonly in sedentary small ruminant flocks where animals are grazed on permanent pastures throughout the year (Sanyal, 1998). In the majority of migratory farmer's flocks no anthelmintic treatment is given unless clinical cases of parasitic gastroenteritis appear. In some flocks tactical anthelmintic treatment is practiced during the months of March-April, June-July, September-December. Strategic drenchings are followed in some institutional stationary flocks with, or without, grazing management (Sanyal, 1998). No other alternative control measures are practiced.

Though anthelmintic resistance is not as rampant as in other tropical countries of southern hemisphere, it is felt that India should develop alternative methods in order to reduce the reliance on the use of chemical anthelmintics (Sanyal, 1998). There is no reason to oppose the use of synthetic chemicals in principle, but they must be a part of integrated control system which minimise the development of resistance with minimal threat to biodiversity. It has now largely been acknowledged that parasite control cannot 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 appears to be one such alternate control strategy.

STUDIES ON BIOLOGICAL CONTROL

In addition to avoiding the problems of drug resistance, food and environmental pollution, the biological control of nematode parasites in ruminants would play an important role in biodynamic farming (organic dairying). In India, the use of *Duddingtonia flagrans* is now considered as a potential biocontrol method against animal parasitic nematodes.

1. Preliminary investigation

Initiated in the year 1999, the research results had demonstrated survival of this locally isolated nematode-trapping fungus, *D. flagrans*, through the gastrointestinal tract of sheep, goat, cattle and buffaloes (Table 1; Sanyal, 2000a) and successful suppression of parasitic nematode larvae on pasture following oral administration of fungal spores to cattle, sheep and goats (Sanyal, 2000b; Sanyal, 2001; Sanyal and Mukhopadhyaya, 2002).

2. Density dependent behaviour

The influence of faecal worm egg counts and varying quantities of fungal chlamydo-spores on the nematode-trapping ability of *D. flagrans* were investigated by studying fungus-larvae interaction using *in vitro* coproculture. Faeces from infected sheep having a mixed nematode infection of *Haemonchus contortus*, *Trichostrongylus* spp. and *Cooperia* spp. was used as a source material. The assay consisted of faeces with low (80 eggs per gram of faeces), medium (350 eggs per gram of faeces) and high (700 eggs per gram of faeces) worm egg density and baited with 0, 100, 1000 and 10000 chlamydo-spores of *D. flagrans* gm⁻¹ faeces. Though larval yield increased with corresponding increase in egg density in control cultures, the yield is correspondingly decreased with increase in chlamydo-spore concentrations (P<0.05). The reduction was not species dependent as all the three nematode genera were equally susceptible to fungal trapping with an efficiency of around 70%.

3. Time of administration

A trial on the time of administration of *D. flagrans* chlamydo-spores to sheep experimentally infected with *H. contortus* was designed. Pasture plots were contaminated with faeces from sheep fed on chlamydo-spores of *D. flagrans* (Plot 1), faeces containing nematode eggs followed a week later with faeces containing chlamydo-spores (Plot 2) and faeces containing nematode eggs only (Plot 3). Pasture larval burdens were monitored for 6 weeks. A general increase of larval numbers, with time to a peak 2-4 weeks post-deposition, was observed in Plot-2 and Plot-3 with the exception of Plot-1, which had low larval numbers throughout the observation period. It was concluded that *D. flagrans* would best work as a bio-control agent against pre-

parasitic stages of *H. contortus* when the chlamyospores are administered to sheep when they are likely to be excreting eggs (Fig. 1).

4. Effect of benzimidazole anthelmintics

Initial *in vitro* growth response studies revealed that *D. flagrans* is susceptible to Carbendazim (a benzimidazole nematocide used in agriculture) even at 0.05 ppm concentration (Fig. 2). *In vivo* experiments are in progress to evaluate faecal recovery of *D. flagrans* in sheep following administration of albendazole using a single dose and through an intraruminal slow release device.

5. Top dressing of feed

Feeding trials were conducted with stall-fed sheep parasitised with *H. contortus*. They were offered 250 gm concentrate feed which had been top dressed with desiccated chlamyospores of *D. flagrans* at 1×10^5 , 5×10^5 , 1×10^6 or 2×10^6 chlamyospores kg^{-1} body weight for 10 days. Pooled faeces from each group were spread on different pasture plots on day 7 of spore feeding. On day 28 of spore feeding, pooled faeces of the group were spread on the same plots. The pasture larval burdens on the plots were monitored for 2 months and *in vitro* faecal cultures were monitored regularly for larval harvest. Spore level of $1 \times 10^6 \text{ kg}^{-1}$ body weight and above virtually eliminated larvae from both the pasture and the faecal cultures. Spore levels as low as $1 \times 10^5 \text{ kg}^{-1}$ body weight had profound impact on larval recovery (Fig. 3). The effect persisted when the spores were fed but not after 4 days following discontinuation of spore feeding. Top dressing supplementary feed with dried chlamyospores offers a potential area of using *D. flagrans* for biological control of the pre-parasitic stages of *H. contortus*.

A pen trial in line with sheep experiment, is in progress involving buffalo calves to understand daily doses of chlamyospores required for a meaningful nematode control on pasture (Fig. 4).

6. Environmental impact

Experiments were conducted to understand the extent *D. flagrans*, could spread once deposited in faeces and alter the organic matter content of the faeces, which could serve as indirect evidences of short-term environmental impact of the fungus. At a central position of a pasture plot, faecal pat containing nematode eggs and fungal chlamyospores was deposited. Faecal pats containing only nematode eggs were deposited in two concentric rings one foot apart. Samples of faeces from these pats were cultured twice weekly for 3 weeks for fungal recovery and pasture clippings from around three deposits were examined for infective third stage larvae. The organic matter content of the faeces were measured fortnightly for 90 days. The findings of these experiments suggest that *D. flagrans* could not spread beyond the faeces on which it

is deposited, survived long enough to trap animal parasitic nematode larvae and was ultimately overpowered by other microbes present in the soil/faeces/plants. The organic matter contents of the faeces remained unaltered. The results provided evidences that the fungus had no significant short-term adverse effect on micro-environment.

7. Farm trial with crossbred cattle

It has been demonstrated that the source of nematode infection in farm-bred cattle in India maintained under tethered husbandry system is within its immediate environment and through fodder fed by cut and carry method from fields manured with dung originating from cattle farms (Sanyal *et al.*, 1992). In similar cattle farms, trials are being initiated involving 50 crossbred cattle which are to be offered *D. flagrans* chlamydospores with a similar group to be kept as fungus unfed controls. Dung from the two groups will be used for manuring different fodder plots. Egg counts and milk yields will be monitored in both the groups for one year.

