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Veterinary Parasitology 103 (2002) 259–265

veterinary  
parasitology

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## Evaluation of *Duddingtonia flagrans* in reducing infective larvae of *Haemonchus contortus* in feces of sheep

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Received 31 July 2001; received in revised form 26 September 2001; accepted 27 September 2001

### Abstract

Consequences of nematode infections due to *Haemonchus contortus* are a serious constraint for the sheep industry worldwide. Development of anthelmintic resistance and increasing concern about the impact of anthelmintic use dictate the need of alternative control. Such an alternative is using the nematode trapping fungus *Duddingtonia flagrans* to reduce infective larvae levels on pasture. Two trials were conducted to determine the effect of *D. flagrans* in reducing infective larvae (predominantly *H. contortus*) in feces. The first trial determined the dose effect of *D. flagrans* in reducing infective larvae in feces. Eighteen ewes were dewormed to remove existing infections and randomly assigned to six treatment groups:  $5 \times 10^4$ ,  $1 \times 10^5$ ,  $2.5 \times 10^5$ ,  $5 \times 10^5$ ,  $1 \times 10^6$  or no (control) spores of *D. flagrans* per kg of body weight mixed in their feed for 7 days. Fecal samples were collected daily from these and from infected donor ewes. Feces from individual-treated ewes were mixed with equal amounts of donor ewe feces, theoretically approximating oral dose spore concentrations of  $2.5 \times 10^4$ ,  $5 \times 10^4$ ,  $1.25 \times 10^5$ ,  $2.5 \times 10^5$ ,  $5 \times 10^5$  and no spores, and were cultured. Across dosages and during the 7 days of fungus feeding, percent reduction of infective larvae ranged from 76.6 to 100.0%. The second trial determined the effect of *D. flagrans* at the dose of  $10^5$  spores per kg body weight on reducing infective larvae in feces from naturally infected lambs. Twenty lambs were randomly assigned to either treatment or control groups based on fecal egg count.

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Treatment lambs were fed spores mixed in feed for 7 days. Feces were collected daily and cultured. During the 7 days of fungus feeding, the percent reduction of infective larvae ranged from 82.8 to 99.7%. Results of these trials demonstrated that the nematode trapping fungus *D. flagrans* was highly effective in reducing infective larvae in sheep feces and should be considered as a biological control agent for integrated nematode control programs. © 2002 Elsevier Science B.V. All rights reserved.

**Keywords:** Nematode; Sheep; Fungus; *Duddingtonia flagrans*; Biological control

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## 1. Introduction

In sheep production, controlling gastrointestinal nematodes can be a challenge. The economic losses and animal health issues that these parasites cause are of constant concern. The traditional method of nematode control has been the use of anthelmintics. When anthelmintics are the primary method of control, resistance becomes widespread (Sangster, 1999). Resistance occurs when nematode populations that are normally susceptible to anthelmintic treatment lose that susceptibility through selection for resistant nematodes. Surviving resistant nematodes then contribute resistance genes to future generations. The problem of anthelmintic resistance is now present for all classes of anthelmintics used to control nematodes; therefore, it is necessary to pursue other control methods.

Biological control is one option where one organism is used to gain control of another target organism (Thamsborg et al., 1999). Nematode trapping fungi are a group of microfungi that can produce a sticky network of mycelia to catch and destroy preparasitic nematodes (Barron, 1977; Nansen et al., 1988; Waller and Faedo, 1993; Gives et al., 1998). The nematophagous fungus that is well suited for gut passage is *Duddingtonia flagrans* because it produces thick-walled chlamyospores (Larsen et al., 1994; Faedo et al., 1997). The question of the number of fungal spores needed to produce substantial larval reduction has also been addressed (Larsen et al., 1998). Several studies have been conducted to determine the larval reduction in feces and pasture areas by *D. flagrans* as summarized by Larsen (1999). Those trials involving sheep have been conducted in areas where infections are predominantly *Teladorsagia (Ostertagia) circumcincta* and *Trichostrongylus* spp., and fecal egg counts (FECs) are relatively low. The objective of this study was to determine the efficacy of *D. flagrans* in reducing infective larvae (predominantly *Haemonchus contortus*) in feces of sheep where relatively high FEC conditions are routinely encountered.

