Original article

Effect of the nematophagous fungus, *Duddingtonia flagrans*, on the larval development of goat parasitic nematodes: a plot study

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Abstract – Effective alternatives to anthelmintic treatment against nematode parasites of goats are required because of the high prevalence of benzimidazole resistance. Towards this objective, the nematophagous fungus, *Duddingtonia flagrans* (Df), was used in a plot study against two main parasitic nematode species of goats, *Teladorsagia circumcincta* (Tcir) and *Trichostrongylus colubriformis* (Tcol). Worm-free, culled goats were experimentally infected with strains of Tcir and Tcol to constitute donors. Half of the animals were periodically given Df chlamydospores at a daily dose of 2.5×10^5 spores/kg BW while the remaining animals were kept as controls. At 5 time periods i.e. March, May, July, September and November 2001, corresponding to the main grazing season in France for goats, faeces were collected from the 6th day of fungus administration for the following 2 days to obtain approximately 1 kg of faeces from each group of animals: Tcir/Control, Tcol/Control, Tcir/Fungus, Tcol/Fungus. For each period and each group, the faeces were deposited on a 1 m^2 grass plot and the grass was cut (3 replicates) on weeks 2, 4, 6, 8 ,12 after deposition, for infective larval recovery. Larvae were counted and the results were expressed as a ratio of larvae/eggs deposited. On the plots with the control faeces deposited in March, July and September, the grass infectivity due to Tcir and Tcol was similar and the maximum number occurred between 2 and 4 weeks post deposition. In May, the maximum numbers of larvae were not recorded until 8 weeks after deposition, due to high daily temperatures and dryness. In November, larval development took place only for Tcir. On the plots with the fungus treated faeces, a significant reduction in grass infectivity occurred for both nematodes and ranged from 50–60% in May, July and November deposits to 80–90% in the September deposit. On the contrary to these findings, no difference was recorded between the fungus and control plots for the March deposit. In conclusion, *D. flagrans* is suitable for reducing the number of infective larvae in the herbage during the main part of the grazing period for the most important digestive nematodes of goats.

goat / biological control / *Duddingtonia flagrans* / nematode parasite

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1. INTRODUCTION

Nematode infections represent an important threat for grazing dairy goat production systems in France. Among domestic ruminants, goats are undoubtedly the less resistant hosts to nematode infection since the intensity of infection does not tend to decrease with age and repeated contact with the parasites in either natural [21] or experimental conditions [14]. As a result, dairy goat flock owners treat their animals with anthelmintics very frequently and consequently resistant nematode populations are prevalent in most farms at least for benzimidazole compounds [1, 3]. In order to decrease the selection pressure on worm populations for the remaining available anthelmintics, some alternatives have to be found to control nematode infections and thus provide an opportunity to reduce anthelmintic use. Biological control with the nematophagous fungus *Duddingtonia flagrans,* represents a possible method to control the development of nematode larvae on pastures [15]. When herbivores (cattle, sheep, horses) are fed *D. flagrans* chlamydospores, these spores survive the passage through the gastrointestinal tract and grow in faeces while forming nematode trapping structures that produce a major reduction in larval numbers [15]. Preliminary results in goats have shown that *D. flagrans* is able to significantly decrease the percentage of larval development of *Teladorsagia circumcincta* in laboratory coprocultures [18]. Nevertheless, these results were obtained in laboratory conditions and data are still lacking about the activity of *D. flagrans* in outdoor situations when exposed to temperature and precipitation prevailing during a whole grazing period in western France. Such plot studies are available for Danish or Australian conditions with sheep or cattle [5, 6, 8].

The objective of this work was thus in a plot study, to subject the most important nematode parasites of goats in France, namely *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* in monospecific infections, to *D. flagrans* in a range of environmental conditions experienced during the grazing season.

