JOURNAL OF ANIMAL SCIENCE
The Premier Journal and Leading Source of New Knowledge and Perspective in Animal Science

Biological control of nematode parasites in sheep

M. Larsen

J Anim Sci 2006. 84:E133.

Biological control of nematode parasites in sheep[1]

M. Larsen[2,3]

Department for Veterinary Pathobiology, Danish Centre for Experimental Parasitology,
The Royal Veterinary and Agricultural University, 100 Dyrlægevej, DK-1870 Frederiksberg C, Denmark

ABSTRACT: In a world in which sheep producers are facing increasing problems due to the rapid spread of anthelmintic resistance, the battle against gastrointestinal parasitic nematodes is a difficult one. One of the potential new tools for integrated control strategies is biological control by means of the nematode-destroying microfungus *Duddingtonia flagrans*. This fungus forms sticky traps that catch developing larval stages of parasitic nematodes in the fecal environment. When resting spores (chlamydospores) of this fungus are fed daily to grazing animals for a period of time, the pasture infectivity and thus, the worm burden of grazing animals are lowered, especially in young lambs. Research has been conducted throughout the world covering many different climates and management systems. An Australian parasite model showed that if the fungus performs efficiently (≥90% reduction in worm burden) for 2 or 3 mo, it should contribute significantly to a reduction in the number of dead lambs otherwise occurring when managed only by anthelmintic treatment and grazing management. Feeding or field trials have clearly demonstrated that dosing with a few hundred thousand spores per kilogram of live BW not only reduced the number of infective larvae but also increased the BW of the lambs compared with controls not given fungus. Initial Australian work with feeding spores by means of a block formulation or a slow-release device has shown some promise, but further work is needed to fully develop these delivery systems. In tropical Malaysia, small paddock trials and field studies resulted in significant improvements, in terms of lower worm burdens and increased live BW, when feeding half a million spores daily to grazing lambs. Additional benefits have been observed when the fungus is employed in combination with a fast rotational grazing system. Research has also demonstrated that spores can be delivered in slightly moist feed block material, but only if such blocks are consumed rapidly, because of their very short shelf life. In the northern, temperate Danish climate it has been demonstrated that daily feeding of half a million spores per kilogram of live BW can lead to significant production benefits, with increased live BW gain in fungus-exposed animals. Biological control of parasitic nematodes in sheep seems to hold promise for the future, but to be able to assist producers, the optimal delivery system needs to be refined and further developed. In addition, more work will be needed to define the best use of this technology in different geographic regions.

Key words: biological control, parasite, nematode, sheep

 J. Anim. Sci. 2006. 84(E. Suppl.):E133–E139

INTRODUCTION

Parasitic nematodes compromise welfare and cause serious production losses in small ruminants on pasture throughout the world (Perry and Randolph, 1999). Anthelmintic resistance in gastrointestinal (**GI**) nematode populations constitutes a major problem to grazing livestock globally (Sangster, 1999; Jackson and Coop, 2000; Besier and Love, 2003; Kaplan, 2004). The time from introduction to resistance appears to be less than 10 yr for all 3 major drug classes (benzimidazoles, imidazothiazoles- tetrahydropyrimidines, and avermectins-milbemycins; kaplan, 2004). Due to the continued threat posed by anthelmintic resistance, work has been carried out to find new supplements or alternatives to chemical treatment. Restrictive legislation regarding the chemical control of parasites in organic livestock production together with a wish to eliminate drug residues in agricultural products are additional driving forces for the interest in alternative parasite control methods. A novel alternative method for control of parasitic nematodes is biological control (**BC**). Smarter use of available broad-spectrum drugs, grazing management, breeding for resistance or tolerance, use of bioac-

[1]Invited review. Presented at the "Management of Gastrointestinal Nematodes in Sheep" symposium held at the American Society of Animal Science Annual Meeting, Cincinnati, OH, July 24–28, 2005.

[2]Corresponding author: aph@post8.tele.dk

[3]Current address: Tyborøn Alle 23B, DK-2720 Vanløse, Denmark.

Received August 24, 2005.

Accepted December 2, 2005.

tive forages, combined with BC can be used against a wide range of nematode species. In contrast to this, narrow-spectrum tools such as certain species-specific anthelmintics, copper oxide wire particles (Burke et al., 2004), the FAMACHA chart (a chart developed on the basis of color of the eye membrane mucosa, grading animals according to level of anemia; van Wyk and Bath, 2002), and vaccines (Knox, 2000) all primarily target infections with the "barber pole" worm, *Haemonchus contortus*. Of BC agents, the group of nematode-destroying microfungi has been investigated almost exclusively, and among those only one, namely *Duddingtonia flagrans*, has shown any real promise when tested in animals.