## 2. Materials and methods

### 2.1. Trial 1—dose titration

#### 2.1.1. Animals and treatments

Eighteen Suffolk ewes were dewormed with doramectin (Dectomax<sup>®</sup>, 0.2 mg/kg) and albendazole (Valbazen<sup>®</sup>, 10 mg/kg) to remove existing infections. Ewes were randomly assigned to six treatment groups and placed in individual concrete floor pens. All animals

were fed a pelleted maintenance ration and water was provided ad libitum. Ewes in the six treatment groups were given  $10^6$  (Group 1),  $5 \times 10^5$  (Group 2),  $2.5 \times 10^5$  (Group 3),  $10^5$  (Group 4),  $5 \times 10^4$  (Group 5) or no (Group 6, control) chlamydospores of *D. flagrans* per kg of body weight for 7 days. Spore material was provided (Christian Hansen A/S, (Copenhagen) Hørsholm, Denmark) in pre-packaged amounts based on the weight of individual animals. Spore material was mixed with approximately 20–25 g of the pelleted maintenance feed to which a small amount of water was added to enhance adherence. Each animal was observed to note consumption of their spore/feed mixture, after which they were given their normal daily ration of the pelleted maintenance feed.

Two additional ewes were used as donors of feces containing nematode eggs.

### 2.1.2. FEC and cultures

Fecal samples were collected directly from the rectum of all animals on a daily basis for 14 days commencing on the first day spores were fed. FEC was determined using the modified McMaster technique (Whitlock, 1948). Feces from the donors were collected daily and pellets were thoroughly mixed together. Three random samples were taken from the fecal mixture and the FEC was determined by the mean of the three counts. Individual animal fecal cultures were performed, in triplicate, where 10 g of fungus-treated or control feces were mixed with 10 g of donor feces in a 100 ml plastic cup which had holes in the bottom for aeration. Theoretically, this gave resulting oral dose spore concentrations of  $5 \times 10^5$ ,  $2.5 \times 10^5$ ,  $1.25 \times 10^5$ ,  $5 \times 10^4$ ,  $2.5 \times 10^4$  and no spores for Groups 1–6, respectively. An approximately equal volume of vermiculite was mixed with the feces and water was then added to make a moist crumbly culture. The top of the cup was wrapped with cheesecloth and turned upside down into a larger cup. The larger cup contained a small amount of water to provide humidity and the cultures were left at room temperature (24 °C). After 10 days, the larger cup was filled with warm water in which the fecal culture material was immersed. After 6 h of baermannization, the small cup containing the culture material was removed and the culture material was discarded. Cups were left standing for a minimum of 1 h to allow infective larvae ( $L_3$ ) to settle and then the water was carefully suctioned off to leave a volume less than 10 ml. The water and residue containing  $L_3$  was thoroughly mixed and then transferred to a 15 ml centrifuge tube. The cup was washed with an additional 3–4 ml of water and added to the tube. The contents of the tube were allowed to sediment for 1–2 h and then the water was carefully siphoned off to a volume of 1 ml. The contents were then thoroughly mixed and two 100  $\mu$ l aliquots were taken and each was placed on a microscope slide and covered with a cover slip. The  $L_3$  were counted by microscope at 100 $\times$  power. The number of  $L_3$  per 100  $\mu$ l was the mean of the two aliquot counts and the total number of  $L_3$  recovered was estimated by extrapolation. The total number of  $L_3$  was divided by 20 to get  $L_3$  per gram of feces. The first 100  $L_3$  encountered in each sample were identified to genus.

### 2.1.3. Percent reduction

The percent reduction for each *D. flagrans* spore dose was calculated as

$$\left[ \frac{\text{control mean } L_3 - \text{fungus mean } L_3}{\text{control mean } L_3} \right] \times 100$$

## 2.2. Trial 2—target dose

### 2.2.1. Animals and treatments

Twenty Suffolk lambs were removed from pasture and placed in individual concrete floor pens. They were fed a growth ration and water was provided ad libitum. The lambs were paired from the lowest to the highest FEC and one of each pair was randomly assigned as either treatment or control. The lambs in the treatment group were fed  $10^5$  *D. flagrans* spores per kg of body weight mixed in their feed for 7 days. The target dose of  $10^5$  spores was selected as being the minimal dose that maintained high reduction in Trial 1.

### 2.2.2. FEC and cultures

Fecal samples were collected directly from the rectum of all animals on a daily basis for 8 days commencing on the first day spores were fed. FEC was determined using the modified McMaster technique. Individual fecal cultures using 10 g of feces were performed on a daily basis as described in Trial 1. After 10 days,  $L_3$  were recovered and counted as described in Trial 1.

### 2.2.3. Percent reduction

The percent reduction was calculated as in Trial 1.