2. MATERIALS AND METHODS

2.1. Animals and experimental infection

Eight culled Alpine dairy goats were purchased from a private farm at the beginning of the experiment. The animals were bred in a zero grazing system and their nematode-free status was checked through repeated coproscopical examinations. The goats were kept on a concrete floor in 4 boxes of 2 animals each and were fed barley (150 g), lucerne pellets (120 g), barley straw and water *ad libitum* daily. Half of the animals were infected orally with third stage larvae (L3) of *T. circumcincta* while the remaining were given L3 of *T. colubriformis,* the infective dose rate ranging from 16 000 to 20 000 L3 per goat. The strains used came from INRA Tours (France) and were of sheep origin. They were chosen since they represent the two most important nematode species of the digestive tract in dairy goats in western France [2] and were used in monospecific infections in order to avoid any interaction between the nematode species in the host and to assess eventual differences in larval development in both control and fungus plots. The goats were infected twice during the whole study in February (start of the experiment) and June. Because of a severe loss of weight, one goat from each nematode group was replaced by another animal infected with the same strain (between July and August).

2.2. Administration of *Duddingtonia flagrans* chlamydospores

The *D. flagrans* Troll A strain of Danish origin (Chr. Hansen A/S, Denmark) was used in this study. One week before each experimental period (March, May, July,

Table I. Estimation of nematode egg deposition on grass plots of 1 m^2 according to the 4 experimental groups and the 5 deposition periods.

Date of faecal deposition	Estimated number of nematode eggs deposited/m^2			
	Trichostrongylus-control (Tr-c)	*Teladorsagia*-control (Te-c)	*Trichostrongylus* fungus (Tr-f)	*Teladorsagia*-fungus (Te-f)
March (28/03/01)	1 683 392	310 096	753 104	487 296
May (23/05/01)	1 220 570	58 872	186 843	474 939
July (25/07/01)	579 133	119 270	913 500	317 310
September (27/09/01)	242 240	212 000	1 370 192	413 600
November (14/11/01)	484 000	16 150	1 182 447	142 576

September, November), half of the animals (2 infected with *Teladorsagia* and 2 infected with *Trichostrongylus*) were given *D. flagrans* chlamydospores daily at a dose rate of 2.5×10^5 spores/kg BW. Fungus spores were mixed with 20 g of mineral powder (P: 30%, Ca: 16%, Mg: 4%) in order to facilitate the intake and given in a trough. The complete ingestion of the mixture was carefully checked before giving the remaining food to the animals. Faecal collection was performed on days 6 and 7.

2.3. Faecal depositions on the pasture

A grass plot (11 m/9 m) that had never been grazed by ruminants was selected and fenced in, in near proximity to the laboratory. The vegetation was comprised mainly of dandelion, clover and grasses. For each of the 5 experimental periods, a row of 4 grass squares of 1 m^2 was defined accordingly: *Trichostrongylus*-control (Tr-c), *Teladorsagia*-control (Te-c), *Trichostrongylus*-fungus (Tr-f), *Teladorsagia*-fungus (Te-f). Each square was separated from the others by a distance of 1 m. Five rows were used during the study which corresponded to the five faecal deposition periods: March (Spring), May (late

Spring – early Summer), July (Summer), September (Autumn), November (late Autumn – early Winter). Before each faecal deposit, grass height was adjusted to about 5–10 cm. At each experimental period, faecal pellets were collected *per rectum* every two hours from each pair of goats during two days in order to obtain about 1 kg of faeces for each group. Nematode faecal egg output was determined by the McMaster technique [20] on three subsamples of the pooled faecal sample to evaluate the mean faecal contamination for each group at each period (see Tab. I). The faecal pellets were then evenly spread on the 1 m^2 square. A grass sampling frame was constructed with horizontal and vertical strings attached every 25 cm, to achieve 16 subsquares for individual sampling.