PRINCIPLES AND SOME BACKGROUND FOR BIOLOGICAL CONTROL

The philosophy behind BC is that by using one of the natural enemies of nematodes, it will be possible to reduce the infection level on pasture to a level at which the grazing animals avoid both clinical and subclinical effects due to parasitic nematodes. Although no BC agent will eliminate the number of infective stages to zero, the grazing animals, such as sheep, will constantly receive a small amount of parasitic larvae and thereby should be able to develop a natural immune response.

To successfully develop a product for use in an integrated BC strategy, a number of criteria have to be met, such as survival through GI tract, able to grow and be active in dung, cheap to produce, and safe to handle. Of these, the ability to survive passage through the GI tract of the animals and subsequently germinate in the voided dung to attack the developing larvae have been the prime goals for the selection of candidates among the nematode-destroying fungi (Larsen et al., 1991, 1992, 1994; Waller and Faedo, 1993; Waller et al., 1994; Faedo et al., 1997; Llerandi-Juárez and Mendoza-de Gives, 1998; Chandrawathani et al., 2002). Unfortunately, some candidate fungi are reported to hold great potential as a BC agent, based only on their performance in laboratory or plot tests, without having fed spores to test animals before performing these tests. This can lead to overly optimistic interpretations with respect to their potential as BC agents. Unless some reliable reduction level can be reached repeatedly after fungal spore material has been through test animals (e.g., cattle or sheep), the potential of such candidates as BC agents cannot be fully evaluated.

The nematode-destroying fungi belong to a heterogeneous group of microfungi that utilize nematodes either as the main source of nutrients, or supplementary to a saprophytic existence (Barron, 1977). These fungi are found worldwide in many different habitats, but are especially frequent in organically rich environments such as compost and feces. Deposited fresh feces are quickly colonized by various species of these fungi (Hay et al., 1997a,b; Bird et al., 1998; Saumell, 1998; Saumell et al., 1999). Fungi are naturally picked up by grazing cattle and sheep and subsequently excreted in the voided feces (Hashmi and Connan, 1989; Larsen et al., 1994; Manueli et al., 1999; Saumell et al., 1999; Chandrawathani et al., 2002).

Duddingtonia flagrans was selected based on its ability to survive passage through the GI tract of ruminants and subsequently to trap the developing parasite larvae in the deposited feces (Larsen et al., 1991, 1992; Peloille, 1991). This fungus is well suited because of its ability to produce an abundance of thick-walled resting spores, the chlamydospores, which are resistant to the stress of the GI tract of livestock. After passing through the animal, the spores germinate in feces, forming specialized, 3-dimensional sticky networks that trap the developing parasite larval stages. This principle has been tested in all livestock species except chickens and was found to be promising in all species (for review, see Larsen, 2000). Research on *D. flagrans* in small ruminants, predominantly sheep, has been carried out on all continents (e.g., Peloille, 1991; Larsen et al., 1998; Mendoza-de Gives et al., 1998; Faedo et al., 1998, 2000, 2002; Chandrawathani et al., 2002, 2003, 2004; Fontenot et al., 2003; Waghorn et al., 2003; Waller et al., 2004), and has demonstrated the potential of this organism as a BC agent against the free-living stages of parasitic nematodes under experimental and seminatural conditions. The research has focused on some of the most important (pathogenic) nematode species: *H. contortus*, *Teladorsagia circumcincta* (both parasites in the abomasum), *Trichostrongylus colubriformis*, and to some extent, *Nematodirus* spp. (both parasites in the small intestine).

In the following sections, selected research performed by scientists under well-defined and distinctly different climates will be presented: Australia in the subtropical summer rainfall zone to temperate uniform or winter rainfall zones; Malaysia representing a tropical climate; and finally, Denmark in the northern temperate climate zone.