## 3. Results

### 3.1. Trial 1—dose titration

For days 1–6 of the trial, the FEC of mixed donor and treated sheep ranged from 6200 to 10 075 eggs per gram, and for days 7–14, the range was from 2966 to 4341 eggs per gram. Recovery of  $L_3$  on day 1 was relatively equal among groups (Table 1). On day 2, reduction of  $L_3$  was greater than 98.5% in all groups except Group 5 (lowest spore concentration) which was 80.9%, and by day 3, reduction was greater than 97.5% in all groups. The reduction remained greater than 93.4% in all groups through day 8 except on day 6, when it was 0% for Group 5. Of the three animals in Group 5 on that day, one had 0  $L_3$ , one had 13  $L_3$  and one had 4250  $L_3$ . On day 10, two days after the fungus feeding stopped, reduction had dropped substantially in all groups and remained low for the remainder of the trial. *Haemonchus* was the predominant genus (97–100%) with a few *Cooperia*.

### 3.2. Trial 2—target dose

FEC remained high and fairly consistent among groups throughout the trial where the fungus-treated group ranged from 27 510 to 60 315 eggs per gram and the control group ranged from 31 300 to 58 495 eggs per gram (Table 2). Percent larval reduction increased by day 2, reached the highest levels on days 4 (96.4%), 5 (99.7%), 6 (94.9%) and 7 (95.3%), and decreased on days 8 and 9 after the fungus feeding was stopped. As in the first trial, *Haemonchus* was the predominant genus (97–100%) with a few *Cooperia*.

Table 1  
Percent reduction<sup>a</sup> (%R) of mean number of infective larvae (L<sub>3</sub>) per gram of feces in a mixture of feces from nematode infected sheep and nematode free sheep fed six different concentrations of *D. flagrans* chlamydispores

Group <sup>b</sup>	Day <sup>c</sup>																										
	1 (L <sub>3</sub> )	2	3	4	5	6	7	8	9	10	11	12	13	14													
	L <sub>3</sub>	%R	L <sub>3</sub>	%R	L <sub>3</sub>	%R	L <sub>3</sub>	%R	L <sub>3</sub>	%R	L <sub>3</sub>	%R	L <sub>3</sub>	%R	L <sub>3</sub>	%R											
1	2600	29	99.0	25	97.5	0	100.0	17	95.8	13	99.0	8	99.7	25	98.9	100	94.8	963	69.2	733	46.0	3263	3.1	2604	10.5	800	27.3
2	1867	8	99.7	0	100.0	0	100.0	0	100.0	8	99.4	88	96.8	13	99.4	92	95.2	971	68.9	592	56.4	2763	17.9	2529	13.0	621	43.5
3	3000	4	99.9	0	100.0	0	100.0	0	100.0	0	100.0	0	100.0	0	100.0	8	99.6	983	68.5	1713	0.0	3746	0.0	2279	21.6	1221	0.0
4	2750	46	98.5	8	99.2	8	99.6	0	100.0	0	100.0	21	99.2	29	98.7	113	94.1	667	78.7	1404	0.0	2558	24.0	2879	1.0	1267	0.0
5	2695	583	80.9	25	97.5	4	99.8	13	96.8	1421	0.0	163	94.1	146	93.4	838	56.6	2646	15.3	1150	15.3	2488	26.1	2808	3.4	708	35.6
6	3813	3050	-	1005	-	2021	-	408	-	1333	-	2763	-	2196	-	1929	-	3125	-	1358	-	3367	-	2908	-	1100	-

<sup>a</sup> [(control L<sub>3</sub> - fungus L<sub>3</sub>)/control L<sub>3</sub>] × 100.

<sup>b</sup> Resulting spore concentration: Group 1:  $5 \times 10^5$  spores per kg body weight; Group 2:  $2.5 \times 10^5$ ; Group 3:  $1.25 \times 10^5$ ; Group 4:  $5 \times 10^4$ ; Group 5:  $2.5 \times 10^4$ ; Group 6: no spores (control) ( $n = 3$  per group).

<sup>c</sup> 1: day fungal spore feeding started; 7: last day fungal spores fed.

Table 2

Mean  $\pm$  SEM FEC (per gram of feces), infective larvae count ( $L_3$ ) per gram of feces and percent larval reduction in feces from lambs ( $n = 10$ ) fed  $10^5$  chlamydo spores per kg body weight of the nematode-trapping fungus, *D. flagrans* compared to feces from control lambs (no spores,  $n = 10$ )

