Biological control of trichostrongylid infections in calves on pasture in Lithuania using *Duddingtonia flagrans*, a nematode-trapping fungus

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Abstract

The effect on the pasture contamination level with infective trichostrongylid larvae by feeding the nematode-trapping fungus, *Duddingtonia flagrans* at two dose levels to first time grazing calves was examined in Lithuania. Thirty heifer-calves, aged 3–6 months, were divided into three comparable groups, A, B and C. Each group was turned out on a 1.07 ha paddock (a, b and c). The paddocks were naturally contaminated with infective trichostrongylid larvae from infected cattle grazing the previous year. Fungal material was fed to the animals daily during a two month period starting 3 weeks after turnout. Groups A and B were given 10^6 and 2.5×10^5 chlamydospores per kg of live weight per day, respectively, while group C served as a non-dosed control group. Every two weeks the heifers were weighed and clinically inspected. On the same dates, faeces, blood and grass samples were collected. From mid-July onwards, the number of infective larvae in grass samples increased markedly ($P < 0.05$) on paddock c, whereas low numbers of infective larvae were observed on paddocks a and b grazed by the fungus treated groups. However, the results indicate that administering fungal spores at a dose of 2.5×10^5 chlamydospores per kg live weight per day did not significantly prevent parasitism in calves, presumably due to insufficient suppression of developing infective larvae in the faeces. In contrast, a dose of 10^6 chlamydospores per kg lowered the parasite larval population on the pasture, reduced pepsinogen levels ($P < 0.05$), and prevented calves from developing parasitosis.

Introduction

During the last 10–15 years, many experiments have been carried out to evaluate the potential of practical biological control of parasitic nematodes in grazing livestock using nematode-trapping fungi. A prerequisite for success using this approach is the availability of fungi that remain viable after passage through the alimentary tract of cattle and subsequently are able to trap the free-living stages of parasitic nematodes in the dung before they migrate onto herbage. As a result of *in vitro* and *in vivo* experiments carried out by Larsen *et al.* (1991, 1992) *Duddingtonia flagrans*, a particularly promising candidate was isolated. Using this, successful trials have been conducted in Denmark to control natural trichostrongylid infections in calves by feeding this isolate to animals (Wolstrup *et al.*, 1994; Larsen *et al.*, 1995; Nansen *et al.*, 1995). It has been shown that feeding chlamydospores of *D. flagrans* to calves during the first 2 or 3 months of the grazing season reduced the numbers of larvae on herbage and lowered the acquisition of parasites in the later part of the season, thus preventing outbreaks of

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clinical parasitic gastroenteritis. However, there is still a need to find an optimal dose of the fungus which will reduce the number of infective stages of gastrointestinal parasites sufficiently to protect grazing animals from suffering production losses. In a plot study by Fernandez *et al.* (1999a) two isolates (CI3 and Troll A) of *D. flagrans* were tested for their ability to reduce the number of infective larvae of cattle gastrointestinal nematodes on herbage. The fungus was used at a dose of 10^6 chlamydospores per kg of body weight. The study showed that a reduction in the number of larvae on the herbage ranged from 30.3% to 95.7% with isolate CI3, while the Troll A isolate reduction varied from 18.1% to 98.1% compared with fungus free control plots. In two recent field experiments performed in Denmark, the efficacy of *D. flagrans* against *Ostertagia ostertagi* was assessed (Fernandez *et al.*, 1999b). First-season calves grazing at two different stocking rates were fed chlamydospores at different dose-levels (10^6 (high dose), 5×10^5 (medium dose) and 2.5×10^5 (low dose) chlamydospores per kg of body weight). These studies demonstrated that a high dose of fungi is required to reduce the number of infective larvae when animals are grazing at high stocking rates while at a low stocking rate the reduction can be accomplished with the lower dose.

Under adverse climatic conditions, such as low temperatures, the fungi may not perform effectively and their ability to trap parasitic larvae could be reduced (Grønvold *et al.*, 1996; Fernandez *et al.*, 1999c). This may be the case occasionally under Lithuanian climatic conditions when the temperature may drop below 10°C in the early part of the grazing season (Šarkūnas *et al.*, 1998). The purpose of the present investigation was to examine the effect of the nematode-trapping fungus *D. flagrans* on a mixed trichostrongylid population when groups of calves, grazing naturally contaminated pasture in Lithuania, were fed two different doses of fungal spores.

Materials and methods

Experimental design and animals

The experiment was conducted in 1997 on a private dairy farm situated 59 km east of Kaunas, in Kaišiadorys District, Lithuania. A permanent pasture which was grazed the preceding year by young stock and was naturally infected with *Ostertagia* spp., *Cooperia* spp. and *Nematodirus* spp., was used for the experiment. The size of the experimental pasture was 3.2 ha. Fertilizer corresponding to 200 kg of N:P:K (25:3:6) per ha was applied during late April. Before the start of the grazing season (May–September), the area was divided into three equal 1.07 ha paddocks, a, b and c.

