Article 31

Biological Control of Helminth Parasites by Predatory Fungi

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Biological control of animal parasites could become a strong arm for Integrated Parasite Control in the very near future. Though various nematode-destroying fungi received attention, predominantly on academic interest, from the 18th Century in Scandinavian countries, work on their application to control animal parasites gathered momentum from 1990's. The philosophy behind biological control is to utilise one or more of the natural enemies of the nematodes, making it possible to reduce the infection on pasture to a level where grazing animals can avoid both clinical and subclinical effects of the parasitic nematodes. The important requirement is the presence of the fungi in the faecal pats where the development of the pre-parasitic larvae takes place. Therefore, to be effective, the fungi should pass through the gastrointestinal tract of the host without loss of viability. The fungi, Duddingtonia flagrans and Verticillium chlamydosporium, which can be isolated from organic environment of India produces thick walled chlamydospores, the stage responsible for their survival during passage through the gut of ruminants following oral administration. The results had indicated survival of the fungus during gastrointestinal transit in grazing animals and successful reduction of numbers of parasitic nematode larvae on pasture. The dose of fungal spores to be given to an animal and the time of administration for effective parasite control has been standardised. The fungus behaves in density dependent manner and appears to be environment-friendly. The challenge lies ahead in its field application.

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INTRODUCTION

During the last 10-15 years there has been an increasing emphasis on the need of development of new alternative to or supplements for chemical control of parasitic nematodes in grazing livestock. The background for this interest is multi-factorial but the major reason is the serious development of anthelmintic resistance in parasitic populations. Anthelmintic resistance involving particularly the gastrointestinal nematodes of small ruminants is escalating globally and is the single most important concern of parasitologists around the world since it threatens the survivability of small ruminant farming as well as the helminthologists. The problem is most severe in the countries of southern hemisphere like South Africa (Van Wyk et al., 1997), Australia (Waller et al., 1995a), New Zealand (Kettle et al., 1983) and many other Latin American countries (Waller et al., 1995b).

India is slowly and steadily emerging as the resistance epicenter of South Asia (Sanyal, 1998). The global tempo of development and extent of anthelmintic resistance in helminths of small ruminants in particular, indicates that the numerous anthelmintics and strategies developed and implemented over the period of last 40-50 years have been incorrectly applied (Van Wyk, 2001). Other reasons include handling of parasite problems in the organic livestock production, regulation of conventional drug use by legislation, political and consumer pressure for reduced chemical residues in products. Sutherst (1986), among others, has talked about the importance of implementing integrated parasite control based upon host resistance, immunization and non-living vaccines to reduce the use of chemotherapy. To become sustainable, parasite control schemes need to be based on the principles of integrated pest management (Waller, 1993). Towards this objective, significant advances have recently been made in the development of worm vaccines (Emery, 1996; Smith, 1997), in the breeding of animals for parasite resistance (Woolaston and Baker, 1996) and biological control exploiting predacious fungi (FAO, 2002).

PARASITE CONTROL MEASURES

Gronvold et al. (1996) have suggested two major groups of control measures that are in use and would be tried in future.

Chemical Control	**Non-Chemical Control**
Chemotherapy	Biological Control
Spraying of Poison	Worm Vaccines
Use of Repellants	Selection for Host Resistance
Use of Pheromones	Grazing Management
	Nutritional Management
	Inter-Specific Competition
	Sterile Male Technique

PRESENT STATUS OF DIFFERENT CONTROL STRATEGIES

Chemical Control

Three crucial reasons for opting alternative parasite control strategies including biological control are drug resistance, residues on food and environmental degradation. Frequent and haphazard use, over-reliance on chemicals are the causes of the drug resistance. As resistance to newer anthelmintics develops, there is a need for control measures alternative to chemotherapy. Chemical residues in foods are now a major concern and a strong driving force for reduced chemical inputs in agriculture. Consumers increasingly demand that food supply should be free from contaminants of all kinds. Unlike organochlorine ectoparasiticides, residues are not a major problem for anthelmintics. The benzimidazoles and their prodrugs are subjected to close scrutiny because some are known teratogens. The environmental impact of anthelmintics has been generally regarded as un-important. Levamisole and the benzimidazoles pose little cause of concern, but there is greater worry regarding the avermectins. There is evidence of adverse effects on a variety of dung colonizing insects.