8. Field trial with buffaloes

Trials are being taken up involving buffaloes in villages where animals are partially tethered and set free for grazing in community grazing areas of the villages every day for a specific time period.

9. Molecular studies

The laboratory is maintaining several local isolates of *D. flagrans* (DF-2507, DF-BJ and DF-2550) which have morphological similarity. DNA fingerprinting of these isolates by the application of RAPD and SRFA techniques, generated 233 different polymorphic alleles. Analyses of data through bootstrapping of 1000 simulates revealed that strains DF-2507 and DF-BJ are closely related to each other, than each one of them with DF-2550. Scanning of the amplicon generated lanes provides unique signature profiles for each one of the isolates. Efforts are being directed to develop molecular probes to monitor the fungus in the environment following application.

CONCLUSION

The impact of globalisation and trade liberalisation has resulted in an increasing awareness among animal farmers of the need to reduce the use of chemicals in animal agriculture. The promising results under experimental conditions on the use of nematophagous fungi have resulted in the development of parasite control technology, alternative to chemotherapy, which hopefully will be available in the near-future. Biological control has many obvious advantages. It could be applicable to a range of worm parasites not only within, but also between livestock

species. It would also provide an opportunity for livestock producers to capitalise on the increasing demands of consumers for chemical-free livestock products (organic farming).

Unlike the developed world, feeding grains to animals is not a regular husbandry practice in India. Therefore, efforts were made to develop a suitable and cheap method for delivering fungus which could be integrated with existing animal husbandry practices without causing problems for the farmers through a novel delivery technology. Supplementary feeding of concentrate feed is a routine ruminant husbandry practice in India. Therefore, incorporation of *D. flagrans* chlamyospores in concentrate feed pellets would have been the ideal choice. However, data generated in our laboratory indicate that shelf lives of such pellets are very poor (Anon., 2001). The fairly long shelf life of desiccated chlamyospores encouraged us to use them for top dressing of concentrate feeds.

The basic objective of an alternative non-chemotherapeutic method for controlling parasites, like biological control, is to reduce the seasonal peaks in the larval availability on pasture rather than to eliminate the larvae, so as to incorporate the procedure in the integrated parasite management programme. The application of 1×10^6 chlamyospores kg^{-1} body weight or more, virtually eliminated pre-parasitic larvae in both *in vitro* faecal cultures and on pasture, and there was a substantial reduction in larval numbers at doses of 1×10^5 or 5×10^5 chlamyospores kg^{-1} body weight. Hence, animals receiving as little as 1×10^5 chlamyospores kg^{-1} body weight would excrete sufficient chlamyospores in their faeces to reduce larval numbers on pasture sufficiently to prevent substantial larval challenge to the grazing livestock. As the nematode parasites are mostly prevalent during monsoon months and such hot and humid conditions are also favourable for the germination of chlamyospores, feeding dried chlamyospores to ruminants as a top dressing component to concentrate feed at the onset of monsoon might be expected to result in a substantial reduction in the seasonal peaks of larval availability on pasture.

In Australia, successful attempts have been made to control the pre-parasitic stages of sheep nematodes using chlamyospores incorporated into nutrient supplement blocks (Waller *et al.*, 2001a) or a fungal controlled release device (Waller *et al.*, 2001b). However, in view of the inherent problem of erratic licking of blocks by ruminants as a whole and restricted shelf life of fungal blocks (Waller *et al.*, 2001a) and also of the cost of the fungal controlled release device (Waller *et al.*, 2001b), top dressing feed on daily basis with desiccated chlamyospores may be an alternative method for achieving effective non-therapeutic parasite control in developing countries.

Epidemiological data on parasitic gastroenteritis in ruminants in India, large ruminants in particular, indicate seasonality of the disease and is suggestive of strategic anthelmintic intervention at the onset of monsoon (Sanyal and Singh, 1995). The proactive biological control

method would have the similar time of application. It remains to be seen to what extent anthelmintics affect the fungal viability and accordingly their strategic application could be fine-tuned.

Economic benefit of the intervention is another important factor to be addressed. Farmers perception of a technology is always measured in terms of its immediate economic benefits, extra litre of milk or kg of wool etc. As biological control is a preventive approach, financial gain would not possibly be immediately apparent. This is particularly true in a country like India where anthelmintic resistance is not as rampant as in other countries in southern hemisphere. The results of on-farm and in-field trials in cattle and buffaloes will provide some insight into this issue. However, the issue needs to be addressed with extensive extension campaign.

The challenge lies ahead in its field application and monitoring.

Table 1: Hour of detection (Mean \pm SD) of *Duddingtonia flagrans* (DF) in animals fed on chlamyospores of *D. flagrans* of sheep and buffalo origin

Animal	Hour of detection	
	DF of sheep origin	DF of buffalo origin
Sheep	36.0 \pm 4.0	34.0 \pm 4.5
Goat	24.0 \pm 6.5	28.0 \pm 3.3
Crossbred cattle	74.0 \pm 3.5	74.0 \pm 2.0
Buffalo	58.7 \pm 3.8	56.0 \pm 6.5

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QUESTION AND ANSWER SESSION

Q: Dr P. Waller

- i) What is the rationale of using anthelmintics and the fungus at the same time?
- ii) With the very good progress with your studies, are there now companies interested in commercial development?
- iii) Are all dairy herds treated for parasite infections in India?

Q: Dr Chandrawathani

- i) How do you scale-up chlamydospore production using liquid fermenters?

A: Dr P.K.Sanyal

Using a dewormer and followed by fungus administration 3 weeks later would have an effect on subsequent adult parasite egg laying worm burdens on their pasture contamination. This trial was necessary to convince to farmers on the benefits of using the fungus. Anthelmintic resistance is not a problem in India and drugs are still commonly used by farmers. However, organic farming is now catching on in popularity, so there is a need to develop the use of fungus for worm control. A pharmaceutical company dealing with organic

products is interested in this venture of using fungal material.

Treatment of dairy heifers is on village basis and not done individually.

The scaling-up of spore production using initially culture rich media and later transferred to nutrient deprived vat, is being done by a commercial partner.

A: Dr M.Larsen

If treatment and fungal material is used, it could it be that the eggs are not going to hatch because of residual bioactivity of the drug. So we will not know if the reduction in larval numbers is due to the fungus, or the anthelmintic.

CONCLUDING REMARKS

In India, the dairy industry is participatory and not subsidy driven. Farmers are willing to try technologies as long as they are proven to be beneficial.