Thirty parasite naive cross-bred Holstein/German Black Pied/Lithuanian Black Pied calves, aged 3–6 months, were turned out on 13 May. Before the start of the experiment, calves were allocated according to weight into three comparable groups (mean weight ±SD of group A: 96 ± 22 kg; group B: 96 ± 24 kg and group C: 96 ± 25 kg). The groups grazed paddocks a, b and c, respectively, at the initial stocking rate of 960 kg per ha. Three weeks after turnout, feeding with fungal material to groups A and B was initiated. Once a day, each of the two groups received fungal material thoroughly mixed with 5 kg of supplement (500 g of ground barley per calf per day). Group A was given 10^6 chlamydospores per kg of live weight (high dose) daily, while group B received 2.5×10^5 chlamydospores per kg of live weight (low dose). The material was given in fodder troughs of sufficient size to allow all animals in the groups to feed at the same time. Group C received an equal amount of supplement but without fungal material. The feeding of all three groups continued for two months. The animals were observed while being fed to check whether they all consumed the material offered.

Fungal material

The *D. flagrans* isolate was cultivated on millet seeds by Christian Hansen BioSystems Ltd., Hørsholm, Denmark. The fungal doses were calculated according to the animal's live weight at turnout and gradually adjusted at an expected individual daily weight gain of approximately 650 g during the fungus feeding period. Batches of fungal material were prepared for each group of ten animals and kept in sealed aluminium foil bags at room temperature. Shortly before feeding the animals, the material was mixed with the supplement.

Parasitological analysis

Herbage samples, collected from each paddock for determination of number of infective trichostrongylid larvae (L3), blood and faecal samples, were all obtained fortnightly. Each grass sample consisted of 200–400 g of grass collected while walking across the paddocks in a W-shaped pattern picking a sub-sample for every ten steps. Grass within 20 cm of dung pats was avoided. Using a modified version of the agar gel technique described by Mwegoha & Jørgensen (1977), but without bile, L3 larvae were isolated, counted, differentiated and the results expressed as numbers of larvae per kg of dried herbage. The number of nematode eggs per gram of faeces (EPG) was determined using a modified McMaster technique (Henriksen & Aagaard, 1976). Third stage larvae for identification of the nematodes to genus, were obtained in July from bulk group faecal samples. Faecal cultures were established using the Henriksen & Korsholm (1983) procedure. Quality control of the chlamydospore material used was performed in May, June and July at the Centre for Experimental Parasitology, Denmark. Subsamples of the spore material from the same batches used for the Lithuanian study were fed to a group of calves at 10^6 chlamydospores per kg live weight (Fernandez *et al.*, 1999b) and the ability to reduce infective larvae in faecal cultures was monitored. Levels of serum pepsinogen were determined by the procedure described by Ross *et al.* (1967).

Other observations and statistical analyses

On each sampling day, calves were weighed on a portable scale and a clinical examination was performed. Data on daily precipitation and average temperature

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were obtained from a meteorological station situated 17 km from the experimental area. For comparison, data on precipitation and average temperatures during a 30 year period (1961–1990) were obtained from the Kaunas Meteorological Station.

Faecal egg counts (log (x + 1) transformed) and blood serum pepsinogen levels (log (x) transformed) were analysed by repeated measures analysis of variance (ANOVA), using SAS version 6.08 (Statistical Analysis Systems, 1991). Thus the results were tested for the between-group effect of pasture treatment, the within-animal effect of time and the time-group interaction.

Results

Fungal effects in faecal cultures, faecal egg counts and herbage larval recovery

A significant effect of the fungus in faecal cultures was recorded from Denmark (Fernandez *et al.*, 1999b) using sub-samples of the fungus from the same batches as used in Lithuania. The reduction in numbers of infective larvae in the faecal cultures in May, June and July was 61%, 97% and 87% respectively, in faeces from calves treated with the high fungus dose (10^6 chlamydospores per kg of live weight per day).