2.4. Grass sampling and laboratory procedures

For each deposition period, 5 grass sampling times were conducted, namely: 2, 4, 6, 8 and 12 weeks after deposition. On each of these occasions, 3 subsquares (replicates) per square were randomly selected for individual grass sampling. The herbage on each selected subsquare was entirely removed up to the ground with scissors and

the samples subsequently were soaked overnight at room temperature in 3 L of tap water containing two drops of detergent. The grass was then removed and washed. The washing and soaking water was then poured into three successive sieves (mesh size: 1000, 125 and 20 μm) to obtain about 100 mL and this was reduced by centrifugation to 20 mL by removal of the supernatant. This latter material containing the recovered larvae was processed according to the technique described by Eysker and Kooyman [4] with conical tubes of 55 mL. Briefly, two successive centrifugations (10 min, 1800 g) were performed, the first one using a Giemsa stained sucrose solution ($d = 1.20$), which enabled the L3 to be removed at the interface between the water and sucrose, followed by a second step using only water which allowed the collection of a pellet containing the L3. The infective larvae were counted after adding a few drops of iodine and the results were expressed as the ratio of the absolute number per subsquare/number of eggs deposited.

2.5. Statistical analysis

The mean numbers of larvae on the plot subsquares (3 replicates) were compared for each species of nematodes i.e. *T. circumcincta* and *T. colubriformis* and between the fungus and control plots at each date of sampling. Comparisons were made using the Mann-Whitney test ($p < 0.05$).

2.6. Meteorological data

Meteorological data were registered at the local meteorological service (Météo-France). Mean rainfall (mm), minimum and maximum temperatures (°C) were expressed per period of ten days.

3. RESULTS

Mean *Teladorsagia* or *Trichostrongylus* larvae recovered from pasture plots (3 rep-

licates) and expressed as a % of L3/number of eggs deposited are presented in Figures 1 and 2. Meteorological data for each sampling period are summarised in Figure 3.