Figure 1: Mean recovery of infective larvae from plots deposited with nematode eggs and *D. Flagrans* Chlamydo spores

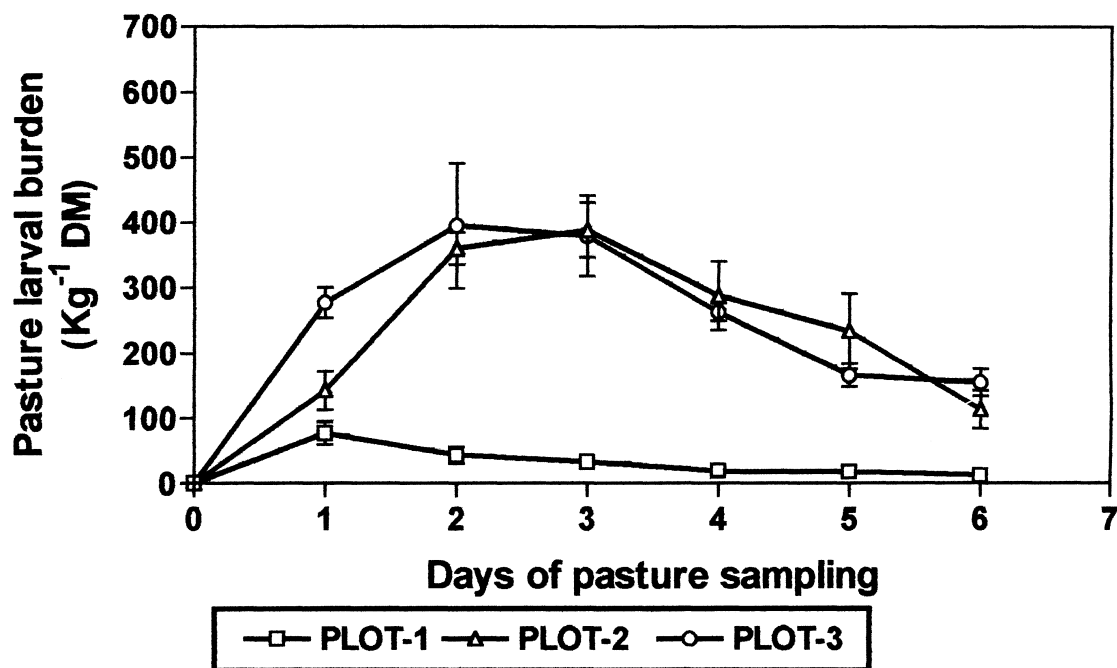


Figure 2: Per cent Inhibition of growth (Mean + Sem) of DF-2550 in agar plates baited with different concentrations (ppm) of carbendazim on different days of incubation

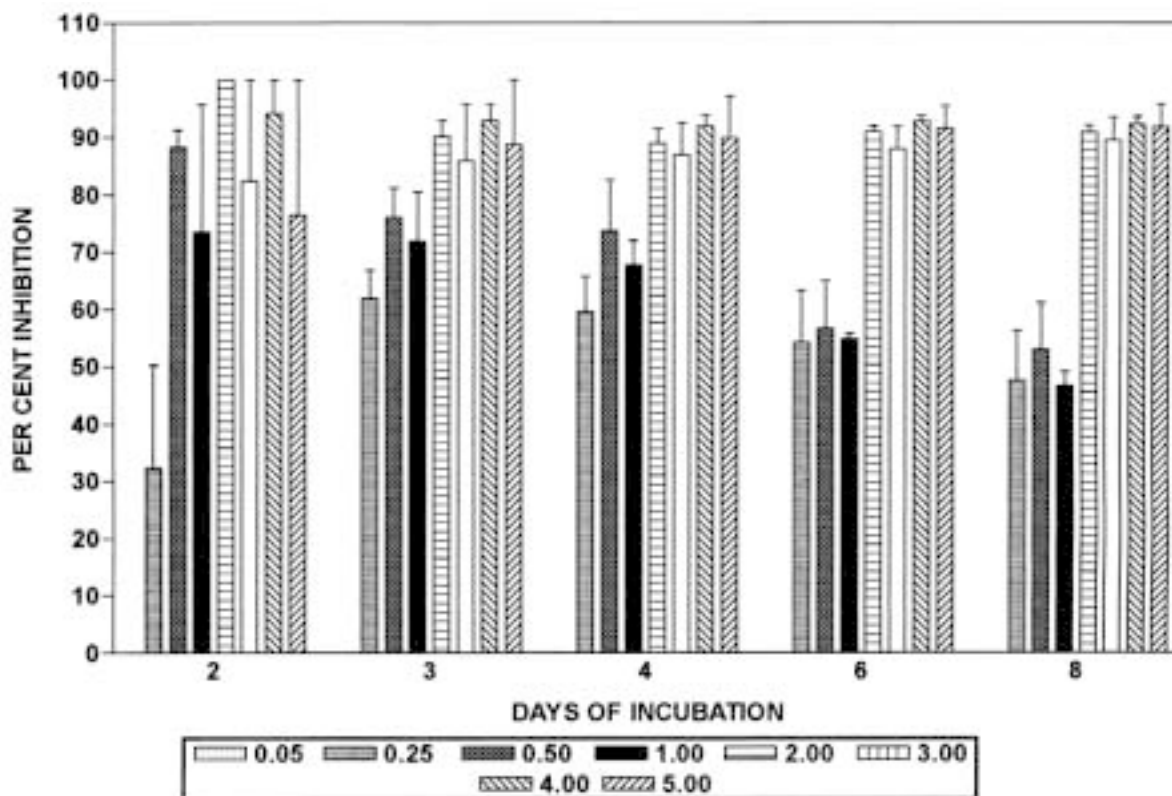


Figure 3: Pasture larval burdens in plots contaminated with faeces of sheep offered with different doses of *D. Flagrans* chlamydo spores