BIOLOGICAL CONTROL OF PARASITIC NEMATODES IN AUSTRALIA IN A SUBTROPICAL TO TEMPERATE CLIMATE

In 1995, Barnes and colleagues used a parasite model developed for *T. colubriformis* infection in the northern New South Wales (winter rainfall zone) to calculate the potential effect of implementing a BC strategy with the fungus (Barnes et al., 1995). Under normal conditions with 3 anthelmintic treatments per year and movement of lambs to safe pasture at weaning, 15 of every 1,000 lambs born would die due to nematode infections. If the fungus was active for 90 d and could reduce the worm burden of the animals by 75% for 90 d, then the estimated number of dead lambs under this management system would be reduced by 73%. If the reduction in worm burden were 90%, then the reduction in dead lambs would be 87 and 43% if the effect of the fungus lasted for 90 or 60 d, respectively. To expect a 90%

reduction in worm burden might be asking a lot, but the example shows that at a reasonable level of worm control due to added fungus (75% reduction in worm burden), the outcome is still significant. With fast spreading multiple resistance in Australia, this might become a valuable adjunct in an integrated control strategy applied to make production sustainable in the long term.

In a dose-titration trial in which the effect of each dose was measured as the percentage development of infective-stage larvae in fecal cultures, Waller et al. (2001b) found that development in cultures with feces from untreated control animals varied between approximately 60 and 100% [number of larvae from measured number of eggs per gram (**EPG**) of feces], whereas the yield dropped to almost zero when feeding a dose of 4, 9, or 13 million spores/animal. With an estimated weight of lambs (6- to 9-mo-old Merino wethers) of 40 kg, this is approximately equivalent to doses of 100,000, 200,000, and 350,000 spores/kg of BW. In a similar trial in the United States, Pena et al. (2002) tested a different strain of *D. flagrans*, and found a >94% reduction in number of developing larvae in cultures from lambs fed ≥250,000/kg of BW. In a dose-titration trial performed with penned goats, Terrill et al. (2004) found ≥80% reduction when feeding 100,000, 250,000, or 500,000 spores/kg of BW.

In a follow-up field trial, Knox and Faedo (2001) tested the effect of feeding 2 million fungal spores/animal daily for 6 mo to 6- to 9-mo-old Merino lambs on pasture. They found that there was huge variation between the intake (mean monthly percentage consumption) in the fungus-treated groups (3 groups/treatment, 10 animals/group), which meant that it was not until after the first 3 to 4 mo that all groups consumed almost equivalent high amounts of supplement with spores, the effect of which was also reflected in the egg excretion from the lambs. Here, the significant difference observed between the treatment groups was most pronounced during the last half of the trial (July to October), when the level in the fungus groups was around 800 EPG, whereas that in the control groups was between 2,000 and 2,400 EPG. The recorded monthly live weight gains were significantly greater (approximately 6 kg for each month from May to October) in the fungus-treated groups compared with the control animals (starting at 6 kg in May and falling to 4 kg in October). These trials, in addition to others testing various means of deploying the spores (blocks and slow-release devices) confirm the potential for implementation of BC by means of *D. flagrans* in at least some climatic zones in Australia.

MALAYSIA—BIOLOGICAL CONTROL OF PARASITIC NEMATODES IN THE TROPICS

In Malaysia, a small paddock trial (Chandrawathani et al., 2003) and 2 large field trials on government farms (Chandrawathani et al., 2004) confirmed the potential for the BC concept in the tropics. In all trials, animals were housed from late afternoon until the following morning, during which time they were fed either supplement or in one case, a small block formulation, with or without fungal spores. In addition, a management strategy was adopted with some form of rotational grazing.

In the paddock trial, 10 *H. contortus*-infected sheep per group were assigned to 1 of 3 treatment groups: a urea molasses block without fungal spores (controls), a urea molasses block with an amount of spores estimated on the expected block consumption to be equivalent to 0.5 million/kg of BW, and a pelleted supplement mix fed to achieve intake of 0.5 million spores/kg of BW. The animals grazed for 5 to 7 d on each of the 3 subplots allocated for each treatment group before being moved to the next subplot. The most significant results were the reduced percentage development of larvae in fecal cultures from fungus-treated animals (from <1% to 20%) compared with what was observed in the cultures from the control animals (development between about 30 to 75%). At the same time, EPG was lowered in the fungus groups. The effect of this was clearly reflected in the 5 sets of back-to-back tracer animals (2 per paddock) that were turned out on the 3 treatment paddocks: The total *H. contortus* burden per tracer animal was very low (from 0 to 56 worms) compared with the tracers on the control paddock (number of worms between 90 and 1,280). Testing a similar principle on a government research farm showed that there was an additional effect of feeding fungal spores daily, adding to the practiced fast rotational grazing system (3 to 4 d/subpaddock, 10 subpaddocks per treatment group, total cycle of 35 d). Because the test was performed under an unusual dry weather for the latter one-half to two-thirds of the trial, the transmission of larvae was kept at a low level, which was reflected in steadily falling levels of EPG in both treatment groups (control group went from ~2,000 to 500 EPG), but for the last 6 mo of the trial, the EPG level was much lower in the fungus-treated group (only 20 to 25% of that observed in the control animals). At the end of the trial, the average BW was greater ($P = 0.054$) in the fungus-treated group (34.6 kg) compared with the control animals (30.8 kg), with both groups having started at the same mean level (21.2 and 20.9 kg, respectively). At the same time, the 17 sets of back-to-back tracer animals used to monitor pasture infectivity showed greater worm burdens after grazing the control paddocks than after grazing the fungus paddocks. The latter showed less than 100 worms/animal, whereas the controls harbored from 150 to 500 worms.