3.1. Numbers of *T. circumcincta* and *T. colubriformis* larvae recovered from control plots

The patterns of larval occurrence in grass plots differed according to the dates of deposition. For the March deposit, the maximum in nematode larvae occurred between 4 and 6 weeks with significant differences between *Teladorsagia* and *Trichostrongylus*, the latter species showing lower values 6 and 8 weeks after faecal deposition. The first 30 days of this period corresponded to low temperatures (mean minimum temperatures around 6 °C and mean maximum temperatures around 15 °C). For the May deposit, no larvae were recovered from the grass until 8 weeks post-deposition when a sharp increase for both species occurred. The first part of this period was characterised by relatively high temperatures (26 °C for the mean maximum temperature during the first ten days) and importantly almost a total absence of rainfall. The occurrence of larval peaks corresponded to heavy rainfall (95 mm for the 10 days period). For July and September deposits, the occurrence of *Trichostrongylus* larvae was earlier and higher compared to *Teladorsagia* ($p < 0.05$) and for both species the larval peaks were between 2 and 4 weeks post-deposition. For both periods, rainfall was relatively high and steady during the first 30 days with values ranging from 25 to 58 mm per 10 day period while mean minimum and maximum temperatures varied from 15 to 25 °C in July deposits to 10 °C to 20 °C in September deposits. For the November deposit, no *Trichostrongylus* larvae were recovered at any time of sampling whereas *Teladorsagia* larval numbers increased slowly from 4 weeks post deposition onwards. The rate of
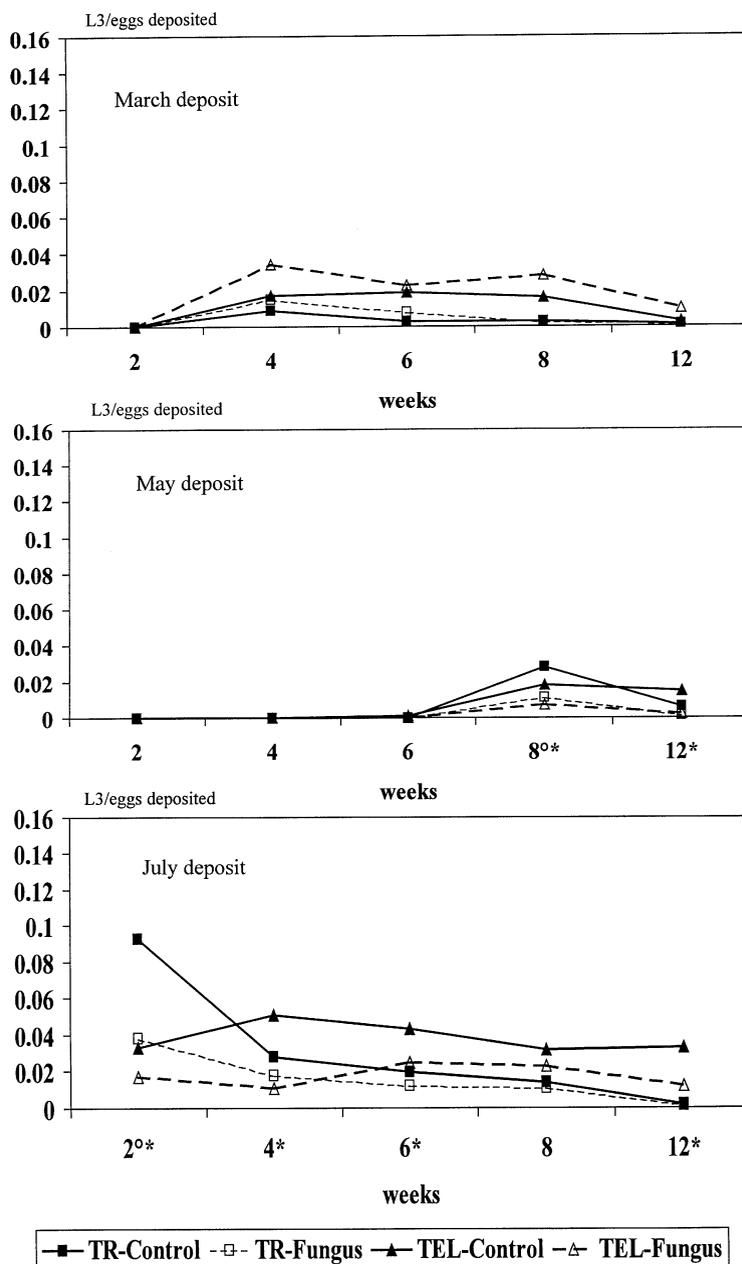
Figure 1. Mean nematode larvae recovered from pasture plots (mean of 3 replicates) according to the strongyle species (*Trichostrongylus*-TR, *Teladorsagia*-TEL) and the presence of *Duddingtonia* spores in faeces (Fungus) or not (Control). Periods of March, May and July.
°,* Significant difference between fungus and control grass samples for *Trichostrongylus* and *Teladorsagia* respectively.
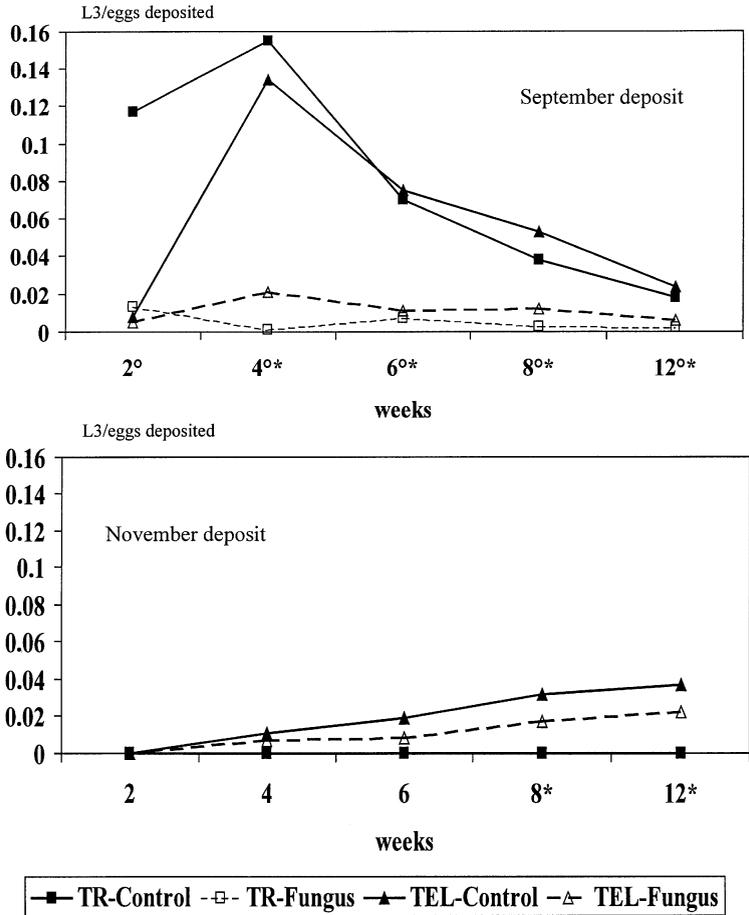
Figure 2. Mean nematode larvae recovered from pasture plots (mean of 3 replicates) according to the strongyle species (*Trichostrongylus*-TR, *Teladorsagia*-TEL) and the presence of *Duddingtonia* spores in faeces (Fungus) or not (Control). Periods of September and November.
°,* Significant difference between fungus and control grass samples for *Trichostrongylus* and *Teladorsagia* respectively.