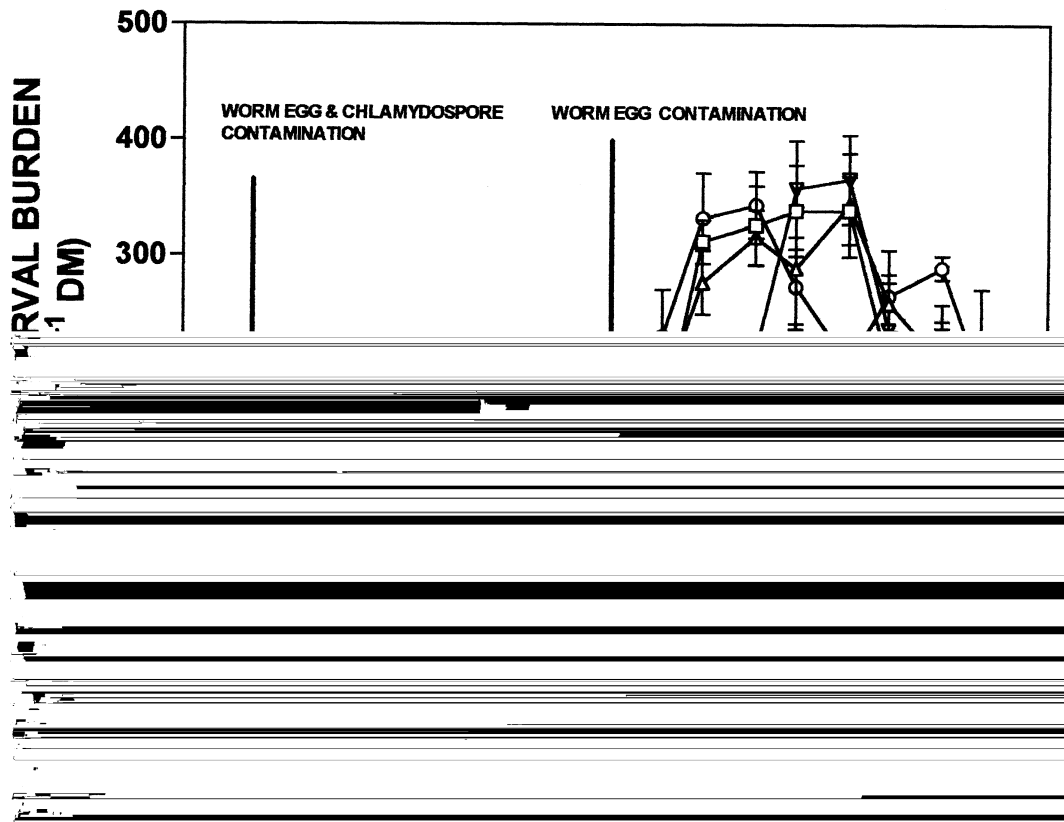
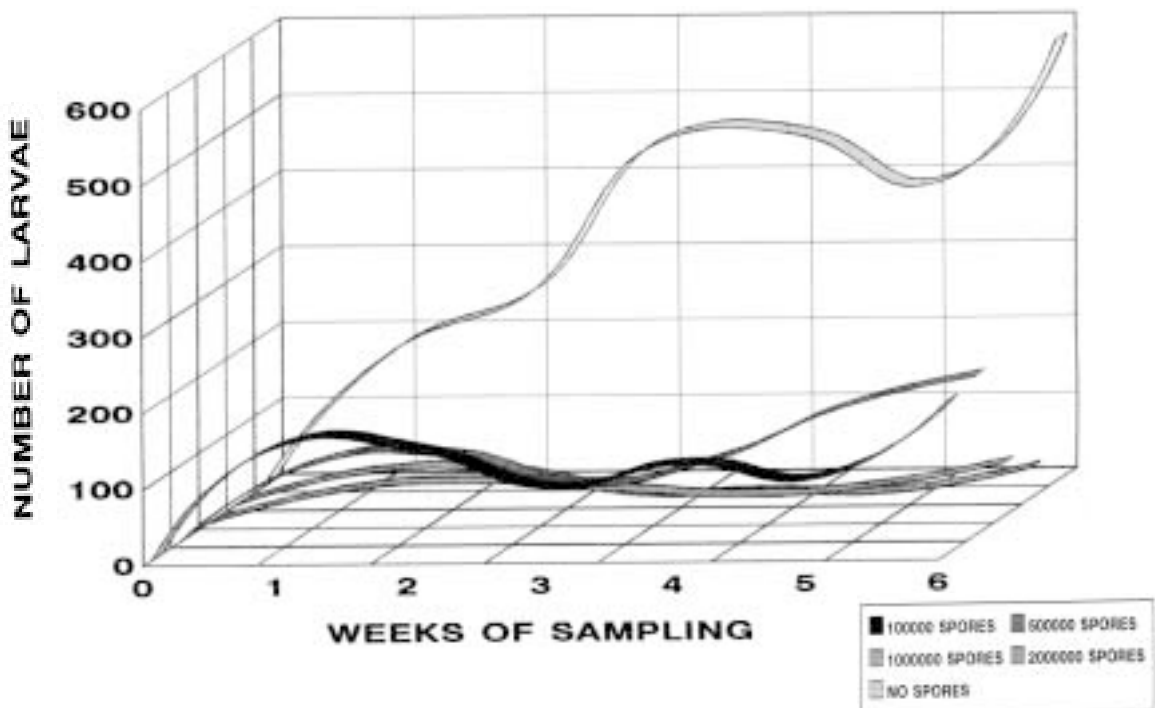


Figure 4: Pasture larval burdens in plots contaminated with faeces of buffaloes offered with different doses of *Duddingtonia Flagrans* chlamydo spores



SURVEY OF NATIVE ISOLATES OF PREDACIOUS FUNGI FOR BIOLOGICAL CONTROL OF GASTROINTESTINAL NEMATODES IN GOATS IN SRI LANKA

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GOAT HUSBANDRY

In Sri Lanka, more than 65% of the estimated population of approximately 500,000 goats are managed under an extensive system mainly by rural, subsistence level farmers in communal grazing areas in the drier parts of the country. The sheep population in the country is only about 20,000 and insignificant as an industry (Ministry of Agricultural Development and Research, 1992).

The goats found in the dry areas are the crosses of Jamnapari, Kottukachchiya (South Indian breed) and Sri Lankan Boer. In general, the goats are turned out in the morning on to the shrub jungles and communal pasture for 6-8 hours and housed in stilted sheds with wooden slatted floors, or mud floors, for the night. It is a common feature of the goat husbandry in the dry areas that the animals encounter stress of nutrition particularly during the dry season, which extends from May to August. All the animals have access to water throughout the day but are not given any supplementary feeding.

The goats raised in these areas are a major source of meat that is 2-3 times as expensive as the beef and chicken available in the country. However, the income generated from these herds is marginal because of the effects of diseases, poor nutrition and management constraints (Hariharan Ravindran, 1992).

IMPORTANCE OF GASTROINTESTINAL NEMATODES

In common with other countries of the tropics, gastrointestinal nematode parasitism in goats is identified as a major constraint to goat farming in Sri Lanka and it has been observed that weight gain benefit of control of gastrointestinal nematodes of goats varies from 30-47% (Faizal et al., 1999; Faizal et al., 2002). *Haemonchus contortus*, *Trichostrongylus colubriformis* and

Oesophagostomum columbianum are the gastrointestinal nematodes frequently encountered in goats in Sri Lanka (Rajapakse et al., 2000; Faizal and Rajapakse, 2001; Faizal et al., 2002) and mixed infections with these species are the commonest (Van Aken et al., 1990; Rajapakse et al. 2000; Faizal and Rajapakse, 2001).