These examples demonstrate that BC can assist in the control of *H. contortus* under the tropical Malaysian climate and grazing management systems. It should be added that providing the pelleted supplement mix to animals for 12 mo will most likely not be economically viable, but timing of strategic use of fungus feeding (e.g., in two 3-mo periods) needs to be tested. The severe situation with widespread multiple anthelmintic resistance on the other hand, does require implementation of additional nonchemical methods to make production of small ruminants sustainable.

BIOLOGICAL CONTROL OF PARASITIC NEMATODES IN DENMARK

Githigia et al. (1997) reported on a field study using *D. flagrans* spores mixed in the daily supplement for control of sheep nematodes. Two groups of set-stocked Dorset-crossbred lambs were turned out on a naturally infected pasture, and allocated to 4 different paddocks (2 fungus-treated and 2 control groups). The fungus-treated groups received 1 million spores/kg of BW daily mixed in 100 g of barley; the other 2 groups received only the barley. In fecal cultures from fungus-treated animals it was found that the percentage development of *Teladorsagia* and *Trichostrongylus* spp. larvae was significantly lower (1 to 28%; $P < 0.05$) compared with the percentage development in cultures from control animals (60 to 80%). Due to outbreak of parasitic gastroenteritis in all groups, all animals were treated with anthelmintics in July. The pasture larval infectivity remained very low (under 1,000 infective larvae/kg of DM) throughout spring and summer until the end of August when levels of *Teledorsagia* and *Trichostrongylus* spp. larvae, as well as *Nematodirus* spp., increased to 4,400 (*Teledorsagia* and *Trichostrongylus* spp.) and 11,600 (*Nematodirus* spp.) larvae/kg of DM, respectively, in mid-September, after which the level dropped off until the end of October. The level of infective larvae was markedly lower on the fungus-treated pastures for both *Teledorsagia—Trichostrongylus* and *Nematodirus* spp. larvae (930 and 7,200 larvae, respectively). In October 4 lambs were turned out as tracers on each of the 4 paddocks. The lambs grazed for 3 wk, were housed for additional 3 wk, and then slaughtered. At the time of slaughter, the mean EPG of the fungus tracers was 283; significantly lower ($P < 0.05$) than for the control tracers (708 EPG). The total worm burden was reduced by 86% ($P < 0.05$) in the fungus tracer lambs, whereas abomasal worm species were reduced by 68% ($P < 0.05$) and *Nematodirus* spp. by over 95% ($P < 0.05$) compared with the control lambs. This trial showed that, during a relatively dry grazing season, feeding the fungus supplement could reduce the number of larvae on pasture. Moreover, the difference in infective larval numbers on pasture had a significant impact on subsequent acquisition of parasites by tracers grazing these paddocks. It is not clear, due to the slightly different transmission pattern compared with other trichostrongyles, whether the observed reduction in numbers of *Nematodirus* spp. larvae is to be expected after such fungus supplement feeding during spring and early summer.

From 2002 to 2004, 3 field trials were carried out as part of a European Union-funded project (Larsen et al., 2005) to test the potential effect of daily fungus treatment by mixing spores with supplement provided to grazing ewes with twin lambs at foot. Ewes are normally housed until late April or early May, depending upon the time of lambing, and subsequently turned out on pasture within a week after lambing. Two treatment groups were used: fungus-fed and controls; both treatment groups were replicated to avoid possible paddock effect. The animals grazed each of 4 paddocks on a 0.6-ha clover grass pasture. Fungus treatment was 0.5 million *D. flagrans* chlamydospores/kg of BW mixed with a small amount of pellet supplement. The length of the fungus treatment was 12, 11, and 10 wk for the 2002, 2003, and 2004 seasons, respectively.