Trichostrongylid egg counts in faeces (fig. 1) exhibited a steep rise from the beginning of June reaching a peak value of approximately 500 EPG in groups B and C at the beginning of July while those of group A remained lower. The EPG in all three groups decreased during July and August to levels of approximately 150, a level maintained during the rest of the grazing season. Results from qualitative analysis of trichostrongylid larvae in faecal cultures showed a predominance of *Cooperia* spp. over *Ostertagia* spp. in all three groups of calves. *Nematodirus* spp. eggs were found in small numbers in all three groups. Other nematode eggs occasionally

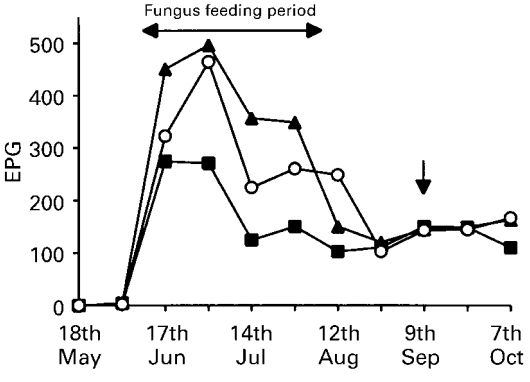

Fig. 1. Mean faecal trichostrongylid egg counts for three experimental groups of calves expressed as the number of eggs per gram of faeces (EPG). ■, Group A (calves given a high fungus dose of 10^6 chlamydospores per kg of live weight per day); ▲, group B (calves given a low fungus dose of 2.5×10^5 chlamydospores per kg of live weight per day). ○, group C (control calves). The vertical arrow indicates the time of removal of two calves from each group.

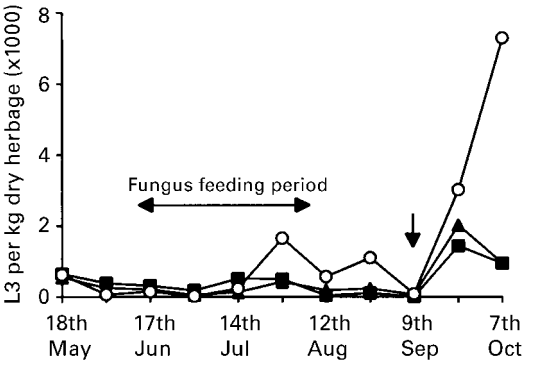

Fig. 2. Mean herbage trichostrongylid larval counts for three experimental plots. ■, Plot a (calves given a high fungus dose of 10^6 chlamydospores per kg of live weight per day); ▲, plot b (calves given a low fungus dose of 2.5×10^5 chlamydospores per kg of live weight per day); ○, plot c (control calves). The vertical arrow indicates the time of removal of two calves from each group.

encountered in small numbers throughout the trial were *Strongyloides* spp. and *Trichuris* spp.

Herbage larval counts were low and comparable in all three groups until mid-July (fig. 2). After this, the number of larvae increased on pasture c, which was grazed by the control calves while numbers of larvae remained low throughout the study in paddocks a and b, grazed by fungus treated calves. In September, the numbers of larvae on herbage exhibited a further steep rise on paddock c.

Performance and clinical observation

The mean live weight gains in all three groups of calves were similar throughout the grazing season (fig. 3). However, the calves in group A treated with the high dose performed slightly better than the other groups from mid-August to early September. After the withdrawal of two animals from each group on 9 September, the growth rate stagnated in group A but increased slightly in groups B and C. The reduction in group sizes followed clinical observations of disease at the beginning of August when most calves in group C (the control animals) and three animals in group B developed loose

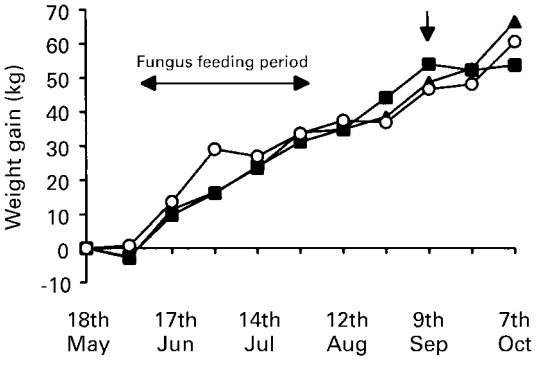

Fig. 3. Mean cumulative body weight gains of the three experimental groups of calves (see fig. 1 for key to symbols).

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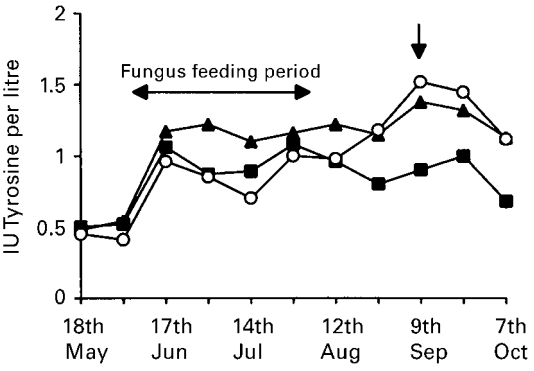

Fig. 4. Mean serum pepsinogen levels of the three experimental groups of calves (see fig. 1 for key to symbols).

stools and started to scour. At the end of August clinical signs, potentially indicative of parasitic gastroenteritis, were obvious in two animals of the control group. They were withdrawn from the experiment and treated with albendazole. In order to ensure comparable stocking rates of the three groups, two animals having similar weight gains were withdrawn from each of the groups A and B. No clinical symptoms were observed among calves in group A treated with the high dose of fungus.