SEASONAL PREVALENCE OF GASTROINTESTINAL NEMATODES

In the area regarded as the dry areas, the climate is bimodal and characterized by a warm rainy season from September to January and another during April, when the conditions are favourable for transmission of infection, resulting in loss of production (Faizal et al., 2002). During the period designated as dry season that extends from May to September, the larval availability on pasture is minimal thus the transmission of gastrointestinal nematodes is very low.

CURRENT CONTROL METHODS AND CONSTRAINTS

Control of gastrointestinal nematodes, which is a major element in ensuring the sustainability of goat production, is currently achieved by the use of anthelmintics. Frequent use of anthelmintics for the control of parasites in goats raised in institutional farms, has thus lead to development of resistance in gastrointestinal nematodes (van Aken *et al.*, 1989; van Aken et al., 1991; Jayasinghe and Faizal, 1993).

Treatment when clinical parasitism is apparent to prevent mortalities, not to control parasites, is the frequently used method in goats raised by the rural, subsistence levels farmers in the drier areas of Sri Lanka. Unfortunately, once clinical disease is seen in an animal, it may take two or three months for it to return to a physiologically normal state, during which time it can be easily overcome by further infections and losses are inevitable.

Strategic treatments, primarily aimed at gastrointestinal nematodes during the end of dry season or just before the rain starts towards the end of September, have been introduced recently for the prevention of build up of larvae awaiting ingestion during the rainy weather transmission season. In some situations, a single de-worming may not be sufficient to keep the level of parasitism below the economic threshold for the entire rainy season.

Alternatively, disseminating the information of grazing management for parasite control and demonstrating its practicability under the existing goat management systems in the dry areas is a problem since it involves costly modifications and additional resources to the extent that anthelmintics are in many ways cheaper.

All these factors emphasize the need to develop other control methods which will be adjuncts to the current methods. An option receiving attention in many countries is the use of predacious fungi as biological control agents which is environmentally friendly and totally compatible with the modern trend towards sustainable agriculture.

ISOLATION AND IDENTIFICATION OF PREDACIOUS FUNGI FROM SRI LANKA

During January 1999 to August 2000, 1029 samples of goat and cattle faecal samples (fresh and aged) were collected from selected locations of dry (average annual rainfall, 520-788 mm), wet (average annual rain fall, 1300-3250 mm) and intermediate (average annual rain fall, 910-2210 mm), zones of Sri Lanka for the isolation of predacious fungi for biological control of goat gastrointestinal nematodes (Table 1).

Of the faecal samples examined, 54 samples yielded 4 species of predacious fungi (Table 1). Of the 54 isolates 36 (66.7%) were *Arthrobotrys oligospora*, 12 (22.2%) were *Monacrosporium asthenopagam*, 5 were *Arthrobotrys anchonia* (9.3%) and 1 (1.8%) was *Arthrobotrys* spp. Aged cattle (13.3%) and goat faecal (6.8%) samples provided comparatively higher number of predacious fungal isolates than the fresh cattle (4.8%) and goat (0.5%) faecal samples.

Duddingtonia flagrans, the well-suited candidate for the biological control of gastrointestinal nematodes of livestock (Larsen, 2000) that has been isolated elsewhere was not found in the present study. Factors that might have led to this observation could be the low number of samples observed, the fact that the sampling was performed at times when chances of isolation of these were low or simply the rare occurrence of this fungus in these areas. Efforts will continue to isolate a local species of this fungus.

Table 1: Species of predacious fungi isolated from cattle and goat faecal samples from different climatic zones in Sri Lanka (The numbers of samples examined are given in parentheses).

Zone	Predacious fungal species	Source faeces			
		Cattle		Goat	
		Fresh	Aged	Fresh	Aged
Dry (n=716)	<i>Arthrobotrys oligospora</i>	9	13	1	5
	<i>Arthrobotrys anchonia</i>	0	2	0	3
	<i>Arthrobotrys</i> spp.	0	1	0	0
	<i>Monacrosporium asthenopagam</i>	1	2	1	5
	Sub total	10 (135)	18 (117)	2 (275)	13 (189)
Intermediate (n=146)	<i>Arthrobotrys oligospora</i>	0	3	0	2
	<i>Arthrobotrys anchonia</i>	0	0	0	0
	<i>Arthrobotrys</i> spp.	0	0	0	0
	<i>Monacrosporium asthenopagam</i>	0	0	0	3
	Sub total	0 (40)	3 (25)	0 (46)	5 (35)
Wet (n=167)	<i>Arthrobotrys oligospora</i>	0	3	0	0
	<i>Arthrobotrys anchonia</i>	0	0	0	0
	<i>Arthrobotrys</i> spp.	0	0	0	0
	<i>Monacrosporium asthenopagam</i>	0	0	0	0
	Sub total	0 (32)	3 (39)	0 (55)	0 (41)
Total number of isolates		10 (207)	24 (181)	2 (376)	18 (265)

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QUESTION AND ANSWER SESSION

Q: Dr P. Waller

- i) The comparison between monthly anthelmintic treatment and the treatment once every 3 months show no difference in weight gains in either the wet or dry season, indicating that 4 treatments a year should suffice.

- ii) Would farmers be treating animals - if mortality rates are as high with 20-30%. Extension services are required and farmers need to be motivated to consider farming as a business in which the aim, of course, is to be profitable.

Q: Dr Rehana Sani

- i) It is interesting to note the high percentage of *Oesophagostomum* in goats of up to 88% prevalence, whereas in Malaysia it is only 25-30%. Is there a difference in the performance between breeds?

A: Dr P.K.Sanyal

This could be due to the age factor. All goats come from India and are dual purpose and weigh about 20-30 kg at slaughter.

Q: Dr A.Nari

Farmers from developing countries may consider mortality rates of 5% as a “normal” and those of 10% even “acceptable”. But when mortality reaches levels of 40%, farmers in any place of the world will not need extension services to realize the importance of the problem because they are facing a real crisis of sustainability in their farming enterprise. Assuming parasitism is a problem in Sri Lanka it would be important to think about validation of Integrated Parasite Control under different management systems. It would be also advisable to choose reliable farmers who are willing to take part in validation trials and demonstrate to others the strengths (or weakness) of one specific integrated control strategy.

Q: Dr P. Waller

Farmers do not realize the problem of helminthiasis – worms may not be the only issue with regards to high mortality in flocks, or herds. . Extension services may not be effective and it is important to demonstrate the positive effects of worm control on a farmer level so that other farmers will be able to relate to the positive results and thus try the same procedures themselves.