In 2002 when a naturally infected pasture was used for turn-out of naturally infected Texel ewes, the limitation of the fungus treatment was observed. The ewes showed relatively high EPG values (between 400 and 1,100) due to the periparturient relaxation shortly after turn-out in May. By mid-June the values for the ewes dropped to practically zero (i.e., they regained their normal immune status). However, the first half of the summer was warm and moist, and the second half was hot; these conditions were favorable for *H. contortus*, which appeared in high numbers in sets of tracer animals as well as in fecal cultures. The greatest counts (more than 9,000 larvae/kg of DM) were found on one of the control paddocks by mid-July, but the level of infectivity on the other control pasture could not be distinguished from the 2 fungus paddocks (<1,000 larvae/kg of DM). By the end of July, all animals had to be treated due to the presence of animals with clinical gastroenteritis in all groups. The lack of difference between the treatment groups for the pasture infectivity was also repeated for the EPG of the lambs (controls: 2,000 to 5,000 EPG; fungus-treated: 3,000 to 5,000 EPG by mid to late July); live weights showed no difference between treatment groups. From the results of the 2002 trial, we concluded that the effect of the fungus treatment is impaired if infected ewes were turned out on infected pasture.

Based upon the 2002 results, it was decided in 2003 and 2004 to use ewes cleared for nematodes by anthelmintic treatment during housing in winter. The same 2 paddocks used for fungus treatment in 2002 were used for this treatment the following 2 years. In 2003, 7 ewes with twin lambs were introduced; in 2004, 10 ewes with twins were used. Fungus dosing (0.5 million spores/kg of BW) was performed for approximately 12 wk beginning shortly after turn-out in May. There were marked differences in climate between the 2 years: the 2003 season was characterized by a very wet May, hot June and July, and dry August to October (with half the normal precipitation); 2004 was characterized by much cooler temperatures (average below normal for June and July), but with a very wet June and July (30% greater precipitation than normal). Therefore, 2003 was very conducive for parasites, which was reflected in greater pasture infectivity. For both years we found greater (but nonsignificant) EPG levels in lambs on control pastures compared with those provided with fungus. Despite the fact that not all parasitological parameters were showing significant differences between treatment groups, we found significant differences in production, namely greater BW gain in the groups grazing pastures where fungus treatment was conducted compared with the control animals in both years. In 2003, the mean BW was 14.5% greater for the lambs on the fungus treatment paddocks ($P = 0.0005$, repeated measures ANOVA, SAS GLM procedure on log-transformed data; SAS Institute, Cary, NC). In 2004, we recorded a 22% greater BW in the fungus-fed lambs ($P < 0.0001$, repeated measures ANOVA, SAS GLM procedure on log-transformed data) compared with the controls.

A preliminary conclusion from these trials must be that feeding fungus to ewes with lambs at foot daily has a positive production benefit. However, when introducing this technology, the focus should be on preventing the overall infection pressure in the early part of the grazing season. In this study, this was achieved by treating housed ewes during the winter so that clean animals were turned out on naturally infected pasture. Despite the fact that the chosen management system (set-stocked with ewes removed at weaning) is not the norm for sheep producers (would move lambs to a safe pasture at weaning), even this high-challenge environment benefited from the implemented fungus supplementation. That similar feeding in early spring can benefit lambs later in the season has been demonstrated in on-farm trials carried out in parallel in the European Union's program under the normal management system used on the island of Gotland in Sweden (Waller et al., 2004).

