## BIOLOGICAL CONTROL OF NEMATODES IN GOATS BY THE NEMATOPHAGOUS FUNGUS DUDDINGTONIA FLAGRANS

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### Introduction

The high prevalence of nematodes resistant to anthelmintics in French dairy goat flocks and the reduction in the number of anthelmintic treatments allowed per year in the organic production system force to develop new schemes for the control of nematode infections.

Different options are currently studied. Amongst them, the biological control method is based on the use of nematophagous fungi that form traps and consume parasitic nematode larvae in faeces. Administered as chlamydospores to sheep or cattle, the predacious fungus *Duddingtonia flagrans* has demonstrated its ability to highly reduce (over 90%) the developing larvae of a large range of nematodes in faeces (Larsen, 2000).

This method has been tested in goats with different aims :- Efficacy of spore administration in goat faeces in laboratory conditions

- Determination of the optimal dose

- Efficacy in field conditions.

## **Material and Methods**

### Animals

For all the laboratory trials, culled dairy goats were purchased in local farms. For the farm trial, a flock of young kids naturally parasite infected was used.

### Fungus

Spores of the Danish Troll A isolate of *D. flagrans* were produced on millet seeds and packaged in aluminium foil bags by Chr. Hansen Biosystems A/S, Denmark.

Experimental schemes and parasitological procedures

For all the trials, half of the animals received the spores at the daily dose rate of  $5 \times 10^5$  spores/kg BW, except for the dose titration trial, while the other half acted as control. After 6 days of administration, faeces were harvested individually. The egg excretions were determined using a modified McMaster technique. Faeces were then cultured for 12 to 14 days at 23 °C in a climatic chamber. At the end of this time, infective larvae were harvested by Baermann technique.

Specific parts of each trial are detailed afterwards.

- Efficacy in laboratory conditions

The efficacy of *D. flagrans* was determined against the 2 main prevalent nematodes in goats: 2 gastro-intestinal species: *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* (experimental infections).

- Dose titration study

A dose effect relation was investigated using both experimental (*T. colubriformis*) and natural mixed infections (*T. colubriformis*, *T. circumcincta* and *Haemonchus contortus*). The tested doses were  $1.25 \times 10^5$ ,  $2.5 \times 10^5$  and  $5 \times 10^5$  spores/kg BW. 2 ways of administration of spores were used: forced administration and administration at trough.

## - Efficacy in farm conditions

Spores were administered daily at trough to half of the goat kids during the grazing season. Different parameters (weight, egg excretion, pasture infectivity and serum pepsinogen) were monitored during the whole grazing season. Kids were weighed at the beginning, middle and end of the grazing season. The egg outputs were measured every 3 weeks. Blood samplings were performed at the beginning, middle and end of the grazing season in order to measure the serum pepsinogen concentration reflecting the infection by abomasal nematodes. Pasture infectivity was evaluated every 3 weeks by washing the sampled grass and extracting the washed larvae by centrifugations using a sucrose solution.

## Statistical analysis

The data were expressed as mean egg output and larval output per group. The percentage of development of larvae from the faecal samples of the different groups was calculated as the ratio between the total number of larvae and the total number of eggs in the faeces×100. The percentage of reduction of larval development was calculated as (1-(percentage of development in the fungus group/percentage of development in the control group))×100.

The comparison of means was made with the nonparametric Mann and Whitney-test using Systat 9.1 for Windows, 1998, SPSS Inc. (Chicago, USA).

# Results

Efficacy in laboratory conditions

Table 1: mean number of eggs per gram (epg) and larvae per gram (lpg) obtained per group, in faeces of goats infected by T. colubriformis or T. circumcincta and receiving or not the spores

	epg	lpg	development
T. colubriformis			
Fungus treated goats	405 <sup>a</sup>	6,28 <sup>1</sup>	1,5
Control goats	384 <sup>a</sup>	62,30 <sup>2</sup>	16,2
T. circumcincta			
Fungus treated goats	167 <sup>a</sup>	3 1	1,8
Control goats	143 <sup>a</sup>	26 <sup>2</sup>	18,2

The reduction of larval development in goat faeces containing *D. flagrans* spores when compared with the larval development in control faeces was 91 % and 90 %, respectively for *T. colubriformis* and for *T. circumcincta* (table 1).

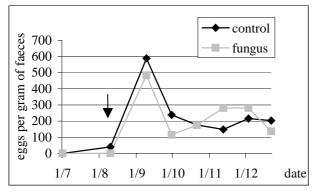
Dose titration study

In laboratory conditions, whatever the dose rate, the larval development reduction was higher than 75 % when compared with a control group and there was no difference between the doses.

On the contrary, with trough administration, a significant increase in the larval development reduction was observed from the  $2.5 \times 10^5$  spores/kg BW/day (83.9 %) to the  $5 \times 10^5$  spores/kg BW/day (97.7 %) when compared to the control. and

#### Efficacy in farm conditions

Figure 1 : evolution of egg outputs in the fungus treated group and in the control group during the grazing season



beginning of administration of spores to kids of the fungus group

None of the parasitological parameters (figure 1 for the egg outputs) showed any statistical difference between the fungus treated and the control groups.

The growth of kids of the 2 groups was the same (mean growth in the 2 groups : 13 kgs).

### Discussion

These trials demonstrated the high ability of Duddingtonia flagrans to trap the infective larvae of gastro-intestinal nematodes in goat faeces. When compared with control faeces, the larval development reduction was 90 % for the 2 species tested, Trichostrongylus colubriformis and **Teladorsagia** *circumcincta* with a daily dose of spores of  $5 \times 10^5$ spores/kg BW. This is in agreement with previous results: with the same dose, Fontenot et al (2003) reported reduction of the larval development of Haemonchus contortus higher than 80 % in sheep faeces.

All the tested doses,  $1.25 \times 10^5$  to  $5 \times 10^5$  spores/kg BW, gave the same reductions in conditions of controlled administration. In goats, Terrill et al (2004) showed a non significant effect with an apparent dose response, the highest dose,  $5 \times 10^5$  spores/kg BW, leading to the highest reduction of the larval development of *H. contortus*, *T. colubriformis* and *Cooperia* spp.. On the contrary, in farm conditions, a dose response was shown in our study.

The minimal dose to recommend appears to be  $5 \times 10^5$  spores/kg BW on a daily basis. A particular attention should be paid to the way of administration in order to limit variations in ingestion: Knox et al. (2001) demonstrated that variations in voluntary consumption may highly influence the efficacy of *D. flagrans* until total failure.

These variations in voluntary consumption may partly explain our results with young kids. When spores were administered at trough during the whole grazing season, we were not able to see any positive effect whatever the parameters. These results agree with other studies in goats : Wright et al., 2003 gave spores to goats at the daily dose rate of  $7.5*10^5$  /kg BW during 26 days. Tracer kids were then introduced during 14 days. The faecal egg counts of the groups receiving or not the spores showed no significant difference nor the pasture larval counts. However, these authors demonstrated an effect of the administration of spores on the latter contamination of the pasture as the worm burdens of the tracer kids were significantly reduced in the group grazing the fungus treated pasture.

### Conclusion

If the efficacy of *D. flagrans* against nematode larvae in controlled conditions of administration and culture has been demonstrated, its efficacy under field conditions should be further evaluated. Cost efficiency and environmental impact of daily feeding of fungal spores should be also evaluated before marketing *D. flagrans* spores as a method of nematode control.

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