Q: Dr B.Joshi

It is ideal to start farmers on a small scale to adopt some control techniques, then for other farmers to emulate.

Q: Dr Chandrawathani

Unlike small holders, commercial farmers tend to be more proactive and often seek advice and are prepared to try out new technologies despite certain costs being involved. This can be seen in Malaysia with the interest being generated by the use of fungal material for worm control.

Q: Dr A.Gillespie

The role of fungus in dry and wet seasons is important, as there is little transmission in the dry season. Germination and spread of the fungus is dependent on reasonably high humidity, as well of course high temperature. The latter is never a limiting factor in the tropics. The same applies to the hatching of worm eggs and the development of the free-living stages of parasites. So, what is good for worm egg hatching and larval development in the free-living stages is also good for the fungus. However there are times when too much rainfall is a bad thing for the fungus, such as following heavy downpours. These have the effect of disintegrating the faecal deposits and therefore the fungal spores are physically separated from the parasite eggs and larvae. Thus it stands to reason that biological control will not be effective under these circumstances.

A: Dr A.C.M. Faizal

The situation in Sri Lankan smallholders needs to be validated and demonstrated to the farmers.

NEMATOPHAGOUS FUNGI AS BIOLOGICAL CONTROL AGAINST GASTROINTESTINAL NEMATODIASIS IN SHEEP AND GOATS IN INDONESIA

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BACKGROUND

Gastrointestinal nematode parasitism is known to be a major problem encountered in small ruminant production in Indonesia and causes a significant loss to producers. Results from village studies indicated that decreases in live-weight gain up to 38 percent and mortality rate especially in lambs up to 28 percent have been recorded. The frequent use of anthelmintic has led to development of resistance to anthelmintics and has raised public concern with respect to residues in animal products and potential negative effects on the environment. Nematophagous fungi is one group of fungi which have the ability to destroy larvae of nematodes by trapping, endo-parasitic, egg parasitic and excrete toxic metabolites. Their activities against nematode larvae can be used as biological control.

This paper is aimed to review the work on nematophagous fungi based on the research carried out in the Research Institute for Veterinary Science since 1996. It seems that due to the limited budget available from the government of Indonesia, activities of nematophagous fungi is not as fast as other countries. It is hoped that this work will yield promising candidate species for the control of gastrointestinal nematode parasites in Indonesia.

PROCEDURE

Faecal samples were collected directly from the rectum of sheep and goats in several parts of Indonesia. Soil samples were taken from the shed, or grazing areas of sheep and goats (Table 1). Furthermore, faecal samples were processed based on the works by Larsen *et al.* (1994) and soil samples by Larsen *et al.* (1991). Isolates were identified based on the morphology characterization (Barnet, 1969, Domsch and Gams, 1972; Barron, 1977; Van Oorschot, 1985). *In vitro* studies followed the procedure of Mendoza-De Gives *et al.* (1992) (Table 2).

RESULTS

Samples of soil and sheep faeces from several parts of Indonesia had been cultured and isolated for their activity as nematophagous fungi i.e. *Arthrobotrys oligospora*, *Cephalosporium* spp, *Duddingtonia flagrans*, *Fusarium* spp, *Gliocladium* spp, *Monacrosporium* spp, *Paecilomyces* spp, *Trichoderma* spp, *Verticillium* spp and endoparasitic fungi (Table 1). However, at the present time, *Duddingtonia flagrans* could not be maintained in the laboratory. Good responses had been shown in several isolates such as *Arthrobotrys oligospora*, *Duddingtonia flagrans*, *Fusarium* sp., *Trichoderma* sp. and *Gliocladium* sp. against *Haemonchus contortus* larvae *in vitro* study (Table 2). Similar results had also been demonstrated in goats, but not in sheep (light) using *Arthrobotrys oligospora* given every day for 14 days (Table 3). Further studies are needed to again isolate *Duddingtonia flagrans*, a promising species of nematophagous fungi from Indonesia.

Table 1: Isolates of nematophagous fungi from faecal and soil samples originated from several parts of Indonesia

Collection				Results		References
Date	Location	Samples	Total	Isolates	Total	
Aug' 95 – Feb' 96	Bogor	Faeces	10	<i>Verticillium</i> spp	2	Ahmad and Beriajaya (1997)
Feb. 97	Bogor	Soil	50	<i>Paecilomyces</i> spp	10	Ahmad (1998)
				<i>Fusarium</i> spp	20	
				<i>Gliocladium</i> spp	10	
July 1999	Kali Gesing, Purworejo, Central Java	Faeces, soil	226	<i>Fusarium</i> spp	143	Beriajaya <i>et al.</i> (2001a)
				<i>Trichoderma</i> spp	12	
				<i>Paecilomyces</i> spp	41	
				<i>Cephalosporium</i> spp	13	
August 1999	Kendal, Central Java	Faeces, soil	18	<i>Fusarium</i> spp	18	Beriajaya <i>et al.</i> (2001a)
				<i>Trichoderma</i> spp	10	
				<i>Paecilomyces</i> spp	2	
August 1999	Sungai Putih, North Sumatera	Faeces, soil	63	<i>Fusarium</i> spp	47	Beriajaya <i>et al.</i> (2001a)
				<i>Trichoderma</i> spp	8	
				<i>Cephalosporium</i> spp	9	
				<i>Paecilomyces</i> spp	7	
				<i>Arthrobotrys oligospora</i>	2	
January 2000	Semarang, Msgelang, Batang, Central Java	Faeces, soil	136	<i>Fusarium</i> spp	78	Beriajaya <i>et al.</i> (2001a)
				<i>Trichoderma</i> spp	6	
				<i>Paecilomyces</i> spp	22	
				<i>Cephalosporium</i> spp	7	

Collection				Results		References
Date	Location	Samples	Total	Isolates	Total	
Feb. 2000	Bogor	Soil	102	<i>Monacrosporium</i> spp <i>Arthrobotrys</i> spp <i>Endoparasit</i>	16 10 6	Ahmad <i>et al.</i> (2001)
March 2000	Sungai Putih, North Sumatera	Soil	30	<i>Fusarium</i> spp <i>Trichoderma</i> spp <i>Cephalosporium</i> spp <i>Paecilomyces</i> spp	28 4 2 6	Berijaya <i>et al.</i> (2001a)
March 2000	Bogor	Soil	15	<i>Fusarium</i> spp <i>Trichoderma</i> spp	15 15	Berijaya <i>et al.</i> (2001a)
Sept 2000 – Mar 2001	Bogor	Soil	200	<i>Arthrobotrys</i> spp <i>Dactylaria</i> spp <i>Dactylella</i> spp	5 1 2	Ahmad (2001)