FORMULATION AND IMPLEMENTATION OF *D. FLAGRANS*

The trials involving *D. flagrans* were performed with spores given with a daily supplement, but initial work on feed blocks (Waller et al., 2001b; Chandrawathani et al., 2002, 2003) as well as slow-release devices (**SRD**; Waller et al., 2001a) highlights the potential of employing these technologies for deployment of spores to grazing animals. Mixing spores into a block (mineral or nutrient block, such as the urea molasses block) could be one approach, but in the case of a mineral block, the daily intake of grazing animals would be very variable, and would depend on the time of the grazing season. In the early part of the grazing season, when animals graze lush fresh growing pasture, the need for (and thus, intake of) block material would be very low, but that is exactly the time when guaranteed uptake of spore material is needed if high levels of contamination are to be prevented later in the season. A nutrient block such as the urea molasses block could be used but then animals would be housed or kept in small enclosures for some time during the day to allow access to the block. Also, due to a relatively high level of moisture in such a block, the shelf life will be extremely short (less than a week), because fungal spores would start to germinate and become vulnerable to destruction during passage through the animals. Waller et al. (2001a) tested an early type of SRD and showed that the reduction in developing larvae could be achieved if enough live material could be contained in such a device for the time needed. However, those authors also showed that there might be huge individual animal variation, something that needs to be covered by the daily level of viable spores released from such a device.

With a fully developed SRD with incorporated fungal spores, BC will become available as an element in sustainable control strategies in which animals are raised in extensive grazing systems. Whether only one fungus SRD will be sufficient to supply spores during the critical period on pasture will depend on both the technology (how many spores can be incorporated in a single SRD) and the local epidemiological situation. In the conventional livestock industry, a combined SRD system containing fungal spores released at a constant rate, and an anthelmintic drug, released once or perhaps twice during the season, could be an option during the first year for introduction of BC on permanent grazed pastures. For the following season or production cycle, a pure fungus bolus could be used. Whether this principle will work in practice will need to be thoroughly tested, and managed according to the local overall epidemiological situation.

In the future, to cope with the problems caused by the parasitic nematodes in grazing sheep in both organic and conventional production systems, it will be necessary to implement integrated, sustainable control strategies. These will have to consist of existing nonchemical options (grazing management), as well some newly developed approaches (FAMACHA, resistant animals, bioactive forages, biological control, and perhaps, vaccines) in combination with appropriate use of existing drugs. Parasites are here to stay, but with BC using the nematode-destroying fungus *D. flagrans* as a tool in integrated control strategies it may be possible to continue to manage parasitic nematodes. This approach will reduce the reliance on chemical treatments, preserving these for when needed in treatment of animals that become sick due to parasites.

IMPLICATIONS

The nematode-trapping fungus *Duddingtonia flagrans* has the potential to reduce the number of infective, parasitic nematode larvae developing on pasture. This will subsequently reduce the contamination of pasture and thereby, the risk of loss due to worm infection in grazing animals such as sheep. Together with grazing management, refined use of existing drugs, and other new tools, biological control can become an important factor in maintaining a sustainable system of sheep production in the future.

LITERATURE CITED

Barnes, E., R. Dobson, and I. Barger. 1995. Worm control and anthelmintic resistance: Adventures with a model. Parasitol. Today 11:56–63.

Barron, G. L. 1977. The nematode-destroying fungi. Topics in Mycobiology. No. 1. Canadian Biological Publications Ltd., Guelph, ON, Canada.

Bird, J., M. Larsen, P. Nansen, H. O. Kragelund, J. Grønvold, S. A. Henriksen, and J. Wolstrup. 1998. Dung-derived biological agents associated with reduced numbers of infective larvae of equine strongyles in fecal cultures. J. Helminthol. 72:21–26.

Besier, R. B., and S. C. J. Love. 2003. Anthelmintic resistance in sheep nematodes in Australia: The need for new approaches. Aust. J. Exp. Agric. 43:1383–1391.

Burke, J. M., J. E. Miller, D. D. Olcott, B. M. Olcott, and T. H. Terrill. 2004. Effect of copper oxide wire particles dosage and feed supplement level on *Haemonchus contortus* infection in lambs. Vet. Parasitol. 123:235–243.

Chandrawathani, P., O. Jamnah, P. J. Waller, J. Höglund, M. Larsen, and W. M. Zahari. 2002. Nematophagous fungi as a biological control agent for nematode parasites of small ruminants in Malaysia: A special emphasis on *Duddingtonia flagrans*. Vet. Res. 33:685–696.

Chandrawathani, P., O. Jamnah, P. J. Waller, M. Larsen, and A. T. Gillespie. 2004. Field studies on the biological control of nematode parasites of sheep in the tropics, using the microfungus *Duddingtonia flagrans*. Vet. Parasitol. 120:177–187.

Chandrawathani, P., O. Jamnah, P. J. Waller, M. Larsen, A. Gillespie, and W. M. Zahari. 2003. Biological control of nematode parasites of small ruminants in Malaysia using the nematophagous fungus *Duddingtonia flagrans*. Vet. Parasitol. 117:173–183.