Worm vaccines

Within the last decade, much effort has been directed at the development of a vaccine especially against Haemonchus contortus, either based upon naturally exposed or hidden antigens (Smith, 1997). Despite promising results and mass appraisal over the years, a commercial product is still to be released. Dictol, based on infective Dictyocaulus viviparous larvae attenuated by irradiation, is the only marketed vaccine against GI nematodes but it has only a very limited distribution.

Grazing Management

Grazing management strategies have been demonstrated to be useful to alleviate the impact of GI nematodes in livestock (Barger, 1999; Stromberg & Averbeck, 1999). Unfortunately, these strategies have not been adopted to their full extent, perhaps due to the ease for the farmer to use drugs and secondly, the increased demand for land, which makes this proposition less likely in many intensive livestock systems. Where it is used it is often in combination with chemotherapy. In organic livestock production these strategies are widely used, but are primarily based upon the availability of herbage rather than an active measure to control problems with GI nematodes (Thamsborg et al 1999).

Breeding for Resistant Host

Breeding for resistance in host animals to GI nematodes has been attempted with some success (Kloosterman et al 1992; Woolaston & Baker, 1996; Gray, 1997), but although breeding programmes are promoted and adopted based upon these principles (e.g. Nemesis in Australia, Worm FEC in New Zealand), they are far from widely implemented.

Nutritional Management

This is now a well-established fact that supplementation of the diet with additional protein does not appear to affect initial establishment of nematode infection but the patho-physiological consequences are generally more severe on lower planes of protein nutrition. The main effect of protein supplementation is to increase the rate of acquisition of immunity and increase resistance to re-infection and this has been associated with an enhanced cellular immune response in the gastro-intestinal mucosa. Studies on the influence of nutrition on the expression of genotype have shown that the benefits of a superior genotype are not lost on a low protein diet whereas a high protein diet can partially ameliorate the disadvantages of an inferior genotype (Coop and Holmes, 1996). Although many aspects of the interaction between nutrition and helminth parasites have been established many features remain to be examined. 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 seems to be one of such alternate control strategy.

CONCEPT OF BIOLOGICAL CONTROL

This area of research has attracted increasing attention, especially within the last 10-12 years. Biological control is defined as the action of natural enemies which maintain a host population at levels lower than would occur in the absence of enemies. This not only includes classical un-exploited organisms but also those that are genetically modified to enhance these properties (Waller and Faedo, 1996). Biological control is divided into two major categories, viz., natural and applied. Natural biological control is affected by native or co-evolved natural enemies in the environment without human intervention. Within the environment, the pre-parasitic stages of nematodes are subjected to a variety of both abiotic and biotic factors that can profoundly influence their development and survival. The most important abiotic factors are temperature; humidity and oxygen-extremes in these can be lethal on the free-living stages. In regard to biotic factors, there exists a vast assemblage of living organisms that can affect the success of worm eggs developing larvae. From these may emerge candidate(s) for biological control of worm parasites. By definition, biological does not assume to be a substitute for chemotherapy, where the expectation, if not the reality, is that parasites may be eradicated by frequent use of drugs with efficacies approaching 100%. Biological control agents really eliminate the target organism, but reduce the number of expectable levels and maintain a balance between the pathogen and the antagonist. In contrast to chemical control of nematode parasites, which is directed entirely at the parasitic stage within the host, biological control will almost certainly be focused on the free-living stages of parasites on pasture.

The intention of using biological control methods is to lower the density of pest population below the clinical level and perhaps below the economic threshold above which production losses are obvious owing to a high parasitic population density.

Agents for Biological Control

All gastro-intestinal nematode parasites of livestock have a life-cycle which involves not only the parasitic stage within the host, but also a free-living or pre-parasitic stage on pasture. The pre-parasitic stages on the pasture are potentially vulnerable to attack by biological control agents. A number of organisms have been identified to exploit the free-living stages of parasites as food source and are likely to be commercially exploited in the near future. These organisms include micro-arthropods, protozoa, predacious nematodes, virus, bacteria and fungi. Although the all are of intrinsic interest, it is from the last group of organism that breakthroughs in biological control of nematode parasites of livestock are likely to emerge.