Table 2: *In vitro* studies on several isolates of nematophagous fungi against *Haemonchus contortus* larvae

Date	Isolates	Larvae	Results	References
July 1997	<i>Arthrobotrys oligospora</i>	<i>Haemonchus contortus</i>	Significant	Berijaya and Ahmad (2002)
July 1999	<i>Gliocladium</i> sp.	<i>Haemonchus contortus</i>	Fair	Dewi (2002)
July 1999	<i>Fusarium</i> sp.	<i>Haemonchus contortus</i>	Significant	Widyaningsih (2002)
July 1999	<i>Trichoderma</i> sp.	<i>Haemonchus contortus</i>	Significant	Achyar (2002)
Sept' 00- Mar' 01	<i>Arthrobotrys oligospora</i> <i>Duddingtonia flagrans</i>	<i>Haemonchus contortus</i>	Significant	Ahmad (2001)

Table 3: *In vivo* studies in sheep and goats using *Arthrobotrys oligospora* (Berijaya *et al.*, 2001b)

Date	Location	Animal	Number	Results
February 2000	Bogor	Sheep	25	Fair
December 1999	Kendal, Central Java	Sheep Goats	41 49	Fair Significant
March 2000	Sungai Putih, North Sumatera	Sheep	23	Significant

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QUESTION AND ANSWER SESSION

Q: Dr P. Waller

- i) The treatment of animals 2 –3 times before the rain is beneficial as shown by the epidemiological picture.

A: Dr Beriajaya

Based on trials, 1-2 anthelmintic treatments a year is sufficient to control helminthiasis i.e. at the beginning of the rainy season. Any delay in treatment could lead to helminthiasis. During the rainy season, nutrition is important as the animals may lose condition. Farmers are advised to increase feed supplementation by not only feeding grass, but also providing legume feed if possible. Anthelmintic resistance is only important on government farms in Indonesia.

THE STATUS OF GASTROINTESTINAL NEMATODE INFECTION OF SHEEP AND ITS CONTROL IN NORTHWEST CHINA, XINJIANG

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Ruminant livestock industries are very important components in the drive for economical development in Xinjiang Province in North West China. Nematode parasites are the major limiting factors which cause economic losses. Since the 1960s, great attention has been paid to research on the epidemiology and control of gastrointestinal nematodes. In investigations of the epidemiology of nematode infection, 2854 sheep in 144 different farms distributed in 79 counties have been conducted. These were based on post mortem examination and identification of gastrointestinal nematodes. In more than 40 years study, 67 Species in 17 Genera of 8 Families have been found in sheep. According to the investigation, infection rate of gastrointestinal nematodes in sheep is 100%; infection intensity is higher in north of Xinjiang than in the south. The dominant genera of sheep gastrointestinal nematodes are *Ostertagia*, *Marshallagia*, *Trichostrongylus*, *Trichocephalus*, *Nematodirus*, *Chabertia* and *Haemonchus*. Their infective rate is 59.6%, 49.1%, 44.2%, 43.4%, 38.0%, 36.8%, and 28.9% respectively.

In the north of Xinjiang, the ecological environment and climate are quite different from the south of the province. It has more annual precipitation and vegetation density as well as a milder temperature. Therefore, the infective rate and intensity, as well as the types of gastrointestinal nematodes, are higher and more prevalent than in the south.

The distribution of nematodes varies with elevation. Within the same district, the nematode species are basically the same at the same elevation. To a certain extent, more rainfall and vegetation is found at higher elevations, which favors the development of free-live stages of infective larvae. Sheep grazing on these pastures will be infected with a greater variety of nematode species and also at a higher infective density.

Investigations of the seasonal dynamics of gastrointestinal nematode infections, show that sheep are infected by third- stage larvae following grazing on summer and autumn pasture. Clinical syndromes and economic losses due to worm infection appears in winter and spring due to the harsh climate and lack of nutrition. In different districts and different sheep age

groups, seasonal dynamics of gastrointestinal nematode infection varies to some degree. In the Urumuqi region, the biggest worm burdens for adult sheep was found in spring, the average worm burden reaches approximately 5,500 and the lowest worm burdens are found in winter, of approximately 2300 parasites. In the Gongnans region, the biggest worm burden appears in spring and winter; the lowest in autumn. However in Zhaosu County, the biggest worm burden of sheep is found in summer, remaining at nearly the same level for the remainder of the year. Seasonal dynamics of gastrointestinal nematodes infection for weaners also varies with farm and district because of the different ecological environment and management. In Urumuqi, seasonal dynamics of gastrointestinal nematodes for weaners is the same as adult sheep, but in the Gongnans, the biggest worm burden found in spring and summer. The seasonal dynamics of gastrointestinal nematodes for weaners in Zhaosu County is quite different, the biggest worm burden is found in spring and autumn, and the lowest worm burdens are found in the summer.

Collecting faecal samples from the rectum to count the number of eggs per gram of faeces by McMaster method is the commonest way to diagnose and quantitate the infection of nematode infection. In research on seasonal dynamics of nematode infection and recovery of alimentary nematodes, main parts of gastrointestinal tract are removed from slaughtered sheep, nematodes are isolated and identified using recommended methods.

Great concern has been paid in effective control of nematode infection in Xinjiang. The basic principle of nematode control is “prevention first and putting more emphasis on prevention than cure”. Grazing management, strategic drenching and adequate nutrition constitute a series of comprehensive preventive measurements of nematode control recommended to sheep farmers in Xinjiang.

In recent years, a strategic drenching programme has been recommended in the northern regions of Xinjiang. This programme consists of two drenches. The first is given when sheep enter winter grazing pasture in December or January, where the worms infected in summer and autumn pasture are removed and the development of nematode eggs will be retarded due to cold weather. The second drench is given before sheep leave summer pasture in August or September in order to prevent the appearance of a peak in nematode infection.

At the present time in Xinjiang, the control of gastrointestinal nematodes in sheep still relies on the use of anthelmintic drugs. In the 1950s, phenothiazine (antiverm) and copper sulphate were used as de-wormers. Trichlorophene was used in the 1960s. Prevention programmes against gastrointestinal nematodiasis in sheep began in 1970s when levamisole, tetramisole and morantel were widely used in China. Albendazole was used for nematode control in 1980s in Xinjiang, which is still the most important and widely used anthelmintic. The use of macrocyclic lactone and its derivatives as anthelmintic started in middle of 1990s. This class

of compounds were first commercially produced in China in 1995 and are now widely used in the control of animal parasite infections. Preparation of tablets, injection, pour-on and pastes made of ivermectin have been used in control of internal parasites and ectoparasites.