Day <sup>a</sup>	Fecal egg count		$L_3$		Percent reduction <sup>b</sup>
	Fungus	Control	Fungus	Control	
1	31 435 $\pm$ 3955	31 970 $\pm$ 7187	6108 $\pm$ 1570	6540 $\pm$ 2051	–
2	27 510 $\pm$ 3543	32 600 $\pm$ 8697	525 $\pm$ 291	3055 $\pm$ 389	82.8
3	33 265 $\pm$ 5476	31 300 $\pm$ 7026	560 $\pm$ 299	4270 $\pm$ 1304	86.9
4	40 185 $\pm$ 6221	40 495 $\pm$ 10 909	245 $\pm$ 151	6740 $\pm$ 1501	96.4
5	56 420 $\pm$ 7852	55 185 $\pm$ 11 003	25 $\pm$ 15	9930 $\pm$ 2260	99.7
6	46 370 $\pm$ 11 384	37 170 $\pm$ 8826	390 $\pm$ 290	7675 $\pm$ 2386	94.9
7	56 070 $\pm$ 14 763	53 765 $\pm$ 13 416	190 $\pm$ 86	4025 $\pm$ 1428	95.3
8	60 315 $\pm$ 12 923	53 515 $\pm$ 16 616	855 $\pm$ 359	3580 $\pm$ 1222	76.1
9	59 655 $\pm$ 15 475	58 495 $\pm$ 18 722	2275 $\pm$ 1125	6720 $\pm$ 1785	66.1

<sup>a</sup> 1: day fungal spore feeding started; 7: last day fungal spores fed.

<sup>b</sup> [(control  $L_3$  – fungus  $L_3$ )/control  $L_3$ ]  $\times$  100.

#### 4. Discussion

Results of these trials demonstrated that spores of *D. flagrans* were effective in reducing  $L_3$  (predominantly *H. contortus*) in sheep feces with relatively moderate to high FECs. Larsen et al. (1998) demonstrated a dose–response relationship where  $3 \times 10^5$  to  $10^7$  spores per day resulted in improvement of the reduction in larval development. In contrast, the results of the first trial did not show a dose–response but that all spore doses achieved a high level of larval reduction by the second day which was maintained during the period of spore feeding. There was one notable exception on day 6 for the  $5 \times 10^4$  dosage where reduction was 0%. This can be accounted for by one animal that had a high larval recovery on that day, and that animal may not have consumed the total dosage consistently as the amount of spore material for that dosage was very small. The decline in larval reduction was noticed within 1–2 days after spore feeding ceased, especially for the lowest dosage, and by 4–5 days essentially all activity was gone. This decline in activity concurs with that reported by Gives et al. (1998) and suggests that daily feeding of spores is necessary to maintain adequate larval reduction levels. This is logical in that spores would be evacuated from the gastrointestinal tract during that time period. This is also supported by the unpublished findings from a recent study with meat goats (S. Husted, Pers. Comm.), when daily feeding spores of *D. flagrans* was compared to feeding at 2- and 3-day intervals. It was quite clear that daily feeding was necessary to keep a constant high reduction. By feeding at 2-day intervals the reduction dropped, and severe fluctuations in larval reductions were observed when feeding the goats at 3-day intervals.

The results of the second trial demonstrated that the target dose of  $10^5$  spores of *D. flagrans* was highly effective in reducing  $L_3$  in feces even under very high FEC conditions. In a study with housed sheep where spores were infused via a rumen cannula, Larsen et al. (1998) found that levels between approximately  $1.5 \times 10^4$  and  $3 \times 10^5$  gave good reduction (>80%) in numbers of *Trichostrongylus colubriformis* larvae developing in

culture. Similarly, Faedo et al. (1997) found that comparable single doses of spores given orally effected high percentage reductions in subsequent fecal cultures. In the latter study, good survival through the gastrointestinal tract and high reduction capability was found only for *D. flagrans*, but not for fungi of the genus *Arthrobotrys* which were used for comparison.

The ultimate goal of using nematophagous fungi is to reduce the number of L<sub>3</sub> present on pasture which would result in decreased nematode burdens. The only published field trial involving the biological control agent *D. flagrans* (Githigia et al., 1997) clearly indicated that indeed it was possible to achieve considerably reduced burdens of most common parasitic nematodes of small ruminants on pasture. However, it also pointed towards some possible limitations in connection with species that dwell a long time inside the egg before or not ever being released onto pasture.

Results of both trials demonstrated that a very high level of larval reduction can be achieved within 2 days of starting fungus feeding that this level can be maintained with daily feeding, and that there is essentially only a day or two of limited residual activity once fungus feeding is discontinued. The nematode trapping fungus *D. flagrans* has potential as a biological control agent for *H. contortus*, one of the most pathogenic gastrointestinal nematodes leading to severe production losses in sheep. At this time, daily mixing of the fungal spores with a supplement feed is the method of choice and is best suited for those operations where such feeding can be implemented. Further work is needed to evaluate other methods of delivery.

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