Faedo, M., E. H. Barnes, R. J. Dobson, and P. J. Waller. 1998. The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep: Pasture plot study with *Duddingtonia flagrans*. Vet. Parasitol. 76:129–135.

Faedo, M., M. Larsen, S. O. Dimander, G. W. Yeates, J. Höglund, and P. J. Waller. 2002. Growth of the fungus *Duddingtonia flagrans* in soil surrounding feces depositied by cattle or sheep fed the fungus as a means of controlling the free-living stages of nematode parasites. Biol. Control 23:64–70.

Faedo, M., M. Larsen, and S. M. Thamsborg. 2000. Effect of different times of administration of the nematophagous fungus *Duddingtonia flagrans* on the transmission of ovine parasitic nematodes on pasture—a plot study. Vet. Parasitol. 94:55–65.

Faedo, M., M. Larsen, and P. J. Waller. 1997. The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep: Comparison between Australian isolates of *Arthrobotrys* spp. and *Duddingtonia flagrans*. Vet. Parasitol. 72:149–155.

Fontenot, M. E., J. E. Miller, M. Larsen, and A. Gillespie. 2003. Efficiency of feeding *Duddingtonia flagrans* chlamydospores to grazing ewes on reducing availability of parasitic nematode larvae on pasture. Vet. Parasitol. 118:203–213.

Githigia, S. M., S. M. Thamsborg, M. Larsen, N. Kyvsgaard, and P. Nansen. 1997. The preventive effect of the fungus *Duddingtonia flagrans* on trichostrongyle infections of lambs on pasture. Int. J. Parasitol. 27:931–939.

Hashmi, H. A., and R. M. Connan. 1989. Biological control of ruminant trichostrongylids by *Arthrobotrys oligospora*, a predacious fungus. Parasitol. Today 5:28–30.

Hay, F. S., J. H. Niezen, C. Miller, L. Bateson, and H. Robertson. 1997a. Infestation of sheep dung by nematophagous fungi and implications for the control of free-living stages of gastro-intestinal nematodes. Vet. Parasitol. 70:247–254.

Hay, F. S., J. H. Niezen, S. Ridley, L. Bateson, C. Milller, and H. Robertson. 1997b. The influence of pasture species and time of deposition of sheep dung on infestation by nematophagous fungi. Appl. Soil Ecol. 6:181–186.

Jackson, F., and R. L. Coop. 2000. The development of anthelmintic resistance in sheep nematodes. Parasitology 120:S95–S107.

Kaplan, R. M. 2004. Drug resistance in nematodes of veterinary importance: A status report. Trends Parasitol. 20:477–481.

Knox, D. P. 2000. Development of vaccines against gastrointestinal nematodes. Parasitology 84:S43–S61.

Knox, M. R., and M. Faedo. 2001. Biological control of field infections of nematode parasites of young sheep with *Duddingtonia flagrans* and effects of spore intake on efficacy. Vet. Parasitol. 101:155–160.

Larsen, M. 2000. Prospects for controlling animal parasitic nematodes by predacious micro fungi. Parasitology 120:S120–S131.

Larsen, M., M. Faedo, and P. J. Waller. 1994. The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep: Survey for the presence of fungi in fresh feces of grazing livestock in Australia. Vet. Parasitol. 53:275–281.

Larsen, M., M. Faedo, P. J. Waller, and D. R. Hennessy. 1998. The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep: Studies with *Duddingtonia flagrans*. Vet. Parasitol. 76:121–128.

Larsen, M., P. Nansen, J. Grønvold, J. Wolstrup, and S. A. Henriksen. 1992. In vivo passage of nematophagous fungi selected for biocontrol of parasitic nematodes in ruminants. J. Helminthol. 66:137–141.

Larsen, M., S. M. Thamsborg, and H. Mejer. 2005. Experiences from Danish field trials to evaluate the effect of the biological control agent *Duddingtonia flagrans*, fed to ewes with lambs at foot. Addendum in Proc. 4th Int. Conf. Novel Approaches to the Control of Helminth Parasites of Livestock, Merida, Yucatan, Mexico. Universidad Autonoma de Yucatan, Merida, Mexico.

Larsen, M., J. Wolstrup, Sv. Aa. Henriksen, C. Dackman, J. Grønvold, and P. Nansen. 1991. In vitro stress selection of nematophagous fungi for biocontrol of pre-parasitic nematode larvae of ruminants. J. Helminthol. 65:193–200.