development for *Teladorsagia* was between 0.03 and 0.04 L3/eggs deposited.

3.2. Numbers of *T. circumcincta* and *T. colubriformis* larvae recovered from fungus plots

No significant difference in larval numbers was seen between fungus plots and

control plots for both *Teladorsagia* and *Trichostrongylus* for the March deposit. In May and July deposits, significantly lower levels in larval numbers were seen in the fungus-treated plots for both nematode species. The reduction in the grass infectivity peaks due to *Duddingtonia flagrans* was about 50–60% and 60–78% in May and July deposits respectively, when
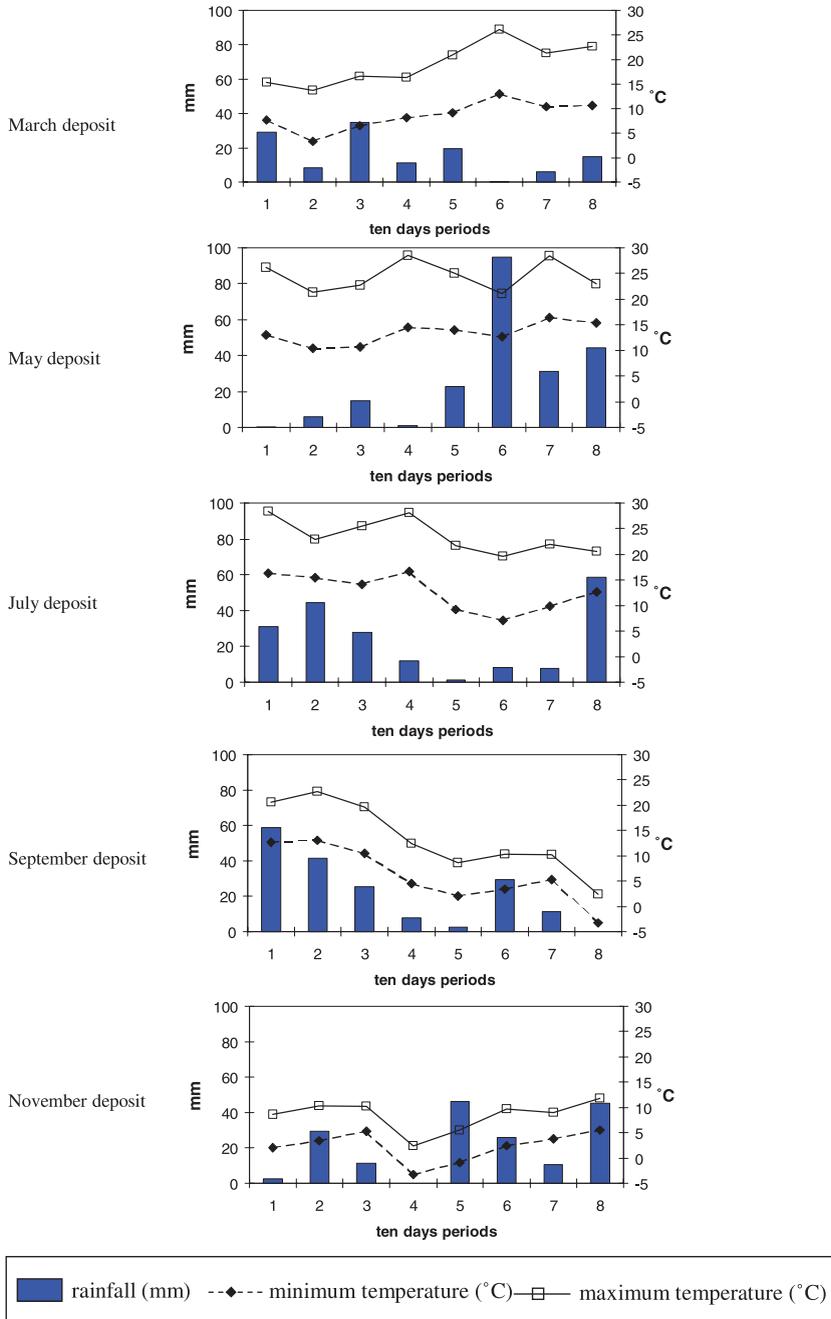
Figure 3. Mean rainfall, minimum and maximum temperatures (period of ten days) during the five periods of study.