Serum pepsinogen levels

Serum pepsinogen levels were relatively low and similar during the experiment in all three groups (fig. 4). From the second part of August and onwards the pepsinogen levels in the high dose group A, were significantly lower than those of the control animals ($P < 0.05$), when tested by repeated measures analysis of variance.

Other recordings

As a consequence of reduced rainfall in the early part of the summer (May) the availability of grass was low in July. Scarcity of grass on all three experimental plots became marked towards the end of August, which also had a lower than normal rainfall (the actual precipitation in August was 52 mm, compared with the average value of 81 mm). The precipitation increased towards the end of September (the actual precipitation in September was 76 mm, compared with the average value of 59 mm), but this did not visibly increase the availability of herbage. The withdrawal of two calves from each of the groups on 9 September reduced grazing pressure in all three paddocks.

Discussion

The present investigation showed that dosing calves with *D. flagrans* spores led to significantly reduced herbage infectivity and prevented clinical outbreaks of parasitic gastroenteritis provided a dose of 10^6 chlamydospores per kg live weight per day was applied. This investigation supports the results reported by Wolstrup *et al.* (1994), Larsen *et al.* (1995), Nansen *et al.* (1995) and Fernandez *et al.* (1999b) in Denmark.

At turn-out, all calves were exposed to a similar level of overwintered herbage larval contamination on all three plots (fig. 2). However, in the second half of July herbage larval contamination in paddock c grazed by the control group started to increase. This is in agreement with observations from an earlier study performed in Lithuania (Šarkūnas *et al.*, 1999). In contrast, herbage larval contamination on both pastures grazed by fungal treated calves remained low throughout the grazing season. Thus, the results suggest that the differences in herbage larval infectivity between control and fungus treated plots may be due to the nematode-trapping effect of the fungus in the faeces. This was supported by the results from *in vitro* faecal cultures performed in Denmark using the same fungal batches (Fernandez *et al.*, 1999b).

During the fungus feeding period the average daily weight gain of the calves was 584 g, which was lower than the expected weight gain of 650 g per day. Thus, the actual fungus spore dose per kg of live weight was higher compared to the dose calculated prior to the start of the study. Irrespective of this, the fungus did not appear to have any obvious effect on the weight gains of the treated calves. In fact, at the beginning of September, the slightly higher weight gain of the calves in group A for some reason was reversed. This is particularly strange as the grazing pressure was reduced by removing two calves from each of the paddocks which seems to have contributed to improved average weight gains of calves in group B and C. If the two animals had not been removed, we would presumably have seen a decrease in average weight gains of control calves as demonstrated by Nansen *et al.* (1995).

At the end of the fungus feeding period, low precipitation was probably responsible for reduced transmission of larvae to the herbage (Grønvold, 1984, 1989; Grønvold & Høgh-Schmidt, 1989) with a subsequent delay in the uptake and development of trichostrongylid infections in the calves. In spite of this, the number of infective larvae on herbage had increased slightly in the paddock grazed by the control animals. A similar suppression of transmission was also seen in August due to reduced precipitation. This was also reflected in a delayed increase in pepsinogen levels in the control group. In general, pepsinogen levels were not very high which could be due to *Cooperia* spp. being predominant over *Ostertagia* spp. as seen in the faecal cultures from all three groups set up in July. Since all animals used in the experiment were owned by a private farmer the calves were not available for post-mortem analysis. Hence, we were not able to determine actual worm burdens in the grazing animals.

This study confirms the results reported by Fernandez *et al.* (1999b) which showed that the number of infective larvae on pasture was more suppressed when calves were given 10^6 chlamydospores compared with 5×10^5 or 2.5×10^5 chlamydospores per kg of body weight. Fungal spores at the dose of 2.5×10^5 chlamydospores per kg live weight per day did not fully suppress the level of parasitism, while at the high dose level (10^6 chlamydospores per kg of live weight per day),

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the fungus suppressed the parasite larval population and prevented clinical outbreaks of trichostrongylid infection in the calves.

In many intensive grazing systems in Lithuania where supplement feeding of cattle is applicable, the biological control of trichostrongylid infections in first-season grazing calves may be a realistic option in an attempt to limit the usage of anthelmintics in the future. However, further experiments are needed to test the effect of the fungi when biological control is integrated into different parasite control strategies. The impact of high and low temperatures on the performance of the fungus exposed to Lithuanian climatic conditions should also be examined.

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