Llerandi-Juárez, R. D., and P. Mendoza-de Gives. 1998. Resistance of chlamydospores of nematophagous fungi to the digestive processes of sheep in Mexico. J. Helminthol. 72:155–158.

Manueli, P. R., P. J. Waller, M. Faedo, and F. Mahommed. 1999. Biological control of nematode parasites of livestock in Fiji: Screening of fresh dung of small ruminants for the presence of nematophagous fungi. Vet. Parasitol. 81:39–45.

Mendoza-de Gives, P., J. Flores Crespo, D. Herrera-Rodriguez, V. M. Vazquez-Prats, E. Liebano Hernandez, and G. E. Ontiveros Fernandez. 1998. Biological control of *Haemonchus contortus* infective larvae in ovine feces by administering an oral suspension of *Duddingtonia flagrans* chlamydospores to sheep. J. Helminthol. 72:343–347.

Pena, M. T., M. E. Fontenot, J. E. Miller, A. Gillespie, and M. Larsen. 2002. Evaluation of the efficacy of *Duddingtonia flagrans* in reducing infective larvae of *Haemonchus contortus* in feces of sheep. Vet. Parasitol. 103:259–265.

Peloille, M. 1991. Selection of nematode-trapping fungi for use in biological control. Pages 13–17 in IOBC/WPRS Bulletin 1991/XIV/2, Working Group "Integrated control of soil pests—Methods for studying nematophagous fungi." B. R. Kerry and D. H. Crump, ed. 10BC/WPRS Bull. 14(2):13–17.

Perry, B. D., and T. F. Randolph. 1999. Improving the assessment of the economic impact of parasitic diseases and of their control in production animals. Vet. Parasitol. 84:145–168.

Saumell, C. A. 1998. Colonização de bolos fecais bovinos e fezes ovinas por fungos nematófagos e sua o corrência em fezes frescas bovinas na Zona de Mata de Minas Gerais (Nematophagous fungi in cattle and sheep feces in the Mata region of Minas Gerais State, Brazil, and their occurrence in fresh cattle feces), Ph.D. Thesis, Universidad Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.

Saumell, C. A., T. Padilha, C. P. Santos, and M. V. C. Roque. 1999. Nematophagous fungi in fresh feces of cattle in the Mata region of Minas Gerais state, Brazil. Vet. Parasitol. 82:217–220.

Sangster, N. C. 1999. Anthelmintic resistance: Past, present and future. Int. J. Parasitol. 29:115–124.

Terrill, T., M. Larsen, O. M. Samples, S. Husted, J. E. Miller, C. Mathews, R. M. Kaplan, and S. Gelaye. 2004. Capability of the nematode-trapping fungus *Duddingtonia flagrans* to reduce infective larvae of gastrointestinal nematodes in goat feces in the southeastern United States: Dose titration and dose time interval studies. Vet. Parasitol. 120:285–296.

Van Wyk, J. A., and G. F. Bath. 2002. The FAMACHA system for managing Haemonchosis in sheep and goats by clinically identifying individual animals for treatment. Vet. Res. 33:509–529.

Waghorn, T. S., D. M. Leathwick, L.-Y. Chen, and R. A. Skipp. 2003. Efficacy of the nematode-trapping fungus *Duddingtonia flagrans* against three species of gastro-intestinal nematodes in laboratory fecal cultures from sheep and goats. Vet. Parasitol. 118:227–234.

Waller, P. J., and M. Faedo. 1993. The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep: In vitro and in vivo studies. Vet. Parasitol. 51:289–299.

Waller, P. J., M. Faedo, and K. Ellis. 2001a. The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep: Towards the development of a fungal controlled release device. Vet. Parasitol. 102:299–308.

Waller, P. J., M. R. Knox, and M. Faedo. 2001b. The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep: Feeding and block studies with *Duddingtonia flagrans*. Vet. Parasitol. 102:321–330.

Waller, P. J., M. Larsen, M. Faedo, and D. R. Hennessy. 1994. The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep: In vitro and in vivo studies. Vet. Parasitol. 51:289–299.

Waller, P. J., O. Schwan, B. L. Ljungström, A. Rydzik, and G. W. Yeates. 2004. Evaluation of biological control of sheep parasites using *Duddingtonia flagrans* under commercial farming conditions on the island of Gotland, Sweden. Vet. Parasitol. 126:299–315.