FUNGI AS BIOLOGICAL CONTROL TOOL

Fungi that exhibit anti-nematode properties have been known for a long time. They consist of a great variety of species characterized by their ability to capture and exploit nematodes either as the main source of nutrients or supplementary to a saprophytic existence. They are divided into three major groups based on their morphology and types of nematode-destroying apparatus (Barron, 1997; Nordbring-Hertz, 1988).

Predacious Fungi

They produce specialized nematode-trapping structures (adhesive knobs, networks, rings etc.) on the mycelium. The idea of possibly using predacious micro-fungi to control animal nematodes arose in the 1930's. It was not until the mid 1980s before thorough and systematic investigations were undertaken and since then, two lines of work can be clearly distinguished.

Trials performed with mainly with *Arthobotrys* spp. (*A. oligospora*) and *Monacrosporium* spp. as biological control agents. A group of Danish researchers, testing the effect of fungus *A. oligospora* primarily against parasitic nematodes in cattle but also in other livestock species. Testing different doses of spores mixed into faeces, 250 and 2500 conidia per gm of faeces was found to significantly lower the number (70 & 99% reduction, respectively) of developing *C. oncophora* larvae in faecal cultures (Gronvold et al. 1985). The trapping activity of the fungus was influenced by the motility of the infective larvae & there is no specificity for the parasitic species (Nansen et al. 1996). Unfortunately various trials performed to test *A. oligospora* mycelium and conidia failed due to the destruction of these structures in the GI tract of the host animals (reported in Gronvold et al. 1993 a, b). A high dose (between 470 & 680 gm of fungal material on millet) of one of the three different fungal species (*A. musiformis, A. tortur, Dactylaria candida*) was fed to housed lambs, harboring a mono infection of either *H. contortus* or *O. circumcincta*. This subsequently led to survival Of *A. tortur* through the GI tract at a level high enough to significantly reduce the number of *H. contortus* in faecal cultures.

The other line of research is with *Duddingtonia flagrans*. This predacious fungus produces three dimensional, sticky networks on its growing hyphae. It also produces an abundance of intercalary thick walled resting spores, chlamydospores. This fungus is relatively slow growing and as with other predacious fungi growth is strongly influenced by temperature (Fernandez et al. 1999e). Many other species of predacious fungi are fast growing but the spores of these fungi are much more sensitive to the stress of the GI tract than that of the chlamydospores of *D. flagrans*. In plot trials *D. flagrans* have shown good reduction of free living larval stages of parasitic nematodes of cattle (Gronvold et al. 1993a, b), sheep (Peloille, 1991) and horses (Fernandez et al. 1997, 1999a; Baudena et al. 1999). These field trials have shown that daily feeding of fungal spores to grazing animals for 3-4 months prevents build-up of dangerous levels of infective larvae on the pasture. In an Australian study Knox and Faedo (2001) found that sheep feed supplement containing *D. flagrans* chlamydospores had lower egg counts and improved liveweight gains compared to untreated animals.

Endo-parasitic Fungi

These invade nematodes either by penetration of cuticle from sticky spores adhering to the cuticle or following ingestion of spores which lodged in the gut. This type of fungi is obligate parasite of nematodes, with very limited capacity to develop outside the prey and density dependent (Jaffe et al, 1993). This has led researchers to believe that there might be better or stronger BC candidate against pest nematodes. *Drechmeria coniospora* is a fungus producing sticky drops on very small conidia, which adhere to the cuticle of the nematode, penetrate the cuticle and destroy the victim. By applying a very high dose (108 conidia per gm of faeces) to faecal cultures, Santos & Charles (1995) found that only infective third stage parasite larvae stripped of the protective extra (second stage) cuticle, became infected by the fungus. Another endoparasitic fungus, *Harposporium anguillulae* produce very small, half moon shaped conidia which lodge in the digestive tract of the feeding nematode and after germination totally digest the victim before finally breaking through the cuticle to produce new conidia on the short conidiophores. In a laboratory study it was found that at a dose of 3 lakh conidia/ gm faeces, the number of *H. contortus* larvae recovered was significantly reduced (Charles et al, 1996). The requirement of spore dispersion or infection to be more or less directly from one infected individual to the next, severely limits or almost excludes the use of this group of fungi as practical BC agents.