In order to improve control efficacy and reduce labour intensity for drenching, some more economical and effective preparations have been developed. Sustained released bolus of albendazole are very efficient in the control of gastrointestinal nematodes of sheep for 100 days and are now available.

Control of gastrointestinal nematodes results in significant economic benefits. It was estimated that average body weight increases 2.6—10.8%, wool production increases 1.7% for each sheep and death rate decreases 3.83—10.02% when a strategic drenching programme was put into use.

In recent years, consumers have increasingly more attention to quality of animal products. In response to the tendency, veterinary researchers and animal producers begin to use alternative methods to reduce the usage of anthelmintic in control of parasite infection. Biological control, integration of anthelmintic with grazing management, rotation of grazing in fenced pastures and monitoring of egg count before deworming, are all these ideals and measurements for parasite control begin to accepted by more and more people.

STUDIES ON BIOLOGICAL CONTROL OF NEMATODE PARASITES OF SHEEP AT URUMUQI

This project was aimed at assessing the effect of providing fungal supplement during the critical late summer and autumn period to sheep grazing on pastures, with the intention of limiting parasitic infections later in the season.

Experimental Site and Animals

The experiment was conducted at a site located 65kms south of Urumuqi. The pastures mainly consisted of Barbate needlegrass (*Festuca valesiaca*) and Smooth bromegrass. On 26 April 2001 an area of uniform topography was fenced off into two equal areas, each of approximately 2.5 ha. One of these plots was designated as the biological control (Fungal) treatment, and the other was designated as the untreated (Control) group. Twenty, crossbred semifine wool sheep, averaging 4.5 months of age were used in both phases of the study. These are allocated 10 per plot and designated as the “Seeder” and “Tracer” animals for the first and second phases of the experiment, respectively. First phase started at end of April. These animals were treated according to the schedules outlined below, and grazed the experimental site for 42 days.

Following the initial 42 days of the trial, the “Seeder” animals were removed from the trial. These were replaced by the “Tracer” group of similarly aged sheep, which received no supplementation and grazed for a further 42 days.

Parasitology

The “Seeder” animals were given avermectin (made in China Agricultural University Pharmaceutical factory containing 10mg/ml avermectin, (0.2ml/10kg bodyweight) 7 days before they entered the plots. Ten days after drug administration, all experimental sheep were artificially infected with 3000 infective larvae consisting predominately of *Haemonchus contortus*, which were derived from faeces of locally infected sheep and cultured by the method described by Henriksen and Korsholm(1983). Faecal samples were collected from all “Seeder” animals every 7 days after larval infection for nematode faecal egg counts according to standard procedures and expressed as eggs per gram of faeces (epg). In addition 3 days after the commencement of fungal feeding, 3g individual faecal samples from all sheep of both groups was pooled and incubated at 26C for 10 days before infective larval recovery, to estimate the success of nematode eggs to develop through to the infective larval stage.

The “Tracer” sheep were also treated with avermectin 5 days prior to allocation to the experimental plots and the same parasitological procedures, described above, were continued. Throughout the study, grass samples from both plots were collected every two weeks by standard procedures and infective larvae were isolated and estimated by method described by Tembely (1998).

Fungal Source and Administration

The Danish isolate of *D. flagrans* was used in these studies. The fungus was grown on autoclaved cultures of moist wheat grains according to the methods described by Larsen and Faedo (1998). Numbers of chlamydospores / g of dried grains were estimated according to the procedures described by Larsen et. al. (1995). On day 45 after artificial infection with third-stage larvae of *H. contortus*, all sheep in the Fungal group were fed the wheat supplement containing 1×10^6 chlamydospores of *D. flagrans*/kg/body weight/day. The control sheep were fed the same amount of plain wheat on a daily basis. Feed supplementation continued for 42 days.

RESULTS

Weather

Rainfall during the experimental period was approximately 1/3 the long-term average and the driest period for 10 years at this experimental site. A summary of the rainfall are shown in Table 1.

Table 1: Rainfall distribution over the period of experiment from May to September in 2001 compared with the 10-year average (1990 –2000) rainfall (mm)

	May	June	July	August	September
10 year average	91.8	104.9	116.1	70.1	56.3
Rainfall in 2001	41.3	35.5	38.7	30.7	21.6

Faecal Egg Counts and Larval Cultures

Average epg for both groups of “Seeder” animals are shown in Table 2. Highest egg counts (> 400 epg) were recorded 28 days post-infection.

Table 2: Faecal egg counts of “Seeder” Animals

Fungus Group											
Sheep No.	R90	R92	R96	R100	R102	R103	R106	R112	R118	R119	Mean
Mean epg	195	765	465	225	0	120	675	1080	195	255	410

Fungus Group											
Sheep No.	Y69	Y73	Y78	Y87	Y88	Y94	Y96	Y98	Y103	Y116	Mean
Mean epg	435	30	150	660	450	405	645	960	300	225	426

Results of the larval cultures showed the numbers of larvae recovered in the Fungal group reduced by 92% compared with the Control group (see Table 3).

Table 3: Mean infective larvae count detected in mixed faeces from two groups after 72hr. of fungi administration.

	EPG	Larvae	% Development
Control group	435	287	66%
Fungal group	405	23	6%

The faecal samples of the “Tracer” animals were very low for both the Fungal and Control plots throughout the whole study.

Unfortunately the very dry weather that prevailed during the course of this investigation had an adverse effect on the potential of *D. flagrans* to attack the free living stages of the nematode parasites of sheep. Virtually no infective larvae were found on the pastures of both the Fungal and Control group. Similar to the dependence on moisture for nematode eggs to develop through a series of free living stages to result in the infective larvae which migrate to herbage

in water films, nematophagous fungi require moisture for germination, and spreading on faecal substrates. However it was demonstrated that the locally produced fungal supplement had the ability to pass through the gut of animals and germinate and capture large numbers of infective larvae in dung samples cultured under ideal conditions of temperature and humidity. This confirms the studies elsewhere and indicates that the potential of nematophagous fungi to be used as a biocontrol agent against nematode parasites of small ruminants is also achievable under conditions found in the Urumuqui region. Further studies are planned to conduct further evaluations of this concept of nematode parasite control of small ruminants in China.

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QUESTION AND ANSWER SESSION

Q: Dr V.Venturina

- i) Factors affecting the worm burden include the stocking rate. In the description of the trial that you conducted, it seemed as though stocking rates were high.

Q: Dr M. Larsen

- i) Would the ideal time for treatment in Xinjiang be from June – Sept as the helminthiasis picture varies from season to season?
- ii) Would experiments need to be repeated at different seasons according to the climate and management?

A: Dr Wang Z.C.

This could be done to assess the effect of helminthiasis during different seasons.