compared with the control plots. In September deposit, the numbers of larvae on the fungus-treated plots were very low throughout the whole period, the differences being significant for all sampling dates except for *Teladorsagia* 2 weeks after deposition. The percentage of reduction in larval numbers at the peak period due to *Duddingtonia* ranged from 84% to 93% for *Teladorsagia* and *Trichostrongylus* respectively. In the November deposit, significant lower larval numbers were seen in the fungus plot for *Teladorsagia* 8 and 12 weeks post deposition giving a reduction percentage compared to the control of about 50%.

4. DISCUSSION

The development of *T. circumcincta* and *T. colubriformis* eggs into larvae in this plot study showed different patterns according to the deposition periods. During the relatively cold months, corresponding to the March and November deposits, the development of *T. colubriformis* eggs was nil, or significantly lower than for *T. circumcincta*. In November, when no *T. colubriformis* egg development occurred, the minimum temperatures were always below 5 °C whereas maximum temperatures never exceeded 10–11 °C. Conversely for the faecal deposits of July and September, when temperatures were higher, the development of *T. colubriformis* eggs was faster than those of *T. circumcincta*. Rossanigo and Gruner [22] showed that the temperature requirements were somewhat different between *Teladorsagia* and *Trichostrongylus*. The former species developed well at temperatures between 5 and 30 °C with an optimum at 23 °C whereas the latter species had a higher temperature optimum at 28 °C with a very low development when the temperatures dropped to 5–10 °C. These differences in temperature requirements for the larval development between the two nematode species is reflected by the epidemiological pattern of nematode infection on pasture in grazing conditions prevailing in the western part of France, with a predominance of *T. circumcincta* larvae in the Spring and two peaks of *T. colubriformis* at the end of the Summer and beginning of the Autumn [13].