<u>Egg-parasitic Fungi</u>

These have the ability to attack the egg stage and may have a role in the control of animal parasites which have a long development and/or survival time in the egg stage in the environment outside host, e.g., *Ascaris*, *Fasciola* spp., amphistomes etc.

Eggs of *Ascaris lumbricoides* collected from naturally infected pigs were used to test the effect of mainly the fungus *Verticillium chlamydosporium* but also other *Verticillium* spp. the fungus was shown to be able to degrade the egg shell enzymatically and infect the eggs (Lysek & Krajci, 1987; Lysek & Sterba, 1991; Kunert, 1992). Short exposure to high temperature or UV- irradiation rendered the eggs more susceptible to fungal attack (Lysek & Bacovsky, 1979). In the USA, Chien and colleagues have shown that *V. chlamydosporium* attacked and destroyed eggs of *Ascaridia galli* and *Parascaris equorum* but only rarely invaded *Trichuris suis*. In Denmark works were done on the predacious fungi *Arthobotrys* spp. and egg parasitic fungi *Paecilomyces lilacinus* for activity against eggs of *T. canis*. *P. lilacinus* showed some activity (16% eggs infected after 7 days) but the predacious species did not attack the eggs.

PRESENT STATUS

<u>Present Global Status</u>

As a result of renewed interest and intensified research in biological control during the last 15 years, a convincing amount of evidence on the potential of this principle has been gathered (Larsen, 2002). Most of this work has been carried out in Europe (Denmark, Sweden, UK and France) and Australia and recently initiated in USA, Latin America, Africa, South-east Asia and Far East. Among the nematode trapping fungi, *Duddingtonia flagrans* has displayed superior abilities with respect to survival through gastro-intestinal transit as well as subsequent destruction of parasitic larvae in faecal pats (Larsen, 2002). Danish scientists first demonstrated through laboratory and field trials, that biological control against pre-parasitic stages of nematodes could be achieved by feeding chlamydospores of this fungus to cattle (Gronvold et al., 1993), horses (Fernandez et al., 1997), pigs (Nansen et al., 1996) and sheep (Githigia et al., 1997). Successful pilot scale trials with *D. flagrans* chlamydospores through feed supplement (Knox and Faedo, 2001), feed blocks (Waller et al., 2001a) and slow release devices (Waller et al., 2001b) provide sufficient euphoria for commercial exploitation of fungal delivery devices in future integrated parasite control programmes. Besides being safe for animals and man, it is imperative that new technologies dealing with BC need to be of no negative impact to the grazing environment. Short time impact studies have shown no negative effect of the fungus on earthworms (Gronvold et al., 2000) and on soil nematodes (Yeates et al.1997; Faedo, 2001). The future appears to be very promising in early outcome of a bio-control product to control nematode parasite of livestock.

<u>Present Indian Status</u>

India initiated the work on biological control of animal nematode parasites using mycological means in 1998 and two species of nematode-trapping fungi, viz., *Arthrobotrys oligospora* and *D. flagrans* and two species of egg parasitic fungi, viz., *Paecilomyces lilacinus* and *Verticillium chlamydosporium* were isolated from organic environment of Gujarat and Chhattisgarh (Sanyal, 2005; Sanyal et al., in press). They were subjected to stringent screening for their suitability as biocontrol agents against nematode parasites of ruminants using growth assay, predatory activity, germination potential and ability to survive ruminant gut passage. The study indicated that the isolates of *D. flagrans* and *V. chlamydosporium* fulfilled all the possible criteria. A strategy is formulated for application of nematode-trapping fungi to control gastrointestinal nematodosis of ruminants (Sanyal et al., 2005). he available classical and alternate technologies should properly be integrated similar to those practiced in the integrated pest control in agriculture. Biological control seems to be one of such alternate control strategy.

CONCLUSION

There is an increasing awareness that in future the parasitic control programme should reduce reliance on chemical anthelmintics. Compared to the other non-chemotherapeutic approaches to parasitic control in ruminant livestock, use of nematode trapping fungi has shown promising results in Denmark, Australia & India. This has been well exemplified through both in vitro and in vivo studies resulting in reduced translation of larvae to the herbage from faecal pats and reduced worm burdens in livestock. As integrated sustainable control strategies would be the modus operandi to control the parasitic gastro-enteritis in livestock both in conventional and organic farming system, mycological control would be arm for integration with both non-chemical and chemical means.

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