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Nematode-Trapping Fungi in Biological Control of *Dictyocaulus viviparus*

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[1]Danish Veterinary Laboratory, Copenhagen, [2]Danish Centre for Experimental Parasitology, Department of Veterinary Microbiology, and [3]Section of Zoology, and [4]Section of Microbiology, Department of Ecology and Molecular Biology, The Royal Veterinary and Agricultural University, Frederiksberg, Denmark.

Henriksen, Sv. Aa., M. Larsen, J. Grønvold, P. Nansen and J. Wolstrup: Nematode-trapping fungi in biological control of *Dictyocaulus viviparus*. Acta vet. scand. 1997, 38, 175-179. – Larvae of the cattle lungworm *Dictyocaulus viviparus* were cultured in experimental units of 200 g cattle faeces placed in semi-transparent trays in the laboratory. In each of 4 experimental series using this experimental unit, chlamydospores (chl) of the nematode-trapping fungus *Duddingtonia flagrans* were admixed to half of the faecal cultures in a concentration of 50.000 chl/g. In all 4 series there was a significant reduction in the development and subsequent release of infective lungworm larvae from faecal cultures containing chlamydospores. The average reduction in larval release, caused by fungal spores, was 86%.

parasites; lungworm; cattle.

Introduction

The nematode-trapping fungus *Duddingtonia flagrans* is able to trap migrating nematodes in three-dimensional adhesive mycelial nets (*Cooke* 1969). *D. flagrans* only produces traps when it is induced to do so, e.g. by physical contact with migrating nematodes.

In field experiments, a *D. flagrans* strain has been able to control free-living stages of a range of parasitic gastrointestinal nematodes of cattle, horse and pig when fungal spores were fed to the host animals (*Grønvold et al.* 1993, *Wolstrup et al.* 1994, *Larsen et al.* 1995, 1996, *Nansen et al.* 1995, 1996). But, sofar, biological control of the cattle lungworm *Dictyocaulus viviparus* by *D. flagrans* has not been tested neither in the laboratory nor in the field.

Earlier on, another nematode-trapping fungus, *Arthrobotrys oligospora,* has been tested against *D. viviparus* larvae in agar cultures in the laboratory (*Nansen et al.* 1988). The results showed that slow-moving nematodes, such as *D. viviparus* larvae, have poor trap-inducing potentials. But if traps were induced by other nematodes, *D. viviparus* larvae were easily trapped in the pre-formed *A. oligospora* nets.

The cattle lungworm *D. viviparus* is ovoviviparous and excretes larvae in faeces. Under normal summer temperatures in Denmark the infective third stage larvae (L_3) is reached within one to 2 weeks in the cow pats. In contrast to gastrointestinal nematodes, it has been indicated that the coprophilous fungi *Pilobolus* spp. may play an important role in transporting the slow-moving infective *D. viviparus* larvae from faeces to herbage from where they can be taken up by susceptible grazing calves (*Jørgensen et al.* 1982).

In relation to a possible biological control effect

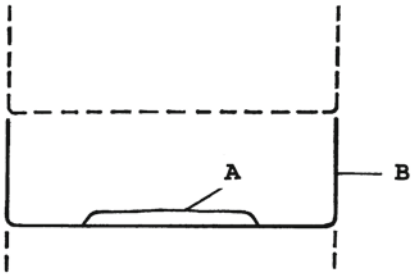

Figure 1. Schematical cross-section of the experimental design. A: Faecal sub-sample containing infective larvae of *Dictyocaulus viviparus* and with or without admixture of the nematode-trapping fungus *Duddingtonia flagrans*. B: Transparent tray.

of *D. flagrans* on *D. viviparus,* the following questions become pertinent:
1) Does the presence of *Pilobolus* spp. mycelium allow the nematode-trapping fungus *D. flagrans* to grow in cattle faeces?, and if it does,
2) can the nematode-trapping fungus subsequently be stimulated by the slow-moving *D. viviparus* L_3-larvae to produce traps that can effectively control the cattle lungworm?

Materials and methods

Experimental animals

Two parasite-naive calves, housed in an experimental unit, were each infected (3 larvae per kg bodyweight) with a Danish strain of *D. viviparus*. After the end of the prepatent period fresh faeces containing lungworm larvae were sampled per rectum.

Pilobolus fungi

No particular measures were taken to ensure that the calves excreted *Pilobolus* spp. spores with their faeces, since it is known that calves fed on stable usually excrete *Pilobolus* spores more or less constantly all year round, because their food (hay, straw etc.) naturally contains spores (*Grønvold & Jørgensen* 1985).

Nematode-destroying fungi

From a culture of *D. flagrans* (strain CI3), thick-walled chlamydospores (chl) were harvested in tap water. The chlamydospore-suspension was adjusted to approximately 10^6 chl/ml.