Faecal material derived from animals fed *D. flagrans* chlamydospores at a dose rate of 2.5×10^5 spores/kg BW, generally resulted in lower numbers of *T. circ* and *T. col* larvae on the pastures, compared with the control plots. This was particularly the case for the faecal deposits of May, July and September with a reduction in larval numbers from 50 to 93% compared to the control. These periods of time probably correspond to the temperature requirements of *D. flagrans* for hyphal growth rate and trap production which are between 15 and 30 °C [9, 11]. However, for the May deposit, no larvae were recovered until 8 weeks after faecal deposition in the control plot, when a sharp increase occurred with a rather low percentage of development of the eggs into L3 (0.018 to 0.028 L3/eggs deposited according to the nematode species). This suggests that the development of the larvae was impaired and took place within the faeces without migration of larvae to the surrounding grass. Such observations have been made by Mounport et al. [17] in the South of France in Mediterranean garrigues where faeces represented the main reservoir of infective larvae during the dry periods of the grazing season. This delay in migration for several weeks could lead to a reduction in the production of the nematode trapping structures by the fungus, that has been reported to occur after 2 or 3 weeks exposure to such conditions in the laboratory [11]. In addition, reduced fungal growth due to the low humidity might further explain the moderate (50–60%) efficacy of *D. flagrans* at that time. Similarly Faedo et al. [6] showed that *D. flagrans* had an unreliable activity on *Nematodirus* because of the delayed hatching of the eggs, when the fungus was no longer present or was no longer able to trap the infective larvae.

When considering the faecal deposits of March and November, the efficacy of *D. flagrans* in reducing larval numbers on grass was irregular, ranging from 0 to 50%. According to Gronvold et al. [11], the development of mycelium and traps is reduced when the temperature is around 10 °C, or below. This situation was prevailing from November when minimal and maximal temperatures were about 3.5 and 10 °C respectively. However significant reductions in *T. circ* larvae due to *D. flagrans* were observed 8 to 12 weeks post deposition. In contrast, the absence of activity of *D. flagrans* in March is difficult to explain since climatic conditions were better for fungus growth and trapping than in November. Such apparent variations in *D. flagrans* activity without a suitable explanation were already pointed out by Fernandez et al. [8] in 3 plot studies with *Ostertagia ostertagi*.

The dose of chlamydospores used in our study i.e. 2.5×10^5/kg BW, gave satisfactory results in various studies where efficacy was determined through coprocultures. For example, Pena et al. [19] showed a 100% reduction on *Haemonchus contortus* larvae. Similarly, Larsen et al. [16] obtained 80% reduction or more on *T. colubriformis* with 1.5×10^4 to 3×10^5 spores given through the rumen cannula. Conversely, results from plot studies with sheep nematodes were variable. Faedo et al. [5] indicated a 43% reduction in grass infectivity with 100 000 to 125 000 chlamydospores/kg BW on *H. contortus* whereas Faedo et al. [6] with 10^6 spores/kg BW found a high reduction (80–90%) on *Teladorsagia/Trichostrongylus*. As a result, when different dosages of spores/kg BW were compared in field studies, the administration of 10^6 spores proved to be very successful in controlling clinical disease in the first-season grazing calves while lower dosages between 2.5 and 5×10^5 spores did not [7, 23]. According to Githigia et al. [10], the dose of 10^6 spores given to lambs infected with *Teladorsagia/Trichostrongylus* during a 4 months grazing period reduced the pasture larval counts by 78% at the end of the experiment. The variability in larval count reduction due to the fungus observed in our study was thus probably due to meteorological factors as well as sub-optimal *Duddingtonia* spore dosage.

The implementation of *D. flagrans* biological control in dairy goat farms needs further investigations, particularly to determine the optimal chlamydospore dosage as it has been done in other ruminants [15]. This factor is crucial since many other factors of variation could occur, such as the heterogeneity of the spore concentration in the food given to the animals, as well as individual variations in food intake. Another important point to consider is to determine an eventual environmental impact on the fauna existing in and around decomposing faeces when treated with the fungus. A preliminary survey seems to indicate an absence of a short-term impact at least for the earthworm, *Aporrectodea longa* [12].

The results of the present study indicate that *D. flagrans* is suitable to reduce the number of infective larvae on the herbage during the main part of the grazing period for the most important gastro intestinal nematodes of goats in France.

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