Experimental design

The present study included 4 test series (I-IV) carried out from October to December 1995. The following design was used for all series: From a thoroughly mixed batch of fresh faeces containing lungworm larvae, 6 sub-samples of 200 g were made. Ten ml chlamydospore-suspension were mixed into each of 3 (experimental) faecal sub-samples, resulting in a fungal concentration of approximately 50,000 chl/g. The 3 other (control) faecal sub-samples received 10 ml of tap water. Each faecal sub-sample was placed in individual semi-transparent polystyrene trays (38×28×12 cm) and formed as a small cow pat (diam.: 20 cm; height: 1 cm) with a smooth surface (Fig. 1).
The trays were stacked randomly and the upper tray was supplied with a lid. The set-up was placed in a room with a window, which allowed daylight to hit the faecal sub-samples. Light is necessary for *Pilobolus* to produce fruit bodies and therefore this arrangement allowed us to register if *Pilobolus* was present in the faeces. The room temperature was approximately 20 °C and the incubation period was 8 days. Daily each cow pat was gently sprayed with tap water leaving a thin film of moisture on the faecal surface.
At the end of the incubation period, the surface of each pat and the inside of each tray were rinsed off gently with approximately 50 ml of tap water. The water from each sample was poured into a test tube. Following centrifugation for 10 min at 1500 rpm, the supernatant was discarded, leaving a remnant of 10 ml. From each remnant 2 sub-samples of 0.5 ml were transfer-

Table 1. The number of infective *Dictyocaulus viviparus* larvae released from cow pats with (+) and without (–) admixture of the nematode-trapping fungus *Duddingtonia flagrans,* in each of the 4 series (I-IV) of experiments.

Series	Fungal material		Reduction (%)	Statistics
	+	–		
	65	494		
I	105	1160		
	95	286		
Mean of serie I	88	647	86	*
	799	3150		
II	1520	1743		
	720	5256		
Mean of serie II	1013	3383	70	*
	138	3360		
III	54	4758		
	90	5454		
Mean of serie III	94	4524	98	*
	216	2247		
IV	320	1950		
	360	3712		
Mean of serie IV	299	2636	89	*
Total mean			86%	

Asteriks indicate significant differences in larval count between the 2 groups of cow pats using a the Mann-Whitney U-test. *: $p<0.05$; **:$p<0.01$; ***:$p<0.001$.

red to slides for counting *D. viviparus* larvae. Subsequently, the total number of infective lungworm larvae, released from each cow pat in 8 days, was calculated.

Statistics

Statistical analyses were made by the Mann-Whitney U-test (*Siegel* 1956). The level of significance was 5%.

Results

Table 1 shows that larval counts from the experimental cow pats, which had received chlamydospores of *D. flagrans,* were significantly lower than counts from the control cow pats in particular in series I, III and IV. On average, the addition of the nematode-trapping fungus resulted in a 86% (range:70%-98%) reduced release of infective lungworm larvae from the faeces.

Pilobolus spp. fungi were observed in all trays, but no quantification was attempted.

Discussion

This paper shows that there was a significant reduction in the number of infective lungworm larvae (*D. viviparus*) released from the cow pats containing the nematode-trapping fungus (*D.*

flagrans). On the average, a nematode-trapping efficacy of 86% was observed. In previous experiments, on gastrointestinal trichostrongyles, similar high reductions in *Ostertagia* and *Cooperia* larval numbers were reached as a result of feeding calves with 200×10^6 chlamydospores per animal per day (*Nansen et al.* 1995).

As *Pilobolus* spp. fungi were observed in all series, the present results indicate that *Pilobolus* fungi and *D. flagrans* can coexist in cattle faeces. Moreover, the results indicate that *D. viviparus* larvae can induce trap-production in *D. flagrans* in cattle faeces, as no other nematodes were observed in the faeces. However, future experiments will have to clarify whether the present results were caused mainly by: 1) reduced release of lungworm larvae caused by reduced *Pilobolus* growth in competition with the nematode-trapping fungus, or by 2) the nematode trapping activity of the latter alone.

If the present laboratory observations reflect the field situation reasonably, which is not necessarily the case, dosing of grazing calves with *D. flagrans* chlamydospores might reduce the herbage contamination, perhabs to a level that will prevent clinical manifestations of lungworm infection. According to *Jørgensen* (1981) strategic anthelmintic treatment of calves 6 and 8 weeks after turn-out onto contaminated pasture, was effective in controlling *D. viviparus*. Thus, given the efficacy of nematode destroying fungi as described above, dosing with fungi during the initial 2 months of the grazing season may be sufficient for practical control of bovine dictyocaulosis.

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Sammendrag

Biologisk kontrol af Dictyocaulus viviparus ved hjælp af rovsvampe.

Larver af kvægets lungeorm, *Dictyocaulus viviparus,* blev dyrket i enheder af 200 g kvæggødning placeret i semi-transparente kasser i laboratoriet. I hver af 4 eksperimentelle serier, hvor ovennævnte opstilling blev anvendt, blev chlamydosporer (chl) af den nematode-fangende svamp *Duddingtonia flagrans* tilblandet halvdelen af opstillingerne i en koncentration på 50.000 chl/g. I alle 4 serier var der en signifikant reduktion i spredningen af infektive lungeormlarver fra kulturer tilblandet chlamydosporer. Den gennemsnitlige reduktion i spredningen, forårsaget af chlamydosporer, var 86%.

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